THE UNIVERSITY *of* EDINBURGH

Tyrosine Derivatives and their Anti-Cancer Applications

Thesis Submitted in Accordance with the Requirements of The University of Edinburgh for the Degree of Doctor of Philosophy

By

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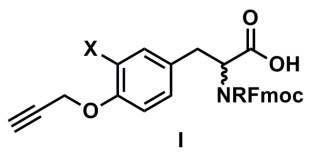
2012

Declaration

I hereby declare that, except where specific reference is made to other sources, the work contained within this thesis is the original work of my own research since the registration of the PhD degree in July 2007, and any collaboration is clearly indicated. This thesis has been composed by myself and has not been submitted, in whole or part, for any other degree, diploma or other qualification.

Sarah Boys

Abstract

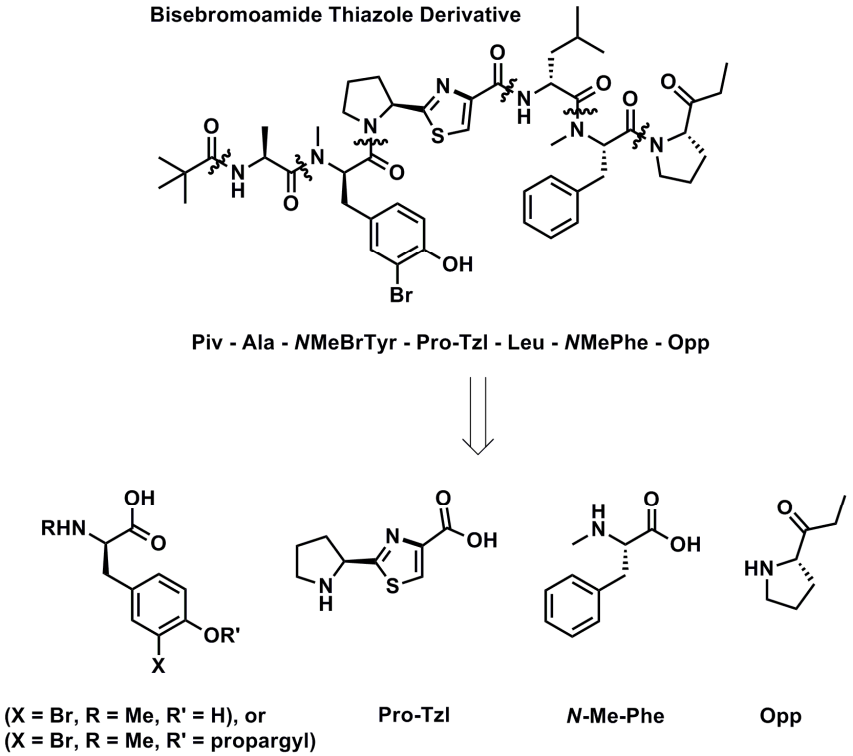


I

The incorporation of a propargyl group to a natural product target allows for a streamlined approach to the investigation of structure activity relationships (SARs) and target identification in forward chemical genetics programmes using a 'click'-based approach. To this end, an efficient synthesis of *O*-propargylated tyrosine derivatives was designed, and these have been used in the construction of peptide motifs both (a) derived from phage display libraries and (b) found in natural products.

The L-tyrosine derivative **Y*** (compound **I**, X=H, R=H) was incorporated into a peptide sequence, PTTIYY, which is known to prevent the inhibition of p53 by the AG-2 protein. **Y*** has been included as both the terminal and the internal tyrosine in the peptide sequence. ELISA assays were carried out to determine how the binding of PTTIY**Y*** and PTTI**Y***Y to AG-2 compared to that of the un-marked PTTIYY sequence. The results of these assays allowed new conclusions to be drawn regarding the important binding features of the peptide and possible sites for further optimisation of the AG-2 binding properties of this peptide through 'click' functionalisation of the modified tyrosine. The binding of the peptides incorporating **Y*** was also assessed using MCF-7 breast cancer cell lysate, known to contain the AG-2 protein. These results confirmed those seen for the purified AG-2 ELISA.

The related bromo-D-tyrosine derivative (compound **I**, X=Br, R=Me) has been prepared and employed towards the synthesis of a bisebromoamide derivative. Bisebromoamide is a newly discovered polypeptide, and a promising anti-cancer agent. The bisebromoamide derivative contains a thiazole unit (Tzl), two *N*-methylated amino acids, and an oxopropyl pyrrolidine (Opp) moiety, which is unique to bisebromoamide in natural products. The activity of this bisebromoamide derivative will be investigated *via* 'click'-based affinity chromatography using a new supported linker recently developed within the Hulme group.


Bisebromoamide Thiazole Derivative
Piv - Ala - NMeBrTyr - Pro-Tzl - Leu - NMePhe - Opp
(X = Br, R = Me, R' = H), or
(X = Br, R = Me, R' = propargyl)
Pro-Tzl
N-Me-Phe
Opp

Acknowledgements

I would like to thank Dr. Alison Hulme for her supervision, patience in assisting me with the preparation of this thesis, and assurance since my time as an undergrad. Thanks to the BBSRC for funding my research.

I am very grateful to Dr. Stefano Sabatini for his generosity in time and helpfulness in teaching me how to make peptides, and for further advice from Dr. JuanJo Diaz-Mochon and Mariona Valles. Thanks to Dr. Antonio Bella and Conny Johansson for assistance with MALDI-TOF MS, and Dr. Miren K. Aiertza Otxotorena for HPLC support, and various other Bradley group members who've looked after their mass spec, which I have relied on heavily. I am also very grateful to the Hupp and Ball groups at the Edinburgh Cancer Research Centre for welcoming me whilst carrying out my biological investigations in their labs. Thank you in particular to Nicolas Petitjean and Dr. Euan Murray for direction, suggestions and tolerance while trying to teach a chemist to do biology.

Thank you to my fellow Hulme group members, especially Felicia and Jill who've been through it all with me, and managed to not become 'third year monsters', if only just! Thanks to the project students I worked with, Claire Mortimer and Alan Healy, good luck to you both in your Ph.D.s. I wish all the best to the rest of the Hulme group; Nico, Helen, Lore, Sarah T, Heather and Kevin. Millions of thanks also to our former post-docs, Emiliano Gemma and Odile Meyer.

Thank you to all the boys in stores, Billy, Stuart the 'glass-bowler', Stuart Mains for often leaving me bewildered with his truly random comments, Alan Taylor and Paul Angus for mass specs, Juraj Bella for NMR help, and for seeing the funny side when I tried to destroy his brand new spectrometer with my tweezers.

Thank you to all the other chemists who've helped to make it an enjoyable experience, including little Ieva and Blondie, Leszek, Yi Wang, Max most-inspiring-presenter von Delius and all of the Lam group lunch crew old and new. In particular, I would like to give Nico a third mention, to say thanks for all your help especially

over the last year, with weekend laboratory entertainment and providing me with numerous bits of vital information at the drop of a hat – h^{ee} heeee!

My eternal gratitude goes to Dr. Stuart Gebbie for all advice, support, mocking and apparently believing in me, also for an unforgettable trip to Boston! Thanks to Dr. Dave Gibson for warning me against doing a Ph.D., but nevertheless encouraging me on my way through and not saying "I told you so" when I've complained! I also appreciate the many cups of tea, biscuits and lunches shared over the last 8 years with my fellow KB residents, divers and good friends Dr. Max Ruffert, Matt Lennox and Nix McDonnell. Thank you to all those in the diving club (especially "the usual suspects") for providing welcome distractions, in particular Paul Bullen for his sense of adventure and always finding some hidden, un-dived corner of Scotland to go and explore, and Gav for being Gav. Mercy buckets again to Max, and to Ben & Helen for putting me up on my trips back up here whilst sorting out my thesis.

I give a humungous thank you, of course, to the most selfless parents in the world for their support, encouragement, interest, advice and for listening throughout. The same goes for my inspirational big sister.

Thank you to Dawn for your immeasurable influences. I certainly wouldn't be who or where I am today without your motivation and support.

To my bestest girly friends in the whole wide world, Alana, Nic and Sheena Bean, thank you for being there despite the considerable miles between us.

Finalement, je rends surtout et beaucoup à mon Benoit. Il ne m'aurait pas été possible de finir sans toi et ton soutien. Tu me rends plus heureuse que je ne l'ai jamais imaginé dans mes plus doux rêves. Je suis vraiment comblée à l'idée de poursuivre ma vie à tes côtés.

List of Abbreviations

^{1}H NMR	Proton Nuclear Magnetic Resonance
1º	Primary
3D	3-Dimensional
Ab	Antibody
ABPP	Activity-Based Protein Profiling
AG-2	Anterior Gradient 2
AIDS	Acquired Immune Deficiency Syndrome
AZT	Azido-thymidine
Boc	*tert*-Butyloxycarbonyl
BOPCl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
BSA	Bovine Serum Albumin
$BzlCl_2$	2,6-Dichlorobenzyl
Cbz	Carboxybenzyl
CHCA	α-Cyano-4-hydroxycinnamic acid
Cp	Cyclopentadiene
CuAAC	Copper Catalysed Azide-Alkyne Cycloaddition
DCB	1,2-Dichlorobenzene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DFT	Density Functional Theory
DIC	*N,N′*-Diisopropylcarbodiimide
DIPEA	*N,N*-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DOS	Diversity Oriented Synthesis
DPPA	Diphenyl phosphoryl azide
EBP1	ErbB3 Binding Protein 1
ECH-1	Enoyl CoA hydratase-1
ECL	Electrochemiluminescence

EDCI	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
ELISA	Enzyme-Linked Immunosorbent Assay
ERK	Extracellular signal Regulated protein Kinase
ESI	Electrospray Ionisation
FDA	U.S. Food and Drug Administration
Fmoc	*9*-Fluorenylmethyloxy carbonyl
Fmoc-OSu	*9*-Fluorenylmethylcarbamate succinimide ester
G	Glycine
GI_{50}	50% Growth Inhibition
GSTO 1-1	Glutathione S-Transferase Omega
HATU	2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate methanaminium
HDAC	Histone deacetylase
HeLa	Human cell line
HIV	Human Immunodeficiency Virus
HMPA	Hexamethylphosphoramide
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole hydrate
HPLC	High Performance Liquid Chromatography
I	Isoleucine
IC_{50}	50% Inhibition Concentration
K	Kelvin
kDa	kiloDaltons
MALDI-TOF	Matrix-Assisted Laser Desorption Ionisation-Time of Flight
MCF-7	Breast cancer cell line
MHz	MegaHertz
NBS	*N*-Bromosuccinimide
n-BuLi	*normal*-Butyllithium
NIH	National Institutes of Health
NIS	*N*-Iodosuccinimide
NMM	*N*-Methylmorpholine
NMR	Nuclear Magnetic Resonance

NRTI	Nucleoside Reverse Transcriptase Inhibitor
Opp	2-(1-Oxopropyl)pyrrolidine
OTf	Triflate
P	Proline
p53	Transcription Factor Protein 53
PBS-T	Phosphate buffered saline with 0.1% Triton
ppm	parts per million
*p*TsOH	*para*-Toluene sulphonic acid
RLU	Relative Luminescence
rpm	Revolutions per minute
RuAAC	Ruthenium Catalysed Azide-Alkyne Cycloaddition
S	Serine
SA	Streptavidin
SAPK	Stress Activated Protein Kinase
SAR	Structure Activity Relationship
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SM	Starting Material
SPE	Solid Phase Extraction
T	Threonine
TBAB	Tetrabutylammonium bromide
TBDMS	t*ert*-Butyldimethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Trimethylsilane
TP53	Transcription Protein 53
TTN	Thallium trinitrate
Tzl	Thiazole
Tzn	Thiazoline
UK	United Kingdom
UV	Ultra Violet
Y	Tyrosine

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Chapter 1 Introduction: p53 as a Drug Target

1.1. Chemical Genetics

Chemical genetics is an increasingly important line of research in several areas of science. It spans the interface between chemistry, biology and medicine. A simple definition of chemical genetics describes that the function of a protein can be altered using a small molecule.[1,2,3] This can be expanded to highlight the main differences between chemical genetics and classical genetics. Where classical genetics uses mutations of genes to affect changes to the proteins they express, and time is taken for this mutation's effects to be seen, in chemical genetics, the effect is instant.[4] This is because the protein itself is directly influenced by a small molecule, rather than the gene that expresses that protein.

Chemical genetics can be used to identify which proteins regulate different biological processes, to help understand how proteins perform their biological functions. The ability to change the function of a protein is vital in understanding diseases and in the development of new treatments and prophylaxes.

There are two approaches, forward and reverse, to the alteration of protein function by chemical genetics. A comparison between forward and reverse approaches for chemical genetics is described in figure 1.[5]

Forward chemical genetics can be carried out with a plate containing many wells of cells.[2,3,6,7] To each well a solution of a different small molecule is added. The cells are then observed to see which shows the resultant phenotype of interest. The small molecule in the well in which this phenotype is seen is known, allowing the protein that it targeted to be identified. This is often an arduous process.[2,6,8] A starting point for development of a new active pharmaceutical ingredient can then be developed based on this identification.

Conversely, reverse chemical genetics takes one specific protein, which is then screened to find a suitable small molecule or ligand to bind, and then the protein-ligand complex is introduced into a cell to observe if and how the phenotype changes from 'normal'.[2,3,6,7]

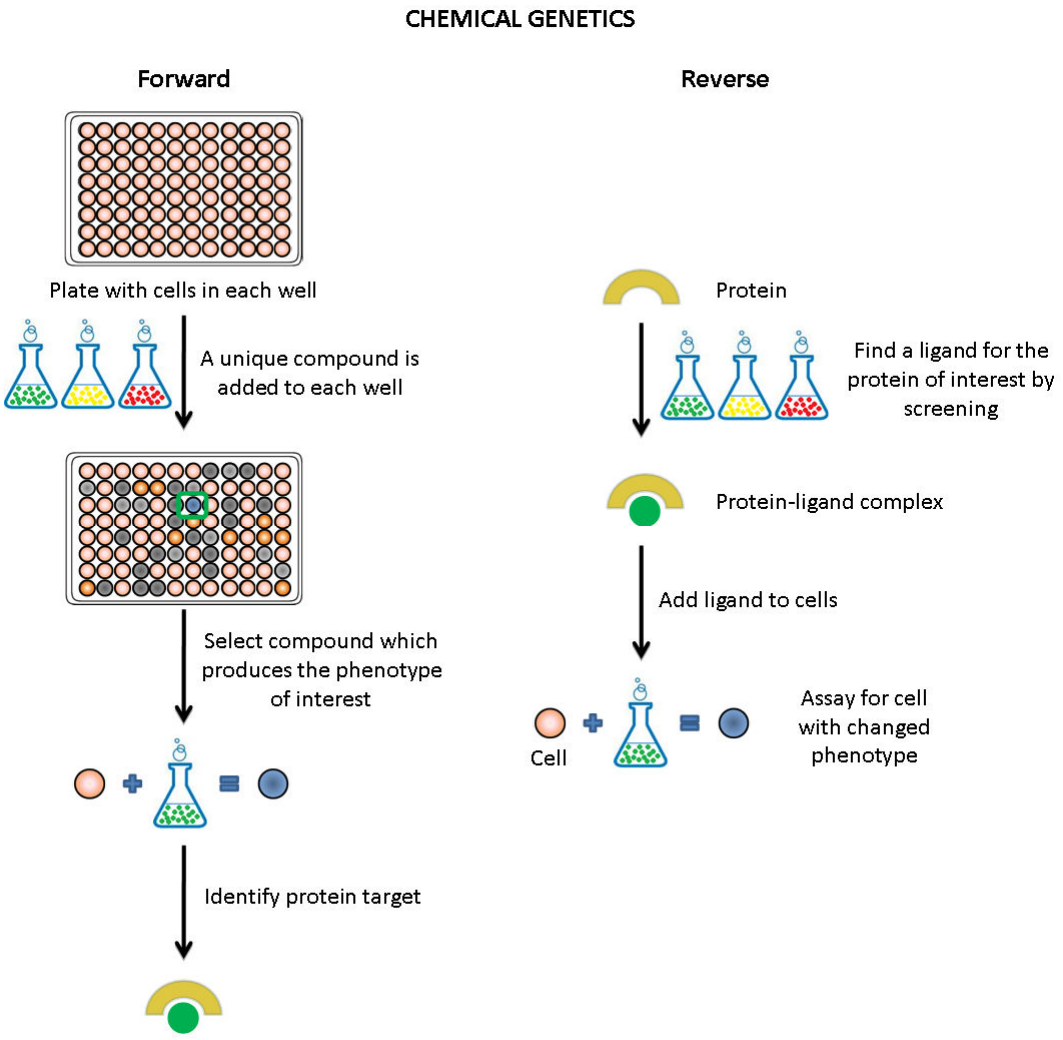


Figure 1. Comparison of Forward and Reverse Chemical Genetics. [5]

For both forward and reverse chemical and classical genetics to affect these changes in the proteins, they require a variety of small molecules to be synthesised.

1.2. Library Strategies for Chemical Genetics Screens

1.2.1. Library Synthesis

Small molecule library synthesis is a well used general protocol for making a large number of often similar molecules. It is frequently carried out to screen for activity of the molecules for a desired target gene product alteration. Once the molecule

within the library which caused the effect, the "hit", has been identified, a commonly challenging deconvolution process of re-synthesis can follow for the effective library member and molecular probe to be successfully and efficiently synthesised (Figure 2, Step 5).[9] The structure of the molecular probe will be based on the active library member with a tag incorporated. The tag will usually allow incorporation of a biotin moiety or a photo- or radio-active label to allow visualisation of the site of interaction of the library member with the biological target, which can then also be identified.

Once the molecular probe has been synthesised, it must then be re-screened to ensure the library member is still active with the incorporated tag (Figure 2, Step 6).[9] Often it is the case that the synthesis to include the tag has altered the molecule's properties, so that it is no longer active, so the design of the molecular probe must begin again. It is clear that this process can be quite laborious.

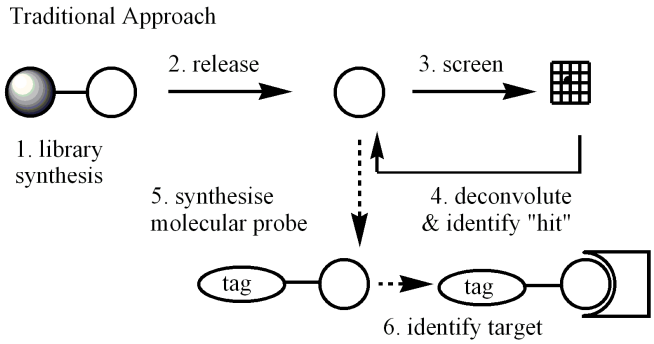


Figure 2. Traditional Approach to Chemical Library Synthesis.[9]

As a result of these challenges there are currently very few published successful target identifications for chemical genetics screens, despite many research groups working in this area. The Hulme group has experienced these difficulties in recent work whilst trying to screen for activity of anisomycin and a small library of its analogues for activation of the stress-activated protein kinase (SAPK) pathways.[9]

1.2.2. "Marked" Libraries

Synthesising a marked library of small molecules offers an accelerated method to identify "hit" compounds. If the library members can all be synthesised to include a small bio-compatible marker, a tag with a complementary functional group can then easily be attached to the marker of those library members shown to be active (Figure 3).

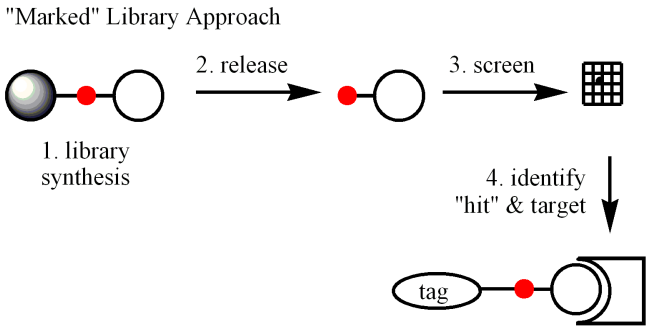


Figure 3. Marked Approach to Chemical Library Synthesis.[9]

This approach would allow the dynamic identification of the "hit" compound, the biological target, and cut out the time-consuming molecular probe synthesis step. As shown in the timeline from the American National Institutes of Health (NIH) (Figure 4), this has the potential to reduce the drug development process by approximately one year.[10]

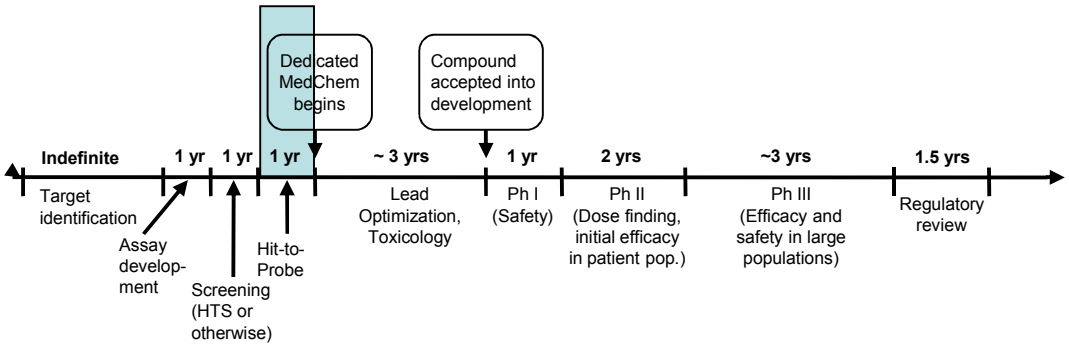


Figure 4. NIH Drug Development Timeline.[10]

This "marked" approach is quite new for the generation of small molecule libraries. However, it has been shown to work well for several kinds of biomolecules.[7,11] Marked sugars and amino acids have been successfully synthesised and incorporated into carbohydrates and peptides, which have then been fluorescently tagged *via* the marker. Markers used have included various functional groups which allow convenient complementary function on a fluorescent portion, making use of the highly effective and versatile Huisgen 1,3-dipolar cycloaddition reaction or Staudinger ligation.[12-14]

1.2.3. Anisomycin Precedent

There are very many fermentation products obtained from various *Streptomyces* species.[15] One compound which has been of particular interest to the Hulme group is anisomycin. It has been shown to have a wide range of roles within organisms. These roles include acting as an antibiotic,[16] an antifungal agent,[17] an antitumour agent,[18] an anticancer agent[17] and as a tool for the activation of the stress activated protein kinase (SAPK) pathway.[9] It is this latter activity which the Hulme group has concerned some of its research with.[19] The target by which anisomycin activates the SAPK pathway is as yet unknown. A small library of anisomycin derivatives were synthesized, many containing markers that might be used in biological studies to find binding partners.[20] Two examples are shown in figure 5.

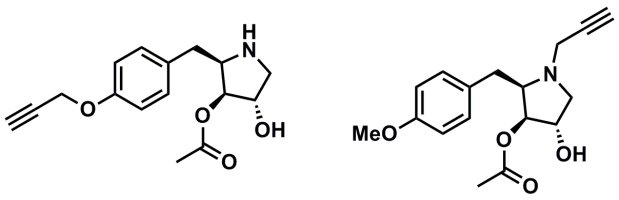


Figure 5. Anisomycin Derivatives from Hulme Group Marked-Anisomycin Library.[20]

These marked library members have been shown to have equal activity in the SAPK pathway compared to anisomycin itself.[19] This demonstrates that a natural product can be marked successfully, whilst retaining biological activity, and the concept can be further pursued.

1.3. Choosing a Bioorthogonal Marker

Tagged, or marked amino acids and sugars have been previously synthesised and successfully incorporated into peptides, glycans and lipids.[7] Generally, markers are required to be small so the risk of them causing steric hindrance to the binding of the biomolecule is minimised. Other requirements are that they are very selective chemically to avoid unintended side reactions of the marker, and that they are stable under physiological conditions to avoid decomposition. There are several main examples of functional groups which have been successfully utilised in this way, (Table 1) and these include aldehydes, ketones, alkynes, phosphines and azides.[7,9,11,21-25]

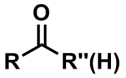
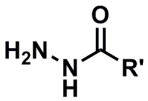
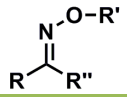
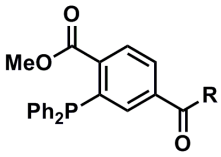
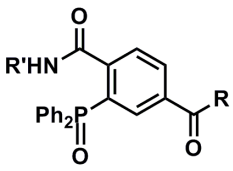
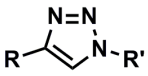
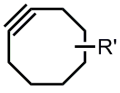
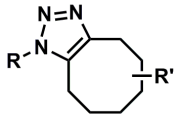
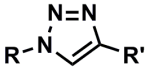
Chemical Reporter	Reactive Partner	Ligation Product	Target (literature reported)
Ketone / Aldehyde 			Protein
	H_2N-O-R'		Glycan
Azide $R'-N_3$			Protein
	R, Cu(I), ligand		Glycan
			Lipid
Terminal Alkyne	N_3-R, Cu(I), ligand		Protein

Table 1. Common Choices for Bio-Orthogonal Markers.[7]

1.3.1. Aldehydes and Ketones

Aldehydes and ketones have been commonly used in marking proteins and glycans.[7] These functional groups contain few atoms and are mildly electrophilic. They are non-native to proteins and glycans, yet can easily be introduced into these and other macromolecules, and are inert in these surroundings. It might be expected that the carbonyl function of the aldehyde or ketone could react with any primary amine present, and form a Schiff base. A primary amine could be present on the side chain of any protein present in a cell, for example, proteins containing asparagine, glutamine, lysine or arginine. However, an equilibrium exists between the carbonyl group and Schiff base, and in aqueous conditions, as it would be inside a cell, this equilibrium favours the carbonyl form.

Aldehydes and ketones are useful as markers when carrying out *in vitro* investigations, however, it is somewhat more complicated for *in vivo* studies. The optimum pH for them to be attached to their complimentary tagging partners is lower than physiological pH, and is often impossible to achieve in cells whilst maintaining life. When using whole organisms for trials the physiology is much more complicated. Aldehydes and ketones are often seen as products of metabolism, therefore where these compounds are used as tags, they may be excreted before they can carry out their intended function.

Ketones were successfully used by Sadamoto's group, to mark bacterial cell wall precursors, and then coupled a hydrazine containing fluorophore for visualisation.[26] Aldehydes and ketones can therefore be very effective as reporters for some large biomolecules, but they should be selected when studying extracellular environments or proteins and glycans involved in cell surfaces to avoid issues of metabolism and pH.

1.3.2. Azides and Phosphines

Azides are much more suitable as general markers. They have been used as tags in three classes of biomolecules; proteins, glycans and lipids, they are not present in these biomolecules, and they have not been found present in any living thing to date,

except one kind of algae.[7] Azides are kinetically stable, but due to the large amount of energy harboured in their structure, they can be conveniently reacted with other bioorthogonal compounds in selective labelling of biomolecules. Like aldehydes and ketones, azides are mildly electrophilic, but in contrast they require soft nucleophiles and harsh conditions in order to react, which is highly unlikely to be supportable within a cell, unless catalysed by some component already present. In certain forms, azides can be toxic, but as an organic azide, and under the mild physiological conditions of a cell, they have been shown to be safe, and are even present in some drug molecules such as azido-thymidine, AZT (Figure 6), a nucleoside reverse transcriptase inhibitor (NRTI). AZT is an inhibitor of HIV-1, delaying the development of AIDS and was approved for clinical use after extensive trials.[27,28]

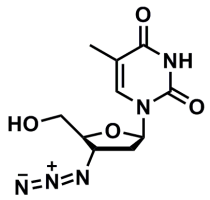
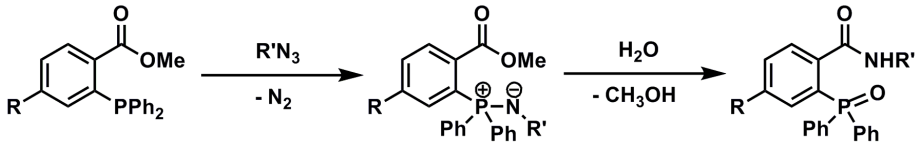


Figure 6. Structure of HIV-1 Inhibitor, AZT.[28]

The complimentary partners most often used with azides are either phosphines or alkynes. Both of these functional groups are foreign to the physiological environment, but generally stable under these conditions, and will react with the azide. Phosphines can react with azides *via* Staudinger ligation to form a stable amide linked product as shown in scheme 1, and this can take place at physiological pH.[29] This approach was discovered by Bertozzi and co-workers in 2000. An azide was attached to sialic acid, which was then incorporated into the cell surface of interest. The azide reacted selectively with a biotinylated triarylphosphine the group had synthesised, allowing visualisation of the cell.

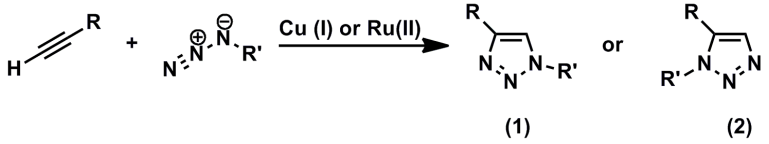


Scheme 1. Staudinger Ligation.

One potential drawback of using a phosphine as the complimentary marker is that it would be susceptible to oxidation by enzymes or just in the presence of air. With this in mind, it would require an excess amount of the phosphine-probe to ensure that there was enough un-oxidised material present for reaction with the azide tag as required.[7]

1.3.3. Azides and Alkynes

Activated alkynes can be employed as the reactive partner for an azide tag *via* the Huisgen 1,3-dipolar cycloaddition reaction. The azide and alkyne react together to form a stable 1,2,3-triazole ring. All three of these species are unusual in a cellular environment, yet biocompatible.[7] This reaction used to require high temperature and pressure, but recent catalytic developments, activating the alkyne, have enabled its progress under milder conditions. These developments have included the use of a copper(I) catalyst[30] to give the 1,4-regioisomer (**1**) selectively or ruthenium(II) catalyst[31] to give the 1,5-regioisomer (**2**) selectively when using a terminal alkyne (Scheme 2).

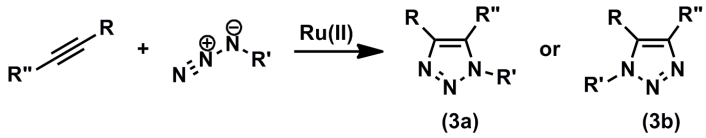


Scheme 2. Formation of 1,4 or 1,5-Triazole Regioisomers.

Even more recently, a method for producing sulfonyl triazoles without metal catalysts has been developed.[32] However, it proceeds selectively with *n*-BuLi at −78ºC, which is not suitable for a cellular environment.

Alkynyl Grignards reacting with organic azides followed by trapping with an electrophile can give the 1,5-disubstituted triazole product (**2**) selectively, but this reaction does not support a wide scope of reagents.[33] Ruthenium(II) catalysts, such as $Cp^*RuCl(PPh_3)_2$ have been used to selectively produce the 1,5-disubstituted triazole product (**2**) with a wider scope, such as primary, secondary and aromatic azides, but not tertiary or aryl azides, where low yields are observed and often several different

by-products.[31] Whereas the Copper Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction is only successful for terminal alkynes, internal alkynes are tolerated for the RuAAC reaction, which can give 1,4,5-trisubstituted 1,2,3-triazole products (**3a & 3b**) (Scheme 3).



Scheme 3. Formation of 1,4,5-Triazole Regioisomers.

However, in a cellular environment, the presence of the 1,4-triazole regioisomer (**1**) is preferred over the 1,5- (**2**) or 1,4,5-triazoles (**3a & 3b**) due its bio-orthogonality stemming from similarity to the amide bond (Figure 7) which is ubiquitous in cells.[34-36]

A triazole ring is relatively planar, and has a strong dipole moment. A triazole ring and amide are similar in their electron distribution and positioning of atoms, and both are capable of H-bonding. The triazole ring can be included in cells without disrupting normal cellular function, and unlike amides, they will not be hydrolysed, making them stable in that environment. This is an excellent trait when looking for a simple way to attach different groups, such as fluorescent tags, to various compounds of interest, such as proteins, *via* a side chain marker. The compatibility of triazole rings in modified peptides, successfully mimicking amides and allowing continuation of their normal function has been previously demonstrated.[35,37,38]

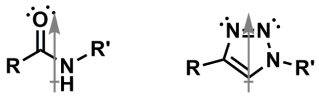


Figure 7. Similarities Between a Peptide Bond and Triazole Ring.[39]

Using the "click" reaction to tag biomolecules has been successful, however, the metals used to catalyse the reaction would be toxic to cells, so investigations are ongoing into further effective catalysts which are not cytotoxic. There are alternative

ways to activate the alkyne, allowing less harsh conditions for the "click" reaction without the use of catalysts. One alternative to a terminal alkyne which requires no activation is to use a cyclooctyne. The reaction between the azide and cyclooctyne is strain-promoted and occurs at room temperature. This bulky group, although not toxic to cells, is more likely to cause disruption to biological activity than the sterically undemanding terminal alkyne. In addition, the reaction between azide and cyclooctyne is slower than the Staudinger ligation and for these reasons the azide/alkyne partnership still appears preferable. A further alternative to catalytically activating the alkyne would be to include an electron withdrawing group in the structure. However, in a cellular environment an electron withdrawing group such as an ester is likely to undergo nucleophilic attack, so this strategy is not likely to be a useful alternative for these biological applications.

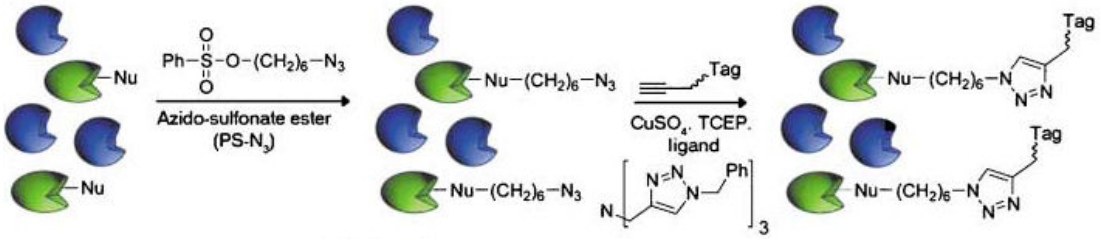


Figure 8. Cravatt's successful demonstration of *in vitro* "click" based ABPP.[40] Nucleophilic group on enzyme reacts with PS-N_3, which undergoes "click" reaction with Tag–≡, forming triazole product; fluorescently labelled enzyme *in vitro*. (PS = phenylsulfonate ester, TCEP = tris(carboxyethyl)phosphines).

The azide-alkyne cycloaddition reaction has been successfully used to label enzymes in complex proteomes, both *in vitro* and *in vivo*.[40,41] Cravatt and co-workers demonstrated the application of azide and alkyne tags in activity-based protein profiling (ABPP). They proved that an alkyne rhodamine species (Rho–≡ or Tag-≡) could react with an azide-sulfonate ester (PS-N_3) bound to its target, distributed throughout several different proteomes (Figure 8), whilst showing comparable sensitivity to that with standard ABPP methods employing a rhodamine-sulfonate ester (PS-Rho). This proved that the concept of using the tagged molecules was as effective as the pre-synthesised fluorescent-sulfonate ester (PS-Rho). Cravatt also showed that the distribution of the azide-sulfonate ester, bound to its target,

throughout the proteome was more extensive than for the rhodamine-sulfonate ester. This confirmed that the pre-synthesised PS-Rho movement around the cell was inhibited by the steric bulk of the rhodamine function attached. This principle was also tested in mice, and these *in vivo* results matched the *in vitro* ones, confirming that the azide and alkyne can work effectively in such applications (Figure 9).

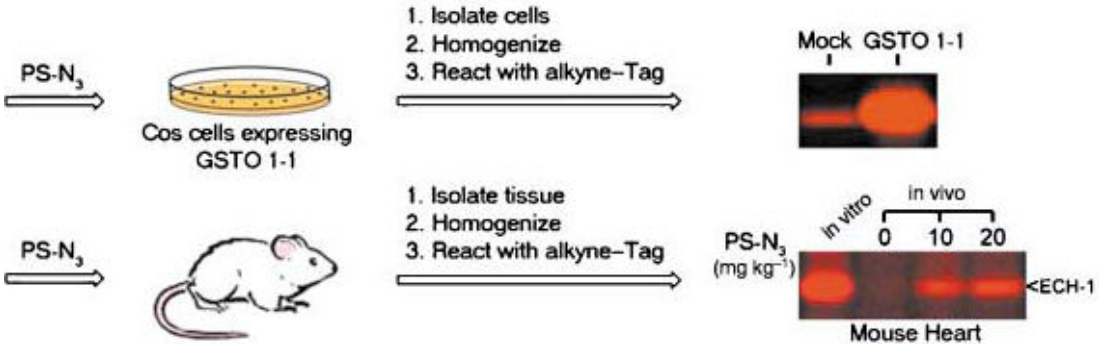


Figure 9. Effective Use of Azide and Alkyne Tags *in vitro* and *in vivo* by Cravatt and Co-workers.[40] Cos cells expressing GSTO 1-1 take up PS-N_3, 1 hour later cells are homogenised, and reacted with Tag–≡. Strong fluorescent signal for GSTO 1-1 shows successful labelling with rhodamine. PS-N_3 was injected into mice, 1 hour later, cells were homogenised, and reacted with Tag–≡. PS-N_3 target ECH-1 showed strong fluorescent signal, proving successful labelling *in vivo*. (GSTO 1-1 = glutathione S-transferase omega class enzyme target, ECH-1 = enoyl CoA hydratase-1 enzyme target).

The azide/alkyne tagging approach has advantages over the azide/phosphine approach. The catalysed "click" reaction is much faster than the Staudinger ligation. The azide and alkyne functionalities could also easily be reversed so that the alkyne could be the biomarker, and the azide the complimentary function to attach a fluorescent tag for example. They provide excellent tools for *in vitro* investigations, and if an alternative catalyst can also be found, then their applications for *in vivo* experiments will be very extensive. For these reasons, and particularly for their ease of incorporation into amino acids, the azide and alkyne tags are often the first choice for peptide-based library syntheses. Their use as tags will be investigated in the specific peptide-protein interaction discussed in section 1.4.

1.4. p53 and AG-2 in Cancer Cells

p53, or transcription factor 53, is a well known protein which is present in all multi-cellular organisms.[42] It is a protein which runs to 53 kDa by SDS-PAGE, hence its name, although from its structure elucidation, it has a molecular mass of 43.7 kDa.[43] The reason for this discrepancy derives from the abundance of proline residues in the protein sequence, which are known to slow down protein migration by SDS-PAGE. p53 is expressed by the anti-oncogene TP53.[44] It has been shown that of all human tumours, around half have been linked to a deficit of effective p53.

When p53 was first discovered, it was considered to be a cancer causing protein as it was found in cancerous cells. However, Vogelstein discovered its true function in cell cycle regulation in 1989.[45] p53 plays an essential role in maintaining healthy cells. If a cell is damaged, the cell can detect that there is a problem during its checkpoint stages of the cell cycle and can increase the stimulation of TP53, leading to increased production of p53.[44] p53 will then relocate to the cell nucleus and ensure integrity of cellular and genetic stability by one of two main routes.[46] If the damage can be repaired, p53 will cause the arrest of the cell cycle, allowing DNA to be repaired before the cell cycle recommences. This ensures that damaged DNA is not replicated, and therefore not passed on to future generations of cells. If the damage cannot be repaired, programmed cell death, apoptosis, will be initiated to eliminate damaged cells. Due to these roles within an organism, preventing the production and/or proliferation of damaged or cancerous cells, p53 is known as a tumour suppressor.[42]

Anterior Gradient-2 (AG-2) is also a protein. It is an androgen inducible secreting protein, regulating the release of the sex hormones; testosterone and oestrogen. AG-2 has been found present in some cancer cells, particularly associated with prostate and breast cancer, as well as Barrett's epithelium, which can lead to oesophageal cancer.[47] More recently, AG-2 has especially been associated with metastatic cancers. Hamburger and co-workers have discovered ErbB3 binding protein 1 (EBP1) which can reduce the expression of AG-2 and as a result, metastatic behaviour is also reduced.[48]

In addition, the use of monoclonal antibodies to detect levels of AG-2 present in cancer cells has been described in the patent literature. This will allow more detailed diagnosis as to the extent of the metastatic behaviour of the cancer, which can help practitioners to decide on the most effective mode of treatment.[47] AG-2 levels also indicate the level of urgency of treatment, and likely length of survival period. This method of detection is much more sensitive than previous diagnoses of metastatic disease, and as such allows earlier detection and earlier treatment.

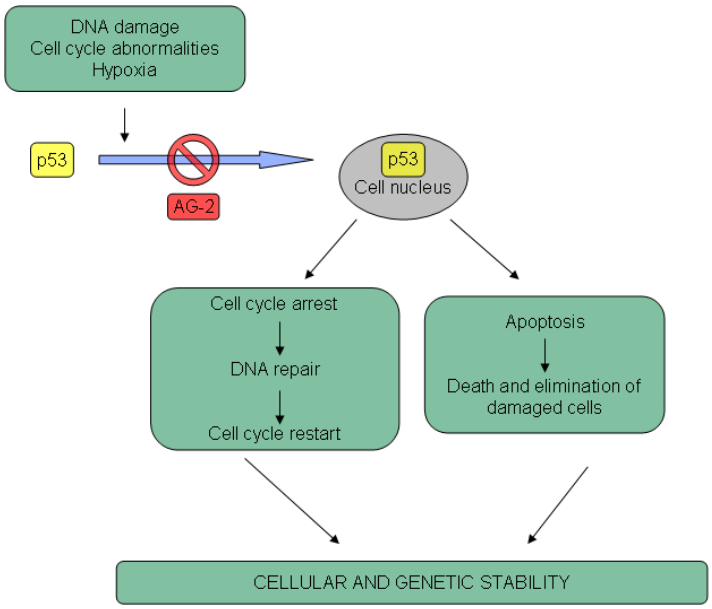
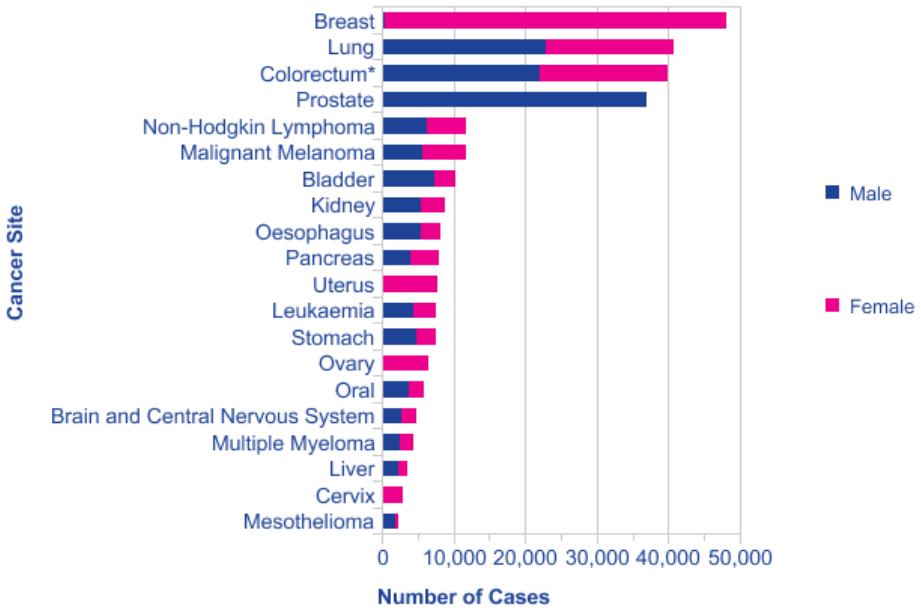


Figure 10. AG-2 is known to inhibit p53, thus reducing cellular and genetic stability.

AG-2 has been shown to inhibit p53 (Figure 10), preventing cell cycle regulation and allowing the proliferation of damaged cells, which can explain the reported presence of AG-2 in cancer cells.[49] However, the mode of action by which AG-2 affects p53 is not yet known. In fact, very little is currently known about AG-2. It has been sequenced[50] however, the 3D structure is as yet unknown, which makes it more challenging to begin designing inhibitory compounds based on binding pocket information and computer simulations. It is possible that AG-2 binds directly to p53, or causes degradation of the protein, or perhaps it acts by inducing defect formation in the TP53 gene, preventing normal expression of p53. Mutation of TP53 is the most common gene mutation to find in human cancers.[44]

Figure 11 shows the latest available data for cancer incidence rates in the UK according to survey results presented by Cancer Research UK.[51] It shows that breast cancer is currently the most commonly diagnosed cancer in the UK. In men, the most commonly diagnosed cancer is prostate cancer, and this is the fourth most commonly diagnosed cancer in the UK. These figures alone show the importance of learning more about AG-2 and discovering how it is interacting with p53 in the propagation of cancer cells; if AG-2 can be inhibited, allowing p53 to be effective in facilitating healthy cell reproduction, perhaps this route for the formation of cancer cells can be deterred.



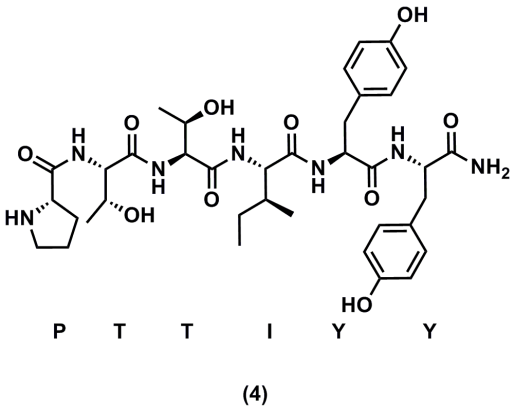
*Colorectum including anus (C18-C21)

Figure 11. The Twenty Most Commonly Diagnosed Cancers, Number of New Cases, by Sex, UK, 2008.[51]

1.5. Identifying AG-2 Binding Partners

The Hupp group have been investigating the AG-2 protein for several years. One of their aims has been the development of a method for diagnostic detection of AG-2 from a clinical biopsy, through affinity chromatography.[49] This requires finding a selective binding partner to facilitate the development of biomarker assays that would allow the selective detection of AG-2 from a complex mixture of cellular

components. Through the use of combinatorial peptide libraries and phage display techniques, Hupp and co-workers have successfully identified a peptide sequence which has a strong binding affinity for AG-2. In addition, when this peptide is present in a cell, it prevents the inhibition of p53, presumably by binding to and inhibiting AG-2 and thereby preventing cancer cell formation, making this peptide a potential candidate as an anti-cancer drug. The peptide identified has the amino acid sequence PTTIYY (**4**) (Figure 12).



(4)

Figure 12. Structure of PTTIYY Peptide (**4**).

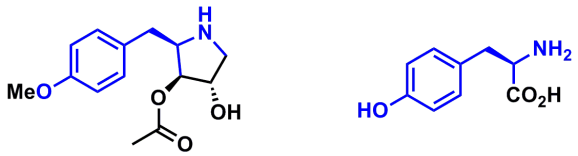
An alanine scan provided further information, that xTxIYY are the amino acids in this sequence, which are essential for binding to AG-2.[49] When the proline was replaced by alanine, the peptide sequence remained active, but when the proline was removed, and the resulting pentamer was tested, the binding was greatly reduced. Although the proline in the sequence is not essential, it is essential that there is an amino acid in that position.

1.6. Tagged Peptide Design

We wanted to synthesis a marked derivative of this peptide to allow further investigation of the relationship between p53 and AG-2, for example by fluorescent tagging. In order to achieve this, we looked for amino acids in the peptide sequence that could be modified to carry a marker that could then be reacted with a complimentary tag carrying a fluorescent species. Ideally, an amino acid side chain

could be functionalised with the marker on the side chain so that neither the *N*- or *C*-terminus of the amino acid would be blocked by a tag. This would allow the amino acid to be anywhere in the sequence, and would also allow the investigation of cyclic peptides.

Tyrosine (**6a**) is the biosynthetic and chemical starting point for the synthesis of many different important compounds, such as adrenaline, morphine, dopamine and various hormones,[52] and has been shown to be the origin of the pyrrolidine core of anisomycin (**5**).[53] Similarities between tyrosine and anisomycin have been highlighted in figure 13.



(5) (6a)

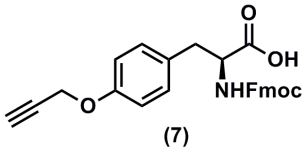
Figure 13. Anisomycin (**5**) Showing its Similarities to Tyrosine (**6a**) in Blue.

Due to tyrosine's biological significance and the convenience of tagging the side chain, it is believed that tyrosine derivatives could provide a good origin for investigations into marked library syntheses for chemical genetics screens. The tyrosine compound could easily be tagged with a terminal alkyne on the phenol side chain or even on the nitrogen, and still allow coupling to further amino acids. Marked tyrosine derivatives therefore have great potential. They could also be very useful to mark the PTTIYY peptide, which has shown promise as an anticancer agent, as well as the potential to make various tyrosine-containing natural product marked derivatives. A tagged tyrosine-containing molecule could then be quickly further investigated by attaching complimentary azide-tagged fluorescent species using the CuAAC reaction, or other functional groups for structure-activity relationship (SAR) studies. In addition, any tyrosine-containing compound could be tagged in this way and by affinity chromatography its target molecule binding investigated. For these reasons, the initial target of this research was to design an efficient synthesis towards a propargyl-tagged tyrosine derivative.

Chapter 2 Results and Discussion: AG-2 Inhibition Studies

2.1. Proposed Synthesis of "Marked" Amino Acid; Route 1

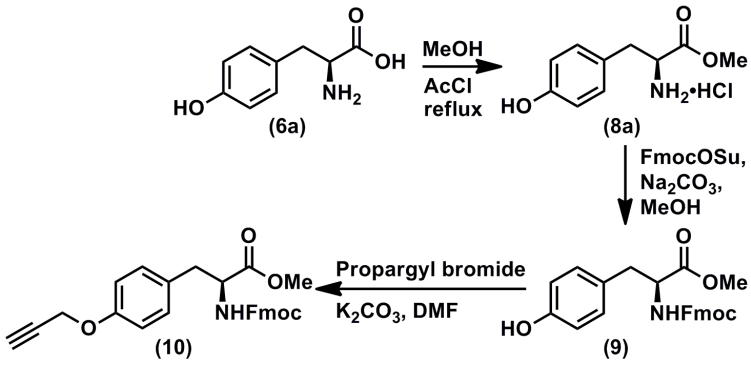
After consideration of the merits of propargyl tagged tyrosine derivatives and their potential for investigating biological activity or biological processes, (discussed in Chapter 1) a synthesis was designed towards a phenol side chain-tagged compound (**7**).



(7)

Figure 14. Target Propargyl-Tagged Tyrosine Derivative.

In order to propargylate the phenol of the tyrosine starting material selectively, it was required to protect the amine and acid functionalities. With a view to preparing the amino acid for subsequent peptide coupling using a Rink amide linker onto solid support, it would be ideal to use the *9*-fluorenylmethylcarbamate (Fmoc) protecting group for amine protection throughout (Scheme 4).[54] Using Rink amide linkers usually involves alcohol side chains being protected as the *tert*-butyl ether, which can be cleaved under the same acidic conditions as cleavage of the final peptide from the solid support. The amine of an amino acid is protected with Fmoc during its coupling to the chain, as this can then be cleaved by base to free the amine ready for coupling of the next amino acid, without disrupting the attachment of the chain to the solid support.



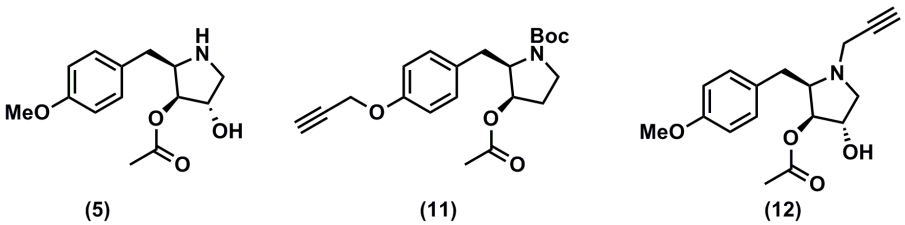
Scheme 4. Proposed Synthesis of Propargyl-Tagged Tyrosine Derivative.

2.1.1. Protection of the Acid and Amine Groups

The acid (**6a**) was successfully protected as the methyl ester under reflux for three hours with acetyl chloride and methanol. After recrystallisation, the desired product was recovered as the white crystalline amine hydrochloride salt (**8a**) in a quantitative yield. The amine was then protected using Fmoc-succinimide ester and sodium carbonate stirring at room temperature overnight. Initially this was carried out in a mixture of 1,4-dioxane and water. However, due to difficulties in removing the solvent even after extended periods under reduced pressure, and the compound's instability on silica during purification by column chromatography,[†] the reaction was instead carried out in methanol. Carrying out the reaction in methanol afforded a sticky off-white film-like crude product, which was difficult to handle, but could be precipitated from minimal ethyl acetate with excess hexane. This gave a clean colourless solid (**9**) in an 88% yield.

2.1.2. Propargylation of the Phenol

There was some precedence of propargylation of anisomycin (**5**) in the Hulme group (Figure 15),[55] including the synthesis of an *O*-propargyl derivative (**11**) and *N*-propargyl derivative (**12**). These compounds were synthesised as "marked" library members which could be used to investigate the role of anisomycin in the stress activated protein kinase (SAPK) pathways.



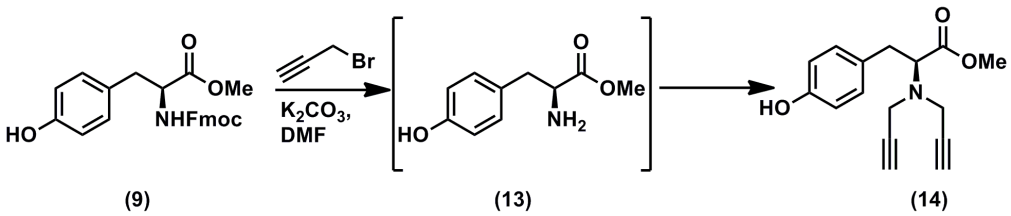
(5) (11) (12)

Figure 15. Anisomycin (**5**) and Propargylated Derivatives (**11** and **12**).[55]

Conditions employed to synthesise the *O*-propargyl derivative (**11**) were propargyl bromide with dry potassium carbonate in DMF, stirred overnight at room

[†] NMR analysis indicated that the decomposition process which occurred on silica involved cleavage of the Fmoc group, and formation of an unknown impurity.

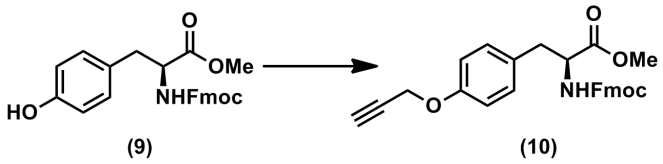
temperature.[55] For the *N*-propargyl derivative (**12**) propargyl bromide was used with piperidinomethyl resin in DCM. It is well known that the Fmoc protecting group is cleaved under basic conditions, but it was thought that potassium carbonate is sufficiently weak as a base, that the Fmoc group would not be cleaved from **9** during propargylation.[56] However, the yield of the desired product was disappointing, and the ^{1}H NMR spectrum showed the integrals of the propargyl protons to be double that expected, and the shifts more consistent with a propargylated amine than a propargyl ether. It is likely that instead of propargylating the alcohol, the Fmoc group was cleaved, and then the amine was doubly propargylated (Scheme 5).[57]



Scheme 5. Formation of Unwanted Doubly Propargylated Product.

It was decided to investigate different bases and dry solvents to see if the mono-propargylation reaction could be successful on Fmoc-Tyr-OMe (**9**) (Table 2). Using adaptations of the method previously employed in the Hulme group with propargyl bromide and potassium carbonate, the solvent was changed to see if any improvement was made. Butan-2-one, acetone and toluene (Table 2, Entries 2–4) all gave results with no improvement from DMF. Different bases were used with DMF, such as potassium *tert*-butoxide and caesium carbonate (Table 2, Entries 5 & 6). Still, the reaction did not yield sufficient product.

Table 2. Attempted Phenolic-Propargylation Conditions.



Entry	Base	Solvent	Additive	Result[a]	Reference
1	K_2CO_3	DMF		45% crude product, 55% (**14**)	Inverarity[55]
2	K_2CO_3	Butan-2-one		35% crude product, 65% (**14**)	Adapted from[55]
3	K_2CO_3	Acetone		30% crude product, 70% (**14**)	Adapted from[55]
4	K_2CO_3	Toluene		35% crude product, 65% (**14**)	Adapted from[55]
5	KO*t*-Bu	DMF		35% crude product, 55% Tyr-OMe	Adapted from[55]
6	$CsCO_3$	DMF		50% (**14**), 50% Tyr-OMe	Adapted from[55]
7	NaH	THF		10% (**14**)	Xiong[58]
8	NaH	DMF		30% (**14**)	Adapted from[58]
9	NaH	DMF	LiI	70% (**14**)	Adapted from[58]
10	Imidazole	DCM		20% crude product, 5% Tyr-OMe, 75% SM	Maryanoff[59]

Reagents and Conditions: **9** (1 eq.), propargyl bromide (3.6 eq.), additive (1 eq.), base (1.3 eq.), solvent. [a] All yields approximate, based on ^{1}H NMR analysis of crude product. (SM = Starting Material).

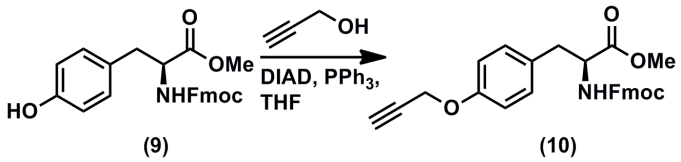
Propargylation was also attempted, despite the strong base, using the procedure published by Xiong,[58] using sodium hydride in THF or in DMF (Table 2, Entries 7 & 8). THF was not polar enough for the reagents to dissolve, and neither of the solvents gave a successful reaction, although when lithium iodide (Entry 9) was used to try to activate the propargyl bromide, 70% of the doubly propargylated material (**14**) was observed.

Finally, the propargylation was attempted using imidazole in DCM (Table 2, Entry 10), which Fmoc had shown to be tolerant to when used in silyl protection of an alcohol by Maryanoff.[59] This was also unsuccessful, and NMR spectra showed ~20% of the desired propargylated product, but mainly starting material.

To confirm that the conditions would work on a simpler more tolerant substrate, propargylation was attempted using the original conditions (Table 2, Entry 1) on 4-

chloro-phenol. The reaction gave the expected propargyl ether product, as confirmed by NMR spectra, showing the conditions are effective when the substrate is not too sensitive.

A final attempt at making the propargyl ether on Fmoc-Tyr-OMe (**9**) was made using Mitsunobu conditions with propargyl alcohol (Scheme 6).[60]

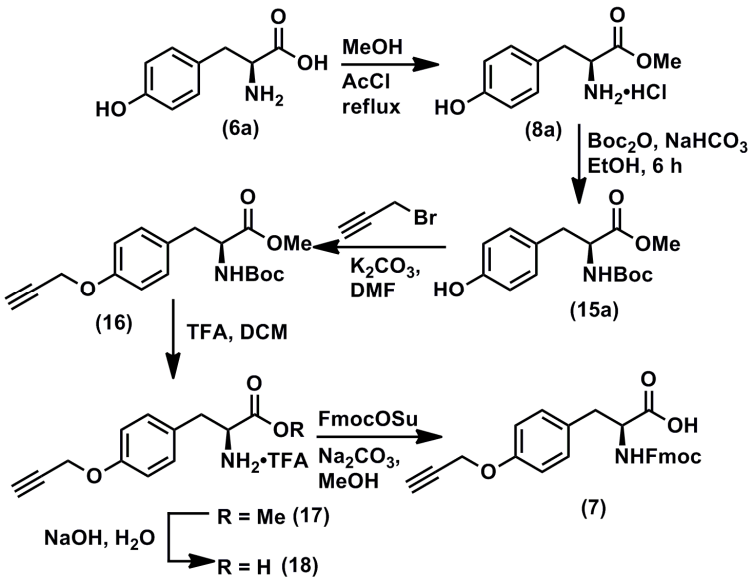


Scheme 6. Attempt at Forming Propargyl Ether Under Mitsunobu Conditions.

This procedure was partially successful in that by NMR analysis, the product could be seen, but as expected, with large amounts of triphenylphosphine oxide. The triphenylphosphine oxide was found to be very difficult to remove, especially as the desired compound (**10**) was not stable to column chromatography. This Mitsunobu approach might be more effective using solid-supported triphenylphosphine oxide, but the cost of this reagent was considered to be prohibitive for large scale synthesis. Due to these challenges, a different route to the target tyrosine derivative was sought.

2.2. Route 2: via Boc-Tyr-OMe

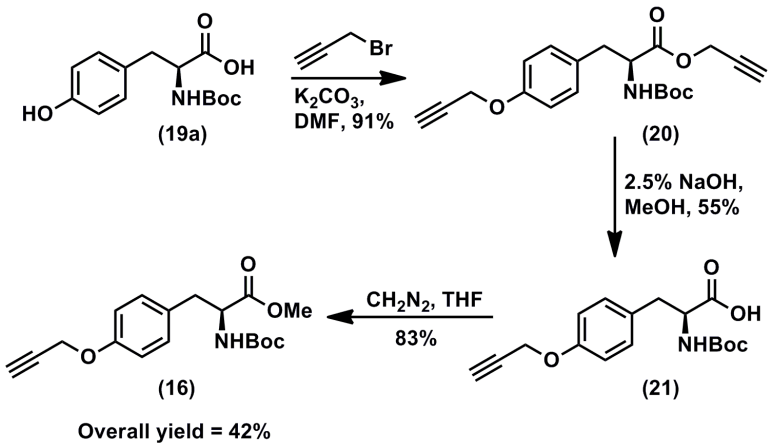
In a second approach, the widely used, base stable, *tert*-butyloxycarbonyl (Boc) protecting group was used instead of the Fmoc, to investigate its suitability for protecting the amine (**8a**) during propargylation.



Scheme 7. Route 2 to Desired Tagged-Tyrosine Derivative **7**.

Tyr-OMe (**8a**) was Boc protected using *tert*-butyloxycarbonyl anhydride in ethanol with sodium bicarbonate.[55] The reaction was found to be complete after six hours at room temperature. The protected compound (**15a**) was purified by column chromatography, and obtained in a 93% yield. Propargylation was then attempted with propargyl bromide and potassium carbonate in DMF, giving Boc-Tyr($CH_2C{\equiv}CH$)-OMe (**16**) in 95% yield.

Shortly after having synthesised this compound (**16**) it was published as a new compound by a group in France.[61] Their starting material had been commercially available Boc protected L-Tyrosine (**19a**), which they reacted to form both the propargyl ether and ester in 91% yield as reported by Schultz.[62] The propargyl ester was hydrolysed before forming the methyl ester with a 42% yield over three steps (Scheme 8). The synthesis developed here (Steps 1-3, Scheme 7), from the cheaper starting material L-tyrosine, has a much higher overall yield of 88% over the three steps.



Scheme 8. The Srivastava Group Route to **16**.[61]

Having successfully made Boc-Tyr($CH_2C{\equiv}CH$)-OMe (**16**), the Boc group was cleaved with TFA in DCM to free the amine in 81% yield; then the methyl ester was hydrolysed using sodium hydroxide in water and methanol. During the work up for this reaction, the pH was adjusted to 7, then water was added and the reaction mixture held at 4°C overnight to promote crystallisation of Tyr($CH_2C{\equiv}CH$)-OH (**18**). Although this procedure was initially successful, allowing the isolation of Tyr($CH_2C{\equiv}CH$)-OH (**18**), it was difficult to reproduce. To adjust the pH to exactly 7 required the use of dilute acid, but a large volume was then needed, and it was more difficult to promote crystallisation. If concentrated acid was used, it was not possible to fine tune the pH to 7. It was decided to use DOWEX 50W-X8 100-200 mesh ion exchange resin in its proton form to adjust the pH to 7. This would allow simple filtration to remove the resin, then methanol removal under reduced pressure. Crystallisation of **18** from the aqueous solution overnight at 4°C, resulted in a good yield (89%). It was found to be necessary to record the NMR spectra of **18** at pH 14 in deuterium oxide, as at neutral pH, and in a vast range of deuterated solvents, the compound was insoluble.

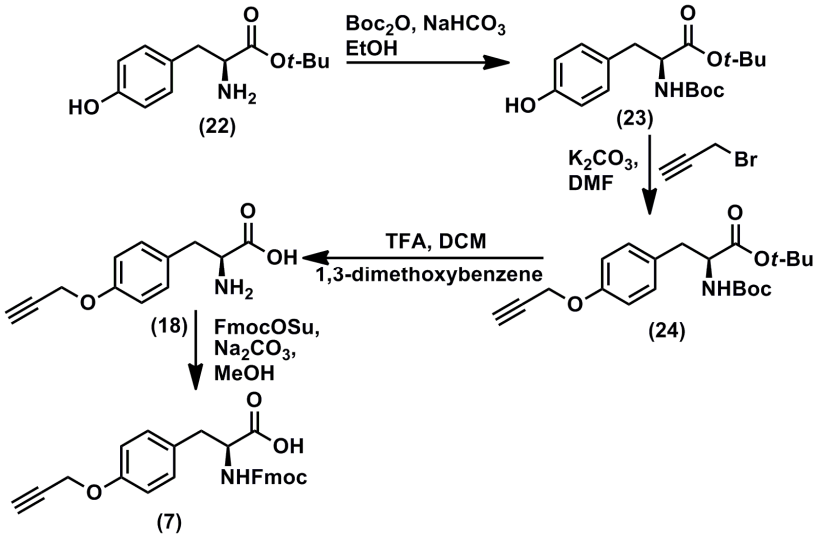
The nitrogen of Tyr($CH_2C{\equiv}CH$)-OH (**18**) was then re-protected with the Fmoc group using Fmoc-succinimide ester and sodium hydrogen carbonate in methanol (85%). This was successful and allowed the completion of the synthesis of Fmoc-Tyr($CH_2C{\equiv}CH$)-OH (**7**) in an overall yield of 54% over six steps.

2.3. Alternative Routes to Fmoc-Tyr-($CH_2C{\equiv}CH$)-OH

Synthesis of the target compound (**7**) had been achieved, but it was felt that it could be improved by using more convenient protecting groups, for example using acid and amine protecting groups that could be cleaved under the same conditions to remove one step, improving efficiency.

Two further alternative routes were designed, one where the acid was protected as the *tert*-butyl ester (Section 2.3.1). Tertiary butyl esters are often cleaved with acidic reagents, such as TFA,[63] which is also used to cleave the Boc protecting group, allowing efficient simultaneous deprotection of both acid and amine groups. The other new proposed route involves using a trifluoroacetyl group to protect the amine (Section 2.3.2).[64] This group can be cleaved by base hydrolysis,[65] and would therefore be an efficient group to use alongside the methyl ester protected acid, as both could be cleaved simultaneously.

2.3.1. Route 3: via Boc-Tyr-Ot-Bu



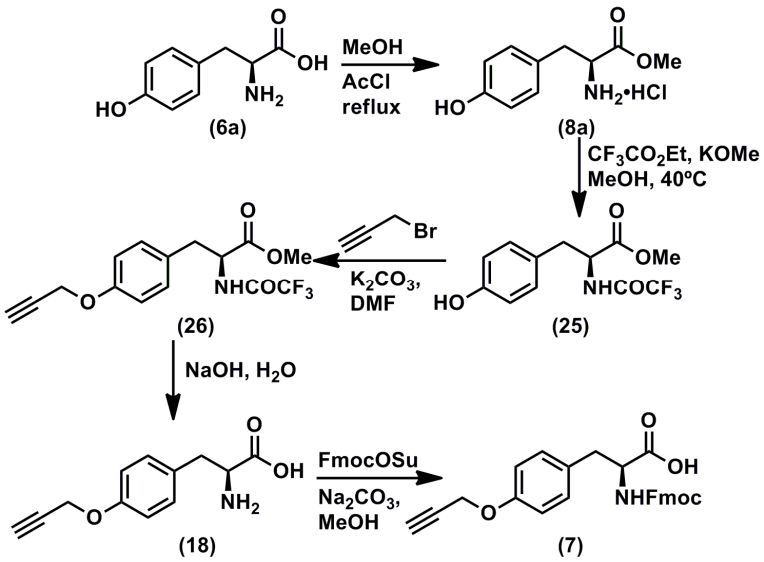
Scheme 9. Route 3 to Target Propargylated Tyrosine Derivative, **7**.

The starting material for the first revised route (Scheme 9) was commercially available L-Tyr-O*t*-Bu (**22**), which was subjected to standard Boc protection conditions with Boc_2O and $NaHCO_3$ to give Boc-Tyr-O*t*-Bu (**23**) in a quantitative

yield. Propargylation was successfully achieved in a 92% yield. Simultaneous cleavage of both the Boc group and *tert*-butyl ester groups was attempted by stirring **24** overnight with TFA in DCM at room temperature. Cleavage of the Boc group was achieved, to give Tyr($CH_2C{\equiv}CH$)-O*t*-Bu, but the ester remained intact. Further attempts to cleave both the *t*-Bu ester and Boc group simultaneously, using neat TFA or TFA and DCM were both tried at room temperature and at reflux. All attempts were unsuccessful. It was assumed that the *tert*-butyl carbocation produced during the cleavage was attacked by any free acid, resulting in reformation of the *tert*-butyl ester. A detailed literature search found some precedent for this, which could be rectified by employing a scavenger.[66] 1,3-Dimethoxybenzene was used in addition to the TFA in DCM, and the mixture stirred for 1.5 h. The crude NMR spectrum revealed that the global deprotection had been successful. It was challenging to isolate the pure product Tyr($CH_2C{\equiv}CH$)-OH (**18**), but precipitation using minimal ethyl acetate and excess hexane gave at best a yield of 78%, though this was not easily reproduced. Fmoc protection was carried out as discussed in section 2.2 to achieve the final compound over four steps in a maximum overall yield of 60%. Route 3 has a slightly higher yield than route 2, and involves one less step. However, it also involves a much more expensive starting material.

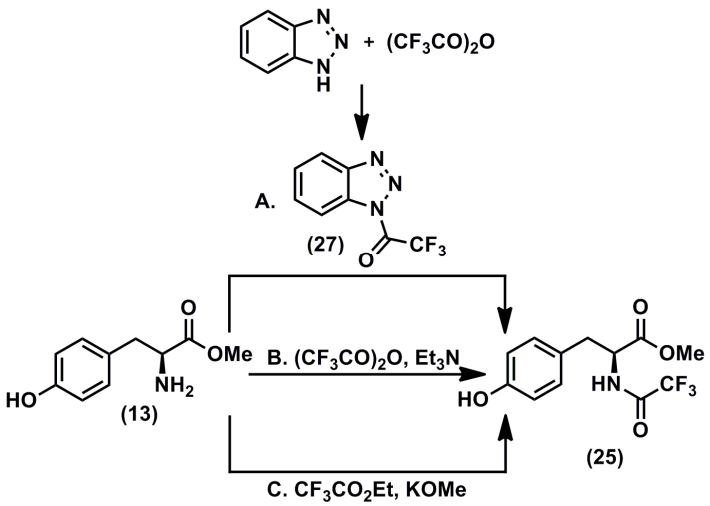
2.3.2. Route 4: via CF_3CO-Tyr-OMe

A final alternative route (Scheme 10) using the trifluoroacetyl ($COCF_3$) protecting group, was also attempted.



Scheme 10. Route 4 to Target Propargylated Tyrosine Derivative, **7**.

Initial attempts to form CF_3CO-Tyr-OMe (**25**) from ester (**8a**) began with synthesis of a (trifluoroacetyl)benzotriazole reagent (**27**) from trifluoroacetic anhydride and benzotriazole in dry THF (Scheme 11, Route A).[67] Despite reports that the reagent was easily formed and stable, in our hands this reagent could not be synthesised in sufficient quantity, neither at room temperature nor with mild heating, in a number of different solvents, or even after prolonged periods. Having failed at this first hurdle, a different method was sought. This time, trifluoroacetic anhydride was used with triethylamine to react with the tyrosine methyl ester (Scheme 11, Route B), a procedure reported by Castro and co-workers.[68] However, once again, this proved unsuccessful. Finally, the amine was protected as the trifluoroacetamide using ethyl trifluoroacetate and potassium methoxide (Scheme 11, Route C), as adapted from a literature procedure.[64] This appeared to be very successful with an excellent yield of 98%; product identification was confirmed by NMR analysis and mass spectrometry.



Scheme 11. Methods attempted towards *N*-trifluoroacetylation of **13**.

Although the yield for this reaction was initially excellent, it was found to be quite variable due to difficulties in isolating the product. It did however prove to be effective as a protecting group, allowing *O*-propargylation to give CF_3CO-Tyr($CH_2C{\equiv}CH$)-OMe (**26**) in an excellent yield (97%), before successful global deprotection under basic conditions (65%). Fmoc protection of Tyr($CH_2C{\equiv}CH$)-OH (**18**) gave the desired final compound (**7**) in an overall yield of 49% over five steps. Route 4 has been successful in achieving the target tagged-tyrosine derivative, and with the cheap L-tyrosine starting material. However, the yields for both route 2 and 3 were higher.

Considering the merits of each of these three successful routes to the desired propargyl-tagged tyrosine derivative (**7**), it was decided to proceed with route 2; *via* Boc-Tyr-OMe. Although the overall yield was very slightly lower than that for route 3 using Boc-Tyr-O*t*-Bu, it was extremely reliable, producing consistent high yields for each step.

Having successfully produced gram quantities of the desired amino acid derivative, it was incorporated into peptides to determine if the desired explorations could be executed with this new tool.

2.4. Peptide Synthesis

With the tagged-amino acid derivative in hand, the synthesis of the hexamer peptide, which has been shown to prevent AG-2 from inhibiting p53 (Section 1.4 – 1.5), was started.

The PTTIYY peptide (**4**) contains two tyrosine amino acids. It was decided to work towards two derivatives of PTTIYY, where in turn either of the two tyrosines was substituted for the new tagged tyrosine derivative (Figure 16). This may indicate which features of each of the tyrosine moieties are important for binding to the AG-2 protein, depending on whether the propargyl group disrupts the binding compared to the original hexamer or not.

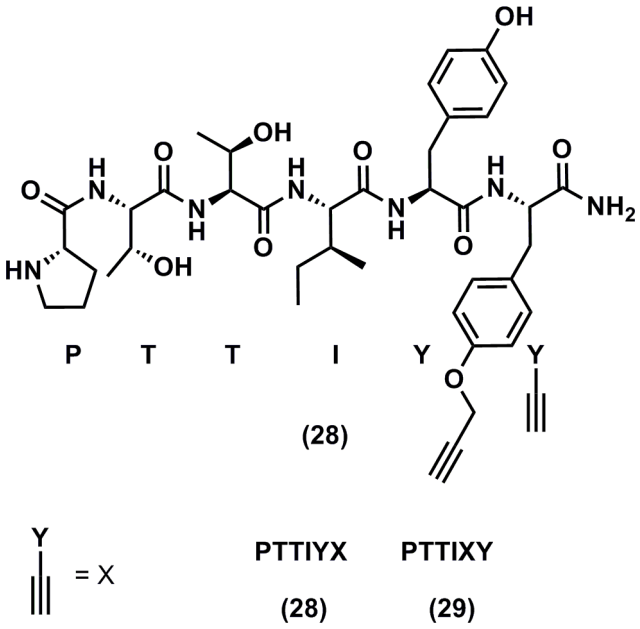
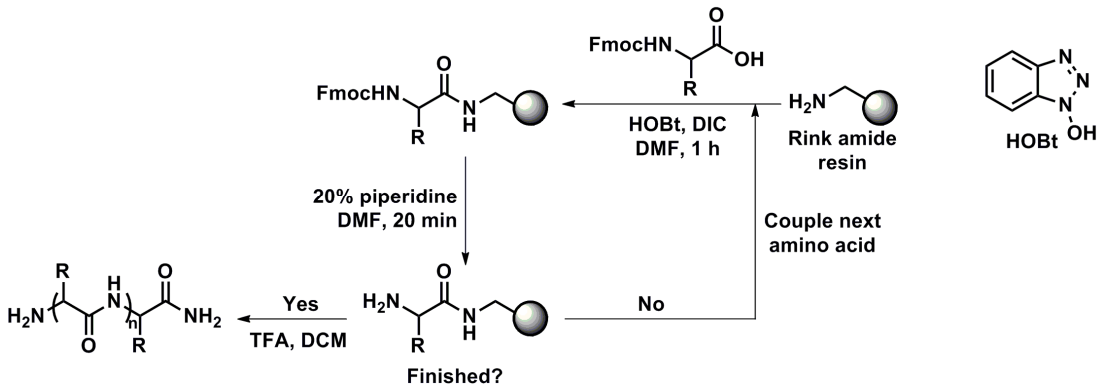


Figure 16. The Two Tagged PTTIYY Derivatives to be Synthesised.

These two peptides were synthesised manually on solid support using SPE tubes and agitation by a Stuart Scientific shaker. Standard Rink amide resin procedures were mostly used (Scheme 12), with a 3-fold excess of Fmoc monomers and coupling agents, HOBt and DIC, compared to the loading of the resin. Excesses were used for the Fmoc-deprotection step with piperidine in DMF, and for the simultaneous cleavage and global deprotection step with TFA in DCM.



Scheme 12. General Peptide Synthesis Procedure on Rink Amide Resin.

After each step throughout the peptide syntheses the Kaïser test was performed to determine whether each coupling or deprotection had been successful. The Kaïser test involves ninhydrin based solutions which change colour depending on the terminal functional group. After coupling an amino acid to the resin, the terminus of the chain should be an Fmoc-protected, secondary amine and this is indicated by the Kaïser test solution and beads turning yellow on heating. After deprotection the terminus should be a free, 1º amine, and the solution and beads should be deep blue. If any test is not the expected colour, it indicates that coupling or deprotection was incomplete, and the reaction can be repeated. Some coupling reactions are notoriously challenging, and it is common to require a repeat, for example, initial coupling to resin with a bulky amino acid, such as tyrosine, or coupling to a secondary amine, such as on proline, or *N*-derivatised amino acids.[69]

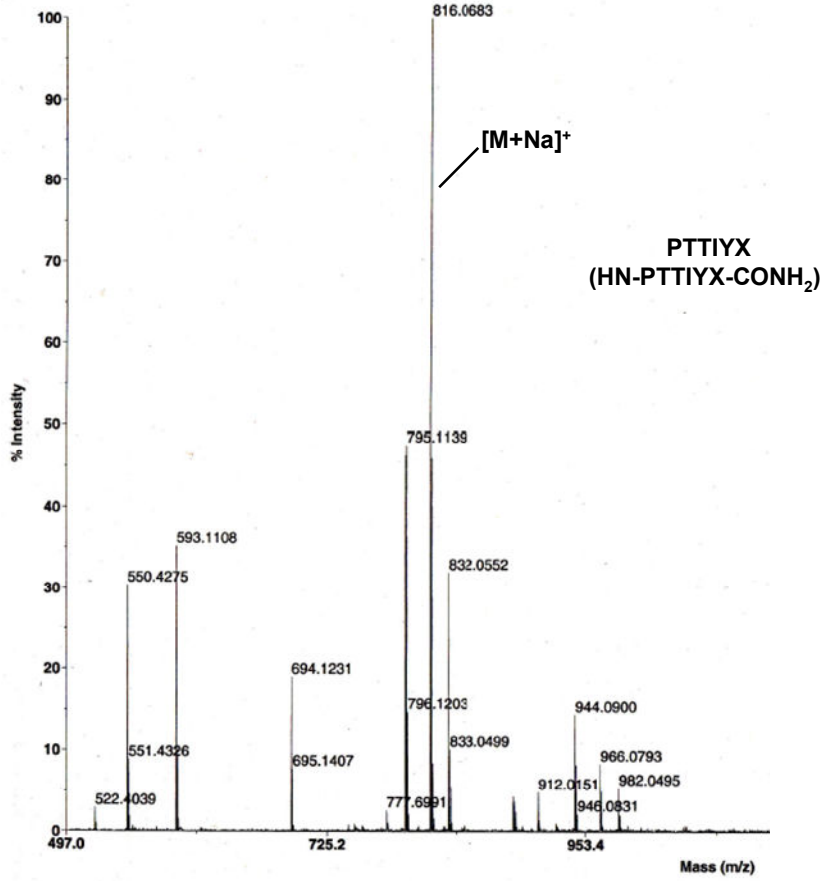
Not surprisingly, for the syntheses of PTTIYX (**28**) and PTTIXY (**29**), the first tyrosine residue and the final proline residue required double couplings in order to achieve completion.

For the simultaneous cleavage from the resin and global deprotection of side chains, the resin was suspended and shaken in TFA:DCM (1:1) for three hours. The deep red solution was filtered, collected and evaporated to near dryness. Ether was added, causing precipitation of the peptide, a colourless solid, which was dried under reduced pressure to give the peptide PTTIYX (**28**).

This procedure was repeated for the second peptide, PTTIXY (**29**). The resultant peptides were analysed by MALDI-TOF mass spectrometry, confirming the expected

mass. The samples were shown to be 98% (**28**) and 96% (**29**) pure by reverse phase HPLC (See figure 17 for **28** analysis. All peptide analysis is shown in Appendix 1).

(a)



(b)

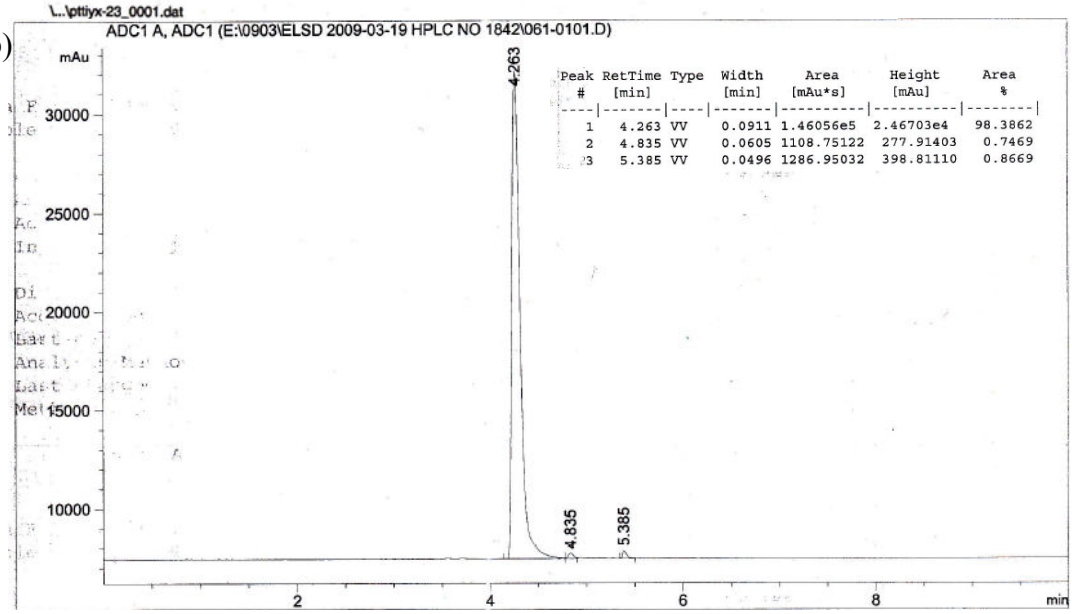


Figure 17. Spectral Analysis of PTTIYX. (a) MALDI-TOF mass spectral analysis. Exact Mass of PTTIYX is 793.40 MALDI Peak found for $[M+Na]^+$ at 816.1. (b) Reverse phase HPLC trace, showing 98% purity.

These procedures proved that the peptides could be successfully synthesised incorporating the propargyl group in either of the tyrosine side chain positions. Following this, investigations into whether the propargyl-derivatised peptides maintain their affinity for AG-2 compared to the normal peptide were carried out. In order to do this, enzyme-linked immunosorbent assay (ELISA) techniques were employed (Section 2.5.1, Figure 19), and required the synthesis of a biotinylated peptide. The full target structures are shown in figure 18.

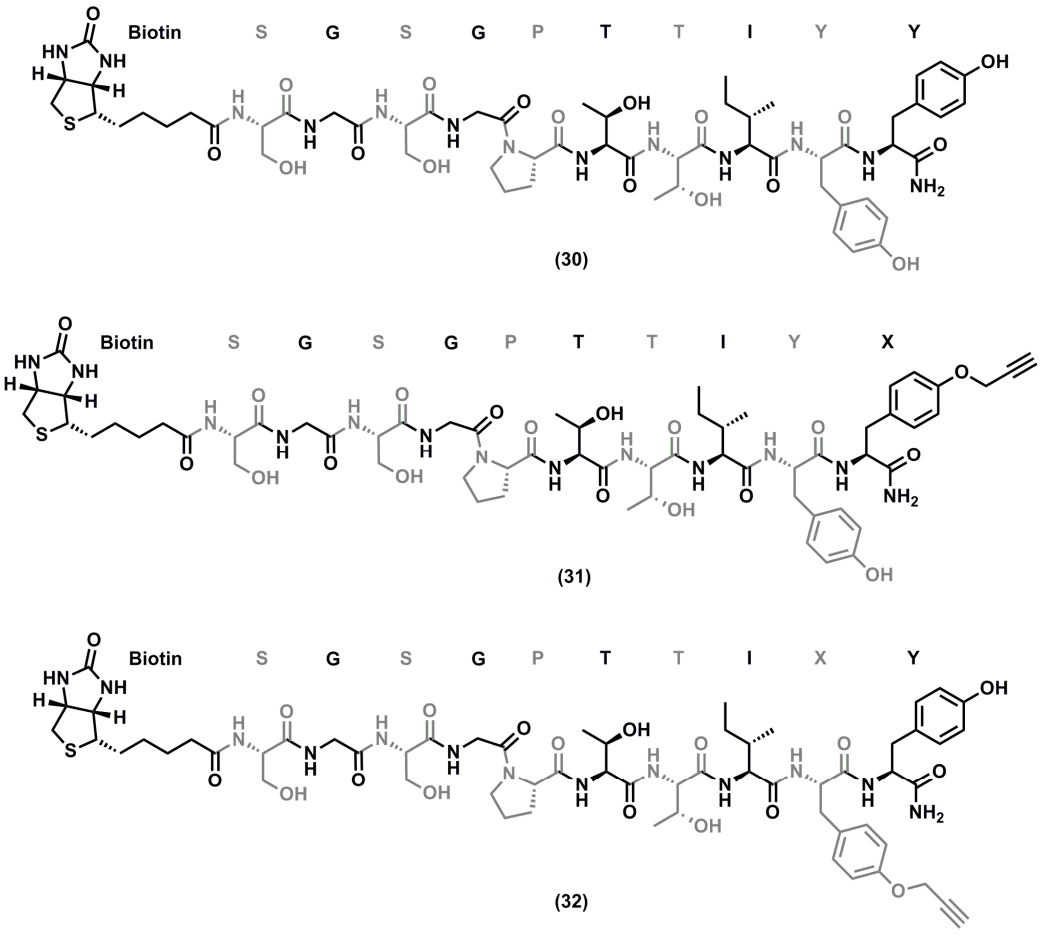


Figure 18. Target Biotinylated Peptide Structures. [Black and grey used to clearly indicate the different amino acid residues].

The biotin moiety is required to take advantage of its strong specific affinity for binding to streptavidin.[70] This relationship is exploited in ELISAs where a multi-well plate is used. Each of the wells on the plate is coated with streptavidin, and then incubated with the biotinylated compound, before further incubations with other

specific compounds which will allow quantification of the binding of the compounds of interest (Figure 19). The target structures include an SGSG "spacer" unit to ensure that the entire peptide is displayed and available for binding to AG-2, to avoid steric interference from the biotin or streptavidin anchor.

BiotinSGSGPTTIYY and derivatives were synthesised manually on Rink amide resin using the same method as shown in figure 12. The first tyrosine residue and the proline residue required double couplings again for each of the peptides, in order to achieve completion. After each of the amino acids had been attached, and whilst the peptide was still attached to the resin, biotin was added to the N-terminus of the peptide by its free acid in another coupling reaction under the same conditions (HOBt, DIC in DMF). Again, simultaneous cleavage from the resin and global deprotection was carried out using TFA/DCM (1:1). The colourless solids (**30**, **31**, **32**) were analysed by HPLC and MALDI-TOF mass spectrometry, confirming the expected masses (Appendix 2). The samples were 70%, 81% and 89% pure by HPLC, respectively. Repeated attempts were made to purify the peptides by preparative HPLC, but these were made very difficult due to extreme lack of solubility, thus further attempts were abandoned. Although several commercial sources[71] recommend >90% purity for quantitative assessment of protein binding, precedent exists of using >70% pure peptides in ELISAs.[72] Personal communication with a colleague at the Edinburgh Cancer Research Centre indicated that 80% purity was sufficient for the intended ELISAs.[73] Although **30** fell below 80% purity, a commercial sample of the same peptide was available, and was used for ELISA alongside the synthetic **31** and **32.**

2.5. Biological Investigations

Having successfully synthesised the three required biotinylated peptide-derivatives, it was time to determine their effectiveness in binding to AG-2, to prevent it from inhibiting the cell-cycle regulation by p53.

2.5.1. Binding of "Marked" Peptides to AG-2 (ELISAs)

ELISAs were carried out to determine the level of binding of the two propargylated versions of the ligand, BiotinSGSGPTTIYX (**31**) and BiotinSGSGPTTIXY (**32**), compared to that of the parent BiotinSGSGPTTIYY peptide (**30**) (Figure 19).

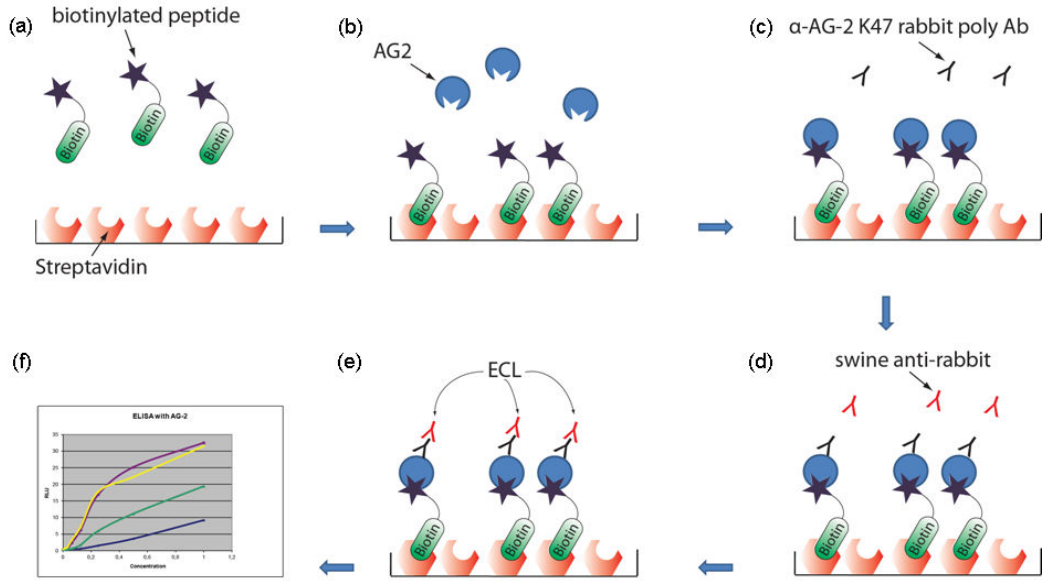
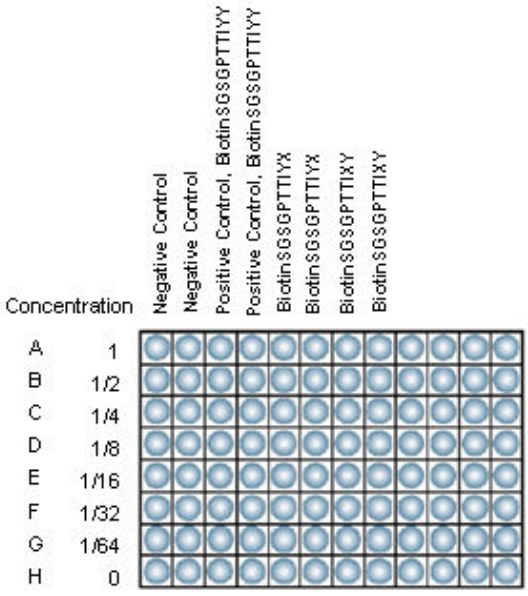


Figure 19. ELISA Process. (a) Each well on a multi-well plate was coated with streptavidin. Wells were then incubated with the four different biotinylated peptides (5 µg in 50 µL DMSO) in the arrangement shown in Figure 20. After washing, all wells were blocked by incubation with 3% bovine serum albumin (BSA) in phosphate buffered saline with 0.1% Triton (PBS-T) to prevent non-specific binding of further species to the streptavidin. (b) After washing, the wells were incubated with solutions of purified AG-2 protein at different relative concentrations made from a 2.5 mg mL^{-1} in 3% BSA in PBS-T stock solution. (c) After washing the wells were incubated with the primary antibody (α-AG-2 K47 rabbit poly) (d) After washing the wells were incubated with secondary antibody (swine anti-rabbit). (e) The wells were incubated with a luminescent probe (ECL), and (f) the relative luminescence (RLU) was measured, which is proportional to the level of binding in each well. A graph was generated displaying these measurements.



	Relative Conc.	Negative Control	BiotinSGSG-PTTIYY	BiotinSGSG-PTTIYX	BiotinSGSG-PTTIXY
A	**1**	**9.1**	**32.5**	**19.4**	**31.5**
B	**1/2**	**3.6**	**25.2**	**11.1**	**22.2**
C	**1/4**	**1.5**	**16.9**	**5.9**	**17.8**
D	**1/8**	**0.6**	**6.2**	**1.7**	**7.2**
E	**1/16**	**0.5**	**2.4**	**0.6**	**3.3**
F	**1/32**	**0.4**	**0.6**	**0.4**	**1.1**
G	**1/64**	**0.3**	**0.4**	**0.4**	**0.7**
H	**0**	**0.3**	**0.4**	**0.3**	**0.5**

Figure 20. ELISA Assay. (a) 96-well plate with peptide and relative concentration arrangements (b) Average RLU Measurement Values (over 2 measurements).

The negative control was a random biotinylated peptide chosen for its lack of affinity for AG-2. This would act as an indicator of the level of non-specific binding to other species present in the wells. The positive control was BiotinSGSGPTTIYY (**30**) with non-derivatised tyrosines. This ELISA procedure was performed in duplicate for each peptide, and the results are shown as averages (Figure 20b) (Raw data can be found in Appendix 3).

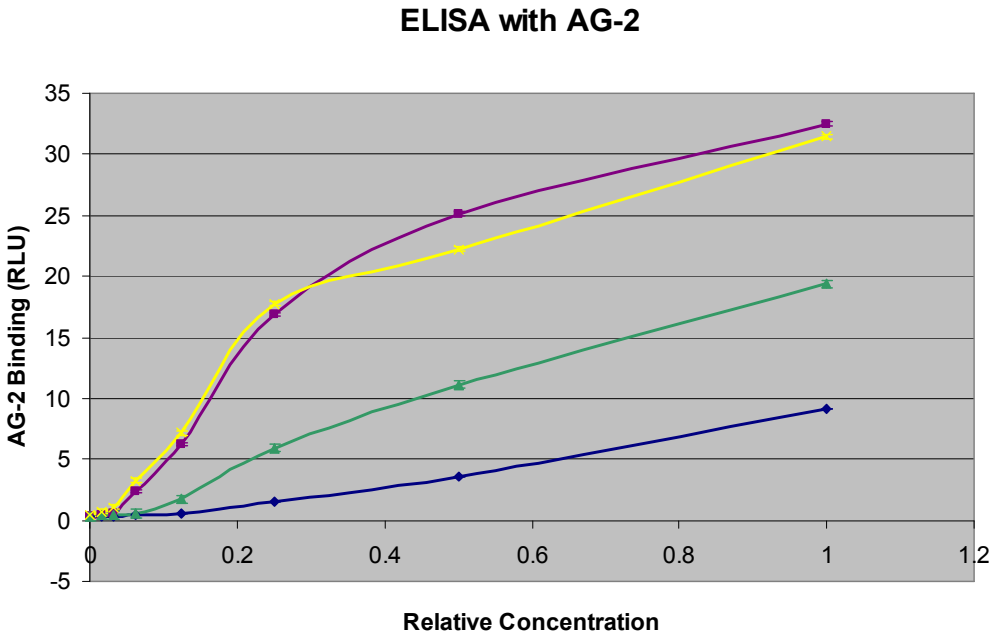


Figure 21. ELISA assay results of peptides', **30**, **31** and **32**, relative binding [proportional to relative luminescence (RLU)] at different concentrations of purified AG-2. Blue = Negative control, random peptide; Purple = Positive control, BiotinSGSGPTTIYY (**30**); Green = BiotinSGSGTTIYX (**31**); Yellow = BiotinSGSGPTTIXY (**32**). (X = Y–propargyl).

The level of non-specific binding, measured by the biotinylated random peptide with no specific affinity for AG-2, and the affinity of the positive control, the non-derivatised biotinSGSGPTTIYY (**30**) to AG-2 are displayed graphically in Figure 21. Relative to these two results, the level of binding of the two tagged peptides to AG-2 can be assessed. The level of binding to AG-2 by the biotinSGSGPTTIYX peptide (**31**), with the propargylated tyrosine in the terminal position is lower than that shown by biotinSGSGPTTIYY (**30**); however, the relative affinity of biotinSGSGPTTIXY (**32**), where the internal tyrosine is propargylated, to AG-2 is at a comparable level. This shows that the propargyl group can be tolerated in the internal tyrosine position, and specific binding to AG-2 occurs from the purified AG-2 protein solution. To further confirm these results, ELISAs were repeated by the same method, but using MCF-7 breast cancer cell lysate. This lysate is known to contain AG-2, but also a wealth of other proteins and cellular components.[74] The

purpose of this ELISA was to determine if the propargylated peptides could show the same result, selectively binding to AG-2, even from a complex mixture. The results of the ELISA with the MCF-7 breast cancer cell lysate are shown in Figure 22 (Full results shown in Appendix 4).

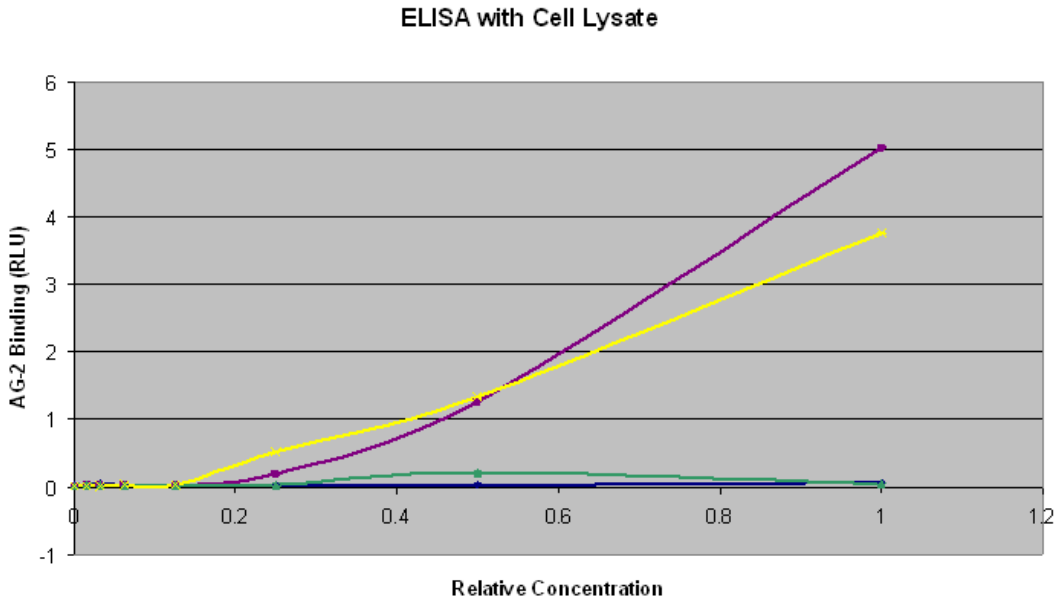


Figure 22. ELISA assay results of peptides, **30**, **31** and **32**, relative binding [proportional to relative luminescence (RLU)] at different concentrations of MCF-7 breast cancer cell lysate. Blue = Negative control, random peptide; Purple = Positive control, BiotinSGSGPTTIYY (**30**); Green = BiotinSGSGTTIYX (**31**); Yellow = BiotinSGSGPTTIXY (**32**).

These results confirm those of the first ELISAs with the purified AG-2 protein. Here the levels of affinity are all comparable to those seen the first time, relative to each other. The overall RLU, or binding affinity appears to be much lower than before, but this is due to the ELISA being carried out with the complex mixture of proteins, rather than just the purified AG-2.

From these results, features of the tyrosine moieties which are important for the binding of the peptide to AG-2 can be recognised. It was previously mentioned (Section 1.5) that an alanine scan of the peptide sequence showed xTxIYY to be the important amino acids in the sequence.[49] For the peptide BiotinSGSGPTTIYX (**31**) the binding affinity was lower than for BiotinSGSGPTTIYY (**30**). This indicates that by converting the phenol into an ether, the binding has been disrupted, and it can be inferred that the terminal phenol is important for binding to the AG-2 protein,

perhaps by hydrogen bonding. The peptide BiotinSGSGPTTIXY (**32**) shows good levels of binding to AG-2 compared to BiotinSGSGPTTIYY (**30**), showing that the propargyl ether is tolerated, and this phenol is not essential for selective binding to AG-2. In this case it is more likely that the aromatic ring is involved in binding to AG-2, perhaps by π-π stacking.

This information is very valuable to future investigations as it is clear that any tagged versions of this peptide, for fluorescent or SAR investigations, should be focused on the PTTIXY derivative (**29**).

2.5.2. Further PTTIYY Biological Investigations

To further confirm the results obtained from the ELISAs, pull-down assays could be performed, to determine the ability of the peptides to bind AG-2 when bound, through biotin, to streptavidin beads. This could again be performed with purified AG-2 and MCF-7 breast cancer cell lysate.

2.6. Future Work

2.6.1. Peptide Display Modes

Affinity chromatography can be used to select targets of biologically active molecules, by purification from a complex biological mixture, but identifying the target has traditionally been challenging due to the harsh conditions often required to separate the target compound from the solid supported system which captured it. The Hulme group recently developed a new affinity chromatography linker (Figure 23),[75] which operates independently from the strength of interaction between the ligand and target. It can be cleaved under mild sodium dithionite conditions, enabling identification of the compound by spectroscopic techniques.

The ligand is attached to the linker *via* "click" chemistry, whilst the other end of the linker is attached to solid support.

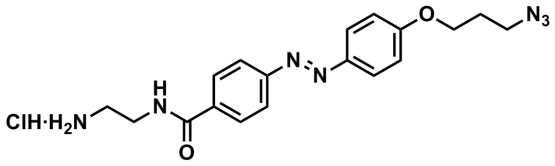


Figure 23. Cleavable Affinity Chromatography Linker Developed by the Hulme Group.[75]

The propargylated peptides discussed in this chapter could be attached to the linker to investigate their binding to AG-2 and how that is affected depending on the way the peptide is displayed. To date, all binding between PTTIYY derivatives (**31** and **32**) and AG-2 has been carried out based on the BiotinSGSG-peptide structure, with display of the peptide from the *N*-terminus (Figure 24a). These peptides could be displayed through side-chain linkage of the *C*-terminus to assess the importance of the SGSG spacer unit, and also to determine if the biotin has any effect on the levels of binding seen. For example, PTTIYX (**28**) or PTTIXY (**29**) could be attached to the linker *via* "click" chemistry and challenged with AG-2 to determine how the binding level to the AG-2 target might change by displaying this peptide with attachment from the *C*-terminus with a triazole[76] (*e.g.* Figure 24b-c).

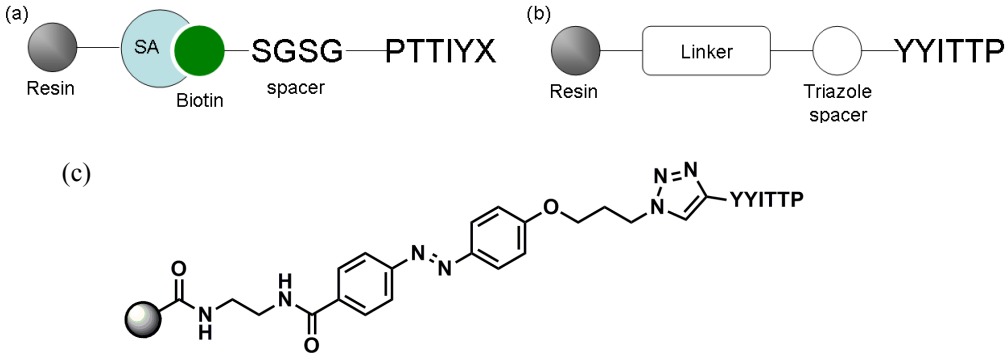


Figure 24. Schematics of PTTIYX displayed using (a) streptavidin-biotin affinity with SGSG spacer, (b) the new Hulme group cleavable affinity chromatography linker (c) Hulme group linker attached to affinity resin with triazole spacer to PTTIYX.

2.6.2. Fluorescent and SAR Investigations with Derivatised Tagged Peptides

Various azido-fluorophore derivatives could be attached to the propargyl tagged peptide *via* "click" chemistry. Amino-fluorescein (**33**) is a known compound,[77] and is an azido-fluorescein precursor, and various azido-rhodamine derivatives including **34** exist,[78] (Figure 25). These could be attached to allow fluorescent imaging of cells containing the derivatised peptide bound to the AG-2 protein. The location of AG-2 in the cell relative to the location of p53 at various stages of the cell cycle, could give a valid insight into their relationship in the formation of cancer cells.[49]

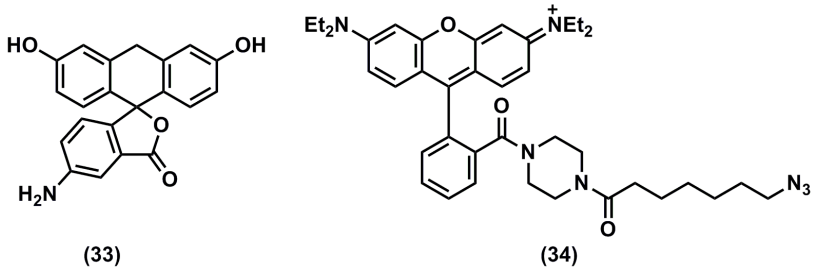


Figure 25. Amino/Azido-Fluorophore Structures.

As alternatives to the azido-fluorophores, other functional groups which might improve the binding affinity of the peptide for AG-2 could be derivatised with an azide group and attached by "click" chemistry. A library of azides could be developed for potential *in situ* "click" chemistry to "grow" the ligand out from this propargyl side chain position, allowing extension of the fragment to cover other accessible sites on the AG-2 surface. Such "click"-based fragment coupling principles to produce modulators of protein-protein interactions has recently been demonstrated by Manetsch and co-workers, having used sulfo-"click" chemistry.[79] This could give more information on the SAR as to preferences for binding environments.

This principle could, of course, be applied to any interesting tyrosine containing peptide or natural product, which supports the tag while maintaining its bioactivity. Investigations in this field are explored further in chapter 4, and could also be combined with the cleavable affinity chromatography linker (Section 2.6.1) to attempt identification of the tagged ligands' biological targets.

Finally, for the particular case of the PTTIYY peptide, the threonine residues could be explored for their tolerance of being tagged with a propargyl group. This would enable similar information to be gathered about their importance towards AG-2 affinity. It is especially likely, in light of the alanine scan showing that the middle threonine is not required to maintain binding, that this position might be suitable for derivatisation.

Chapter 3 Introduction: Natural Occurrence and Synthetic Utility of Halogenated-Tyrosine Derivatives

3.1. Tyrosine-Containing Natural Products (by known structure/activity)

Tyrosine is a very important compound. It is essential to our survival, although it is not one of the essential amino acids as it can be synthesised within the body from phenylalanine.[80] Tyrosine derivatives feature in many different naturally occurring biologically active compounds, including hormones and neurological transmitters such as adrenaline[81] (**35**) and dopamine[82] (**36**) (Figure 26).

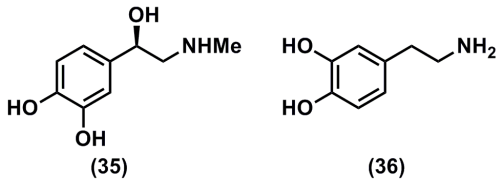


Figure 26. Tyrosine Related Structures of Adrenaline and Dopamine.

In addition to these examples, tyrosine is also found in natural products isolated from marine sponges and bacterial fermentation, some of which have been shown to have potential uses as anticancer,[17] antitumour,[18] antibiotic medicines[16] and more. Tyrosine found and used in the human body to make proteins and neurotransmitters is the natural enantiomer, L-tyrosine. Sometimes during post translational modifications this is converted to D-tyrosine.[83] Both isomers are found in tyrosine containing secondary metabolites.

Tyrosine oligomers are also very important compounds, and there are several different naturally occurring forms, such as dityrosine, isodityrosine, trityrosine and pulcherosine (Figure 27).[84] These compounds are found in various surroundings, including plant and fungal cell wall proteins, proteins such as collagen and elastin, and in insect and sea urchin egg envelopes.[85,86] These poly-tyrosine moieties are also associated with proteins found in various diseases such as Alzheimer's and

Parkinson's disease, as well as atherosclerosis and cystic fibrosis.[86] It is thought that the cross-linking between tyrosines in these structures adds stability and strength to proteins. A link has been made between the level of tyrosine cross-linking and oxidative stress in cells. Extensive cross-linking of tyrosine moieties is used diagnostically as an indicator of protein and cellular damage. There are many natural products also containing tyrosines, sometimes cross-linked, these include RP 66453,[87] chloropeptin,[88] bouvardins,[89] geodiamolides[90] and bisebromoamide.[91] It is unclear whether these naturally occurring cross-linked functionalities are present as a result of two neighbouring tyrosine residues being oxidatively coupled together in the cell, or if they were directly incorporated into the structure already as isodityrosine.[92]

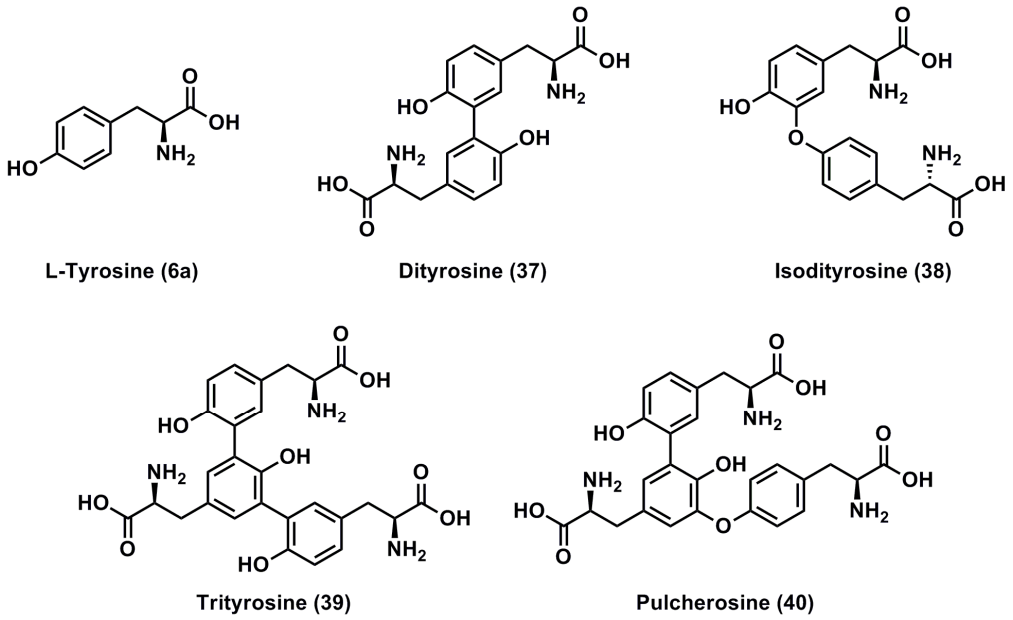
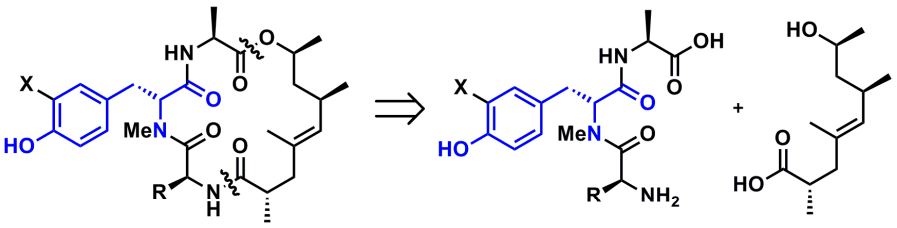


Figure 27. Structures of Tyrosine Oligomers.[86]

There are several feasible ways of synthesising such tyrosine derivatives, including Stille or Suzuki couplings, Ullmann condensations, copper-mediated biaryl coupling. Some of these methods require halogenated-tyrosine derivatives in order to carry out these transformations. Some examples of such procedures, as applied to natural product syntheses are described below.

3.1.1. Geodiamolides

Geodiamolides are a family of cyclodepsipeptide natural products isolated from *Geodia* species of marine sponge.[90] They have been shown to have anti-fungal activity against *Candida albicans*. Their structures (Figure 28) generally contain a tripeptide and a polypropionate unit. The tripeptide unit contains an unnatural *ortho*-halo-*N*-methyl-D-tyrosine derivative.

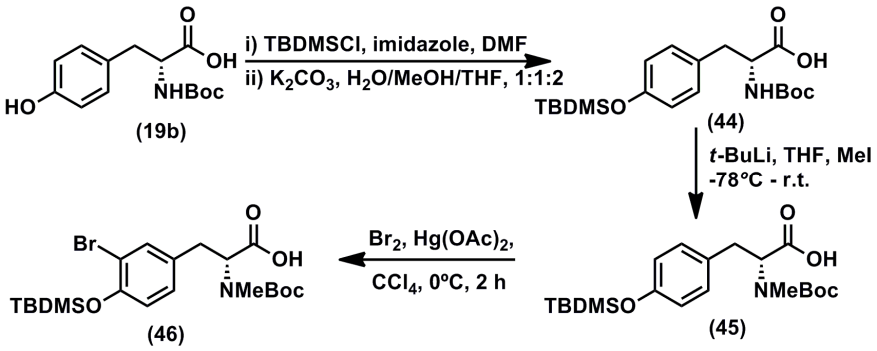


Geodiamolide A, X = I, R = Me
Geodiamolide B, X = Br, R = Me
Geodiamolide C, X = Cl, R = Me
Geodiamolide D, X = I, R = H
Geodiamolide E, X = Br, R = H
Geodiamolide F, X = Cl, R = H

A **(41)** X = I, R = Me
B **(42)** X = Br, R = Me
D **(43)** X = I, R = H

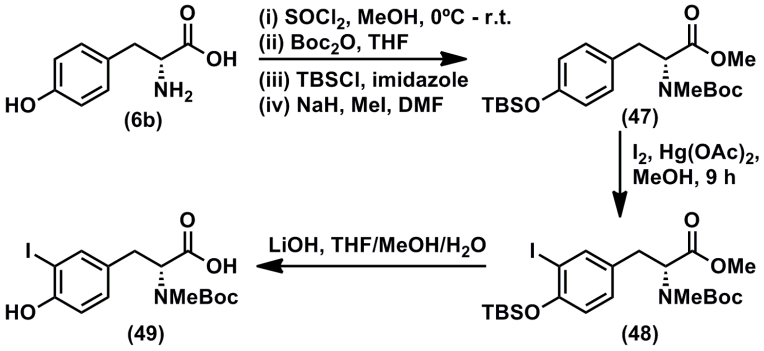
Figure 28. Structure and Retrosynthesis of the Geodiamolide Family of Compounds.[93]

The first total synthesis of Geodiamolide B was carried out by Perez-Medrano and Grieco largely by peptide coupling of fragments, and by macrolactonisation to complete the structure of the final compound.[94] The tyrosine derivative was synthesised (Scheme 13) starting with protection of the phenol (**19b**) as the TBDMS ether (**44**). *N*-methylation was conducted with *tert*-BuLi and methyl iodide in 71% yield, followed by selective mono-bromination in the *ortho*-position using bromine and mercuric acetate in carbon tetrachloride with an 80% yield. **46** was then ready for coupling to the two further amino acid fragments, completing the tripeptide unit (**42**) in 15% yield. **42** was coupled to the nonenoic acid successfully, and completion of Geodiamolide B was achieved in four further steps.



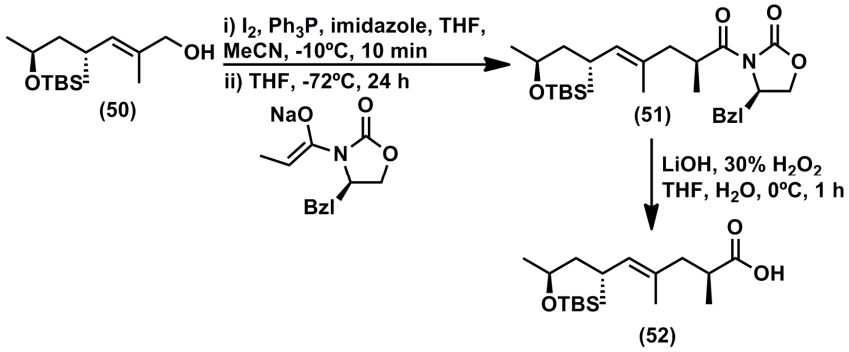
Scheme 13. Perez-Medrano's Synthesis of the Tyrosine Fragment of Geodiamolide B.[94]

In 1993, the Bhandari group completed the total synthesis of Geodiamolide D.[95] Their approach (Scheme 14) to this compound was similar to that of Perez-Medrano's approach to Geodiamolide B (Scheme 13). They carried out methyl ester protection of D-tyrosine (**6b**), followed by Boc protection, alcohol protection as the silyl ether, and *N*-methylation with sodium hydride and methyl iodide in DMF with a yield of 58% over these four steps to **47**. Mono-*ortho*-iodination using iodine and mercuric acetate was performed, followed by basic ether and ester hydrolysis to give Boc*N*Me-3-I-Tyr-OH (**49**) in an overall yield of 46% in six steps. The tyrosine derivative (**49**) was coupled to the other amino acids forming intermediate (**43**), which was coupled to the nonenoic acid fragment, ready for formation of the macro-structure. The Bhandari group had difficulty with the final macrolactonisation, but achieved it with DCC/DMAP/TFA in refluxing chloroform in a 7% yield for this final step.



Scheme 14. Bhandari Group's Approach to the Tyrosine Feature of Geodiamolide D.[95]

Shioiri and co-workers completed a unique approach to the synthesis of Geodiamolide A, focussing on three main steps which they believed to allow large scale production of the natural product.[96] These main steps include synthesising the polypropionate unit by an asymmetric alkylation using Evans' chiral oxazolidinone (Scheme 15), then for the initial coupling of the tripeptide and polypropionate units, they used a Mitsunobu reaction, and finally macrolactamisation to complete the cyclic structure.



Scheme 15. Evans' Asymmetric Alkylation as Employed by Shioiri and Co-Workers.[96]

For their synthesis of the tyrosine derivative, the Shioiri group performed *N*-methylation on Boc-Tyr(P)-OH, where P is either the $BzlCl_2$ or TBS ether, using sodium hydride and methyl iodide in THF.[96] The Shioiri group then coupled together the three amino acids forming the tripeptide unit before carrying out selective mono-*ortho*-iodination with iodine and mercuric acetate.

3.1.2. Bouvardins

Bouvardin (**53**) and related compounds such as deoxybouvardin (**54**) and RA-VII (**55**) (Figure 29) are a family of bicyclic hexapeptides which have been isolated from the *Bouvardia ternifolia* plant over the last thirty years.[97] Bouvardin and deoxybouvardin were initially shown to have anti-neoplastic disease activity both *in vitro* and *in vivo*, and have since also been shown to be exceptionally potent anti-tumour and antibiotic agents which can inhibit protein synthesis.[92]

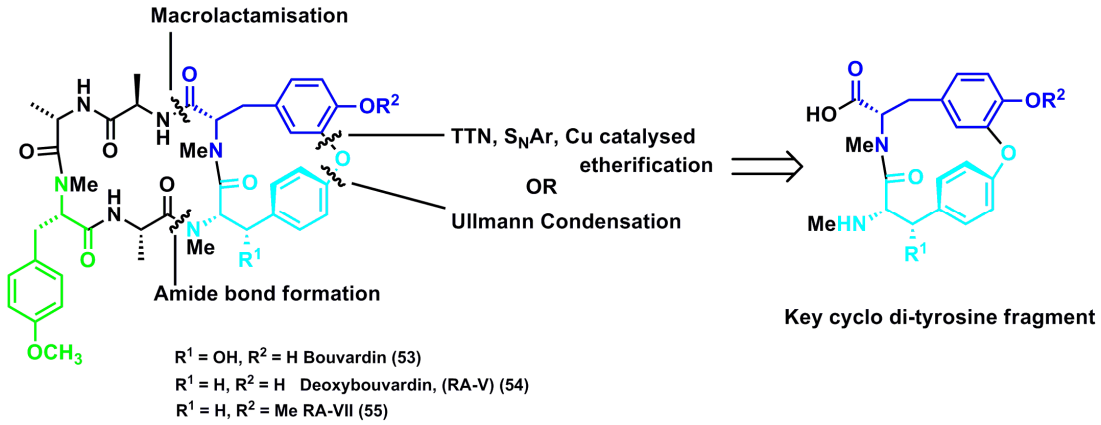
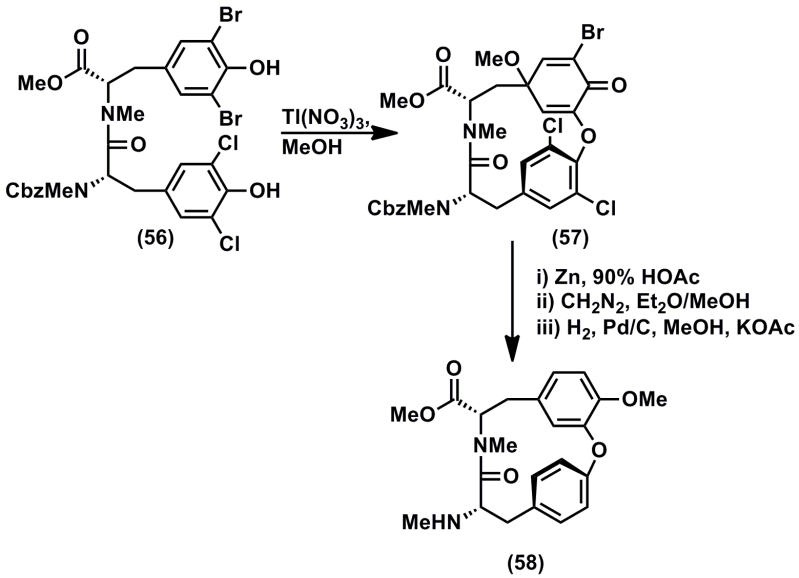


Figure 29. Structure of Some of the Bouvardin Family of Natural Products and Retrosynthetic Approaches.[89,92,98-101]

Several synthetic routes towards various members of this family of natural products have been published, based on similar retrosynthetic approaches.[89,92,98-101] All of the approaches involve cleaving the dityrosine unit from the tetrapeptide, synthesising the two fragments independently then forming the final bicyclic structure *via* macrolactamisation. A number of different methods have been used in order to form the dityrosine unit.

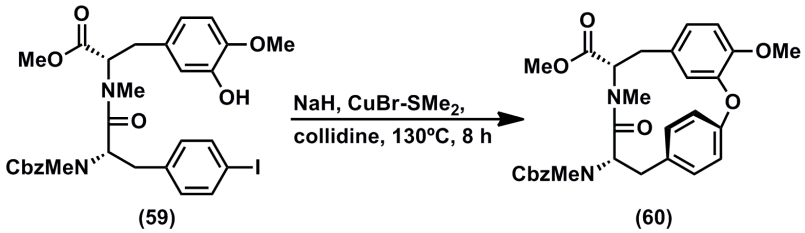
All of the tyrosine units in this family of structures are *N*-methylated. All groups whose syntheses are discussed carried out this transformation using sodium hydride and methyl iodide in THF, usually with DMF as a co-solvent, and obtained a yield around 90%.[89,92,98-101]

In the first total synthesis of deoxybouvardin (**54**) and RA-VII (**55**), Ogura and co-workers decided to synthesise this biaryl unit (**58**) by a thallium trinitrate (TTN) intramolecular oxidative coupling (Scheme 16).[98] They did successfully achieve the isodityrosine unit by this method (5% yield), and complete the coupling to the tetrapeptide unit to synthesise deoxybouvardin and RA-VII, but in very poor overall yields (<1%).



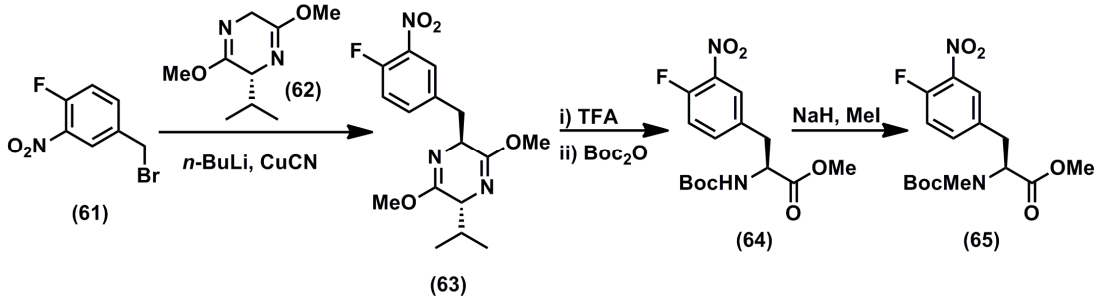
Scheme 16. Ogura and Co-workers' TTN Approach to the Cyclo Dityrosine Fragment.[98]

The Boger group undertook various studies towards the total synthesis of these bouvardin structures, and they focused on forming the 14-membered cyclo dityrosine unit by employing an Ullmann type reaction in order to achieve the cyclisation by intramolecular condensation (Scheme 17).[99] This was more successful than the TTN approach previously used, as it achieved higher yields and the starting materials used were more common amino acids, rather than di-halo derivatives. From a previously reported L-Dopa-type tyrosine derivative[102] coupled *via* amide bond to the Cbz-protected methyl ester of 4-iodo-phenylalanine, the intramolecular Ullmann condensation was completed as shown in scheme 17 in 24-30% yield.[99] It was then coupled to the tetrapeptide fragment with appropriate protecting group manipulations to afford the target deoxybouvardin (**54**) or RA-VII (**55**) compounds. The overall yield (2%) was an improvement on the TTN method, but still not very remarkable.



Scheme 17. Ullmann Biaryl Coupling Approach to the Key Cyclo Dityrosine Fragment, Used by Boger and Co-Workers.[99]

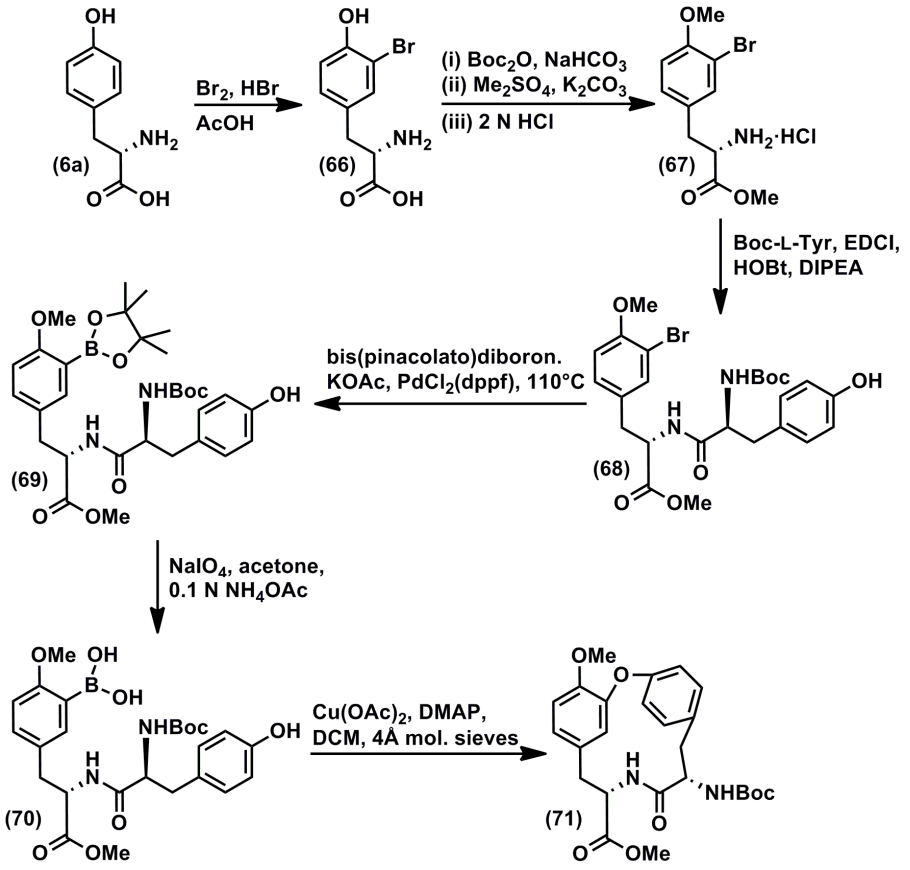
A few years later, the Boger group had managed to make significant progress in this respect, and achieved a moderate (54%) yield of **60**, in closure of this 14-membered isodityrosine unit.[89] They employed a 4-fluoro-3-nitro-phenylalanine substrate (**65**) that the Zhu group used in the synthesis of Chloropeptin I and RP 66453[103] (Scheme 18). The nitro group could be removed later by reduction, then elimination.



Scheme 18. Synthesis of 4-Fluoro-3-Nitro-Phenylalanine Fragment.[103]

This substrate was *N*-methylated using NaH in THF, in a 95% yield, and then they formed the isodityrosine unit with a simple intramolecular nucleophilic aromatic substitution procedure, in a yield of 50-61%. Around the same time, Zhu and co-workers published a similar application of this previously developed procedure, also towards the bouvardin family structures.[100] They reported a 54% yield for this biaryl unit, which in five further steps was manipulated into an intermediate (**58** in Scheme 16) used in the synthesis published by Ogura and colleagues.[98]

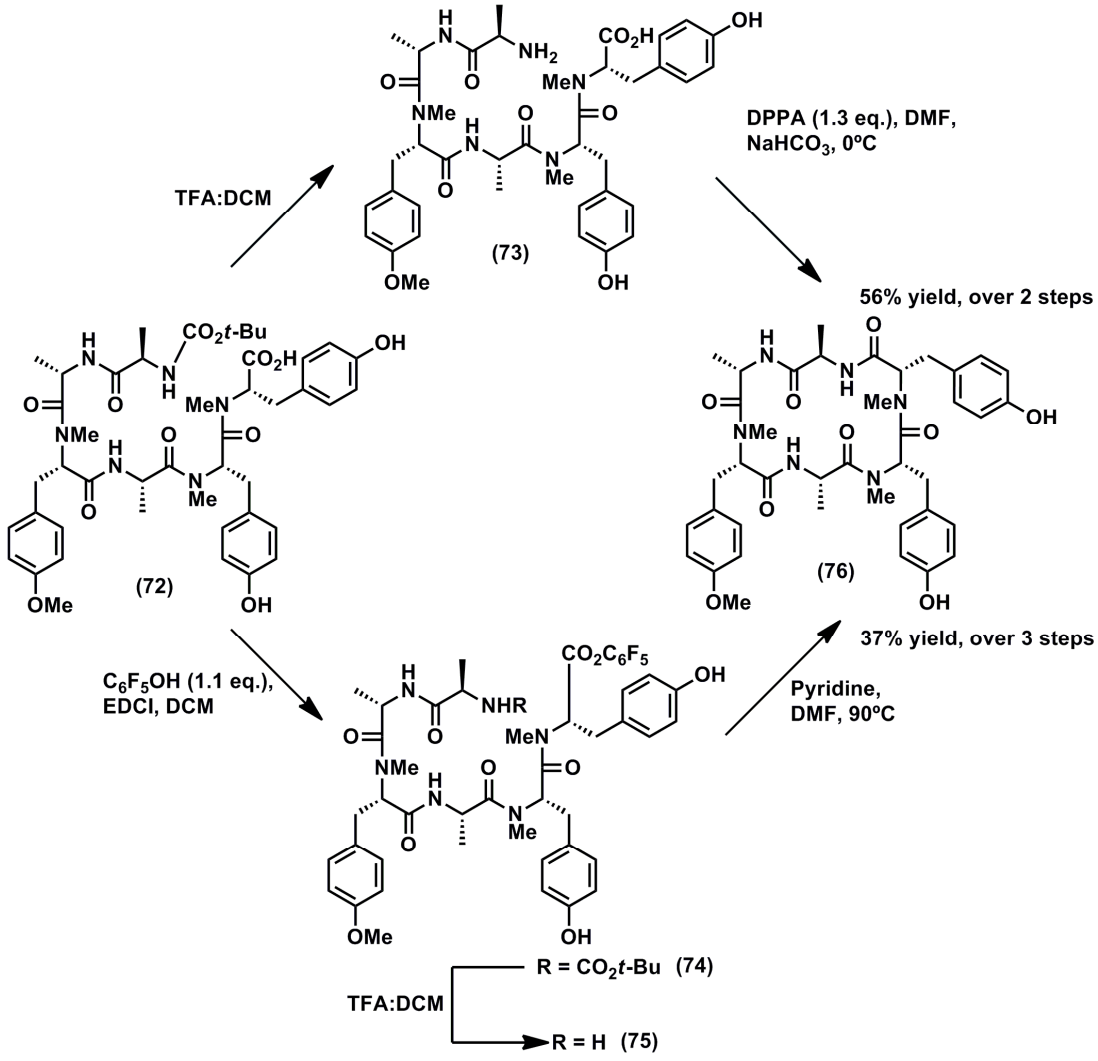
Recently, Soundararajan and collaborators published an improved synthesis of the 14-membered cyclo dityrosine ring component of deoxybouvardin (**54**) and RA-VII (**55**).[101] They synthesised a 3-bromo-tyrosine derivative (**67**) from tyrosine with bromine and hydrogen bromide, and achieved mono-brominated product (**66**) in 95% yield (Scheme 19). This bromo-derivative (**66**) was incorporated into dityrosine derivative (**68**), which was later converted to a boronic acid (**70**). Copper mediated coupling of the dimer with $Cu(OAc)_2$ and DMAP, selectively achieved the desired cyclisation product (**71**) in a yield of 60%, higher than any of the TTN, Ullmann, or nucleophilic aromatic substitution reactions previously used.



Scheme 19. Soundararajan Group's Approach to Cyclo Dityrosine.[101]

The synthesis of this biaryl dityrosine unit has been the main challenge of these syntheses. Boger and co-workers have been working towards the syntheses of these compounds, and other related dityrosine compounds, for more than twenty years, which reflects the difficulty of achieving such units efficiently. They published a paper in 1988 which included investigations into the cyclisation of the core peptide structure, without the biaryl coupling between the two tyrosine components.[92] They intended to find out if the extended efforts into this functionality were necessary for the bioactivity of the bouvardin family of compounds. Boger and co-workers carried out macrolactamisation of the hexapeptide core, carefully choosing the location for the final amide bond formation. They considered that the rate of amide bond formation would be affected by substitution at the amine, for instance it could be slowed down by coupling to an *N*-Me group, but the rate could also be increased by coupling to the *N*-terminus of a D-amino acid rather than an L-isomer. With these considerations in mind, they chose to close the ring between the D-alanine and L-

tyrosine. They tried with two different groups at the *C*-terminus; the free acid (**73**), coupling with DPPA and $NaHCO_3$, and also with the pentafluorophenyl ester (**75**) using pyridine to affect the cyclisation (Scheme 20). Both methods were successful, but the free acid/DPPA method was one step shorter and gave a higher overall yield. Having successfully synthesised the core structure (**76**) of the bouvardin family of natural products, Boger and colleagues tested the bioactivity of the compound and found that it showed no appreciable bioactivity, therefore proving that the biaryl-link between the two tyrosine components is essential for activity. There remains room for improvement for the synthesis of this biaryl unit.



Scheme 20. Boger's Approaches to the Non-Cyclised Dityrosine Bouvardin Derivative.[92]

3.1.3. Chloropeptins I and II

Chloropeptins I (**77**) and II (**78**) (complestatin), are related molecules, extracted from *Streptomyces* species of bacteria, which have proven activity against HIV-1.[104] They are remarkable because they have been shown to act in two locations against HIV, thereby making it much more difficult for the virus to evolve to become resistant to them. The two compounds differ only by the attachment of the indole to the central phenyl segment, ring 'D' (Figure 30). The left-hand macrocycle contains an *N*-methyl-tyrosine derivative.

Several syntheses exist for these two compounds; most employ a nucleophilic aromatic substitution to form the left hand macrocycle, and either Stille or Suzuki coupling, or a Larock annulation to form the right hand macrocycle.[88,104-106] Formation of the rest of the structure is usually based on peptide couplings. The focus of this section will be the generation of the tyrosine derivative and the two macrocycles.

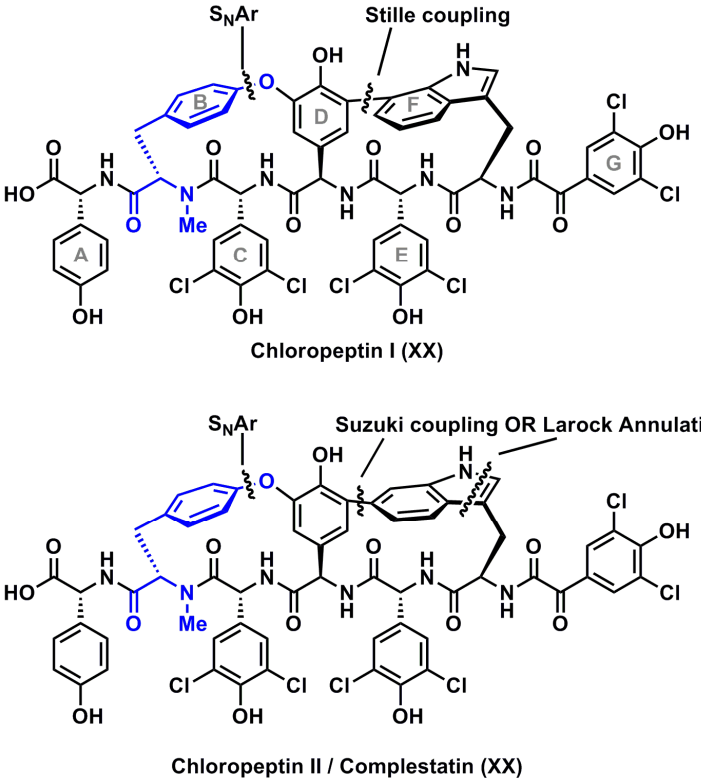
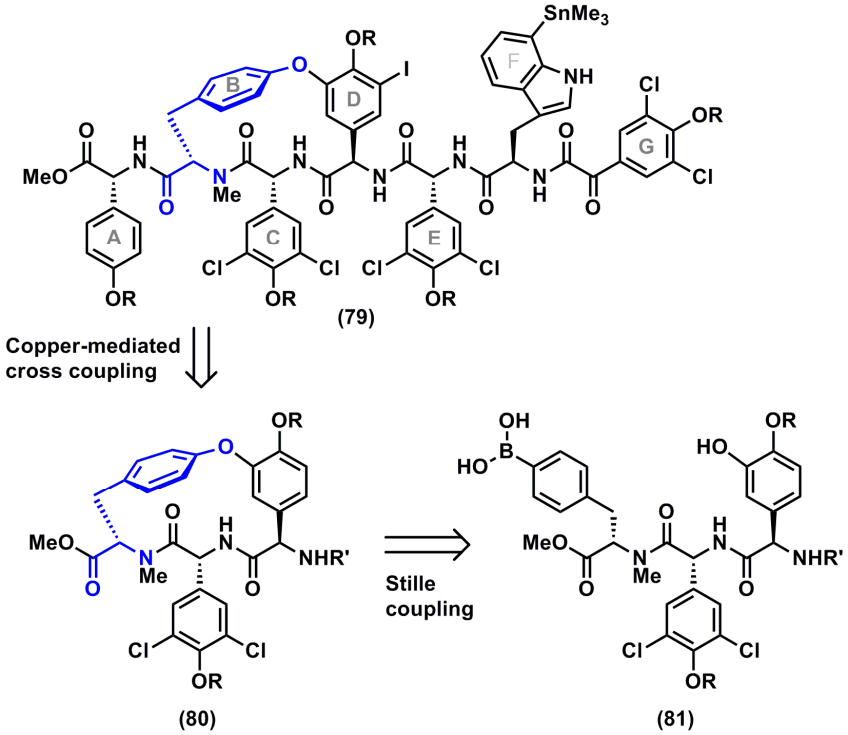


Figure 30. Retrosynthetic Approaches Toward the Structures of the Chloropeptin Compounds.[88,104-106]

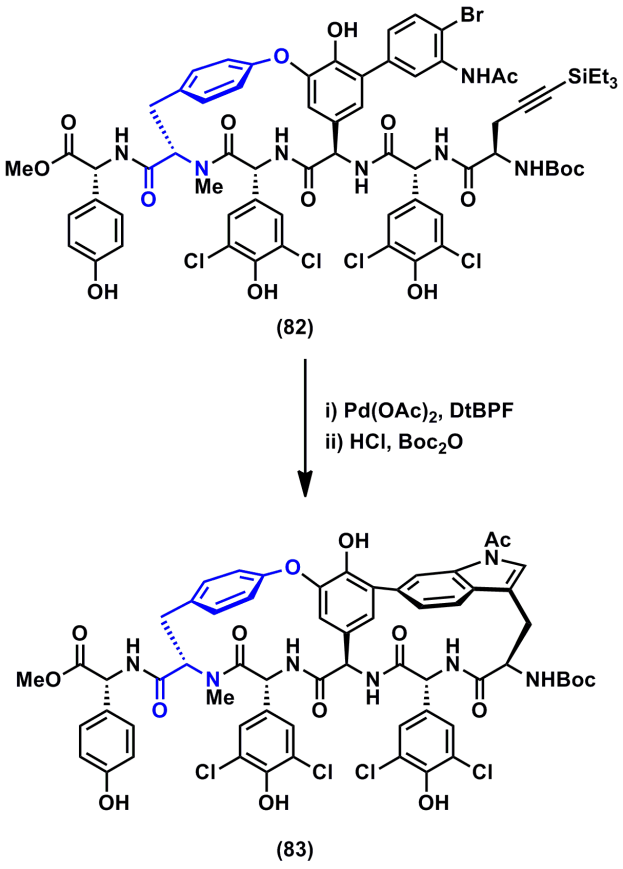
Snapper and Hoveyda published the first synthesis of chloropeptin I, and found that with their chosen conditions, in order to form the desired atropisomer, they needed to carry out the formation of the left hand macrocycle first (Scheme 21).[105] They achieved this *via* copper-mediated biaryl ether formation (50% yield), this then led to the desired atropisomer when carrying out a Stille coupling to form the phenyl-indole bond (40% yield, **80**).



Scheme 21. Snapper and Hoveyda's Approach to Chloropeptin I.[105]

Snapper and Hoveyda then tried a similar approach to the synthesis of chloropeptin II, with a Suzuki-Miyaura borylation coupling to form the phenyl-indole bond after the biaryl ether was in place. However, this resulted in the unnatural atropisomer of chloropeptin II. Further work using the Suzuki-Miyaura approach has been more successful towards chloropeptin II synthesis in the hands of Zhu group members.[106] In order for successful formation of the correct atropisomer, they formed the right hand macrocycle by Suzuki-Miyaura coupling in 66% yield, and later nucleophilic aromatic substitution (62% yield) to form the left hand macrocycle.[103]

Boger *et al.* carried out the first synthesis of chloropeptin II in 2009.[88] They decided to take a different approach to the formation of the indole and employed an intramolecular Larock annulation (Scheme 22), successfully demonstrating the reaction's first use in macrocyclisation with a yield of 56%. This step was carried out before the nucleophilic substitution to form the biaryl ether (81% yield), and successfully gave the desired atropisomer (**83**) in 4:1 selectivity, contrary to the selectivity shown by the Suzuki-Miyaura approach by Snapper and Hoveyda. In later studies by the same group, they managed to optimise the synthesis, finding that by forming the left hand macrocycle first, they achieved greater selectivity, 20:1, for the Larock annulation product (**83**) when forming the right hand macrocycle, achieving a 56% yield of the single desired atropisomer (Scheme 22).[104]



Scheme 22. Larock Annulation Method for Right Hand Macrocycle Formation of Chloropeptin II.[88]

The tyrosine derivative appearing in the left hand macrocycle of the chloropeptin structures is *N*-methylated. This was achieved using methyl iodide and sodium

hydride, by all three groups discussed.[88,105,106] However, none of the approaches used tyrosine as the starting material for this portion, instead they employed the method reported in the synthesis of the similar natural compound, teicoplanin aglycon (Scheme 18, Section 3.1.2).[107]

It was also discovered that by treating chloropeptin II (**78**) with acid, it could be easily converted into chloropeptin I (**77**).[108] This conversion was most efficient when using TFA at 50 ºC for five minutes.

3.1.4. RP 66453

RP 66453 (**84**) is a neurotensin antagonist, a cyclic peptide containing pulcherosine.[86] It was first isolated from a *Streptomyces* species of bacteria as a secondary metabolite in 1998.[87] It can be used to treat psychosis, Alzheimer's and Parkinson's diseases.[86]

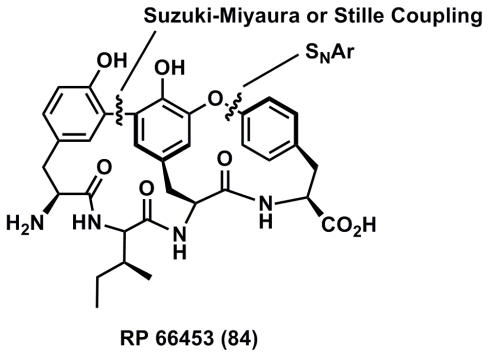
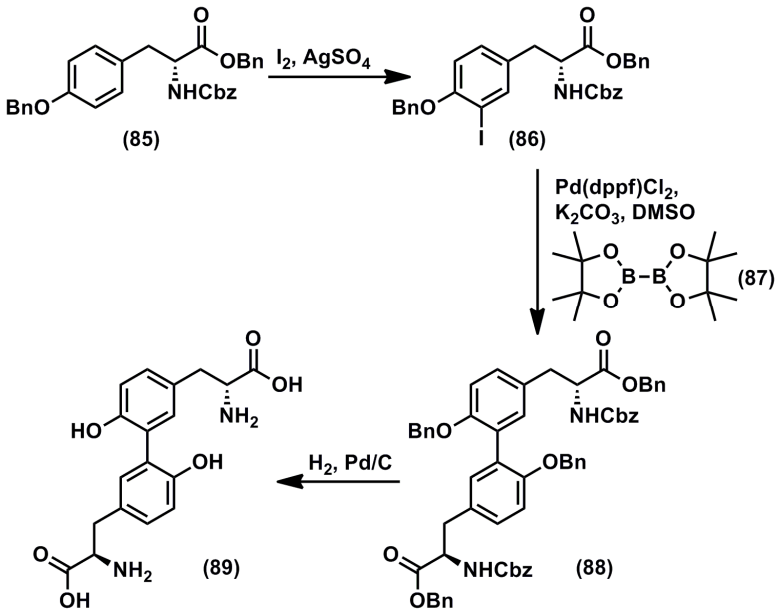


Figure 31. Structure of Tyrosine-Containing Neurotensin Antagonist RP 66453, and Retrosynthetic Approaches.[85,86,103,109]

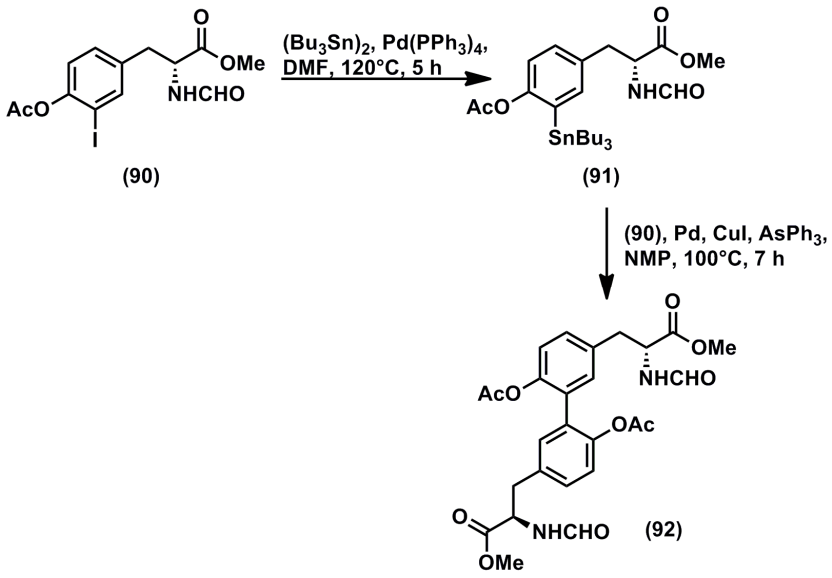
The stereochemistry of RP 66453 was determined by Zhu and co-workers during their total synthesis in 2003.[103] Their approach to the formation of RP 66453 involved solution phase coupling of the amino acids and nucleophilic aromatic substitution to form the 14-membered ring by ether formation using the same 4-fluoro-3-nitro-phenylalanine type of substrate (**65**) approach as the group also used in the synthesis of chloropeptin II.[106] This was followed by Suzuki-Miyaura borylation coupling of a 3-iodo-tyrosine derivative (**86**) to afford the 15-membered macrocycle in 40% yield, completing the core structure of RP 66453. Skaff and co-workers had

first published this tandem Suzuki-Miyaura borylation coupling method of forming the dityrosine, 15-membered ring section of the molecule earlier the same year.[85] They used iodine and silver sulfate to form the 3-iodo-tyrosine derivative (**86**) (Scheme 23), in a 90% yield, that was then used with $Pd(dppf)Cl_2$, K_2CO_3 and bis(pinacolato)diboron in DMSO to give the biaryl compound (**88**) in 70% yield, which underwent global deprotection with H_2, Pd/C to give the final dityrosine desired product (**89**).



Scheme 23. Suzuki-Miyaura Borylation Biaryl Coupling to Form Dityrosine.[85]

Stille couplings have also been successfully employed by Velay and Achab to form a similar bi-aryl dityrosine derivative in 56% yield for the coupling of **90** to **91** (Scheme 24), as demonstrated during their formal total synthesis of the antibiotic, Hazimycin.[109]



Scheme 24. Stille Biaryl Coupling to Form Dityrosine Derivative as used by Velay and Achab.[109]

3.1.5. Bisebromoamide

In October 2009, a new tyrosine containing natural product's discovery was published, bisebromoamide (**93**).[91] It was isolated from the *Lyngbya sp.* of a marine cyanobacterium found in Japan. It is a novel cytotoxic linear peptide based compound, which has sparked interest due to its anti-proliferative activity at nanomolar levels. Particularly interesting features of this compound include a thiazoline ring, which is methylated in the α-position, attached to a 4-methyl-pyrrole ring. It also contains a 3-bromo-D-tyrosine derivative, with two *N*-methyl amino acids, and the 2-(1-oxopropyl)pyrrolidine (Opp) moiety, which had never previously been seen in a natural product.

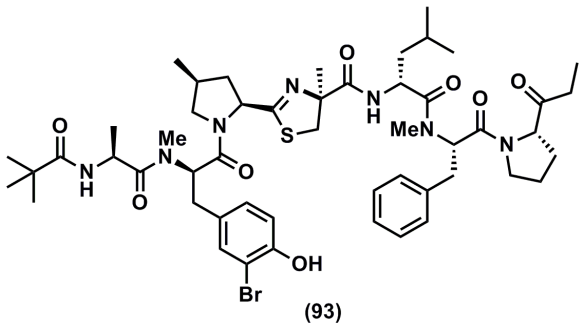


Figure 32. Proposed Structure of the Natural Product Bisebromoamide.[91]

The Ye group published the first total synthesis of bisebromoamide in 2010.[110] They approached the synthesis by creating four main fragments as shown in figure 33.

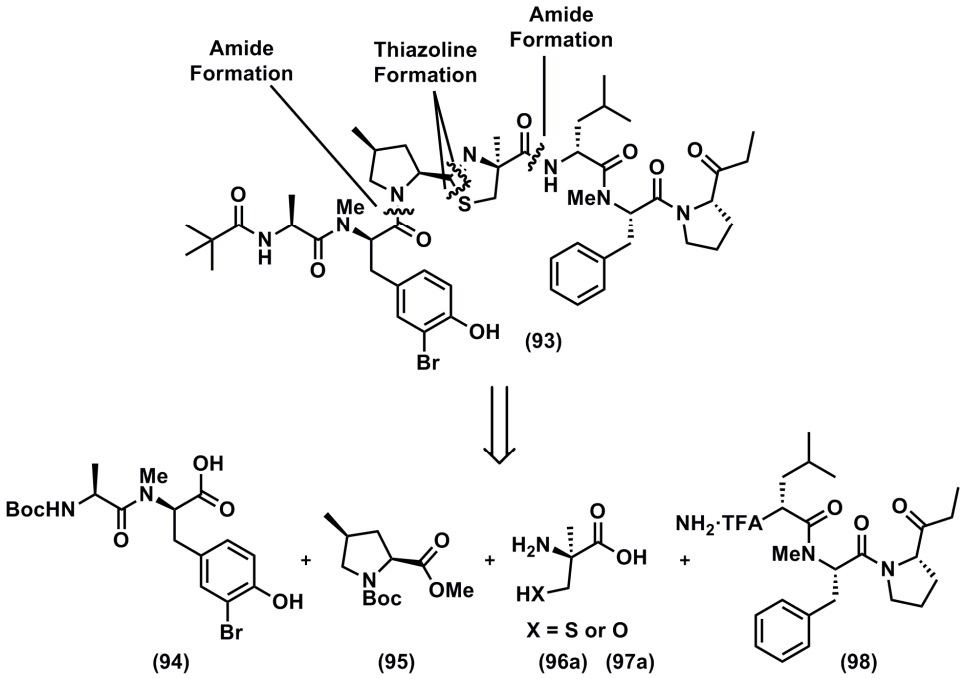
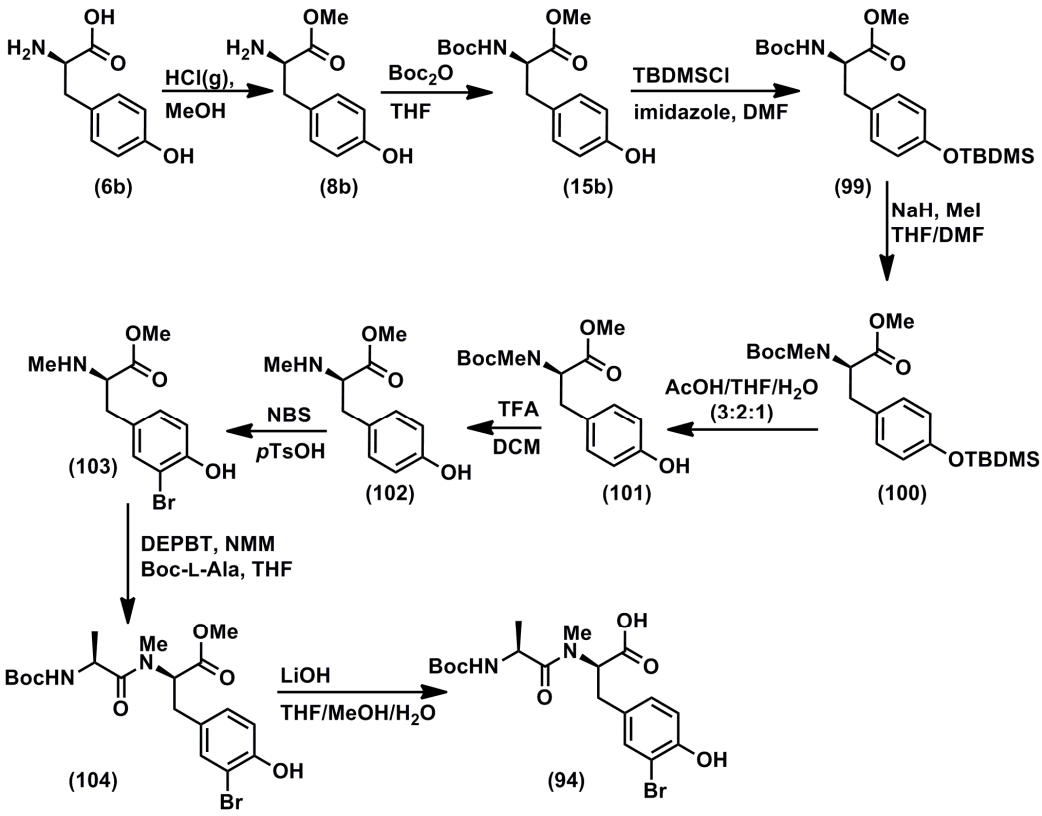


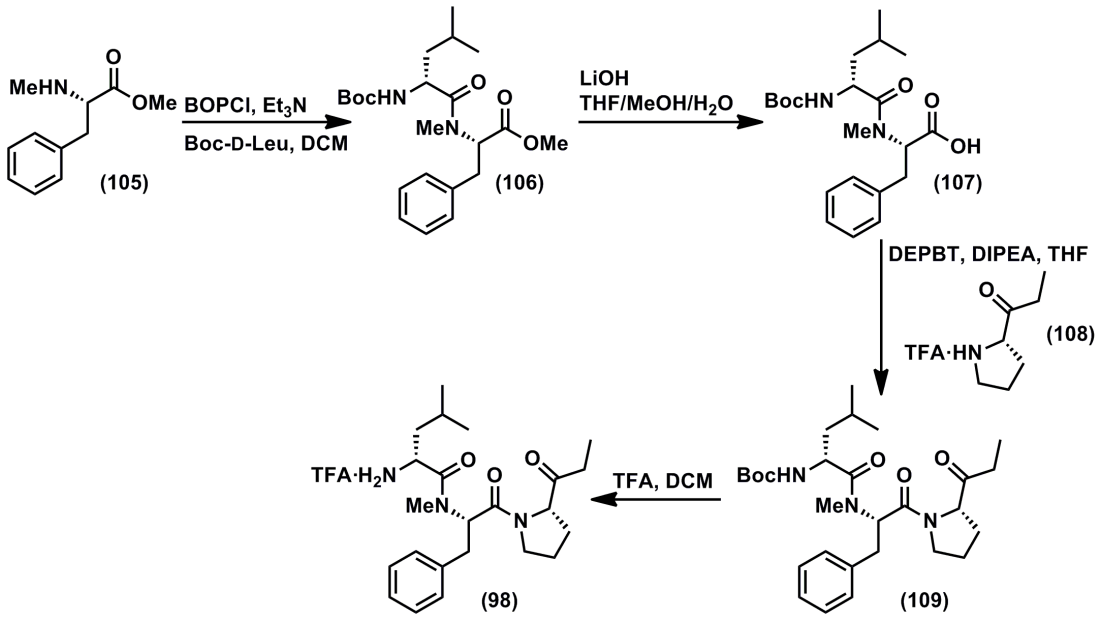
Figure 33. Ye Group's Retrosynthetic Approach to the Proposed Structure of Bisebromoamide, Showing Key Target Fragments.[110]

The Ye group hypothesised that the thiazoline feature might be sensitive, and should be established late on in the synthesis, to reduce the chances of its epimerisation.[110] The group began their synthesis by developing fragment **94** from D-tyrosine (Scheme 25). They employed methodology published by the Boger group for the synthesis of the antipode (Scheme 25)[92] to achieve compound **102**. They then carried out selective mono-*ortho*-bromination using *N*-bromosuccinimide and *para*-toluene sulfonic acid in acetonitrile with an 87% yield.[110] This gave compound **103** in a yield of 27% over seven steps from D-tyrosine. This sequence was followed by coupling to Boc protected L-alanine using 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT), the product (**104**) of which underwent base hydrolysis to give fragment **94**.



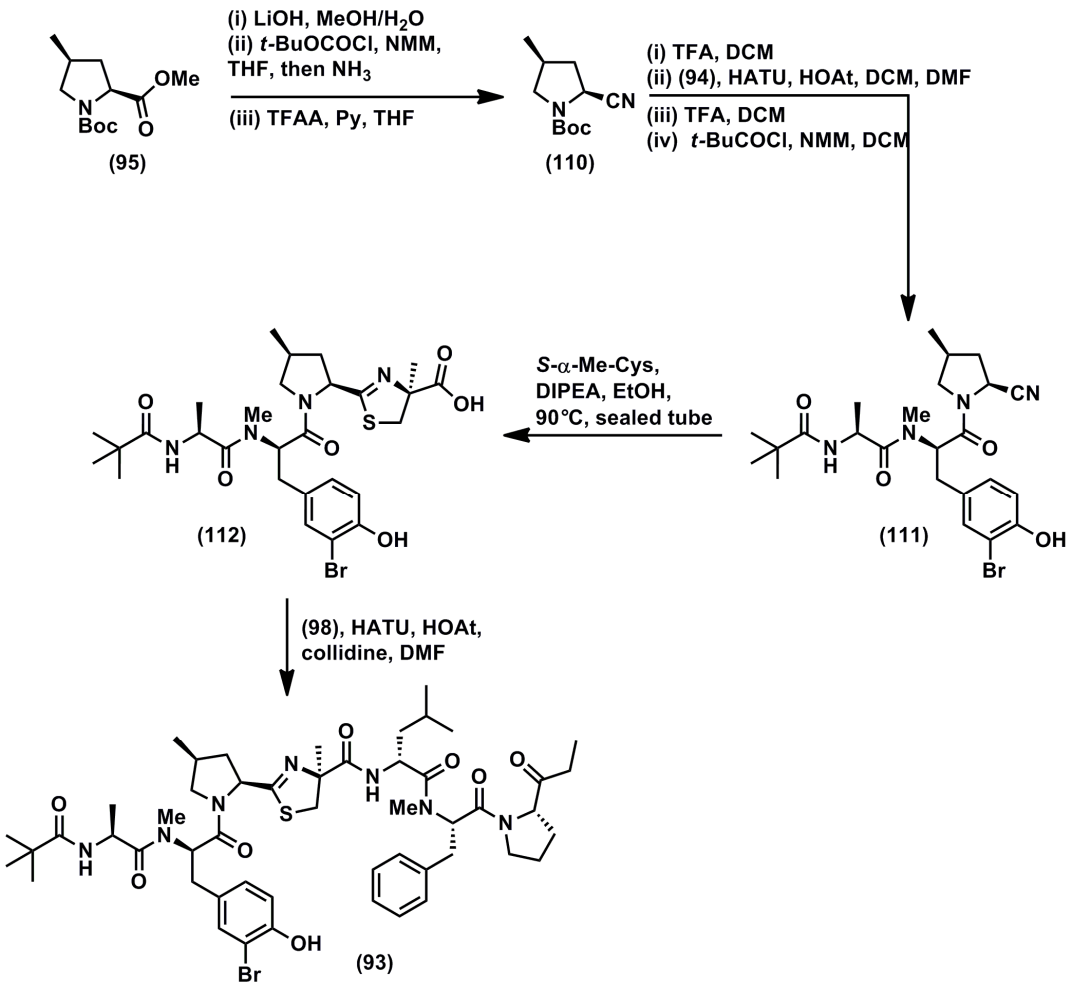
Scheme 25. The Ye Group Approach to Fragment **94** of Bisebromoamide.[110]

The group obtained the known phenylalanine derivative (**105**) of fragment (**98**)[106] and coupled it to Boc-D-leucine using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) and triethylamine. The dipeptide product (**106**) was then coupled with the 2-(1-oxopropyl)pyrrolidine (Opp) fragment (**108**), prepared from a literature procedure,[111] using DEPBT (Scheme 26).[110]



Scheme 26. The Ye Group Approach to Fragment **98** of Bisebromoamide.[110]

Ye and co-workers then attempted to construct the thiazoline ring from α-methyl serine coupled to fragment (**107**) and then through cyclodehydration of a β-hydroxy thioamide derivative of the 4-methyl pyrrole moiety.[110] This was unsuccessful, and they assumed the reason was due to steric hindrance between the thioamide and the α-methyl serine quaternary centre. They revised their approach to involve cyclocondensation between a nitrile derivative of the 4-methyl pyrrole moiety (**110**) and *S*-α-methyl cysteine (**96a**) (Scheme 27). This approach was effected by converting Boc-(4Me)-Pro-OMe (**95**) to the corresponding nitrile (**110**) using a literature procedure.[112] The Boc group was then removed, ready for coupling to Boc-Ala-*N*Me-Br-Tyr-OH (**94**) using HATU and HOAt.[110] The Boc group was removed from the resultant compound, and the free amine coupled with pivalic acid, giving **111**. The thiazoline ring was formed by reaction with *S*-α-methyl cysteine (**96a**)[110] in DIPEA and EtOH at 90°C in a sealed tube. The final step of coupling **98** with **112** was carried out employing HATU and HOAt, to afford the proposed structure of the bisebromoamide natural product (**93**).

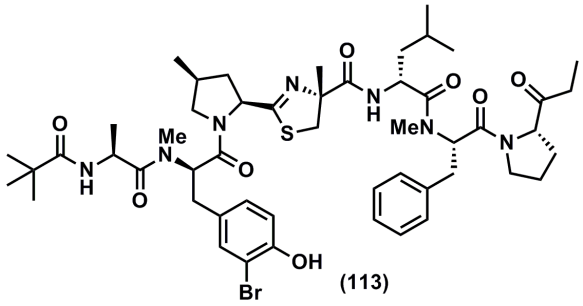


Scheme 27. The Ye Group's Formation of Bisebromoamide's Proposed Structure.[110]

On analysis of the experimental data, it was determined that the NMR data and optical rotation value of the synthetic product did not match those of the natural product sample.[110] Through careful observation of that data, it was proposed that the quaternary centre of the thiazoline ring had been wrongly assigned. Employing the same methodology, but using *R*-α-methyl cysteine (**96b**), the epimer was synthesised, and found to match the natural product exactly. The true stereochemistry of bisebromoamide is shown in figure 34.

Having synthesised two epimers of bisebromoamide, the Ye group investigated their bioactivity.[110] Investigations showed that both epimers showed similar levels of activity, and the IC_{50} values of *epi*-bisebromoamide (**113**) were comparable to those of bisebromoamide (**93**) across three cancer cell lines. This result indicates that the

stereochemistry of the α-position of the thiazoline is not significant for the biological efficacy of the compound.



(113)

Figure 34. Revised Structure of Bisebromoamide Natural Product. [110]

The Ma group completed the total synthesis of bisebromoamide in 2011.[113] In a method similar to the Ye group, they decided to install the thiazoline as late as possible in the synthesis, due to concern over the stability of this group. Ma and co-workers elected to form the thiazoline last, leaving two main fragments, one bearing a nitrile derivative of 4-methyl pyrrole (**111**), and the other with *S*-α-methyl cysteine (**114**). These two fragments could be further broken down to individual amino acid fragments (Figure 35). This design is convergent, and would easily allow adaptation of the individual amino acids to make analogues efficiently.

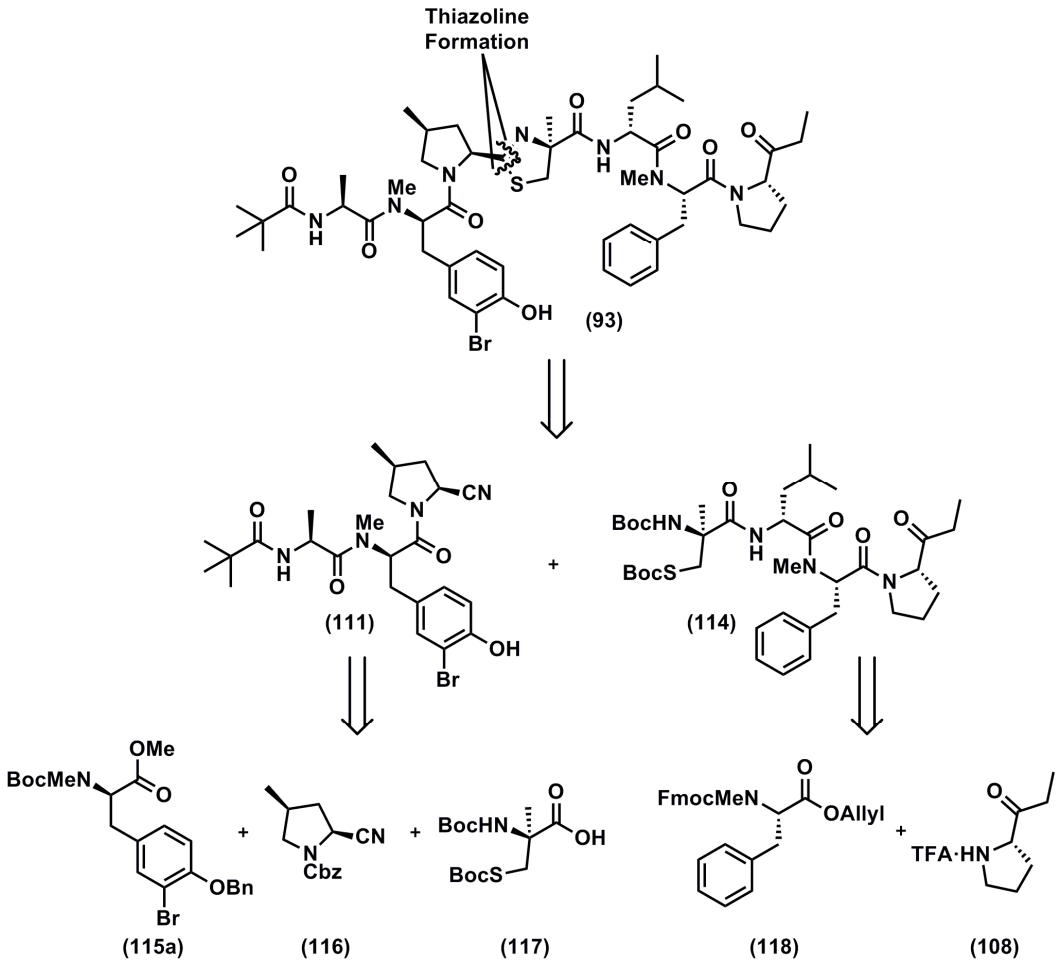
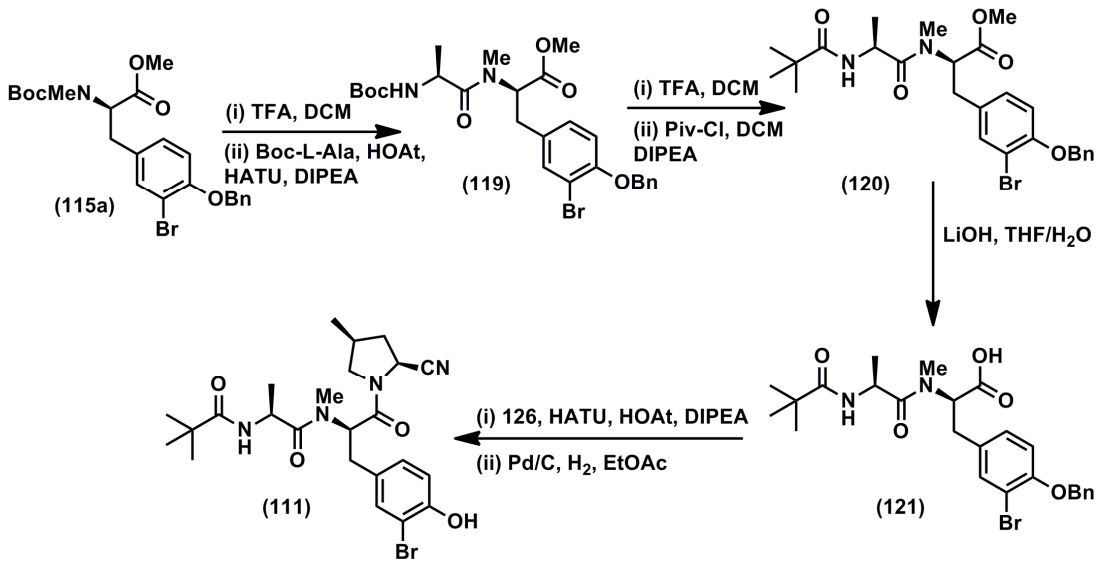


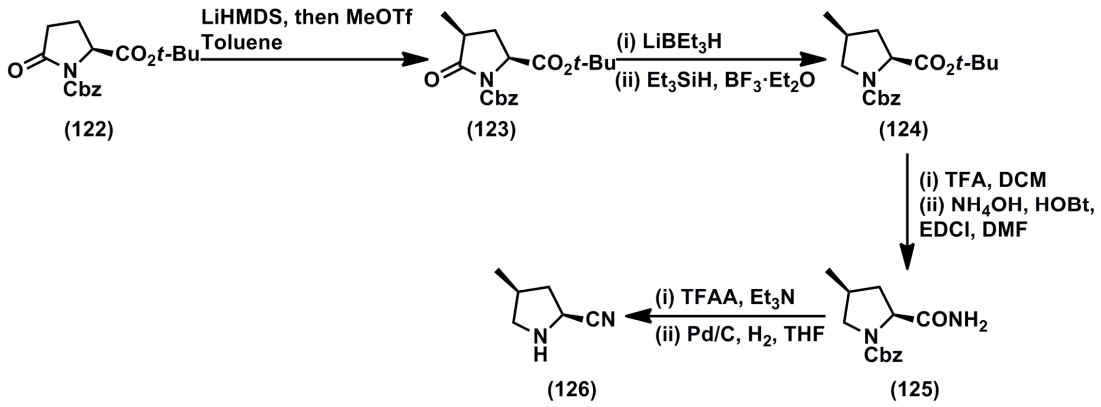
Figure 35. The Ma Group's Key Fragments and Retrosynthetic Route to the Proposed Structure of Bisebromoamide.[113]

The Ma group started off synthesising the left hand fragment (**111**) (Scheme 28). Despite the very high cost, they chose the commercially available Boc-*N*Me-D-Tyr(Bn)-OH as the starting point for their tyrosine derivative. They converted it to the methyl ester using $TMSCHN_2$, and then achieved selective mono-*ortho*-bromination with *N*-bromosuccinimide in DMF to give **115a** in an excellent yield of 98%. This brominated-tyrosine derivative was then Boc deprotected and coupled with Boc-L-Ala using HOAt and HATU, before further Boc deprotection and coupling with the terminal pivaloyl group. The tyrosine methyl ester (**120**) underwent base hydrolysis to give the free acid (**121**) ready to be coupled later.



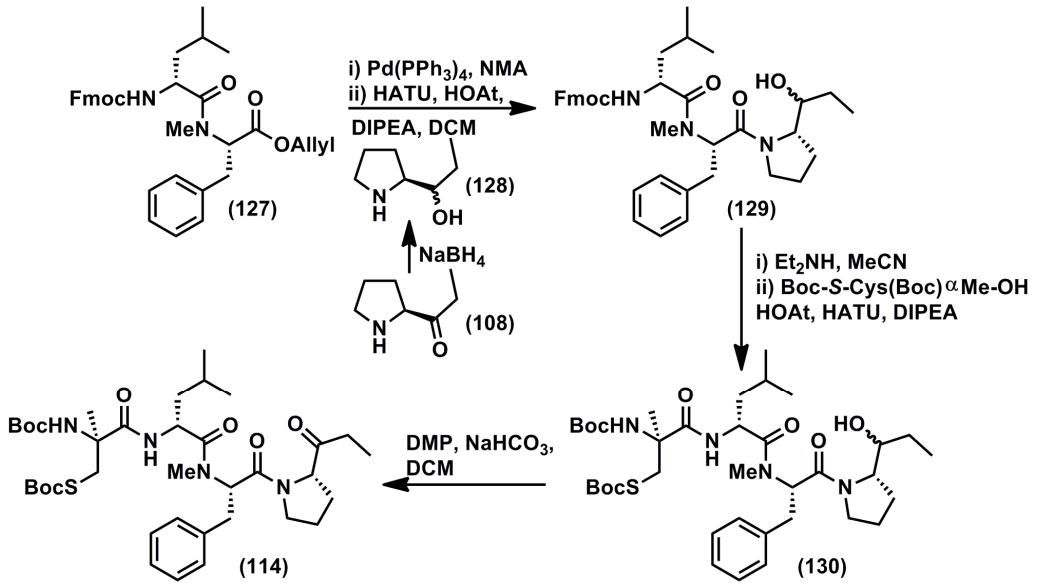
Scheme 28. The Ma Group's Approach to the Left-Hand Fragment of Bisebromoamide.[113]

The 4-methyl pyrrole derived fragment (**126**) was synthesised starting from a known literature compound (**122**).[114] This pyroglutamic acid derivative was methylated in the 4-position using LiHMDS then treated with methyl triflate to selectively give the *syn* diastereomer of **123**, which was reduced in two steps by using $LiBEt_3H$, and then Et_3SiH with $BF_3{\cdot}Et_2O$, affording **124** (Scheme 29).[113] The *tert*-butyl ester was cleaved with TFA, and did not require a scavenger. The acid was converted to the amide (**125**) through coupling with ammonium hydroxide, HOBt and EDCI. Dehydration was then carried out with trifluoroacetic anhydride, followed by Cbz deprotection by hydrogenation, affording the target fragment (**126**). The pyrrole derivative was then coupled to the rest of the tyrosine containing dipeptide derivative (**121**) using HATU, HOAt and DIPEA (Scheme 28). Hydrogenation was carried out under Pd/C, H_2 with ethyl acetate, achieving a low yield of the desired compound (**111**). If this hydrogenation step was carried out in a protic solvent, loss of the bromine was observed.



Scheme 29. The Ma Group's Approach towards the 4-Methyl Pyrrole Fragment, **126**.[113]

In undertaking the synthesis of the right hand segment of the natural product, Ma *et al.* started from Fmoc-*N*Me-Phe-OAllyl (**118**).[113] The Fmoc group was removed under basic conditions, and then the phenylalanine derivative coupled with Fmoc-D-Leu using HATU and DIPEA to give **127** (Scheme 30). The allylic group was removed with $Pd(PPh_3)_4$ and NMA, before coupling the amino alcohol (**128**) to the acid with HATU, HOAt and DIPEA. The amino alcohol was prepared through $NaBH_4$ reduction of the known compound **108**.[115] Having achieved **129**, the Fmoc group was removed, followed by coupling of the free acid of Boc-*S*-Cys(Boc)αMe-OH (**117**), again using HOAt, HATU and DIPEA, which was then oxidised to convert the amino alcohol (**130**) back to the ketone, the right hand segment of the natural product. (**114**).[113]



Scheme 30. Synthesis of the Right Hand Fragment of Bisebromoamide by the Ma group.[113]

The Boc protecting groups were removed from the right hand segment with TFA, then coupling to the left hand segment took place employing sodium hydrogen carbonate and methanol in a pH 6 buffered solution at 70ºC.[113] This successfully achieved formation of the thiazoline ring, and completed the synthesis of the proposed structure of bisebromoamide (**93**). The analytical data did not agree with that of the natural sample. The Ma group synthesised the compound with an epimeric thiazoline quaternary centre (**113**), and this agreed with the spectroscopic data of the natural product; thus supporting the conclusions of the Ye group after they completed their total synthesis.[110]

Making use of the convergent nature of their synthetic route, the Ma group decided to investigate some of the other stereocentres in the bisebromoamide structure.[113.] The group made a bisebromoamide epimer containing the alternative, L-enantiomer of the tyrosine fragment (**115b**), another containing D-alanine, and another without the methyl group in the 4-position of the central pyrrole fragment (**126**). These isomers together with the two different thiazoline isomers (**93** and **113**) were all tested to see how their IC_{50} values against HeLa S_3 cell lines compared. It was discovered that the unnatural thiazoline epimer of bisebromoamide (**93**) is only slightly less cytotoxic (IC_{50} 165 ng mL^{-1}) than the natural compound (IC_{50} 40 ng mL^{-}

[1]), as is the analogue with 4Me-Pro replaced by Pro (134 ng mL^{-1}), indicating that these features are not essential for activity. Changing the enantiomer of the tyrosine fragment (**115b**) caused the cytotoxicity to drop slightly (396 ng mL^{-1}), and changing the enantiomer of alanine destroyed all cytotoxicity (no activity at 8 μg mL^{-1}). The L-alanine stereochemistry is therefore essential to maintaining bioactivity.

Suenaga and co-workers have more recently published further investigations into bisebromoamide and some of its analogues.[116] They have isolated norbisebromoamide, an analogue where the α-methyl group from the thiazoline is replaced by a hydrogen (Figure 36, R^1=H). From the parent bisebromoamide structure (**113**), they have also developed several more analogues as shown in figure 36. The Suenaga group carried out investigations to measure the derivatives' IC_{50} values against HeLa S_3 cells, and found that all these analogues have cytotoxicity of a similar magnitude as the parent compound (40 – 91 ng mL^{-1}). This information means that the functional groups involved in all of the analogues are not essential for biological activity, as changing them has not reduced the IC_{50} values. Figure 36 shows a summary of all the adaptations made to create the analogues, and indicates the features of the molecule that can and cannot be modified in further investigations which might try to develop more cytotoxic analogues, or attach probes to learn more about the actions of the natural product and derivatives.

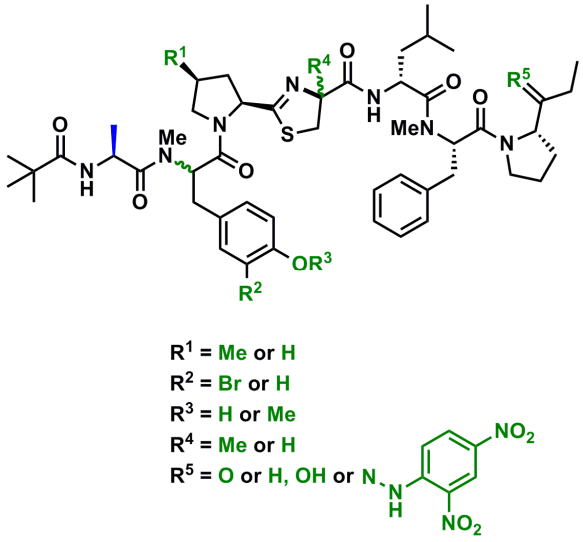


Figure 36. Tolerance of Biological Activity to Changing Structural Features of Bisebromoamide, as Shown by the Ma and Suenaga Groups.[113,116] Green – Tolerated. Blue – Essential for Bioactivity.

3.2. Biological Activity of Bisebromoamide

Further biological details were determined regarding the activity of bisebromoamide.[91] When tested against a selection of human cancer cell lines, bisebromoamide showed an average 50% growth inhibition (GI_{50}) value of 40 nM for all 39 cell lines assessed. It was also shown that the natural product could selectively inhibit the phosphorylation of a specific protein kinase (extracellular signal regulated protein kinase, ERK), and it did not show any inhibition of phosphorylation for other related proteins tested. Bisebromoamide therefore, is potentially selectively targeting the ERK signalling pathways.

3.2.1. Targeting Actin Filaments

Uesugi and co-workers have undertaken some investigations into actin filament stabilisers and destabilisers.[117] Observing changes in cell morphology, they used a library of natural products to identify compounds which had an effect on actin filaments in HeLa cells. The library used consisted of 400 purified natural products, which were a mixture of compounds with and without known biological activity. Of these 400 compounds, nine were identified, at concentrations between 0.1-1000 μM, as "hit" molecules after the treatment of HeLa cells for one hour produced an observable protrusion of the cell's nucleus. These nine compounds were cytochalasin D (**131**), mycalolide B (**132**), latrunculin A (**133**), swinholide A (**134**), jasplakinolide (**135**), doliculide (**136**), seragamide A (**137**), which were all previously known to have actin-targeted activity, plus miurenamide A (**138**) and of particular interest to our studies, bisebromoamide (**113**), whose activity was previously unknown (Figure 37).

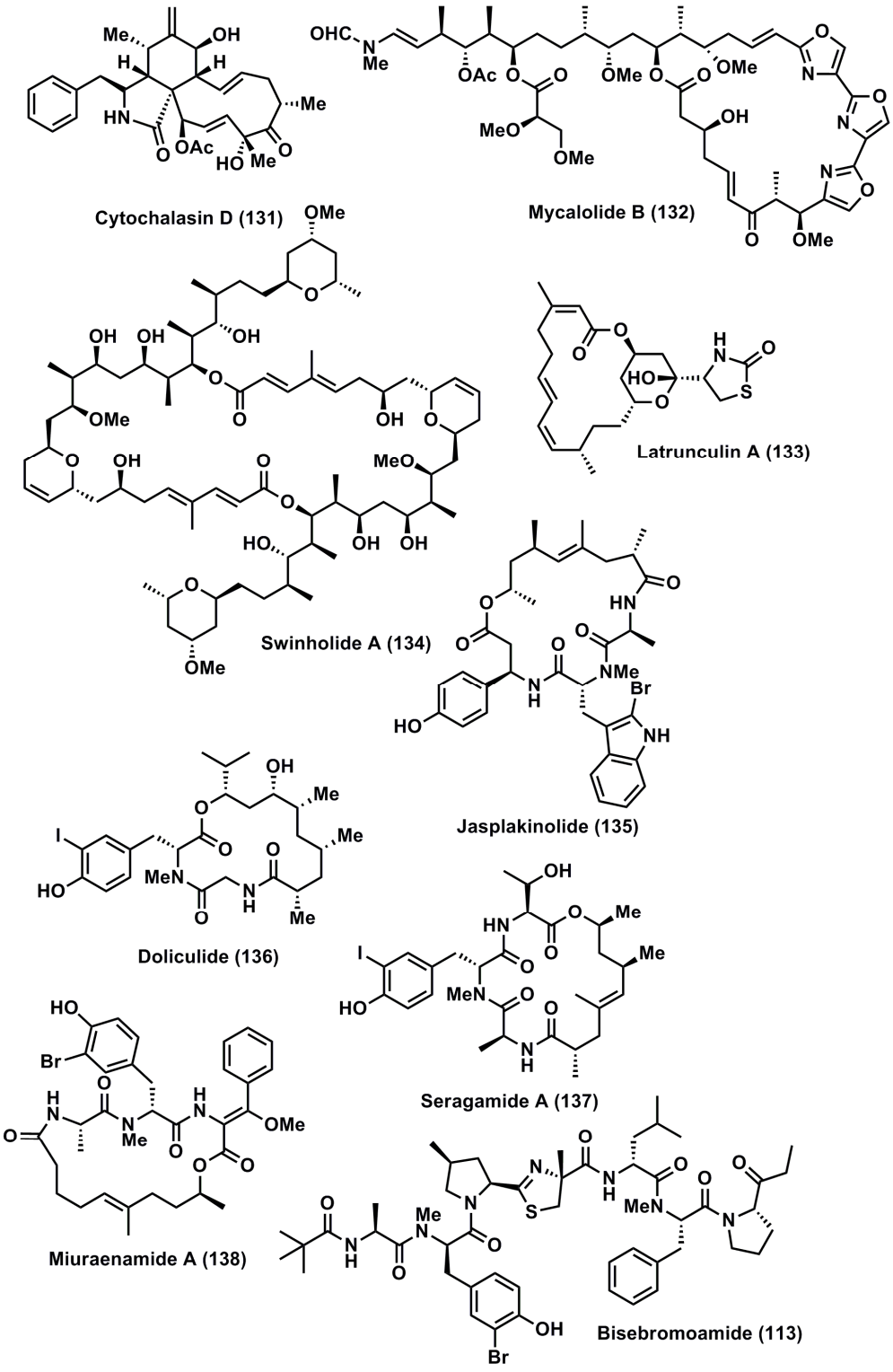


Figure 37. Structures of Nine Natural Products "Hits" with Actin-Targeted Bioactivity from the Uesugi Group Investigations.[117]

Of the nine marine-derived natural product "hits", four (**113**, **136**-**138**) contain *ortho*-halogenated-tyrosine derivatives. It was already known that cytochalasin D (**131**), mycalolide B (**132**), latrunculin A (**133**) and swinholide A (**134**) are actin filament destabilising compounds, and that jasplakinolide, doliculide and seragamide are actin filament stabilising compounds. Two of the morphological changes that were observed with both of these classes of actin-targeting compounds, were that the area of cytoplasm in the cell reduced, and the centroid of the nucleus became further from the centroid of the whole cell upon treatment (Figure 38a-c).[117] A graphical plot of the effects of each of the natural products tested on these two parameters was generated (Figure 38d).

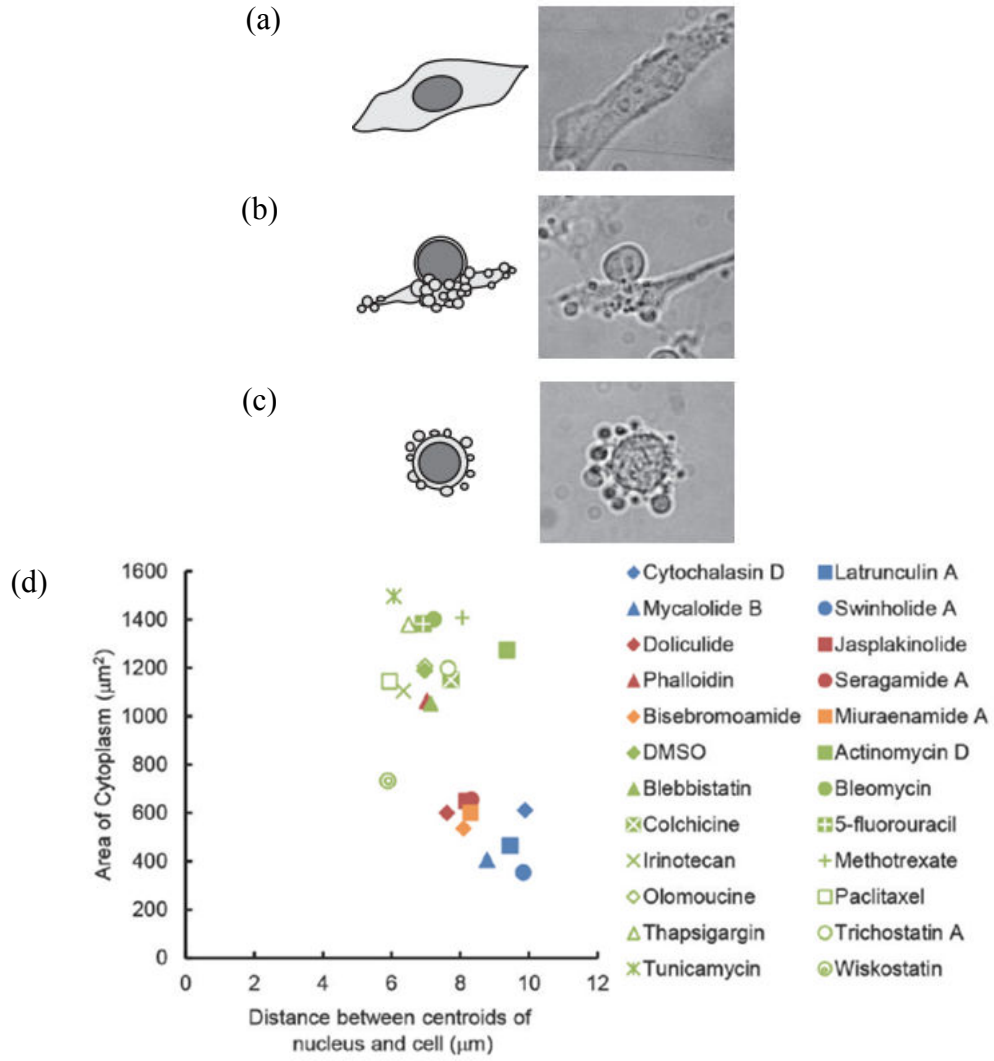


Figure 38. Examples of the morphological changes observed in the HeLa cells on treatment with the library's "hit" natural products. (a) No morphological change or weak blebbing of the plasma membrane (b) Nuclear-protruded morphology (c) Completely retracted cytosol, and only the nucleus is visible. (d) Extent of natural products' effects on cell morphology. Blue = known actin destabiliser, Red = known actin stabiliser, Orange = unknown biological activity, Green = non-actin targeting compound.[117]

Interestingly, the majority of the actin targeting compounds can be seen in the same bottom right area of the graph (Figure 38d), and the tyrosine-containing ones are especially close together. From the clustering of the two new natural product hits, bisebromoamide (**113**) and miuraenamide A (**138**), it could be estimated that they are actin filament stabilisers as their morphological effects are most similar to those of

the known actin filament stabilisers doliculide (**136**), jasplakinolide (**135**) and seragamide (**137**). In order to confirm this hypothesis, actin-polymerisation and depolymerisation assays were carried out for bisebromoamide (**113**) and miuraenamide A (**138**). These two compounds were shown to enhance polymerisation, and inhibit depolymerisation, which are characteristics further indicating that the compounds are actin filament stabilisers. This was further confirmed when a bisebromoamide derivative with fluorescein attached (**139**, Figure 39a) *via* a terminal oxopropylpyrrolidine moiety derivative with linker, was observed within HeLa cells. It was noted that the potency reduced significantly with the linker-fluorescein attached, presumably due to permeability problems, but it was clearly seen on fluorescent images that the small amount of compound that did enter the HeLa cells was specifically targeting the actin filaments (Figure 39b). The reduction in potency of bisebromoamide with fluorescein attached was confirmed to be due to permeability rather than loss of activity due to derivatisation, as bisebromoamide with the linker, without terminal fluorescein was shown to be as active bisebromoamide. In addition, attachment of the linker and fluorescein in this position showed that the terminal ketone of the bisebromoamide structure is not required for biological activity.

It is still not understood how the protrusion of the cell nucleus is caused by the actin-targeting molecules, but it is clear that these compounds have a positive effect and could be potential drug candidates, with their activity at nanomolar levels. This simple and effective newly developed assay for chemical genetics screens could be used in further screens as new natural compounds are discovered. Bisebromoamide (**113**) and Miuraenamide A (**138**) could be further explored to see if slight structure modifications could make them even more potent.

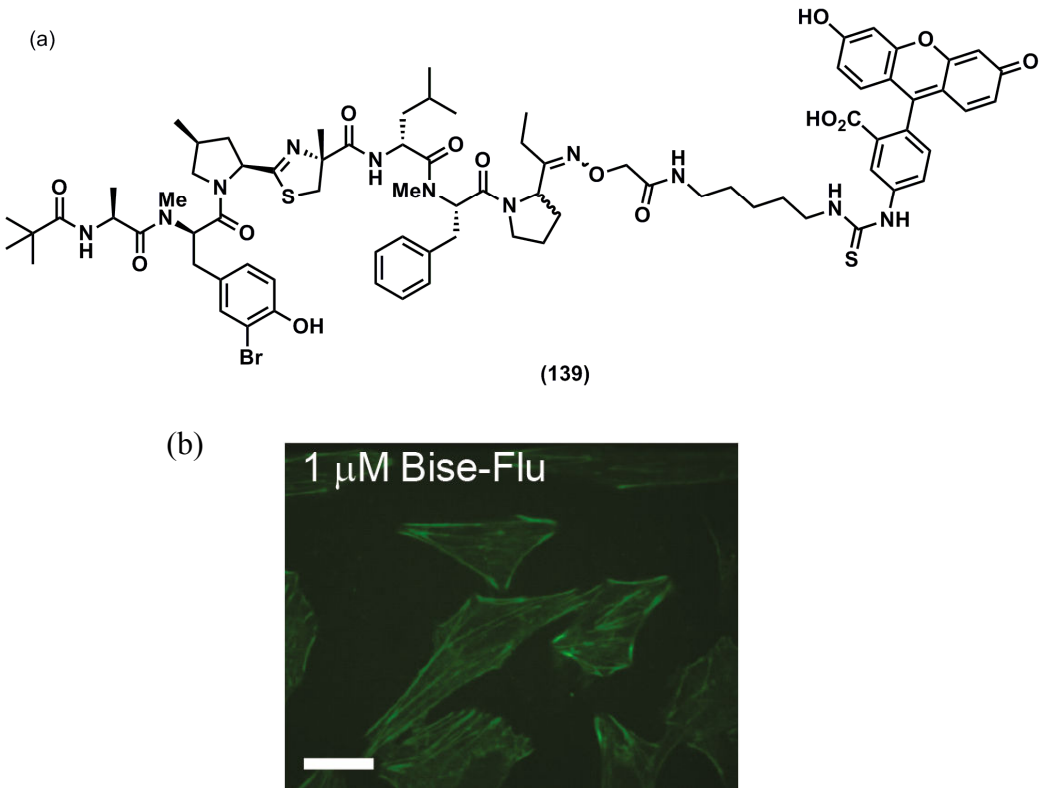


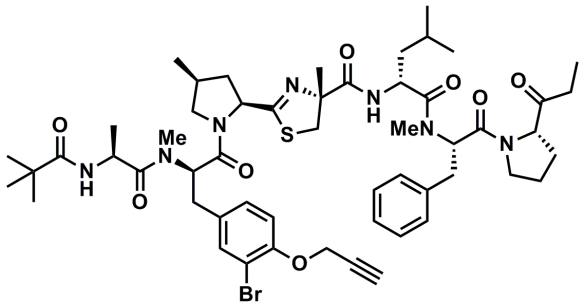
Figure 39. (a) Structure of Bisebromoamide-Fluorescein (**139**)[117] (b) Targeted Actin Filaments Within HeLa Cells, Highlighted by **139**.

3.2.2. Bisebromoamide as a Target for Screening

The Hulme group has shown an interest in tyrosine derivatives over a number of years.[55] This new bisebromoamide natural product was thus identified as an excellent target to bring together several different projects. Previous work carried out to synthesise the tagged tyrosine-containing peptide, PTTIYY (Chapter 2) could be applied to the synthesis of the tyrosine derivative in bisebromoamide, to create a tagged derivative (**140**). This probe could then be used to investigate the biological targets of bisebromoamide using the new azide-based affinity chromatography linker developed by the group.[75] An attempt towards a step-wise first total synthesis of bisebromoamide and tagged analogues was undertaken as described in Chapter 4. A step-wise approach would allow facile derivatisation of the natural product, including

the possibility of rapidly carrying out an alanine scan to determine important functional aspects of the natural product.

During the course of the synthetic work discussed in this thesis, the Ye group completed the synthesis of bisebromoamide,[110] and their chosen methods had some significant similarities to our own.



(140)

Figure 40. Propargyl Tagged Bisebromoamide Derivative Target with Revised Stereochemistry.

Chapter 4 Results and Discussion: Towards the Synthesis of Bisebromoamide and Derivatives

4.1. Retrosynthetic Analysis

Having identified potential synthetic targets in bisebromoamide **113** and a propargylated analogue **140**, a retrosynthetic approach was designed (Scheme 41). Our approach involves cleavage of the six amide bonds, creating seven amino acid-like fragments. Of these seven fragments, three are the commercially available pivalic acid (**142**), L-alanine (**143**), and D-leucine (**146**), and the remaining four fragments required synthesis (**144**, **145**, **147, 148**).

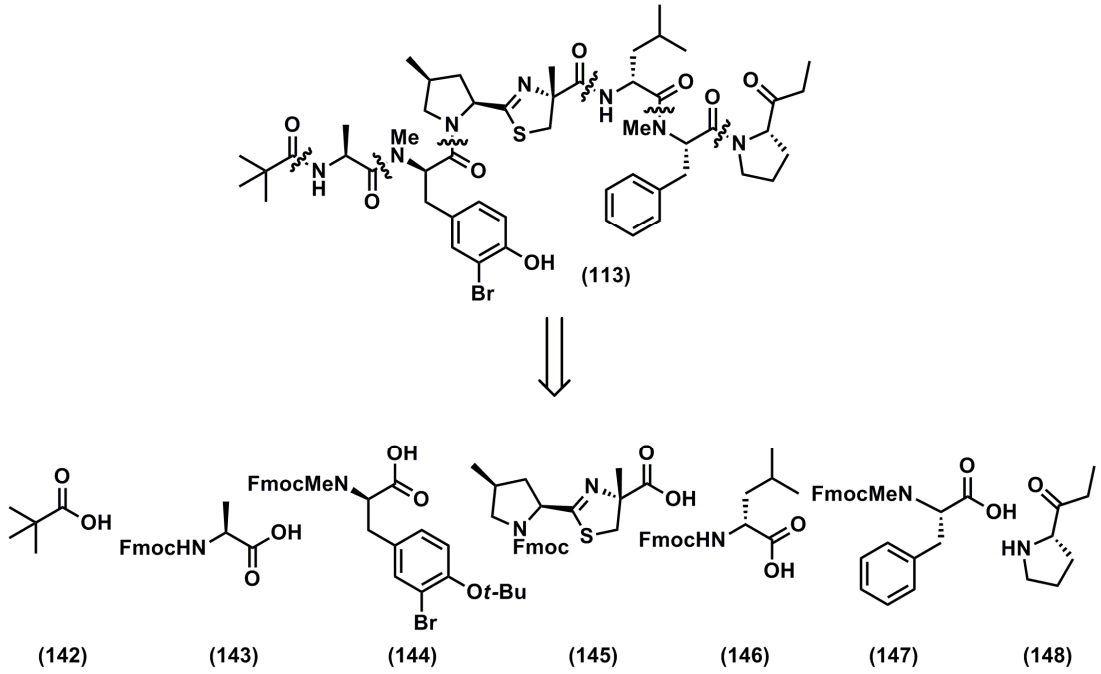


Figure 41. Retrosynthetic Approach Towards Bisebromoamide and Tagged-Analogues.

This approach, creating amino acid-like fragments, allows great flexibility when constructing the natural product. Each amino acid can be derivatised independently, enabling the facile synthesis of various analogues of bisebromoamide. The amino acids could be coupled together on solid support using standard peptide synthesis procedures, allowing construction of the complete compound.

For synthesis on solid support protecting groups will be employed to ensure coupling in the desired position. Orthogonality between side chain protecting groups, *C*-terminus and *N*-terminus protecting groups, and solid support attachment must be chosen carefully. For the approach to bisebromoamide, it was anticipated to begin by coupling the Fmoc protected phenylalanine fragment (**147**) to Wang resin. Sequential coupling of the other fragments would be continued until reaching the pivalic acid terminus. The peptide could then be cleaved from the resin using TFA which would also cleave side chain protecting groups. Cleavage from the Wang resin would result in the carboxylic acid at the *C*-terminus. The final fragment, Opp (**148**), could then be coupled in solution phase to result in the natural product.

In previous syntheses of bisebromoamide, groups installed the thiazoline fragment late-on to reduce any risk of epimerisation at the 2-position of the proline,[110,113] although literature precedent does exist for thiazoline functionalities surviving solid phase synthesis.[118] The Ye and Suenaga groups showed that the chirality at the α-carbon in α-Me-Cys does not affect the bioactivity of the compound.[91,110] In addition, the methyl group in the 4-position of the proline was shown not to affect the bioactivity.[113] With these things in mind, and taking advantage of the flexible nature of our approach towards this natural product, it was decided to work towards the synthesis of another analogue, replacing the 4-Me-Pro with commercial Pro and the thiazoline (**145**) with a thiazole (**150**). The heteroaryl derivative might be expected to be less susceptible to racemisaton of the adjacent proline stereocentre, and would also be more straightforward for synthesis (see section 4.2.3).

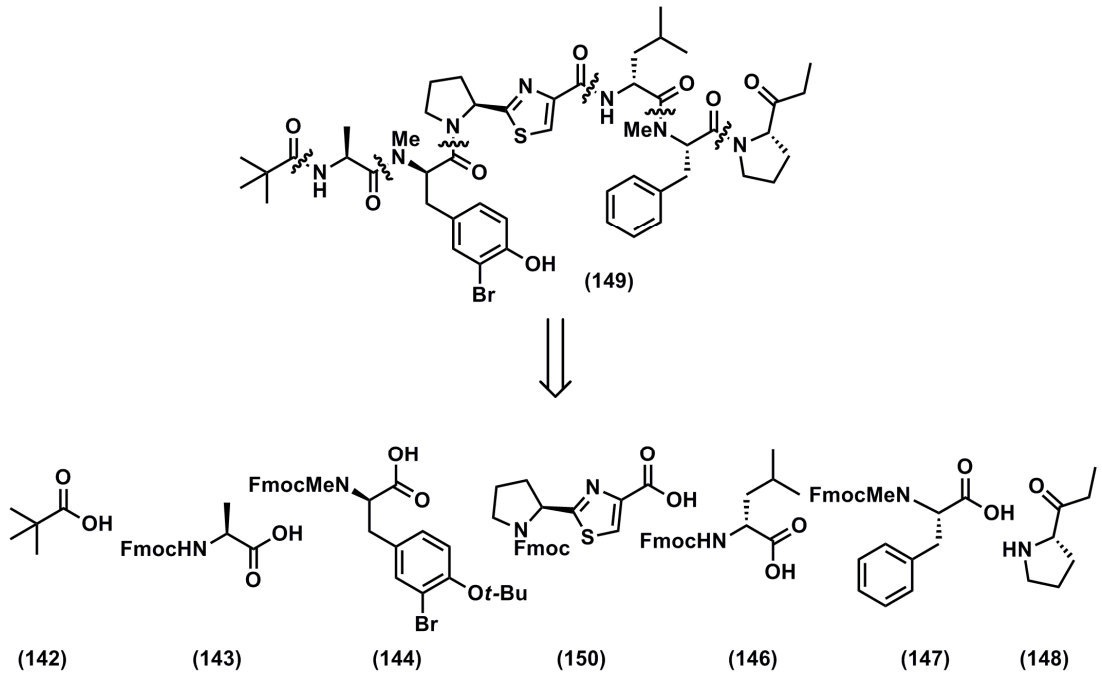
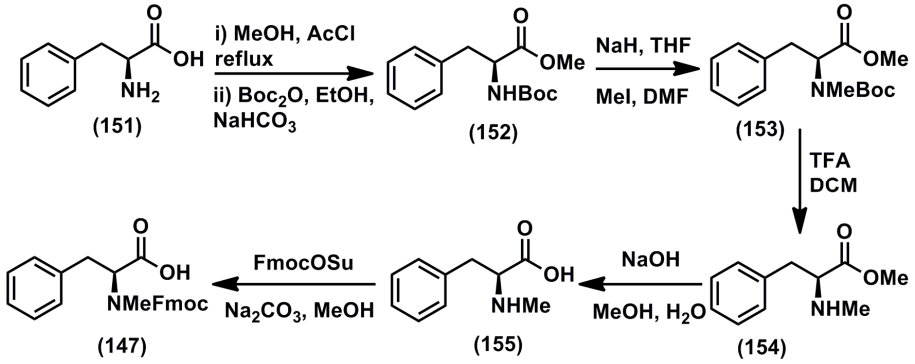


Figure 42. Retrosynthetic Approach Toward the Thiazole Analogue of Bisebromoamide.

4.2. Fragment Syntheses

4.2.1. N-Methyl-Phenylalanine (NMeFmoc-Phe-OH)

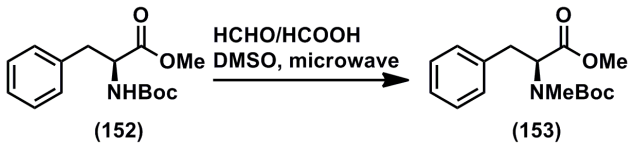
The *N*-methyl-phenylalanine fragment (**147**) required the free acid, and Fmoc-protected amine to be compatible with solid phase synthesis using Wang resin. The proposed route towards this fragment (Scheme 31) was based on the chemistry employed in synthesising the tyrosine fragment (**7**) previously used for the PTTIYY peptide (Chapter 2), with the addition of an *N*-methylation step.



Scheme 31. Synthetic Route Toward Phenylalanine Fragment, **147**.

L-phenylalanine (**151**) was converted to the methyl ester with acetyl chloride and methanol at reflux in a quantitative yield, then the amine was Boc-protected with Boc-anhydride and sodium hydrogen carbonate in ethanol to give **152** in a 97% yield.

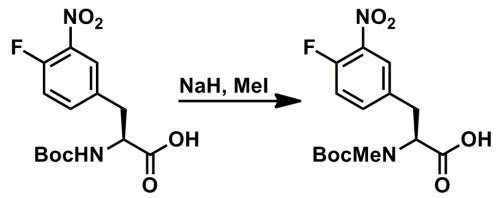
Initial attempts to methylate the amine involved using formaldehyde and formic acid in DMSO in a Eschweiler-Clarke-type microwave reaction on **152** (Scheme 32).[119] However, no identifiable product was recovered from column chromatography.



(152) (153)

Scheme 32. Eschweiler-Clarke Type Attempt at *N*-Methylation.[119]

Boger *et al.* had used sodium hydride and methyl iodide to *N*-methylate a phenylalanine-like compound (Scheme 33) in their syntheses of Chloropeptin I and II,[88] so this method was attempted on the phenylalanine derivative (**152**). This protocol proved to be equally successful in our hands on the phenylalanine substrate, giving the desired product **153** in a 95% yield.



(156) (157)

Scheme 33. Boger's *N*-methylation Procedure.[88]

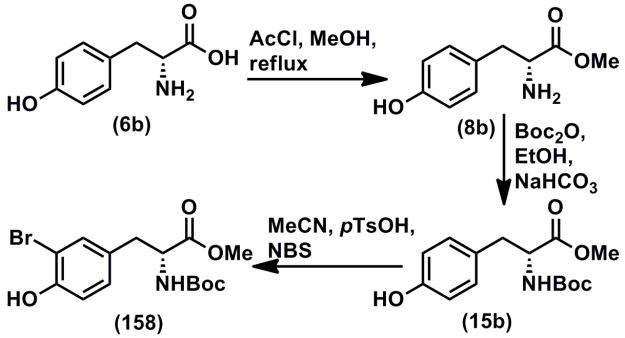
Protecting group manipulations were then carried out to remove the Boc-protecting group under standard TFA conditions (89% yield), and to convert the ester to the free acid (**155**) by base hydrolysis (89% yield). The final step was then to protect the amine with the Fmoc group, preparing the fragment (**147**) for solid phase synthesis. The Fmoc protection was achieved with Fmoc-OSu and sodium bicarbonate in methanol. The product (**147**) was visible in the NMR analysis and confirmed by mass spectrometry, but purification was very challenging. Crystallisation was

attempted with various solvent combinations, but always resulted in an impure sticky oil. Column chromatography was also attempted, but despite one TLC spot being isolated, it quickly decomposed, becoming four spots by TLC. Since the product was clearly present, and the main contaminant appeared to be cleaved Fmoc, it was decided to proceed with the fragment in this condition.

4.2.2. Ortho-*Bromo-Tyrosine (*N*MeFmoc-Br-Tyr(*t*-Bu)-OH and* N*MeFmoc-Br-Tyr(Propargyl)-OH)*

In order to synthesise the natural product (**113**), the D-tyrosine derivative must have a free phenol. To maintain this group during solid phase synthesis, the alcohol requires protection. A *tert*-butyl ether was the group of choice since it can be cleaved by TFA, so side chain deprotection could be achieved under the same conditions as for cleavage of the peptide from the resin, therefore making the synthesis as efficient as possible. The target tyrosine derivative fragment is therefore **144** with an Fmoc-protected methyl amine. For the same reasons as discussed in Chapter 2, the Boc protecting group is employed for the amine during fragment synthesis, as basic conditions are used for most transformations, and these would prematurely deprotect the amine if Fmoc was used as the initial *N*-protecting group.

Much of the chemistry required to achieve the desired fragment (**144**) involves transformations already investigated and discussed in Chapter 2. In addition to these, the fragment required *ortho*-bromination, *N*-methylation and protection of the phenol.

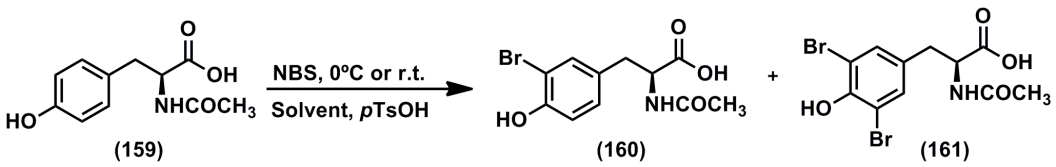


Scheme 34. Towards the Synthesis of Tyrosine Derivative Fragments.

By the same methodology as for the phenylalanine fragment (**147**), and the L-tyrosine derivative used in peptide PTTIYY (**7**), D-tyrosine was converted to the methyl ester in quantitative yield, then Boc-protected in an excellent yield (97%). Bromination in the *ortho*-position to give the target fragment (**158**) was then attempted.

†First attempts at mono-*ortho*-bromination were made using *N*-bromosuccinimide (NBS) and indium triflate ($In(OTf)_3$) as the catalyst in acetonitrile.[120] The reaction proceeded very rapidly, and di-bromination was complete in minutes. In attempts to slow and control the reaction, it was carried out in the dark and at 0ºC, but di-bromination was still the main reaction taking place.

Leykajarakul's group published a procedure for selective mono-*ortho*-halogenation of tyrosine derivatives, with their most successful conditions reported as NBS in acetonitrile with *para*-toluene sulphonic acid (*p*TsOH) at room temperature for 18 hours (Scheme 35).[121] This reaction achieved mono-brominated **160** in 96% yield, di-brominated **161** in 2% yield, and 2% starting material was recovered. It was decided to attempt this procedure on our substrate (**15b**).



Scheme 35. Leykajarakul Group's Bromination Procedure.[121]

In accordance with the literature procedure, 1 equivalent of *p*TsOH was stirred with **15b** in acetonitrile for five minutes before NBS (1 equiv.) was added, and the reaction mixture was stirred for 18 hours, becoming pale yellow.[121] Mass spectrometric analysis of the reaction mixture after 10 minutes showed small peaks for di-brominated product and some starting material in addition to two very large peaks for the bromine isotopes of the desired mono-brominated product (**158**). This

† Work towards *ortho*-bromination of tyrosine derivatives was carried out in collaboration with project student Alan Healy.

analysis was very similar 18 hours later. The work up procedure involved extraction of the acetonitrile (MeCN) reaction mixture with ethyl acetate (EtOAc) from a 5% sodium thiosulphate solution. Unfortunately, after the work up, less than 20% crude material had been obtained. Further washings of the remaining aqueous layers did not significantly improve the recovery. It appeared that problems were arising from the miscibility of MeCN with water, from which it proved difficult to extract the product.

This reaction was repeated, and rather than attempt work up on the reaction mixture in MeCN, first the solvent was removed under reduced pressure. The remaining oil was partitioned between ethyl acetate and 5% aqueous $Na_2S_2O_3$ solution, washed with water and brine, but again the recovery of product was very poor.

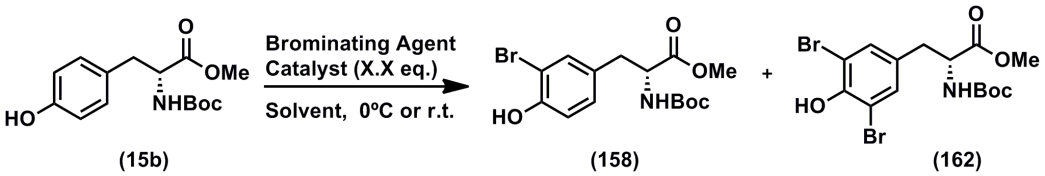
Concurrent attempts at bromination were made using bromine and hydrogen bromide in acetic acid.[122] Although mono-*ortho*-bromination was initially successful on a small scale, it proved difficult to reproduce, and the toxicity and difficulty in handling bromine made it an undesirable route to proceed with.

It was decided to persist with the NBS and *p*TsOH route,[121] investigating different solvents in an attempt to improve the recovery of the product.

On milligram scale, using EtOAc and *p*TsOH (1 equiv.) with NBS, the desired product was obtained over 4 hours in a 90% yield after work up, including washing with $Na_2S_2O_3$ (5% aq. solution), water then brine. This method was repeated on multi-gram scale. The reaction was observed to be proceeding at a much slower rate, and was left overnight. After this time, a white precipitate had formed in the reaction mixture. The solid was removed by filtration and identified as the Boc-deprotected, *p*TsOH salt by ^{1}H, ^{13}C NMR and mass spectrometric analysis.

The reaction was repeated again on 2 g scale, this time using 0.1 equivalents of *p*TsOH in EtOAc, to reduce the formation of the de-protected salt side product. The reaction proceeded more slowly again, but after 30 hours, a good yield of 85% mono-brominated product had been achieved.

Table 3. Attempted Mono-*Ortho*-Bromination Conditions

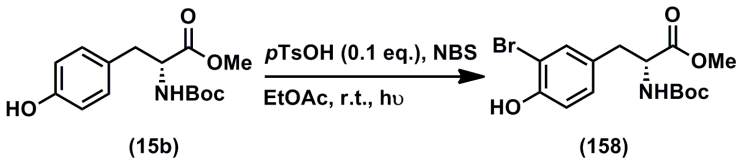


Entry	Brominating Agent	Catalyst	Solvent	Conditions	Products.
1	NBS	$In(OTf)_3$	MeCN	r.t.	~45% **162**
2	NBS	$In(OTf)_3$	MeCN	Dark, r.t.	~45% **162**
3	NBS	$In(OTf)_3$	MeCN	Dark, 0ºC	~45% **162**
4	NBS	pTsOH (1 eq.)	MeCN	18 h, r.t	~20% **158** isolated
5	NBS	pTsOH (1 eq.)	MeCN	r.t., Remove MeCN before EtOAc work up	~20% **158** isolated
6	Br_2, HBr		AcOH	r.t.	**158**, not reproducible.
7	NBS	pTsOH (1 eq.)	EtOAc	Milligram, 4 h, r.t.	90% **158** isolated yield.
8	NBS	pTsOH (1 eq.)	EtOAc	Multi-gram, 20 h, r.t.	Boc deprotected sulfonate salt
9	NBS	pTsOH (0.1 eq.)	EtOAc	Mutli-gram, 30 h, r.t.	85% **158**
10	NBS	-	MeCN	15 mins, hυ, r.t.	~45% **162**
11	NBS	pTsOH (0.1 eq.)	MeCN	40 mins, hυ, r.t.	~20% **158** isolated
12	NBS	pTsOH (0.1 eq.)	EtOAc	40 mins, hυ, r.t.	99% **158** isolated

Reaction conditions: Substrate **15b** in solvent with catalyst was stirred for 5 minutes before addition of NBS.

Around this time, Leykajarakul and co-workers published further results regarding this reaction.[123] They proposed a mechanism for the involvement of *p*TsOH in the reaction, conjugating to the phenol, and directing bromination to the *para* position. However, if the phenol is already *para*-substituted, the bromination is directed to the *ortho*-position, and selectivity for mono-bromination is observed. Leykajarakul and Chhattise both proposed that such bromination reactions proceed *via* a radical mechanism,[123,124] and could be accelerated using ultraviolet light (hυ).

With our desired substrate, **15b**, under hυ light, in acetonitrile with NBS, and without *p*TsOH, complete conversion to di-brominated product was observed within 15 minutes. This method was repeated with *p*TsOH (0.1 equiv.), and mono-brominated product observed after 20 minutes. It was left stirring for an extra 20 minutes, and no-di-brominated product was observed. Once again, the use of acetonitrile as solvent caused trouble with product isolation, so the reaction was repeated replacing the acetonitrile solvent with EtOAc. This led to the successful recovery of a quantitative yield of mono-*ortho*-brominated product **158** after 40 minutes, which is a vast improvement on the initial result.



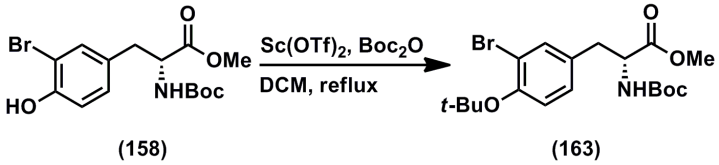
(15b) (158)

Scheme 36. Successful Mono-*Ortho*-Bromination Conditions.

From this point, two reaction schemes were pursued; one to form the *tert*-butyl ether tyrosine derivative, and one to form the propargyl ether derivative.

The *tert*-butyl ether is required to prevent methylation of the alcohol during the *N*-methylation step, or side reactions occurring during the amino acid coupling stages. It can be removed at the same time as cleavage from the solid support once the natural product peptide skeleton is complete. Protecting an alcohol as a *tert*-butyl ether used to require harsh conditions, often strongly acidic and requiring the use of isobutene gas.[125] A strong acid would not be compatible with our substrate, as it

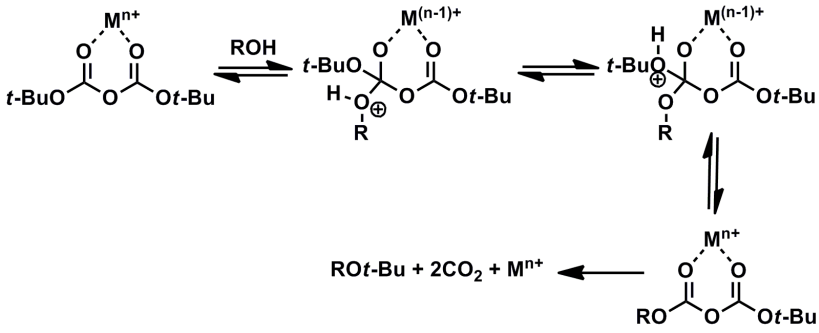
would be likely to cleave the Boc protecting group. In addition, the standard conditions used for introduction of the *tert*-butyl group usually leads to Friedel-Crafts alkylation when used with aromatic substrates.[126]



(158) (163)

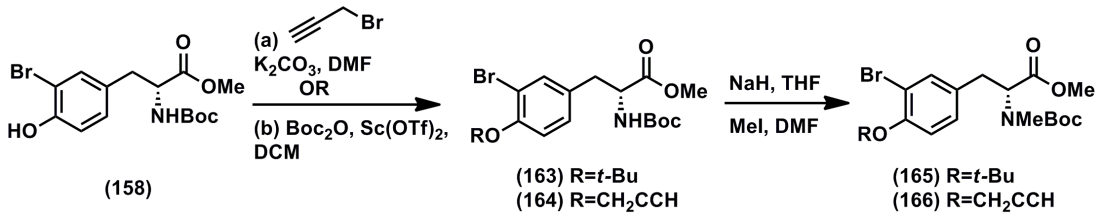
Scheme 37. *Tert*-Butyl Ether Formation.[126]

Instead, an adaptation of a recent publication, using mild conditions with Boc anhydride (Boc_2O) and scandium triflate ($Sc(OTf)_2$) to protect alcohols including a range of phenols was used to protect our substrate (**158**).[126] The Sambri group had expected that these conditions would form the *tert*-butyl carbonate, but instead they observed high yields of *tert*-butyl ether. In reproducing this method, it was found to require, extra additions of Boc_2O at intervals throughout the reaction. Over time the Boc_2O decomposed, before the reaction was complete, so 5 portions were added over the course of the reaction, achieving a 72-77% yield. This reaction is proposed to proceed *via* chelation of the scandium to the anhydride, forming *tert*-butyl alcohol, and forming an anhydride with the phenol, then 2 equivalents of carbon dioxide are liberated and the *tert*-butyl ether is formed.[126]



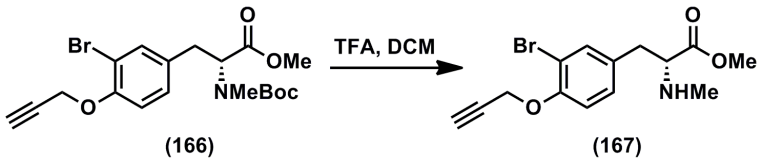
Scheme 38. Proposed Mechanism for t*ert*-Butyl Ether Formation.[126]

The propargyl ether (**164**) was obtained in an excellent yield (98%) from phenol (**158**), using propargyl bromide and potassium carbonate in DMF, by the same method used for the tyrosine derivative in Chapter 2.



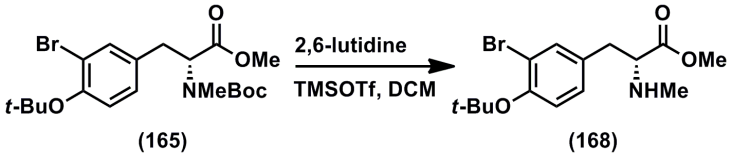
Scheme 39. Towards the Syntheses of Bromo-Tyrosine Derivative Fragments.

Following ether formation, *N*-methylation was attempted using the same conditions as had been successful for forming the phenylalanine fragment. NaH with MeI again proved fruitful, giving **166** in an excellent yield of 99% for the propargyl ether and **165** in 95% a yield for the *tert*-butyl ether. Having installed all the required functional groups, protecting group manipulation remained in order to prepare the fragments for solid phase synthesis.



Scheme 40. Boc Deprotection of Propargyl Ether Tyrosine Derivative.

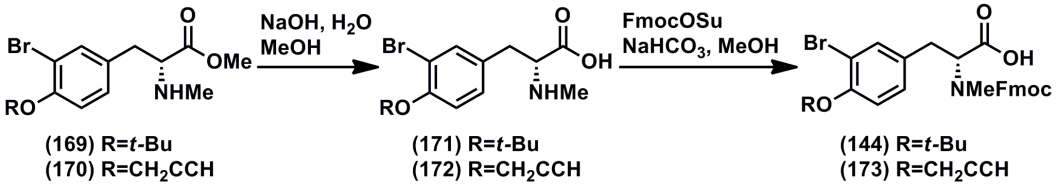
For the propargyl ether (**166**), Boc-deprotection was carried out under standard TFA conditions (Scheme 40). For the *tert*-butyl ether, TFA could not be used, as this would also cleave the *tert*-butyl protected phenol. A literature procedure was used to successfully carry out the selective Boc-deprotection, using 2,6-lutidine and trimethylsilyl triflate to give **168** in an 86% yield (Scheme 41).[127]



Scheme 41. Boc Deprotection of t*ert*-Butyl Ether Tyrosine Derivative.[127]

This selective deprotection of the Boc group of **165**, and standard Boc deprotection of **166,** was followed by ester hydrolysis under basic conditions, to give the free acids (**171** and **172**), then Fmoc-protection of the methyl amine to afford the desired

target fragments (**144** and **173**). Unfortunately, as with the phenylalanine fragment (**147**), the final Fmoc-protected compounds were very challenging to purify. Crystallisation and column chromatography were unsuccessful. Having confirmed the presence of the products through NMR and mass spectral analysis, the impure final compounds were used as isolated.



Scheme 42. Protecting Group Manipulations Toward Tyrosine Derivatives.

Having successfully synthesised these fragments towards bisebromoamide and a propargyl-tagged derivative, the iodo equivalent fragments were also synthesised. The methodology used to form these iodo fragments was based on the synthetic route used for the bromo-derivatives. The *ortho*-halogenation was carried out using *N*-iodosuccinimide (NIS) rather than NBS, and this reaction took longer, requiring 4 hours. Otherwise, the route to form these fragments was the same.

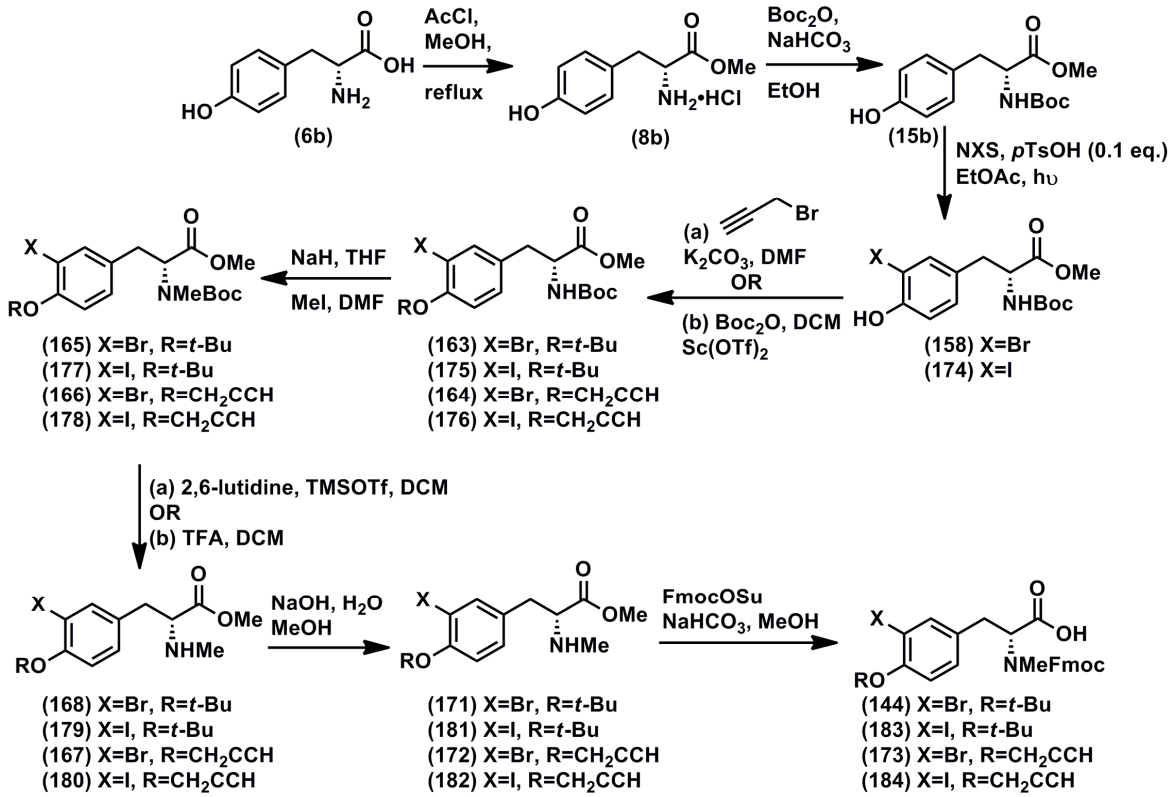
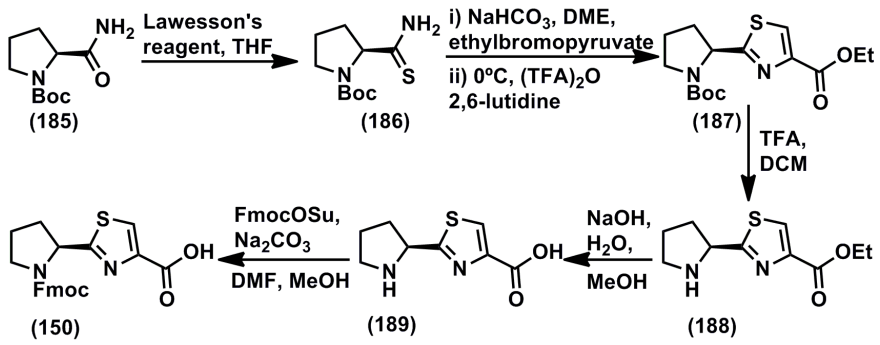


Figure 43. Syntheses of the Four Final Tyrosine Derivatives.

Each of the four fragments was formed over 8 steps, with an overall yield of 48% for *N*MeFmoc-3-Br-Tyr($CH_2C{\equiv}CH$)-OH (**173**), 35% for *N*MeFmoc-3-Br-Tyr(*t*-Bu)-OH (**144**), 43% for *N*MeFmoc-3-I-Tyr($CH_2C{\equiv}CH$)-OH (**184**) and 30% for *N*MeFmoc-3-I-Tyr(*t*-Bu)-OH (**183**).

4.2.3. Thiazole Fragment (Fmoc-Pro-Tzl-OH)

The thiazole **150**, was synthesised as an alternative fragment to the thiazoline, **145**. This was due to concerns raised by the Ye and Ma groups over potential racemisation of the thiazoline fragment at the 2-position of the proline. The thiazole fragment was synthesised using literature procedures (Scheme 44).[128,129]



Scheme 44. Synthesis of Thiazole Fragment, **150**.[128]

L-Prolinamide (**185**) was converted to the thioamide (**186**) in a quantitative yield, using the very mild and high yielding, Lawesson's reagent under anhydrous conditions. In carrying out this reaction on test substrates, the Ko group found by HPLC analysis, that the majority of amino acid compounds converted to the thioamides by this procedure were achieved in >95% enantiomeric purity and in very good or excellent yields.[130]

The thiazole ring was then formed according to a modified Hantzsch procedure in a two step reaction; using sodium hydrogen carbonate and ethylbromopyruvate to form the five membered ring, which was then converted to the thiazole (**187**) in a second step with triflic anhydride and 2,6-lutidine.

Standard conditions allowed for Boc deprotection, ester hydrolysis and finally, Fmoc protection to afford Fmoc-Pro-Tzl-OH (**150**) as required for solid phase peptide synthesis in an overall yield of 79%.

4.2.4. Thiazoline Unit (4-Me-Pro-α-Me-Tzl-OH)

Although the thiazole has been identified as a key analogue of bisebromoamide due to its enhanced stability to the conditions employed in solid phase synthesis, work has been undertaken towards the synthesis of the parent thiazoline-containing subunit as outlined below.

The thiazoline unit of bisebromoamide can be further broken down into two amino acid-like fragments (Figure 43), by cleavage of the thiazoline ring, to give 4-methyl proline (4-Me-Pro, **190**) and α-methyl cysteine (α-Me-Cys, **191**), as the starting fragments.

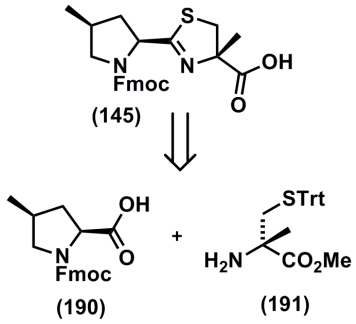
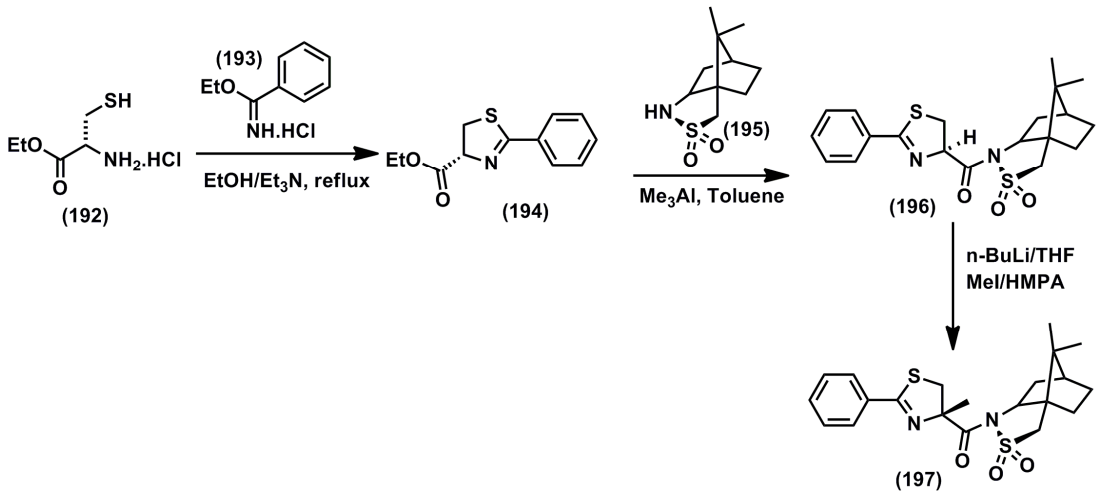


Figure 43. Retrosynthetic Approach Toward Thiazoline Fragment.

4.2.4.1. α-Methyl Cysteine (α-Me-Cys)

The synthesis of the α-methyl-cysteine fragment was begun according to a procedure published by Singh and co-workers (Scheme 45).[131]

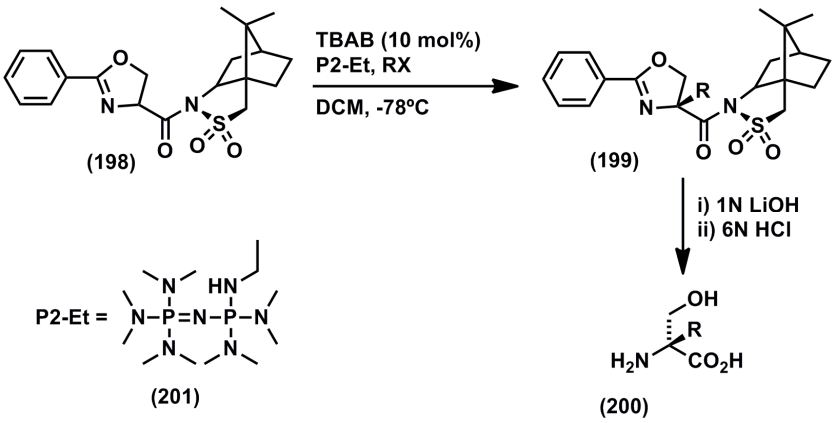


Scheme 45. Route Towards α-Methyl-Cysteine by Singh *et al.*[131]

The above method towards α-Me-Cys was chosen for its expected ease of handling the crystalline intermediates, and use of commercially available reagents. Indeed, the first two steps were completed conveniently and efficiently; *R*-cysteine ethyl ester (**192**) was converted to the thiazoline (**194**) and used without purification. The (1*S*)-(-)-2,10-camphorsultam was installed forming the amide (**196**), and this bulky group would direct the methylation, but purification became challenging at this point. Product isolation by column chromatography was possible, but labourious due to co-elution of the product with an impurity. It was discovered that by repeated precipitation with EtOAc from a minimal amount of hexane, pure product could be isolated. Although it required repeated precipitations, this was more efficient than the challenging column chromatography otherwise required.

The key step was then attempted, with methylation at the α-position of **196**, using MeI, *n*-BuLi and HMPA. This reaction appeared to proceed successfully, achieving the enantioselective methylation, but purification was again very challenging. Great difficulty was experienced in the removal of the HMPA. At this point, due to concerns over this purification step, and the use of undesirable HMPA required for the methylation, it was decided to explore an alternative methodology for this step towards the α-Me-Cys, involving the use of a phase transfer catalyst, and this work was carried out by another member of the Hulme group.

α-Alkyl-serine (**200**) had been synthesised by the Jew group, using a phase transfer catalyst called P2-Et (**201**), with TBAB and an alkyl halide using a substrate very similar to the camphor sultam (**198**), but with a pendant oxazoline rather than thiazoline (Scheme 46).[132]



Scheme 46. Use of P2-Et Phase Transfer Catalyst for α-Alkyl-Serine Synthesis.[132]

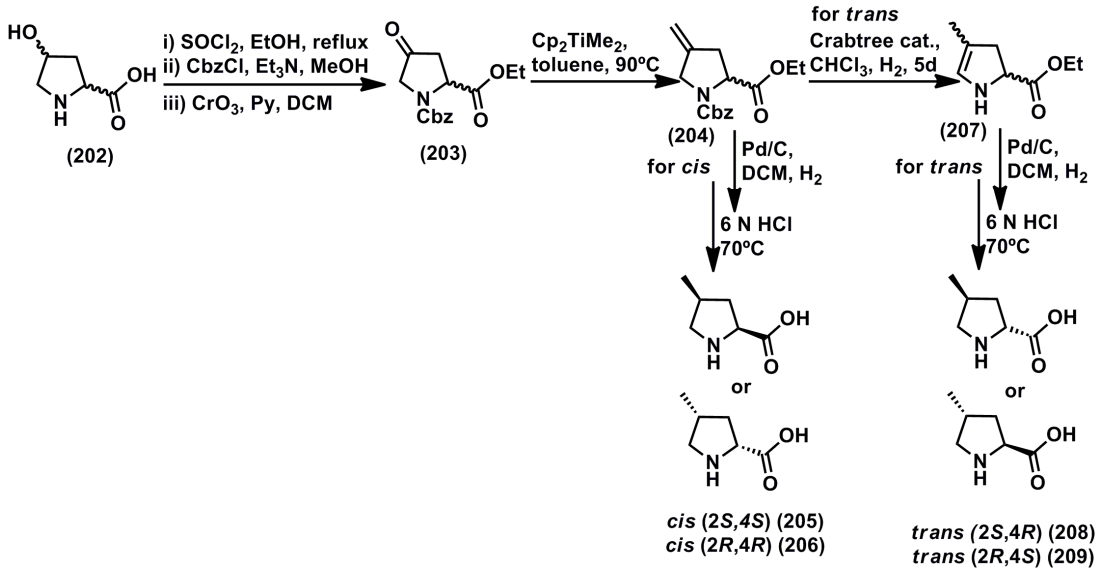
Heather Johnston then applied this method to our thiazoline equivalent (**196**), and successfully and rapidly afforded the desired enantiomer (**197**). Purification was achieved by column chromatography giving **197** in a 92% yield. This step was followed by removal of the camphorsultam by base hydrolysis, and cleavage of the thiazole ring using 6 N HCl at reflux. The α-Me-Cys was then purified by column chromatography over ion exchange resin to achieve 57% pure product, over these 3 steps.

The final step required was trityl protection required for the formation of the thiazoline fragment (**191**), this was carried out using triphenylmethanol and BF_3-OEt_2 cooled to 0ºC with sodium acetate added, according to Cheng's procedure.[133] The product was obtained by extraction, and then purified by successive hexane washes to achieve STrt-α-Me-Cys-OH (**191**) in a 94% yield.

4.2.4.2. 4-Methyl Proline (4-Me-Pro)

To achieve enantioselective methylation in the 4-position of proline was considered to be a challenging transformation. A literature procedure exists for producing each of the four stereoisomers of 4-Me-Pro selectively (Scheme 47).[134] The reaction schemes published by Munro boast being concise, showing good selectivity, and with good overall yields. However, some of the steps do involve the use of

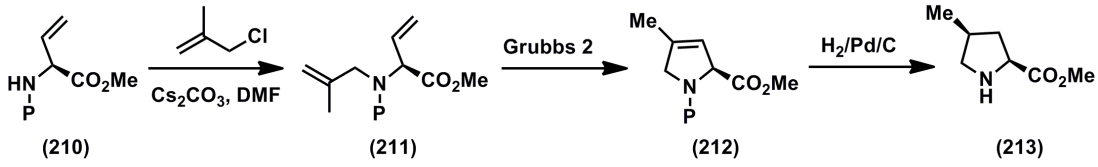
unfavourable reagents, such as chromium VI, which is highly carcinogenic, and titanium species which are notoriously difficult to handle.



Scheme 47. Munro's Selective Syntheses of the 4 Stereoisomers of 4-Me-Pro.[134]

4-Me-Pro is commonly found in natural products, and prolines have been shown to be important for intramolecular binding for such compounds. Often prolines are key to the three-dimensional structure of peptides, and therefore, it is of great importance to be able to synthesise them effectively and efficiently. It was felt that there is room for improvement in terms of the synthesis of these compounds, and investigations into this area were initiated.

Early strategy focussed on producing alkylated linear amines that could be cyclised by ring closing metathesis by a route such as that shown in Scheme 48.



Scheme 48. The Hulme Group's First Proposed Route Towards 4-Me-Pro.

Unfortunately, this scheme failed at the first hurdle of making vinyl glycine. Despite several literature procedures existing for the synthesis,[135-137] they could not be

reproduced. However, it was felt that the general approach towards the 4-Me-Pro fragment had promise, so with a re-design of the initial idea, a few routes were designed for further investigations (Figure 44), and work towards this aim is ongoing in the Hulme group.

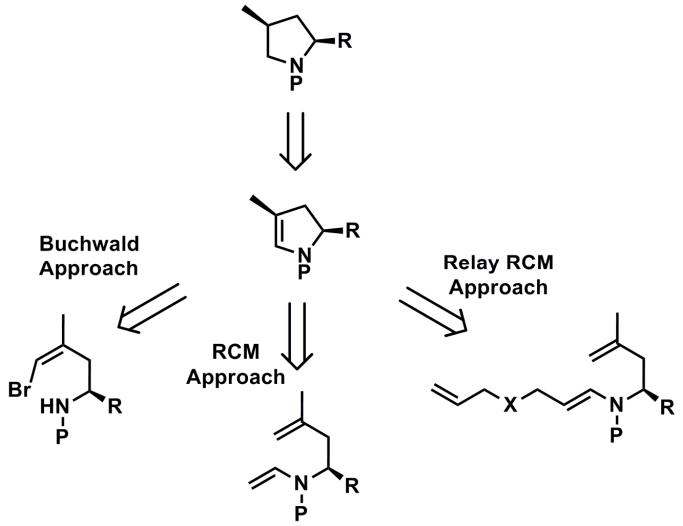
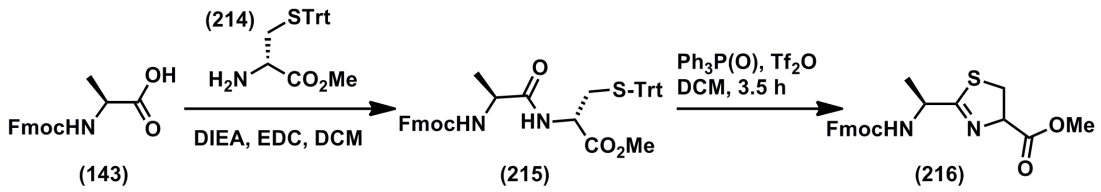


Figure 44. Revised Approaches Towards 4-Me-Pro Synthesis.

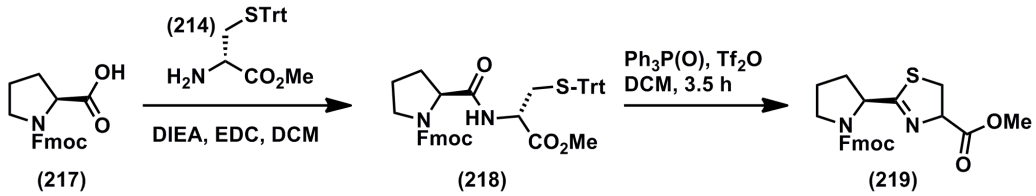
Once 4-Me-Pro has been successfully synthesised, it will be combined with α-Me-Cys to form the thiazoline by a literature procedure modified from forming a thiazoline between Cys(Trt)-OMe and Fmoc-Ala-OH (Scheme 49).[138]



Scheme 49. Literature Procedure Used for Basis of Formation of Thiazoline from Component Cys and Ala.[138]

Preliminary investigations into forming the thiazoline ring have been successfully carried out with similar substrates (Scheme 50), showing promise for this approach in the future. Fmoc-Pro-OH (**217**) was coupled to commercially available Cys(Trt)-OMe, followed by the use of $(Ph_3)_3O$ and triflic anhydride allowed formation of the

simplified thiazoline ring structure. Products were confirmed as present by crude NMR and mass spectroscopy.

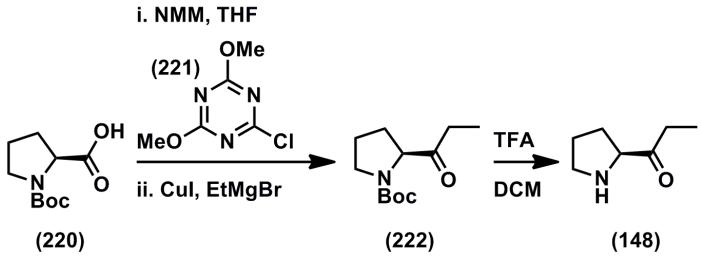


Scheme 50. Thiazoline Formation Test Reactions Using Simplified Substrates.

The successful formation of **219**, has given confidence that when 4-Me-Pro (**190**) and α-Me-Cys (**191**) syntheses have been completed within the Hulme group, the steps shown in scheme 50 can be readily applied for formation of the thiazoline ring. After formation of the thiazoline ring (**145**), solid phase coupling could take place to attempt the synthesis of the parent compound bisebromoamide (**113**), and propargyl tagged-derivatives (**140**). This would then determine whether the Ye and Ma groups' epimerisation concerns[110,113] were fair, or if, as in Nicas' work[118], the thiazoline can be successfully incorporated into the natural product on solid support without epimerisation.

4.2.5. Oxopropyl Pyrrolidine (Opp)

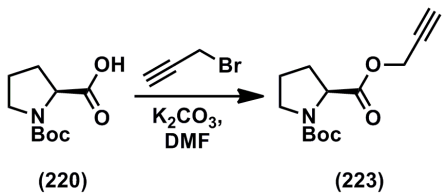
The Opp fragment (**148**) is a unique natural product feature of bisebromoamide. The fragment was derived from proline according to a procedure published by De Luca and co-workers.[139] L-Proline was Boc protected under standard conditions, with $NaHCO_3$ and Boc anhydride in ethanol in a 93% yield (**220**). De Luca's procedure was then employed, creating the activated ester under anhydrous conditions, and then reducing it to the ketone with copper and a Grignard reagent (Scheme 51).



Scheme 51. De Luca Route Towards Opp Fragment.[139]

The success of this procedure was found to be very dependent on the apparatus set up. It is essential for the conditions to be dry and under an inert atmosphere. This is challenging, as after the initial step forming the activated ester, a filtration is required. In this instance, oven-dried glassware was used, and an argon atmosphere. An adapter containing a sinter was used to remove the precipitate by filtration. It was found that using a vacuum pump introduced air into the system, and the subsequent step was unsuccessful. Instead, the filtrate was forced through the sinter, and into an attached reaction flask, using a positive pressure of argon. Copper iodide and Grignard reagent were then added to the filtrate. This resulted in the desired ketone product **148** which was purified by column chromatography (91% yield).

Boc deprotection was successfully carried out under standard TFA conditions, giving a near-pure volatile product (83% crude yield). Improvement of the purity was attempted by column chromatography, however, the product was unstable and decomposed on silica. Instead, the crude de-protection product was used without further purification.



Scheme 52. Synthesis of Boc-Opp-OProp (**223**) Fragment.

A similar propargylated version of the Opp fragment was also synthesised (**223**). This fragment is the propargyl ester of L-proline. Boc-protected proline (**220**) was propargylated using propargyl bromide and potassium carbonate under the same

conditions as employed for the tyrosine propargyl ether derivatives. Propargylation was successful in a 96% yield. This compound (**223**) suffered similar volatility and decomposition issues as the Opp fragment, so Boc-deprotection using TFA was carried out immediately before use, and the crude product used without further purification.

4.3. Coupling

Having successfully synthesised all fragments required, the solid phase coupling of the components to form the thiazole derivative of bisebromoamide was attempted. Two analogues were to be attempted initially; bisebromoamide with the thiazoline replaced by thiazole, and with the tyrosine with a free phenol (**149**), the second analogue would be bisebromoamide with the thiazoline replaced by thiazole and with the propargyl-tagged tyrosine (**224**).

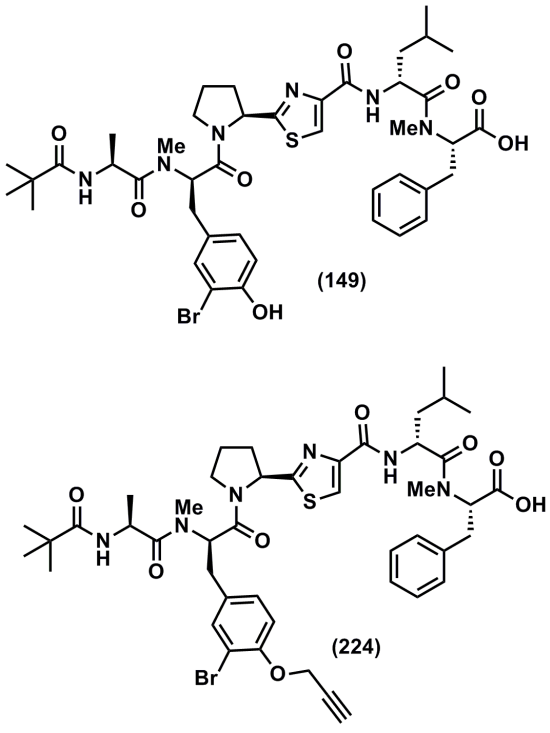
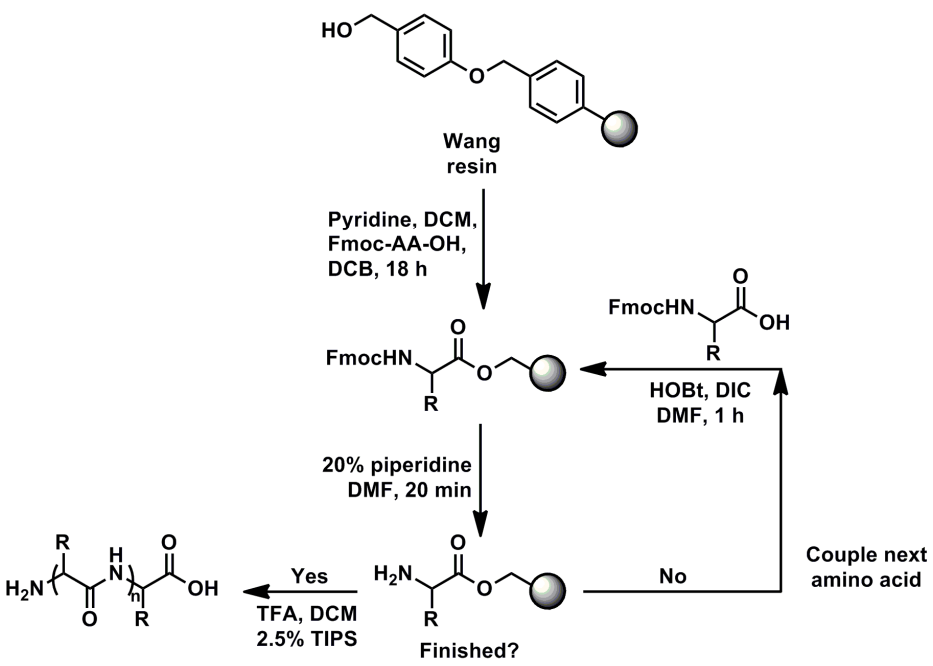


Figure 45. Initial Bisebromoamide Analogue Targets.

The first step was to attach the first fragment, *N*MeFmoc-Phe-OH (**147**), to Wang resin (Scheme 53). This was achieved by shaking swelled Wang resin with 2,6-dichlorobenzoyl chloride (DCB) with pyridine and fragment **147** in DMF for 18 hours. This procedure was selected as despite its relatively long reaction time, minimal epimerisation and formation of impurities is observed.[140] Fmoc deprotection of the Phe fragment (**147**) was carried out using 20% piperidine in DMF. The chloranil test was carried out to confirm the presence of a terminal secondary amine, with the beads turning green as a positive result. Having confirmed the successful deprotection, the next amino acid fragment could be coupled. Fmoc-D-Leucine-OH was added to the resin using HOBT, and DIC in DMF, shaken at room temperature for 1 hour. After this time, the chloranil test was performed, but showed some green beads still present, indicating the coupling was incomplete. The coupling reaction was repeated under the same conditions, and completed coupling was confirmed by the chloranil test showing colourless beads. Following Fmoc-deprotection of Leu, confirmed by observing dark blue beads with the Kaiser test, the thiazole fragment (**150**) was coupled, using the same conditions; HOBT and DIC in DMF. This process was repeated to deprotect and couple each amino acid; *N*MeFmoc-Br-Tyr(*t*-Bu)-OH (**144**), Fmoc-Ala-OH (required triple coupling), and pivalic acid, until the terminus was reached. After successful coupling of pivalic acid to the *N*-terminus, the peptide was cleaved from the resin using TFA:DCM (1:1), in the presence of 2.5% triisopropyl silane as a scavenger for the *tert*-butyl carbocation which formed upon simultaneous cleavage from the tyrosine of the *tert*-butyl ether side chain.



Scheme 53. General Protocol for Solid Supported Amino Acid Coupling on Wang Resin.

This process was repeated in order to produce an analogue with a propargyl-tagged tyrosine side chain. The same procedure was used, double coupling was again required for leucine attachment, the *N*MeFmoc-Br-Tyr(*t*-Bu)-OH was replaced by *N*MeFmoc-Br-Tyr(Prop)-OH (**173**), and the alanine again required three couplings before it was complete. Cleavage of the peptide from the resin was carried out with TFA:DCM (1:1).

Following cleavage, the filtrates for each peptide were collected separately, and evaporated to near dryness before precipitation with cold ether. The colourless solids were collected and analysed by mass spectrometry.

It was with great disappointment that the thiazole bisebromoamide analogue precursor, with free phenol (**149**) was not isolated following peptide coupling. The product could not be identified by mass spectrometry.

The second thiazole analogue of bisebromoamide, containing the propargyl-tag (**224**), appeared to be present by mass spectrum analysis, but in a very small quantity. The major peaks of the mass spectrum could not be assigned.

It is possible that the inability to purify the Phe and Tyr–derivatives had an effect on the synthesis and disrupted the amino acid coupling, however, from chloranil and Kaiser tests, the coupling steps appeared to proceed as expected. In order to clarify where the problem is arising from, it will be required to obtain more material and re-attempt the coupling process, as well as identify the compounds which were made during the peptide coupling.

One possible approach to improving the purity of Fmoc-Phe-OH and Fmoc-Tyr-OH derivatives could be to attempt Fmoc protection twice. Double couplings are often employed in peptide synthesis when a bulky, or cyclic amino acid is challenging to couple to. Indeed, during the coupling steps, it was seen that it was very difficult to couple to the tyrosine derivatives, as triple couplings were required in order to complete the attachment of the alanine. If repeated Fmoc-protection allowed a purer product to be synthesised, this may aid with efficient amino acid attachment.

4.4. Biological Investigations (Future Work)

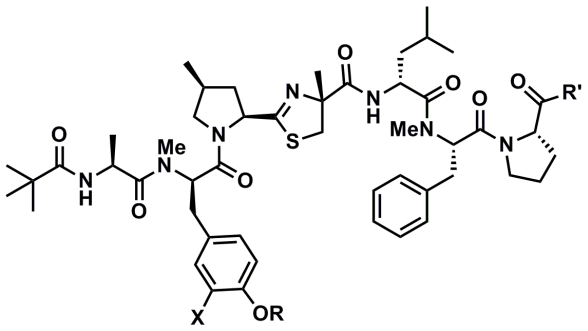
The first attempts at the synthesis of bisebromoamide thiazole analogues, have been disappointing. Confidence remains that this approach can be successful, but more material is required in order to re-attempt the coupling of the fragments. The further efforts will involve repeating the process of solid supported coupling towards the bromo-thiazole analogues of bisebromoamide.

The synthesis of the α-Me-Cys fragment has now been successfully completed, and that of 4-Me-Pro is nearing conclusion. Test reactions have been carried out to confirm that coupling of STrt-Cys-OMe and Fmoc-Pro-OH by the proposed method (Scheme 50) can be successful, and this will be applied to α-Me-cys and 4-Me-Pro when both are available to make the thiazoline fragment. It will then be attempted to use solid supported amino acid coupling to incorporate the thiazoline to the bisebromoamide structure without epimerisation. Ye and Ma groups did show concern over the stability of the stereocentres of this fragment,[110,113] but some

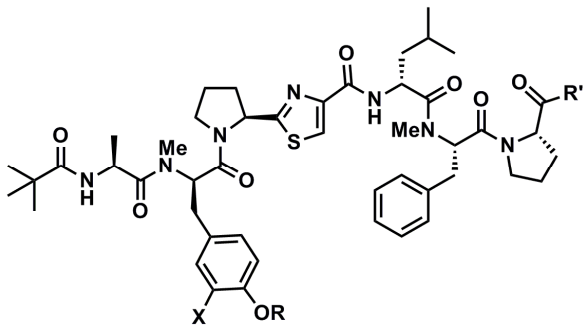
literature precedent does exist where thiazolines have been successfully incorporated into peptidic structures on solid support.[118]

If the synthesis of the natural product with the thiazoline is successful on solid support, the propargylated version will also be synthesised in this way. Iodo-equivalents of the bromo-tyrosine fragments have already been synthesised, and can readily be incorporated into the natural product analogues for any of the thiazole or thiazoline structures, both for the free phenol of tyrosine, and the propargyl-tagged derivative as well.

In addition, the propargyl-Opp (**223**) fragment has also been synthesised, and could also be readily incorporated into more natural product analogues. The synthesis of the hydrazone derivative by the Suenaga group[116] suggests that this is a suitable point for attachment to resin or a fluorophore.



(113) X=Br, R=H, R'=CH_2CH_3
(140) X=Br, R=CH_2CCH, R'=CH_2CH_3
(225) X=Br, R=H, R'=OCH_2CCH
(226) X=I, R=H, R'=CH_2CH_3
(227) X=I, R=CH_2CCH, R'=CH_2CH_3
(228) X=I, R=H, R'=OCH_2CCH



(149) X=Br, R=H, R'=CH_2CH_3
(224) X=Br, R=CH_2CCH, R'=CH_2CH_3
(229) X=Br, R=H, R'=OCH_2CCH
(230) X=I, R=H, R'=CH_2CH_3
(231) X=I, R=CH_2CCH, R'=CH_2CH_3
(232) X=I, R=H, R'=OCH_2CCH

Figure 46. Potential Bisebromoamide Analogues Towards which Progress has been made.

This will complete a small library of bisebromoamide analogues which can be screened against HeLa cells, to compare their bioactivity to the natural sample of the compound, already tested by the Suenaga group.[91] This will lead to important information regarding which sites of the natural product can tolerate derivatisation, whilst maintaining activity. With this information in hand, propargyl-tagged bisebromoamide derivatives which are efficacious can be attached *via* "click" chemistry to the new cleavable affinity chromatography linker developed in the Hulme group,[75] in the same manner as proposed for the PTTIYY peptide in Chapter 2 (Section 2.6.1). This could allow for bisebromoamide binding targets to be

identified, allowing greater understanding of their biological activity, and their potential uses.

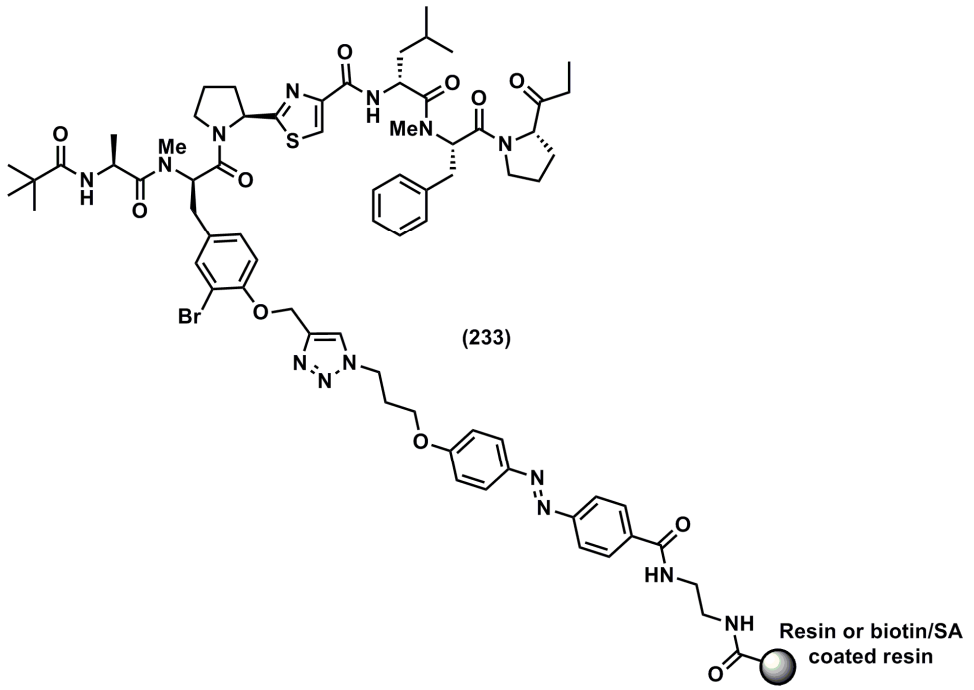


Figure 47. Thiazole Analogue (**224**) of Bisebromoamide Attached to Hulme Group Cleavable Affinity Chromatography Linker.[75]

Chapter 5 Experimental

5.1 General Experimental Details

^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 298 K or 323 K on Bruker AC250 (250 MHz), DPX360 (360 MHz), AVA400 (400 MHz) and AVA500 (500 MHz) instruments. The data is presented as follows: chemical shift (in ppm scale relative to δ_{TMS}= 0), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (measured in Hz) and interpretation. ^{13}C NMR spectra were recorded at 298 K or 323 K on Bruker DPX360 (90.6 MHz), AVA400 (100.6 MHz), AVA500 (125.8 MHz) Fourier transform instruments and were referenced to the solvent carbon peak or 'spiked' with MeOH for samples run in D_2O. The data is presented as follows: chemical shift (in ppm on the δ scale) and assignment; and were confirmed by DEPT 90 and DEPT 135. ^{19}F NMR spectra were recorded at ambient temperature on Bruker AVA400 (376.3 MHz) fourier transform instrument. The data is presented as follows: chemical shift (in ppm on the δ scale) and assignment.

Infra-red spectra were recorded on a Shimadzu IR Affinity-1 spectrometer. The wavelengths of maximum absorbance (υ_{max}) are quoted in cm^{-1}.

Melting points were determined on a Gallenkamp Electrothermal Melting Point apparatus and are uncorrected.

Electrospray ionisation (ESI) mass spectra were recorded on a Finnigan LCQ or Micromass Platform instrument at The University of Edinburgh. The parent ion or relevant fragment is quoted, followed by significant fragments and their

percentages. MALDI-TOF mass spectra were recorded on a Voyager-DE STR Biospectrometry Workstation at COIL, School of Biology, University of Edinburgh., using α-cyano-4-hydroxycinnamic acid (CHCA) matrix.

TLC was performed on Merck 60 F_{254} (0.25 mm) aluminium silica plates and visualised by ultraviolet (UV) light, potassium permanganate or vanillin stains.

Flash chromatography was carried out on Fluka Kieselgel 60 under positive pressure by means of a hand pump. Eluent compositions are quoted as ratios.

Optical rotations were measured on an AA-1000 polarimeter with a path length of 1.0 dm at the sodium D line (589 nm) and are reported as follows: $[\alpha]_{D,}$ concentration (c in g/ 100 cm^3), and solvent. All optical rotations were measured at room temperature.

Reagents were purified by standard techniques. Triethylamine (Et_3N) was distilled from calcium hydride and stored over calcium hydride. Tetrahydrofuran (THF) and dichloromethane (DCM) were passed through activated alumina columns using a solvent purification system from Glass Contour Solvent Systems. *N*-Bromosuccinimide and copper iodide were recrystallised from water. All other reagents were used as supplied.

General Reaction Procedures

General Procedure A: Methyl ester formation

Methanol (14 cm^3) was cooled to 0 ºC (ice bath) and acetyl chloride (11.3 mmol, 3.00 eq.) was added slowly (CARE: gas evolved). The solution was stirred at 0 ºC for 15 minutes before carboxylic acid (3.77 mmol, 1.00 eq.) was added portion-wise. The colourless solution was warmed to room temperature before heating to reflux for 3 h.

The reaction mixture was cooled to room temperature, then the solvent evaporated under reduced pressure. The resultant solid was recrystallised from methanol, affording the colourless solid methyl ester.

General Procedure B: Boc-protection

Amine (6.71 mmol, 1.00 eq.) was dissolved in ethanol (25 cm^3) with $NaHCO_3$ (20.1 mmol, 3.00 eq.) and Boc_2O (6.71 mmol, 1.00 eq.). The colourless suspension was stirred at room temperature for 6 h, before vacuum filtration, and removal of the solvent under reduced pressure, affording the Boc-protected amine. The oil was purified by column chromatography.

General Procedure C: Propargyl ether formation

Alcohol or carboxylic acid (4.65 mmol, 1.00 eq.) was dissolved in dry DMF (30 cm^3) under a nitrogen atmosphere. K_2CO_3 (9.29 mmol, 2.00 eq.) was added, followed by propargyl bromide (80% in toluene, 16.7 mmol, 3.60 eq.). The reaction

was carried out in the dark (foil-wrapped flask), and stirred at room temperature for 16 h.

The reaction was quenched with water (20 cm^3) with cooling (ice bath), and extracted with ether (3 × 20 cm^3). The combined ether layers were washed with brine (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure to afford the crude propargyl ether, which was purified by column chromatography.

General Procedure D: Standard Boc-deprotection

Boc-protected amine (6.49 mmol, 1.00 eq.) was dissolved in DCM (30 cm^3) with TFA (64.9 mmol, 10.0 eq.). The pale yellow solution was stirred at room temperature for 16 h.

The reaction mixture was quenched with $NaHCO_3$ (30 cm^3, sat. aq. soln.) added gradually, with cooling (ice bath). The mixture was left for bubbling to cease (~ 30 minutes), then extracted with DCM (3 × 30 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure to afford the TFA salt of the free amine. The crude product was purified by column chromatography.

General Procedure E: Ester hydrolysis (with DOWEX)

Methyl ester (11.7 mmol, 1.00 eq.) was dissolved in methanol (10 cm^3) and water (40 cm^3) with NaOH (12.2 mmol, 1.1 eq.). The pale yellow reaction mixture was stirred at room temperature for 3 h.

The pH was adjusted to 7 (DOWEX ion-exchange resin, 50W-X8 100-200 mesh, hydrogen form), then the resin was removed by vacuum filtration, and organic solvent removed under reduced pressure. The residual aqueous suspension was cooled (4 ºC) overnight to afford the crude free acid which was removed by vacuum filtration. No further purification was carried out.

General Procedure F: Ester Hydrolysis

Methyl ester (11.7 mmol, 1.00 eq.) was dissolved in methanol (10 cm^3) and water (40 cm^3) with NaOH (12.2 mmol, 1.1 eq.). The pale yellow reaction mixture was stirred at room temperature for 3 h.

The pH was adjusted to 7 (1 N HCl aq.) resulting in precipitate formation. The colourless solid was collected by vacuum filtration. No further purification was carried out.

General Procedure G: Fmoc-protection

Amine (11.5 mmol, 1.00 eq.) was dissolved in methanol (90 cm^3) and DMF (10 cm^3) with Na_2CO_3 (34.5 mmol, 3.00 eq.) and Fmoc-OSu (11.5 mmol, 1.00 eq.). The colourless suspension was stirred at room temperature for 16 h.

The pH was adjusted to 2 (10 N HCl), and the mixture extracted with ether (3 × 50 cm^3), washed with brine (50 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure to form the crude Fmoc-protected amine.

General Procedure H: Global deprotection (Boc and *t*-Bu ester)

Boc-protected amine (9.45 mmol, 1.00 eq.) was dissolved in DCM (20 cm^3) with TFA (94.5 mmol, 10.0 eq.) and 1,3-dimethoxybenzene (14.2 mmol, 1.50 eq.). The deep red solution was stirred at room temperature for 3 h.

The reaction mixture was quenched with $NaHCO_3$ (30 cm^3, sat. aq. soln.) gradually added with cooling (ice bath). The mixture was left for bubbling to cease (~ 30 minutes), then extracted with DCM (3 × 30 cm^3), dried ($MgSO_4$), and the solvent

removed under reduced pressure to afford the TFA salt of the free amine. Precipitate formed from minimal EtOAc on addition of hexane (excess).

General Procedure I: Amino Acid Attachment to Rink Amide Resin

Rink amide resin (0.09 mmol, 1.00 eq.) was swollen in DCM (2 cm^3) for 30 minutes, then washed with DMF (3 × 3 cm^3). The first Fmoc-protected amino acid residue (0.27 mmol, 3.00 eq.) was then coupled by addition of HOBt (0.27 mmol, 3.00 eq.) and DMF (3 cm^3), which was shaken for 10 minutes before addition of DIC (0.27 mmol, 3.00 eq.). The reaction mixture was shaken for 1 h at room temperature, before being washed (3 × shaken with DMF (3 cm^3) then vacuum filtered, 3 × shaken with MeOH (3 cm^3) then vacuum filtered, 3 × shaken with DCM (3 cm^3) then vacuum filtered). The resin was then dried under reduced pressure for 2 minutes.

General Procedure J: Amino Acid Attachment to Wang Resin

Wang resin (0.09 mmol, 1.00 eq.) was swollen in DCM (2 cm^3) for 30 minutes, then washed with DMF (3 × 3 cm^3). The first Fmoc-protected amino acid residue (0.45 mmol, 5.00 eq.) was coupled to the resin by addition of pyridine (0.74 mmol, 8.25 eq.), and shaking until completely dissolved. DCB (0.45 mmol, 5.00 eq.) was added, and the mixture gently shaken for 18 h. The resin was then washed (3 × shaken with DMF (3 cm^3) then vacuum filtered, 3 × shaken with MeOH (3 cm^3) then vacuum filtered, 3 × shaken with DCM (3 cm^3) then vacuum filtered). The resin was then dried under reduced pressure for 2 minutes.

General Procedure K: Amino Acid Coupling

Fmoc-protected amino acid (0.27 mmol, 3.00 eq.), HOBt (0.27 mmol, 3.00 eq.) and DMF (3 cm^3) were added to the resin (0.09 mmol, 1.00 eq.) and shaken at room temperature for 10 minutes. DIC (0.27 mmol, 3.00 eq.) was added, and the mixture shaken at room temperature for 1 h. It was then filtered under reduced pressure, and then washed (3 × shaken with DMF (3 cm^3) then vacuum filtered, 3 × shaken with MeOH (3 cm^3) then vacuum filtered, 3 × shaken with DCM (3 cm^3) then vacuum filtered). The resin was then dried under reduced pressure for 2 minutes.

General Procedure L: Fmoc-Deprotection

The Fmoc-protected peptide on solid support was shaken in a 20% solution of piperidine in DMF (3 cm^3) for 20 minutes. It was then filtered under reduced pressure and washed (3 × shaken with DMF (3 cm^3) then vacuum filtered, 3 × shaken with MeOH (3 cm^3) then vacuum filtered, 3 × shaken with DCM (3 cm^3), before being dried under reduced pressure for 2 minutes. The Kaiser test was performed. If the result was yellow, deprotection was repeated, if blue, the next Fmoc-protected amino acid was coupled according to the above procedure.

General Procedure M: Cleavage of Peptide from Resin

After addition and, if necessary, Fmoc-deprotection of the final residue to the resin, global side-chain deprotection and cleavage from the resin was performed.

The solid supported peptide sequence was shaken in DCM:TFA (3 cm^3, 1:1) for 3 h. The deep red solution was filtered and collected, then evaporated to dryness under nitrogen. Ether (15 cm^3) was added forming a colourless precipitate. The suspension was cooled (dry ice) to ensure complete precipitation. The suspension was

centrifuged (5000 rpm), and the ether poured off before fresh ether (15 cm^3) was added. Sonication was used to re-mobilise the precipitate. The suspension was centrifuged (5000 rpm) and the ether poured off, before the colourless solid was dried at 40 ºC under reduced pressure to afford the free peptide sequence. Any *tert*-butyl-protected side chains present were also cleaved during this procedure.

General Procedure N: *t*-Bu ether formation

Alcohol (6.24 mmol, 1.00 eq.) was dissolved in dry DCM (10 cm^3) with $Sc(OTf)_3$ (0.31 mmol, 0.05 eq.) and Boc_2O (74.9 mmol, 12.0 eq. added in 2 eq. portions as required, [indicated by absence on TLC]), under nitrogen. It was stirred at 25 ºC and the reaction monitored by TLC at hourly intervals, until complete (stained with vanillin). The reaction mixture was then cooled to room temperature, water (10 cm^3) was added, then extracted with DCM (3 × 10 cm^3), dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the crude *tert*-butyl ether which was purified by column chromatography.

General Procedure O: *N*-methylation

Boc-protected amine (5.50 mmol, 1.00 eq.) was dissolved in dry THF (30 cm^3) under a nitrogen atmosphere at 0 ºC (ice bath). NaH (60% dispersion in mineral oil, 8.25 mmol, 1.50 eq.) was carefully added and the suspension stirred at 0 ºC for 15 minutes before DMF (5 cm^3) and MeI (44.0 mmol, 8.00 eq.) were added. The pale yellow reaction mixture was stirred at 0 ºC for 1 h, then at room temperature for 16 h.

The reaction mixture was then diluted with EtOAc (30 cm^3), and $NaHCO_3$ (30 cm^3 sat. aq. soln.) was carefully added. The aqueous layer was extracted with EtOAc (3 × 30 cm^3). The combined organic layers were washed with brine (30 cm^3), dried

($MgSO_4$), and the solvent removed under reduced pressure to afford the crude N-methylated compound. The crude product was purified by column chromatography.

General Procedure P: Selective Boc Deprotection

Boc protected amine-*tert*-butyl ether (5.93 mmol, 1.00 eq.) was dissolved in DCM (20 cm^3) at 0 ºC (ice bath) with 2,6-lutidine (29.6 mmol, 5.00 eq.). TMSOTf (23.7 mmol, 4.00 eq.) was added, and the reaction mixture stirred at 0 ºC (ice bath) for 15 minutes. The reaction mixture was warmed to room temperature, then stirred for 95 minutes.

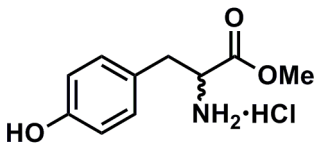
The reaction mixture was again cooled to 0 ºC (ice bath), and cold NH_4Cl (80 cm^3 sat. aq. soln.) was added. The reaction mixture was extracted with EtOAc (2 ×20 cm^3), washed with $NaHCO_3$ (20 cm^3), then brine (20 cm^3), dried ($MgSO_4$) and the solvent removed under reduced pressure. The free amine-*tert*-butyl ether was purified by column chromatography.

General Procedure Q: $COCF_3$ Protection

Amine (7.30 mmol, 1.00 eq.) was dissolved in methanol (3.3 cm^3) with KOMe (14.6 mmol, 2.00 eq.) and then ethyl trifluoroacetate (14.6 mmol, 2.00 eq.). The reaction mixture was stirred at 40 ºC for 18 h.

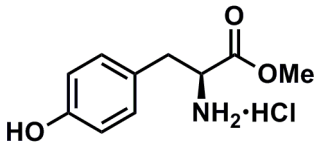
The solution was then cooled (ice bath), quenched into HCl (2.5 N, 10 cm^3) and extracted with EtOAc (20 cm^3). The organic phase was washed with acidic brine (10% brine in 1 N HCl, 50 cm^3) and the solvent removed under reduced pressure. The product was used without further purification.

Methyl 2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride (8)



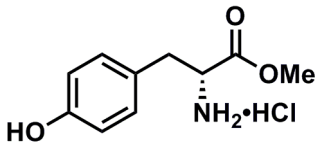
Using **General Procedure A**, tyrosine (49.9 g, 276 mmol) was reacted with acetyl chloride (58.9 cm^3, 827 mmol) in methanol (850 cm^3). The resultant solid was recrystallised from methanol, affording methyl ester **8** as a colourless solid (63.3 g, 99%).

Methyl (*S*)-2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride (8a)



R_f (DCM:MeOH, 99:1) = 0.00; **mp** 125-127 °C, (lit.[141] 128-131 °C); **$[\alpha]_D$** = +33.9 (c 1.07, EtOH), [lit.[141] $[\alpha]_D$ +28.0 (c 0.1, EtOH)]; **IR** (neat) 3335 (OH), 1746 (C=O, ester); **^{1}H NMR** (400 MHz, CD_3OD) δ 7.08 (2H, d, *J* = 8.5, Ar*H*), 6.80 (2H, d, *J* = 8.5, Ar*H*), 4.26 (1H, t, *J* = 6.6, C*H*NH_2), 3.80 (3H, s, OCH_3), 3.18 (1H, dd, *J* = 14.5, 6.1, C$H_A$$H_B$Ar), 3.11 (1H, dd, *J* = 14.5, 7.1, $CH_A$$H_B$Ar); **^{13}C NMR** δ (125.8 MHz, CD_3OD) 170.6 (C=O), 158.4 (C), 131.5 (2 ×CH), 125.6 (C), 116.9 (2 × CH), 55.4 (CH), 53.6 (CH_3), 36.6 (CH_2); ***m/z*** (ESI+, MeOH) 391 ($[2M+H]^+$, 10%), 196 ($[M+H]^+$, 100).

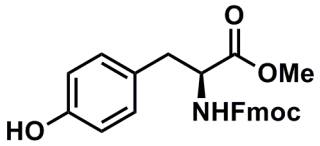
Methyl (*R*)-2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride (8b)



R_f (DCM:MeOH, 99:1) = 0.00; **mp** 175-177 °C, (lit.[142] 176 °C); **$[\alpha]_D$** = −37.8 (c 0.98, EtOH), [lit.[142] $[\alpha]_D^{23}$ −27.7 (c 0.1, EtOH)]; **IR** (neat) 3335 (OH), 1746 (C=O, ester); **^{1}H NMR** (400 MHz, CD_3OD) δ 7.08 (2H, d, *J* = 8.5, Ar*H*), 6.80 (2H, d, *J* = 8.5, Ar*H*), 4.26 (1H, t, *J* = 6.6, C*H*NH_2), 3.80 (3H, s, OCH_3), 3.18 (1H, dd, *J* = 14.5, 6.1, CH_AH_BAr), 3.11 (1H, dd, *J* = 14.5, 7.1, CH_AH_BAr); **^{13}C NMR** δ (125.8 MHz, CD_3OD) 170.6 (C=O), 158.4 (C), 131.5 (2 × CH), 125.6 (C), 116.9 (2 × CH), 55.4 (CH), 53.6 (CH_3), 36.6 (CH_2); ***m/z*** (ESI+, MeOH) 391 ($[2M+H]^+$, 10%), 196 ($[M+H]^+$, 100).

Spectroscopic data is in good agreement with the literature.[141,142]

Methyl 2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (9)



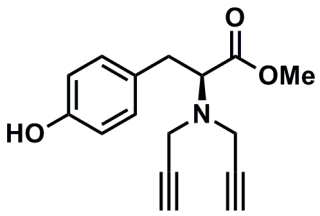
Using **General Procedure G,** amine **8a** (0.500 g, 2.16 mmol) was reacted with Fmoc-OSu (0.728 g, 2.16 mmol) to give **9** as a colourless solid (0.793 g, 88%).

R_f (Hexane:EtOAc, 3:1) = 0.09; **mp** 122-125 ºC, (lit.[143] 122-123 °C); **$[\alpha]_D$** = -7.00 (c 1.11, DMF), [lit.[143] $[\alpha]_D$ −19 (c 5.0, DMF)]; **IR** (neat) 3318 (OH), 1724 (C=O, ester), 1688 (C=O, carbamate); **^{1}H NMR** δ (360 MHz, $CDCl_3$) 7.76 (2H, d, *J* = 7.5, Ar*H*), 7.56 (2H, dd, *J* = 7.3, 3.0, Ar*H*), 7.40 (2H, t, *J* = 7.4, Ar*H*), 7.30 (2H, td, *J* = 7.4, 1.0, Ar*H*), 6.95 – 6.80 (2H, m, Ar*H*), 6.74 – 6.68 (2H, m, Ar*H*), 6.00 (1H, br s, O*H*), 5.35 (1H, d, *J* = 8.3, N*H*), 4.64 (1H, q, *J* = 14.1, 5.9, C*H*NH), 4.43 – 4.36 (2H, m, OCH_2), 4.20 (1H, t, *J* = 7.0, CH_2CHAr), 3.73 (3H, s, OCH_3), 3.04 (2H, qd, *J* = 14.1, 6.0, ArCH_2CH); **^{13}C NMR** δ (90.6 MHz, $CDCl_3$) 172.4 (C), 156.0 (C), 155.3

(C), 144.0 (2 × C), 141.5 (2 × C), 130.6 (2 × CH), 127.9 (2 × CH), 127.5 (2 × CH), 127.3 (2 × CH), 125.3 (2 × CH), 120.2 (2 × CH), 115.7 (2 × CH), 67.2 (CH_2), 55.2 (CH), 52.6 (CH_3), 47.3 (CH), 37.6 (CH_2); ***m/z*** (ESI+, MeOH) 418.6 ($[M+H]^+$, 1%), 166 (61), 165 (84), 91 (100).

Spectroscopic data is in good agreement with the literature.[143]

Methyl (*S*)-2-(di(prop-2-ynyl)amino)-3-(4-hydroxyphenyl)propanoate (14)



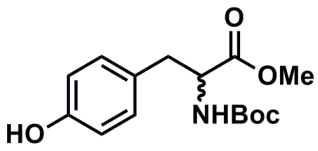
Amino carbamate **9** (0.304 g, 0.902 mmol) was dissolved in DMF (10 cm^3) with NaH (60% mineral oil dispersion, 0.028 g, 1.17 mmol), LiI (0.121 g, 0.902 mmol) and propargyl bromide (80% in toluene, 0.362 cm^3, 3.25 mmol). The reaction was carried out in the dark (foil-wrapped flask) and stirred at room temperature for 16 h.

The reaction was quenched with water (20 cm^3) with cooling (ice bath), and extracted with ether (3 × 20 cm^3). The combined ether layers were washed with brine (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure to afford crude **14** (70% crude yield by NMR analysis).

^{1}H NMR δ (360 MHz, $CDCl_3$) 7.08 (2H, d, *J* = 7.5, Ar*H*), 6.78 (2H, d, *J* = 7.5, Ar*H*), 3.90 (1H, t, *J* = 6.4, C*H*CH_2Ar), 3.72 (3H, s, OCH_3), 3.28 (1H, dd, *J* = 13.2, 6.4, C$H_A$$H_B$Ar), 3.17 (1H, dd, *J* = 13.2, 6.4, C$H_A$$H_B$Ar), 3.35 (2H, s, NC$H_2$), 3.34 (2H, s, NC$H_2$), 2.59 (1H, s, HC≡C), 2.56 (1H, s, HC≡C); **^{13}C NMR** δ (90.6 MHz, $CDCl_3$) 171.3 (C=O), 157.8 (C), 131.9 (2 × CH), 129.6 (C), 116.3 (2 × CH), 77.4

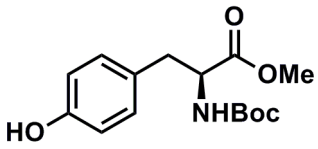
(C), 77.3 (C), 73.5 (CH), 73.2 (CH), 67.8 (CH), 52.5 (CH_3), 39.8 (CH_2), 39.7 (CH_2), 34.9 (CH); ***m/z*** (ESI–, MeOH) 270.2 ([M–H]$^-$, 36%), 416 (16), 207 (24), 183 (70).

Methyl 2-(*tert*-butoxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (15)



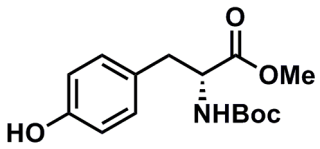
Using **General Procedure B**, amine hydrochloride **8** (63.2 g, 324 mmol) was reacted with Boc_2O (70.7 g, 324 mmol) and $NaHCO_3$ (81.6 g, 971 mmol) in ethanol (800 cm^3). The off-white solid was purified by column chromatography (Hexane:EtOAc, 9:1 to 3:2) to give Boc protected amine **15** as a colourless crystalline solid (78.0 g, 97%).

Methyl (*S*)-2-(*tert*-butoxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (15a)



R_f (Hexane:EtOAc, 3:1) = 0.17; **mp** 107-109 ºC, (lit.[144] 106-107 °C); **$[\alpha]_D$** = +57.3 (c 1.10, $CHCl_3$), [lit.[145] $[\alpha]_D$ +51.4 (c 1.0, $CHCl_3$)]; **IR** (neat) 3368 (OH), 1738 (C=O, ester), 1688 (C=O, carbamate); **^{1}H NMR** (400 MHz, $CDCl_3$, 323 K) δ 6.99 (2H, d, *J* = 8.5, Ar*H*), 6.74 (2H, d, *J* = 8.5, Ar*H*), 4.94 (1H, s, N*H*CH), 4.52 (1H, s, C*H*NH), 3.71 (3H, s, OCH_3), 3.04 (1H, dd, *J* = 13.9, 5.8, CH_AH$_B$Ar), 2.96 (1H, dd, *J* = 13.9, 6.0, CH$_A$$H_B$Ar), 1.43 (9H, s, OC(C$H_3$)$_3$); **^{13}C NMR** (125.8 MHz, $CDCl_3$, 298 K) δ 172.7 (C=O), 155.4 (C=O), 155.0 (C), 130.6 (2 × CH), 128.0 (C), 115.6 (2 ×CH), 80.2 (C), 54.7 (CH), 52.4 (CH_3), 37.7 (CH_2), 28.5 (3 × CH_3); ***m/z*** (ESI+, MeOH) 318 ([M+Na]$^+$, 100%).

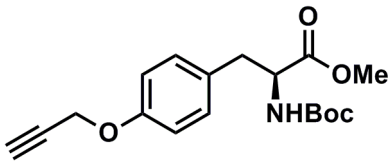
Methyl (*R*)-2-(*tert*-butoxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (15b)



R_f (Hexane:EtOAc, 3:1) = 0.17; **mp** 107-108 ºC, (lit.[146] 102-109 °C); **$[\alpha]_D$** = −59.5 (c 1.08, $CHCl_3$), [lit.[147] $[\alpha]_D$ −52.6 (c 1.0, $CHCl_3$)]; **IR** (neat) 3368 (OH), 1738 (C=O, ester), 1688 (C=O, carbamate); **^{1}H NMR** (400 MHz, $CDCl_3$, 323 K) δ 6.99 (2H, d, *J* = 8.5, Ar*H*), 6.74 (2H, d, *J* = 8.5, Ar*H*), 4.94 (1H, s, N*H*CH), 4.52 (1H, s, C*H*NH), 3.71 (3H, s, OCH_3), 3.04 (1H, dd, *J* = 13.9, 5.8, C$H_A$$H_B$Ar), 2.96 (1H, dd, *J* = 13.9, 6.0, C$H_A$$H_B$Ar), 1.43 (9H, s, OC(C$H_3$)$_3$); **^{13}C NMR** (125.8 MHz, $CDCl_3$, 298 K) δ 172.7 (C=O), 155.4 (C=O), 155.0 (C), 130.6 (2 × CH), 128.0 (C), 115.6 (2 ×CH), 80.2 (C), 54.7 (CH), 52.4 (CH_3), 37.7 (CH_2), 28.5 (3 × CH_3); ***m/z*** (ESI+, MeOH) 318 ($[M+Na]^+$, 100%).

Spectroscopic data is in good agreement with the literature.[144,145,146,147]

Methyl (*S*)-2-*tert*-butoxycarbonylamino-3-[4-(prop-2-ynyloxy)phenyl]propanoate (16)



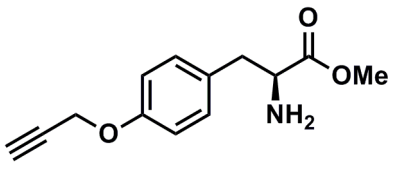
Using **General Procedure C**, phenol **15a** (1.50 g, 5.08 mmol) was reacted with propargyl bromide (2.04 cm^3, 18.3 mmol) and potassium carbonate (1.40 g, 10.2

mmol) in DMF (40 cm^3). The resultant yellow oil was purified by column chromatography (Hexane:EtOAc, 3:1) to give propargyl ether **16** as a pale yellow oil (1.62 g, 95%).

R_f (Hexane:EtOAc, 3:1) = 0.11; **$[\alpha]_D$** = +36.4 (c 1.10, DCM), [lit[148] +35.7 (c 1.00, DCM)]; **IR** (neat) 3291 (NH), 1711 (C=O, carbamate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.05 (2H, d, *J* = 7.0, Ar*H*), 6.90 (2H, d, *J* = 7.0, Ar*H*), 5.03 (1H br s, N*H*), 4.67 – 4.62 (2H, m, CH_2C≡CH), 4.58-4.49 (1H, m, C*H*NH), 3.70 (3H, s, CH_3), 3.08-2.94 (2H, m, CH_2Ar), 2.53-2.52 (1H, m, *H*C≡CCH_2), 1.41 (9H, s, C(CH_3)$_3$); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 172.4 (C), 156.7 (C), 155.5 (C), 130.3 (2 × CH), 129.0 (C), 115.0 (2 × CH), 80.0 (C), 78.6 (C), 75.5 (CH), 55.8 (CH_2), 54.2 (CH), 52.3 (CH_3), 37.4 (CH_2), 28.3 (3 × CH_3); ***m/z*** (ESI+, MeOH/DCM) 689 ($[2M+Na]^+$, 100%), 667 ($[2M+H]^+$, 5), 356.0 ($[M+Na]^+$, 94), 334 ($[M+H]^+$, 3).

Spectroscopic data is in good agreement with literature.[148]

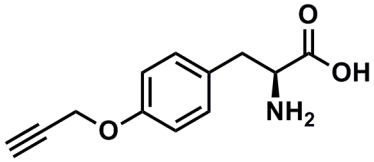
Methyl (*S*)-2-amino-3-[4-(prop-2-ynyloxy)phenyl]propanoate (17)



Using **General Procedure D**, Boc protected amine **16** (0.360 g, 1.06 mmol) was reacted with TFA (0.790 cm^3, 10.6 mmol) to give amine **17** as a pale yellow crystalline solid (0.200 g, 81%).

R_f (Hexane:EtOAc, 3:1) = 0.78; **mp** 57-59 ºC; **$[\alpha]_D$** = -27.0 (c 1.01, MeOH); **IR** (neat) 3285 (NH); **^{1}H NMR** δ (250 MHz, $CDCl_3$) 7.09 (2H, d, *J* = 8.6, Ar*H*), 6.89 (2H, d, *J* = 8.6, ArH), 4.64 (2H, d, *J* = 2.4, HC≡CCH_2), 3.68 (3H, s, OCH_3), 3.37 - 3.65 (1H, m, C*H*NH_2), 3.00 (1H, dd, *J* = 10.8, 4.1, C$H_A$$H_B$Ar), 2.79 (1H, dd, *J* = 10.8, 6.1, C$H_A$$H_B$Ar), 2.50 (1H, t, *J* = 2.4, *H*C≡C), 1.71 (2H, br s, NH_2); **^{13}C NMR** δ (62.9 MHz, $CDCl_3$) 176.4 (C), 157.5 (C), 131.3 (2 × CH), 131.2 (C), 116.0 (2 × CH), 79.6 (C), 76.5 (CH), 56.2 (CH), 56.1 (CH_2), 53.0 (CH_3), 41.1 (CH_2); ***m/z*** (ESI+, MeOH) 234.1 ($[M+H]^+$, 69%), 149 (100); **HRMS** (ESI+, MeOH) $[M+H]^+$ found 234.1124, $C_{13}H_{16}O_3N$ requires 234.1125.

(*S*)-2-Amino-3-[4-(prop-2-ynyloxy)phenyl]propanoic acid (18)



Using **General Procedure E**, methyl ester **17** (0.500 g, 2.14 mmol) was reacted with NaOH (94.3 mg, 2.36 mmol) in MeOH (10 cm^3) and water (10 cm^3) to afford carboxylic acid **18** as colourless precipitate (0.419 g, 89%).

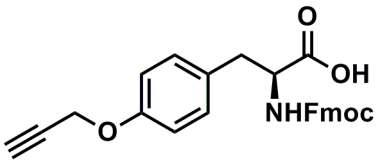
Using **General Procedure H**, Boc protected amine, *tert*-butyl ester **24** (0.500 g, 2.14 mmol) was reacted with TFA (1.59 cm^3, 21.4 mmol) and 1,3-dimethoxybenzene (0.42 cm^3, 3.21 mmol) in DCM (5 cm^3) to afford carboxylic acid **18** as colourless precipitate (0.367 g, 78%).

R_f (Hexane:EtOAc, 3:1) = 0.00; **mp** 202 ºC (dec.); **$[\alpha]_D$** = +24.4 (c 1.00, aq. NaOH)**; IR** (neat) 3285 (OH), 2122(C≡C), 1725 (C=O, carboxylic acid); **^{1}H NMR** δ (360 MHz, $CDCl_3$) 7.20 (2H, d, *J* = 8.4, Ar*H*), 6.93 (2H, d, *J* = 8.3, Ar*H*), 4.78 (2H, d, *J*

= 2.0, CH_2C≡CH), 3.60 (1H, d, *J* = 14.6, C*H*NH), 3.11 (2H, dd, *J* = 14.3, 3.6, ArCH_2), 2.82 (1H, q, *J* = 14.2, 8.1, C≡C*H*); **^{13}C NMR** δ (90.6 MHz, $CDCl_3$) 176.8 (C), 157.4 (C), 131.8 (2 × CH), 131.7 (C), 116.1 (2 × CH), 80.9 (C), 79.6 (CH), 57.1 (CH_2), 56.8 (CH), 38.0 (CH_2); ***m/z*** (ESI+, MeOH) 220.1 ([M+H^+], 12%).

Spectroscopic data is in good agreement with the literature.[62]

(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino-3-(4-prop-2-ynyloxyphenyl)propanoic acid (7)



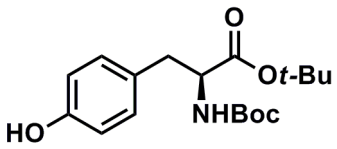
Using **General Procedure G**, amine **18** (0.300 g, 1.37 mmol) was reacted with Fmoc-OSu (0.462 g, 1.37 mmol) to give Fmoc protected amine **7** as a colourless solid (0.513 g, 85%).

R_f (Hexane:EtOAc, 3:1) = 0.30; **mp** 128-132 ºC; **$[\alpha]_D$** = +7.96 (c 1.01, EtOH); **IR** (neat) 3285 (OH), 2122 (C≡C); **^{1}H NMR** δ (360 MHz, $CDCl_3$) 7.77 (2H, d, *J* = 7.6, Ar*H*), 7.56 (2H, dd, *J* = 6.8, 4.4, Ar*H*), 7.40 (2H, t, *J* = 7.2, Ar*H*), 7.35 – 7.29 (2H, m, Ar*H*), 7.07 (2H, d, *J* = 8.4, Ar*H*), 6.90 (2H, d, *J* = 8.4, Ar*H*), 5.24 (1H, d, *J* = 8.1, N*H*), 4.51 (2H, d, *J* = 2.2, CH_2C≡CH), 4.45 (1H, d, *J* = 7.1, C*H*NH), 4.21 (1H, t, J = 6.7, C*H*Ar), 3.12 (2H, qd, *J* = 32.1, 14.0, 5.6, ArCH_2) 2.49 (1H, t, *J* = 5.6, C≡C*H*); **^{13}C NMR** δ (90.6 MHz, $CDCl_3$) 176.0 (C), 157.3 (C), 156.3 (C), 144.2 (2 × C), 141.9 (2 × C), 131.0 (2 × CH), 129.1 (C), 128.3 (2 × CH), 127.9 (2 × CH), 125.6 (2 × CH), 120.6 (2 × CH), 115.6 (2 × CH), 79.1 (C), 76.1 (CH), 67.6 (CH_2), 56.4

(CH_2), 55.2 (CH), 47.7 (CH), 37.5 (CH_2); ***m/z*** (ESI+, MeOH) 442.1 ($[M+H]^+$, 9%), 257.0 (100).

NMR data is in good agreement with the literature.[149]

***tert*-Butyl (*S*)-2-*tert*-butoxycarbonylamino-3-(4-hydroxyphenyl)propanoate (23)**

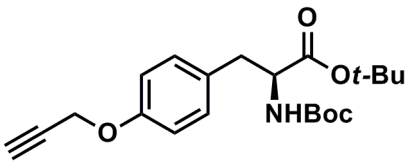


Using **General Procedure B**, amine **22** (2.00 g, 8.43 mmol) was reacted with Boc_2O (1.84 g, 8.43 mmol) and $NaHCO_3$ (2.13 g, 25.3 mmol) in ethanol (30 cm^3) to give Boc-protected amine **23** as a colourless solid (2.82 g, 99%).

R_f (Hexane:EtOAc, 3:1) = 0.34; **mp** 111-113 °C, (lit.[150] 113 ºC); **$[\alpha]_D$** = +7.33 (c 0.96, EtOH), [lit.[150] +8.00 (c 1.00, EtOH)]; **IR** (neat) 3360 (OH), 1693 (C=O, carbamate); **^{1}H NMR** (360 MHz, $CDCl_3$) δ 7.01 (2H, d, *J* = 8.1, Ar*H*), 6.73 (2H, d, *J* = 8.2, Ar*H*), 5.92 (1H, br s, O*H*), 5.02 (1 H, d, *J* = 8.2, N*H*), 4.40 (1H, dd, *J* = 13.8, 5.9, C*H*NH), 2.96 (2H, m, CH_2Ar), 1.43 (9H, s, C(CH_3)$_3$) 1.40 (9H, s, C(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$) 171.3 (C), 155.4 (C), 155.1 (C), 130.6 (2 × CH), 127.8 (C), 115.3 (2 × CH), 82.2 (C), 80.0 (C), 55.1 (CH), 37.7 (CH_2), 28.4 (3 × CH_3), 28.0 (3 × CH_3); ***m/z*** (ESI-, MeOH) 336.2 ($[M-H]^-$, 31%), 218 (66), 105 (100).

Spectroscopic data is in good agreement with literature.[150]

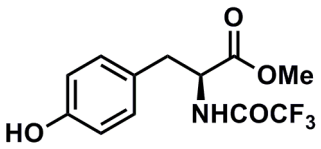
tert-Butyl (_S_)-2-_tert_-butoxycarbonylamino-3-[4-(prop-2-ynyloxy)phenyl]propanoate (24)



Using **General Procedure C**, phenol **23** (560 mg, 1.66 mmol) was reacted with propargyl bromide (711 mg, 5.97 mmol) and potassium carbonate (459 mg, 3.32 mmol) in DMF (15 cm^3) to give propargyl ether **24** as a pale yellow oil (575 mg 92%).

R_f (Hexane:EtOAc, 3:1) = 0.55; **$[\alpha]_D$** = +6.25 (c 1.12, EtOH); **IR** (neat) 1709 (C=O, ester), 1610 (C=O, carbamate); **^{1}H NMR** (360 MHz, $CDCl_3$) δ 7.07 (2H, d, *J* = 8.6, Ar*H*), 6.87 (2H, d, *J* = 8.5, Ar*H*), 4.95 (1H, d, *J* = 7.7, N*H*), 4.64 (2H, d, *J* = 2.4, HC≡CCH_2), 4.38 (1H, dd, *J* = 7.7, 6.1, C*H*NH), 3.03-2.90 (2H, m, CH_2Ar), 2.48 (1H, t, *J* = 2.4, *H*C≡CCH$_2$), 1.39 (9H, s, C(CH_3)$_3$), 1.37 (9H, s, C(CH_3)$_3$); **^{13}C NMR** (125.8 MHz, $CDCl_3$) δ 171.0 (C), 156.5 (C), 155.1 (C), 130.0 (2 × CH), 129.4 (C), 114.8 (2 × CH), 82.0 (C), 79.6 (C), 78.6 (C), 75.5 (CH), 55.8 (CH_2), 54.9 (CH), 37.7 (CH_2), 28.3 (3 × CH_3), 28.0 (3 × CH_3); ***m/z*** (ESI+, MeOH) 376 ($[M+H]^+$, 62%), 259 (55), 146 (100).

Methyl (_S_)-3-(4-hydroxy-phenyl)-2-(2,2,2-trifluoroacetylamido)propanoate (25)

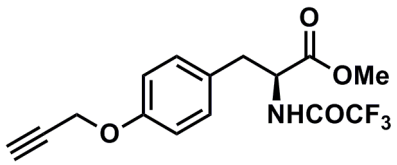


Using **General Procedure Q**, amine **8** (1.43 g, 7.30 mmol) was reacted with potassium methoxide (1.02 g, 14.6 mmol) in methanol (3.3 cm^3) and ethyl trifluoroacetate (8.32 cm^3, 14.6 mmol) to afford trifluoroacetamide **25** as a colourless solid (2.08 g, 98%).

R_f (Hexane:EtOAc, 3:1) = 0.08; **mp** 144-147 °C, (lit.[151] 141-143 ºC); **$[\alpha]_D$** = -37.3 (c 0.97, $CDCl_3$); **IR** (neat) 3279 (OH), 1697 (C=O, carbamate); **^{1}H NMR** (400 MHz, MeOD) δ 7.01 (2H, d, *J* = 8.6, Ar*H*), 6.71 (2H, d, *J* = 8.6, Ar*H*), 4.63 (1H, td, *J* = 9.9, 5.0, C*H*N), 3.72 (3H, s, OCH_3), 3.17 (1H, dd, *J* = 14.0, 5.3, C$H_A$$H_B$Ar), 2.92 (1H, dd, *J* = 14.0, 9.8, C$H_A$$H_B$Ar); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$) 174.2 (C), 159.1 (q, *J* = 37.3, C), 156.3 (C), 132.0 (2 × CH), 129.6 (C), 118.2 (q, *J* = 286.7, CF_3), 117.7 (2 × CH), 56.6 (CH), 52.6 (CH_3), 37.8 (CH_2); **^{19}F NMR** (376.3 MHz, $CDCl_3$) δ -77.2 (3F, s, C); ***m/z*** (ESI-, MeOH) 290 ($[M-H]^-$, 86%), 276 (100).

Melting point data is in good agreement with literature.[151]

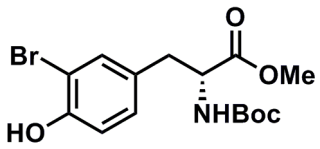
Methyl (S)-3-[4-(prop-2-ynyloxy)phenyl]-2-(2,2,2-trifluoroacetamido)propanoate (26)



Using **General Procedure C**, phenol **25** (1.00 g, 3.43 mmol) was reacted with propargyl bromide (1.38 g, 12.4 mmol) and potassium carbonate (0.95 g, 6.87 mmol) in DMF (30 cm^3), to afford propargyl ether **26** as a pale yellow oil (1.10 g, 97%).

R_f (Hexane:EtOAc, 3:1) = 0.37; **$[\alpha]_D$** = -87.7 (c 1.43, EtOH); **IR** (neat)1746 (C=O, ester), 1699 (C=O, carbamate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.18-7.10 (2H, m, Ar*H*), 7.01– 6.89 (2H, m, Ar*H*), 4.86-4.70 (1H, m, N*H*), 4.67 (2H, d, *J* = 2.4, HC≡ CCH_2), 4.51 – 4.25 (1H, m, C*H*NH), 3.75 (3H, s, CH_3), 3.45 – 3.24 (2H, m, CH_2Ar), 2.51 (1H, t, *J* = 2.4, *H*C≡CCH_2); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ 171.2 (C), 156.7 (C), 156.4 (q, *J* = 44.5, C), 130.1 (2 × CH), 129.7 (C), 115.8 (q, *J* = 287.9, CF_3), 115.2 (2 × CH), 78.4 (C), 76.0 (CH), 62.3 (C), 60.4 (CH_2), 55.9 (CH), 53.0 (CH_3), 33.2 (CH_2); **^{19}F NMR** (376.3 MHz, $CDCl_3$) δ -69.6 (3F, s, CF_3); ***m/z*** (ESI-, MeOH) 328 ($[M-H]^-$ 100%).

Methyl (*R*)-3-(3-bromo-4-hydroxyphenyl)-2-(*tert*-butoxycarbonylamino)propanoate (158)

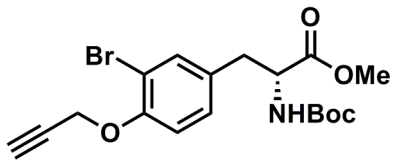


Phenol **15b** (3.00 g, 10.2 mmol) was dissolved in ethyl acetate (100 cm^3) with *p*-TsOH (0.193 g, 1.02 mmol) under UV radiation (365 nm) at room temperature. *N*-Bromosuccinimide (1.81 g, 10.2 mmol) was added, and the very pale yellow solution was stirred for 40 minutes. The reaction mixture was washed with $Na_2S_2O_3$ (5% aq., 3 × 200 cm^3), water (3 × 200 cm^3), brine (200 cm^3), dried ($MgSO_4$) and the solvent removed under reduced pressure. The dark orange oil was purified by column chromatography (Hexane:EtOAc, 95:5) to give *ortho*-bromo-phenol **158** as a colourless crystalline solid (3.42 g, 90%).

R_f (Hexane:EtOAc, 3:1) = 0.12; **mp** 121-123 ºC; **$[\alpha]_D$** = −58.6 (c 1.11, $CHCl_3$); **IR** (neat) 3360 (OH), 1742 (C=O, ester), 1688 (C=O, carbamate); **^{1}H NMR** (400 MHz, $CDCl_3$, 323 K) δ 7.24 (1H, d, *J* = 2.0, Ar*H*), 6.98 (1H, dd, *J* = 8.3, 2.0, Ar*H*), 6.92

(1H, d, *J* = 8.3, Ar*H*), 5.52 (1H, s, O*H*), 4.96 (1H, br s, N*H*CH), 4.50 (1H, br s, C*H*NH), 3.72 (3H, s, OC*H*$_3$), 3.04 (1H, dd, *J* = 13.8, 5.8, C*H*$_A$H$_B$Ar), 2.94 (1H, dd, *J* = 13.8, 5.8, CH$_A$*H*$_B$Ar), 1.43 (9H, s, OC(C*H*$_3$)$_3$); **^{13}C NMR** (125.8 MHz, $CDCl_3$, 298 K) δ 172.3 (C=O), 155.2 (C=O), 151.6 (C), 132.9 (CH), 130.5 (C), 130.1 (CH), 116.3 (CH), 110.2 (CBr), 80.3 (C), 54.6 (CH), 52.5 (CH_3), 37.3 (CH_2), 28.4 (3 × CH_3); ***m/z*** (ESI+, MeOH) 398 ([M^{81}Br+Na]$^+$, 99%), 396 ([M^{79}Br+Na]$^+$, 100), 342 (25), 340 (30).

Methyl (*R*)-3-(3-bromo-4-(prop-2-ynyloxy)phenyl)-2-(*tert*-butoxycarbonylamino)propanoate (164)

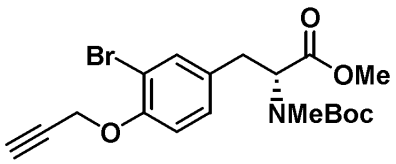


Using **General Procedure C**, phenol **158** (2.29 g, 6.12 mmol) was reacted with K_2CO_3 (1.69 g, 12.2 mmol) and propargyl bromide (2.5 cm^3, 22.0 mmol) in DMF (40 cm^3). The crude product was purified by column chromatography (Hexane:EtOAc, 90:10) to afford propargyl ether **164** as a colourless solid (2.47 g, 98%).

R$_f$ (Hexane:EtOAc, 3:1) = 0.29; **mp** 66 ºC; **[α]$_D$** = -41.1 (c 1.24, $CHCl_3$); **IR** (neat) 3435 (NH), 3304 (C≡C−H), 2122 (C≡C), 1738 (C=O, ester), 1708 (C=O, carbamate); **^{1}H NMR** δ (400 MHz, $CDCl_3$, 323 K) 7.32 (1H, d, *J* = 2.1, Ar*H*), 7.03 (1H, dd, *J* = 8.4, 2.1, Ar*H*), 6.97 (1H, d, *J* = 8.4, Ar*H*), 4.97 (1H, br s, N*H*CH), 4.72 (2H, d, *J* = 2.4, HC≡CC*H*$_2$), 4.50 (1H, br s, C*H*NH), 3.70 (3H, s, OC*H*$_3$), 3.05 (1H, dd, *J* = 13.8, 5.8, C*H*$_A$H$_B$Ar), 2.93 (1H, dd, *J* = 13.8, 5.8, CH$_A$*H*$_B$Ar), 2.51 (1H, t, *J* = 2.4, *H*C≡CCH$_2$) 1.41 (9H, s, OC(C*H*$_3$)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 298 K) 172.1 (C=O), 155.1 (C=O), 153.2 (C), 134.4 (C), 130.9 (CH), 129.2 (CH), 114.2

(CH), 112.3 (CBr), 80.1 (C), 78.0 (C), 76.3 (CH), 57.0 (CH_2), 54.5 (CH), 52.4 (CH_3), 37.2 (CH_2), 28.4 (3 × CH_3); ***m/z*** (ESI+, MeOH) 436 ([$M^{81}Br$+Na]$^+$, 21%), 434 ([$M^{79}Br$+Na]$^+$, 24), 356 (100), 300 (70); **HRMS** (ESI+, MeOH) [$M^{79}Br$+Na]$^+$ found 434.0572, $C_{18}H_{22}{}^{79}BrNNaO_5$ requires 434.0574.

Methyl (*R*)-3-(3-bromo-4-(prop-2-ynyloxy)phenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoate (166)

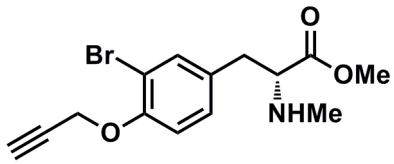


Using **General Procedure O,** Boc protected amine **164** (2.08 g, 5.05 mmol) was reacted with NaH (60% mineral oil dispersion, 0.303 g, 7.58 mmol) in dry THF (25 cm^3) and DMF (6 cm^3) with MeI (2.5 cm^3, 40.4 mmol). The crude product was purified by column chromatography (Hexane:EtOAc, 100:0 to 85:15) to afford Boc protected methyl amine **166** as a colourless oil (2.13 g, 99%).

R_f (Hexane:EtOAc, 3:1) = 0.20; **$[\alpha]_D$** = +23.5 (c 1.02, $CHCl_3$)**; IR** (neat) 3292 (C≡C−H), 2122 (C≡C), 1740 (C=O, ester), 1688 (C=O, carbamate); **^{1}H NMR** δ (500 MHz, $CDCl_3$, 298 K) 7.39 (1H, d, *J* = 4.7, Ar*H*), 7.09 (1H, dd, *J* = 8.2, 4.7, Ar*H*), 6.98 (1H, d, *J* = 8.2, Ar*H*), 4.85 (0.44H, dd, *J* = 10.5, 5.3, NCH_3CH), 4.74 (2H, s, $HC{\equiv}CCH_2$), 4.54 (0.56H, dd, *J* = 10.5, 4.3, NCH_3CH), 3.75 (1.68H, s, OCH_3), 3.73 (1.32H, s, OCH_3), 3.22 (1.12H, m, CH_2Ar), 2.94 (0.88H, m, CH_2Ar), 2.73 (1.68H, s, NCH_3CH), 2.70 (1.32H, s, NCH_3CH), 2.51 (1H, t, *J* = 2.4, $HC{\equiv}CCH_2$), 1.39 (3.96H, s, $OC(CH_3)_3$), 1.34 (5.04H, s, $OC(CH_3)_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 298 K) major rotamer (56%) 171.4 (C=O), 155.0 (C=O), 153.0 (C), 134.0 (CH), 132.6 (C), 129.1 (CH), 114.3 (CH), 112.5 (CBr), 80.6 (C), 78.0 (C), 76.3 (CH), 61.4 (CH), 57.1 (CH_2), 52.4 (CH_3), 34.5 (CH_2), 32.2 (CH_3), 28.3 (3 × CH_3), minor rotamer (44%) 171.7 (C=O), 155.9 (C=O), 152.9 (C), 134.0 (CH),

132.4 (C), 128.9 (CH), 114.3 (CH), 112.3 (CBr), 80.4 (C), 78.0 (C), 76.2 (CH), 59.6 (CH), 57.1 (CH_2), 52.4 (CH_3), 34.0 (CH_2), 32.4 (CH_3), 28.4 (3 × CH_3); ***m/z*** (ESI+, MeOH) 450 ($[M^{81}Br+Na]^+$, 96%), 448 ($[M^{79}Br+Na]^+$, 100), 394 (52), 392 (55); **HRMS** (ESI+, MeOH) $[M^{79}Br+Na]^+$ found 448.0728, $C_{19}H_{24}{}^{79}BrNNaO_5$ requires 448.0730.

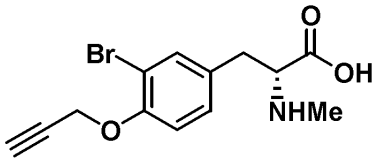
Methyl (*R*)-3-(3-bromo-4-(prop-2-ynyloxy)phenyl)-2-methylaminopropanoate (167)



Using **General Procedure D,** Boc protected methyl amine **166** (4.45 g, 10.4 mmol) was reacted with TFA (8.00 cm^3, 104 mmol) in DCM (50 cm^3). The crude product was purified by column chromatography (DCM) to give methyl amine **167** as a colourless solid (3.06 g, 90%).

R_f (DCM:MeOH, 99:1) = 0.11; **mp** 80-82 ºC; **$[\alpha]_D$** = +14.3 (c 1.05, $CHCl_3$); **IR** (neat) 3291 (C≡C−H), 2122 (C≡C), 1732 (C=O, ester); **^{1}H NMR** δ (500 MHz, $CDCl_3$) 7.35 (1H, d, *J* = 2.1, Ar*H*), 7.05 (1H, dd, *J* = 8.4, 2.1, Ar*H*), 6.95 (1H, d, *J* = 8.4, Ar*H*), 4.71 (2H, d, *J* = 2.4, HC≡CCH_2), 3.65 (3H, s, OCH_3), 3.37 (1H, t, *J* = 6.8, *CH*NH), 2.86 (1H, dd, *J* = 12.9, 5.6, C$H_A$$H_B$Ar), 2.82 (1H, dd, *J* = 12.9, 6.1, C$H_A$$H_B$Ar), 2.52 (1H, t, *J* = 2.4, *H*C≡CCH_2), 2.33 (3H, s, NHCH_3); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$) 174.7 (C=O), 152.9 (C), 134.1 (CH), 132.1 (C), 129.1 (CH), 114.1 (CH), 112.3 (CBr), 78.1 (C), 76.2 (CH), 64.5 (CH), 57.0 (CH_2), 51.8 (CH_3), 38.2 (CH_2), 34.8 (CH_3); ***m/z*** (ESI+, MeOH) 350 ($[M^{81}Br+Na]^+$, 21%), 348 ($[M^{79}Br+Na]^+$, 22), 328 ($[M^{81}Br+H]^+$, 96), 326 ($[M^{79}Br+H]^+$, 100); **HRMS** (ESI+, MeOH) $[M^{79}Br+H]^+$ found 326.0386, $C_{14}H_{17}{}^{79}BrNO_3$ requires 326.0386.

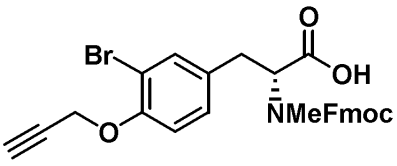
(*R*)-3-(3-Bromo-4-prop-2-ynyloxyphenyl)-2-methylaminopropanoic acid (172)



Using **General Procedure F**, methyl ester **167** (4.33 g, 13.3 mmol) was reacted with NaOH (0.585 g, 14.6 mmol) in H_2O (54 cm^3) and MeOH (12 cm^3) to afford carboxylic acid **172** as a colourless solid (3.69 g, 89%).

R_f (DCM:MeOH, 99:1) = 0.00; **mp** 204-206 ºC (subl.); **$[\alpha]_D$** = −14.0 (c 0.93, aq. NaOH); **IR** (neat) 3437 (OH); **^{1}H NMR** δ (400 MHz, D_2O, pD 14) 7.35 (1H, d, *J* = 1.8, Ar*H*), 7.07 (1H, dd, *J* = 8.5, 1.8, Ar*H*), 6.99 (1H, d, *J* = 8.5, Ar*H*), 4.71 (2H, s, HC≡CC*H*$_2$), 3.05 (1H, t, *J* = 6.8, C*H*NH), 2.72-2.66 (2H, m, C*H*$_2$Ar), 2.12 (3H, s, NHC*H*$_3$); **^{13}C NMR** δ (125.8 MHz, D_2O, pD 14) 181.7 (C=O), 152.5 (C), 134.5 (CH), 134.3 (C), 130.2 (CH), 115.4 (CH), 112.0 (CBr), 78.6 (C), 67.5 (CH), 67.4 (CH), 57.8 (CH_2), 38.5 (CH_2), 34.0 (CH_3); ***m/z*** (ESI-, MeOH) 647 ([2M^{81}Br-2H+Na]$^-$, 12%), 645 ([M^{81}Br+M^{79}Br-2H+Na]$^-$, 27), 643 ([2M^{79}Br-2H+Na]$^-$, 13), 312 ([M^{81}Br-H]$^-$, 100), 310 ([M^{79}Br-H]$^-$, 97); **HRMS** (ESI-, MeOH) [M^{81}Br-H]$^-$ found 312.0065, $C_{13}H_{14}{}^{81}BrNO_3$ requires 312.0053, [M^{79}Br-H]$^-$ found 310.0085, $C_{13}H_{14}{}^{79}BrNO_3$ requires 310.0073.

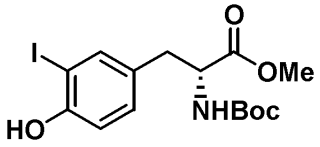
(*R*)-3-(3-Bromo-4-prop-2-ynyloxyphenyl)-2-[(9H-fluoren-9-ylmethoxycarbonyl) methylamino]propanoic acid (173)



Using **General Procedure G**, 3-(3-Bromo-4-prop-2-ynyloxy-phenyl)-2-methylamino-propionic acid **172** (3.68 g, 11.8 mmol) was reacted with Fmoc-OSu (3.98 g, 11.8 mmol) and Na_2CO_3 (3.75 g, 35.4 mmol) in MeOH (90 cm^3) and DMF (20 cm^3) to afford Fmoc-protected amine **173** as a yellow oil (4.53 g, 72%), which was used without further purification.

R_f (Hexane:EtOAc, 3:1) = 0.05; **^{1}H NMR** δ (400 MHz, $CDCl_3$) 7.58-7.52 (2H, m, Ar*H*), 7.37-7.32 (2H, m, Ar*H*), 7.27-7.25 (2H, m, Ar*H*), 7.25-7.17 (3H, m, Ar*H*), 7.12-7.08 (2H, m, Ar*H*), 4.83-4.55 (1H, m, C*H*NMe), 4.55-4.53 (2H, m, CH_2C≡CH), 4.53-4.52 (1H, m, CH_2C*H*Ar), 4.46-4.45 (2H, m, CH_2O), 3.24 (3H, s, NCH_3), 3.04-2.86 (2H, m, CH_2NH), 2.86 (1H, s, C≡C*H*); **^{13}C NMR** δ (100.6 MHz, $CDCl_3$) 172.4 (C=O), 155.2 (C=O), 154.8 (C), 145.0 (2 × C), 138.7 (2 × C), 134.2 (CH), 132.7 (C), 129.9 (CH), 128.8 (2 × CH), 127.1 (2 × CH), 124.1 (2 × CH), 122.9 (2 × CH), 114.7 (CH), 112.7 (CBr), 80.2 (C), 78.6 (CH), 67.7 (CH_2), 59.8 (CH), 57.8 (CH_2), 48.2 (CH), 34.0 (CH_2), 32.5 (CH_3); ***m/z*** (ESI+, MeOH) 1093 ([2M^{81}Br+Na]$^+$, 46%), 1091 (M^{81}Br+M^{79}Br+Na]$^+$, 72), 1089 ([2M^{79}Br+Na]$^+$, 43), 558 ([M^{81}Br+Na]$^+$, 100), 556 ([M^{79}Br+Na]$^+$, 92), 1709 (43), 1142 (41), 622 (57), 447 (54); **HRMS** (ESI+, MeOH) [M^{81}Br+Na]$^+$ found 558.0712, $C_{28}H_{24}{}^{81}BrNNaO_5$ requires 558.0710, [M^{79}Br+Na]$^+$ found 556.0717, $C_{28}H_{24}{}^{79}BrNNaO_5$ requires 556.0730.

Methyl (*R*)-3-(3-iodo-4-hydroxyphenyl)-2-(*tert*-butoxycarbonylamino)propanoate (174)

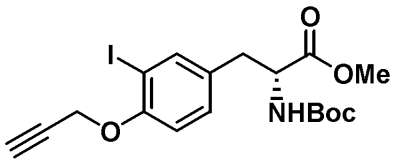


Phenol **15b** (3.00 g, 10.2 mmol) was dissolved in ethyl acetate (100 cm^3) with *p*-TsOH (0.193 g, 1.02 mmol) under UV radiation (365 nm) at room temperature. *N*-Iodosuccinimide (2.29 g, 10.2 mmol) was added, and the very pale yellow solution was stirred for 11 hours. The reaction mixture was washed with $Na_2S_2O_3$ (5% aq. soln., 3 × 200 cm^3), water (3 × 200 cm^3), brine (200 cm^3), dried ($MgSO_4$) and the solvent removed under reduced pressure. The dark orange oil was purified by column chromatography (Hexane:EtOAc, 95:5) to give *ortho*-iodo-phenol **174** as a colourless crystalline solid (3.59 g, 84%).

R_f (Hexane:EtOAc, 3:1) = 0.13; **mp** 112-114 ºC, (lit.[152] 110-112 °C); **$[\alpha]_D$** = −49.1 (c 1.14, $CHCl_3$), [lit.[152] (for (*S*)-enantiomer) $[\alpha]_D$ +48.8 (c 0.57, $CHCl_3$)]; **IR** (neat) 3368 (OH), 1738 (C=O, ester), 1686 (C=O, carbamate); **^{1}H NMR** δ (400 MHz, $CDCl_3$, 323 K) 7.43 (1H, d, *J* = 2.0, Ar*H*), 7.00 (1H, dd, *J* = 8.3, 2.0, Ar*H*), 6.89 (1H, d, *J* = 8.3, Ar*H*), 5.30 (1H, s, O*H*), 4.96 (1H, br s, N*H*CH), 4.50 (1H, br s, C*H*NH), 3.72 (3H, s, OCH_3), 3.03 (1H, dd, *J* = 13.9, 5.8, CH_AH$_B$Ar), 2.92 (1H, dd, *J* = 13.9, 5.8, CH$_A$$H_B$Ar), 1.43 (9H, s, OC(C$H_3$)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 323 K) 172.3 (C=O), 155.2 (C=O), 154.3 (C), 139.1 (CH), 131.2 (CH), 130.4 (C), 115.2 (CH), 85.6 (CI), 80.3 (C), 54.8 (CH), 52.4 (CH_3), 37.3 (CH_2), 28.5 (3 × CH_3); ***m/z*** (ESI+, MeOH) 444 ($[M+Na]^+$, 100%).

Spectroscopic data is in good agreement with the literature.[152]

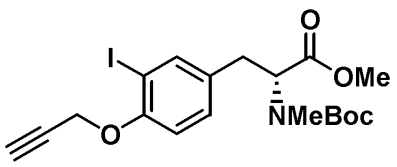
Methyl (*R*)-3-(3-iodo-4-(prop-2-ynyloxy)phenyl)-2-(*tert*-butoxycarbonylamino)-propanoate (176)



Using **General Procedure C**, phenol **174** (2.54 g, 6.03 mmol) was reacted with K_2CO_3 (1.67 g, 12.1 mmol) and propargyl bromide (2.4 cm^3, 21.7 mmol) in DMF (40 cm^3). The crude product was purified by column chromatography (Hexane:EtOAc, 90:10) to afford propargyl ether **176** as a colourless solid (2.58 g, 93%).

R_f (Hexane:EtOAc, 3:1) = 0.26; **mp** 73-75 ºC; **$[\alpha]_D$** = −50.7 (c 1.01, $CHCl_3$); **IR** (neat) 3377 (NH), 3292 (C≡C−H), 2122 (C≡C), 1742 (C=O, ester), 1709 (C=O, carbamate); **^{1}H NMR** δ (500 MHz, $CDCl_3$, 323 K) 7.55 (1H, d, *J* = 2.1, Ar*H*), 7.07 (1H, dd, *J* = 8.4, 2.1, Ar*H*), 6.90 (1H, d, *J* = 8.4, Ar*H*), 4.97 (1H, br s, N*H*CH), 4.71 (2H, d, *J* = 2.4, HC≡CCH_2), 4.50 (1H, br s, C*H*NH), 3.71 (3H, s, OCH_3), 3.03 (1H, dd, *J* = 13.9, 5.8, C$H_A$$H_B$Ar), 2.93 (1H, m, CH$_A$$H_B$Ar), 2.51 (1H, t, *J* = 2.4, *H*C≡CCH_2), 1.42 (9H, s, OC(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 323 K) 172.1 (C=O), 155.8 (C=O), 155.1 (C), 140.6 (CH), 131.7 (C), 130.3 (CH), 113.3 (CH), 86.8 (CI), 80.2 (C), 78.2 (C), 76.2 (CH), 57.3 (CH_2), 54.7 (CH), 52.3 (CH_3), 37.2 (CH_2), 28.5 (3 × CH_3); ***m/z*** (ESI+, MeOH) 482 ($[M+Na]^+$, 100%), 426 (53), 359 (28), 356 (49), 300 (27); **HRMS** (ESI+, MeOH) $[M+Na]^+$ found 482.0432, $C_{18}H_{22}INNaO_5$ requires 482.0435.

Methyl (*R*)-3-(3-iodo-4-(prop-2-ynyloxy)phenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoate (178)

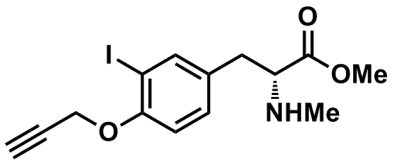


Using **General Procedure O,** Boc protected amine **176** (3.30 g, 7.12 mmol) was reacted with NaH (60% mineral oil dispersion, 0.431 g, 10.8 mmol) in dry THF (35

cm^3) and DMF (8 cm^3) with MeI (3.6 cm^3, 57.5 mmol). The crude product was purified by column chromatography (Hexane:EtOAc, 100:0 to 85:15) to afford Boc protected methyl amine **178** as a colourless oil (3.16 g, 93%).

R_f (Hexane:EtOAc, 3:1) = 0.21; **$[\alpha]_D$** = +38.9 (c 1.24, $CHCl_3$); **IR** (neat) 3291 (C≡C−H), 2122 (C≡C), 1740 (C=O, ester), 1688 (C=O, carbamate); **^{1}H NMR** δ (400 MHz, $CDCl_3$, 323 K) 7.63 (1H, d, *J* = 1.9, Ar*H*), 7.12 (1H, br s, Ar*H*), 6.91 (1H, d, *J* = 8.4, Ar*H*), 4.82 (0.45H, br s, C*H*NCH_3), 4.72 (1.1H, s, HC≡CCH_2), 4.71 (0.9H, s, HC≡CCH_2), 4.53 (0.55H, br s, C*H*NCH_3), 3.74 (3H, s, OCH_3), 3.20 (1H, dd, *J* = 14.5, 5.1, CH_AH_BAr), 2.93 (1H, m, CH_AH_BAr), 2.73 (3H, br s, CHNCH_3), 2.50 (1H, t, *J* = 2.4, *H*C≡CCH_2), 1.38 (9H, br s, OC(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 298 K) major rotamer (55%) 171.5 (C=O), 155.3 (C=O), 155.0 (C), 140.1 (CH), 133.0 (C), 130.2 (CH), 113.1 (CH), 86.8 (CI), 80.6 (C), 78.1 (C), 76.3 (CH), 61.4 (CH), 57.1 (CH_2), 52.4 (CH_3), 34.3 (CH_2), 32.2 (CH_3), 28.4 (3 × CH_3), minor rotamer (45%) 171.7 (C=O), 155.9 (C=O), 155.3 (C), 140.1 (CH), 132.8 (C), 130.0 (CH), 113.1 (CH), 86.5 (CI), 80.3 (C), 78.2 (C), 76.2 (CH), 59.6 (CH), 57.1 (CH_2), 52.4 (CH_3), 33.8 (CH_2), 32.4 (CH_3), 28.4 (3 × CH_3); ***m/z*** (ESI+, MeOH) 496 ($[M+Na]^+$, 100%), 440 (73), 374 (27); **HRMS** (ESI+, MeOH) $[M+Na]^+$ found 496.0596, $C_{19}H_{24}INNaO_5$ requires 496.0602.

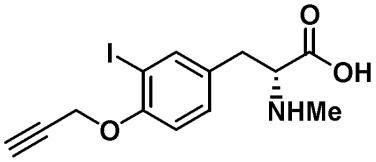
Methyl (*R*)-3-(3-iodo-4-prop-2-ynyloxyphenyl)-2-methylaminopropanoate (180)



Using **General Procedure D**, Boc protected methyl amine **178** (2.61 g, 5.51 mmol) was reacted with TFA (4.2 cm^3, 55.1 mmol) in DCM (25 cm^3). The crude product was purified by column chromatography (DCM) to give methyl amine **180** as a colourless solid (1.71 g, 83%).

R_f (DCM:MeOH, 99:1) = 0.09; **mp** 93-94 °C; **$[\alpha]_D$** = −27.6 (c 1.09, $CHCl_3$)**; IR** (neat) 3294 (C≡C−H), 2122 (C≡C), 1732 (C=O, ester); **^{1}H NMR** δ (500 MHz, $CDCl_3$) 7.60 (1H, d, *J* = 2.2, Ar*H*), 7.11 (1H, dd, *J* = 8.4, 2.2, Ar*H*), 6.90 (1H, d, *J* = 8.4, Ar*H*), 4.72 (2H, t, *J* = 2.4, HC≡CCH_2), 3.68 (3H, s, OCH_3), 3.38 (1H, t, *J* = 6.8, C*H*NH), 2.86 (1H, dd, *J* = 12.7, 5.6, C$H_A$$H_B$Ar), 2.83 (1H, dd, *J* = 12.7, 5.6, C$H_A$$H_B$Ar), 2.52 (1H, t, *J* = 2.4, *H*C≡CCH_2), 2.36 (3H, s, NCH_3); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$) 174.7 (C=O), 155.4 (C), 140.3 (CH), 132.6 (C), 130.2 (CH), 112.9 (CH), 86.7 (CI), 78.2 (C), 76.2 (CH), 64.6 (CH), 57.1 (CH_2), 51.9 (CH_3), 38.1 (CH_2), 34.8 (CH_3); ***m/z*** (ESI+, MeOH) 374 ($[M+H]^+$, 100%), 388 (31), 314 (30); **HRMS** (ESI+, MeOH) $[M+H]^+$ found 374.0248, $C_{14}H_{17}INO_3$ requires 374.0248.

(*R*)-3-(3-Iodo-4-prop-2-ynyloxyphenyl)-2-methylaminopropanoic acid (182)

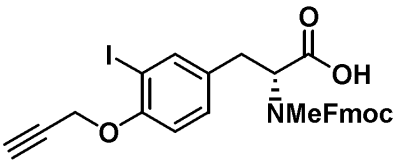


Using **General Procedure F**, methyl ester **180** (1.51 g, 4.05 mmol) was reacted with NaOH (0.194 g, 4.86 mmol) in H_2O (16 cm^3) and MeOH (5 cm^3) to afford carboxylic acid **182** as a colourless solid (1.45 g, 97%).

R_f (DCM:MeOH, 99:1) = 0.00; **mp** 197-198 ºC (dec.); **$[\alpha]_D$** = −17.1 (c 1.06, aq. NaOH); **IR** (neat) 3409 (OH); **^{1}H NMR** δ (500 MHz, D_2O, pD 14) 7.07 (1H, s, Ar*H*), 6.56 (1H, d, *J* = 8.4, Ar*H*), 6.26 (1H, d, *J* = 8.4, Ar*H*), 4.16 (2H, s, HC≡CCH_2), 2.63 (1H, t, *J* = 6.7, NHC*H*), 2.35 (1H, dd, *J* = 13.4, 3.9, C$H_A$$H_B$Ar), 2.11 (1H, dd, *J* = 13.4, 8.3, C$H_A$$H_B$Ar), 1.64 (3H, s, NHC$H_3$); **^{13}C NMR** δ (125.8 MHz, D_2O, pD 14) 181.7 (C=O), 155.0 (C), 140.6 (CH), 134.7 (C), 131.3 (CH), 114.5 (CH), 86.8 (CI), 78.8 (CH), 77.8 (C), 67.6 (CH), 57.9 (CH_2), 38.3 (CH_2), 34.0 (CH_3); ***m/z*** (ESI+, MeOH) 795 ($[2M-H+2K]^+$, 36%), 785 ($[2M-2H+3Na]^+$, 19), 773

(46), 404 ([M-H+2Na]$^+$, 24), 382 ([M+Na]$^+$, 32), 360 ([M+H]$^+$, 100), 314 (48); **HRMS** (ESI-, MeOH) [M-H]$^-$ found 357.9946, $C_{13}H_{14}INO_3$ requires 357.9935.

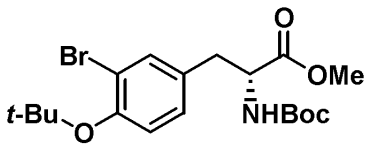
(*R*)-3-(3-Iodo-4-prop-2-ynyloxyphenyl)-2-[(9H-fluoren-9-ylmethoxycarbonyl) methylamino]propanoic acid (184)



Using **General Procedure G**, methyl amine **182** (2.06 g, 5.74 mmol) was reacted with Fmoc-OSu (1.94 g, 5.74 mmol) and Na_2CO_3 (1.83 g, 17.2 mmol) in MeOH (45 cm^3) and DMF (10 cm^3) to afford Fmoc-protected methyl amine **184** (2.57 g, 77%).

R$_f$ (Hexane:EtOAc, 3:1) = 0.07; **^{1}H NMR** δ (400 MHz, $CDCl_3$) 7.58-7.52 (2H, m, Ar*H*), 7.36-7.34 (2H, m, Ar*H*), 7.25-7.18 (2H, m, Ar*H*), 7.18-7.15 (3H, m, Ar*H*), 7.15-7.10 (2H, m, Ar*H*), 4.85-4.75 (1H, m, C*H*NMe), 4.62-4.53 (2H, m, CH_2C≡CH), 4.53-4.49 (1H, m, CH$_2$C*H*Ar), 4.47-4.45 (2H, m, CH_2O), 2.93 (3H, s, NCH_3), 3.02-2.84 (2H, m, CH_2NH), 2.56 (1H, s, C≡C*H*); **^{13}C NMR** δ (100.6 MHz, $CDCl_3$) 172.5 (C=O), 155.3 (C=O), 154.7 (C), 145.2 (2 × C), 138.7 (2 × C), 134.2 (CH), 132.8 (C), 129.9 (CH), 128.8 (2 × CH), 127.1 (2 × CH), 124.1 (2 × CH), 122.9 (2 × CH), 113.7 (CH), 87.0 (CI), 80.4 (C), 78.3 (CH), 68.0 (CH_2), 59.8 (CH), 57.7 (CH_2), 48.2 (CH), 34.3 (CH_2), 32.3 (CH_3); ***m/z*** (ESI+, MeOH) 604 ([M+Na]$^+$, 87%), 714 (49), 667 (100), 360 (54), 316 (79), 197 (50); **HRMS** (ESI+, MeOH) [M+Na]$^+$ found 604.0605, $C_{28}H_{24}INNaO_5$ requires 604.0602.

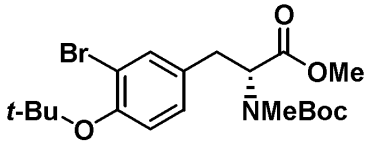
Methyl (*R*)-3-(3-bromo-4-*tert*-butoxyphenyl)-2-*tert*-butoxycarbonylaminoprop-anoate (163)



Using **General Procedure N**, phenol **158** (2.33 g, 6.24 mmol) was reacted with Boc_2O (10.9 g, 49.9 mmol) and $Sc(OTf)_3$ (0.154 g, 0.311 mmol) in DCM (10 cm^3). The crude product was purified by column chromatography (Hexane:EtOAc, 98:2 to 88:12) to afford *tert*-butyl ether **163** as a colourless solid (2.07 g, 77%).

R_f (Hexane:EtOAc, 3:1) = 0.39; **mp** 78-79 °C; **$[\alpha]_D$** = −47.2 (c 1.17, $CHCl_3$); **IR** (neat) 3368 (NH), 1745 (C=O, ester), 1709 (C=O, carbamate); **^{1}H NMR** δ (500 MHz, $CDCl_3$, 323 K) 7.32 (1H, d, *J* = 2.1, Ar*H*), 7.01 (1H, d, *J* = 8.3, Ar*H*), 6.96 (1H, dd, *J* = 8.3, 2.1, Ar*H*), 4.97 (1H, br s, N*H*CH), 4.51 (1H, br s, C*H*NH), 3.69 (3H, s, OCH_3), 3.03 (1H, dd, *J* = 13.8, 5.7, C$H_A$$H_B$Ar), 2.93 (1H, dd, *J* = 13.8, 6.3, $CH_A$$H_B$Ar), 1.42 (9H, s, OC(C$H_3$)$_3$), 1.41 (9H, s, OC(C$H_3$)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 323 K) major rotamer (52%) 172.2 (C=O), 155.1 (C=O), 152.6 (C), 134.3 (CH), 132.4 (C), 128.8 (CH), 123.7 (CH), 119.1 (CBr), 81.4 (C), 80.2 (C), 54.7 (CH), 52.3 (CH_3), 37.8 (CH_2), 29.2 (3 × CH_3), 28.5 (3 × CH_3), minor rotamer (48%) 172.5 (C=O), 154.8 (C=O), 152.6 (C), 134.3 (CH), 129.8 (C), 128.8 (CH), 124.1 (CH), 119.1 (CBr), 81.0 (C), 78.4 (C), 54.7 (CH), 52.3 (CH_3), 37.8 (CH_2), 29.2 (3 × CH_3), 28.5 (3 × CH_3); ***m/z*** (ESI+, MeOH) 885 ($[2M^{81}Br+Na]^+$, 5%), 883 ($[M^{81}Br+M^{79}Br+Na]^+$, 10), 881 ($[2M^{79}Br+Na]^+$, 6), 454 ($[M^{81}Br+Na]^+$, 92), 452 ($[M^{79}Br+Na]^+$, 100), 374 (76); **HRMS** (ESI+, MeOH) $[M^{79}Br+Na]^+$ found 452.1042, $C_{19}H_{28}{}^{79}BrNNaO_5$ requires 452.1043.

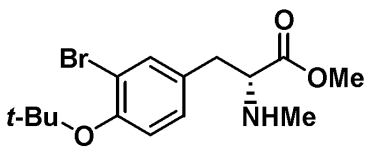
Methyl (*R*)-3-(3-bromo-4-*tert*-butoxyphenyl)-2-*tert*-butoxycarbonylmethylaminopropanoate (165)



Using **General Procedure O**, Boc protected amine **163** (1.35 g, 3.14 mmol) was reacted with NaH (60% mineral oil dispersion, 0.188 g, 4.71 mmol) in dry THF (15 cm^3) and DMF (4 cm^3) with MeI (1.6 cm^3, 25.1 mmol). The crude product was purified by column chromatography (Hexane:EtOAc, 100:0 to 85:15) to afford Boc protected methyl amine **165** as a colourless oil (1.33 g, 95%).

R_f (Hexane:EtOAc, 3:1) = 0.37; **$[\alpha]_D$** = +31.8 (c 1.07, $CHCl_3$); **IR** (neat) 1745 (C=O, ester), 1693 (C=O, carbamate); **^{1}H NMR** δ (400 MHz, $CDCl_3$, 323 K) 7.38 (1H, s, Ar*H*), 7.01 (2H, br s, Ar*H*), 4.81 (0.48H, br s, C*H*NCH_3), 4.53 (0.52H, br s, C*H*NCH_3), 3.73 (3H, s, OCH_3), 3.20 (0.96H, m, CH_2Ar), 2.96 (1.04H, m, CH_2Ar), 2.71 (3H, br s, NCH_3) 1.57 (9H, s, OC(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 298 K) major rotamer (52%) 171.5 (C=O), 155.0 (C=O), 152.0 (C), 133.9 (C), 133.7 (CH), 128.7 (CH), 123.9 (CH), 119.2 (CBr), 81.4 (C), 80.6 (C), 61.5 (CH), 52.4 (CH_3), 34.6 (CH_2), 32.3 (CH_3), 29.1 (3 × CH_3), 28.4 (3 × CH_3), minor rotamer (48%) 171.8 (C=O), 155.9 (C=O), 152.2 (C), 133.9 (C), 133.8 (CH), 128.5 (CH), 123.9 (CH), 119.1 (CBr), 81.3 (C), 80.3 (C), 59.7 (CH), 52.4 (CH_3), 34.1 (CH_2), 32.7 (CH_3), 29.0 (3 × CH_3), 28.4 (3 × CH_3); ***m/z*** (ESI+, MeOH) 913 ([2M^{81}Br+Na]$^+$, 8%), 911 ([M^{81}Br+M^{79}Br+Na]$^+$, 21), 909 ([2M^{79}Br+Na]$^+$, 10), 468 ([M^{81}Br+Na]$^+$, 92), 466 ([M^{79}Br +Na]$^+$, 100), 454 (49), 452 (53), 374 (32); **HRMS** (ESI+, MeOH) [M^{79}Br+Na]$^+$ found 466.1199, $C_{20}H_{30}BrNNaO_5$ requires 466.1200.

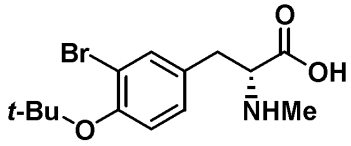
Methyl (*R*)-3-(3-bromo-4-*tert*-butoxyphenyl)-2-methylaminopropanoate (168)



Using **General Procedure P,** Boc protected methyl amine **165** (1.38 g, 3.11 mmol) was reacted with TMSOTf (2.30 cm^3, 12.4 mmol) and 2,6-lutidine (1.80 cm^3, 15.5 mmol) in dry DCM (8 cm^3). The crude product was purified by column chromatography (Hexane:EtOAc, 4:1 to 3:2) to afford methyl amine **168** as a colourless oil (0.92 g, 86%).

R_f (Hexane:EtOAc, 3:1) = 0.04; **$[\alpha]_D$** = −14.3 (c 0.98, $CHCl_3$); **IR** (neat) 3347 (NH), 1736 (C=O, ester); **^{1}H NMR** δ (400 MHz, $CDCl_3$, 323 K) 7.34 (1H, s, Ar*H*), 6.98 (1H, s, Ar*H*), 6.98 (1H, s, Ar*H*), 3.61 (3H, s, OCH_3), 3.36 (1H, t, *J* = 6.8, C*H*NH), 2.90-2.75 (2H, m, CH_2Ar), 2.34 (3H, s, NCH_3), 1.39 (9H, s, OC(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 298 K) 174.7 (C=O), 152.1 (C), 133.8 (CH), 133.5 (C), 128.6 (CH), 123.7 (CH), 119.0 (CBr), 81.2 (C), 64.6 (CH), 51.6 (CH_3), 38.5 (CH_2), 34.8 (CH_3), 29.0 (3 × CH_3); ***m/z*** (ESI+, MeOH) 346 ([M^{81}Br+H]$^+$, 95%), 344 ([M^{79}Br+H]$^+$, 100); **HRMS** (ESI+, MeOH) [M^{79}Br+H]$^+$ found 344.0856, $C_{15}H_{23}{}^{79}BrNO_3$ requires 344.0856.

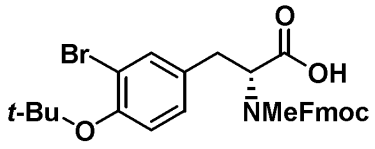
(*R*)-3-(3-Bromo-4-*tert*-butoxyphenyl)-2-methylaminopropanoic acid (171)



Using **General Procedure F**, methyl ester **168** (0.670 g, 1.94 mmol) was reacted with NaOH (0.093 g, 2.33 mmol) in H_2O (8 cm^3) and MeOH (2 cm^3) to afford carboxylic acid **171** as a colourless solid (0.559 g, 87%).

R_f (Hexane:EtOAc, 3:1) = 0.00; **mp** 219-220 ºC (subl.); **$[\alpha]_D$** = −12.4 (c 1.05, aq. NaOH); **IR** (neat) 3446 (OH); **^{1}H NMR** δ (400 MHz, D_2O, pD 14) 7.26 (1H, d, *J* = 1.3, Ar*H*), 6.91-6.89 (2H, m, Ar*H*), 2.94 (1H, t, *J* = 6.8, C*H*NH), 2.60 (1H, dd, *J* = 12.1, 5.3, CH_AH$_B$Ar), 2.55 (1H, dd, *J* = 12.1, 5.3, CH$_A$$H_B$Ar), 1.99 (3H, s, NHC$H_3$), 1.12 (9H, s, OC(C$H_3$)$_3$); **^{13}C NMR** δ (100.6 MHz, D_2O, pD 14) 181.6 (C=O), 151.0 (C), 136.3 (C), 134.3 (CH), 129.8 (CH), 125.1 (CH), 119.0 (CBr), 83.5 (C), 67.4 (CH), 38.5 (CH_2), 34.0 (CH_3), 28.9 (3 × CH_3); ***m/z*** (ESI−, MeOH) 683 ([2M^{81}Br−2H+Na]$^-$, 15%), 681 (M^{81}Br+M^{79}Br−2H+Na]$^-$, 31), 679 ([M^{79}Br-2H+Na]$^-$, 15), 330 ([M^{81}Br−H]$^-$, 93), 328 ([M^{79}Br−H]$^-$, 100); **HRMS** (ESI-, MeOH) [M^{81}Br-H]$^-$ found 330.0534, $C_{14}H_{20}{}^{81}BrNO_3$ requires 330.0522 , [M^{79}Br-H]$^-$ found 328.0555, $C_{14}H_{20}{}^{79}BrNO_3$ requires 328.0543.

(*R*)-3-(3-Bromo-4-*tert*-butoxyphenyl)-2-[(9H-fluoren-9-ylmethoxycarbonyl)methylamino]propanoic acid (144)

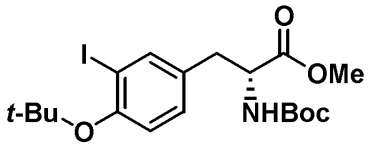


Using **General Procedure G**, methyl amine **171** (0.500 g, 1.51 mmol) was reacted with Fmoc-OSu (0.511 g, 1.51 mmol) and Na_2CO_3 (0.482 g, 4.54 mmol) in MeOH (5 cm^3) and DMF (15 cm^3) to afford a pale yellow oil which was used as the crude Fmoc-protected methyl amine **144** (0.617 g, 74%).

R_f (Hexane:EtOAc, 3:1) = 0.02; **^{1}H NMR** δ (500 MHz, $CDCl_3$) 7.75-7.73 (2H, m, Ar*H*), 7.66-7.61 (1H, m, Ar*H*), 7.52-7.50 (2H, m, Ar*H*), 7.42 (1H, br s, Ar*H*), 7.38 (2H, br s, Ar*H*), 7.29-7.26 (2H, m, Ar*H*), 7.03-7.00 (1H, m, Ar*H*), 4.55 (1H, br s, C*H*NCH$_3$), 4.34 (2H, m, OCH_2CH), 4.20 (1H, br s, OCH$_2$C*H*), 3.31-3.04 (2H, m,

C*H*$_2$Ar), 2.81-2.77 (3H, m, NCH$_3$), 1.37-1.35 (9H, m, O(CH$_3$)$_3$); **^{13}C NMR** δ (125.8 MHz, CDCl$_3$) 172.0 (C=O), 155.3 (C=O), 153.1 (C), 142.9 (2 × C), 137.3 (2 × C), 134.2 (C), 133.7 (CH), 129.2 (CH), 128.2 (2 × CH), 127.2 (2 × CH), 124.2 (2 × CH), 123.8 (2 × CH), 119.3 (CH), 116.2 (CBr), 77.7 (C), 66.9 (CH$_2$), 59.7 (CH), 48.1 (CH), 34.1 (CH$_2$), 32.7 (CH$_3$), 27.9 (3 × CH$_3$); ***m/z*** (ESI+, MeOH) 576 ([M^{81}Br+Na]$^+$, 94%), 574 ([M^{79}Br+Na]$^+$, 100), 1347 (15), 663 (25), 274 (22); **HRMS** (ESI+, MeOH) [M^{81}Br+Na]$^+$ found 576.1177, $C_{29}H_{30}{}^{81}BrNNaO_5$ requires 576.1179, [M^{79}Br+Na]$^+$ found 574.1195, $C_{29}H_{30}{}^{79}BrNNaO_5$ requires 574.1200.

Methyl (*R*)-2-*tert*-butoxycarbonylamino-3-(4-*tert*-butoxy-3-iodophenyl)propanoate (175)

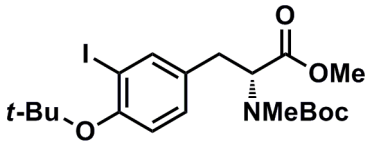


Using **General Procedure N**, phenol **174** (2.40 g, 5.70 mmol) was reacted with Boc$_2$O (9.95 g, 45.6 mmol) and Sc(OTf)$_3$ (0.224 g, 0.456 mmol) in dry DCM (15 cm^3). The crude product was purified by column chromatography (Hexane:EtOAc, 98:2 to 88:12) to afford *tert*-butyl ether **175** as a colourless solid (1.96 g, 72%).

R$_f$ (Hexane:EtOAc, 3:1) = 0.43; **mp** 87-89 ºC; **[α]$_D$** = −45.8 (c 1.18, CHCl$_3$); **IR** (neat) 3440 (NH), 1744 (C=O, ester), 1706 (C=O, carbamate); **^{1}H NMR** δ (500 MHz, CDCl$_3$) 7.54 (1H, br s, Ar*H*), 7.00 (1H, dd, *J* = 8.2, 1.9, Ar*H*), 6.98 (1H, d, *J* = 8.2, Ar*H*), 5.02 (1H, br d, *J* = 7.8, N*H*CH), 4.51 (1H, br d, *J* = 7.1, C*H*NH), 3.69 (1.65H, s, OC*H*$_3$), 3.67 (1.35H, s, OC*H*$_3$), 3.03-2.89 (2H, m, C*H*$_2$Ar), 1.44 (4.95H, s, OC(C*H*$_3$)$_3$), 1.41 (4.05H, s, OC(C*H*$_3$)$_3$), 1.40 (4.05H, s, OC(C*H*$_3$)$_3$), 1.31 (4.95H, s, OC(C*H*$_3$)$_3$); **^{13}C NMR** δ (125.8 MHz, CDCl$_3$) major rotamer (55%), 172.3 (C=O),

155.3 (C=O), 154.5 (C), 140.2 (CH), 132.4 (C), 129.8 (CH), 124.3 (CH), 94.8 (CI), 81.4 (C), 78.5 (C), 54.5 (CH), 52.4 (CH_3), 37.3 (CH_2), 29.4 (3 × CH_3), 28.4 (3 × CH_3), minor rotamer (45%), 172.6 (C=O), 155.2 (C), 155.1 (C=O), 140.2 (CH), 130.9 (C), 129.8 (CH), 121.5 (CH), 94.8 (CI), 80.1 (C), 80.0 (C), 54.6 (CH), 52.2 (CH_3), 37.9 (CH_2), 29.4 (3 × CH_3), 28.9 (3 × CH_3); ***m/z*** (ESI+, MeOH) 500 ($[M+Na]^+$, 100%), 444 (64); **HRMS** (ESI+, MeOH) $[M+Na]^+$ found 500.0902, $C_{19}H_{28}INNaO_5$ requires 500.0904.

Methyl (*R*)-2-(*tert*-butoxycarbonylmethylamino)-3-(4-*tert*-butoxy-3-iodophenyl) propanoate (177)

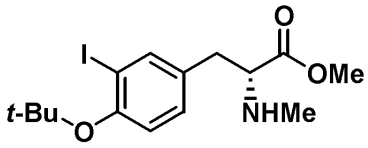


Using **General Procedure O**, Boc protected amine **175** (2.18 g, 4.58 mmol) was reacted with NaH (60% mineral oil dispersion, 0.275 g, 6.87 mmol) in dry THF (22 cm^3) and DMF (5 cm^3) with MeI (2.28 cm^3, 36.6 mmol). The crude product was purified by column chromatography (Hexane:EtOAc, 100:0 to 85:15) to afford Boc protected methyl amine **177** as a colourless oil (2.16 g, 96%).

R_f (Hexane:EtOAc, 3:1) = 0.26; **$[\alpha]_D$** = +41.7 (c 1.20, $CHCl_3$); **IR** (neat) 1745 (C=O, ester), 1696 (C=O, carbamate); **^{1}H NMR** δ (500 MHz, $CDCl_3$, 323 K) 7.62 (1H, br s, Ar*H*), 7.09-7.03 (1H, m, Ar*H*), 6.97 (1H, d, *J* = 8.3, Ar*H*), 4.80 (0.45H, br s, NHC*H*), 4.52 (0.55H, br s, NHC*H*), 3.73 (3H, s, OCH_3), 3.19-2.89 (2H, m, CH_2Ar), 2.71 (3H, br s, NCH_3), 1.44 (4.95H, s, OC(CH_3)$_3$), 1.39 (4.95H, s, OC(CH_3)$_3$), 1.37 (4.05H, s, OC(CH_3)$_3$), 1.32 (4.05H, s, OC(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$, 323 K) major rotamer (55%), 171.5 (C=O), 155.2 (C=O), 155.1 (C), 139.9 (CH),

134.0 (C), 129.5 (CH), 121.5 (CH), 94.8 (CI), 81.3 (C), 78.3 (C), 59.9 (CH), 52.2 (CH_3), 34.4 (CH_2), 32.4 (CH_3), 29.4 (3 × CH_3), 28.5 (3 × CH_3), minor rotamer (45%), 171.7 (C=O), 155.9 (C=O), 154.4 (C), 139.9 (CH), 132.6 (C), 129.5 (CH), 124.2 (CH), 94.8 (CI), 80.5 (C), 80.3 (C), 61.6 (CH), 52.2 (CH_3), 34.1 (CH_2), 32.4 (CH_3), 29.4 (3 × CH_3), 29.0 (3 × CH_3); ***m/z*** (ESI+, MeOH) 514 ($[M+Na]^+$, 100%), 458 (24), 388 (29); **HRMS** (ESI+, MeOH) $[M+Na]^+$ found 514.1064, $C_{20}H_{30}INNaO_5$ requires 514.1061.

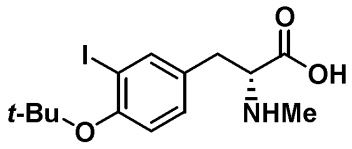
Methyl (*R*)-3-(4-*tert*-butoxy-3-iodophenyl)-2-methylaminopropanoate (179)



Using **General Procedure P,** Boc protected methyl amine **177** (2.23 g, 4.55 mmol) was reacted with TMSOTf (3.29 cm^3, 18.2 mmol) and 2,6-lutidine (2.65 cm^3, 22.7 mmol) in dry DCM (10 cm^3). The crude product was purified by column chromatography (Hexane:EtOAc, 4:1 to 3:2) to afford methyl amine **179** as a colourless oil (1.60 g, 90%).

R_f (Hexane:EtOAc, 3:1) = 0.06; **$[\alpha]_D$** = −15.9 (c 1.20, $CHCl_3$); **IR** (neat) 3332 (NH), 1733 (C=O, ester); **^{1}H NMR** δ (500 MHz, $CDCl_3$) 7.56 (1H, s, Ar*H*), 7.01 (1H, dd, *J* = 8.2, 2.2, Ar*H*), 6.95 (1H, d, *J* = 8.2, Ar*H*), 3.62 (3H, s, OCH_3), 3.36 (1H, t, *J* = 6.8, C*H*NH), 2.83 (2H, m, CH_2Ar), 2.34 (3H, s, NCH_3), 1.42 (9H, s, OC(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$) 174.7 (C=O), 155.0 (C), 139.9 (CH), 133.5 (C), 129.7 (CH), 121.4 (CH), 94.9 (CI), 81.3 (C), 64.6 (CH), 51.7 (CH_3), 38.3 (CH_2), 34.8 (CH_3), 29.3 (3 × CH_3); ***m/z*** (ESI+, MeOH) 414 ($[M+Na]^+$, 15%), 392 ($[M+H]^+$, 100), 336 (50), 276 (32); **HRMS** (ESI+, MeOH) $[M+H]^+$ found 392.0712, $C_{15}H_{23}INO_3$ requires 392.0717.

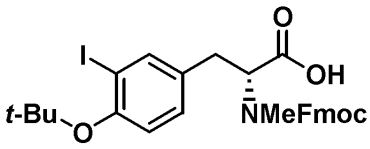
(*R*)-3-(4-*tert*-Butoxy-3-iodophenyl)-2-methylaminopropanoic acid (181)



Using **General Procedure F**, methyl ester **179** (1.45 g, 3.70 mmol) was reacted with NaOH (0.178 g, 4.45 mmol) in H_2O (14 cm^3) and MeOH (4 cm^3) to afford carboxylic acid **181** as a colourless solid (1.22 g, 87%).

R_f (Hexane:EtOAc, 3:1) = 0.00; **mp** 210-211 ºC (subl.); **$[\alpha]_D$** = −16.4 (c 1.16, aq. NaOH); **IR** (neat) 3381 (OH); **^{1}H NMR** δ (500 MHz, D_2O, pD 14) 7.22 (1H, s, Ar*H*), 6.69 (1H, d, *J* = 8.3, Ar*H*), 6.52 (1H, d, *J* = 8.3, Ar*H*), 2.75 (1H, t, *J* = 6.8, C*H*NH), 2.47 (1H, dd, *J* = 13.5, 5.3, CH_AH_BAr), 2.26 (1H, dd, *J* = 13.5, 8.0, CH_AH_BAr), 1.76 (3H, s, NCH_3), 0.89 (9H, s, $OC(CH_3)_3$); **^{13}C NMR** δ (125.8 MHz, D_2O, pD 14) 181.5 (C=O), 154.3 (C), 140.5 (C), 136.4 (CH), 130.9 (CH), 123.0 (CH), 95.4 (CI), 83.1 (C), 67.6 (CH), 38.5 (CH_2), 34.1 (CH_3), 29.6 (3 × CH_3); ***m/z*** (ESI−, MeOH) 775 ($[M-2H+Na]^-$, 13%), 376 ($[M-H]^-$, 100); **HRMS** (ESI-, MeOH) $[M-H]^-$ found 376.0416, $C_{14}H_{20}INO_3$ requires 376.0404.

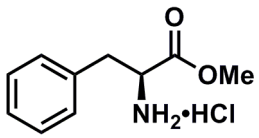
(*R*)-3-(4-*tert*-Butoxy-3-iodophenyl)-2-[(9H-fluoren-9-ylmethoxycarbonyl)methylamino]propanoic acid (183)



Using **General Procedure G**, methyl amine **181** (0.749 g, 1.98 mmol) was reacted with Fmoc-OSu (0.670 g, 1.98 mmol) and Na_2CO_3 (0.631 g, 5.95 mmol) in MeOH (15 cm^3) and DMF (5 cm^3) to afford a pale yellow oil which was used as the crude Fmoc-protected amine **183** (0.819 g, 69%).

R_f (Hexane:EtOAc, 3:1) = 0.07; **^{1}H NMR** δ (500 MHz, $CDCl_3$) 7.75-7.73 (2H, m, Ar*H*), 7.66-7.61 (1H, m, Ar*H*), 7.52-7.50 (2H, m, Ar*H*), 7.42 (1H, br s, Ar*H*), 7.38 (2H, br s, Ar*H*), 7.29-7.26 (2H, m, Ar*H*), 7.03-7.00 (1H, m, Ar*H*), 4.55 (1H, br s, C*H*NCH_3), 4.34 (2H, m, OCH_2CH), 4.20 (1H, br s, OCH_2C*H*), 3.31-3.04 (2H, m, CH_2Ar), 2.81-2.77 (3H, m, NCH_3), 1.37-1.35 (9H, m, $O(CH_3)_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$) 172.0 (C=O), 157.4 (C=O), 155.4 (C), 145.0 (2 × C), 140.0 (CH), 139.1 (2 × C), 134.6 (C), 129.1 (CH), 127.2 (2 ×CH), 126.3 (2 × CH), 124.5 (2 ×CH), 123.2 (2 × CH), 117.4 (CH), 88.8 (CI), 77.7 (C), 67.0 (CH_2), 59.4 (CH), 48.0 (CH), 34.0 (CH_2), 32.7 (CH_3), 27.7 (3 ×CH_3); ***m/z*** (ESI+, MeOH) 622 ($[M+Na]^+$, 100%), 1347 (16), 782 (20), 697 (28), 644 (37), 511 (49), 392 (44), 378 (36), 192 (33); **HRMS** (ESI+, MeOH) $[M+Na]^+$ found 622.1076, $C_{29}H_{30}INNaO_5$ requires 622.1061.

Methyl (*S*)-2-amino-3-phenylpropanoate hydrochloride (151)



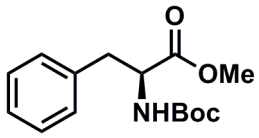
Using **General Procedure A**, L-phenylalanine (3.00 g, 18.2 mmol) was reacted with acetylchloride (3.9 cm^3, 54.8 mmol) in methanol (50 cm^3). The resultant solid

was recrystallised from methanol, affording methyl ester **151** as a colourless solid (3.91 g, 99%).

R_f (DCM:MeOH, 99:1) = 0.00; **mp** 156-159 °C, (lit.[153] 158-161 °C); **$[\alpha]_D$** = +37.1 (c 1.05, EtOH), [lit.[153] $[\alpha]_D$ +37 (c 2, EtOH)]; **IR** (neat) 3402 (1º amine), 1748 (C=O, ester); **^{1}H NMR** δ (400 MHz, CD_3OD) 7.40 – 7.28 (5H, m, Ar*H*), 4.35 (1H, t, *J* = 6.7 Hz, C*H*NH_2), 3.79 (3H, s, OCH_3) 3.28 (1H, dd, *J* = 13.6, 5.8 Hz, C$H_A$$H_B$Ar), 3.23 (1H, dd, *J* = 13.6, 6.3 Hz, CH_AH_BAr); **^{13}C NMR** δ (125.8 MHz, CD_3OD), 170.5 (C=O), 135.3 (C), 130.4 (2 × CH), 130.2 (2 × CH), 129.0 (CH), 55.2 (CH), 53.6 (CH_3), 37.4 (CH_2); ***m/z*** (ESI+, MeOH) 180 ($[M+H]^+$, 100%).

Spectroscopic data is in good agreement with the literature.[153]

Methyl (*S*)-2-(*tert*-butoxycarbonylamino)-3-phenylpropanoate (152)



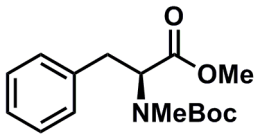
Using **General Procedure B**, amine hydrochloride **151** (2.00 g, 11.2 mmol) was reacted with Boc_2O (2.44 g, 11.2 mmol) and $NaHCO_3$ (2.81 g, 33.5 mmol) in ethanol (30 cm^3). The crude product was purified by column chromtography (Hexane:EtOAc 9:1 to 3:1) to give Boc protected amine **152** as a colourless crystalline solid (2.52 g, 97%).

R_f (Hexane:EtOAc, 3:1) = 0.43; **mp** 39-40 °C, (lit.[154] 39-41 °C); **$[\alpha]_D$** = +62.6 (c 1.22, $CHCl_3$), [lit.[147] $[\alpha]_D$ +60.0 (c 0.8, $CHCl_3$)]; **IR** (neat) 3435 (2º amine), 3335 (2º

amine), 1744 (C=O, ester), 1705 (C=O, amide), 1611 (2º amine); **^{1}H NMR** δ (400 MHz, 323 K, $CDCl_3$) 7.30 – 7.12 (5H, m, Ar*H*), 5.00 (1H, br s, N*H*CH), 4.57 (1H, br s, C*H*NH), 3.69 (3H, s, OCH_3), 3.12 (1H, dd, *J* = 13.6, 5.8 Hz, CH_AH$_B$Ar), 3.03 (1H, dd, *J* = 13.6, 6.0 Hz, CH$_A$$H_B$Ar), 1.42 (9H, s, OC(C$H_3$)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$), 172.4 (C=O), 155.1 (C=O), 136.1 (C), 129.3 (2 × CH), 128.5 (2 × CH), 127.0 (CH), 79.8 (C), 54.5 (CH), 52.2 (CH_3), 38.3 (CH_2), 28.3 (CH_3); ***m/z*** (ESI+, MeOH) 302 ($[M+Na]^+$, 100%).

Spectroscopic data is in good agreement with the literature.[147,154]

Methyl (*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-phenylpropanoate (153)



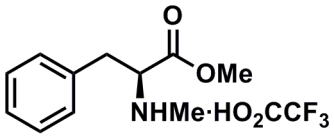
Using **General Procedure O**, Boc protected amine **152** (2.69 g, 9.62 mmol) was reacted with NaH (60% mineral oil dispersion, 0.58 g, 14.4 mmol) and MeI (4.80 cm^3, 76.9 mmol), in dry THF (45 cm^3) and dry DMF (9 cm^3). The crude product was purified by column chromatography (Hexane:EtOAc, 3:1) to give the Boc protected methyl amine **153** as a colourless oil (2.82 g, 95%).

R_f (Hexane:EtOAc, 3:1) = 0.56; **$[\alpha]_D$** = −48.8 (c 1.03, $CHCl_3$), [lit.[155] $[\alpha]_D$ −54 (c 0.04, $CHCl_3$)]; **IR** (neat) 1742 (C=O, ester), 1692 (C=O, amide); **^{1}H NMR** δ (400 MHz, 323 K, $CDCl_3$) 7.30 – 7.19 (5H, m, Ar*H*), 4.93 (0.45H, br s, C*H*NMe), 4.75 (0.55H, br s, C*H*NMe), 3.74 (3H, s, OCH_3), 3.32-3.27 (1H, m, CH_AH$_B$Ar), 3.03 (1H, dd, *J* = 14.3, 10.6 Hz, CH$_A$$H_B$Ar), 2.73 (3H, s, NC$H_3$), 1.37 (9H, s, OC(CH$_3$)$_3$); **^{13}C**

NMR δ (125.8 MHz, 323 K, $CDCl_3$), major rotamer (55%); 171.6 (C=O), 154.9 (C=O), 137.7 (C), 129.0 (2 × CH), 128.5 (2 × CH), 126.6 (CH), 80.2 (C), 61.7 (CH), 52.1 (CH_3), 35.5 (CH_2), 32.6 (CH_3), 28.2 (3 × CH_3), minor rotamer (45%); 171.9 (C=O), 155.8 (C=O), 137.4 (C), 128.9 (2 × CH), 128.4 (2 × CH), 126.5 (CH), 79.9 (C), 59.6 (CH), 52.1 (CH_3), 35.0 (CH_2), 31.9 (CH_3), 28.2 (3 × CH_3); ***m/z*** (ESI+, MeOH) 316 ($[M+Na]^+$, 100%).

Spectroscopic data is in good agreement with the literature.[155]

Methyl (*S*)-2-(methylamino)-3-phenylpropanoate trifluoroacetate (154)

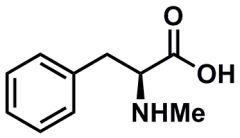


Using **General Procedure D**, methyl amine **153** (2.52 g, 8.59 mmol) was reacted with TFA (6.60 cm^3, 86.2 mmol) in DCM (30 cm^3). The TFA salt **154** was obtained as an off-white solid, which was used without further purification (2.49 g, 89%).

R_f (DCM:MeOH, 99:1) = 0.11; **mp** 73-75 °C, (lit.[156] 75-76 ºC); **$[\alpha]_D$** = +39.2 (c 1.25, $CHCl_3$); **IR** (neat) 1748 (C=O, ester), 1668 (C=O, HO_2CCF_3); **^{1}H NMR** δ (400 MHz, $CDCl_3$) 7.28 – 7.11 (5H, m, Ar*H*), 4.13 (1H, t, *J* = 6.7 Hz, C*H*NHMe), 3.69 (3H, s, OCH_3) 3.29 (1H, dd, *J* = 14.3, 6.2 Hz, C$H_A$$H_B$Ar), 3.22 (1H, dd, *J* = 14.3, 7.3 Hz, C$H_A$$H_B$Ar), 2.72 (3H, s, NC$H_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$), 168.5 (C=O), 162.4 (q, *J* = 35.5 Hz, C=O), 133.9 (C), 129.2 (2 × CH), 129.1 (2 × CH), 128.0 (CH), 116.6 (q, *J* = 292.0 Hz, CF_3), 62.4 (CH), 53.0 (CH_3), 35.9 (CH_2), 32.1 (CH_3); ***m/z*** (ESI+, MeOH) 194 ($[M+H]^+$, 100%), 208 (45).

Melting point data is in good agreement with the literature.[156]

(*S*)-2-(methylamino)-3-phenylpropanoic acid (155)

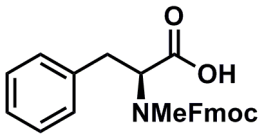


Using **General Procedure E**, the trifluoroacetate salt of methyl amine trifluoroacetate salt **154** (2.14 g, 11.1 mmol) was reacted with NaOH (0.49 g, 12.2 mmol) in methanol (10 cm^3) and water (45 cm^3). The carboxylic acid **155** was obtained as a colourless solid (1.77 g, 89%).

R_f (DCM:MeOH, 99:1) = 0.00; **mp** 255-256 °C (dec.), (lit.[157] 253-254 °C); **$[\alpha]_D$** = +46.7 (c 1.50, aq. NaOH), [lit.[158] $[\alpha]_D$ +47.0 (c 0.5, aq. NaOH)]; **IR** (neat) 3391 (OH); **^{1}H NMR** δ (400 MHz, D_2O, pDH 14) 7.36 – 7.02 (5H, m, Ar*H*), 3.11 (1H, t, *J* = 6.4 Hz, C*H*NHCH$_3$), 2.79 (1H, dd, *J* = 12.1, 5.0 Hz, CH_AH$_B$Ar), 2.75 (1H, dd, *J* = 12.1, 6.0 Hz, CH$_A$$H_B$Ar), 2.12 (3H, s, NHC$H_3$), 1.82 (1H, s, N*H*CH$_3$); **^{13}C NMR** δ (125.8 MHz, D_2O, pD 14), 182.1 (C=O), 163.6 (q, *J* = 35.4, C=O), 138.9 (C), 130.0 (2 × CH), 129.2 (2 × CH), 127.3 (CH), 117.0 (q, *J* = 291.9, CF_3), 67.5 (CH), 39.7 (CH_2), 34.0 (CH_3); ***m/z*** (ESI+, MeOH) 180 ($[M+H]^+$, 42%).

Spectroscopic data is in good agreement with the literature. [157,158]

(*S*)-2-[(9H-fluoren-9-ylmethoxycarbonyl)methylamino]-3-phenylpropanoic acid (147)

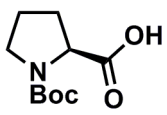


Using **General Procedure G**, methyl amine **155** (2.16 g, 12.0 mmol) was reacted with FmocOSu (4.06 g, 12.0 mmol) and Na_2CO_3 (3.83 g, 36.1 mmol) in DMF (10 cm^3) and MeOH (50 cm^3), which afforded a yellow oil, which was sued as the crude Fmoc protected methyl amine **147** as a yellow oil (4.24 g, 88%).

R_f (DCM:MeOH, 99:1) = 0.04; **^{1}H NMR** δ (400 MHz, $CDCl_3$) 7.79-7.74 (2H, m, Ar*H*), 7.64-7.61 (1H, m, Ar*H*), 7.52-7.48 (2H, m, Ar*H*), 7.44-7.37 (2H, m, Ar*H*), 7.34-7.32 (2H, m, Ar*H*), 7.30-7.26 (2H, m, Ar*H*), 7.24-7.22 (2H, m, Ar*H*), 4.95-4.68 (1H, br, m , C*H*NCH_3), 4.48-4.44 (“H, m, OCH_2CH), 4.32-4.27 (1H, m, OCH_2C*H*), 3.43-3.10 (2H, m, ArCH_2CH), 2.73-2.71 (3H, m, NCH_3); **^{13}C NMR** δ (100.6 MHz, $CDCl_3$), 171.4 (C=O), 156.5 (C=O), 144.0 (2 × C), 141.2 (2 × C), 137.3 (C), 129.8 (2 × CH), 128.8 (2 × CH), 128.7 (2 × CH), 128.6 (2 × CH), 127.9 (CH), 126.9 (2 ×CH), 125.0 (2 ×CH), 67.8 (CH_2), 60.3 (CH), 47.4 (CH), 34.7 (CH_2), 32.7 (CH_3); ***m/z*** (ESI+, MeOH) 424 ($[M+Na]^+$, 100%), 857 (41), 847 (48), 456 (30), 392 (51).

Spectroscopic data is in good agreement with the literature.[159]

(*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carboxylic acid (220)

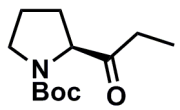


Using **General Procedure B**, L-proline (15.0 g, 130 mmol) was reacted with Boc_2O (28.5 g, 130 mmol) and $NaHCO_3$ (32.9 g, 391 mmol) in ethanol (300 cm^3). The off-white solid was purified by column chromatography (Hexane:EtOAc, 9:1 to 3:2) to give Boc protected amine **220** as a colourless crystalline solid (26.1 g, 93%).

R_f (Hexane:EtOAc, 3:1) = 0.01; **mp** 134-136 °C, (lit.[160] 133 °C); **$[\alpha]_D$** = −111.6 (c 0.95, $CHCl_3$), [lit.[160] $[\alpha]_D$ −80.0 (c 1.38, $CHCl_3$)]; **IR** (neat) 3100 (br, C=O), 1749 (C=O, acid), 1697 (C=O, carbamate); **^{1}H NMR** δ (400 MHz, $CDCl_3$) 11.38 (1H, s, O*H*), 4.39-4.25 (0.43H, m, C*H*NBoc), 4.25-4.13 (0.57H, m, C*H*NBoc), 3.60-3.20 (2H, m, CH_2NBoc), 2.33-1.71 (4H, m, $CH_2CH_2CH_2CH$), 1.42 (3.87H, s, OC(CH_3)$_3$), 1.37 (5.13H, s, OC(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$), major rotamer (57%); 178.4 (C=O), 154.1 (C=O), 80.5 (C), 59.2 (CH), 46.5 (CH_2), 30.9 (CH_2) , 28.5 (3 × CH_3) , 23.8 (CH_2), minor rotamer (43%); 175.8 (C=O), 156.0 (C=O), 81.1 (C), 59.2 (CH), 47.0 (CH_2), 29.1 (CH_2), 28.5 (3 × CH_3), 24.4 (CH_2); ***m/z*** (ESI−, MeOH) 451 ($[2M-2H+Na]^-$, 23%), 214 ($[M-H]^-$, 100).

Spectroscopic data is in good agreement with the commercially available material.[160]

(*S*)-*tert*-Butyl 2-propionylpyrrolidine-1-carboxylate (222)

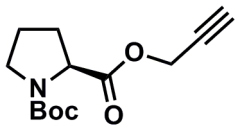


Carboxylic acid **220** (0.40 g, 1.86 mmol) was reacted with CDMT (0.392 g, 2.23 mmol) and NMM (0.613 cm^3, 5.57 mmol) in THF (5 cm^3) at room temperature for 1 h. A colourless precipitate formed and was removed by positive pressure by argon through a sintered adapter. CuI (0.354 g, 1.86 mmol) was added, then the reaction mixture cooled to 0 ºC (ice bath), and EtMgBr (1 M in THF, 1.86 cm^3, 1.86 mmol) was added slowly. The reaction mixture was stirred for a further 3 h at room temperature, before being quenched by NH_4Cl (sat. aq. soln., 10 cm^3). The mixture was extracted with Et_2O (3 × 15 cm^3) and the organic layers washed with Na_2CO_3 (sat. aq. soln., 15 cm^3), followed by HCl (1 M, 15 cm^3), and then brine (15 cm^3). The organic layer was dried ($MgSO_4$), and the solvent removed under reduced pressure. The yellow/brown liquid was purified by column chromatography (Hexane:EtOAc, 9:1), affording ketone **222** as a colourless oil (0.385 g, 91%).

R_f (Hexane:EtOAc, 3:1) = 0.24; **$[\alpha]_D$** = −59.1 (c 1.03, $CHCl_3$), [lit.[115] $[\alpha]_D$ −62.7 (c 1.1, $CHCl_3$)]; **IR** (neat) 1728 (C=O, ketone), 1694 (C=O, carbamate); **^{1}H NMR** δ (400 MHz, $CDCl_3$) 4.30-4.16 (1H, m, C*H*N), 3.48-3.33 (2H, m, CH_2N), 2.49-2.29 (2H, m, C$H_2$$CH_3$), 2.15-2.06 (2H, m, C$H_2$CHN), 1.83-1.73 (2H, m, CH_2C$H_2$$CH_2$), 1.39 (9H, s, OC($CH_3$)), 1.02-0.97 (3H, m, CH_2CH_3); **^{13}C NMR** δ (100.6 MHz, $CDCl_3$), 173.5 (C=O), 154.1 (C=O), 80.0 (C), 61.1 (CH), 59.4 (CH_2), 46.5 (CH_2), 31.1 (CH_2), 28.5 (3 × CH_3), 23.8 (CH_2), 14.5 (CH_3); ***m/z*** (ESI+, MeOH) 477 ($[2M+Na]^+$, 100%), 250 ($[M+Na]^+$, 58).

Spectroscopic data is in good agreement with the commercially available material.[115]

(*S*)-1-*tert*-Butyl 2-prop-2-ynylpyrrolidine-1,2-dicarboxylate (223)

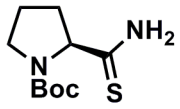


Using **General Procedure C**, carboxylic acid **220** (2.11 g, 9.80 mmol) was reacted with K_2CO_3 (2.71 g, 19.6 mmol) and propargyl bromide (3.93 cm^3, 35.3 mmol) in DMF (60 cm^3). The crude product was purified by column chromatography (Hexane:EtOAc, 90:10) to afford propargyl ester **223** as a colourless solid (2.38 g, 96%).

R_f (Hexane:EtOAc, 3:1) = 0.28; **mp** 53-55 °C, (lit.[161] 52-54 °C); **$[\alpha]_D$** = –64.6 (c 1.32, $CHCl_3$), [lit.[161] $[\alpha]_D$ −68.5 (c 1.0, $CHCl_3$)]; **IR** (neat) 3250 (C≡CH), 2127 (C≡C), 1748 (C=O, ester), 1697 (C=O, carbamate); **^{1}H NMR** δ (400 MHz, $CDCl_3$) 4.72-4.46 (2H, m, HC≡CCH_2), 4.28-4.18 (0.38H, m, C*H*NBoc), 4.18-4.07 (0.62H, m, C*H*NBoc), 3.52-3.16 (2H, m, CH_2NBoc) 2.42 (0.62H, t, *J* = 2.4, CH_2C≡C*H*), 2.40 (0.38H, t, *J* = 2.3, CH_2C≡C*H*), 2.24-1.63 (4H, m, $CH_2CH_2CH_2$Ar), 1.33 (3.42H, s, OC(CH_3)$_3$), 1.29 (5.58H, s, OC(CH_3)$_3$); **^{13}C NMR** δ (125.8 MHz, $CDCl_3$) major rotamer (62%), 172.4 (C=O), 153.7 (C=O), 80.0 (C), 77.4 (C), 75.2 (CH), 58.9 (CH), 52.2 (CH_2), 46.3 (CH_2), 30.8 (CH_2), 28.3 (3 × CH_3), 23.6 (CH_2), minor rotamer (38%), 172.1 (C=O), 154.4 (C=O), 79.9 (C), 77.4 (C), 75.0 (CH), 58.6 (CH), 52.4 (CH_2), 46.5 (CH_2), 29.8 (CH_2), 28.4 (3 × CH_3), 24.3 (CH_2); ***m/z*** (ESI+, MeOH) 276 ($[M+Na]^+$, 100%).

Spectroscopic data is in good agreement with the commercially available material.[161]

(*S*)-*tert*-Butyl-2-carbamothioylpyrrolidine-1-carboxylate (186)

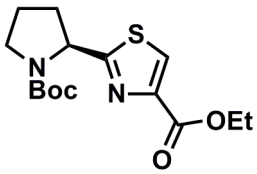


Boc-L-Prolinamide **185** (2.95 g, 14.0 mmol) was dissolved in dry THF (18 cm^3), then Lawesson's reagent (2.87 g, 7.00 mmol) was added portionwise. The reaction mixture was stirred at room temperature under argon for 3.5 h. The solvent was removed under reduced pressure, and column chromatography (DCM:MeOH, 20:1), afforded **186** as an off-white solid.(3.01 g, 95%).

R_f (DCM:MeOH, 20:1) = 0.37; **mp** 190-193 °C, (lit.[130] 192-193 ºC); **$[\alpha]_D$** = -99.4 (c 0.90, $CHCl_3$), [lit.[162] $[\alpha]_D$ −103.4 (c 1.0, $CHCl_3$)]; **IR** (neat) 1672 (C=O, carbamate), 1167 (C=S); **^{1}H NMR** 7.71 (2H, br s, NH_2), 4.66-4.63 (1H, C*H*N), 3.48 (2H, br s, CH_2N), 2.20 (2H, br s,, CH_2CHN), 1.87 (2H, m, CH_2CH_2N), 1.45 (9H, s, OC(CH_3)$_3$); **^{13}C NMR** δ (100.6 MHz, $CDCl_3$), 194.3 (C=S), 155.7 (C=O), 80.7 (C), 62.0 (CH), 47.2 (CH_2), 33.4 (CH_2), 28.4 (3 × CH_3), 24.5 (CH_2); ***m/z*** (ESI+, MeOH) 253 ($[M+Na]^+$, 100%), 667 (37), 639 (42), 413 (28).

Spectroscopic data is in good agreement with the commercially available material.[130,162]

Ethyl (*S*)-2-(1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl)thiazole-4-carboxylate (187)



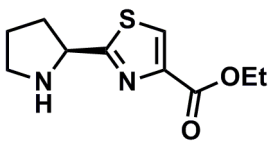
Thioamide **186** (1.79 g, 7.78 mmol) and $NaHCO_3$ (5.23 g, 62.2 mmol) were suspended in DME (40 cm^3) and stirred at room temperature for 10 minutes. Ethyl

bromopyruvate (3.25 cm^3, 23.3 mmol) was added dropwise, and the reaction mixture stirred at room temperature for 30 minutes before cooling to 0 ºC (ice bath). A solution of trifluoroacetic anhydride (4.33 cm^3, 31.1 mmol) and 2,6-lutidine (7.70 cm^3, 66.1 mmol) was added dropwise over 10 minutes. The reaction was stirred at 0 ºC for 30 minutes, before the solvent was removed under reduced pressure. The reaction mixture was partitioned between chloroform (40 cm^3) and HCl (1 N, 40 cm^3). The organic layer was dried ($MgSO_4$), and the solvent removed under reduced pressure. The resultant oil was purified by column chromatography (Hexane:EtOAc 10:1 to 2:1), affording **187** as a yellow solid (1.82 g, 72%).

R_f (DCM:MeOH, 10:1) = 0.70; **mp** 110-111 °C (lit.[163] 102-104 ºC); **$[\alpha]_D$** = -78.8 (c 1.32, DMF), [lit.[163] $[\alpha]_D$ −96.7 (c 0.85, DMF)]; **IR** (neat) 1734 (C=O, ester), 1699 (C=O, carbamate); **^{1}H NMR** δ (400 MHz, $CDCl_3$) 8.06 (1H, s, C=C*H*S), 5.20 (1H, br s, C*H*N), 4.42 (2H, dd, *J* = 13.8, 6.7, CH_3CH_2), 3.66-3.44 (2H, m, CH_2N), 2.40-2.20 (2H, m, CH_2CH_2N), 1.96-1.85 (2H, m, CH_2CHN), 1.40 (3H, t, *J* = 7.0, CH_2CH_3), 1.31 (9H, s, $OC(CH_3)3$); **^{13}C NMR** δ (100.6 MHz, $CDCl_3$), 176.4 (C=N), 160.7 (C=O), 153.6 (C=O), 146.5 (C), 126.3 (CH), 79.8 (CH), 60.7 (CH), 59.0 (CH_2), 46.2 (CH_2), 33.7 (CH_2), 27.7 (3 × CH_3), 22.6 (CH_2), 13.8 (CH_3); ***m/z*** (ESI+, MeOH) 349 ($[M+Na]^+$, 100%), 675 (23).

Spectroscopic data is in good agreement with the commercially available material.[163]

Ethyl (*S*)-2-(pyrrolidin-2-yl)thiazole-4-carboxylate (188)

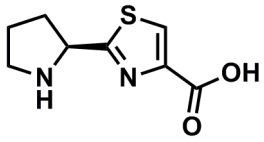


Using **General Procedure D**, Boc protected amine **187** (2.41 g, 7.38 mmol) was reacted with TFA (5.65 cm^3, 73.8 mmol) in DCM (10 cm^3). The resultant oil was purified by column chromatography methyl amine **188** as a yellow oil (1.25 g, 98%).

R_f (DCM:MeOH, 10:1) = 0.00; **mp** 167-169 ºC, (lit[163] 169-171 ºC); **$[\alpha]_D$** = -15.0 (c 1.61, DMF), [lit.[163] $[\alpha]_D$ –21.2 (c 1.02, DMF)]; **IR** (neat) 3523 (NH), 1729 (C=O, ester); **^{1}H NMR** δ (400 MHz, $CDCl_3$) 8.08 (1H, s, C=C*H*S), 5.20 (1H, br s, C*H*N), 4.42 (2H, dd, *J* = 13.8, 6.7, CH_3CH_2), 3.63-3.44 (2H, m, CH_2N), 2.40-2.22 (2H, m, CH_2CH_2N), 1.96-1.85 (2H, m, CH_2CHN), 1.40 (3H, t, *J* = 7.0, CH_2CH_3); **^{13}C NMR** δ (100.6 MHz, $CDCl_3$), 176.2 (C=N), 160.7 (C=O), 146.5 (C), 126.3 (CH), 79.8 (CH), 60.7 (CH), 59.0 (CH_2), 46.6 (CH_2), 33.7 (CH_2), 22.4 (CH_2), 13.8 (CH_3); ***m/z*** (ESI+, MeOH) 227 ($[M+H]^+$, 100%), 908 (13), 293 (23), 249 (22), 239 (23).

Spectroscopic data is in good agreement with the commercially available material.[163]

(S)-2-(Pyrrolidin-2-yl)thiazole-4-carboxylic acid (189)

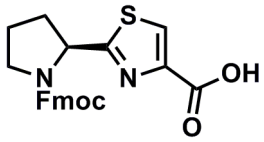


Using **General Procedure F**, ethyl ester **188** (1.71 g, 7.56 mmol) was reacted with NaOH (0.363 g, 9.07 mmol) in H_2O (3 cm^3) and MeOH (3 cm^3). The resultant solid was carboxylic acid **189,** a colourless solid (1.34 g, 89%).

R_f (DCM:MeOH, 10:1) = 0.00; **mp** 250-253 °C, (lit.[163] 255-256 ºC); **$[\alpha]_D$** = -18.8 (c 1.03, MeOH), [lit.[163] $[\alpha]_D$ -19.3 (c1.08, MeOH)]; **IR** (neat); 1681 (C=O, acid) **^{1}H NMR** δ (400 MHz, D_2O, pD 14) 8.08 (1H, s, C=C*H*S), 5.18 (1H, br s, C*H*N), 3.66-3.47 (2H, m, CH_2N), 2.40-2.22 (2H, m, CH_2CH_2N), 1.96-1.85 (2H, m, CH_2CHN); **^{13}C NMR** δ (100.6 MHz, D_2O, pD 14) 176.2 (C=N), 167.7 (C=O), 146.5 (C), 126.3 (CH), 79.8 (CH), 60.7 (CH), 46.6 (CH_2), 33.7 (CH_2), 22.4 (CH_2); ***m/z*** (ESI+, MeOH) 221 ([M+Na]$^+$, 41%), 1482 (100), 649 (32), 639 (28), 451 (64), 301 (34), 243 (95).

Spectroscopic data is in good agreement with the commercially available material.[163]

(*S*)-2-(1-[(9H-fluoren-9-ylmethoxycarbonyl)pyrrolidin-2-yl]thiazole-4-carboxylic acid (150)



Using **General Procedure G**, secondary amine **189** (2.16 g, 12.0 mmol) was reacted with FmocOSu (4.06 g, 12.0 mmol) and Na_2CO_3 (3.83 g, 36.1 mmol) in DMF (10 cm^3) and MeOH (50 cm^3). The resultant yellow gum, crude Fmoc protected methyl amine **150,** was purified by column chromatography to obtain a colourless solid (1.25 g, 98%).

R_f (DCM:MeOH, 10:1) = 0.00; **$[\alpha]_D$** = -12.7 (c 1.13, MeOH); **IR** (neat); 1661 (C=O, acid) **^{1}H NMR** δ (400 MHz, $CDCl_3$) 12.9 (1H, br s, COO*H*), 8.08 (1H, s, C=C*H*S),

5.18 (1H, br s, C*H*N), 3.66-3.47 (2H, m, CH_2N), 2.40-2.22 (2H, m, CH_2CH$_2$N), 1.96-1.85 (2H, m, CH_2CHN); **^{13}C NMR** δ (100.6 MHz, $CDCl_3$) 176.8 (C=N), 161.2 (C=O), 157.4 (C=O), 149.0 (C), 144.8 (2 × C), 139.4 (2 × C), 132.3 (C), 128.9 (2 × CH), 127.5 (2 × CH), 124.0 (2 × CH), 122.9 (2 × CH), 67.4 (CH_2), 52.5 (CH), 48.1 (CH), 47.1 (CH_2), 33.7 (CH_2), 25.2 (CH_2); ***m/z*** (ESI+, MeOH) 349 ($[M+Na]^+$, 100%), 675 (23).

Biological Methods

Solution Preparation

PBS (***Phosphate buffered saline***) solution was made up with 100 PBS tablets in distilled H_2O (1 L). The PBS solution was stored at room temperature.

PBS-T solution was made up of PBS solution with 0.1% tween-20. PBS-T was stored at room temperature.

3% BSA (bovine serum albumin) was made up of BSA (3 g), TBS-tween (99 cm^3), β-glycerophosphate (1 M, 1 cm^3). It could be stored at 4°C for 3 weeks.

ECL solution 1 was made up of luminol (250 mM, 1 cm^3) with p-coumaric acid (90 μM, 440 cm^3, Tris pH 8.5 (1 M, 10 cm^3), and made up to 100 cm^3 with distilled H_2O. The solution was stored at 4°C,wrapped in foil.

ECL solution 2 30% H_2O_2 (64 μL), Tris pH 8.5 (1 M, 10 cm^3), was made up to 100 cm^3 with distilled H_2O. The solution was stored at 4 ºC,wrapped in foil.

ELISA Methodology

ELISA with purified AG-2.

Each well on a multi-well plate was coated with streptavidin (20 μg mL^{-1}) by incubation at 37ºC overnight. Wells were then each washed with PBS-T (4 x 200 μL). Two columns of wells were then incubated with each of the four different biotinylated peptides, negative control, positive control BiotinSGSGPTTIYY (**30**), BiotinSGSGPTTIYX (**31**) and BiotinSGSGPTTIXY (**32**) (5 μg in 50 μL DMSO) for 1 h at room temperature, to create duplicate results for each column. Wells were then each washed with PBS-T (6 x 200 μL), before blocking by 1 h incubation with 3% BSA in PBS-T (200 μL per well). Excess BSA was removed, then wells were incubated for a further 1 h at room temperature with purified AG-2 protein at a range of concentrations in 3% BSA in PBS-T (50 μL). The relative concentrations used were 1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 0, where 1 = 2.5 mg AG-2 in 1 mL 3% BSA in PBS-T, and 0 was 3% BSA in PBS-T.

After washing all wells with PBS-T (6 x 200 μL), they were incubated for 1 h at room temperature with primary antibody α-AG-2 K47 rabbit poly (50 μL, 1:1000 dilution in 3% BSA in PBS-T). The wells were again washed with PBS-T (6 x 200 μL) before 1 h incubation at room temperature with the secondary antibody swine anti-rabbit (50 μL, 1:1000 dilution in 3% BSA in PBS-T). The wells were washed a final time with PBS-T (6 x 200 μL), ECL (50 μL) was added, and the relative luminescence measured by a Finstruments Micro-plate reader using Fluoroscan Ascent software, giving a relative luminescence value for each well.

ELISA with cell lysate.

Each well on a multi-well plate was coated with streptavidin (20 μg mL^{-1}) by incubation at 37ºC overnight. Wells were then each washed with PBS-T (4 x 200 μL). Two columns of wells were then incubated with each of the four different biotinylated peptides, negative control, positive control BiotinSGSGPTTIYY (**30**), BiotinSGSGPTTIYX (**31**) and BiotinSGSGPTTIXY (**32**) (5 μg in 50 μL DMSO) for 1 h at room temperature, to create duplicate results for each column. Wells were

then each washed with PBS-T (6 x 200 μL), before blocking by 1 h incubation with 3% BSA in PBS-T (200 μL per well). Excess BSA was removed, then wells were incubated for a further hour at room temperature with MCF-7 cell lysate at a range of concentrations in 3% BSA in PBS-T (50 μL). The relative concentrations used were 1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 0, where 1 = 2.5 mg AG-2 in 1 mL 3% BSA in PBS-T, and 0 was 3% BSA in PBS-T.

After washing all wells with PBS-T (6 x 200 μL), they were incubated for 1 h at room temperature with primary antibody α-AG-2 K47 rabbit poly (50 μL, 1:1000 dilution in 3% BSA in PBS-T). The wells were again washed with PBS-T (6 x 200 μL) before 1 h incubation at room temperature with the secondary antibody swine anti-rabbit (50 μL, 1:1000 dilution in 3% BSA in PBS-T). The wells were washed a final time with PBS-T (6 x 200 μL), ECL (50 μL) was added, and the relative luminescence measured by a Finstruments Micro-plate reader using Fluoroscan Ascent software, giving a relative luminescence value for each well.

References

(1) Khersonsky, S. M.; Chang, Y. T. *ChemBioChem* **2004**, 5, 903.

(2) Spring, D. R. *Chemical Society Reviews* **2005**, 34, 472.

(3) O'Connor, C. J.; Laraia, L.; Spring, D. R. *Chemical Society reviews* **2011**, 40, 4332.

(4) Ahn, Y. H.; Chang, Y. T. *Accounts of Chemical Research* **2007**, 40, 1025.

(5) Stockwell, B. R. *Nature Reviews Genetics* **2000**, 1, 116.

(6) Huang, S.-M. A.; Cheung, A. K.; Cong, F. *Annual Review of Pharmacology and Toxicology* **2012**, 52, 57.

(7) Prescher, J. A.; Bertozzi, C. R. *Nature Chemical Biology* **2005**, 1, 13.

(8) Chang, Y. T.; Leong, C.; Su, D.; Xu, W.; Zhai, D.; Ghosh, K.; Samanta, A.; Das, R. K. *Interdisciplinary Bio Central* **2011**, 3, 1.

(9) Inverarity, I. A.; Hulme, A. N. *Organic & Biomolecular Chemistry* **2007**, 5, 636.

(10) Austin, C. P.; Brady, L. S.; Insel, T. R.; Collins, F. S. *Science* **2004**, 306, 1138.

(11) Agard, N. J.; Baskin, J. M.; Prescher, J. A.; Lo, A.; Bertozzi, C. R. *ACS Chemical Biology* **2006**, 1, 644.

(12) Boettcher, T.; Pitscheider, M.; Sieber, S. A. *Angewandte Chemie-International Edition* **2010**, 49, 2680.

(13) Eirich, J.; Orth, R.; Sieber, S. A. *Journal of the American Chemical Society* **2011**, 133, 12144.

(14) Slack, J. L.; Causey, C. P.; Luo, Y.; Thompson, P. R. *ACS Chemical Biology* **2011**, 6, 466.

(15) Sobin, B. A.; Tanner, F. W. *Journal of the American Chemical Society* **1954**, 76, 4053.

(16) Hall, S. S.; Loebenberg, D.; Schumacher, D. P. *Journal of Medicinal Chemistry* **1983**, 26, 469.

(17) Iino, Y.; Ishii, M.; Ohsumi, K.; Tsuji, T.; Ajinomoto Co., Inc.

(18) Hosoya, Y.; Kameyama, T.; Naganawa, H.; Okami, Y.; Takeuchi, T. *Journal of Antibiotics* **1993**, 46, 1300.

(19) Inverarity, I. A.; Viguier, R. F. H.; Cohen, P.; Hulme, A. N. *Bioconjugate Chemistry* **2007**, 18, 1593.

(20) Rosser, E. M.; Morton, S.; Ashton, K. S.; Cohen, P.; Hulme, A. N. *Organic & Biomolecular Chemistry* **2004**, 2, 142.

(21) Chin, J. W.; Cropp, T. A.; Anderson, J. C.; Mukherji, M.; Zhang, Z. W.; Schultz, P. G. *Science* **2003**, 301, 964.

(22) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *Journal of the American Chemical Society* **2004**, 126, 15366.

(23) Link, A. J.; Tirrell, D. A. *Journal of the American Chemical Society* **2003**, 125, 11164.

(24) Nonaka, H.; Tsukiji, S.; Ojida, A.; Hamachi, I. *Journal of the American Chemical Society* **2007**, 129, 15777.

(25) Rabuka, D.; Hubbard, S. C.; Laughlin, S. T.; Argade, S. P.; Bertozzi, C. R. *Journal of the American Chemical Society* **2006**, 128, 12078.

(26) Sadamoto, R.; Niikura, K.; Ueda, T.; Monde, K.; Fukuhara, N.; Nishimura, S. I. *Journal of the American Chemical Society* **2004**, 126, 3755.

(27) Broder, S. *Antiviral Research* **2010**, 85, 1.

(28) Griffin, R. J. *Progress in Medicinal Chemistry* **1994**, 31, 121.

(29) Saxon, E.; Bertozzi, C. R. *Science* **2000**, 287, 2007.

(30) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angewandte Chemie-International Edition* **2002**, 41, 2596.

(31) Zhang, L.; Chen, X. G.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. C. *Journal of the American Chemical Society* **2005**, 127, 15998.

(32) Meza-Avina, M. E.; Patel, M. K.; Lee, C. B.; Dietz, T. J.; Croatt, M. P. *Organic Letters* **2011**, 13, 2984.

(33) Krasinski, A.; Fokin, V. V.; Sharpless, K. B. *Organic Letters* **2004**, 6, 1237.

(34) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *European Journal of Organic Chemistry* **2006**, 51.

(35) Holub, J. M.; Kirshenbaum, K. *Chemical Society Reviews* **2010**, 39, 1325.

(36) Mamidyala, S. K.; Finn, M. G. *Chemical Society Reviews* **2010**, 39, 1252.

(37) Appendino, G.; Bacchiega, S.; Minassi, A.; Cascio, M. G.; De Petrocellis, L.; Di Marzo, V. *Angewandte Chemie-International Edition* **2007**, 46, 9312.

(38) van Maarseveen, J. H.; Horne, W. S.; Ghadiri, M. R. *Organic Letters* **2005**, 7, 4503.

(39) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *Journal of the American Chemical Society* **2003**, 125, 4686.

(40) Speers, A. E.; Cravatt, B. F. *ChemBioChem* **2004**, 5, 41.

(41) Speers, A. E.; Cravatt, B. F. *Chemistry & Biology* **2004**, 11, 535.

(42) Bargonetti, J.; Manfredi, J. J. *Current Opinion in Oncology* **2002**, 14, 86.

(43) Levine, A. J.; Oren, M. *Nature Reviews Cancer* **2009**, 9, 749.

(44) Patrick, G. L. *An Introduction to Medicinal Chemistry*, Oxford University Press, **2005**.

(45) Baker, S. J.; Fearon, E. R.; Nigro, J. M.; Hamilton, S. R.; Preisinger, A. C.; Jessup, J. M.; Vantuinen, P.; Ledbetter, D. H.; Barker, D. F.; Nakamura, Y.; White, R.; Vogelstein, B. *Science* **1989**, 244, 217.

(46) Fourtouna, A.; Murray, E.; Nicholson, J.; Maslon, M. M.; Pang, L. Y.; Dryden, D. T. F.; Hupp, T. R. *Current Chemical Biology* **2009**, 3, 124.

(47) Barraclough, R.; Barraclough, D. L.; Rudland, P. S. 2011; Vol. US2001/0052586 A1.

(48) Zhang, Y. X.; Ali, T. Z.; Zhou, H.; D'Souza, D. R.; Lu, Y.; Jaffe, J.; Liu, Z. Q.; Passaniti, A.; Hamburger, A. W. *Cancer Research* **2010**, 70, 240.

(49) Murray, E.; McKenna, E. O.; Burch, L. R.; Dillon, J.; Langridge-Smith, P.; Kolch, W.; Pitt, A.; Hupp, T. R. *Biochemistry* **2007**, 46, 13742.

(50) Zhang, J. S.; Gong, A.; Cheville, J. C.; Smith, D. I.; Young, C. Y. F. *Genes Chromosomes & Cancer* **2005**, 43, 249.

(51) In Based on data prepared by Cancer Research UK, Original Data Sources: 1 Office for National Statistics. Cancer Statistics: Registrations Series MB1. http://www.statistics.gov.uk/statbase/Product.asp?vlnk=8843. 2. Welsh Cancer Intelligence and Surveillance Unit. http://www.wcisu.wales.nhs.uk. 3. Information Services Division Scotland. Cancer Information Programme. www.isdscotland.org/cancer. 4. N. Ireland Cancer Registry. www.qub.ac.uk/nicr. Page last updated:20/04/2011, page accessed: 23/04/2011.; Available from: http://info.cancerresearchuk.org/cancerstats/incidence/commoncancers/.

(52) Shurtleff, D.; Thomas, J. R.; Schrot, J.; Kowalski, K.; Harford, R. *Pharmacology Biochemistry and Behavior* **1994**, 47, 935.

(53) Kim, J. H.; Curtis-Long, M. J.; Seo, W. D.; Ryu, Y. B.; Yang, M. S.; Park, K. H. *Journal of Organic Chemistry* **2005**, 70, 4082.

(54) Fields, G. B.; Noble, R. L. *International Journal of Peptide and Protein Research* **1990**, 35, 161.

(55) Inverarity, I. A. Ph.D. Thesis, University of Edinburgh, 2007.

(56) Zajdel, P.; Subra, G.; Bojarski, A. J.; Duszynska, B.; Tatarczynska, E.; Nikiforuk, A.; Chojnacka-Wojcik, E.; Pawlowski, M.; Martinez, J. *Bioorganic & Medicinal Chemistry* **2007**, 15, 2907.

(57) Ben-Efraim, D. A. *Tetrahedron* **1973**, 29, 4111.

(58) Xiong, Y. F.; Xia, H. J.; Moore, H. W. *Journal of Organic Chemistry* **1995**, 60, 6460.

(59) Maryanoff, B. E.; Greco, M. N.; Zhang, H. C.; Andradegordon, P.; Kauffman, J. A.; Nicolaou, K. C.; Liu, A. J.; Brungs, P. H. *Journal of the American Chemical Society* **1995**, 117, 1225.

(60) Mitsunobu, O.; Yamada, M. *Bulletin of the Chemical Society of Japan* **1967**, 40, 2380.

(61) dos Anjos, J. V.; Sinou, D.; de Melo, S. J.; Srivastava, R. M. *Synthesis-Stuttgart* **2007**, 2647.

(62) Deiters, A.; Cropp, T. A.; Mukherji, M.; Chin, J. W.; Anderson, J. C.; Schultz, P. G. *Journal of the American Chemical Society* **2003**, 125, 11782.

(63) Premdjee, B.; Adams, A. L.; Macmillan, D. *Bioorganic & Medicinal Chemistry Letters* **2011**, 21, 4973.

(64) Jass, P. A.; Rosso, V. W.; Racha, S.; Soundararajan, N.; Venit, J. J.; Rusowicz, A.; Swaminathan, S.; Livshitz, J.; Delaney, E. J. *Tetrahedron* **2003**, 59, 9019.

(65) Schaffert, D.; Badgujar, N.; Wagner, E. *Organic Letters* **2011**, 13, 1586.

(66) Kocienski, P. J., *Protecting Groups*, Georg Thieme Verlag, **2003**.

(67) Katritzky, A. R.; Yang, B. Z.; Semenzin, D. *Journal of Organic Chemistry* **1997**, 62, 726.

(68) Baker, R.; Castro, J. L. *Journal of the Chemical Society-Perkin Transactions 1* **1990**, 47.

(69) Amblard, M.; Fehrentz, J. A.; Martinez, J.; Subra, G. *Molecular Biotechnology* **2006**, 33, 239.

(70) Weber, P. C.; Ohlendorf, D. H.; Wendoloski, J. J.; Salemme, F. R. *Science* **1989**, 243, 85.

(71) In http://www.altabioscience.bham.ac.uk/services/peptide/purity.shtml Page accessed: 24/09/2011.

http://www.genscript.com/recommended_peptide_purity.html Page accessed: 24/09/2011. http://www.sigmaaldrich.com/life-science/cell-biology/peptides-and-proteins/peptides-proteins/technical-resource/recommended-peptide.html Page accessed: 24/09/2011.

(72) Houghten, R. A. *Proceedings of the National Academy of Sciences of the United States of America* **1985**, 82, 5131.

(73) Murray, E., Edinburgh Cancer Research Centre.

(74) Innes, H. E.; Liu, D.; Barraclough, R.; Davies, M. P. A.; O'Neill, P. A.; Platt-Higgins, A.; Rudland, S. D.; Sibson, D. R.; Rudland, P. S. *British Journal of Cancer* **2006**, 94, 1057.

(75) Landi, F.; Johansson, C. M.; Campopiano, D. J.; Hulme, A. N. *Organic & Biomolecular Chemistry* **2010**, 8, 56.

(76) Zamolo, L.; Salvalaglio, M.; Cavallotti, C.; Galarza, B.; Sadler, C.; Williams, S.; Hofer, S.; Horak, J.; Lindner, W. *Journal of Physical Chemistry* **2010**, 114, 9367.

(77) Sigmund, H.; Pfleiderer, W. *Helvetica Chimica Acta* **2003**, 86, 2299.

(78) Charron, G.; Tsou, L. K.; Maguire, W.; Yount, J. S.; Hang, H. C. *Molecular Biosystems* **2011**, 7, 67.

(79) Kulkarni, S. S.; Hu, X. D.; Doi, K.; Wang, H. G.; Manetsch, R. *ACS Chemical Biology* **2011**, 6, 724.

(80) Fuerst, P.; Stehle, P. *Journal of Nutrition* **2004**, 134, 1558S.

(81) Foster, G. A.; Schultzberg, M.; Goldstein, M.; Hokfelt, T. *Journal of Comparative Neurology* **1985**, 236, 348.

(82) Gauchy, C.; Agid, Y.; Glowinsk.J; Cheramy, A. *European Journal of Pharmacology* **1973**, 22, 311.

(83) Buczek, O.; Yoshikami, D.; Bulaj, G.; Jimenez, E. C.; Olivera, B. M. *Journal of Biological Chemistry* **2005**, 280, 4247.

(84) Brady, J. D.; Sadler, I. H.; Fry, S. C. *Phytochemistry* **1998**, 47, 349.

(85) Hutton, C. A.; Skaff, O. *Tetrahedron Letters* **2003**, 44, 4895.

(86) Skaff, O.; Jolliffe, K. A.; Hutton, C. A. *Journal of Organic Chemistry* **2005**, 70, 7353.

(87) Helynck, G.; Dubertret, C.; Frechet, D.; Leboul, J. *Journal of Antibiotics* **1998**, 51, 512.

(88) Garfunkle, J.; Kimball, F. S.; Trzupek, J. D.; Takizawa, S.; Shimamura, H.; Tomishima, M.; Boger, D. L. *Journal of the American Chemical Society* **2009**, 131, 16036.

(89) Boger, D. L.; Zhou, J. C. *Journal of Organic Chemistry* **1996**, 61, 3938.

(90) Tinto, W. F.; Lough, A. J.; McLean, S.; Reynolds, W. F.; Yu, M.; Chan, W. R. *Tetrahedron* **1998**, 54, 4451.

(91) Teruya, T.; Sasaki, H.; Fukazawa, H.; Suenaga, K. *Organic Letters* **2009**, 11, 5062.

(92) Boger, D. L.; Yohannes, D. *Journal of Organic Chemistry* **1988**, 53, 487.

(93) De Silva, E. D.; Andersen, R. J.; Allen, T. M. *Tetrahedron Letters* **1990**, 31, 489.

(94) Grieco, P. A.; Perezmedrano, A. *Tetrahedron Letters* **1988**, 29, 4225.

(95) Rao, A. V. R.; Gurjar, M. K.; Nallaganchu, B. R.; Bhandari, A. *Tetrahedron Letters* **1993**, 34, 7085.

(96) Imaeda, T.; Hamada, Y.; Shioiri, T. *Tetrahedron Letters* **1994**, 35, 591.

(97) Cole, J. R.; University Patents Inc.: Patent No. US 4226856A.

(98) Inaba, T.; Umezawa, I.; Yuasa, M.; Inoue, T.; Mihashi, S.; Itokawa, H.; Ogura, K. *Journal of Organic Chemistry* **1987**, 52, 2957.

(99) Boger, D. L.; Yohannes, D. *Journal of the American Chemical Society* **1991**, 113, 1427.

(100) Bigot, A.; Beugelmans, R.; Zhu, J. P. *Tetrahedron* **1997**, 53, 10753.

(101) Ghosh, S.; Kumar, A. S.; Mehta, G. N.; Soundararajan, R. *Synthetic Communications* **2010**, 40, 2389.

(102) Boger, D. L.; Yohannes, D. *Journal of Organic Chemistry* **1987**, 52, 5283.

(103) Bois-Choussy, M.; Cristau, P.; Zhu, J. P. *Angewandte Chemie-International Edition* **2003**, 42, 4238.

(104) Shimamura, H.; Breazzano, S. P.; Garfunkle, J.; Kimball, F. S.; Trzupek, J. D.; Boger, D. L. *Journal of the American Chemical Society* **2010**, 132, 7776.

(105) Deng, H. B.; Jung, J. K.; Liu, T.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *Journal of the American Chemical Society* **2003**, 125, 9032.

(106) Wang, Z.; Bois-Choussy, M.; Jia, Y.; Zhu, J. *Angewandte Chemie-International Edition* **2010**, 49, 2018.

(107) Boger, D. L.; Kim, S. H.; Mori, Y.; Weng, J. H.; Rogel, O.; Castle, S. L.; McAtee, J. J. *Journal of the American Chemical Society* **2001**, 123, 1862.

(108) Jayasuriya, H.; Salituro, G. M.; Smith, S. K.; Heck, J. V.; Gould, S. J.; Singh, S. B.; Homnick, C. F.; Holloway, M. K.; Pitzenberger, S. M.; Patane, M. A. *Tetrahedron Letters* **1998**, 39, 2247.

(109) Achab, S.; Velay, L. *Tetrahedron Letters* **2005**, 46, 2627.

(110) Gao, X.; Liu, Y.; Kwong, S.; Xu, Z.; Ye, T. *Organic Letters* **2010**, 12, 3018.

(111) De Luca, L.; Giacomelli, G.; Taddei, M. *Journal of Organic Chemistry* **2001**, 66, 2534.

(112) Gardelli, C.; Nizi, E.; Muraglia, E.; Crescenzi, B.; Ferrara, M.; Orvieto, F.; Pace, P.; Pescatore, G.; Poma, M.; Ferreira, M. d. R. R.; Scarpelli, R.; Homnick, C. F.; Ikemoto, N.; Alfieri, A.; Verdirame, M.; Bonelli, F.; Paz, O. G.; Taliani, M.; Monteagudo, E.; Pesci, S.; Laufer, R.; Felock, P.; Stillmock, K. A.; Hazuda, D.; Rowley, M.; Summa, V. *Journal of Medicinal Chemistry* **2007**, 50, 4953.

(113) Li, W.; Yu, S.; Jin, M.; Xia, H.; Ma, D. *Tetrahedron Letters* **2011**, 52, 2124.

(114) Deguest, G.; Bischoff, L.; Fruit, C.; Marsais, F. *Tetrahedron-Asymmetry* **2006**, 17, 2120.

(115) Conrow, R.; Portoghese, P. S. *Journal of Organic Chemistry* **1986**, 51, 938.

(116) Sasaki, H.; Teruya, T.; Fukazawa, H.; Suenaga, K. *Tetrahedron* **2011**, 67, 990.

(117) Sumiya, E.; Shimogawa, H.; Sasaki, H.; Tsutsumi, M.; Yoshita, K. i.; Ojika, M.; Suenaga, K.; Uesugi, M. *ACS Chemical Biology* **2011**, 6, 425.

(118) Lee, J. H.; Griffin, J. H.; Nicas, T. I. *Journal of Organic Chemistry* **1996**, 61, 3983.

(119) Harding, J. R.; Jones, J. R.; Lu, S. Y.; Wood, R. *Tetrahedron Letters* **2002**, 43, 9487.

(120) Zhou, C.-Y.; Li, J.; Peddibhotla, S.; Romo, D. *Organic Letters* **2010**, 12, 2104.

(121) Bovonsombat, P.; Khanthapura, P.; Krause, M. M.; Leykajarakul, J. *Tetrahedron Letters* **2008**, 49, 7008.

(122) Prieto, M.; Mayor, S.; Rodriguez, K.; Lloyd-Williams, P.; Giralt, E. *Journal of Organic Chemistry* **2007**, 72, 1047.

(123) Bovonsombat, P.; Ali, R.; Khan, C.; Leykajarakul, J.; Pla-on, K.; Aphimanchindakul, S.; Pungcharoenpong, N.; Timsuea, N.; Arunrat, A.; Punpongjareorn, N. *Tetrahedron* **2010**, 66, 6928.

(124) Chhattise, P. K.; Ramaswarny, A. V.; Waghmode, S. B. *Tetrahedron Letters* **2008**, 49, 189.

(125) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; 2nd Edition ed.; John Wiley & Sons, Inc.: United States, **1991**.

(126) Bartoli, G.; Bosco, M.; Carlone, A.; Dalpozzo, R.; Locatelli, M.; Melchiorre, P.; Sambri, L. *Journal of Organic Chemistry* **2006**, 71, 9580.

(127) Bastiaans, H. M. M.; vanderBaan, J. L.; Ottenheijm, H. C. J. Journal of *Organic Chemistry* **1997**, 62, 3880.

(128) Deng, S. J.; Taunton, J. *Journal of the American Chemical Society* **2002**, 124, 916.

(129) Bruno, P.; Pena, S.; Just-Baringo, X.; Albericio, F.; Alvarez, M. *Organic Letters* **2011**, 13, 4648.

(130) Brain, C. T.; Hallett, A.; Ko, S. Y. *Journal of Organic Chemistry* **1997**, 62, 3808.

(131) Singh, S.; Rao, S. J.; Pennington, M. W. *Journal of Organic Chemistry* **2004**, 69, 4551.

(132) Lee, J.; Lee, Y. I.; Kang, M. J.; Lee, Y. J.; Jeong, B. S.; Lee, J. H.; Kim, M. J.; Choi, J. Y.; Ku, J. M.; Park, H. G.; Jew, S. S. *Journal of Organic Chemistry* **2005**, 70, 4158.

(133) Zeecheng, K. Y.; Cheng, C. C. *Journal of Medicinal Chemistry* **1970**, 13, 414.

(134) Murphy, A. C.; Mitova, M. I.; Blunt, J. W.; Munro, M. H. G. *Journal of Natural Products* **2008**, 71, 806.

(135) Hanessian, S.; Sahoo, S. P. *Tetrahedron Letters* **1984**, 25, 1425.

(136) Bartley, D. M.; Coward, J. K. *Journal of Organic Chemistry* **2005**, 70, 6757.

(137) Marchand, D.; Martinez, J.; Cavelier, F. *European Journal of Organic Chemistry* **2008**, 3107.

(138) Hara, S.; Makino, K.; Hamada, Y. *Tetrahedron Letters* **2006**, 47, 1081.

(139) De Luca, L.; Giacomelli, G.; Porcheddu, A. *Organic Letters* **2001**, 3, 1519.

(140) Sieber, P. *Tetrahedron Letters* **1987**, 28, 6147.

(141) Watanabe, K.; Yamada, Y.; Goto, K. *Bulletin of the Chemical Society of Japan* **1985**, 58, 1401.

(142) Boyle, T. P.; Bremner, J. B.; Coates, J.; Deadman, J.; Keller, P. A.; Pyne, S. G.; Rhodes, D. I. *Tetrahedron* **2008**, 64, 11270.

(143) Gerstenberger, B. S.; Konopelski, J. P. *Journal of Organic Chemistry* **2005**, 70, 1467.

(144) Jackson, R. F. W.; Rilatt, I.; Murray, P. J. *Organic & Biomolecular Chemistry* **2004**, 2, 110.

(145) Sarkar, A.; Roy, S. R.; Parikh, N.; Chakraborti, A. K. *Journal of Organic Chemistry* **2011**, 76, 7132.

(146) Kolodziejczyk, A. M.; Manning, M. *Journal of Organic Chemistry* **1981**, 46, 1944.

(147) Zheng, J.; Yin, B.; Huang, W.; Li, X.; Yao, H.; Liu, Z.; Zhang, J.; Jiang, S. *Tetrahedron Letters* **2009**, 50, 5094.

(148) dos Anjos, J. V.; Sinou, D.; de Melo, S. J.; Srivastava, R. M. *Synthesis-Stuttgart* **2007**, 2647.

(149) Reitz, S.; Cebi, M.; Reiss, P.; Studnik, G.; Linne, U.; Koert, U.; Essen, L.-O. *Angewandte Chemie-International Edition* **2009**, 48, 4853.

(150) Schlienger, N.; Peyrottes, S.; Kassem, T.; Imbach, J. L.; Gosselin, G.; Aubertin, A. M.; Perigaud, C. *Journal of Medicinal Chemistry* **2000**, 43, 4570.

(151) Lutz, W. B.; Witkop, B.; Daly, J. W.; Crevelin.Cr; Goldberg, L. I. *Journal of Medicinal Chemistry* **1972**, 15, 795.

(152) Chalker, J. M.; Wood, C. S. C.; Davis, B. G. *Journal of the American Chemical Society* **2009**, 131, 16346.

(153) Tandon, V. K.; Yadav, D. B.; Singh, R. V.; Chaturvedi, A. K.; Shukla, P. K. *Bioorganic & Medicinal Chemistry Letters* **2005**, 15, 5324.

(154) Jahani, F.; Tajbakhsh, M.; Golchoubian, H.; Khaksar, S. *Tetrahedron Letters* **2011**, 52, 1260.

(155) Dinh, T. Q.; Du, X. H.; Armstrong, R. W. *Journal of Organic Chemistry* **1996**, 61, 6606.

(156) Park, J. D.; Lee, K. J.; Kim, D. H. *Bioorganic & Medicinal Chemistry* **2001**, 9, 237.

(157) Meyer, W. L.; Templeton, G. E.; Grable, C. I.; Jones, R.; Kuyper, L. F.; Lewis, R. B.; Sigel, C. W.; Woodhead, S. H. *Journal of the American Chemical Society* **1975**, 97, 3802.

(158) Warnke, J. G.; Young, G. T. *Journal of the Chemical Society, Perkin Transactions 1* **1980**, 2797.

(159) Di Gioia, M. L.; Leggio, A.; Liguori, A.; Perri, F. *Journal of Organic Chemistry* **2007**, 72, 3723.

(160) Huy, P.; Neudoerfl, J.-M.; Schmalz, H.-G. *Organic Letters* **2011**, 13, 216.

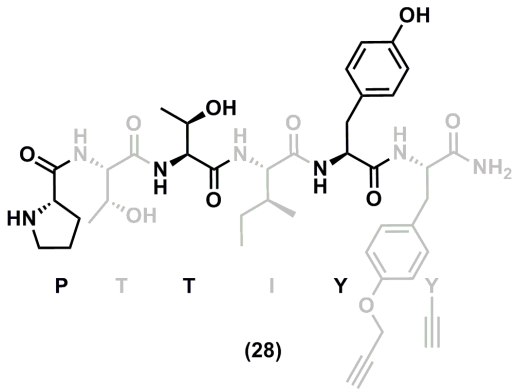
(161) Bew, S. P.; Hiatt-Gipson, G. D. *Journal of Organic Chemistry* **2010**, 75, 3897.

(162) Just-Baringo, X.; Bruno, P.; Albericio, F.; Alvarez, M. *Tetrahedron Letters* **2011**, 52, 5435.

(163) Jayaprakash, S.; Pattenden, G.; Viljoen, M. S.; Wilson, C. *Tetrahedron* **2003**, 59, 6637.

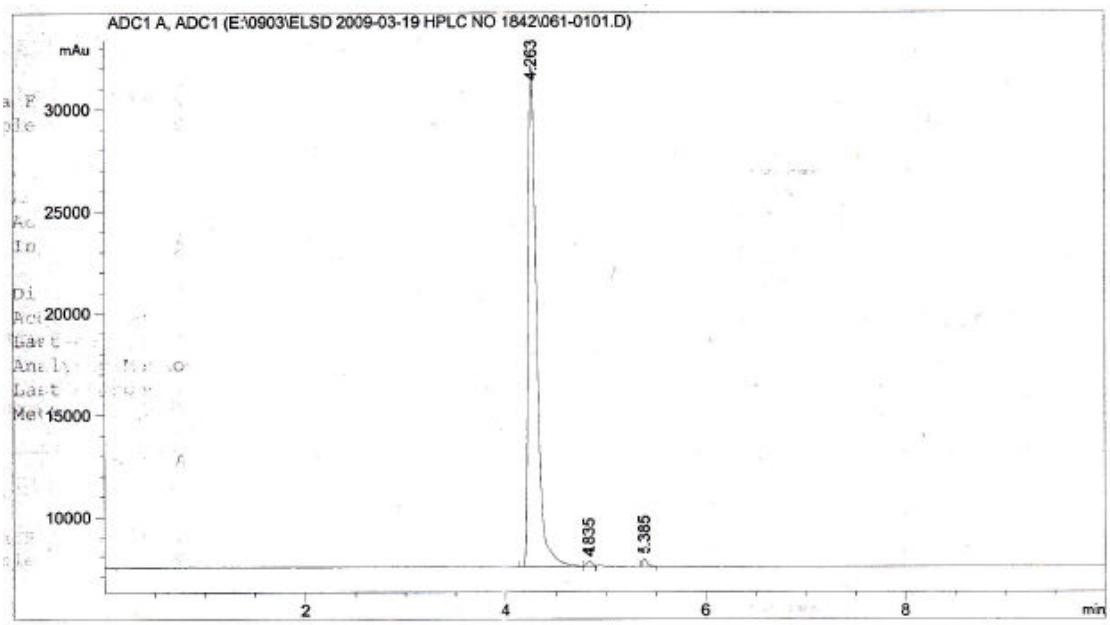
Appendix 1: PTTIYX and PTTIXY Analysis

PTTIYX (28)

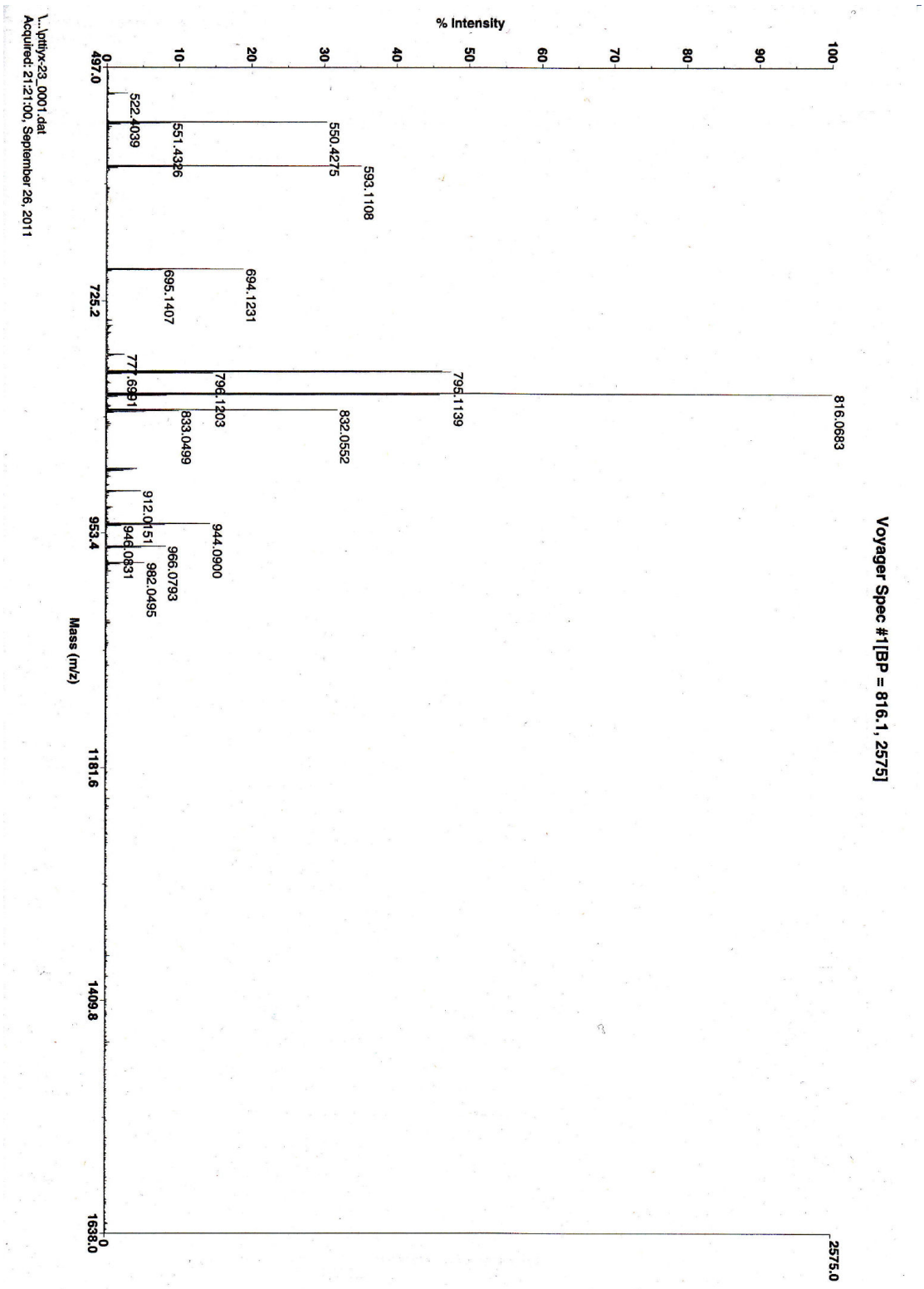


(28)

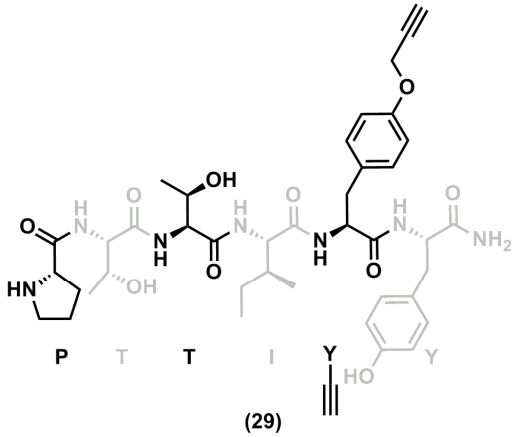
Chemical Formula: $C_{40}H_{55}N_7O_{10}$
Exact Mass: 793.40
Molecular Weight: 793.91



Peak #	RetTime [min]	Type	Width [min]	Area [mAu*s]	Height [mAu]	Area %
1	4.263	VV	0.0911	1.46056e5	2.46703e4	98.3862
2	4.835	VV	0.0605	1108.75122	277.91403	0.7469
3	5.385	VV	0.0496	1286.95032	398.81110	0.8669
Totals :				1.48452e5	2.53470e4	

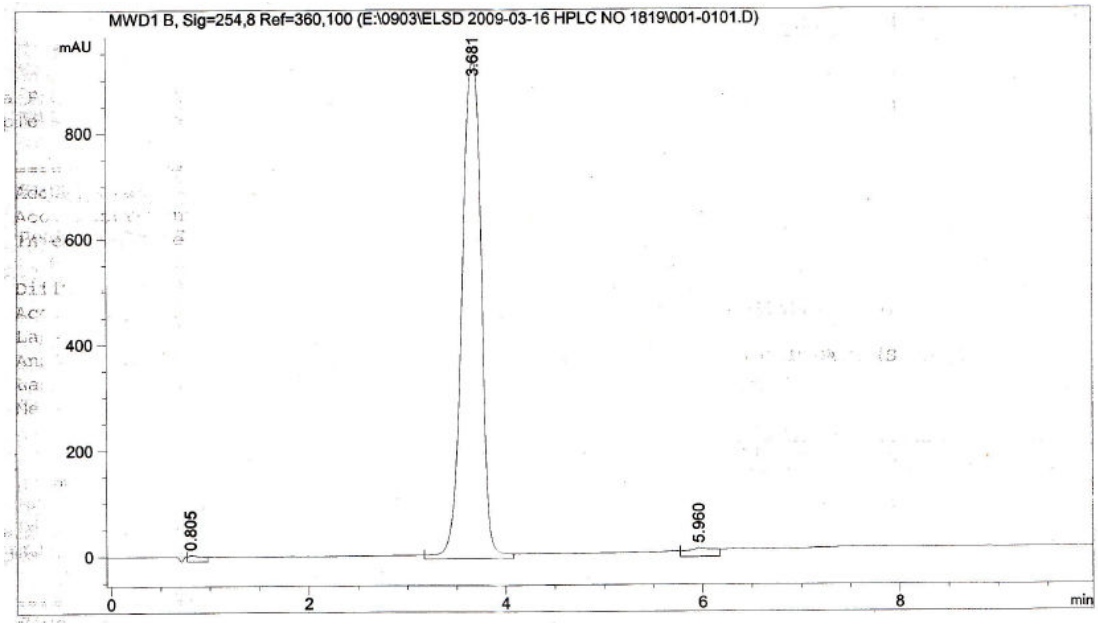

% Intensity
0
10
20
30
40
50
60
70
80
90
100
497.0
522.4039
551.4326
550.4275
593.1108
725.2
695.1407
694.1231
777.6991
796.1203
795.1139
816.0683
833.0499
832.0552
953.4
912.0151
944.0900
948.0831
966.0793
982.0495
1181.6
1409.8
1638.0
2575.0
Mass (m/z)
Voyager Spec #1[BP = 816.1, 2575]
\...\ptttyx-23_0001.dat
Acquired: 21:21:00, September 26, 2011

PTTIXY (29)



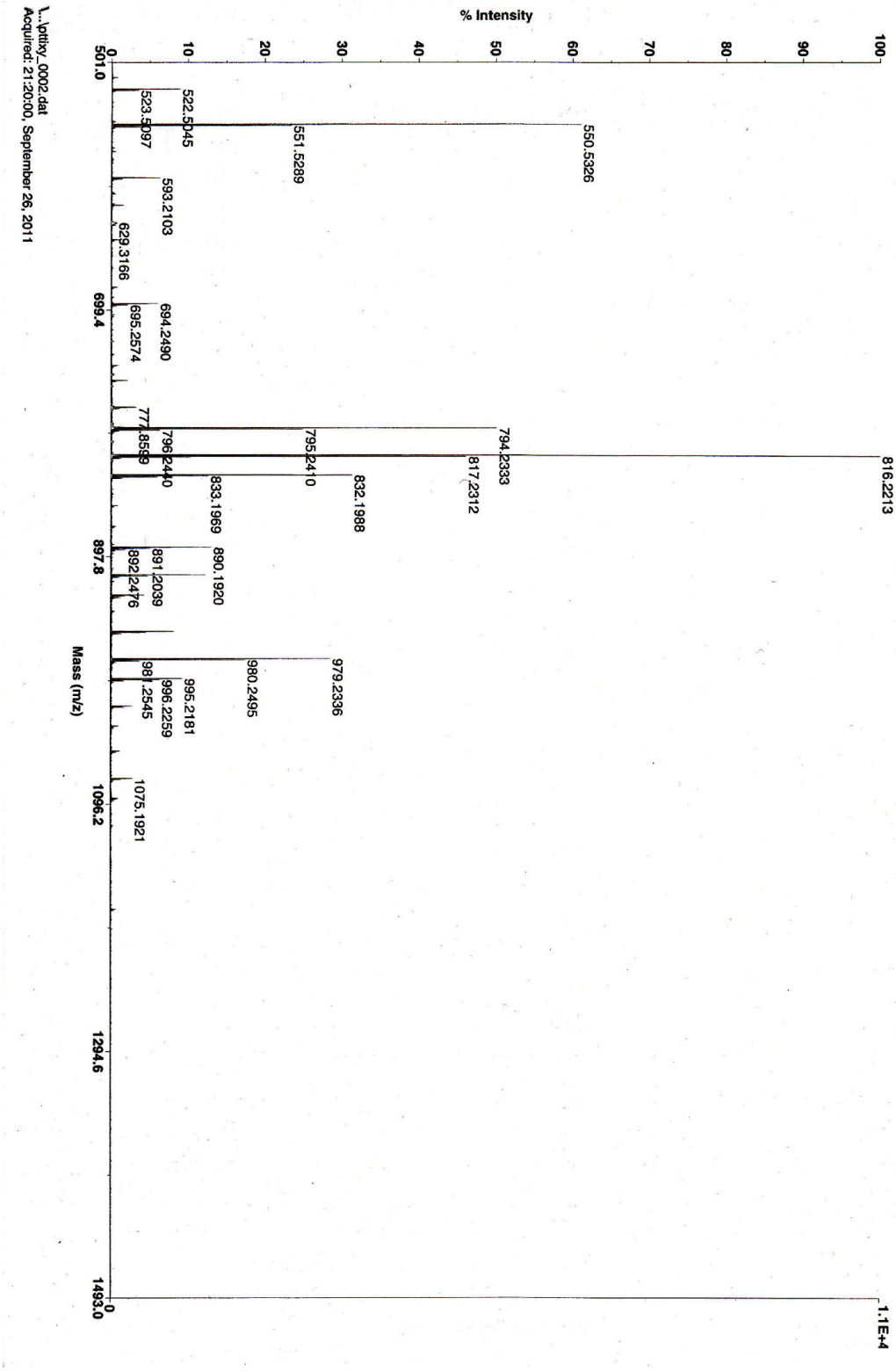
(29)

Chemical Formula: $C_{40}H_{55}N_7O_{10}$
Exact Mass: 793.40
Molecular Weight: 793.91



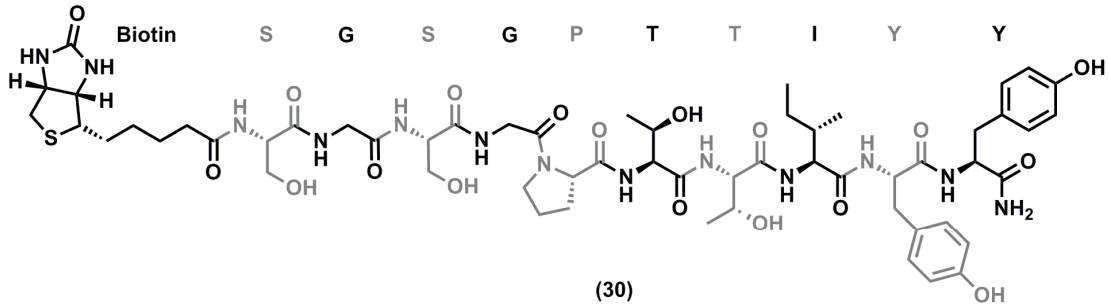
Signal 1: MWD1 B, Sig=254,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.805	VV	0.1320	122.26089	11.69282	0.9191
2	3.681	VV	0.2175	1.28503e4	939.91730	96.6005
3	5.960	VV	0.2533	329.95984	16.78825	2.4804
Totals :				1.33026e4	968.39837	


% Intensity
Mass (m/z)
Voyager Spec #1[BP = 816.2, 10874]
\...\ptitxy_0002.dat
Acquired: 21:20:00, September 26, 2011
816.2213
794.2333
817.2312
550.5326
795.2410
832.1988
833.1969
890.1920
979.2336
980.2495
995.2181
996.2259
1075.1921
522.5045
551.5289
593.2103
694.2490
1.1E+4

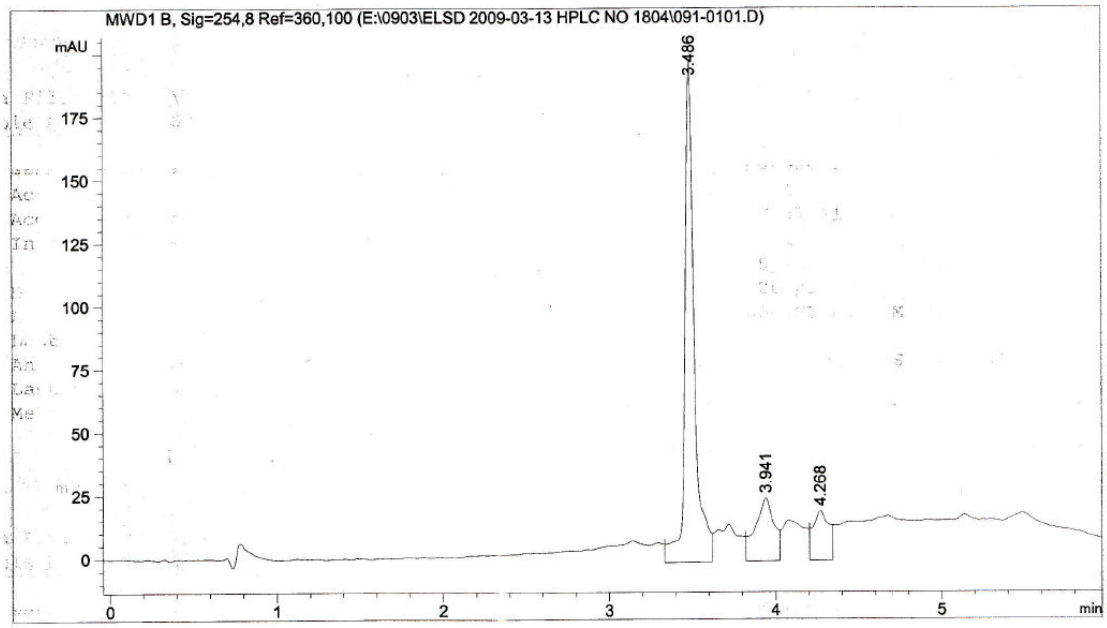
Appendix 2: Biotinylated Peptide Analysis

BiotinSGSGPTTIYY (30)



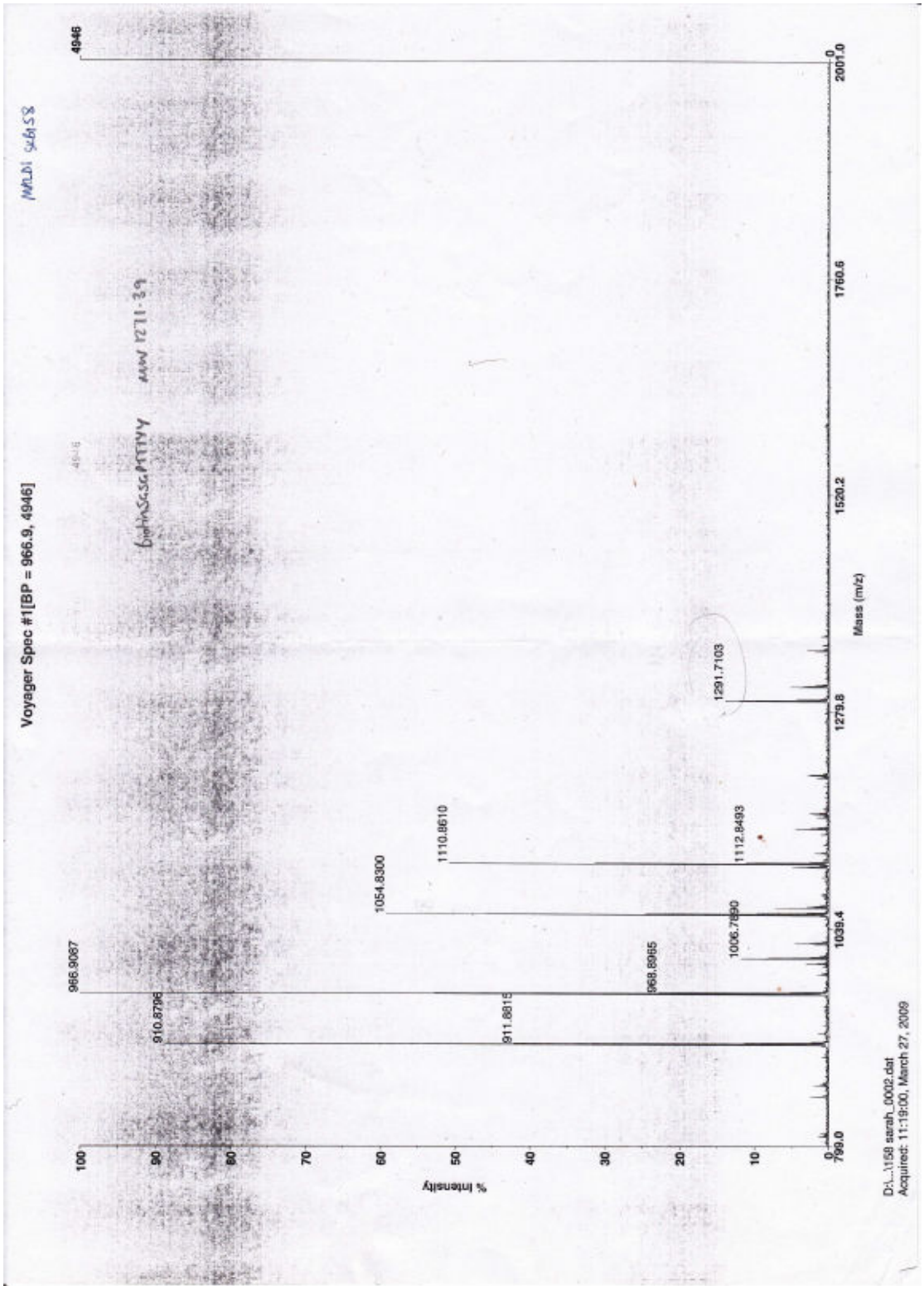
(30)

Chemical Formula: $C_{57}H_{83}N_{13}O_{18}S$
Exact Mass: 1269.57
Molecular Weight: 1270.41

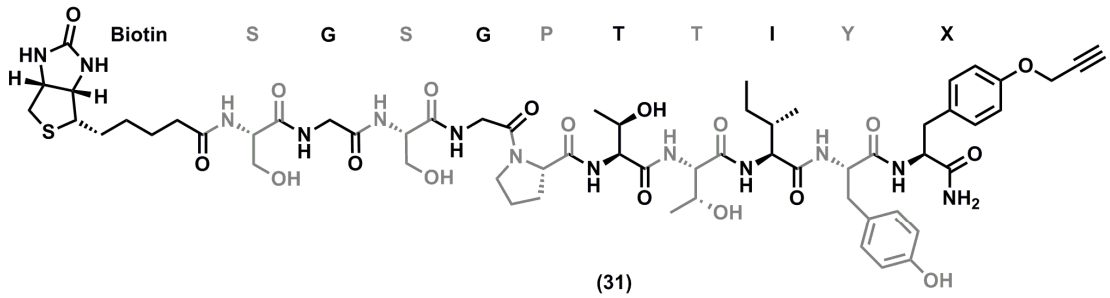


Signal 1: MWD1 B, Sig=254,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.486	VV	0.0597	777.75891	198.73679	70.4887
2	3.941	VV	0.1032	195.03444	24.96169	17.6761
3	4.268	VV	0.0918	130.58696	19.57156	11.8352
Totals :				1103.38031	243.27003	

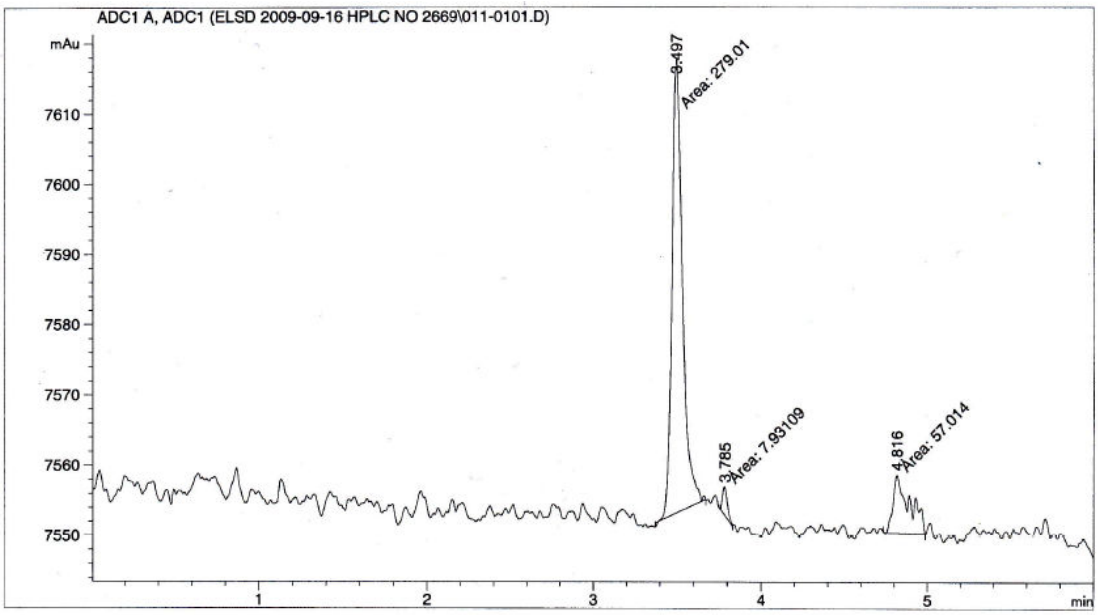

Voyager Spec #1[BP = 966.9, 4946]
MALDI 96458
4946
966.9067
910.8796
911.8815
968.8965
1054.8300
1110.8610
1112.8493
1006.7890
1291.7103
% Intensity
Mass (m/z)
799.0
1039.4
1279.8
1520.2
1760.6
2001.0
D:\...\158 sarah_0002.dat
Acquired: 11:19:00, March 27, 2009

BiotinSGSGPTTIYX (31)

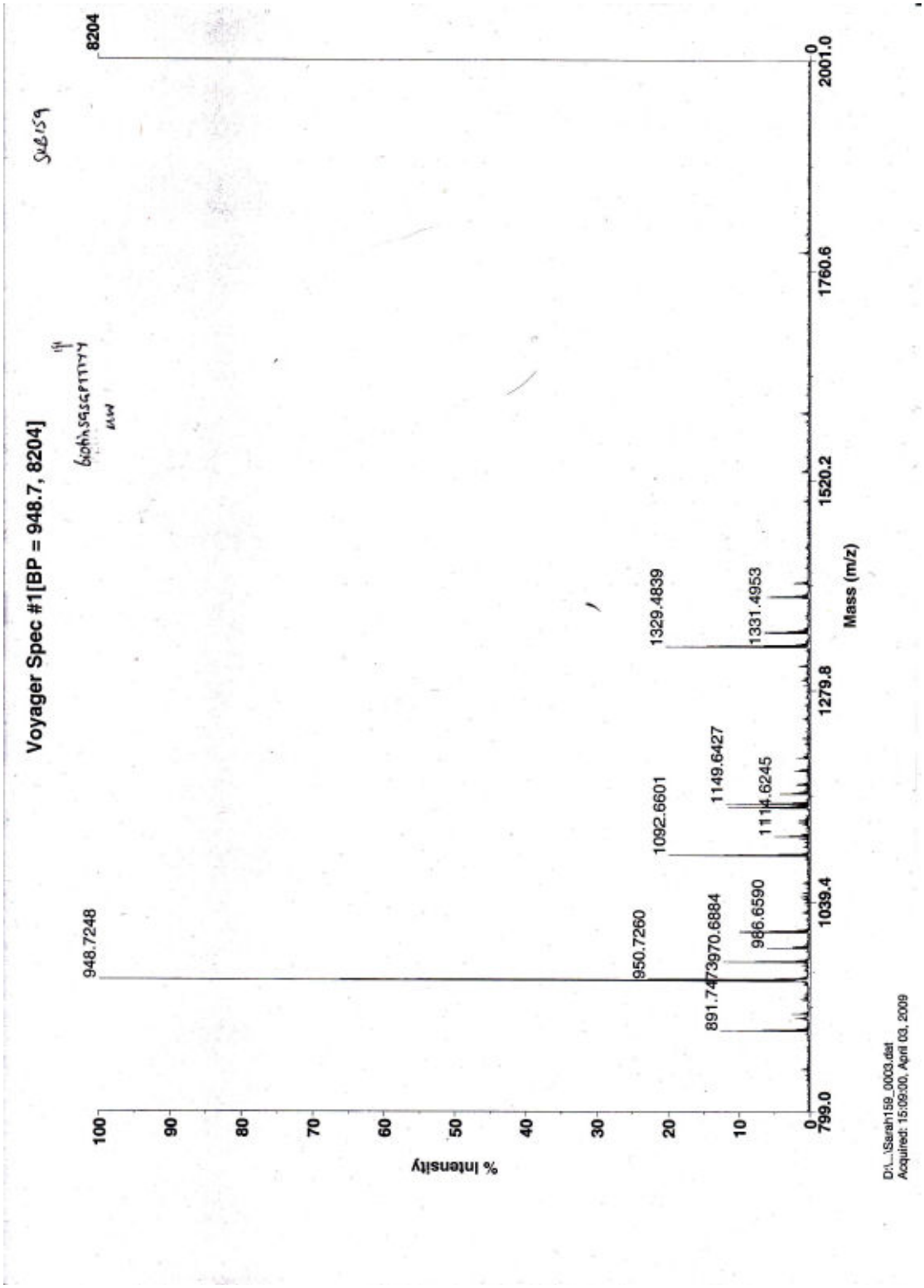


(31)

Chemical Formula: $C_{60}H_{85}N_{13}O_{18}S$
Exact Mass: 1307.59
Molecular Weight: 1308.46



Peak #	RetTime [min]	Type	Width [min]	Area [mAu*s]	Height [mAu]	Area %
1	3.497	MM	0.0718	279.01004	64.72198	81.1181
2	3.785	MM	0.0328	7.93109	4.03099	2.3059
3	4.816	MM	0.1159	57.01400	8.20033	16.5760
Totals :				343.95513	76.95330	


Voyager Spec #1[BP = 948.7, 8204]
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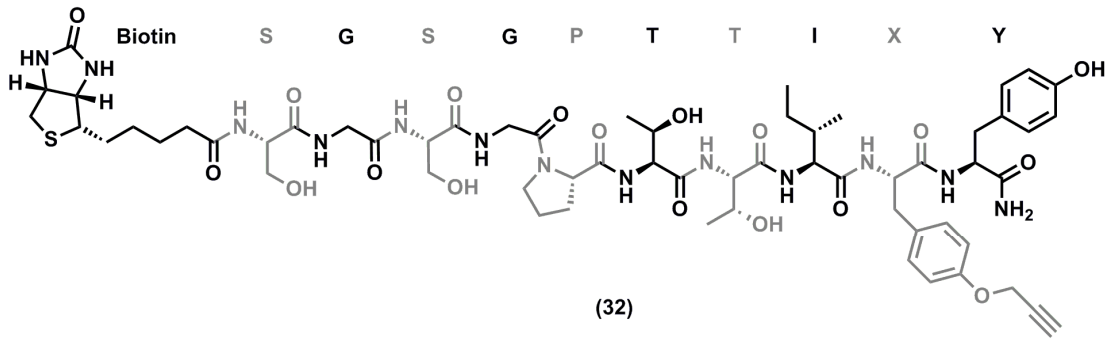
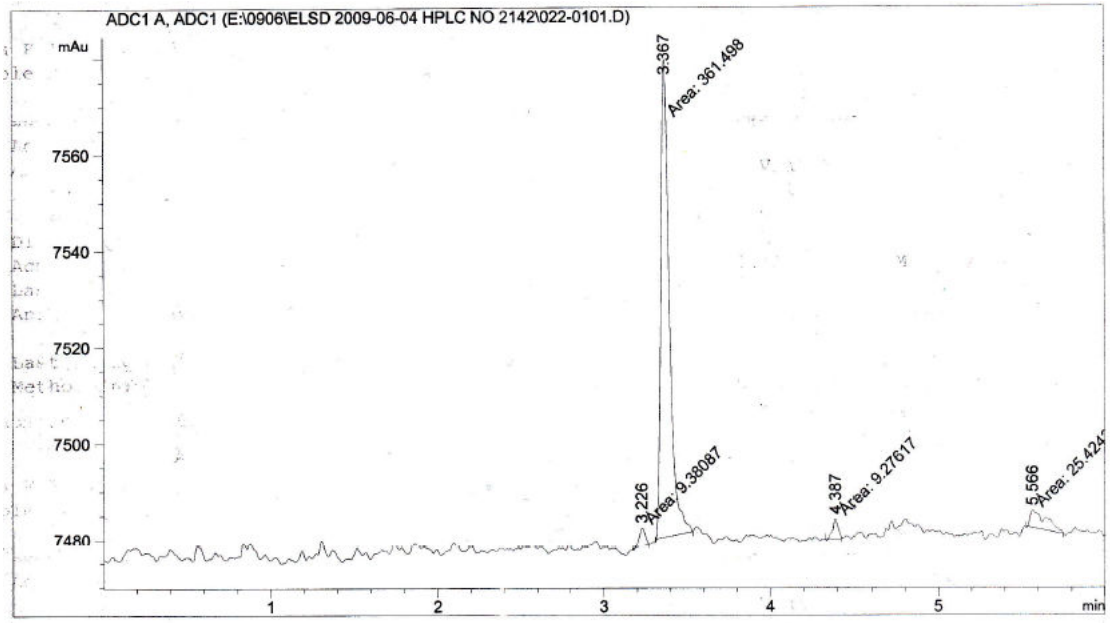
948.7248
950.7260
891.7473
970.6884
986.6590
1092.6601
1149.6427
1114.6245
1329.4839
1331.4953
% Intensity
Mass (m/z)
799.0
1039.4
1279.8
1520.2
1760.6
2001.0
D:\...\Sarah159_0003.dat
Acquired: 15:09:00, April 03, 2009

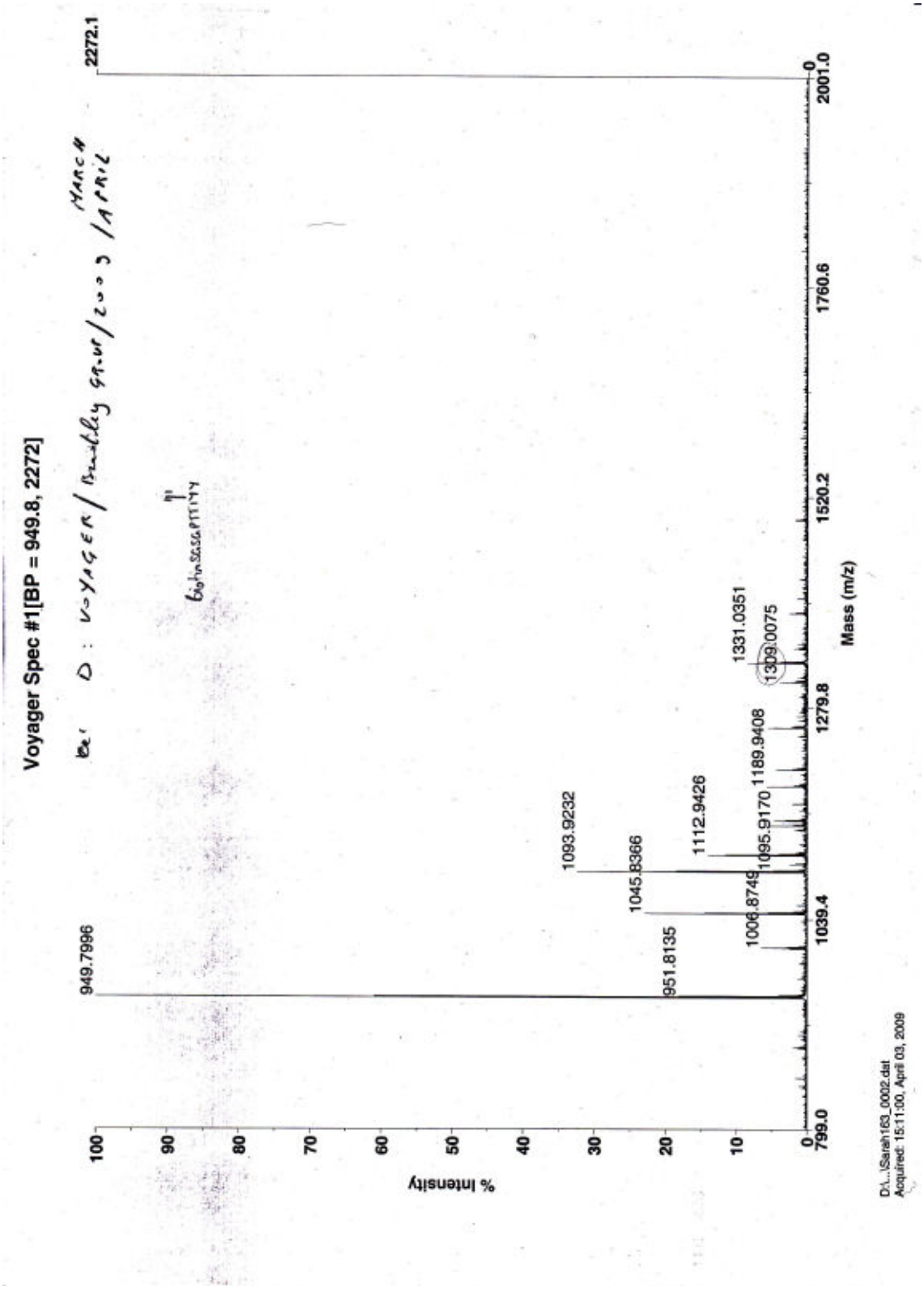
BiotinSGSGPTTIXY (32)



Chemical Formula: $C_{60}H_{85}N_{13}O_{18}S$
Exact Mass: 1307.59
Molecular Weight: 1308.46



Peak #	RetTime [min]	Type	Width [min]	Area [mAu*s]	Height [mAu]	Area %
1	3.226	MM T	0.0404	9.38087	3.87414	2.3130
2	3.367	MM T	0.0608	361.49789	99.04558	89.1313
3	4.387	MM T	0.0372	9.27617	4.15342	2.2871
4	5.566	MM T	0.1199	25.42416	3.53379	6.2686
Totals :				405.57909	110.60693	


Voyager Spec #1[BP = 949.8, 2272]
949.7996
951.8135
1006.8749
1045.8366
1093.9232
1095.9170
1112.9426
1189.9408
1309.0075
1331.0351
2272.1
% Intensity
Mass (m/z)
D:\...\Sareh163_0002.dat
Acquired: 15:11:00, April 03, 2009

Appendix 3: Results of ELISA for Peptide Binding to AG-2

An ELISA was run of 4 peptides measuring their relative binding to purified AG-2. Samples were run in duplicate, and averages taken to present data graphically.

Peptides used:

Column 1: Negative control. A random peptide used to measure non-specific binding to AG-2.

Column 2: Duplicate of column 1.

Column 3: Positive control. Commercially supplied BiotinSGSGPTTIYY (**30**).

Column 4: Duplicate of column 3.

Column 5: Synthetic Propargylated Peptide. BiotinSGSGPTTIYX (**31**).

Column 6: Duplicate of column 5.

Column 7: Synthetic Propargylated Peptide. BiotinSGSGPTTIXY (**32**).

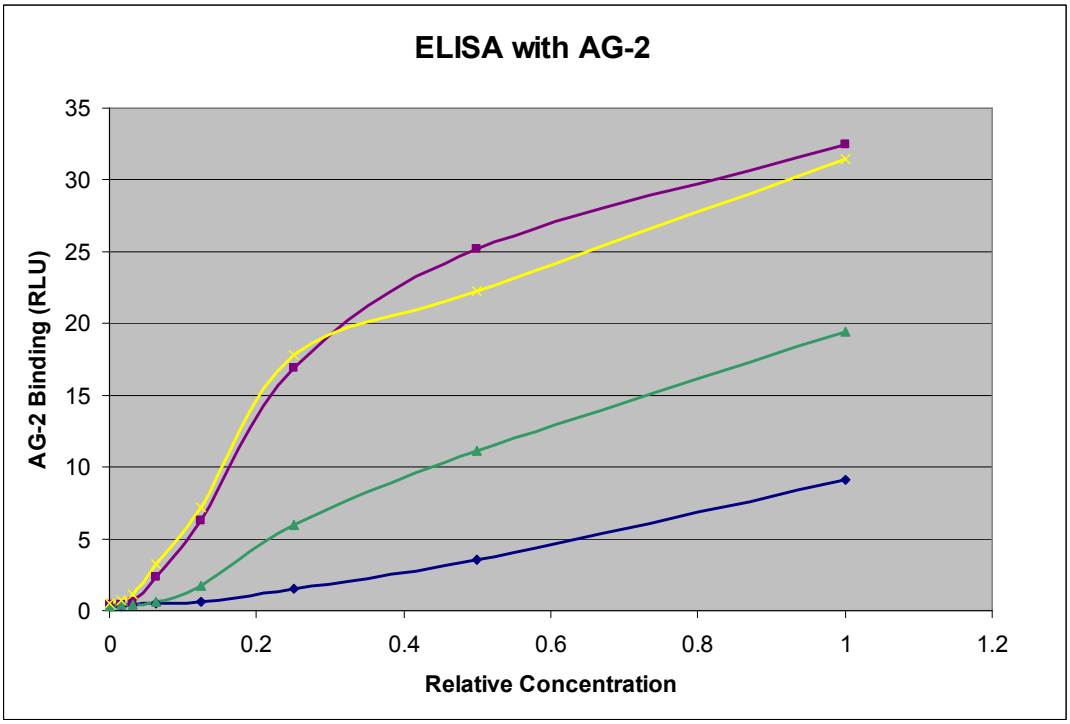
Column 8: Duplicate of column 7.

Readout from the “Plate reader” machine, using Fluoroscan Ascent software.

	Conc.	1 (-)	2 (-)	3 (**30**)	4 (**30**)	5 (**31**)	6 (**31**)	7 (**32**)	8 (**32**)
A	1	9.5	8.8	32.3	32.7	20.0	18.7	32.0	30.9
B	1/2	3.6	3.6	24.2	26.1	12.3	10.0	21.7	22.7
C	1/4	1.2	1.8	16.5	17.2	6.6	5.2	17.4	18.1
D	1/8	0.5	0.6	6.2	6.3	2.0	1.4	6.7	7.7
E	1/16	0.4	0.5	2.5	2.2	0.5	0.6	2.7	3.8
F	1/32	0.3	0.4	0.6	0.6	0.4	0.5	1.0	1.1
G	1/64	0.3	0.3	0.4	0.4	0.3	0.4	0.6	0.8
H	0	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.6

Average reading over 2 duplicate runs

	Conc.	(-)	(+)	159	163
A	1	9.1	32.5	19.4	31.5
B	1/2	3.6	25.1	11.1	22.2
C	1/4	1.5	16.9	5.9	17.8
D	1/8	0.6	6.2	1.7	7.2
E	1/16	0.5	2.4	0.6	3.3
F	1/32	0.4	0.5	0.4	1.1
G	1/64	0.3	0.4	0.4	0.7
H	0	0.3	0.4	0.3	0.5


ELISA with AG-2
AG-2 Binding (RLU)
35
30
25
20
15
10
5
0
0
0.2
0.4
0.6
0.8
1
1.2
Relative Concentration

Appendix 4: Results of ELISA for Peptide Binding to AG-2 in MCF-7 Breast Cancer Cell Lysate

An ELISA was run of 4 peptides measuring their relative binding to AG-2 within MCF-7 breast cancer cell lysate. Samples were run in duplicate, and averages taken to present data graphically.

Peptides used:

Column 1: Negative control. A random peptide used to measure non-specific binding to components present in MCF-7 cell lysate.

Column 2: Duplicate of column 1.

Column 3: Positive control. Commercially supplied BiotinSGSGPTTIYY (**30**).

Column 4: Duplicate of column 3.

Column 5: Synthetic Propargylated Peptide. BiotinSGSGPTTIYX (**31**).

Column 6: Duplicate of column 5.

Column 7: Synthetic Propargylated Peptide. BiotinSGSGPTTIXY (**32**).

Column 8: Duplicate of column 7.

Readout from the “Plate reader” machine, using Fluoroscan Ascent software.

Average reading over 2 duplicate runs:

	Conc.	1 (-)	2 (+)	3 (159)	4 (163)
A	1	0.061	5.029	0.041	3.759
B	1/2	0.039	1.266	0.214	1.325
C	1/4	0.028	0.188	0.025	0.528
D	1/8	0.030	0.027	0.024	0.024
E	1/16	0.037	0.028	0.026	0.037
F	1/32	0.041	0.027	0.024	0.020
G	1/64	0.038	0.024	0.024	0.022
H	0	0.031	0.019	0.020	0.015