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New Methods for the Rapid Synthesis of Thiazoles

A thesis submitted to University of Sussex

By

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In Candidature of

Doctor of Philosophy

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School of Life Sciences

University of Sussex

Declaration

I hereby declare that the work presented in this dissertation was carried out at the University of Sussex under the supervision of Prof. Mark Bagley between the dates of January 2017 and May 2021. The work presented in this thesis is my own, unless otherwise stated, and has not been submitted in whole or in part form for the award of another degree.

Yousef Mukhrish

May 2021.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الرَّحْمَنُ تِلْكَ آيَاتُ الْكِتَابِ الْمُبِينِ ﴿1﴾ إِنَّا أَنْزَلْنَاهُ قُرْآنًا عَرَبِيًّا لَعَلَّكُمْ تَعْقِلُونَ ﴿2﴾ نَحْنُ نَقُصُّ عَلَيْكَ أَحْسَنَ الْقَصَصِ بِمَا أَوْحَيْنَا إِلَيْكَ هَذَا الْقُرْآنَ وَإِنْ كُنْتَ مِنْ قَبْلِهِ لَمَنِ الْعَافِينَ ﴿3﴾

Alif, Lām, Rā. These are the verses of the clear Book (1) Indeed, We have sent it down as an Arabic Qur'ān that you might understand (2) We relate to you, [O Muḥammad], the best of stories in what We have revealed to you of this Qur'ān although you were, before it, among the unaware (3)

12th chapter of the Quran

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Abstract

Chapter 1 provides an overview on, thiazole-containing natural products, cyclic peptides, their biological activities and methods for their synthesis based upon the Hantzsch approach and modifications of the Hantzsch thiazole synthesis. It discusses the application of modern alternative automated reaction platforms in for the synthesis of thiazoles, such as solid-phase peptide synthesis, microwave-assisted synthesis and flow chemistry.

Chapter 2 aims of the project.

Chapter 3 describes the preparation of amide and thioamide derivatives, and treatment of ethyl bromopyruvate with thioamides in the absence of catalysts under microwave irradiation has been developed as a convenient method for the synthesis of thiazoles. The products were formed rapidly and in high yields.

Chapter 4 describes a new route that have been developed for the synthesis of chiral thiazole-containing amino acids in the liquid phase using Robinson-Gabriel cyclisation, and the potential of these methods to be automated in the solid phase so they can be integrated into automated natural product synthesis.

Chapter 5 describes a new microwave-assisted method for the synthesis of dihydropyrazinones is achieved by mixed anhydride coupling reaction of 2,2-diethoxy-ethanamine with Fmoc-protected amino acids followed by cyclisation reactions via acid catalyzed.

Abbreviations

Ac	Acetyl
AcOH	Acetic acid
Ala	Alanine
Ar	Unspecified aryl substituent
aq.	Aqueous solution
Boc	tert-Butoxycarbonyl
Bn	Benzyl
Brine	Saturated sodium chloride solution
bp	Boiling point
Bu	Butyl
cat.	Catalytic/catalyst
Cbz	Benzyloxycarbonyl
CDI	1,1'-Carbonyldiimidazole
DBU	1,8-Diaza[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DME	1,2-Dimethoxyethane
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DPPA	Diphenylphosphoryl azide
<i>ee</i>	Enantiomeric excess
El	Electron Impact
Enz	Enzyme
eq.	Equivalent

equiv.	Equivalent
Et	Ethyl
EtOAc	Ethyl acetate
Fmoc	9-Fluorenylmethoxycarbonyl
g	Grams
GC-MS	Gas chromatography mass spectrometry
Gly	Glycine
h	Hour(s)
HATU	<i>O</i> -(7-Azabenzotriazol-1-yl)- <i>N, N, N', N'</i> -tetramethyluronium hexafluorophosphate
HBTU	<i>O</i> -(Benzotriazol-1-yl)- <i>N, N, N', N'</i> -tetramethyluronium hexafluorophosphate
Hex	Hexane
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
IBCF	Isobutyl chloroformate
IR	Infrared
<i>J</i>	Coupling constant (in Hz)
kg	Kilograms
Leu	Leucine
lit.	Literature
LRMS	Low Resolution Mass Spectrometry
Lu	Lutidine
M	Molar
Me	Methyl-
MeCN	Acetonitrile
MeOH	Methanol

mg	Milligrams
min	Minutes
mL	Millilitres
Mol	Mole
mp	Melting point
MS	Mass Spectrometry
Ms	Mesyl (methanesulfonyl)
MW	Microwave
NMR	Nuclear magnetic resonance
OAc	Acetate
PG	Protecting group
Ph	Phenyl
PhMe	Toluene
ppm	Parts per million
Pr	Propyl
Pro	Proline
py	Pyridine
PTSA	<i>p</i> -Toluenesulfonic acid monohydrate
R	Specified substituent
<i>rac</i>	Racemic
R _f	Retention factor
r.t.	Room temperature
Sat.	Saturated
Ser	Serine
SPPS	Solid-phase peptide synthesis
TBS	<i>tert</i> -Butyldimethylsilyl

TEAP	Triethylammonium phosphate
<i>Tert</i>	Tertiary
TES	Triethylsilyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
UV	Ultraviolet
Val	Valine

Table of Contents

Acknowledgements.....	iii
Abstract.....	iv
Abbreviations	v
Table of Contents	ix
Chapter 1 Introduction.....	1
1.1. Heterocyclic compounds and their applications.....	1
1.2. Thiazole-containing natural products.....	1
1.3. Thiopeptide antibiotics: Occurrence and biological activity	3
1.4. Methods for the synthesis of thiazoles.....	5
1.4.1. Thiazole synthesis.....	5
1.4.2. The Hantzsch thiazole synthesis.....	5
1.4.3. Racemization of thiazoles with side chains derived from natural amino acids.....	7
1.4.4. Holzapfel modification of the Hantzsch thiazole synthesis.....	8
1.4.5. Meyers modification of the Hantzsch thiazole synthesis.....	10
1.4.6. Nicolaou modification of the Hantzsch thiazole synthesis	12
1.4.7. Bagley-Merritt modification of the Hantzsch thiazole synthesis.....	13
1.5. Methods for the synthesis of cyclic peptides.....	16
1.5.1. Dolastatin 3	16
1.5.2. (-)-Bistratamide C	18
1.5.3. (+)-Nostocyclamide	20
1.5.4. Micrococcin PI.....	22
1.5.5. Thiostrepton	26
1.6. Robinson-Gabriel synthesis of oxazoles	30
1.6.1. Oxazole.....	30
1.6.2. Background	31
1.7.1. Microwave-Assisted synthesis.....	33
1.7.2. Automated Platforms in synthesis: Peptides	34
Chapter 2 Aims of the project.....	38
Chapter 3: Results and Discussions.....	39

3.1. Develop new reaction conditions for rapid Hantzsch thiazole synthesis	39
3.1.1 Preparation of amide derivatives	41
3.1.2 Preparation of thioamide derivatives	42
3.2. Modification of the Hantzsch thiazole synthesis	45
Chapter 4: Results and Discussions	49
4.1. New methods for the synthesis of chiral thiazole-containing amino acids	49
4.1.1. Preparation of anilide derivatives	51
4.1.2. Preparation of thioanilide derivatives	54
4.1.3 Preparation of thiobenzotriazole derivatives	59
4.2. Synthesis of thiazoles	61
4.2.1. Preparation of Ethyl 2-amino-3,3-diethoxypropanoate	62
4.2.2 Preparation of Thioamide Cyclization Precursors	65
4.2.3. Optimization of the cyclisation reaction conditions	68
4.2.4. Microwave-Assisted Robinson-Gabriel Thiazole Synthesis	70
4.3 Solid Phase Peptide Synthesis (SPPS)	74
4.3.1. Peptides Synthesis	74
4.3.2. First residue attachment	76
4.3.3. Fmoc Removal	76
4.3.4. Coupling Methods	77
4.3.5. Developing an Automated Method for the Synthesis of Thiazoles	78
4.4 Conclusion	81
Chapter 5: Results and Discussions	83
5.1. A novel protocol for the synthesis of dihydropyrazinones via acid catalysed cyclisation reaction	83
5.1.1 Preparation of amide Cyclization Precursors	86
5.1.2 Optimization of the cyclisation reaction conditions	89
5.2 Conclusion	92
Chapter 6: Experimental	93
6.1. Materials and Methods	93
6.1.1. General method for synthesis of thiazole 3.9. (A)	93
6.1.2. General method for synthesis of thiazole 3.9. (B)	94
6.2 Experimental Procedures	95
6.2.1. 1-(α-N-Fmoc-L-valinamido)-2-amino-5-nitrobenzene (4.7a)	95
6.2.2. 1-(α-N-Fmoc-L-alaninamido)-2-amino-5-nitrobenzene (4.7b)	96

6.2.3.1-(α - <i>N</i> -Fmoc-L-leucinamido)-2-amino-5-nitrobenzene (4.7c).....	97
6.2.4. 1-(α - <i>N</i> -Fmoc-L-phenylalaninamido)-2-amino-5-nitrobenzene (4.7d)	98
6.2.5. 1-(α - <i>N</i> -Fmoc-glycinamido)-2-amino-5-nitrobenzene (4.7e).....	99
6.2.6.1-(α - <i>N</i> -Fmoc-L-valinthioamido)-2-amino-5-nitrobenzene (4.8a).....	101
6.2.7. 1-(α - <i>N</i> -Fmoc-L-alaninthioamido)-2-amino-5-nitrobenzene (4.8b)	102
6.2.8. 1-(α - <i>N</i> -Fmoc-L-leucinthioamido)-2-amino-5-nitrobenzene (4.8c)	103
6.2.9. 1-(α - <i>N</i> -Fmoc-L-phenylalaninthioamido)-2-amino-5-nitrobenzene (4.8d).....	104
6.2.10. 1-(α - <i>N</i> -Fmoc-L-glycinthioamido)-2-amino-5-nitrobenzene (4.8e).....	106
6.2.11. 1-(<i>N</i> -Fmoc-L-thiovalinoyl)-6-nitrobenzotriazole (4.9a).....	107
6.2.12. 1-(<i>N</i> -Fmoc-L-thioalaninoyl)-6-nitrobenzotriazole (4.9b)	108
6.2.13. 1-(<i>N</i> -Fmoc-L-thioleucinoyl)-6-nitrobenzotriazole (4.9c)	109
6.2.14. 1-(<i>N</i> -Fmoc-L-thiophenylalaninoyl)-6-nitrobenzotriazole (4.9d).....	110
6.2.15. 1-(<i>N</i> -Fmoc-L-thioglycinoyl)-6-nitrobenzotriazole (4.9e)	111
6.2.16. Ethyl 2-bromo-3,3-diethoxypropanoate (4.29)	112
6.2.17. Ethyl 2-azido-3,3-diethoxypropanoate (4.30)	112
6.2.18. Ethyl 2-amino-3,3-diethoxypropanoate (4.10b).....	113
6.2.19. Ethyl 2-(<i>N</i> -Fmoc-L-valinthioamido)-3,3-diethoxypropanoate (4.11a).....	114
6.2.20. Ethyl 2-(<i>N</i> -Fmoc-L-alaninthioamido)-3,3-diethoxypropanoate (4.11b).....	115
6.2.21. Ethyl 2-(<i>N</i> -Fmoc-L-leucinthioamido)-3,3-diethoxypropanoate (4.11c).....	116
6.2.22. Ethyl 2-(<i>N</i> -Fmoc-L-phenylalaninthioamido)-3,3-diethoxypropanoate (4.11d)	117
6.2.23. Ethyl 2-(<i>N</i> -Fmoc-glycinthioamido)-3,3-diethoxypropanoate (4.11e).....	119
6.2.24. 1-(<i>N</i> -Fmoc-L-valinthioamido)-2,2-diethoxyethane (4.11f)	120
6.2.25. 1-(<i>N</i> -Fmoc-L-alaninthioamido)-2,2-diethoxyethane (4.11g)	121
6.2.26. 1-(<i>N</i> -Fmoc-L-leucinthioamido)-2,2-diethoxyethane (4.11h).....	122
6.2.27. 1-(<i>N</i> -Fmoc-L-phenylalaninthioamido)-2,2-diethoxyethane (4.11i).....	123
6.2.28. <i>N</i> -Fmoc-L-valinamide (3.7a)	124
6.2.29. <i>N</i> -Fmoc-L-alaninamide (3.7b).....	125
6.2.30. <i>N</i> -Fmoc-L-leucinamide(3.7c)	126
6.2.31. <i>N</i> -Fmoc-L-phenylalaninamide (3.7d).....	127
6.2.32. <i>N</i> -Fmoc-L-tryptophanamide (3.7e)	128
6.2.33. <i>N</i> -Fmoc-glycinamide (3.7f).....	129
6.2.34. <i>N</i> -Fmoc-L-valinthioamide (3.8a)	130
6.2.35. <i>N</i> -Fmoc-L-alaninthioamide (3.8b).....	131
6.2.36. <i>N</i> -Fmoc-L-leucinthioamide (3.8c).....	132
6.2.37. <i>N</i> -Fmoc-L-phenylalaninthioamide (3.8d)	133

6.2.38. <i>N</i> -Fmoc-L-tryptophanthioamide (3.8e).....	134
6.2.39. <i>N</i> - Fmoc-glycynthioamide (3.8f)	135
6.2.40. (<i>RS</i>) Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)-2-methylpropyl]thiazole-4-carboxylate (3.9a).....	136
6.2.41. (<i>RS</i>)-Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)ethyl]thiazole-4-carboxylate (3.9b) ...	137
6.2.42. (<i>RS</i>)-Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)-3-methylbutyl]thiazole-4-carboxylate (3.9c)	138
6.2.43. (<i>RS</i>)-Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)-2-phenylethyl]thiazole-4-carboxylate (3.9d).....	139
6.2.44. Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)methyl]thiazole-4-carboxylate (3.9e)	140
6.2.45. (<i>RS</i>)-Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)-2-(3-indolyl)ethyl]thiazole-4-carboxylate (3.9k).....	141
6.2.46. (<i>RS</i>)-2-[1-(Fluorenylmethyloxycarbonylamino)-2-methylpropyl]thiazole (3.9f).....	142
6.2.47. (<i>RS</i>)-2-[1-(Fluorenylmethyloxycarbonylamino)ethyl]thiazole (3.9g)	143
6.2.48. (<i>RS</i>)-2-[1-(Fluorenylmethyloxycarbonylamino)-3-methylbutyl]thiazole (3.9h)	144
6.2.49. (<i>RS</i>)-2-[1-(Fluorenylmethyloxycarbonylamino)-2-phenylethyl]thiazole (3.9i)	145
6.2.50. {2-[(1 <i>RS</i>)-1-(Fluorenylmethyloxycarbonylamino)ethyl]thiazol-4-yl}carbonyl-L-valine (4.46).	146
6.2.51. 1-(<i>N</i> -Fmoc-L-valinamido)-2,2-diethoxyethane (5.19a)	146
6.2.52. 1-(<i>N</i> -Fmoc-L-alaninamido)-2,2-diethoxyethane (5.19b).....	147
6.2.53. 1-(<i>N</i> -Fmoc-L-leucinamido)-2,2-diethoxyethane (5.19c).....	149
6.2.54. 1-(<i>N</i> -Fmoc-L-phenylalaninamido)-2,2-diethoxyethane (5.19d)	150
6.2.55. 1-(<i>N</i> -Fmoc-L-tryptophanamido)-2,2-diethoxyethane (5.19e).....	151
6.2.56. 1-(<i>N</i> -Fmoc-glycinamido)-2,2-diethoxyethane (5.19f)	153
6.2.57. (3 <i>S</i>)- <i>N</i> ⁴ -Fmoc-3-(2-propyl)-3,4-dihydropyrazin-2-one (5.20a).....	154
6.2.58. (3 <i>S</i>)- <i>N</i> ⁴ -Fmoc-3-methyl-3,4-dihydropyrazin-2-one (5.20b)	155
6.2.59. (3 <i>S</i>)- <i>N</i> ⁴ -Fmoc-3-(1-[2-methyl]propyl)-3,4-dihydropyrazin-2-one (5.20c)	156
6.2.60. (3 <i>S</i>)- <i>N</i> ⁴ -Fmoc-3-(phenylmethyl)-3,4-dihydropyrazin-2-one (5.20d)	157
6.2.61. (3 <i>S</i>)- <i>N</i> ⁴ -Fmoc-3-(indol-3-ylmethyl)-3,4-dihydropyrazin-2-one (5.20e).....	158
6.2.62. <i>N</i> ⁴ -Fmoc-3,4-dihydropyrazin-2-one (5.20f).....	159
References	160

Chapter 1 Introduction

1.1. Heterocyclic compounds and their applications

Heterocyclic chemistry is one of the largest branches of organic chemistry due to the biological and industrial importance of these compounds.¹ There are many additives and modifiers such as plastics, reprographic materials, and cosmetics that are heterocycles; in addition, many biologically active agrochemicals and clinical medicines are heterocycles.¹ Heterocycles have played a major role in the biological and pharmaceutical disciplines and so methods for their synthesis can find widespread application. Nature also utilizes heterocycles due to their ability to participate in a wide range of reaction types.¹ The production of many synthetic drugs has involved the use of heterocycles, including antiviral, anticancer, antibacterial and antifungal agents.

1.2. Thiazole-containing natural products

As an aromatic five membered ring, thiazole is a heterocyclic compound containing both a sulfur and nitrogen atom as part of the aromatic system. These 1,3-azoles and related compounds (nitrogen and one other heteroatom in the ring) are numbered as shown in **(Figure 1.1)**.

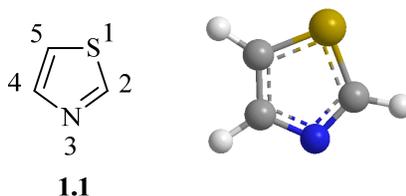


Figure 1.1. Thiazole numbering system and structure.

With a 116-118 °C boiling point and a specific gravity of 1.2, thiazole is a clear or pale-yellow liquid, slightly soluble in water, but completely soluble in ether and alcohol. The thiazole motif can be found

as an intermediate in, or target for, the synthesis of drugs, fungicides, dyes and is present in naturally occurring vitamins like Vitamin B1 (thiamin) (**Figure 1.2**).

In general thiazoles are a significant component of biologically-active heterocyclic compounds that are present in many drugs, such as Tiazofurin (antineoplastic drug), Abafungin (antifungal drug), Sulfathiazole (antimicrobial drug) and Ritonavir (antiretroviral drug) (**Figure 1.2**).² The presence of a thiazole-containing moiety can elucidate a variety of biological responses such as antifungal or antimicrobial,³ anticancer,⁴ anticonvulsant,⁵ Syk inhibitor,⁵ anti-inflammatory,⁶ antihypertensive,⁷ anti-HIV⁸ and antidiabetic⁹ properties. Accordingly, medicinal chemists have devoted considerable interest to this ring system and new synthetic methods for the preparation of this heteroaromatic ring continue to be developed.

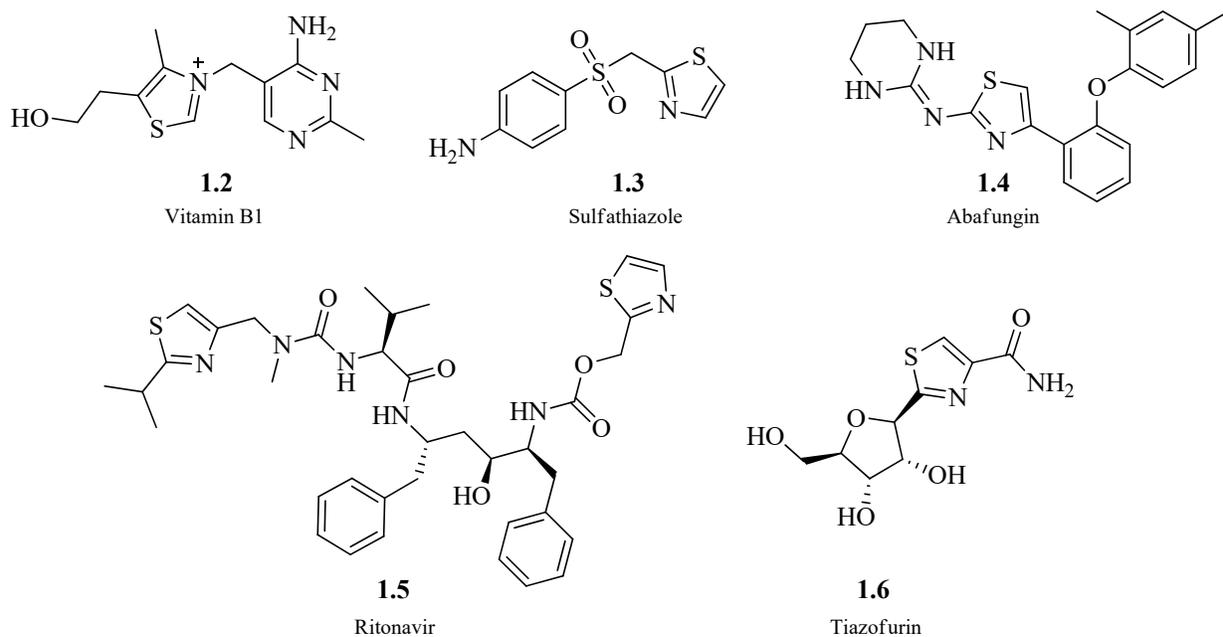


Figure 1.2: The structure of biologically active thiazole-containing agents.

1.3. Thiopeptide antibiotics: Occurrence and biological activity

The thiopeptides are heterocyclic and macrocyclic peptide antibiotics composed of highly modified amino acid building blocks. These natural products are produced by bacteria; they possess antibiotic activity against gram-positive bacteria and no activity against gram-negative bacteria.^{10, 10} Thiopeptides contain an arrangement of L- and D- amino acids and feature a core pyridine domain that serves as a scaffold for a macrocycle that includes amino acids transformed to a range of heterocyclic rings derived from threonine, serine, and cysteine residues.¹¹ The thiopeptides are classified into five structurally distinct series depending on the oxidation state and heterocyclic core, although only a small number of the peptide have been well studied and their data stored in genomic libraries or genome databases. They include a range of characteristic features including thiazoles, oxazoles, indoles, and dehydroamino acids.¹⁰

Thiostrepton (**1.7**) (**Figure 1.3**) is an oligopeptide antibiotic from the thiopeptide group normally obtained from strains of Streptomyces. The main application of thiostrepton is found in veterinary medicine for the treatment of mastitis that develops as a result of gram-negative organisms, and it's mainly utilized in complex ointments such as nystatin. Thiostrepton has been investigated in the treatment of breast cancer as it shows activity against cancer cells and targets factor forkhead box M1 (FOXO1).^{12,13}

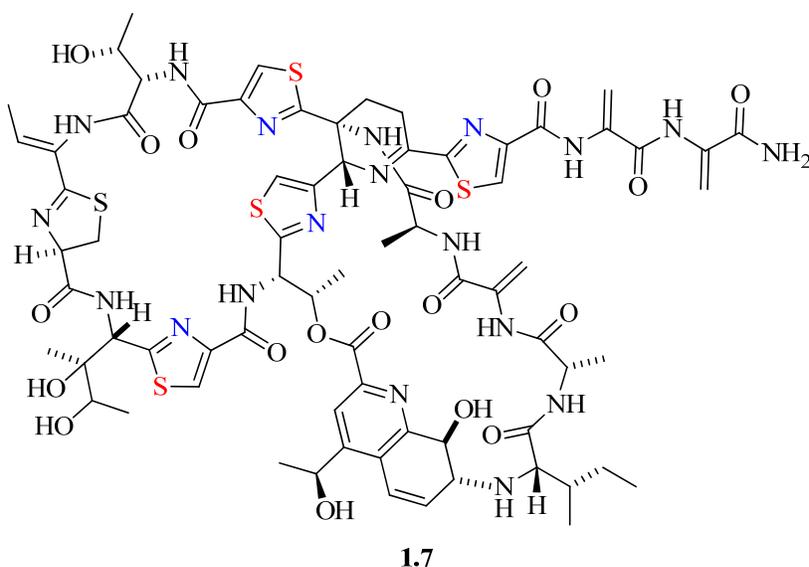


Figure 1.3. Thiostrepton (1.7).

Nocathiacin, which is placed in the E group of Thiopeptides, exhibits multi-drug resistant pathogens both in vivo and in vitro. Nocathiacin has poor water solubility; thus, efforts to study this promising molecule have been hindered, but biotransformation and semi-synthetic approaches have been adopted to generate new derivatives. Biosynthetic manipulation has been the only means through which one can create derivatives with improved properties.¹⁴ Analysis of thiopeptide properties has enabled researchers to exploit the gene cluster of Nocathiacin efficiently. This biologically active thiopeptide is produced by actinomycetes, but in general the thiopeptides have been grouped into around 29 antibiotic families with almost 76 structural differences.¹⁴ The chemical and taxonomic differences do not alter dramatically the activity of most thiopeptides; they are usually active against gram-positive bacteria and dormant when exposed to gram-negative bacteria.¹⁵

Thiopeptides have been shown to exhibit activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), which is a bacterium known to resist many conventional treatments. Current studies of bacteria have enabled researchers to learn more about thiopeptides by understanding their biological features and mode of actions that naturally inhibits bacterium protein development.¹⁶ Other studies

have revealed biosynthetic mechanisms, sources of resistance determinants, and new approaches for chemical synthesis and modification.

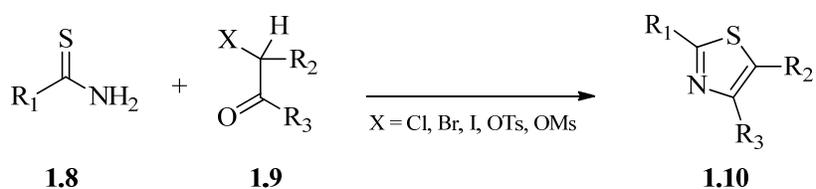
1.4. Methods for the synthesis of thiazoles

1.4.1. Thiazole synthesis

Once an antimicrobial compound has been isolated typically in a small quantity from the natural source, it can then be synthesized in larger bulk for testing and sometimes structure and stereochemical elucidation or validation. Biological optimization can take place making the structure more drug-like such as reducing molecular weight and sometimes improving physiological stability. In the case of amino acid-derived thiazoles, since most are derived from a chiral source, the thiazole building blocks are also chiral and so any method of thiazole synthesis may require to operate under enantiocontrol (usually using a single enantiomer as starting material). However, in many cases and without due care being taken the thiazole moieties can be formed as a racemic or a partially racemized mixture. Drug enantiomers are known for their potential to elicit a distinct biological response.¹⁷ In the synthesis of chiral-thiazole derived peptides a synthesis that proceeds with enantiocontrol is important, as without it yields may be compromised and a means to separate enantiomers might be needed, which can be challenging especially upon a large scale.

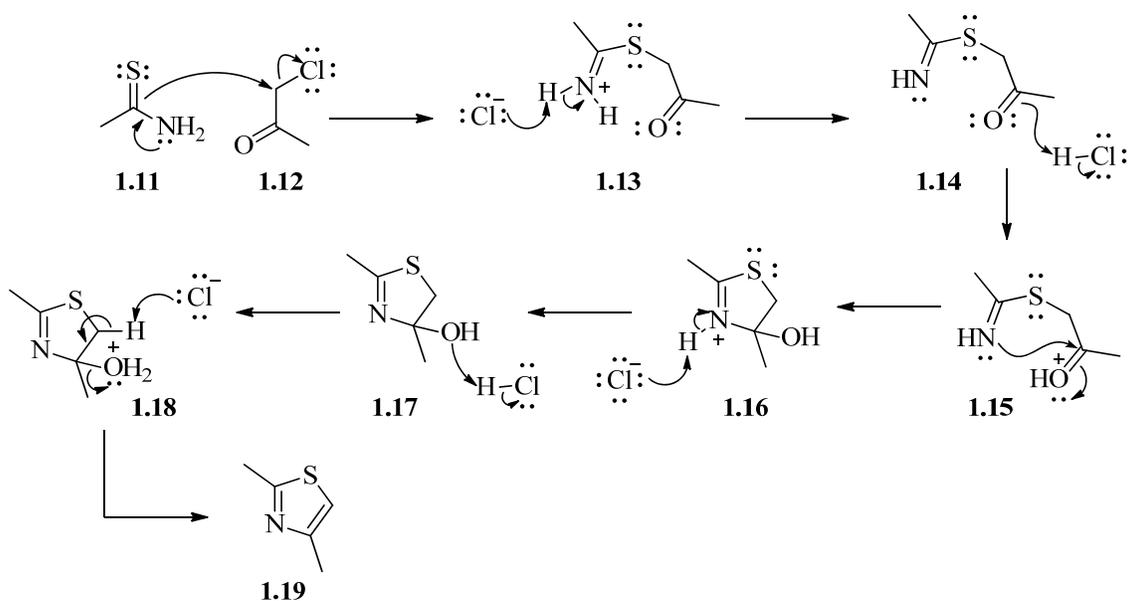
1.4.2. The Hantzsch thiazole synthesis

The Hantzsch thiazole synthesis was developed originally in the late 1880s, using the reacting components as shown in (**Scheme 1.1**)¹⁸ It has been described as one of the most useful and versatile methods for thiazole production.



Scheme 1.1: The Hantzsch thiazole synthesis

The method uses a bis-nucleophile (N-C=S) and reacts it (in ethanol at reflux traditionally) with a two-carbon unit (C-C) with a halogen leaving group, such as an α -halocarbonyl compound by a S_N2 process. This intermediate then cyclises through condensation.¹⁸ The general mechanism is outlined below (**Scheme 1.2**).

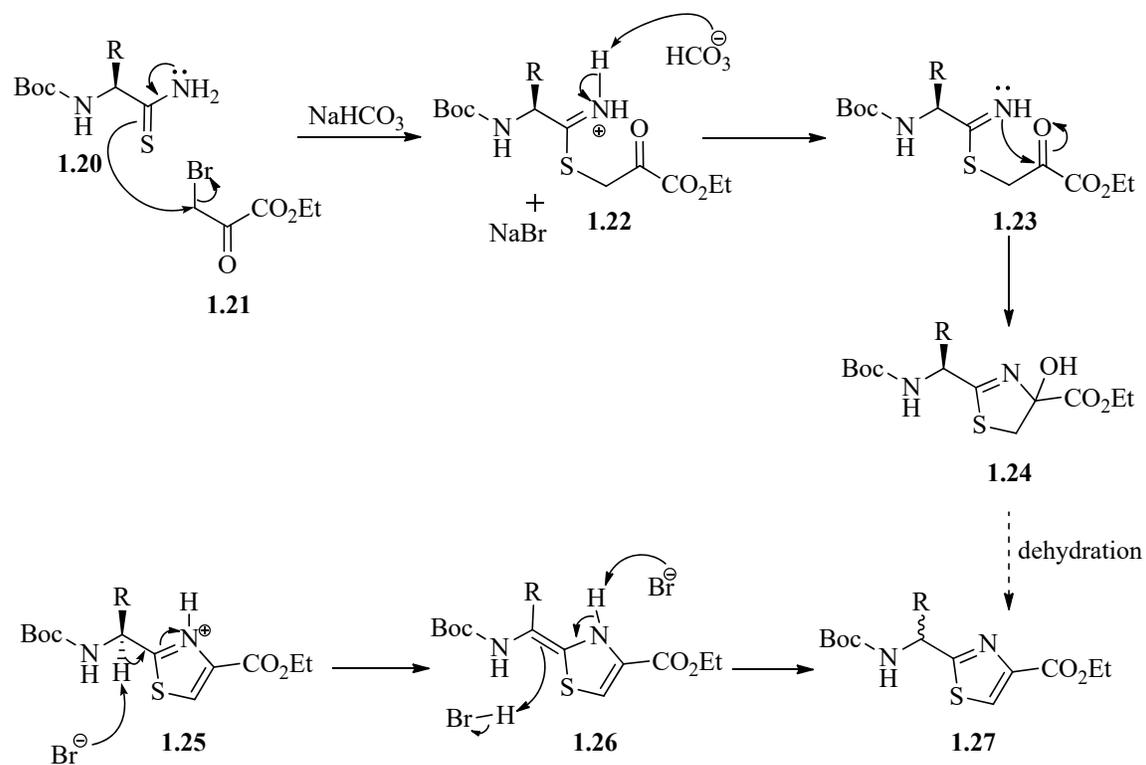


Scheme 1.2: The mechanism of Hantzsch thiazole synthesis.

From this process was developed an amino acid derived thiazole synthesis, showing the S_N2 reaction between a thioamide and an α -chlorocarbonyl compound, followed by dehydration to yield the thiazole **1.19**.^{19, 20}

1.4.3. Racemization of thiazoles with side chains derived from natural amino acids.

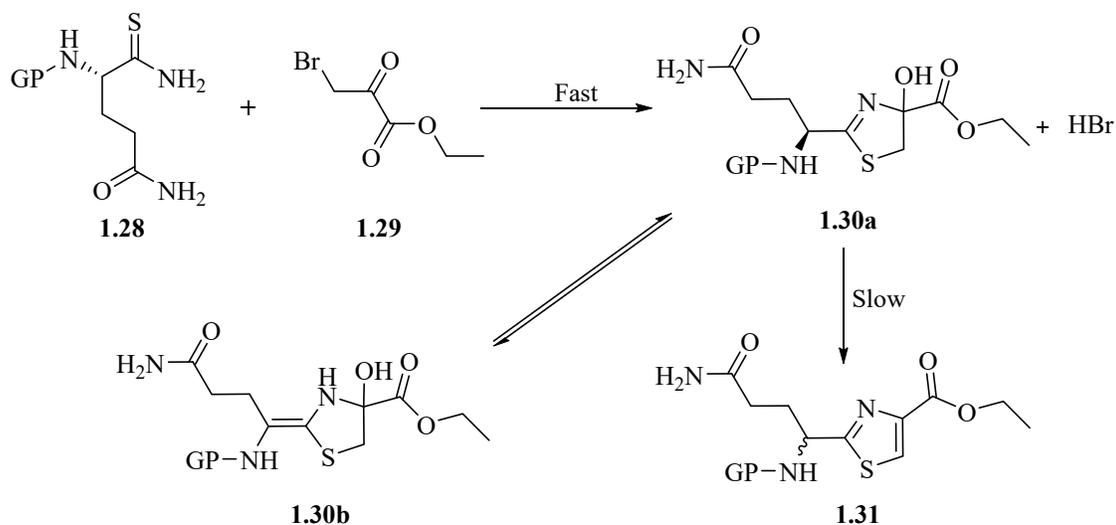
The total synthesis of complex natural products are one of the most difficult and challenging endeavours in organic chemistry. For targets such as micrococcin P1 (**1.54**) (**Figure 1.7**), a compound containing thiazoles with side chains derived from natural amino acids, any synthesis is dependent on the ability to prepare the structural units in high yield and with high enantiomeric purity. The thiazole component would be most easily synthesized by the Hantzsch method. The relevant thioamide precursors are prepared relatively easily, essentially as a single enantiomer, from commercially available starting materials. However, in any process it is necessary to circumvent the problematic racemization produced by the concomitant release of HBr. (**Scheme 1.3**). The reaction in the presence of a base neutralizes the acid produced, but the intermediate hydroxythiazoline **1.24** needs to be isolated and necessarily activated in order to undergo dehydration to the thiazole (**Scheme 1.3**) a process that ordinarily would be done with acid but which is incompatible for the synthesis of these chiral thiazoles.



Scheme 1.3: Effect of the acid and base on the Hantzsch thiazole synthesis.

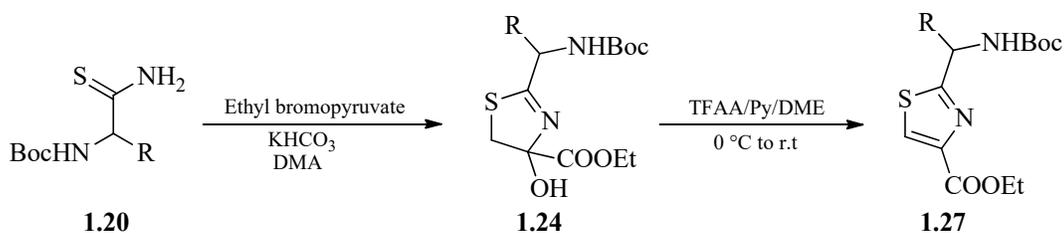
1.4.4. Holzapfel modification of the Hantzsch thiazole synthesis

In 1990, Holzapfel modified the Hantzsch thiazole synthesis with the aim of reducing racemization in the formation of amino acid-derived thiazoles, after working on approaches towards dolastatin 3 (**1.33**) in 1985. Holzapfel suggested an acid-catalysed imine-enamine style equilibration occurred before the aromatization step converting the hydroxythiazoline intermediate to the thiazole (**Scheme 1.4**). This interconversion was deemed to be the cause of racemization.^{21, 22}



Scheme 1.4: Holzapfel's racemization proposal²³

To prevent this issue, the reaction was carried out under basic (KHCO_3) conditions for the $\text{S}_{\text{N}}2$ step and then the reaction temperature lowered to $0\text{ }^\circ\text{C}$ for the dehydration step facilitated through trifluoroacetylation as can be seen in **(Scheme 1.5)**.²²



Scheme 1.5: Holzapfel's Hantzsch modification.²²

Holzapfel also noted the importance of the key intermediate hydroxythiazoline **1.24**, as shown in **(Scheme 1.5)**. Without isolating this intermediate, the thiazole was formed by activating the hydroxythiazoline **1.24** using TFAA in pyridine. This step was reported to proceed with complete retention of configuration, due to the complete suppression under essentially neutral

reaction conditions of the acid-catalyzed imine-enamine interconversion. The base prevented HBr from catalyzing the interconversion assisted by operating at a lower reaction temperature.²²

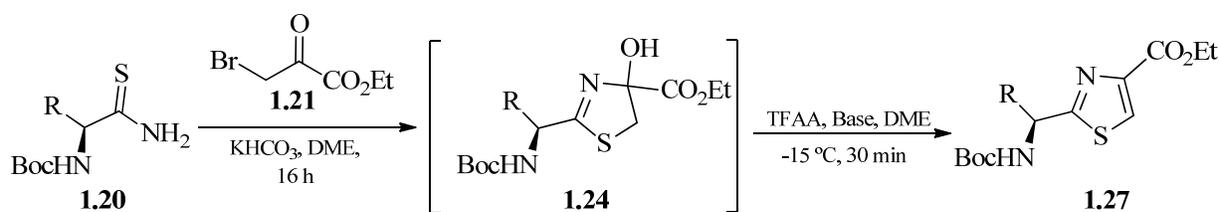
The same protocol was applied to the formation of different amino acid derived thiazoles, all of which were produced with far higher 'efficacy', (as stated in the paper, about retention of configuration) than that achieved by comparable methods of synthesizing similar structures.²⁴

Schmidt, in contrast - in the least cumbersome of the two methods he tested in their 1986 paper followed the Hantzsch method more closely and reacted *N*-Boc-protected thioamides with ethyl bromopyruvate in acetone at -10 °C.^{22, 24} It was noted that this avoided producing the resultant thiazoles in *ee* of 40-60% when the solvent used was ethanol, and also avoided complete racemization observed when the solvent was dioxane or toluene. 1,2-Epoxybutane was added to trap the HBr and prevent the racemization processes shown in (**Scheme 1.4**) and (**Scheme 1.5**). Schmidt then isolated the dihydrothiazole (the thiazoline) and carefully dehydrated this intermediate with TFAA to give the desired thiazoles. The method gave low levels of racemization (no more than 16%) but Holzapfel reported that their method gave complete retention of configuration by comparison.²²

The difference in the retention of configuration achieved between Holzapfel's method and Schmidt's method was Holzapfel's use of base to neutralise the HBr produced. By contrast, Schmidt used 1,2-epoxybutane to trap the HBr and lower temperature to reduce the rate of racemization, but not did manage to suppress the process sufficiently to give near total retention of configuration.²²

1.4.5. Meyers modification of the Hantzsch thiazole synthesis

In 1994 Meyers reinvestigated the modified Hantzsch thiazole synthesis with amino acid derived thioamides. Holzapfel's modification generally provided moderate yield and excellent enantiocontrol of the desired thiazoles.^{25, 26} Although, when Holzapfel's method was utilised for certain amino acid derived thioamides the enantiomeric excess of the product thiazole lowered dramatically as racemization takes place. In order to get to accomplish the stereo-controlled synthesis of alanine-derived thiazoles, Meyers changed Holzapfel's modification by simply varying the base from pyridine to lutidine and lowering the dehydration reaction temperature to -15 °C (**Scheme 1.6**). The isolated yield of the product needed was reported to have improved to 96%, and the enantiomeric excess was higher than 98%.²⁶ The results reported in his paper are shown below in table 1.1.²⁶



Scheme 1.6: The Meyers Hantzsch modification.

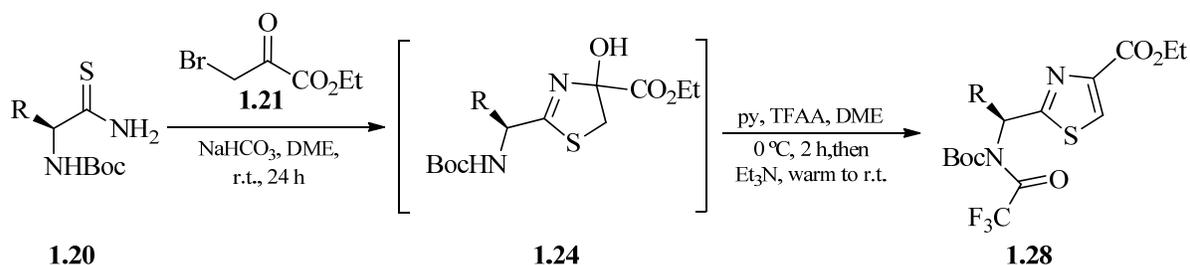
Compd. No	R group	Method	Base	Temp. (°C)	Yield (%)	ee (%)
1	$\text{CH}_2\text{CH}_2\text{CO}_2\text{Bn}$	Holzapfel	Pyridine	0	53	98
2	<i>i</i> - C_3H_7	Holzapfel	Pyridine	0	84	94
3	PhCH_2	Holzapfel	Pyridine	0	69	>98
4	Ph	Holzapfel	Pyridine	0	87	2
5	CH_3	Holzapfel	Pyridine	0	69	48
6	CH_3	Meyers	Lutidine	-15	96	98

Table 1.1: Meyers synthesis of amino acid derived thiazoles.²⁷

The Meyers modification of the synthesis of Hantzsch Thiazole has been successfully used in the synthesis of several natural products including (+)-nostocyclamide and promothiocin A.^{28, 29} In the synthesis of both (+)-nostocyclamide and promothiocin A, the Meyers-Hantzsch reaction conditions employed differ slightly from those reported by Meyers in that the addition of ethyl bromopyruvate in the first step, and the addition of the TFAA - 2,6 -lutidine solution in dehydration step was stirred at -40 °C, and then it was heated to -20 °C, and then it was stirred for the times indicated in (**Scheme 1.6**). Despite this method being highly effective in this case it remains a challenging and involved procedure, which is highly substrate dependent requires a large excess of the bromopyruvate and multiple purification steps and is poorly reproducible.²⁷ New improved methods that are more facile and reliable are still urgently required.

1.4.6. Nicolaou modification of the Hantzsch thiazole synthesis

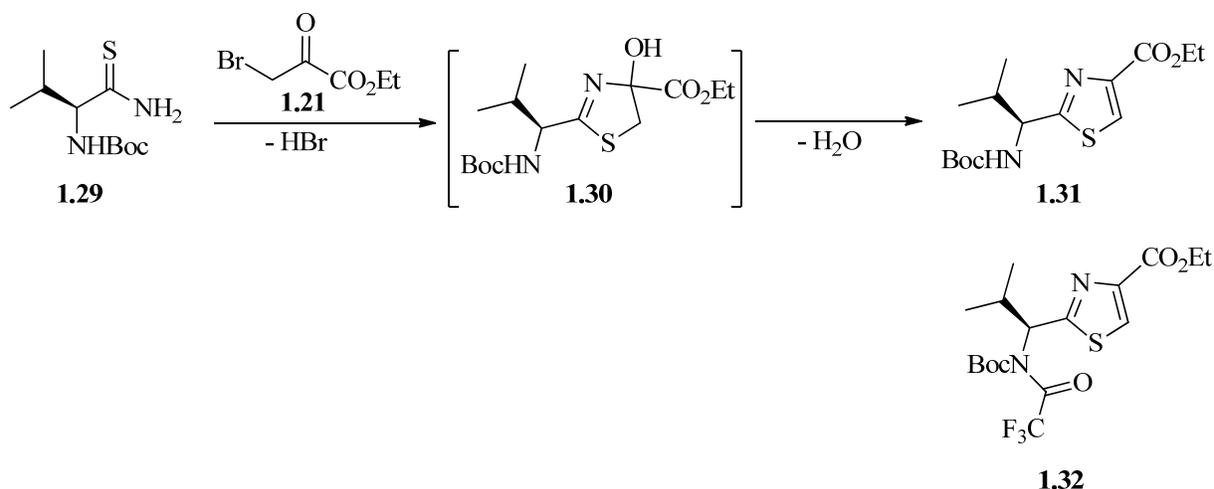
As mentioned, Nicolaou further investigated the Hantzsch thiazole synthesis in 2005 as part of his total synthesis of Thiostrepton and processes developed by Holzapfel and Meyers were reviewed and revisited.²⁶ The Nicolaou-Hantzsch thiazole synthesis was developed to solve some of the many problems with the Meyers-Holzapfel approach. Nicolaou investigated changes in the base and a significant increase in the reaction temperature (**Scheme 1.7**) was replaced 2,6-Lutidine as used by Holzapfel with pyridine which was then used with TFAA at 0 °C for 2 h, not as a pre-mixed solution as used by Holzapfel and Meyers.³⁰ The Nicolaou-Hantzsch thiazole synthesis led to the trifluoroacetylation of any free NH groups in the target molecule, a problem that can accompany the Meyers modification.



Scheme 1.7: The Nicolaou-Hantzsch thiazole synthesis.

1.4.7. Bagley-Merritt modification of the Hantzsch thiazole synthesis

Merritt and Bagley used the Hantzsch thiazole synthesis as part of the methodology for the synthesis of micrococcin PI **1.54** - with Nicolaou's modification used in the stereo selective synthesis of one thiazole component (**Scheme 1.7**) although simple Hantzsch method was appropriate for the synthesis of an a chiral thiazole building block.²⁵ Micrococcin PI **1.54** has a chiral valine-derived thiazole present in the cyclic peptide structure. In the 2007 paper by Merritt and Bagley, the Holzapfel, Meyers and Nicolaou modifications were tested and compared with the original Hantzsch method (Table 1.2) for the development of a valine-derived thiazole without significant loss of enantiopurity (**Scheme 1.8**).³¹



Scheme 1.8: Formation of valine-derived thiazoles **1.31** and **1.32**.

Entry	Method	Quantity Of 5 (g)	Solvent	Step 1		Step2		Yield (%)	ee ^a (%)
				Temp (°C)	Base	Temp (°C)	Reagents		
1	Hantzsch		EtOH	Reflux	-	Reflux	-	0 ^b	-
2	Holzapfel	0.050	DME	0	KHCO ₃	0	TFAA, 2,6-Lu ^c	33	>98
3	Meyers	0.22	DME	-40 to- 20	KHCO ₃	-40 to -20	TFAA, 2,6-Lu ^c	9	>98
4	Meyers	1.0	DME	-40 to -20	KHCO ₃	-40 to -20	TFAA, 2,6-Lu ^c	59	88
5	Nicolaou	0.3	DME	r.t.	NaHCO ₃	0	TFAA, py ^d , Et ₃ N	85	88 ^e

a) Determined using chiral HPLC (stationary phase ChiralPac AD column)
b) No product isolated
c) 2,6-Lu: 2,6-lutidine
d) py: pyridine
e) Only **1.32** isolated; *ee* determined following solvolysis to **1.31**

Table 1.2: Investigation of literature methods for the synthesis of thiazole **1.31**.³¹

As tabulated below, Merritt and Bagley compared the Hantzsch thiazole synthesis using the Holzapfel, Meyers and Nicolaou modifications for the conversion of thioamide **1.29** to thiazole **1.31** (Table 1.2).

³¹ The traditional Hantzsch method (entry 1) was shown to be totally unsuitable for this substrate. The acidic conditions generated in the method were suspected by Merritt and Bagley to have caused the HBr-catalysed cleavage of the Boc- protecting group, resulting in loss of product in the work-up and purification steps. Holzapfel (entry 2) and Meyers (entries 3 and 4) modifications did deliver as the thiazole product **1.31** but either in low yield when carried out on small scale (entries 2 and 3) or with loss of retention of configuration in a larger scale test (Entry 4), which was as Merritt and Bagley noted, in accordance with Pattenden's observations.³² The Nicolaou modification (Entry 5) yielded

only the trifluoroacetylated thiazole product **1.32**. The trifluoroacetyl group was removed from the Boc-amide nitrogen through solvolysis.³¹ This led to an extended procedure but was the only method that approached the original claims of Meyers in terms of yield and enantiopurity.

As none of the tests provided a product of adequate quality of both yield and enantiomeric excess, Merritt and Bagley developed further modifications of the existing Hantzsch modifications, focusing on Meyers and Nicolaou methodologies.³¹ Keeping the other conditions in the Nicolaou modification the same whilst replacing the pyridine used in Nicolaou's method with 2,6-lutidine as used in other modifications, resulted in a mixture of **1.31** and **1.32** (overall yield 88%). The formation of mixed thiazole products was less than desirable despite the 91% enantiomeric excess found for both products. The Meyers method was then repeated at -18 °C which drastically improved the yield from 59% to 97%, though oddly this increase in temperature gave rise to the trifluoroacetamide **1.32** instead of the expected **1.31**. Nicolaou's solvolysis treatment using NaHCO₃ released the desired thiazole **1.32** which was found as a near single enantiomer at >98% enantiomeric excess. It was also found that the hydroxythiazoline intermediate **1.30** could be synthesized as a single enantiomer at room temperature using ethyl bromopyruvate, KHCO₃, DME after 2 h - showing that under basic conditions, racemization in the Hantzsch synthesis occurs in the elimination step as shown in (**Scheme 1.3**).³¹

Attempts were made to avoid the formation of trifluoroacetamide **1.32** by changing the dehydration conditions in order to avoid a lengthy reaction scheme.³¹ Methanesulfonyl chloride and triethylamine were applied in the dehydration step but though they varied the MsCl equivalents, the temperature and the reaction time utilised, it was not possible to find conditions that gave both high yield and enantiomeric purity. Excellence could be achieved in one property, but at the cost of the other i.e. use of 1.1 equivalents of MsCl used at room temperature for 1 hour without work-up gave 70% yield but

only 38% ee, whilst the same equivalence of MsCl used whilst raising the temperature from 0 °C to ambient with a reaction time of 2 hours gave excellent retention of configuration at >98% ee, but 33% yield before work-up, which reduced to 15% yield after the work-up. Even increasing the reaction time to 48 hours did nothing to improve the yield.

It was recognised that the racemization was exothermic but that this exothermic reaction was also responsible for providing a respectable yield.³¹ Merritt and Bagley investigate other methods, but the use of a microwave synthesiser gave only moderate yield (68%) and poor ee (12%). Other thermal conditions achieved the synthesis of a single enantiomer of **1.31**, but in only 26% yield.³¹ It was reported that tests with *p*-toluenesulfonyl chloride as the activating agents did not result in any thiazole formation at all regardless of the conditions used and it was consequently concluded by Merritt and Bagley that the Meyers-Nicolaou modification was the most effective method, despite the hazards of the reagents multistep process, purification demands and route via the problem of the trifluoroacetamide **1.32**. This a simple approach to chiral thiazole products directly in high enantiopurity that can operate reliably and reproducibly at scale remains unsolved.

1.5. Methods for the synthesis of cyclic peptides

1.5.1. Dolastatin 3

Dolastatin 3 (**1.33**) is a cyclic peptide anticancer agent isolated from the Indian Ocean sea hare *Dolabella auricularia*³³ (**Figure 1.4**).³⁴ This structure is the revised arrangement.³⁵ The cyclic peptide is important as a cell growth inhibitor of murine P388. lymphocytic leukaemia cells.³³

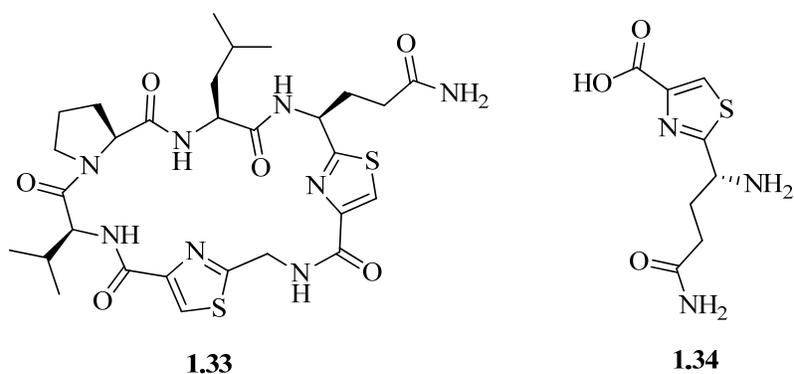
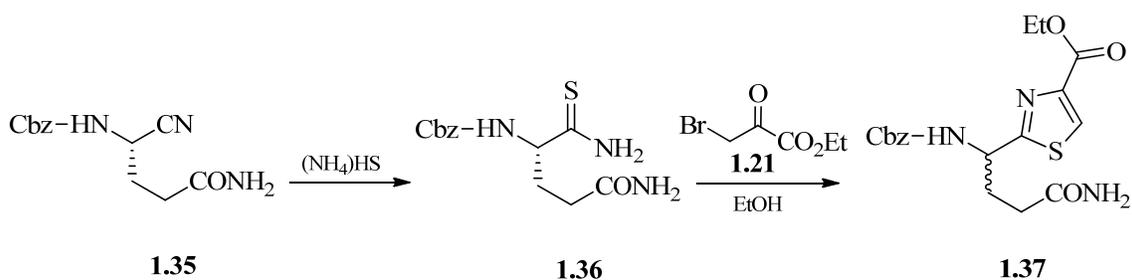


Figure 1.4: Dolastatin 3 (**1.33**) and (Gln)-Thz derivative **1.34**.^{33, 35}

The need to develop a synthesis of the thiazole component (Gln)-Thz **1.34** from L-glutamate led to modifications of the Hantzsch method. Initially, the synthesis of the precursor for the Hantzsch method, thioamide glutamate-derived was achieved by reacting the corresponding nitrile **1.35** with ammonium hydrosulfide. Condensation of the resulting thioamide **1.36** with ethyl bromopyruvate **1.21** by reflux in ethanol for 15 minutes gave the corresponding thiazole **1.37** but the method resulted in complete racemization (**Scheme 1.9**). This was attributed to the slow rate of acid catalysed dehydration of the intermediate hydroxythiazoline, facilitating complete racemization through an acid-catalysed imine-enamine style equilibration.^{21, 22}



Scheme 1.9: Synthesis of thiazole **1.37**.^{21, 22}

A method that did not lead to racemization was not achieved in the work published in 1985, however a new Hantzsch modification was established under far milder conditions that was used to synthesise

the *N*-Boc-DL-(Gln)Thz ethyl ester.²¹ This modification reacted the thioamide with an excess of ethyl bromopyruvate in DMF (a polar aprotic solvent) for 40 minutes at room temperature, followed by dilution with ethyl acetate. Holzapfel did go on to develop a modification of the Hantzsch method that could provide excellent retention of configuration in the synthesis of the (Gln)-Thz derivative **1.34** referred to as the Holzapfel Modification.

1.5.2. (-)-Bistratamide C

(-)-Bistratamide C **1.38** (Figure 1.5) is a cytotoxic cyclic peptide with two valine-derived amino acid residues, originally isolated from *Lissoclinum bistratum*, containing an oxazole and two thiazole groups.³⁶

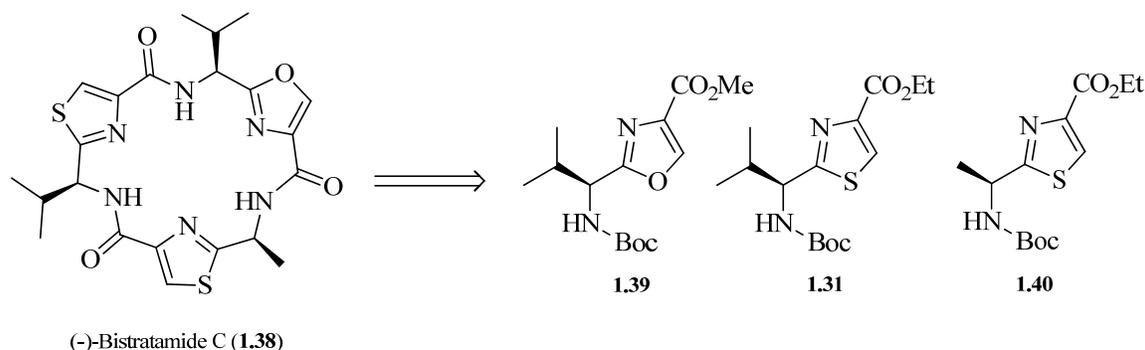
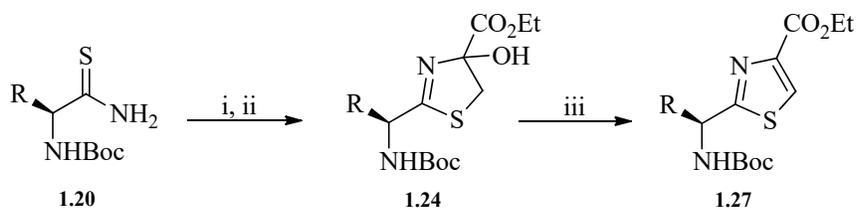


Figure 1.5: Retrosynthesis of **1.38**.

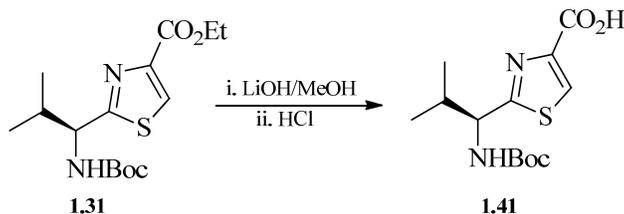
Meyers carefully developed and published a synthesis of this cyclic peptide in 1994,^{27,37} and based the synthesis of the thiazole precursors, residues **1.24** and **1.27** (Scheme 1.10)³⁷ on the Holzapfel modification of the Hantzsch thiazole synthesis. The synthesis of these thiazoles proceeded as shown below.



Scheme 1.10: Synthesis of thiazoles of the type **1.27**.

Reagents and conditions: i) KHCO_3 , DME, $-15\text{ }^\circ\text{C}$; ii) ethyl bromopyruvate; then the isolation of the intermediate and removal of heterogeneous base; iii) TFAA, 2,6-Lutidine, DME, $-15\text{ }^\circ\text{C}$.^{27,31}

These conditions were found to be optimal both in terms of yield and stereoselectivity. The optimization of these precise variables was discussed in Meyers modification. Ethyl ester **1.31** then underwent basic hydrolysis (LiOH , MeOH) to give the free acid **1.41** after acidic work up (**Scheme 1.11**).



Scheme 1.11: Hydrolysis of ester **1.31** to **1.41**.

The linear peptide was prepared via mixed anhydride methodology³⁷ similar to the process for nostocyclamide synthesis.²⁸ The oxazole and thiazole-containing residues were activated by treatment with chloroformate and reacted with free amine tert-butoxycarbonyl deprotection through in situ generation of HCl (step ii) and reaction with the next activated residue built up the linear peptide macrolactamization by C-terminus deprotection using base hydrolysis followed by treatment with DPPA under condition of high dilution gave (-)-bistratamide C **1.38** in 70% yield.³⁷

1.5.3. (+)-Nostocyclamide

(+)-Nostocyclamide **1.42** (**Figure 1.6**) is an anticyanobacterial and antialgal cyclopeptide which shows toxicity against *Brachinus Calyciflorus*.²⁹ Originally found to be synthesized by the marine-based dinitrogen-fixing cyanobacteria, Nostoc 31, this secondary metabolite contains alanine, valine, glycine, threonine and two possible cysteine amino acid residues that have been modified by the organism. The cyclic hexapeptide's amino acid residues have been modified upon cyclodehydration to form the thiazole and oxazole substructures following ribosomal protein synthesis in the cell.²⁹

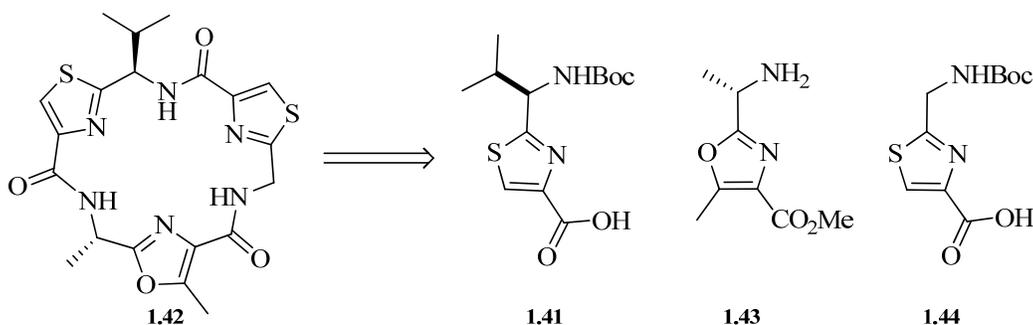
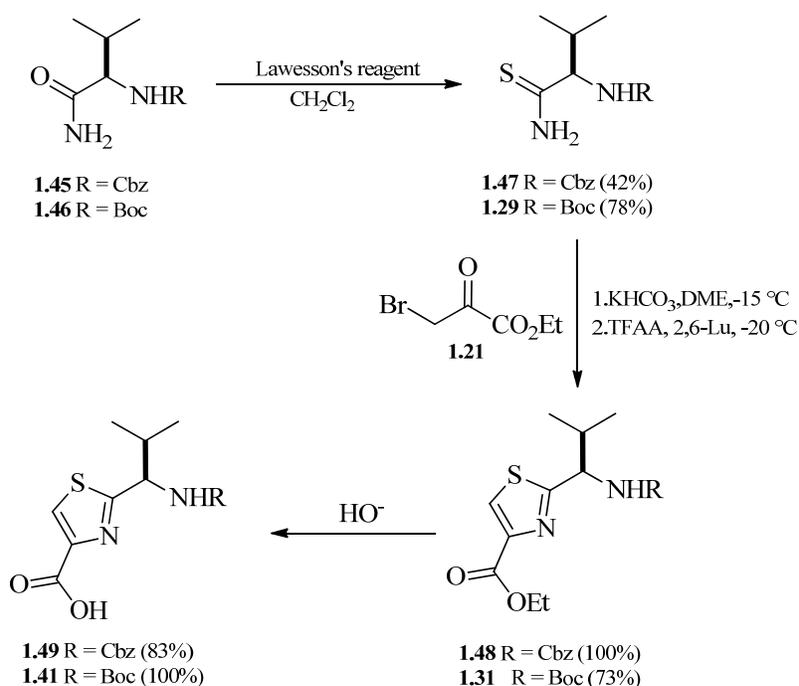


Figure 1.6: Retrosynthesis of (+)-Nostocyclamide **1.42**.

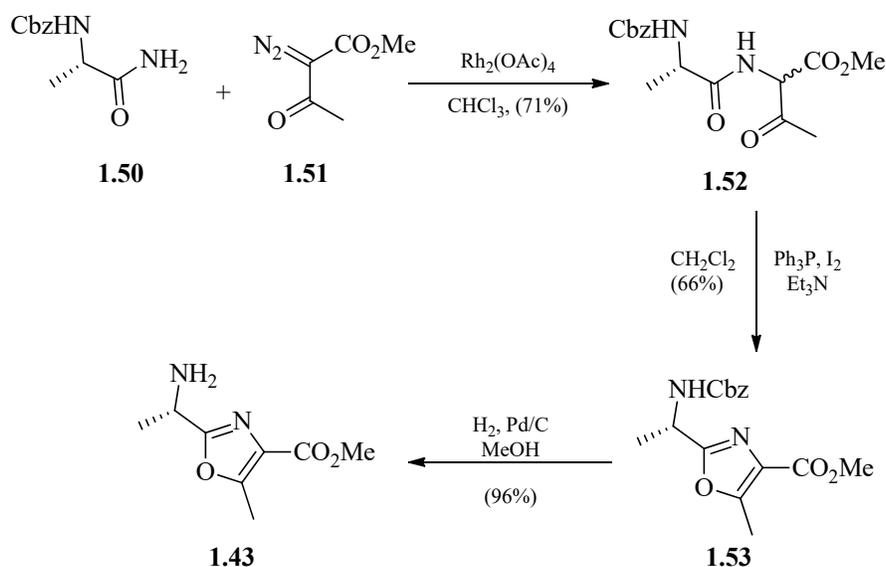
In 1998, Bagley and Moody published the first synthesis of (2*S*, 12*R*)-nostocyclamide using a Boc protecting strategy and the disconnections shown (**Figure 1.6**) to provide appropriate precursors.²⁸ Thiazole-containing building blocks **1.41** and **1.44** were synthesized using a modified Hantzsch reaction following on from work done by Meyers in the synthesis of the cyclic peptide, (-)-bistratamide C **1.38** which in turn was previously based on modifications developed by Holzapfel. Initially Cbz was used as *N*-protecting group during the synthesis of thiazole **1.49**, but low yields in the thionation step (**Scheme 1.12**) led to the replacement of the Cbz group with the more sterically encumbered Boc protecting group **1.46**. Thioamide **1.29** was reacted with EBPY **1.21** under basic conditions at -15 °C followed by dehydration using Trifluoroacetic anhydride

and 2,6-lutidine to give the Thiazole-4-carboxylic acid ethyl ester **1.31**. The thiazole amino acid **1.41** was provided by hydrolysis with Sodium hydroxide in aqueous Tetrahydrofuran. Whilst the glycine-derived thiazole **1.44** did not need a stereoselective route, it was successfully synthesized using the same methodology to prevent the acid mediated deprotection of the Boc protecting group.²⁸



Scheme 1.12: Synthesis of ester **1.49** to **1.41**.

The 5-methyloxazole-4-carboxylic acid **1.43** was readily prepared using a rhodium (II) acetate $\text{Rh}_2(\text{AcO})_4$, as catalysed reaction of Cbz-alaninamide **1.50** with methyl diazoacetoacetate **1.51**, resulting in chemoselective *N-H* insertion to give the keto amide **1.52**, The N-protected oxazole ester **1.53** was cyclodehydrated using the triphenylphosphine iodine-triethylamine procedure.³⁸



Scheme 1.13: Synthesis of 5-methyloxazole-4-carboxylic acid **1.43**.

Finally, the required oxazole amino ester **1.43** was deprotected by catalytic hydrogenolysis over palladium-on-carbon (**Scheme 1.13**). With the residues prepared, the thiazole-4-carboxylic acid **1.41** was activated with isobutyl chloroformate and NMM in THF at 0 °C, and reacted with the oxazole-derived amine **1.43**, followed by *N*-deprotection under acidic condition and a second coupling with the glycine was carried out by pentafluorophenyl ester methodology, which on basification gave the free base and cyclized to give the desired (2*S*, 12*R*)-nostocyclamide cyclic peptide in reasonable yield.²⁸

1.5.4. Micrococcin PI

This multi-thiazole containing natural product inhibits the viral attachment of the Hepatitis C virus and can be used as a combinatorial therapeutic agent.³⁹ The cyclic peptide Micrococcin PI (**1.54**) (**Figure 1.7**)⁴⁰ interferes with HCV entry at an attachment stage, and inhibits HCV spread by prevents cell-to-cell transmission and prevents cell-free infection, without affecting the secretion of infectious virions.³⁹

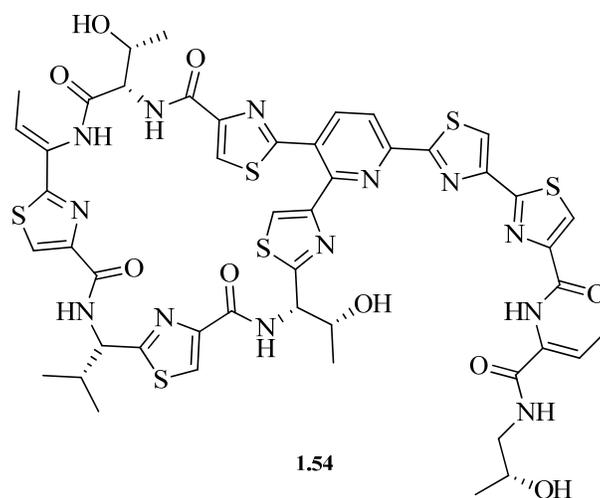


Figure 1.7: Micrococcin P1.

Micrococcin was the first thiopeptide antibiotic ever discovered⁴¹ - found by Su in a sewage sample from Oxford, England, and reported without any structural analysis.⁴⁰ Micrococcin P1 (**1.54**) was obtained from a strain of *Bacillus pumilus* in the mid-1950s by Fuller using a sample of soil from East Africa.²³ It was deemed identical to the sample Su had isolated earlier with the exception of the optical rotation. It was found that Fuller's sample existed as a 7:1 mixture of micrococcin P1 and micrococcin P2.⁴⁰ Micrococcin P1 (**1.54**) has also been found as a metabolite of foodborne *Staphylococcus equorum*.^{39, 40} Micrococcin demonstrates cytotoxicity; inhibiting ribosomal protein synthesis by selectively binding to the GTPase-associated centre (GAC) of the ribosome, inhibiting translation factor function.⁴⁰ Bagley and Merritt developed a convergent synthesis of the micrococcin P1 (**1.54**) central heterocyclic domain **1.55** (**Figure 1.8**) in 2007 using Nicolaou's Hantzsch modification of methodology to form the various thiazole moieties.^{25, 26} This alternative approach to the thiazole units was adopted due to difficulties with the conventional Meyers methodology.

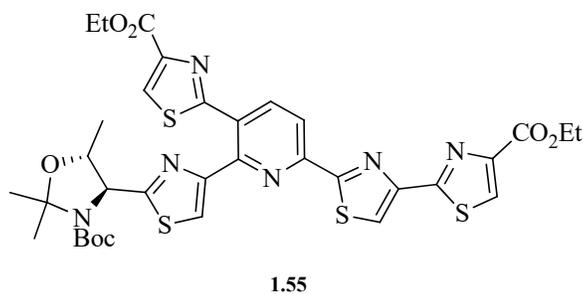


Figure 1.8: Synthesis of Micrococcin PI central heterocyclic domain **1.55** by Bagley and Merritt.

The Bagley approach established the central domain through Bohlmann-Rahtz pyridine synthesis. The thiazole group in the two designated precursors, enamine **1.57** and propynone **1.58** (**Figure 1.9**) were synthesized through two different Hantzsch modifications.²⁵

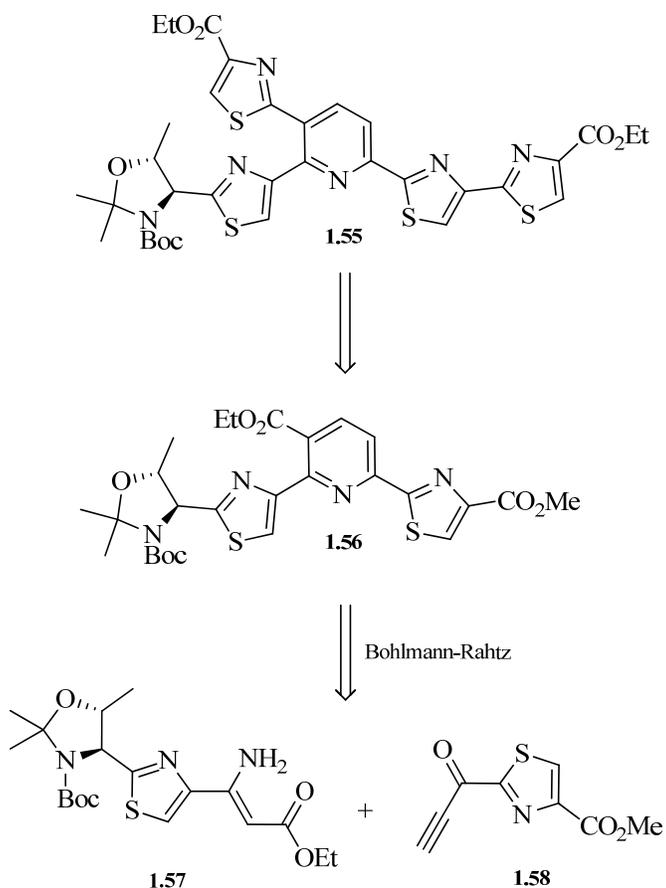
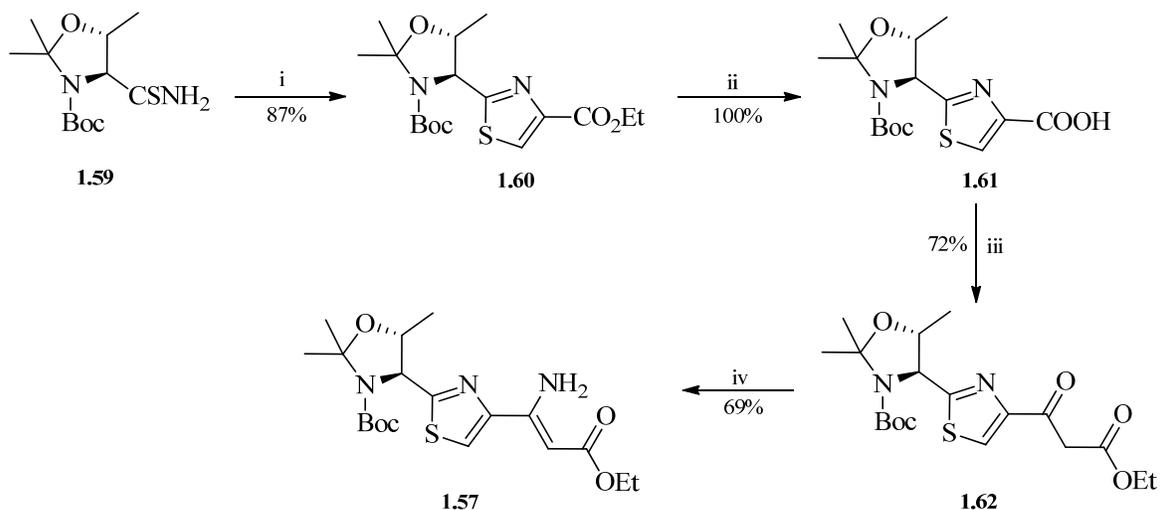


Figure 1.9: Synthesis of Micrococcin PI.

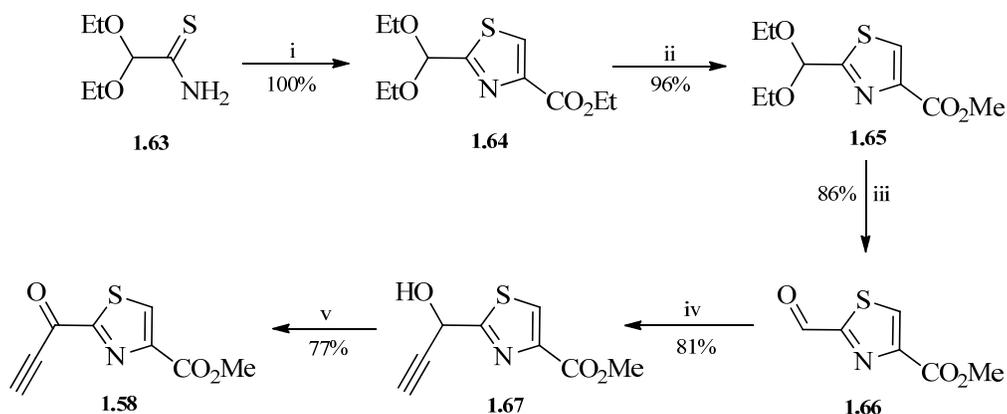
The thiazole component **1.57** of **1.55** was synthesized as shown in (Scheme 1.14) using Nicolaou based methodology.²³ The thioamide was synthesized via a threonine-derived nitrile reacting with ammonium sulfide with 100% yield and no need for column chromatography purification.



Reagents and conditions: **i:** ethyl bromopyruvate, NaHCO₃, DME, r.t., 24 h, then TFAA, pyridine, 2 h then Et₃N. **ii)** LiOH, MeOH–H₂O, r.t., 3 h, 100%. **iii:** EtOCOCl, Et₃N, THF, 0 °C, 1 h, then potassium ethyl malonate–methylmagnesium bromide mixture in THF, 2 h, 72%. **iv:** NH₄OAc, toluene–AcOH, microwave irradiation, 150 °C (300 W initial power), 15 min, 69%.²⁵

Scheme 1.14: Synthesis of thiazole component **1.57** using Nicolaou based methodology.²³

The thiazole component in **1.58** was synthesized as shown in (Scheme 1.15) using the following and simpler Hantzsch method.



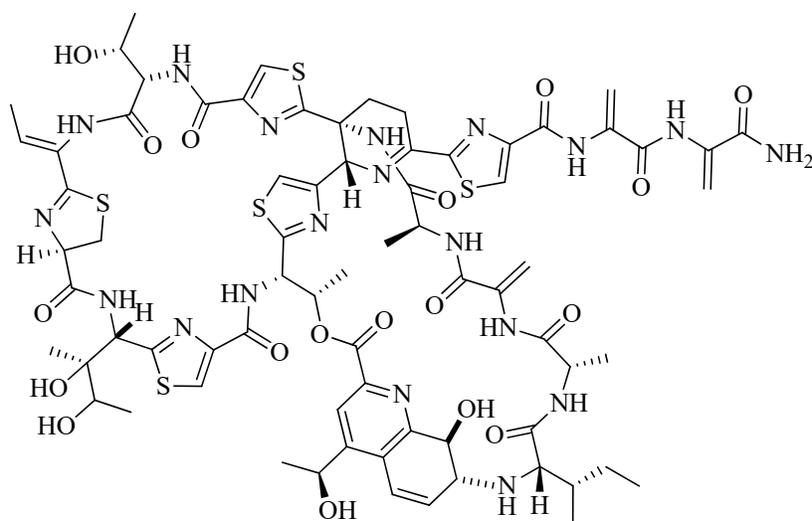
Reagents and conditions: **i:** ethyl bromopyruvate, 4Å molecular sieve, EtOH, reflux, 1 h with 100% yield. **ii:** PTSA, MeOH, reflux, 24 h, 96%. **iii:** 2 M aq HCl, acetone, reflux 2h, 86% **iv:** ethynylmagnesium bromide, THF, 0 °C, 1 h, 81%. **v:** activated MnO₂, CH₂Cl₂, r.t., 2 h, 77%.²⁵

Scheme 1.15: Synthesis of thiazole component **1.58** using Nicolaou based methodology.²³

Enamine **1.57** and Michael acceptor **1.58** were reacted together in a Bohlmann-Rahtz reaction to give an aminodienone intermediate, cyclodehydration of which was achieved successfully using iodine as a catalyst to give pyridien **1.56**. The formation of two additional thiazole units as shown in (**Figure 1.9**) was achieved in a two-directional multistep process to give the central heterocyclic domain **1.55** of Micrococcin P1 (**1.54**).²⁵

1.5.5. Thiostrepton

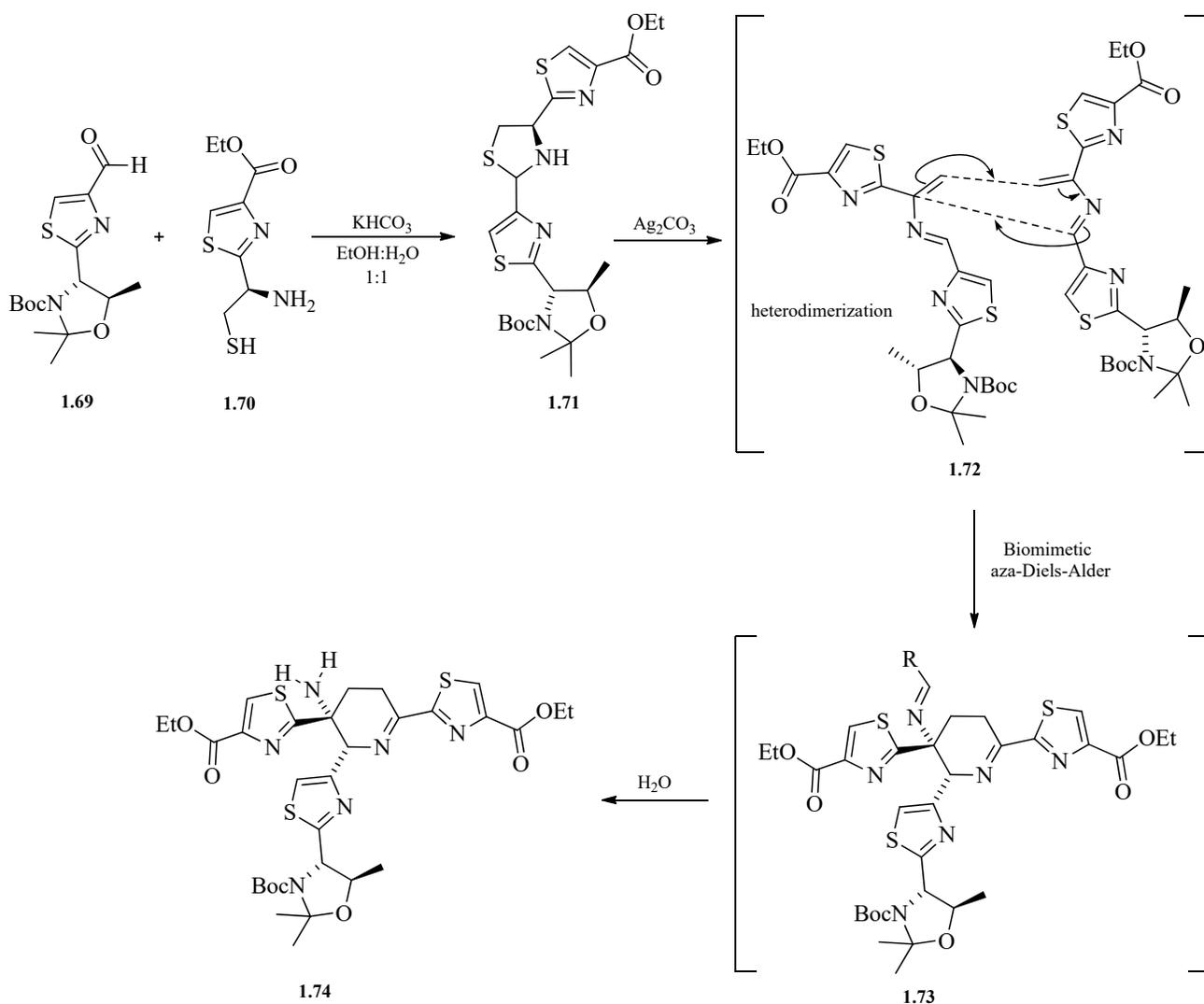
Thiostrepton (**1.7**) is a natural antibiotic often viewed as the parent of the thiopeptide class and is derived from streptomycetes which is the most extensive set of the actinobacteria.



1.7

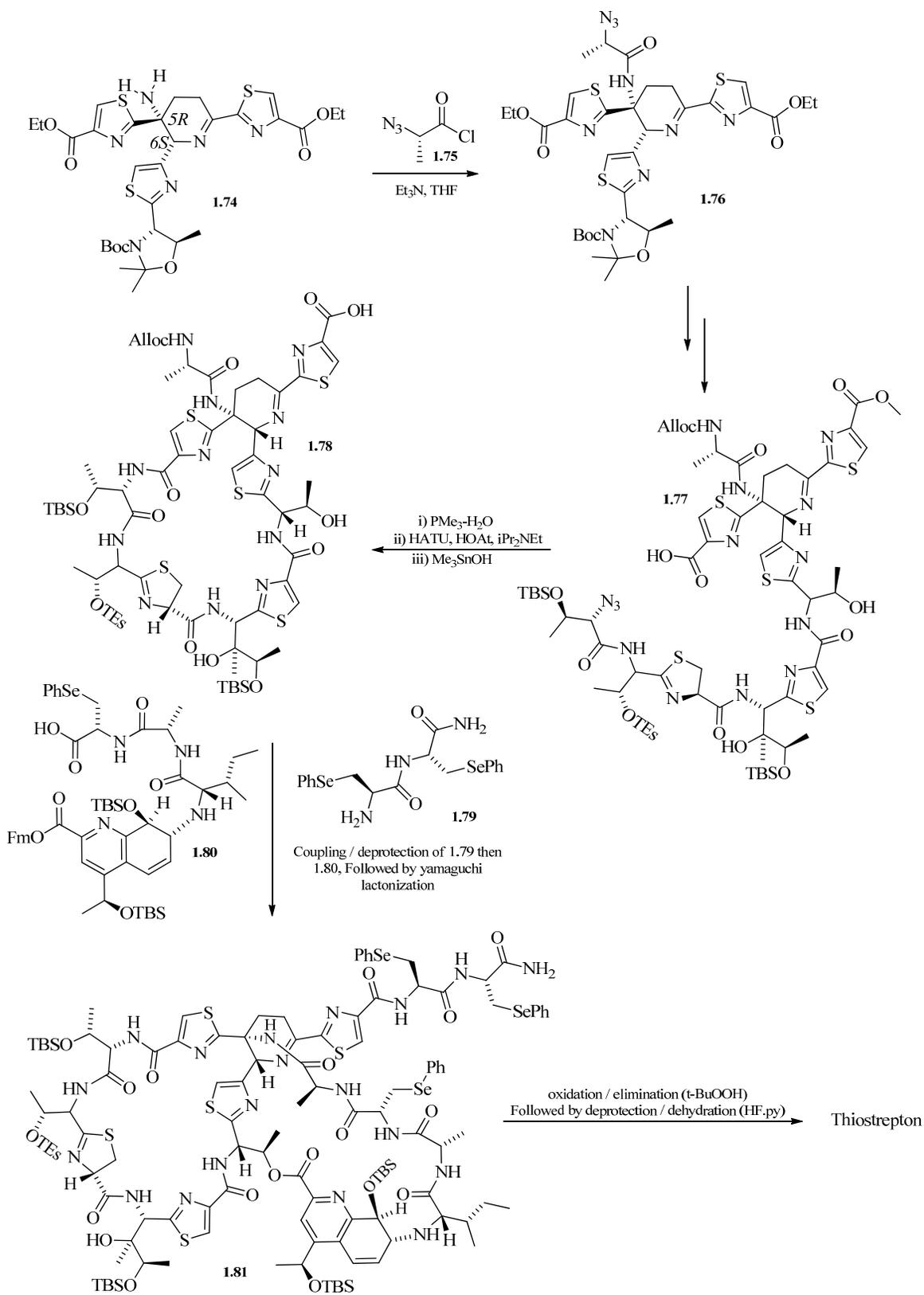
Figure 1.10: Thiostrepton (1.7).

The antibacterial properties of thiostrepton were discovered in 1955 by Donovan, and numerous studies have been undertaken about this antibiotic and its properties.²⁶ Thiostrepton has found a number of applications, including the development of a veterinary medicine, use in complex ointments, application in topical steroids, and use in molecular biology to determine the right selection of genes involved in nucleotide metabolism.⁴² A large portion of the thiostrepton structure was established through the use of x-ray crystallography, together with evaluative studies conducted by various scholars in the 1970s.⁴³ The total synthesis of thiostrepton has been achieved and involves multiple steps, for this is a highly complex thiopeptide antibiotic. Retrosynthetic analysis of thiostrepton shows that it requires a variety of building blocks **1.74**, **1.75**, **1.79**, **1.80** and thiazoline-thiazole subunits.^{42,26} The key step in Nicolaou's approach involves the construction of tetrahydropiperidine **1.74** by a biomimetic strategy based on the dimerization of an azadiene system (**Scheme 1.16**).



Scheme 1.16: Biomimetic synthesis of the piperidine domain of thiostrepton.

The method involves coupling labile dehydropiperidine building block **1.74** to a stable peptide **1.76** by capturing a reactive alanine equivalent **1.75** under specific conditions that avoided the ring contraction process (**Scheme 1.17**). Dehydroalanine tail **1.79** and quinaldic acid **1.80**, together with thiazoline-thiazole subunit **1.78** was developed by a complex series of stereoselective procedures.⁴⁴ Thiostrepton synthesis was finally complete with an oxidation of three compounds present in the phenyl seleno-groups, which brought about selenoxide syn elimination. Utilising the above process led to the synthesis of a thiostrepton with similar features to an authentic sample.



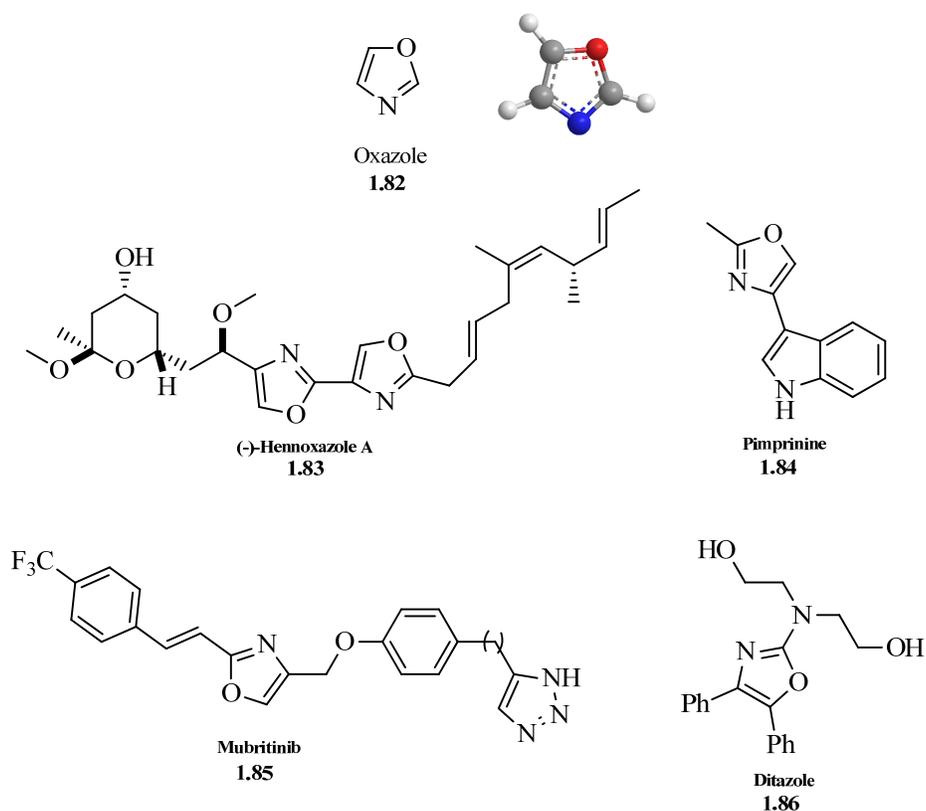
Scheme 1.17: Total synthesis of thiostrepton (**1.7**).

1.6. Robinson-Gabriel synthesis of oxazoles

1.6.1. Oxazole

Oxazole is a planar five membered heterocycle which is peculiar in its structure (**Figure 1.11**) and the scaffold is a component of numerous natural products such as (-)-Hennoxazole A (antiviral) ⁴⁵ and Pimperinine (alkaloid) ⁴⁶ with a good biological activity. Oxazole-bearing compounds have been extensively used as diabetes II treatment e.g. Aloglitazar, ⁴⁷ as COX-2 inhibitors such as Oxaprozin ⁴⁸ platelets aggregation inhibitor e.g. ditazole, ⁴⁹ as part of tyrosine kinase inhibitor such as mubritinib.

50



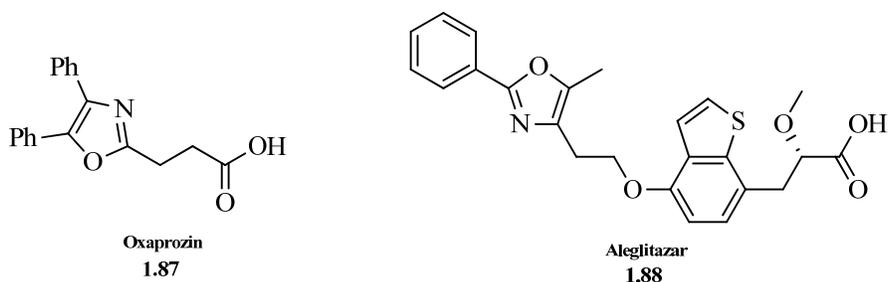
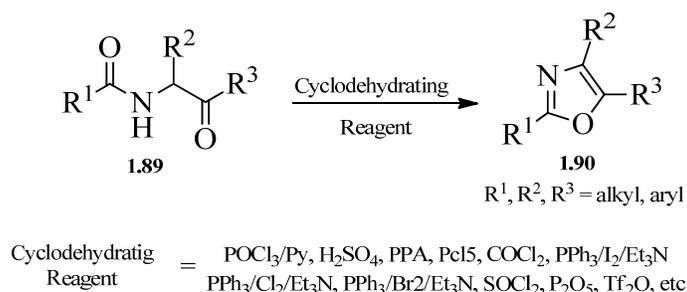


Figure 1.11: Structure of oxazole and its derivatives found in nature and in some drugs.

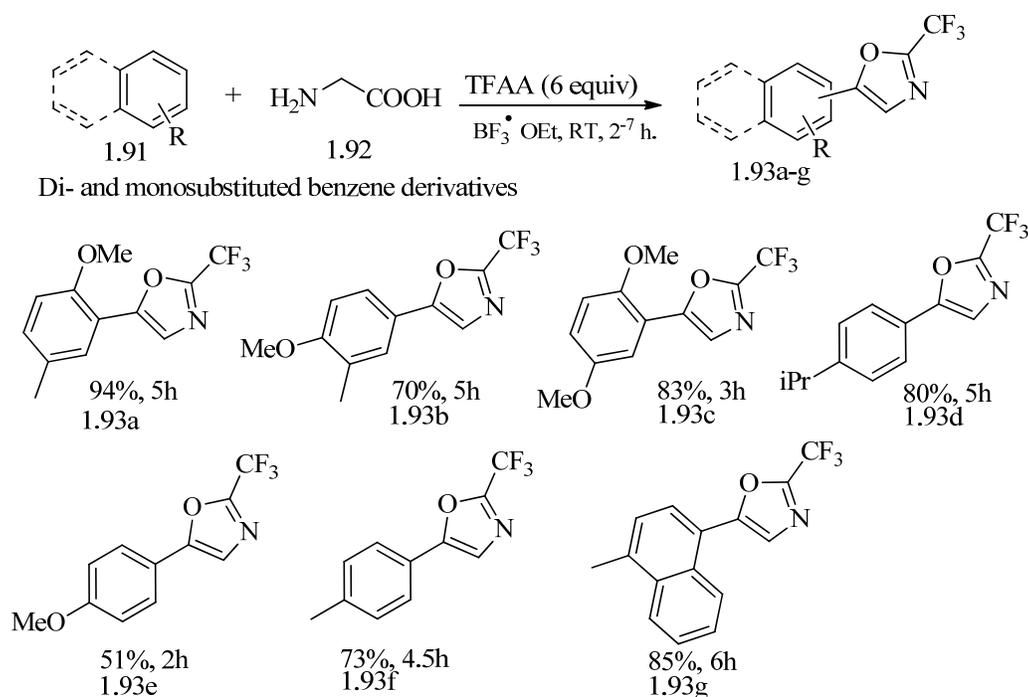
1.6.2. Background

Gabriel in 1907⁵¹ and Robinson⁵² in 1909 reported a novel protocol to synthesise 2,5- and 2,4,5-substituted oxazole derivatives via intramolecular condensation and subsequent dehydration of *N*-acyl α -aminoketones in the presence of strong dehydrating agents like PCl_5 and sulfuric acid. The reaction is also known as Robinson-Gabriel synthesis,⁵³ Robinson-Gabriel dehydration,⁵⁴ Robinson-Gabriel reaction,⁵⁵ Robinson-Gabriel oxazole synthesis,⁵⁶ Robinson-Gabriel cyclodehydration⁵⁷ and Robinson-Gabriel cyclisation.^{38, 57} A variety of dehydrating agents have been used in this reaction however polyphosphoric acid has been found most effective.⁵⁸ The general reaction scheme is outlined below.



Scheme 1.18: General reaction scheme of Gabriel-Robinson synthesis of oxazoles.

Isotopic labeling experimental studies⁵⁸ provided a detailed insight about the progress of the reaction. The reaction involves the protonation of the ketone carbonyl, formation of dihydrooxazolol as a result



Scheme 1.20: Synthesis of oxazoles reported by Ilangoan et al. 2020,⁵⁹

1.7. Technology Platforms for Synthesis

1.7.1. Microwave-Assisted synthesis.

Microwave-Assisted Organic Synthesis attains effective heat transfer via dielectric heating, which primarily depends upon a reagent's capability to absorb microwave energy. The usage of microwave energy is continuously becoming a common passage when employing innovative applications in nanotechnology, polymer chemistry, biochemical premises, peptide and organic synthesis, and materials science.⁶⁰ There are peculiar properties that enable Microwave-Assisted organic synthesis a key tactic in the organic synthesis. The advantages it offers are easy attainment of elevated temperatures, quick & safe synthesis. It also enables analysts to control the energy input whilst executing a chemical reaction. Other key advantages offered by microwave assisted organic synthesis include utilisation of lesser amounts of solvents, environmental benignity and affordability.⁶¹ This

technique also enables analysts to selectively activate catalysts and a complete control over reaction specifications such as safety and practical utility. The technique i.e., microwave assisted synthesis can provide best results if the reaction is performed at optimum temperature, appropriate volume and concentration of the solution and use of a magnetic stirring bar for homogeneous mixing.

1.7.2. Automated Platforms in synthesis: Peptides

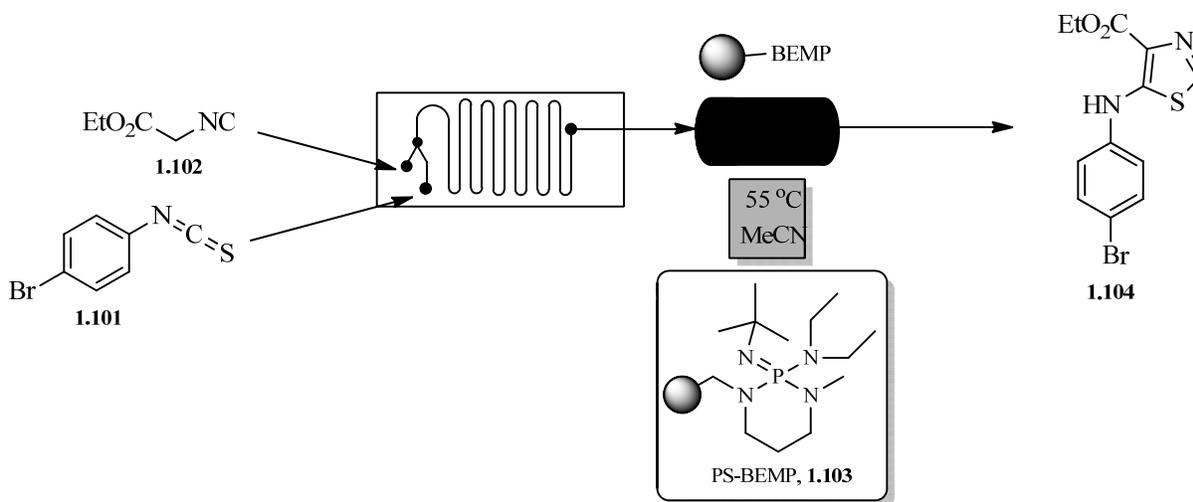
The regulatory & control techniques and procedures for a range of biological methods depend upon the synthesis of the peptides and proteins from their fundamental amino acids. In addition, peptides and their derivatives are extensively used in this day and age to obtain medicines of extreme significance.^{62, 63} Medicines obtained via peptide or their derivatives can be used as anti-cancer agents, to control blood pressure and as an antibiotic. Owing to the superior features of peptides and amino acids, they gained supreme importance in numerous fields of chemistry, medicine, biochemistry and biotechnology.⁶⁴ The easy commercial availability of peptides and their respective derivatives for scientists and members of the scientific community is due to the fact that solid phase peptide chemistry can be easy automated hence attracting more research interest from the scientific community. On the other hand, the automation of other fields of synthesis is at early stage in spite of the value of many of these biologically active compounds.^{65, 63}

In the solid-phase peptide synthesis, the central concept is to attach the peptide chain to a polymeric resin hence allowing allows the operator to get rid of the unreacted reagents without removing the peptide.⁶⁶ The carboxyl (COOH) group of the amino acids can be activated and derivatised and ultimately bounded to the growing peptide chain upon the resin. The competing peptide formation can be avoided by protecting the incoming alpha-amino group. Moreover, the peptide group is removed via a new synthetic route and the peptide is extended by reiteration. Lastly, the peptide is detached from the resin and can be analysed for quality purposes.⁶⁶

1.7.3. Automated platforms in synthesis: Thiazoles

Automated process is the necessity of chemical synthesis and is widely used in pharmaceutical industry. Techniques like microwave assisted synthesis, synthesis via flow methods and solid-supported reagents are of significance as they open doors for new discoveries, save time and attain process optimization with minimal scientist interference.

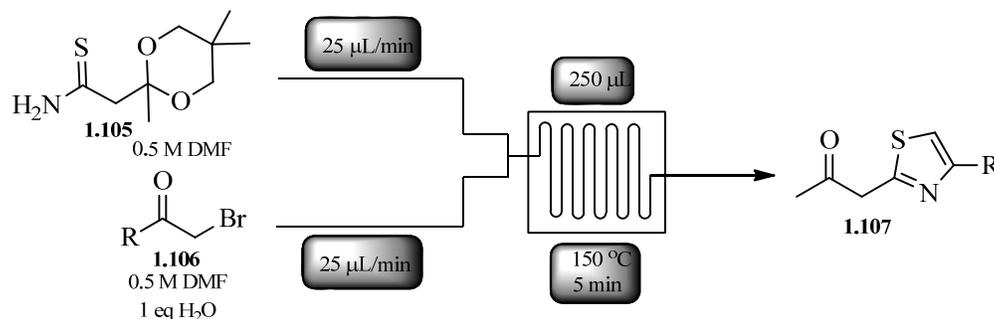
To our surprise, thiazole synthesis via automated processes is underexplored besides few reported methods. Steven Ley et al. reported a scalable process for accessing 4,5-disubstituted thiazoles through a modular flow microreactor. The process uses the microfluidic reaction chips and packed immobilised-reagent columns to impact the bifurcation of the reaction path.⁶⁷ They used the molecule **1.102** and **1.101** and PS-BEMP **1.103** at 55 °C to obtain 4,5-disubstituted thiazoles **1.104** in good to excellent yields.



Scheme 1.21: Synthesis of 4,5-disubstituted thiazoles using flow microreactor.

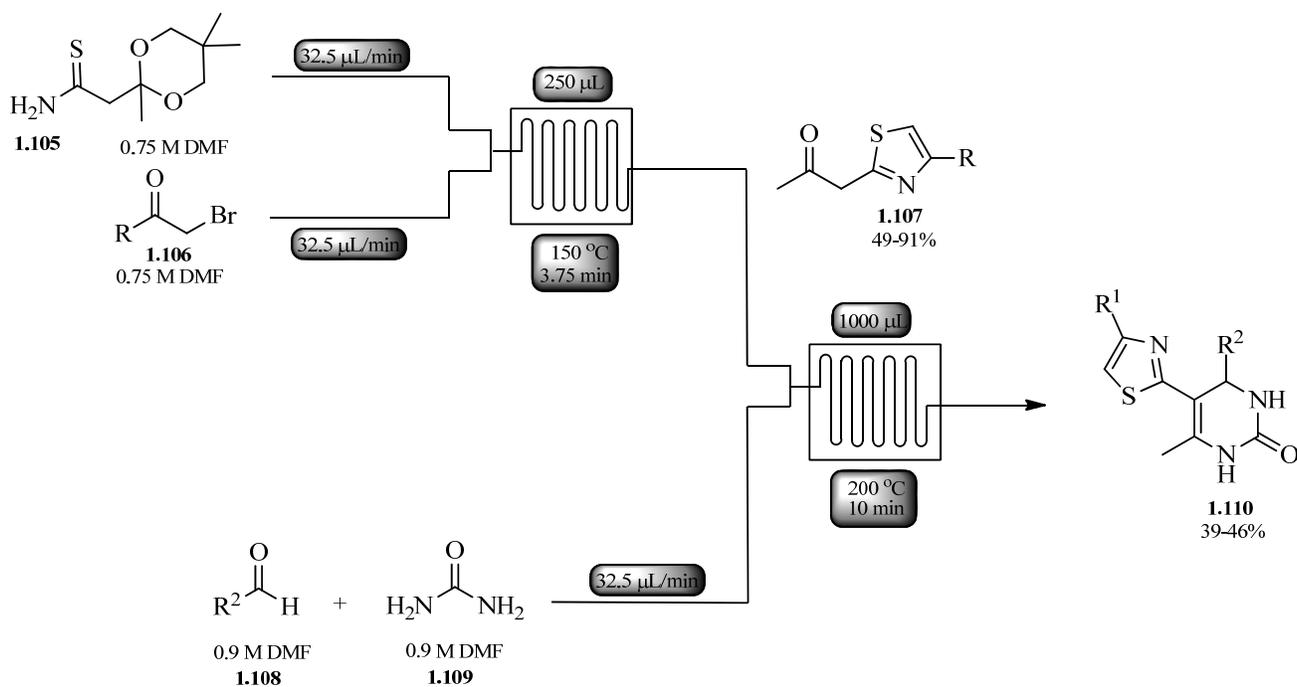
Later on, N. Pagano and colleagues reported another example of a sequential thiazole formation reaction using an automated continuous flow microreactor.⁶⁸ Thioamides like **1.105** and α -halogenated

ketones **1.106** were reacted in a microflow reactor at 150 °C for 5 minutes at a flow rate of 25 $\mu\text{L}/\text{min}$ to afford β -ketothiazole **1.107**.



Scheme 1.22: Microfluidic synthesis of β -ketothiazole.

N. Pagano et al. reported a novel synthesis of indolyl thiazoles **1.110** via a multistep continuous flow based upon Hantzsch thiazole synthesis, deketalisation, and Fischer indole synthesis.⁶⁹ The work engineered successive heterocycle construction reactions using an automated continuous flow process toward indolylthiazoles of the type **1.110**. Their approach involved the synthesis of β -ketothiazole **1.107** based upon the aforementioned method. However, aldehyde **1.108** and urea **1.109** were reacted to afford an intermediate which underwent reaction with β -ketothiazole **1.107** to afford thiazole **1.110** in moderate yield. (**Scheme 1.23**)



Scheme 1.23: Continuous flow process of indolylthiazole synthesis.⁶⁹

Chapter 2 Aims of the project

Given the value of thiazole-containing compounds in previous reports, the stereoselective construction of a thiazole ring containing an adjacent chiral carbon centre has particular significance in natural product total synthesis.^{70, 71} To deliver these compounds for structural confirmation or biological evaluation, the synthesis of substituted thiazoles in a stereo- and enantioselective manner has gained huge interest over recent years. Developing a feasible and highly stereoselective route to thiazoles bearing a stereogenic centre prone to racemization is challenging.

To that end, the goals of this project were: -

- Establish the extent of racemization that occurs in classic Hantzsch thiazole synthesis in the presence of acids or bases under ambient conditions. A range of thioamides derived from α -amino acids would be used in this initial study and methods for analysis established.
- Develop new reaction conditions for the rapid microwave-assisted synthesis of thiazole containing amino acids and establish if these similarly result in racemization of an α -centre.
- Develop a new route for the synthesis of thiazole-containing amino acids that has the potential to be automated on the solid phase so it can be integrated into automated natural product synthesis.
- Investigate if approaches for the synthesis of thiazoles could be extended to the synthesis of oxazoles to provide new routes to these alternative building blocks.

Chapter 3: Results and Discussions

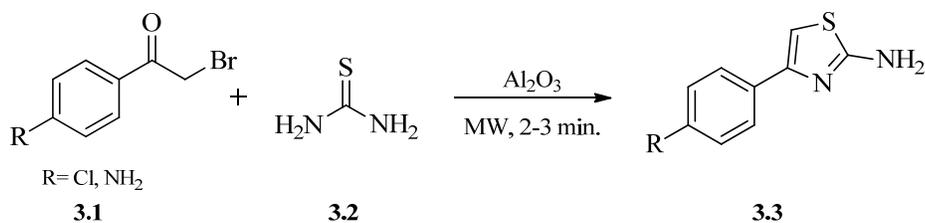
3.1. Develop new reaction conditions for rapid Hantzsch thiazole synthesis

The synthesis of thiazoles and derivatives has attracted considerable interest in recent decades due to their wide range of biological and pharmaceutical properties. If this process could be automated, using fast reaction kinetics and a simple procedure, it could find immediate application in library synthesis or in the pharmaceutical industry. Microwave-assisted organic synthesis of heterocyclic compounds has proven to be a successful method for producing new heterocyclic scaffolds rapidly and effectively for drug development but has seen little application in the synthesis of thiazoles.

A new method for the preparation of thiazoles could have particular significance. Thiazoles derived from amino acids are a common component in biologically active substances like thiopeptide antibiotics.^{72, 73} Furthermore, thiazole forming reactions are important for the synthesis of pharmaceuticals and industrial products.⁷³ In addition to the reactions listed in chapter 1, there are a number of known procedures for the rapid preparation of thiazoles under microwave irradiation, described below.

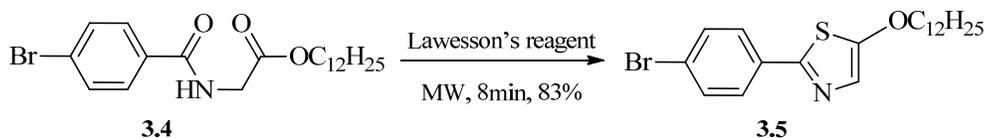
Kidwai and coworkers reported a solvent-free microwave assisted synthesis of 2-aminothiazole **3.3**.

2-Bromoacetophenone **3.1** and thiourea **3.2** were reacted in presence of Al_2O_3 under microwave-irradiation, which gave 2-aminothiazole **3.3** in 90-96% yields. (**Scheme 3.1**).⁷⁴



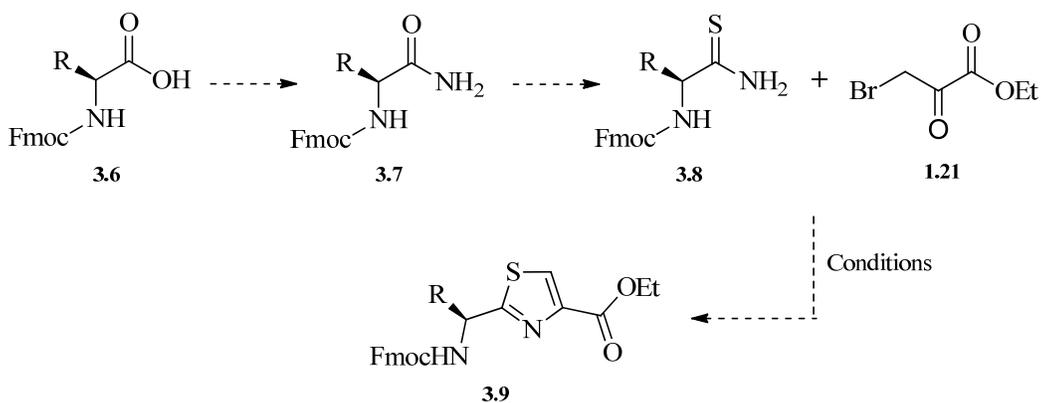
Scheme 3.1: Microwave assisted to prepare 2-aminothiazole **3.3**.⁷⁴

Kiryarov and coworkers have reported a solvent-free, Lawesson's reagent-mediated cyclization of 1,4-dicarbonyl compound **3.4** under microwave irradiation, which after 8 minutes gave thiazole **3.5**, isolated in excellent yield (**Scheme 3.2**).⁷⁵ The process was originally designed to make alkoxythiophenes as intermediates in liquid crystal syntheses, but it was later expanded to make 5-alkoxy-1,3-thiazole and thiadiazoles, which were then converted to liquid crystals using traditional methods.⁷⁵



Scheme 3.2: cyclization of 1,4-dicarbonyl compounds to produce thiazoles **3.5**.⁷⁵

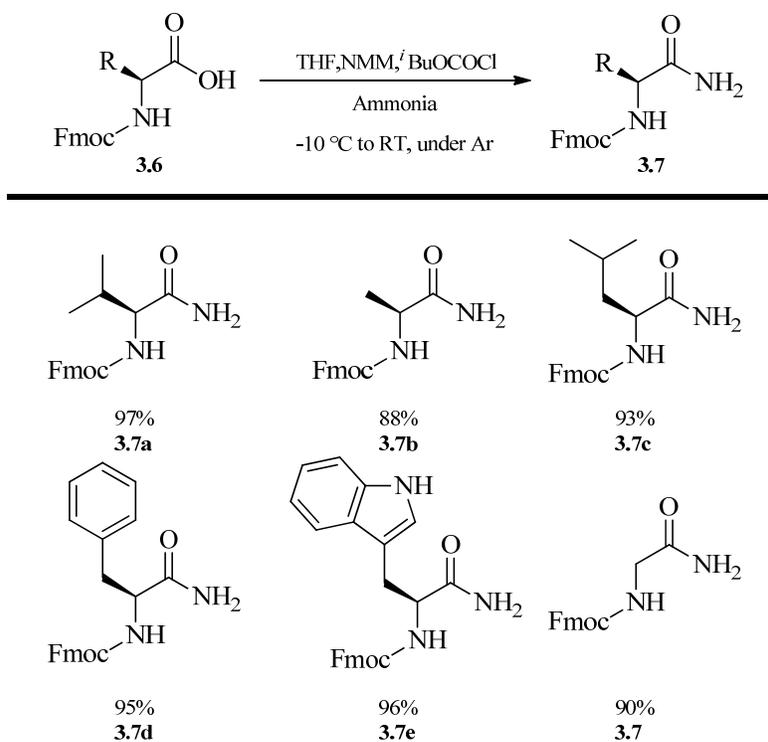
Given these precedents, a rapid microwave-assisted route to thiazoles bearing an aliphatic group at C-2 seemed feasible based upon Hantzsch methods but, for the synthesis of chiral thiazoles, the extent of racemization that occurs under acidic or basic conditions would need to be established. The simplest approach towards amino acid derived chiral thiazoles would start with *N*-Fmoc protected amino acids **3.6** to give amide **3.7** upon amidation, followed by the thionation step to provide thioamide **3.8**. This thioamide upon reaction with ethyl bromopyruvate (**1.21**) under modified Hantzsch conditions would provide chiral thiazoles **3.9** as shown (**Scheme 3.3**) so that the extent of racemization could be established.



Scheme 3.3: Optimization condition for Hantzsch thiazole synthesis.

3.1.1 Preparation of amide derivatives

As a first step, a *N*-protected amino acid was activated and reacted with ammonia in order to obtain the corresponding amide using a known literature procedure.⁷⁶ For the activation step, NMM and isobutyl chloroformate were added to a solution of the Fmoc-protected amino acid **3.6** in THF stirred in an ice bath at -10 °C to produce mixed anhydride, and then ammonia solution (7 N) in methanol was added. The fluorenylmethyloxycarbonyl protecting group was chosen as it was acid stable and so would be able to withstand acid produced in the Hantzsch process, but also would be compatible with Fmoc peptide coupling strategies and so had the potential to be readily automated. After stirring overnight at room temperature, the solvent was removed under reduced pressure and the residue was extracted with aqueous base and recrystallized to produce the corresponding amide **3.7** (**Scheme 3.4**).



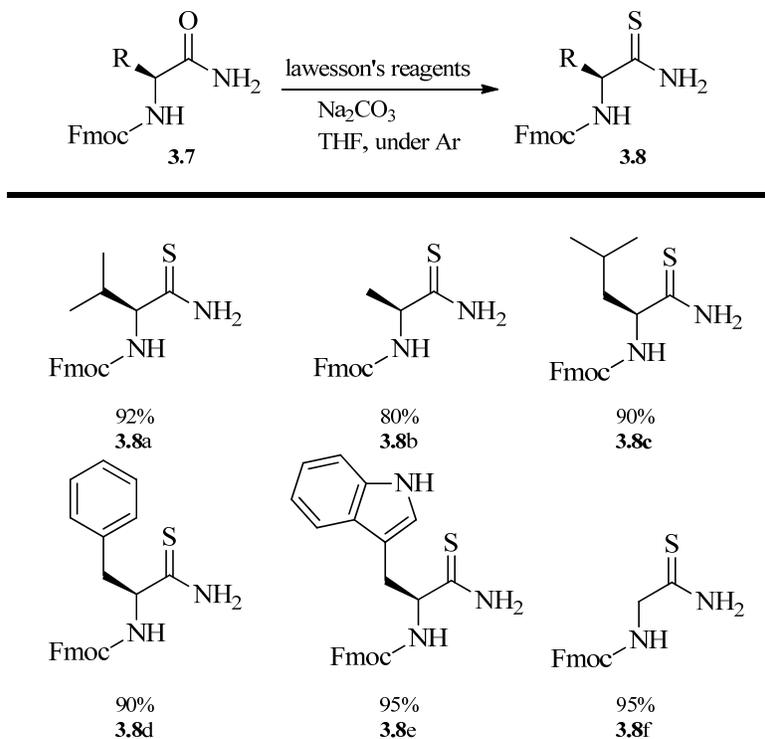
Scheme 3.4: Synthesis of amides.

A range of alkyl/aryl substituents were tested in the above-mentioned reaction. The corresponding amides were produced in good to excellent yields of 88-97 %, even after purification. Structure elucidation was confirmed by analysis of ^1H NMR spectroscopic, mass spectrometric and melting point data. For example, for amide **3.7a**, the new resonant frequency of the proton from the amide (NH_2) appeared as broad singlets at δ 7.02 and 7.32 ppm. Mass spectrometric data confirmed the mass and identity of the product as the sodium adduct $[\text{M}+\text{Na}]^+$ was observed at m/z 361.1528. Furthermore, the melting point at 195-196 $^\circ\text{C}$, consistent with literature values⁷⁷ and successful amide formation.

3.1.2 Preparation of thioamide derivatives

Next step was the thionation of the newly synthesized amides **3.8a-f**, and for this purpose, a literature procedure was followed.⁷⁸ To a solution of Lawesson's reagent and Na_2CO_3 in anhydrous THF, the

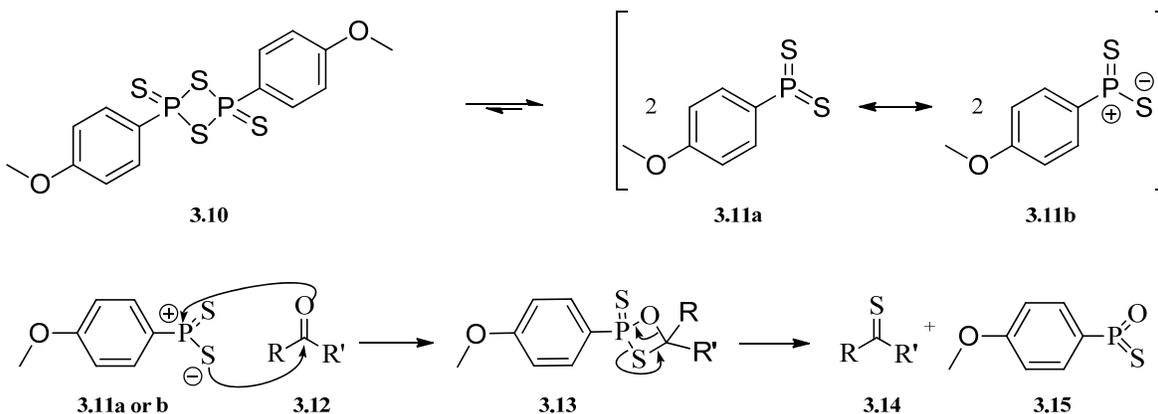
amide was added and the mixture was stirred under slow argon flow at room temperature. The reaction was stirred overnight. Upon completion, the solution of the reaction was filtered through Celite and the filtrate was evaporated to dryness. Following aqueous extraction, a series of thioamides **3.8a-f** was produced in good to excellent yield (**Scheme 3.5**).



Scheme 3.5: Thionation reaction by Lawesson's reagent.

Thionation using Lawesson's reagent **3.10** proceeds by dissociation of the reagent into **3.11a** and **3.11b**. The decomposition product **3.11a** or **3.11b** can then react with Lewis basic functional groups such as carbonyl **3.12** to form four-membered ring **3.13**, which decomposes to the corresponding thioketone **3.14**. The driving force is the formation of a stable P=O bond **3.15** in a cycloreversion step that resembles a portion of the mechanism known for the Wittig Reaction (**Scheme 3.6**).⁷⁹ The general mechanism of thionation is outlined below.

Lawesson's Reagent in solution is in equilibrium



Scheme 3.6: General mechanism of thionation by Lawesson's reagent.

Structure elucidation was confirmed by analysis of ^1H and ^{13}C NMR spectroscopic and mass spectrometric data. For example, comparing **3.7a** and **3.8a**, major differences were observed upon thionation: in the ^1H NMR spectrum the broad thioamide NH_2 proton resonances shifted in their resonant frequency from δ 7.02 and 7.32 ppm on the amid to δ 9.22 and 9.63 ppm on the thioamide in a characteristic downfield shift (**Figure 3.1**).

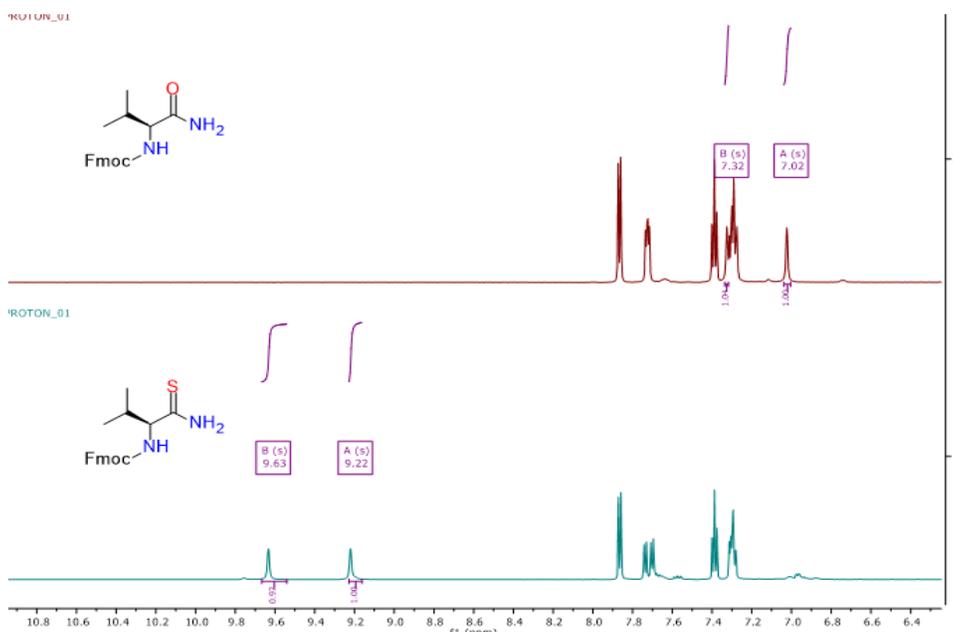
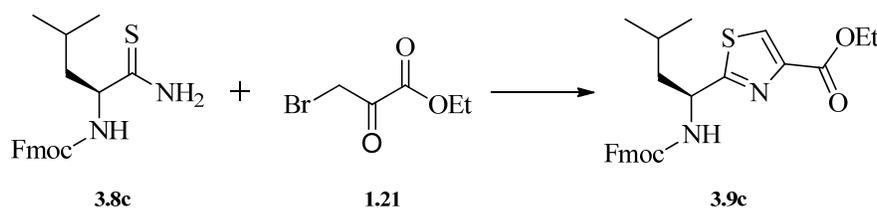


Figure 3.1: the major differences between amid and thioamid in ^1H NMR spectrum.

Further confirmation was obtained from the ^{13}C NMR spectrum where the resonant frequency of the carbon from the thiocarbonyl group moved significantly from δ 173.6 to 208.0 ppm. Mass spectrometric data confirmed the mass and identity of the product as a protonated molecular ion (MH^+) was observed at m/z 355.1489 consistent with successful thionation.

3.2. Modification of the Hantzsch thiazole synthesis

With a library of thioamides in hand, it was possible to explore a range of conditions and a variety of susceptibilities to racemization in the Hantzsch thiazole synthesis. As discussed in the Introduction, different amino acid derived thiazoles can racemize in modified Hantzsch conditions to different extents. At the outset of optimizations, we chose thioamide **3.8c** as a standard substrate to benchmark different conditions. The reaction was performed in two different solvents, DME (the standard solvent for Holzapfel/Myers modified methods) and MeCN, at 25 °C with Na_2CO_3 or under neutral conditions in the first step (which would become acidic as the reaction progressed) and catalysed by citric acid and formic acid in the second step. It was hoped that by shifting to a milder acid (than HBr) and alternative solvents that an efficient and enantioselective process could be found. However, although the reaction was successful under ambient conditions (**Table 3.1, entries 1-3**) and thiazole **3.9c** was produced in good to excellent yield, unfortunately all the products were racemic mixtures.



Scheme 3.7: Optimization of Hantzsch synthesis using *N*-protected thioamide **3.8c** under different conditions.

Entry	solvent	Reagent		Time		Temp. (°C)		Yield (%)	ee ^f
		Step1	Step2	Step1	Step2	Step1	Step2		
1	DME	Na ₂ CO ₃ ^a	Citric acid ^b	1h	2h	25	25	83	<2%
2	DME	Mg ^c	Citric acid ^b	24h	2h	25	25	75	<2%
3	MeCN	Na ₂ CO ₃	Formic acid ^d	1h	24h	25	25	85	<2%
4	MeCN	Without reagent		3 min ^e		70		90	<2%
5	DME	Without reagent		3 min ^e		70		85	<2%

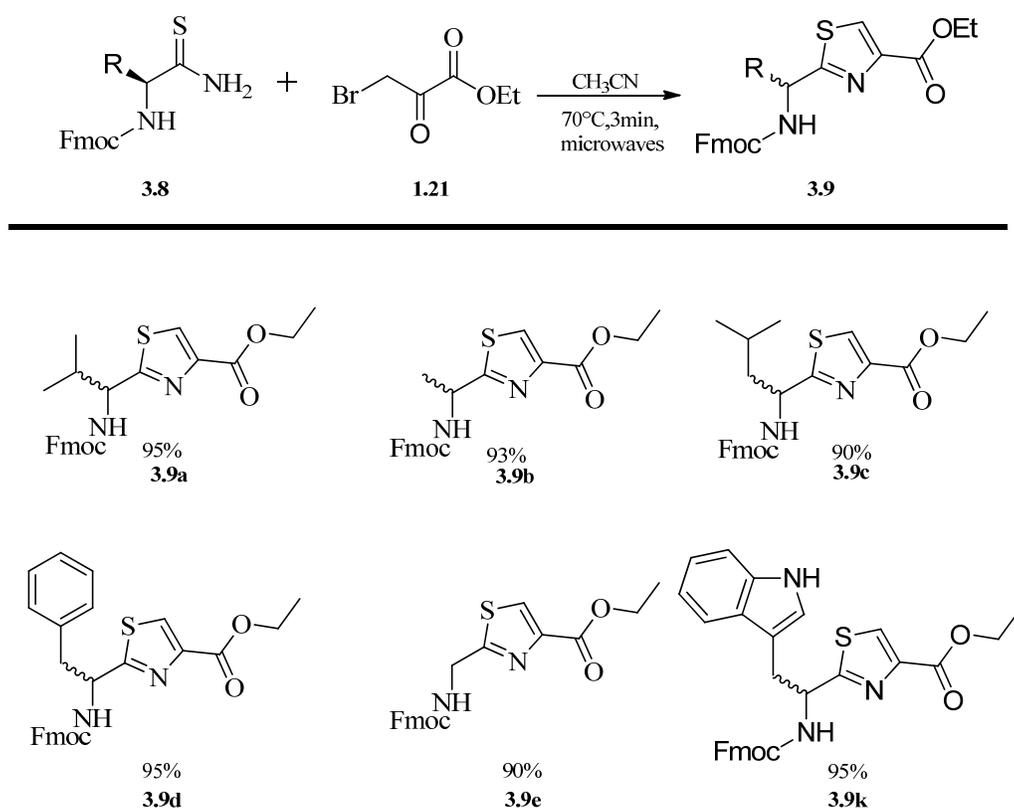
a) Na₂CO₃ (1 equiv.)
b) Citric acid (2 equiv.)
c) Mg (6 equiv.)
d) acetonitrile containing 30% (v/v) of formic acid
e) by microwave
f) Determined using HPLC analysis on a chiral stationary phase (CHIRALPAK® AD column)

Table 3.1: Hantzsch synthesis using *N*-protected thioamide **3.8c** under different conditions.

Next, we decided to perform the reaction without added reagents but at elevated temperature i.e. 70 °C under a microwave heating and shortening the reaction time to 3 min. To our delight, the reaction worked exceptionally well and gave 90% yield of the target thiazole in 3 minutes in acetonitrile solvent (**Table 3.1 entry 4**). Keeping this in view, we decided to change the solvent, gratifying 85% of the product was obtained when the reaction was processed in dimethoxyethane solvent. (**Table 3.1 entry 5**).

In order to confirm that similar results would be obtained for a number of different amino acid derivatives, the library of thioamides **3.8** was subjected to the microwave-assisted conditions in acetonitrile. In all cases, thiazoles **3.9** were produced in very good to excellent yields, but also all the

products were racemic mixtures, indicating that standard Hantzsch conditions under microwave irradiation did indeed cause racemization regardless of the amino acid involved. Flash microwave heating for a short time in a polar aprotic solvent was harsh enough to cause the rapid racemization of an α -centre. The study did provide racemic samples of these thiazoles for use in analytical studies, quantified the extent of the challenge and provided a rapid route to achiral thiazole **3.9e** in excellent yield.



Scheme 3.8: Thiazole-containing Fmoc-protected amino acids prepared under microwave heating.

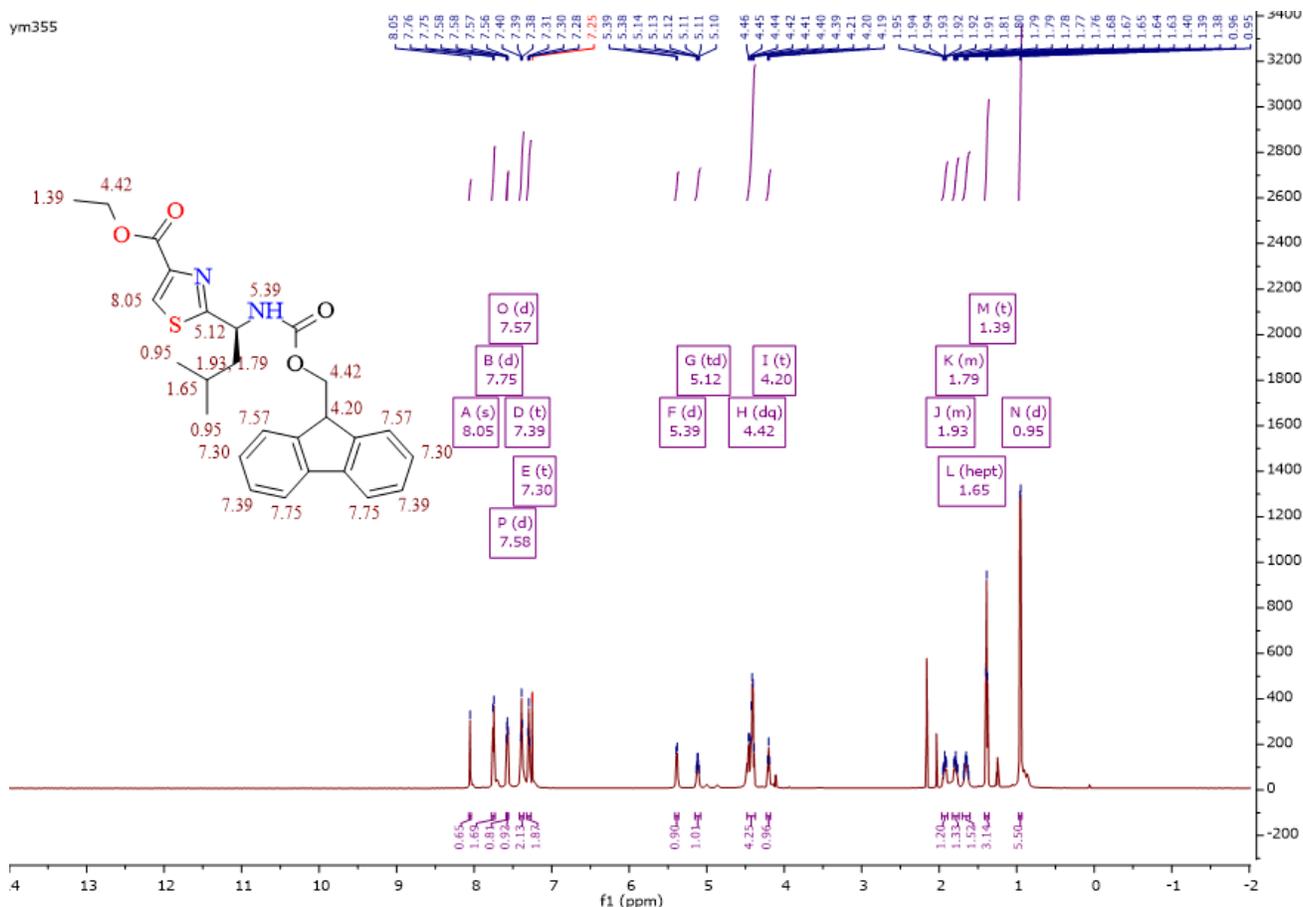


Figure 3.2: ^1H NMR spectrum of thiazole **3.9c**.

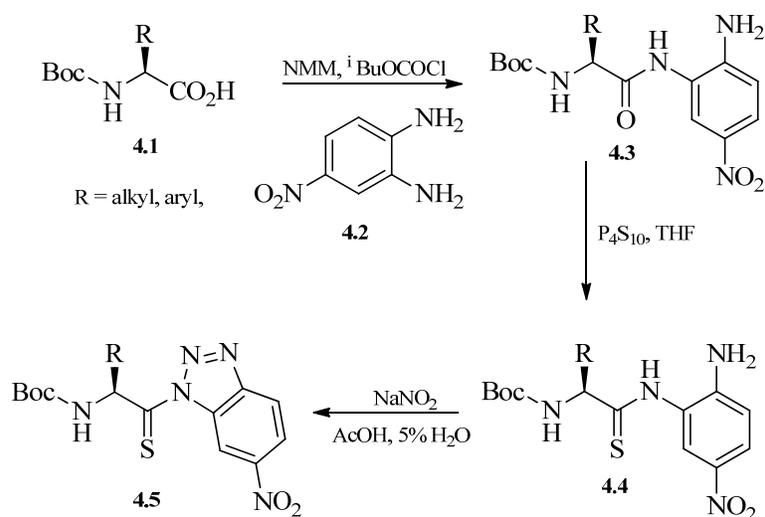
Structure elucidation was confirmed by analysis of ^1H and ^{13}C NMR spectroscopic and mass spectrometric data. For example, thiazole **3.9c** where the resonant frequency of the proton from the starting materials (NH_2) disappeared and new resonance was observed from the proton of the thiazole ring (5-H), which appeared at δ 8.05 ppm. Analysis of the ^{13}C NMR spectrum demonstrated that the resonant frequency of the carbon from the thiocarbonyl group disappeared and new quaternary and methine resonances from the thiazole ring appeared at δ 173.5 (2-C) and 127.0 ppm (5-C), respectively. Mass spectrometric data confirmed the mass and identity of the product as a protonated molecular ion (MH^+) was observed at m/z 465.1827, consistent with successful thiazole formation.

Chapter 4: Results and Discussions

4.1. New methods for the synthesis of chiral thiazole-containing amino acids

Many natural products contain chiral thiazole building blocks,⁸⁰ but their synthesis can prove problematic in terms of the isolated yield or enantiopurity of the product. This was demonstrated in Chapter 3, where racemization in the Hantzsch synthesis of thiazoles was established. New improved methods are still urgently required to access these valuable targets. Our goal was to discover a facile route to chiral thiazoles that had the potential to be automated on the solid phase so it could be integrated into an automated method for natural product synthesis. Initial studies would explore a novel route in the liquid phase to validate a solid-phase approach.

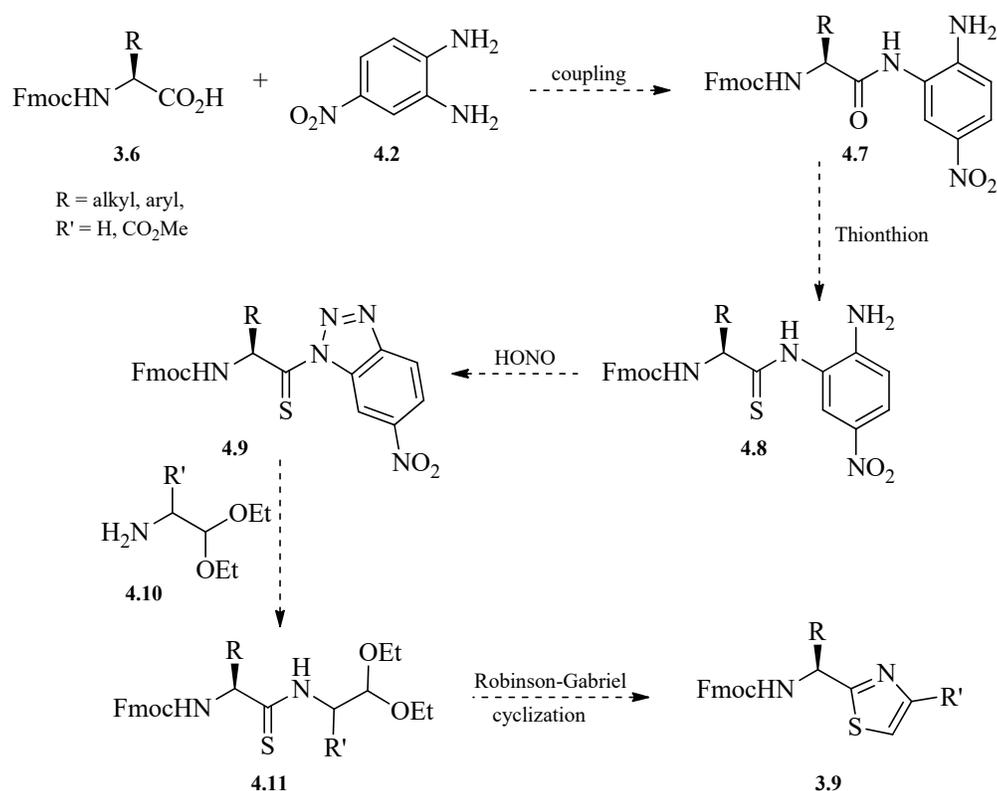
Keeping in view the methods reported in literature to access a range of oxazoles through Robinson-Gabriel cyclisation, we decided to develop a novel protocol to obtain by Robinson-Gabriel cyclisation of a thioamide. Key to that approach would be the need to introduce a thioamide into a growing peptide chain at a specific amino acid site such that cyclization to the thiazole could be controlled. One such approach for specific thioacylation of an amino group uses benzotriazolyl thioacylated compounds (**Scheme 4.1**), which can be prepared from the corresponding α -*N*-Boc-L-amino acids using methods described in the literature.⁷⁶ We envisaged that coupling commercially available *N*-protected α -amino acids with aromatic amines would generate the corresponding amide which after thionation and reaction with HONO would produce benzotriazolyl thioacylated compounds that could be used as thioacylating reagents in peptide synthesis.



Scheme 4.1: Benzotriazole thioacylating reagents **4.5** prepared from amino acids.⁷⁶

The method for preparing benzotriazole thioacylating agents, using mixed carbonic anhydride methodology for anilide bond formation: 4-nitro-1,2-phenylenediamine and the *N*-Boc amino acid can be coupled in THF at 0 °C.⁷⁶ After isolation, this method provides crystalline anilides **4.3** in 90-92% yield. With a mixture of P₄S₁₀ and anhydrous Na₂CO₃ in THF, direct thionation of **4.3** has been reported after a total of 3 hours at 0 °C and RT; the reaction went smoothly to afford thioamides **4.4** in excellent yield (86-88%). When thionation of **4.3** was carried out with limited P₄S₁₀ (40-50 mol%), a better yield of **4.4** was obtained and only the corresponding thioamide was produced in the reaction. The benzotriazoles **4.5** were produced in 72-83% yield by intramolecular cyclization of **4.4** with nitrous acid formed in situ with NaNO₂ in AcOH. These compounds are stable orange solids that can be kept at 0 °C for months without decomposition (**Scheme 4.1**).⁷⁶ As such this approach looked ideal to produce a thioacylating agent for use in thiazole synthesis that was compatible with automated strategies.

We chose to employ amino acids with a Fmoc protecting group as this moiety shows high stability to acidic conditions, more so than *N*-Boc-protected amino acids. A Fmoc protection strategy would be compatible with both automated Fmoc peptide synthesis and acid mediated thiazole synthesis. A general approach to thiazoles using benzotriazole-derived thioacylating agents **4.9**, to be carried out in the solution phase, is described below (**Scheme 4.2**).

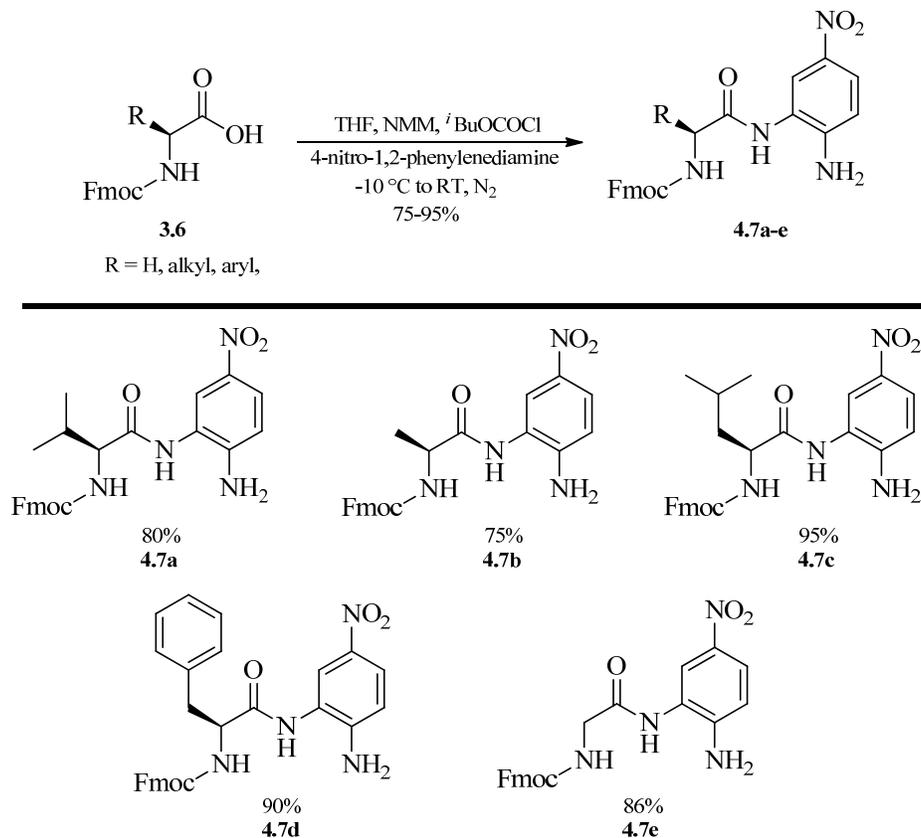


Scheme 4.2: Solution phase approach to chiral thiazoles.

4.1.1. Preparation of anilide derivatives

As a first step, the carboxyl group of amino acid was amidated with 4-nitro-1,2-phenylenediamine, in order to obtain a latent benzotriazole in accordance with the known literature procedure.⁷⁸ To a solution of amino acid in THF stirred in an ice bath at $-10\text{ }^\circ\text{C}$, NMM and isobutyl chloroformate were added to generate the corresponding mixed anhydride. 4-Nitro-1,2-phenylenediamine was added in a separate

step to react with the activated acid (**Scheme 4.3**). After stirring overnight at room temperature, the solvent was removed under reduced pressure and the residue were extracted with aqueous base. Purification by recrystallization (EtOAc-hexane) gave the corresponding anilides **4.7a-e** in 75-95% yield.



Scheme 4.3: Synthesis of anilides by mixed anhydride methodology.

Structure elucidation was confirmed by analysis of ^1H NMR spectroscopic, mass spectrometric and melting point data. For example, for anilide **4.7a** a new proton resonance from the anilide NH appeared at δ 9.40 ppm in the ^1H NMR spectrum of the recrystallized product (**Figure 4.1**). Mass spectrometric data confirmed the mass and identity of this product as sodium adduct $[\text{M}+\text{Na}]^+$ was observed at m/z 497.1797. The melting point at 170-172 $^\circ\text{C}$, consistent with successful anilide formation.

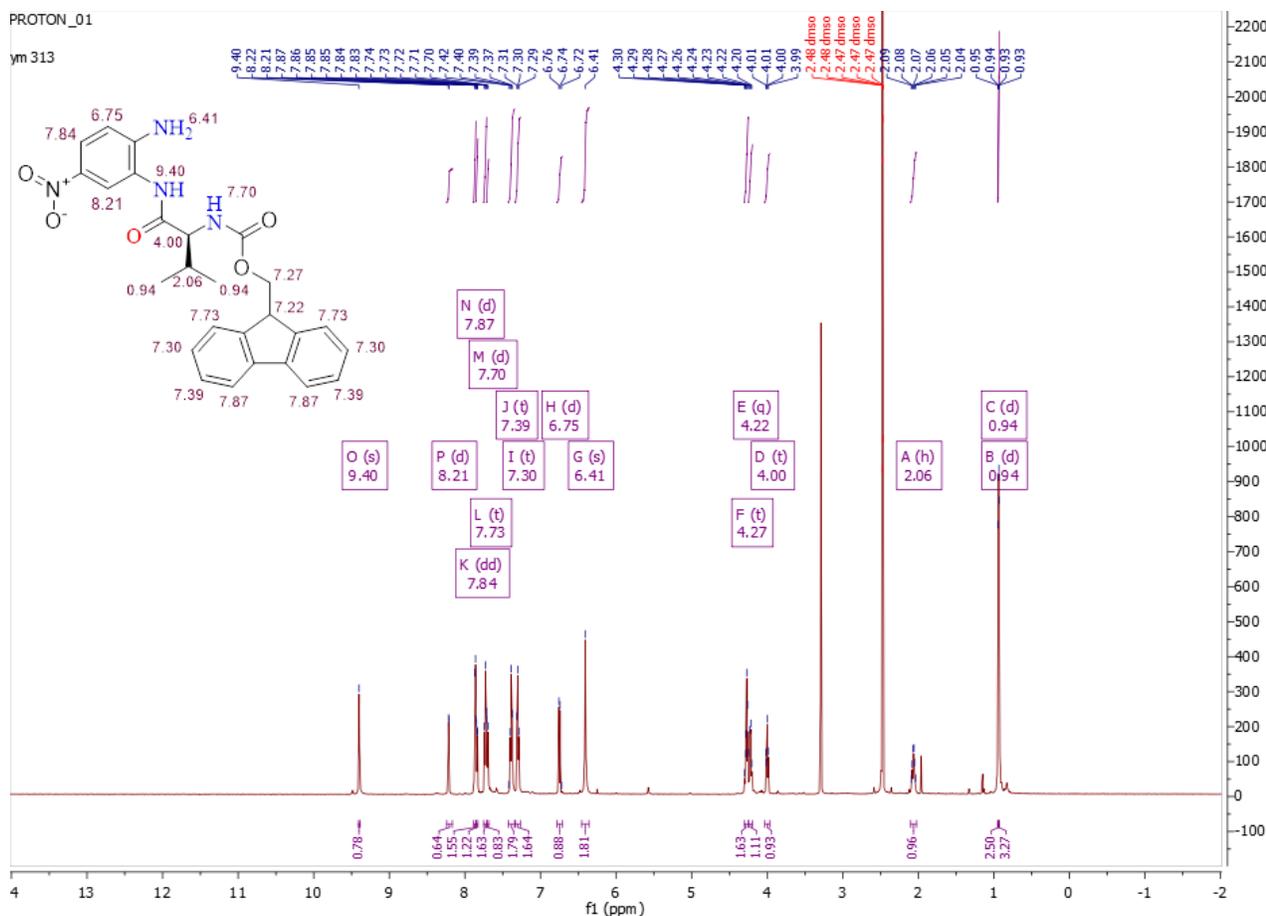
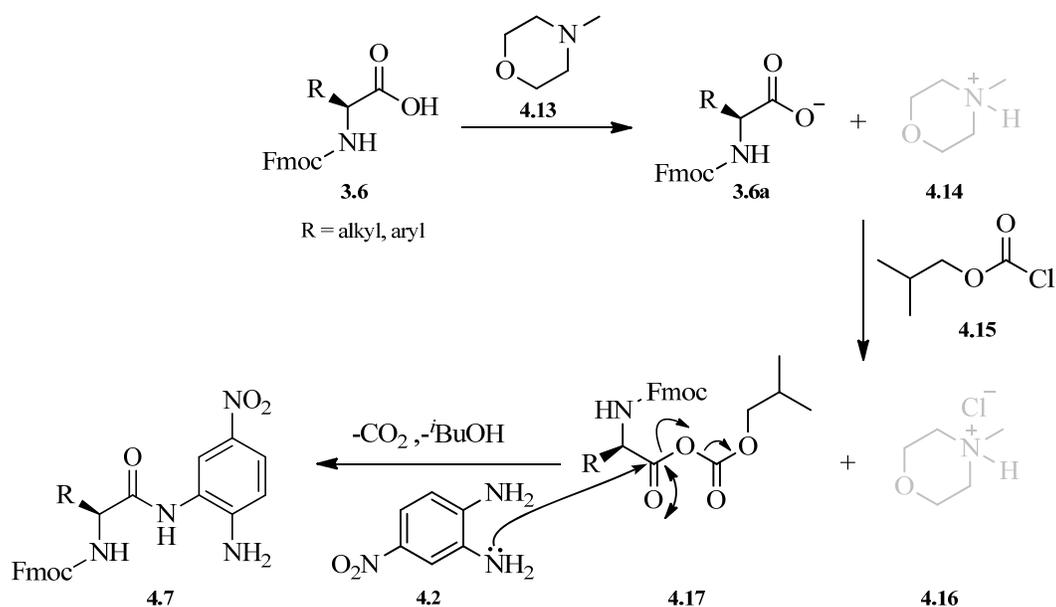


Figure 4.1: ^1H NMR spectrum of anilide **4.7a**.

A range of alkyl/ aryl substituents were tested in the aforementioned reaction. The corresponding anilides were obtained in good to excellent yields 75-95 %. It was noticed that the amino acids containing bulkier alkyl/ aryl groups proved to be effective substrates as they provided corresponding anilides in excellent yields i.e. **4.7c** & **4.7d**. The small variation in yield can probably be attributed to the efficiency of the purification process. A general mechanism of the amidation reaction is outlined as follows (**Scheme 4.4**).



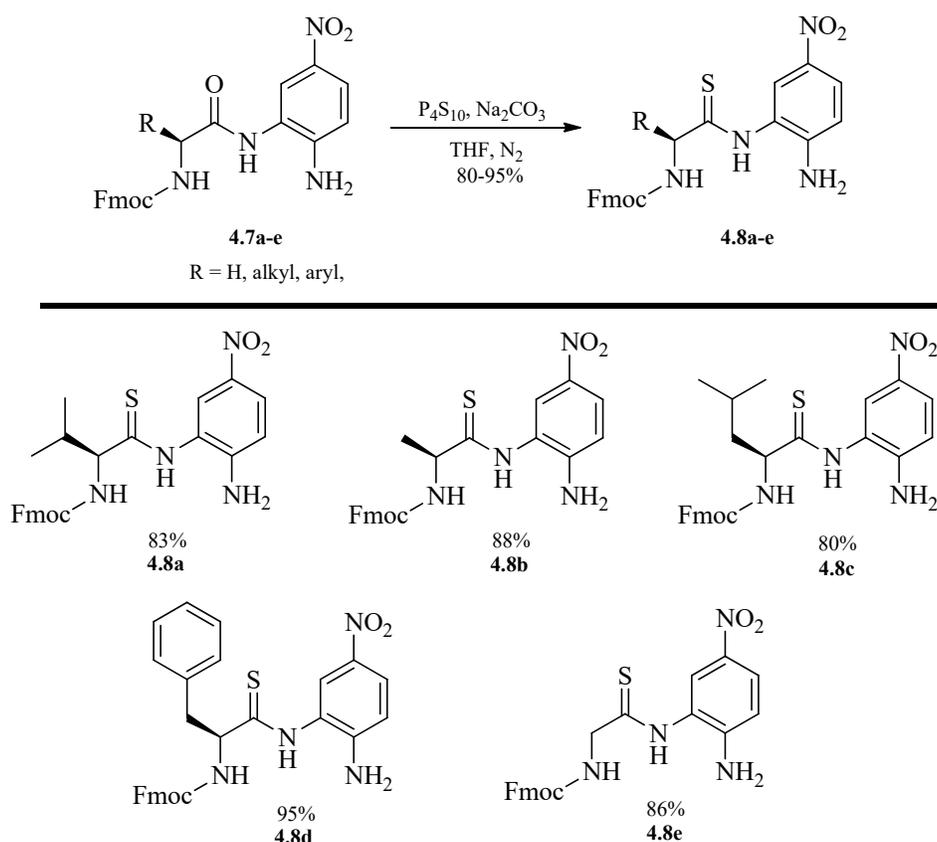
Scheme 4.4: A general mechanism for the synthesis of anilides by NMM, *t*-BuOCOC1.⁷⁶

Mechanistically speaking, the chiral amino acid **3.6** was converted to corresponding carboxylate anion by the action of NMM (**4.13**) via deprotonation to afford the conjugate acid **4.14** and a carboxylate nucleophile which subsequently reacted with chloroformate **4.15** to yield the mixed anhydride **4.17**. The anhydride **4.17** underwent further attack by the more basic amino group of aromatic amine **4.2** to give the desired amide **4.7** along with carbon dioxide and isobutanol as side products.

4.1.2. Preparation of thioanilide derivatives

Next step was the thionation of the newly synthesized anilides **4.7a-d**. For this purpose, we followed an already reported literature procedure.⁷⁸ It was found that the thionation of *N*-Fmoc-nitroanilide with Lawesson's reagent resulted in either low conversion at room temperature or formation of significant amounts of a benzimidazole side product at 70 °C. On the contrary, direct thionation by P₄S₁₀ was high yielding with minimal side product formation (**Scheme 4.5**). Berzelius synthesized P₄S₁₀ via a violent reaction of white phosphorus and sulfur. However, Berzelius reagent i.e. P₄S₁₀ can also be prepared by a more controlled reaction using red phosphorus. It can also be designed by reaction of elemental sulfur

or pyrite (FeS₂) with ferrophosphorus (Fe₂P).⁷⁹ In order to obtain thioanilides **4.8**, nitroanilide was added to a solution of P₄S₁₀ and Na₂CO₃ in anhydrous THF and stirred at room temperature under slow argon flow. The reaction was monitored by TLC analysis to follow the consumption of nitroanilide. Upon completion, the reaction solution was filtered through Celite, and the filtrate was evaporated to dryness. Following aqueous extraction, a series of thioanilides **4.8a-e** was produced in very good to excellent yield. Although purification of P₄S₁₀ by Soxhlet extraction is often recommended, it was found to be unnecessary and high yields were obtained with commercial P₄S₁₀ stored at room temperature and used directly.



Scheme 4.5: Thionation reaction by Berzelius reagent P₄S₁₀.

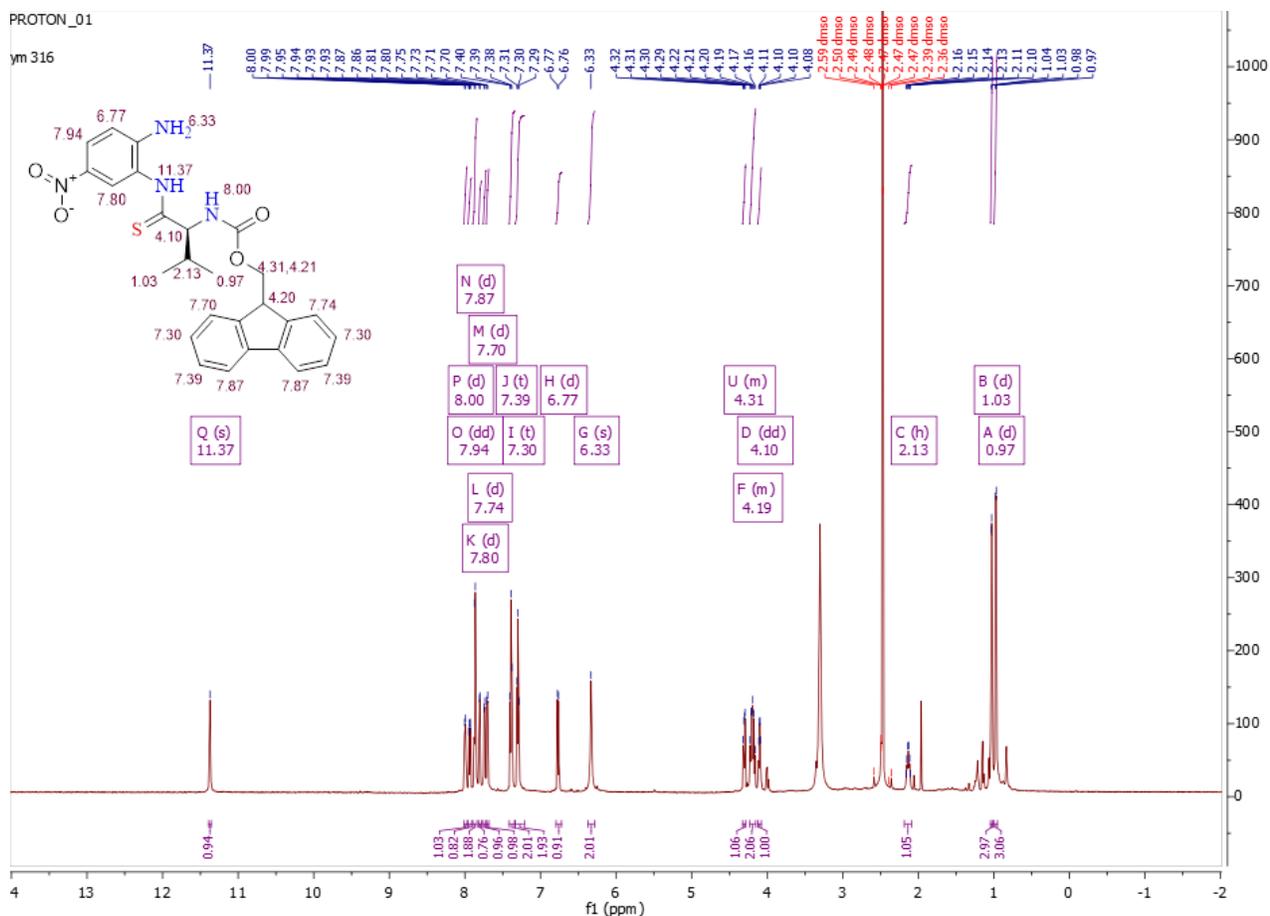
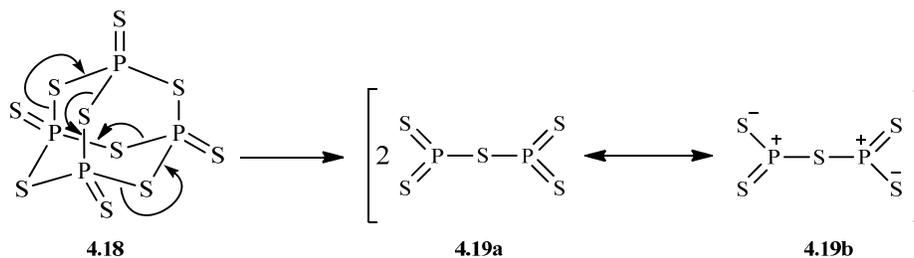


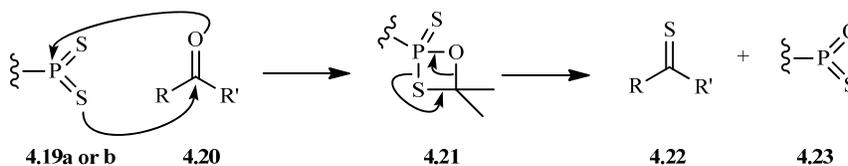
Figure 4.2: ¹H NMR spectrum of thioanilide **4.8a**.

A general mechanism of the above reaction is outlined as follows (**Scheme 4.6**). Firstly, P₄S₁₀ dissociates into **4.19a** and **4.19b**. The decomposition product **4.19a** or **4.19b** can then react with functional groups such as carbonyl **4.20** to form four-membered ring **4.21**, which decomposes to the corresponding thioketone **4.22** and to the thermodynamically more stable product **4.23**, having a P=O bond (**Scheme 4.6**).⁷⁹ The general mechanism of thionation is outlined below.

Dissociation of P₄S₁₀



Thionation mechanism by P₄S₁₀



Scheme 4.6: Mechanism of thionation reaction by P₄S₁₀.

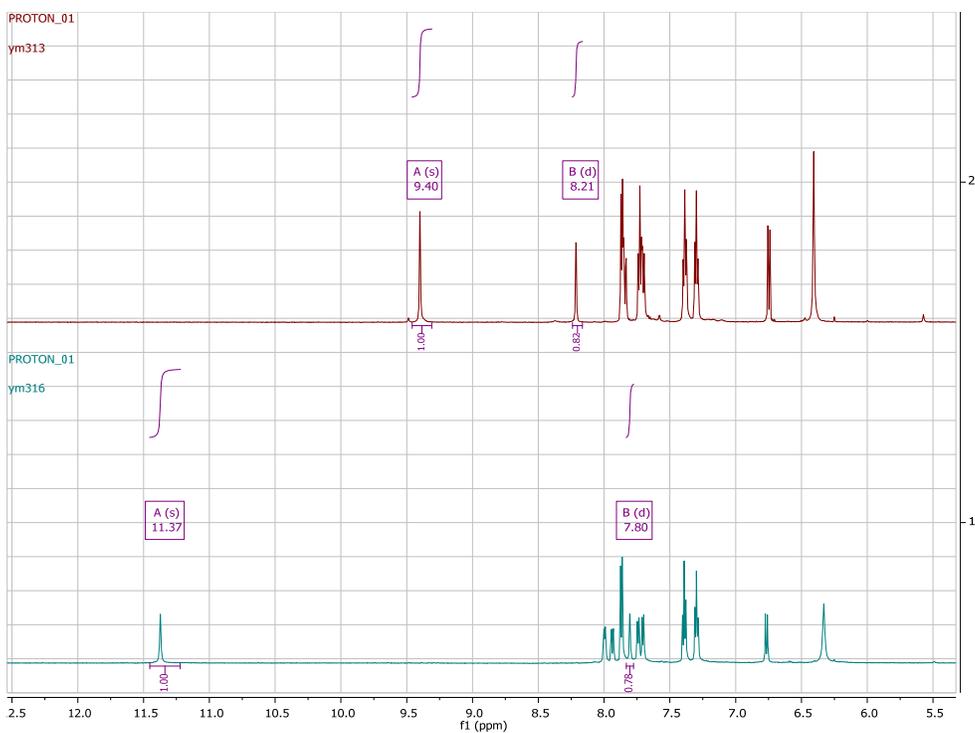


Figure 4.3: major differences between anilide and thioanilide in ¹H NMR spectrum.

Structure elucidation was confirmed by analysis of ¹H and ¹³C NMR spectroscopic and mass spectrometric data. For example, comparing **4.7a** and **4.8a** major differences were observed upon

thionation: in the ^1H NMR spectrum the NH proton connected to the thiocarbonyl group shifted in its resonant frequency downfield from δ 9.40 to 11.37 ppm and CH-6 on the aromatic ring the peak shifted from δ 8.21 to high field at 7.80 ppm.

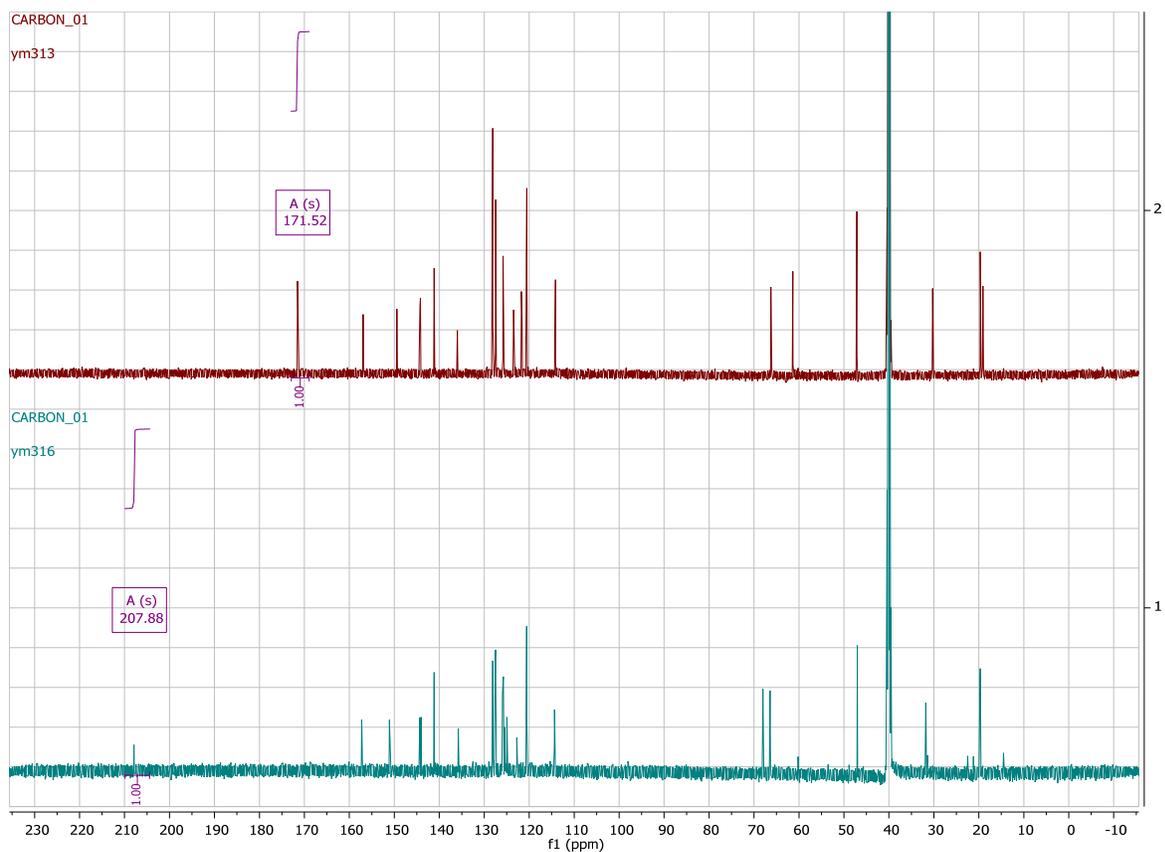
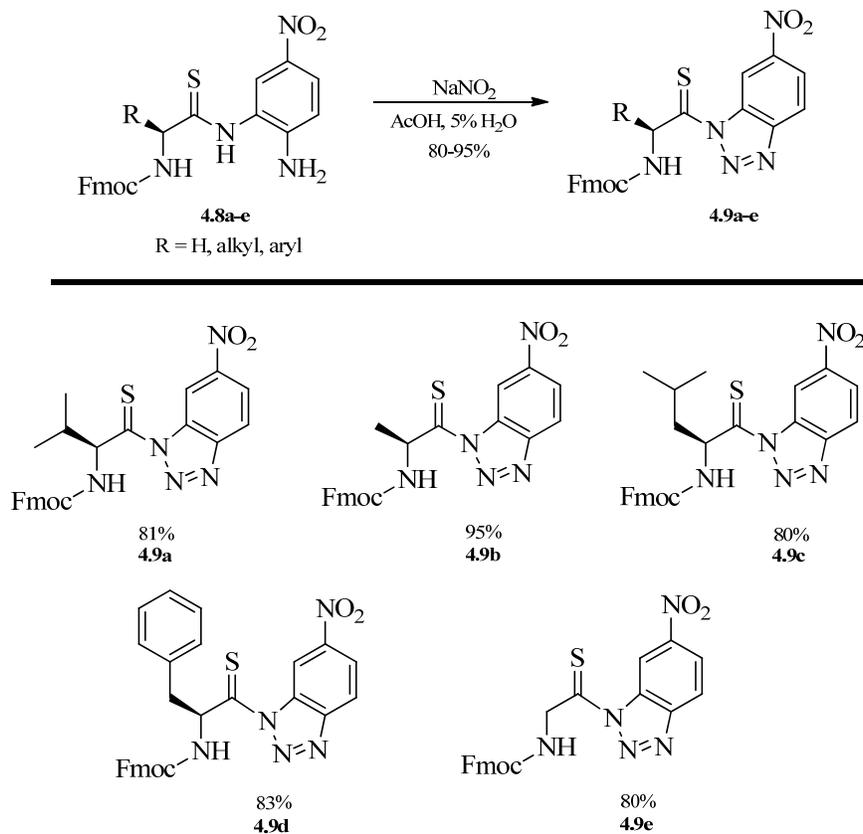


Figure 4.4: major differences between anilide and thoanilide in carbon-13 NMR.

Further confirmation was obtained from the ^{13}C NMR spectrum where the resonant frequency of the carbon from the thiocarbonyl group moved significantly from δ 171.5 to 207.9 ppm. Mass spectrometric data confirmed the mass and identity of the product as a protonated molecular ion (MH^+) was observed at m/z 591.1733 consistent with successful thionation.

4.1.3 Preparation of thiobenzotriazole derivatives

With a library of precursors in hand, intramolecular mediated cyclisation of thioanilides **4.8a-e** was investigated using nitrous acid generated in situ by protonation of NaNO_2 with AcOH to give the corresponding benzotriazoles **4.9a-e** (Scheme 4.7). The benzotriazole forming reaction reached completion in 30 minutes and the products precipitated upon the addition of cold water to the reaction mixture. The precipitated products were filtered, washed with water and dried in vacuo at room temperature overnight and then at $50\text{ }^\circ\text{C}$ for 4 h to afford benzotriazoles **4.9a-e** in 80-95% yield.



Scheme 4.7: Intramolecular mediated cyclisation of thioanilides.

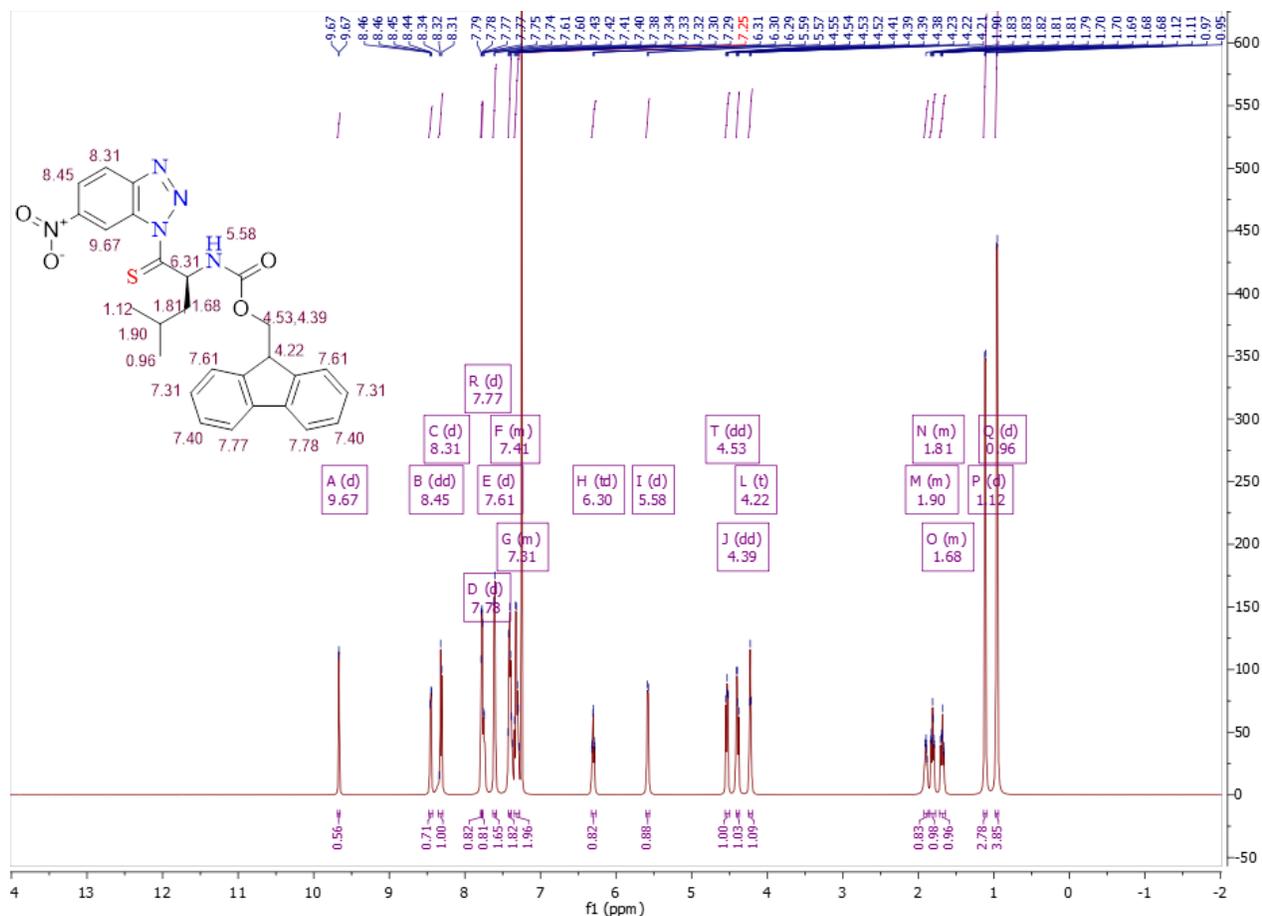
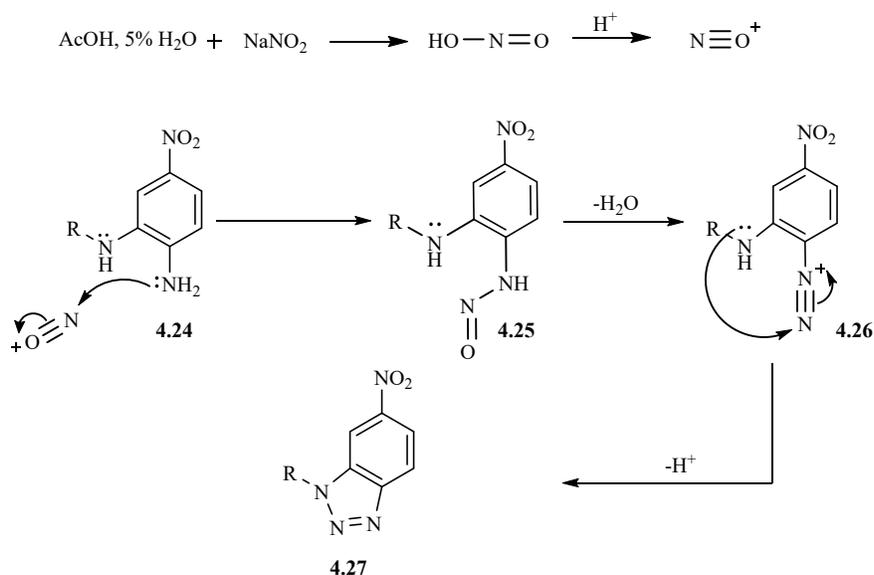


Figure 4.5: ^1H NMR spectrum of benzotriazole **4.9c**.

Structure elucidation was confirmed by analysis of ^1H NMR spectroscopic and mass spectrometric data. For example, for benzotriazole **4.9c**, in the ^1H NMR spectrum the NH proton connected to the thioacyl group disappeared as predicted after cyclisation and the resonant frequency of the protons from the aromatic ring of the benzotriazole were shifted downfield at δ 9.67, 8.45 and 7.32 ppm. Mass spectrometric data confirmed the mass and identity of the product as sodium adduct $[\text{M}+\text{Na}]^+$ was observed at m/z 538.1521, consistent with successful benzotriazole formation.

Mechanistically speaking, the amino group was converted into the nitrosylated derivative **4.25** by reacting with the nitronium ion, produced by the action of acetic acid on sodium nitrite, which

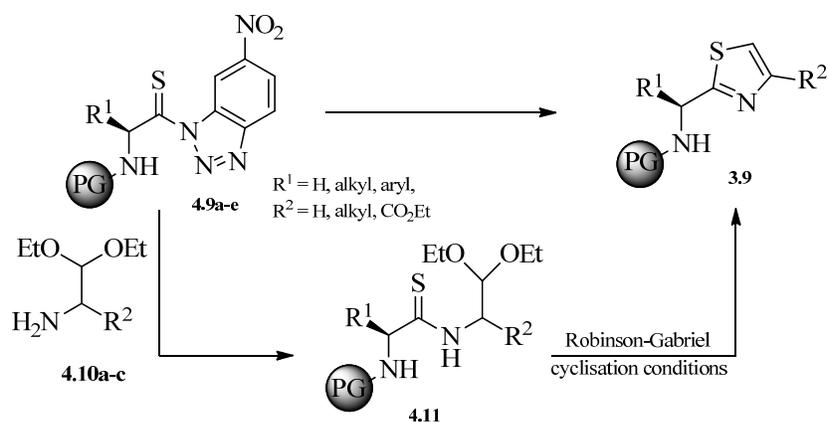
undergoes reaction with the anilino nucleophile rather than the less basic thioamide (**Scheme 4.8**). Cyclization is probably mediated by dehydration to the more reactive diazonium electrophilic species **4.26** which can then undergo intramolecular nucleophilic attack by the amine at the terminal nitrogen. Lastly, loss of a proton gives the desired product.



Scheme 4.8: Mechanism of diazonium-mediated cyclisation of thioanilides **4.24**.

4.2. Synthesis of thiazoles

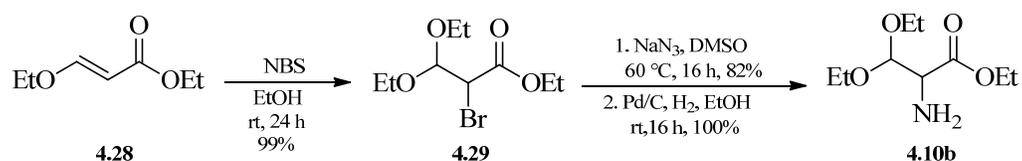
Our approach to access different substituted chiral thiazoles was to couple the aforementioned benzotriazolyl thioacylated compounds **4.9a-e** with various amines **4.10a-c** to generate the amide intermediate **4.11** bearing a thiocarbonyl group at a specific position and protected functionality (originally present in the amine nucleophile) to react with the thiocarbonyl group. This intermediate would then be subjected to the different modified Robinson-Gabriel cyclisation reaction conditions to furnish the desired chiral thiazole. If successful this approach might be suitable for thiazole introduction at a particular amino acid position of a growing peptide chain.



Scheme 4.9: Strategy for the synthesis of thiazole.

4.2.1. Preparation of Ethyl 2-amino-3,3-diethoxypropanoate

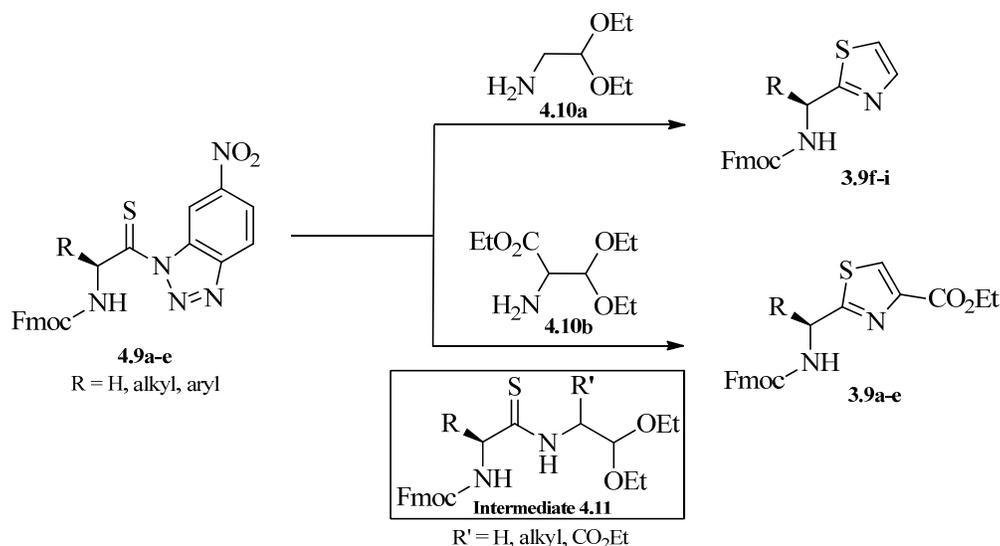
We began our investigation by choosing two different amines **4.10a-b** containing suitable protected functionality and sourcing routes for their synthesis. Amine **4.10a** ($R = \text{H}$) was commercially available and so would be suitable for testing methodology. Amine **4.10b** ($R = \text{CO}_2\text{Et}$) would be appropriate for the synthesis of longer chain peptide derivatives and was prepared by following known literature procedures.^{81, 82, 83} In short, compound **4.29** was prepared by the reacting the appropriate α,β -unsaturated ester **4.28** with NBS in ethanol. The obtained product **4.29** was subsequently converted to its corresponding azide and ultimately afforded the corresponding amine **4.10b** upon treatment with Pd/C and H_2 gas in ethanol.



Scheme 4.10: Synthesis of amine **4.10b**.

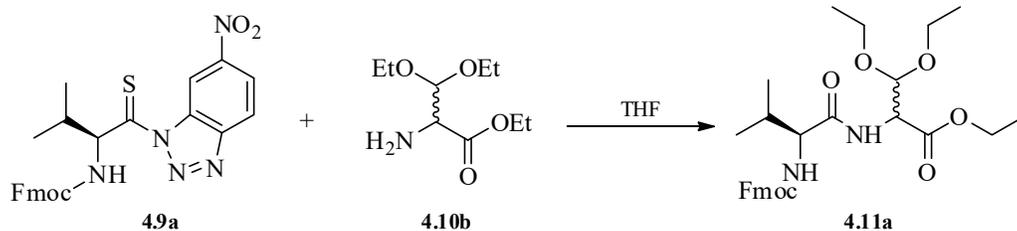
After having the amines in hand, they were reacted with various benzotriazolyl thioacylated compounds **4.9a-e** to afford the targeted chiral thiazole derivatives **3.9**. Coupling with 2,2-

diethoxyethylamine **4.10a** should give the corresponding *N*-(2,2-diethoxyethyl)thioamide **4.11**. The cyclisation of which under acidic conditions by cyclodehydration should give the corresponding monosubstituted thiazole in good to excellent yields. This new method provides a facile route to chiral thiazoles in the solution, which on further development could be automated on the solid phase.



Scheme 4.11: Synthesis of substituted thiazoles **3.9**.

The thiobenzotriazole derivatives **4.9a-e** were coupled with Ethyl 2-amino-3,3-diethoxypropanoate **4.10b** in THF to give corresponding *N*-functionalized thioamide 3,3-diethoxy-2-amino-propionic acid ethyl ester **4.11a** in quantitative yield. However, at the outset of the project there was limited access to a chiral stationary phase for HPLC analysis so a conventional methods were employed to confirm that amide **4.11a** was present as a mixture of diastereoisomers in a 1:1 ratio as expected. Since the amine **4.10b** was synthesized as a racemic sample, a mixture of diastereoisomers was expected for amide **4.11a** (**Figure 4.6**) but further data on the enantiopurity of this derivative was required.



Scheme 4.12: Synthesis of intermediate amide **4.11a**.

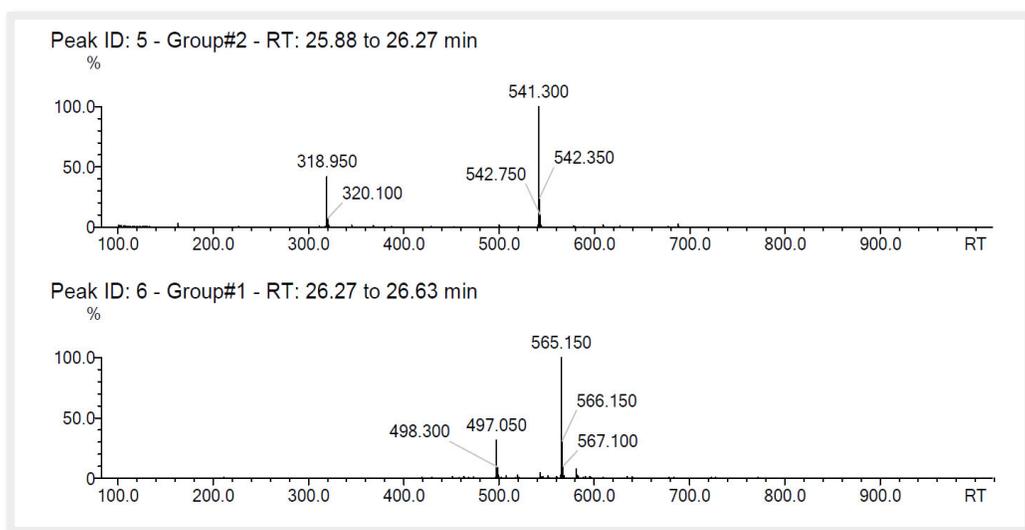
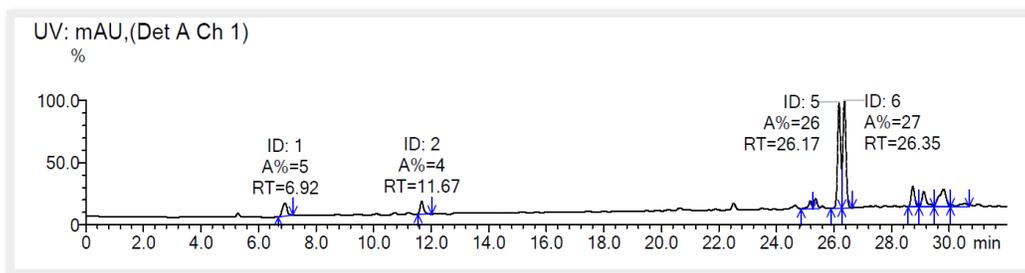
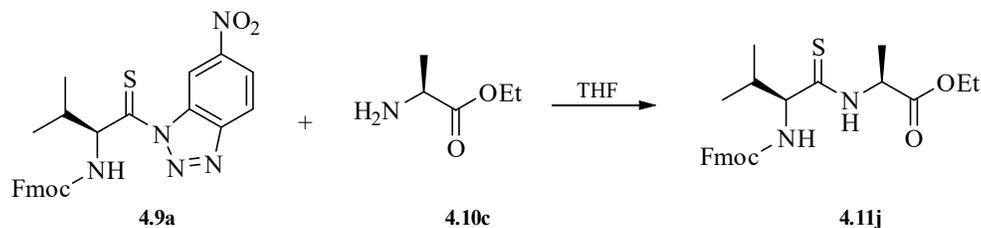


Figure 4.6: HPLC analysis using racemic sample as the amine component.

To overcome the lack of enantiopurity data at that stage (**Scheme 4.12**), further experiments were carried out with commercially available L-alanine ethyl ester **4.10c** as the amine component to confirm that thioacylation for this alternative substrate proceeded without racemization. The valine-derived benzotriazole **4.9a** was reacted with L-alanine ethyl ester **4.10c** under identical conditions to afford

thioamide **4.11j** in quantitative yield (**Scheme 4.13**). The HPLC analysis of the product dipeptide **4.11j** showed only one peak suggesting the presence of a single diastereoisomer of the corresponding amide **4.11j** (**Figure 4.7**), confirming that within the limits of HPLC analysis racemization had not occurred.



Scheme 4.13: Synthesis of intermediate amide **4.11j**.

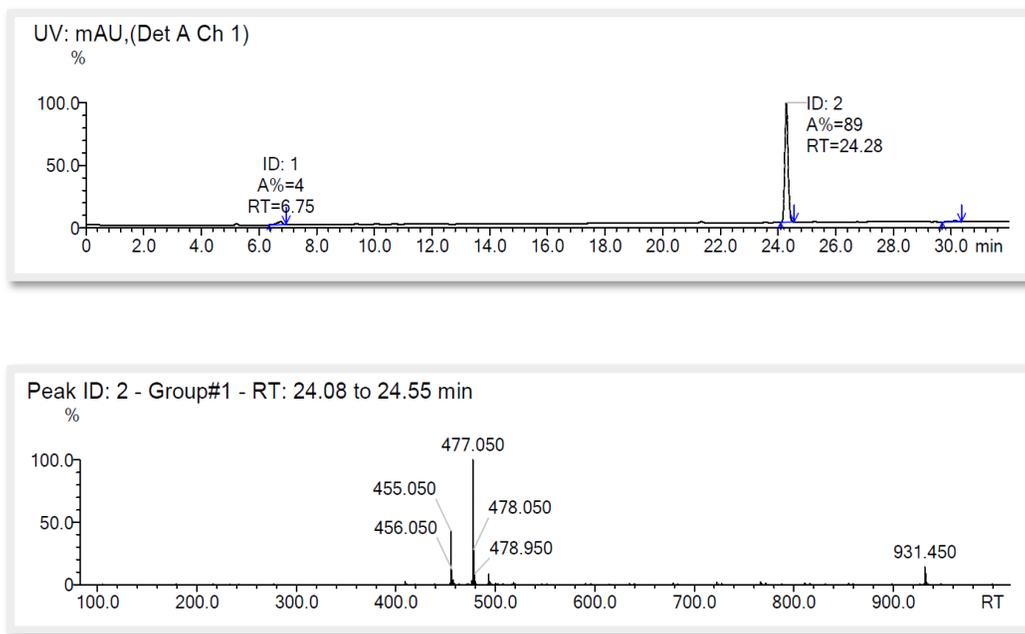
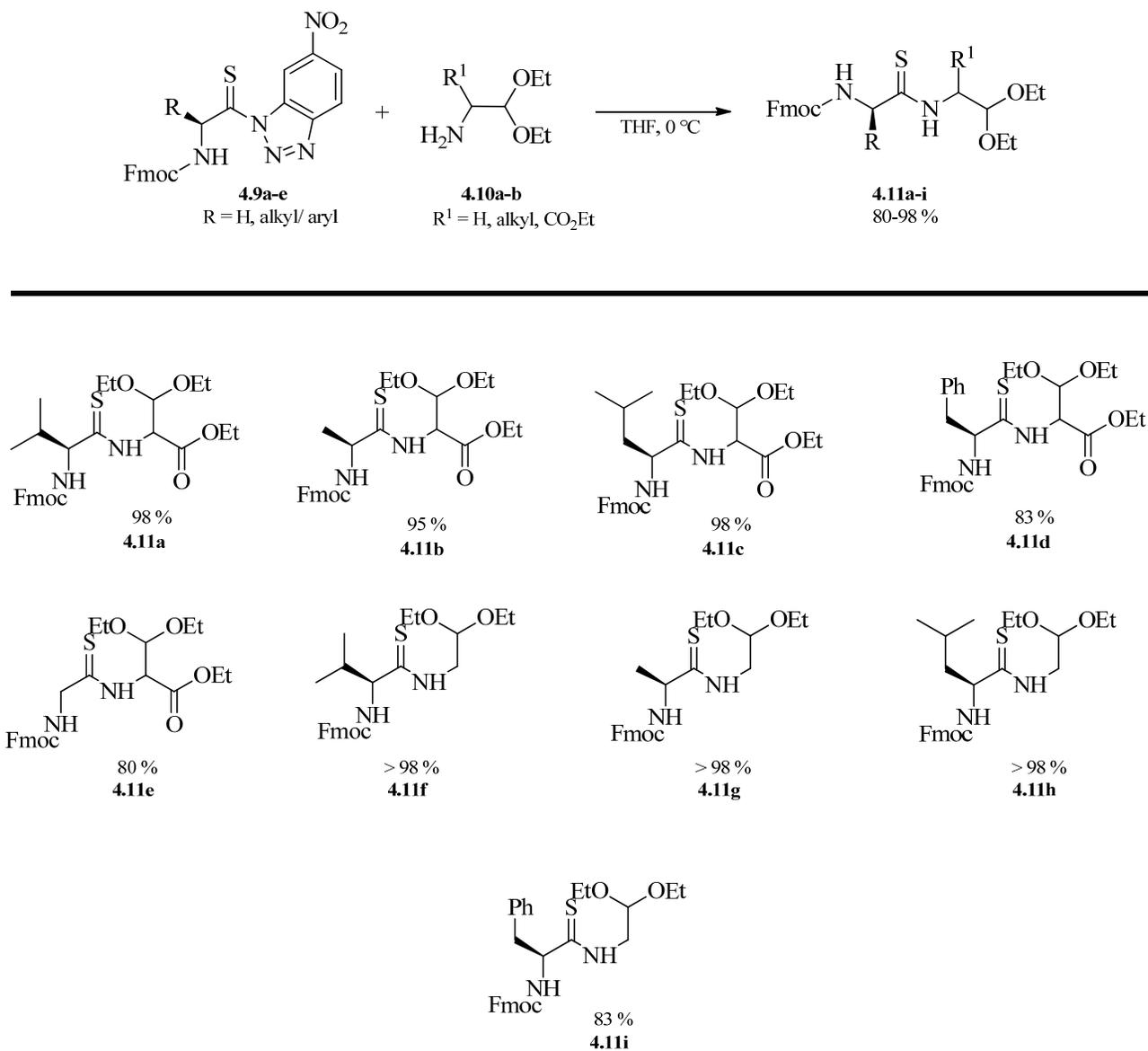


Figure 4.7: HPLC analysis using single enantiomer sample as the amine component.

4.2.2 Preparation of Thioamide Cyclization Precursors

Having developed a method for the synthesis of amides thioacylated at a specific position that appeared to proceed in an enantiospecific fashion, we turned our intention to synthesise different amide

precursors required for thiazole synthesis to establish the scope of the thioacylating process. To achieve this, the two diethoxyamine precursors **4.10a-b** were coupled with a range of chiral amino acid derived thiobenzotriazoles **4.9a-e** to obtain the desired thioamides **4.11a-i** in good to excellent yields. Overall, the reaction showed excellent effectiveness with this range of R substituents. The general reaction scheme is outlined below.



Scheme 4.14: Synthesis of thioamide derivatives.

Amides **4.11a-d** and **4.11f-h** were obtained in excellent yields. However, amide **4.11e** was formed in slightly lower yield perhaps due to reduced efficiency in the isolation process rather than as a consequence of reduced reactivity.

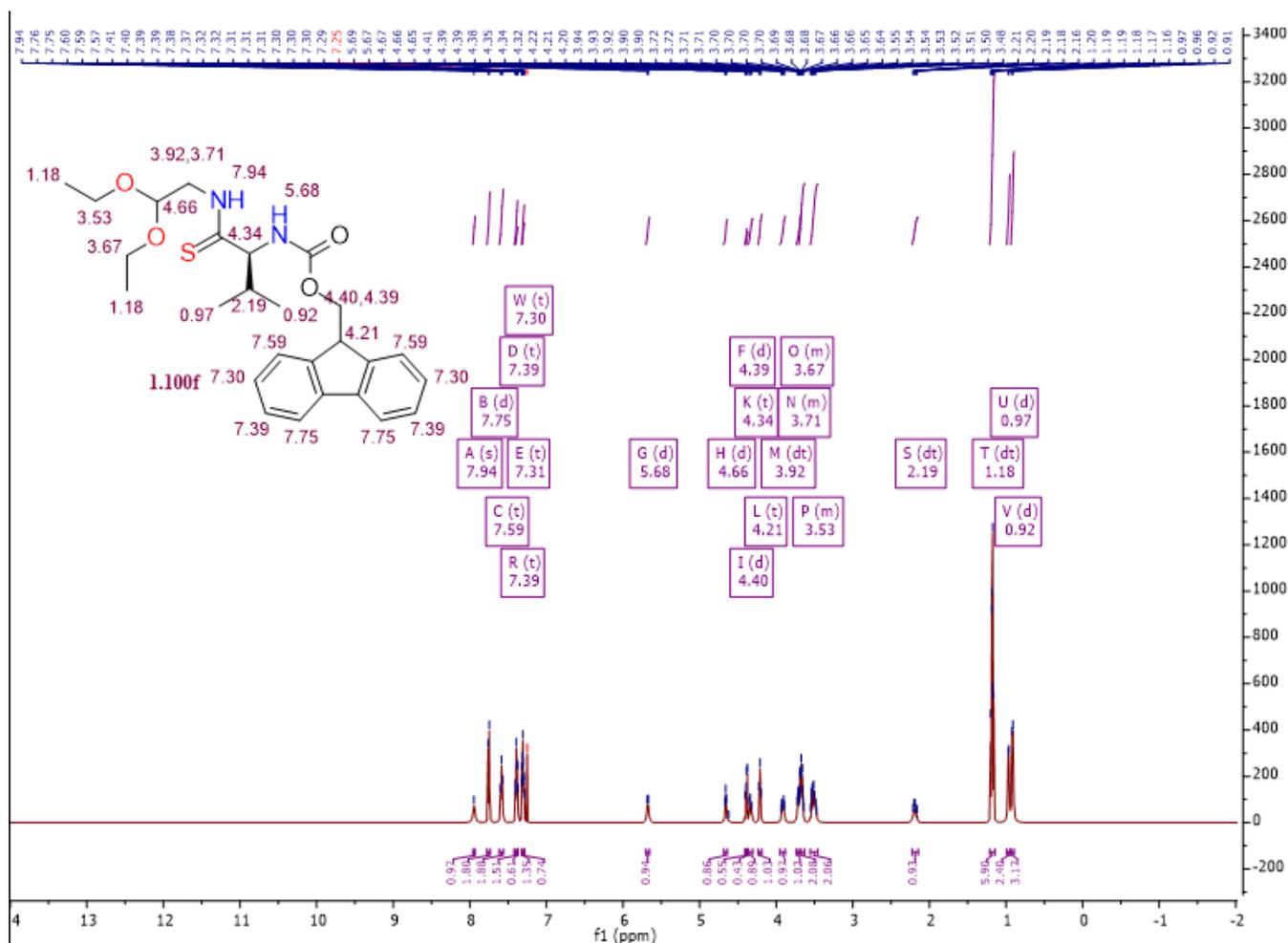
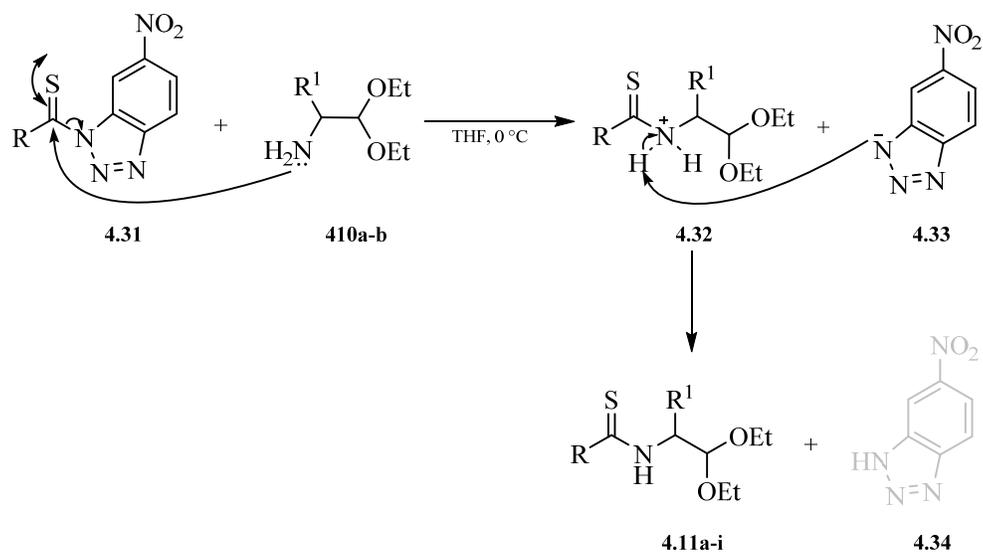


Figure 4.8: ^1H NMR spectrum of *N*-Fmoc-L-valinthioamido derivative **4.11f**.

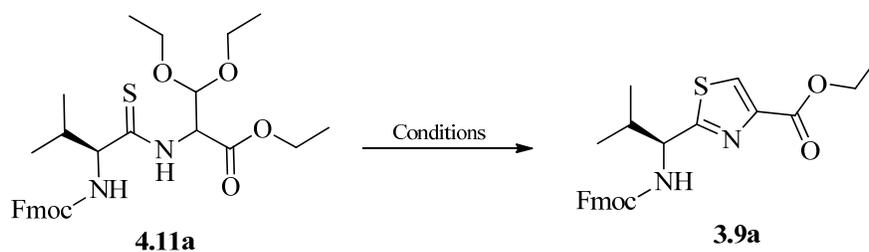
Mechanistically speaking, the nucleophilic nitrogen of the amino group of compounds **4.10a-b** attacks the carbon atom of thioamide **4.31** in an addition-elimination process to afford intermediate **4.32** and **4.33**. A rapid proton transfer from **4.32** to the negatively charged nitrobenzotriazole anion **4.33** would give the desired amides **4.11a-i** and the leaving group side product benzotriazole **4.34**. The reaction mechanism is shown below (**Scheme 4.15**).



Scheme 4.15: Mechanism for the synthesis of thioamide derivatives.

4.2.3. Optimization of the cyclisation reaction conditions

Having synthesized thioamide derivatives **4.11a-i**, this range of substrates was subjected to oxonium ion-mediated cyclisation reactions catalyzed by different acids at variable temperatures in various solvents. The results of our investigations are summarized below (**Table 4.3**).



Scheme 4.16: Robinson-Gabriel cyclisation of *N*-protected thioamide-ester **4.11a**.

Entry	Solvent	Reagent	Time	Temp. / °C	Yield %	ee (%) ^h
1	CH ₂ Cl ₂	Acetic acid ^a	24 h	25	N.D. ^g	
2	MeCN	Acetic acid ^b	24 h	25	N.D. ^g	
3	CHCl ₃	Amberlyst 15	24 h	25	N.D. ^g	
4	CHCl ₃	Amberlyst 15	35 min ^f	120	20%, Product lost	8%
5	MeCN	Formic acid ^d	3h	90	60	<2%
6	MeCN	Formic acid ^d	35 min ^f	120	96	<2% ^c
7	MeCN	Formic acid ^d	24 h	25	Mixture	
8	CH ₂ Cl ₂	PPh ₃ / I ₂ / Et ₃ N ³⁸	24 h	25	N.D. ^g	
9	MeCN	H ₃ PO ₄ ^e	30 min ^f	120	80	<2%
10	Toluene	H ₂ SO ₄ ^c	30 min ^f	120	80	<2%

a) DCM containing 20% to 50% (v/v) of acetic acid

b) acetonitrile containing 20% to 50% (v/v) of acetic acid

c) toluene containing 30% (v/v) of sulfuric acid

d) acetonitrile containing 30% (v/v) of formic acid

e) acetonitrile containing 30% (v/v) of phosphoric acid

f) by microwave

g) Only unreacted starting material was observed upon spectroscopic analysis of the crude material

h) Determined using HPLC analysis on a chiral stationary phase (CHIRALPAK® AD column)

Table 4.3: Optimization of the cyclisation of *N*-protected thioamide-ester **4.11a**.

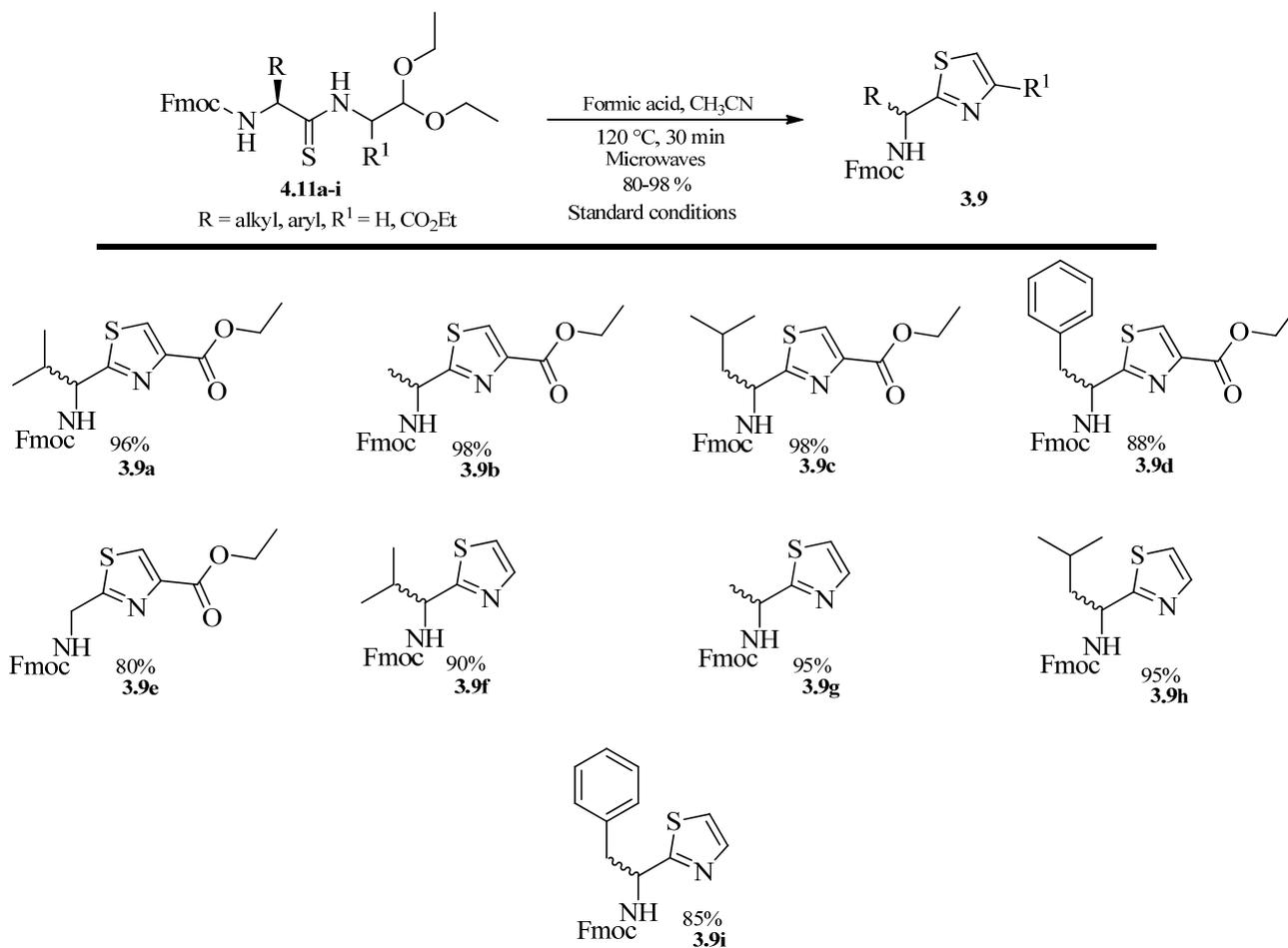
At the outset of optimizations, we chose thioamide **4.11a** as a standard substrate. Assuming that the amide **4.11a** would undergo smooth cyclisation in the presence of a dehydrating agent like sulfuric acid or phosphoric acid at optimum temperatures. To our surprise, the reaction failed to produce any product even after prolonged duration i.e. 24 hours. The reaction was performed in solvents like DCM and chloroform at 25 °C for 24 hours catalyzed by acetic acid and Amberlyst-15 however none of these reactions gave any product (**Table 4.3 entries, 1-3 & 8**). In the case of entries 1-3, almost all of the starting material was recovered, however, a mixture of starting material and product was obtained when

reaction was performed in acetonitrile mediated by formic acid at 25 °C (**entry 7**). The reaction failed to proceed when performed with PPh₃/ I₂/ Et₃N³⁸ conditions which would generally produce oxazoles from keto precursors (**Table 4.3, entry 8**). Further attempts to optimize the reaction under ambient conditions were neglected in favour of more rigorous conditions to perform the reaction.

Next, we attempt to do the reaction at high temperatures i.e. 90 °C. was investigated Surprisingly, we got 60 % conversion of the starting amide to its corresponding chiral thiazole (**Table 4.3 entry, 5**) along with the formation of intermediate **4.37** and unreacted starting material. This proved that the reaction is indeed temperature dependent and would work better when performed more forcing conditions i.e. high reaction temperature, strong dehydrating agent and appropriate solvent. It was decided to investigate the reaction under microwave irradiation in a hope to improve the conversion. To our delight, the reaction worked exceptionally well and gave 96% of the targeted thiazole in 35 minutes when mediated by formic acid in acetonitrile solvent (**Table 4.3 entry, 6**). Keeping this in view, we decided to change the solvent and the acid required for catalysation, gratifying 80% of the product was obtained when sulfuric acid and phosphoric acid mediated the reaction in toluene and acetonitrile solvents. (**Table 4.3 entries, 9-10**). Surprisingly, diminished yield was achieved when the reaction was mediated by Amberlyst-15 in chloroform solvent under microwave irradiation. It was concluded that the Amberlyst-15 have immobilized or degraded the product. Attempts to recover the target thiazole by successively washing Amberlyst-15 with sodium bicarbonate solution were in vain as only 20% yield of product could be recovered.

4.2.4. Microwave-Assisted Robinson-Gabriel Thiazole Synthesis

Our optimization experimentation found the that reaction worked best under microwave irradiation and in the presence of strong acids like sulfuric acid and phosphoric acid at 120 °C. Since the reaction worked much better under microwave irradiation and moved to completion in a short period of time, it was decided to carry out further cyclisation reactions under these conditions to appreciate the scope of the process. A range of novel *N*-Fmoc protected chiral thiazoles were synthesized. Formic acid was chosen as dehydrating agent, acetonitrile as standard solvent and 120 °C as standard temperature for all the transformations. The corresponding thiazoles and their yields are given as follows (**Scheme 4.17**).



Scheme 4.17: Thiazole-containing amino acids prepared by optimized method.

Thiazoles **3.9a-c**, **g-h** were obtained in excellent yield i.e. >90 % from their corresponding amides including thiazoles containing no substituent at C-4 which were also synthesized in good yield **3.9f-i**. Overall, reaction showed great functional group (alkyl/aryl) tolerance when performed under microwave irradiation in the presence of formic acid as a dehydrating agent. However all the cyclisation products were obtained in racemic form.

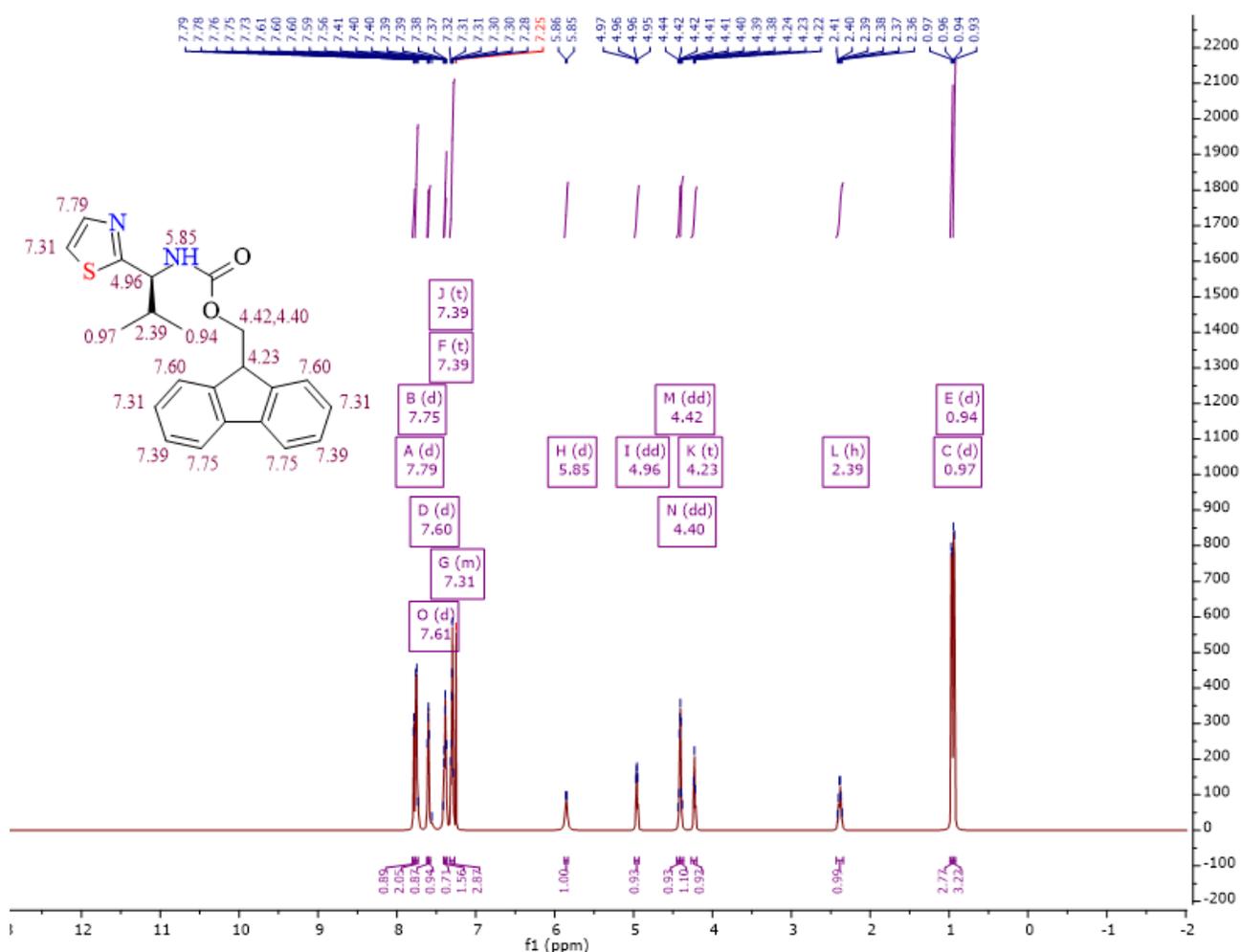
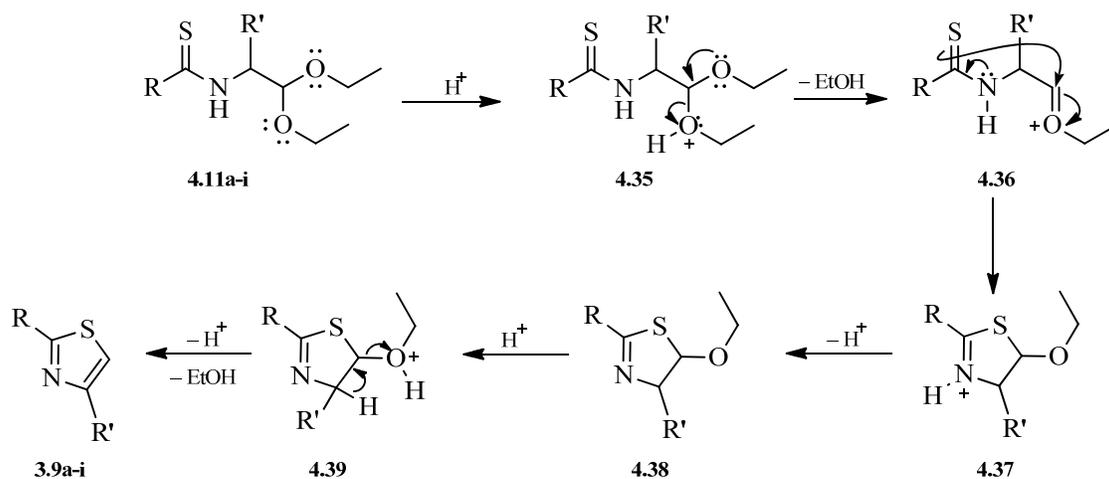


Figure 4.9: ¹H NMR spectrum of *N*-Fmoc-L-Val- derived thiazole **3.9f**.

Structure elucidation was confirmed by analysis of ¹H and ¹³C NMR spectroscopic and mass spectrometric data. For example, thiazole **3.9f** where the resonance frequency of the proton from the diethoxy group in starting materials disappeared and new resonances from the thiazole ring appeared at

δ 7.31 and 7.79 ppm. This was supported by data from the ^{13}C NMR spectrum where the resonant frequency of the carbon from the thiocarbonyl group disappeared and a new resonance appeared from the thiazole ring (2-C) at δ 171.6 ppm. Mass spectrometric data confirmed the mass and identity of the product as a protonated molecular ion (MH^+) was observed at m/z 379.1479, consistent with successful thiazole formation.

Mechanistically, the acid protonates one of the ethoxy substituent of the starting amide to produce oxonium ion **4.36**. This electrophile undergoes intramolecular attack to produce ethoxythiazoline **4.38** after deprotonation of the cyclization product **4.37**. In the final step, ether **4.38** is protonated and eliminates water with the loss of a proton at C-4 to produce the aromatic heterocycle **4.12a-i** and ethanol as a by-product.



Scheme 4.18: General mechanism of amide cyclisation to afford thiazoles.

Although it was hoped that a Robinson-Gabriel type approach under catalysis by a relatively weak acid such as formic acid might not suffer from the same challenges of racemization as the Hantzsch thiazole synthesis, where HBr is produced in the process, this was found not to be the case. Thus, despite this new approach being an extremely efficient route to the target thiazoles, it was not in its current form

able to deliver the targets with enantiocontrol. However, if alternative conditions could be found for cyclization that were compatible with an α -stereocentre, this would offer a valuable new approach especially if the benzothiazole-containing thioacylating reagents could be stored and used on demand. With the hope that future work would be able to deliver this goal, efforts turned to studying if the thioacylation-cyclization technology could be incorporated into an automated method.

4.3 Solid Phase Peptide Synthesis (SPPS)

4.3.1. Peptides Synthesis

The regulatory and control mechanisms and measures for a majority of biological processes depend on the production of peptides and proteins from their constituent amino acids.^{66, 84} Moreover, a majority of medicines produced in the current age are produced from peptides and in some cases are derivatives of peptides.⁶⁶ Amongst the clinical treatments derived from peptides include anti-cancer agents, peptide-based blood pressure control drugs, and antibiotics. Peptide and amino acid chemistry have become a crucial part of many fields of chemistry and medicine, including organic chemistry, biotechnology, medicinal chemistry, and biochemistry. Synthetic peptides and their respective derivatives are now accessible by many scientists and members of the entire scientific community because the process of solid phase peptide synthesis is readily automated, and more improvements and research work is being implemented in these processes. Conversely, the automation of other branches of synthesis is at very preliminary stage despite the value of many of these biologically active compounds.^{63, 85, 86}

The main concept in solid-phase peptide synthesis is the attachment of the peptide chain to a polymeric resin. This approach allows the operator to remove unreacted reagents without washing away the

peptide. The carboxyl (COOH) moiety of every amino acid is activated through derivatization and then is bound to the growing peptide chain on the resin. The incoming alpha-amino group is protected to prevent competing peptide formation. A new synthesis cycle removes the protecting group and the peptide chain extended through reiteration. Finally, the peptide is cleaved from the resin and analysed to verify its quality.^{63, 85, 87} The most common and reliable strategy for accessing simple peptides that is readily automated involves use of the Fmoc-protecting group and a nascent peptide that is built up one amino acid at a time on the resin through immobilization at the C-terminus (**Figure 4.10**).

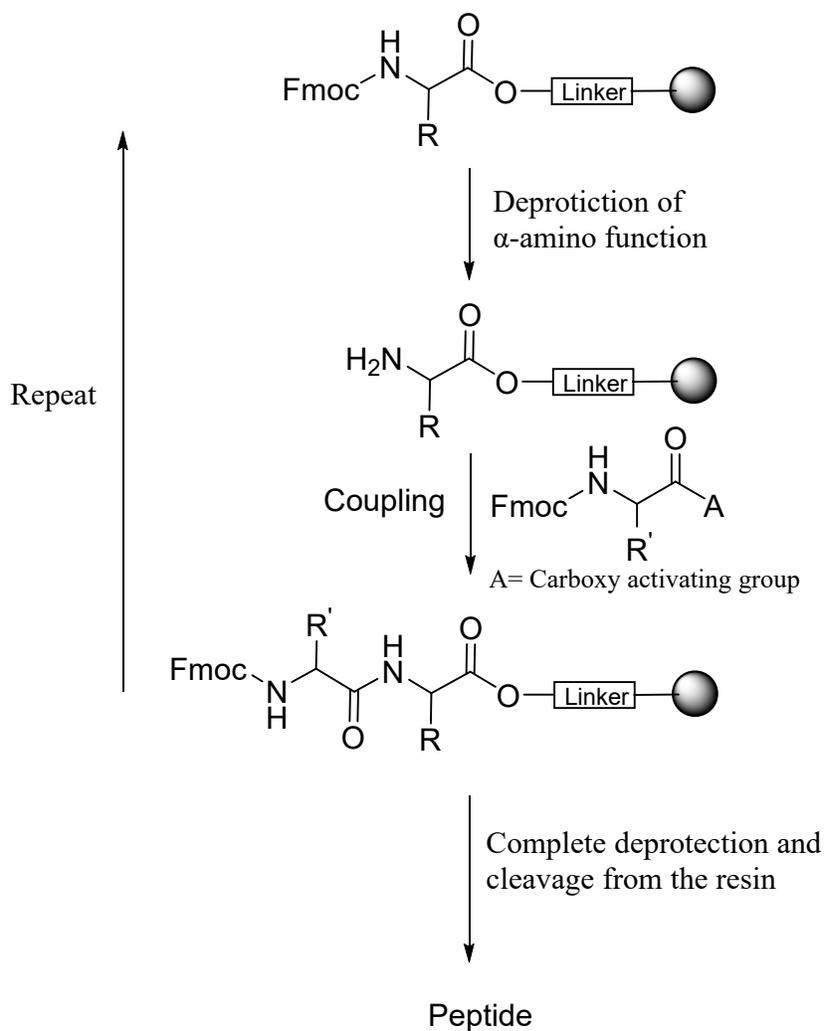


Figure 4.10: General scheme of solid phase peptide synthesis.^{84, 87}

4.3.2. First residue attachment

Attachment of the first residue to the solid resin is crucial as it ultimately determines the purity of the final peptide product. The type of resin that a researcher decides to use in the peptide synthetic process will determine the procedure that must be followed to ensure reliable and efficient first residue attachment.⁸⁴ The first residue attachment entails the anchoring of a protected terminal amino acid residue to a compact or synthesis support through the use of an ester or amide bond, but it entirely depends on the functional group of a specific peptide that is likely to be acid or amide. C-terminal amino acids anchored to support now contain permanent protection through the carboxy group. All the reactive functional groups present in each amino acid must be masked with protecting groups if they are to be rendered inert to reaction conditions present during the peptide chain assembly.^{84, 63, 85, 88}

4.3.3. Fmoc Removal

Fmoc removal occurs when one can separate the Fmoc group from the N-terminal of the resin-bound peptide chain.^{89, 84} Fmoc removal is regularly utilized in combination with piperidine as a solution in DMF. The most crucial step in Fmoc removal is the deprotonation of a fluorene ring to develop an aromatic pentadiene type intermediate. The pentadiene type intermediate later forms the dibenzofulvene, which is recaptured by piperidine to form an adduct that prevents the alkalinizing of the new amino acid group. The electron-withdrawing influence of the fluorene ring system of the Fmoc group makes the hydrogen of the beta-carbon acidic and easy to remove by weak bases. Beta elimination leads to the formation of stable adducts, which results from trapping dibenzofulvene by cleavage agents. The Fmoc group is swiftly removed by the use of primary and secondary amines that include cyclohexylamine, piperidine, ethanolamine, and piperazine and slowly removed by the tertiary amines that include triethylamine and N-diisopropylethylamine. Removal of the Fmoc group also

occurs fast in a polar medium that contains formamide or N-methylpyrrolidone in comparison to solvents such as dichloromethane, which form a non-polar medium.^{89, 84}

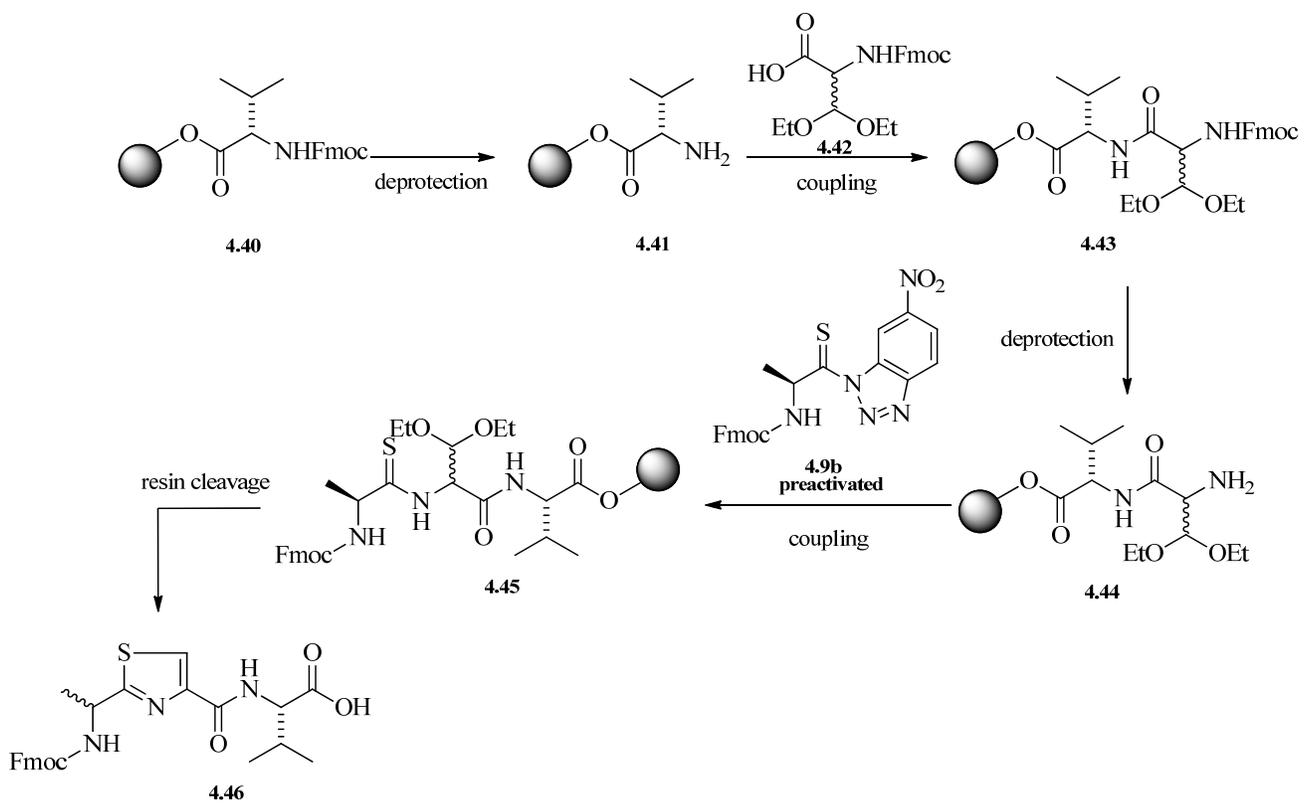
4.3.4. Coupling Methods

The coupling methods refer to the introduction to protected amino acids, which entail an in situ carboxy activation of new and incoming groups of amino acids with activating reagents.^{63, 84} In the coupling process, the HATU and HBTU reagents are used for the synthesis of peptides since the two active reagents are efficient and result in minimum racemization/epimerization in comparison to other reagents that can be utilized for the same process.⁹⁰ Peptide bond development requires the activation of a carboxyl group, after which aminolysis of the activated carbonyl occurs. The perfect strategy must be utilized by the researcher to ensure isomerization is avoided. Approaches and conditions should be selected to minimize aggregation that might interfere with the coupling process. A coupling reagent is used to ensure the proper coupling of two amino acids. A researcher can select from a variety of coupling reagents that include carbodiimides, phosphonium, uranium, and aminium reagents. Recent studies show that adding a hydrogen bond acceptor to the iminium coupling reagent can lead to increased performance. The current studies on the reagents used during the coupling of amino acids aim at ensuring that the process is less dangerous and has little waste.^{63, 85}

4.3.5. Developing an Automated Method for the Synthesis of Thiazoles

The work describes a study of a new approach to the synthesis of substituted thiazoles on solid phase using automated platform as a configuration for the future practice of compound assembly. The main goal of this work was to prepare thiazole in an automated way and establish if the fast kinetics and high efficiency observed in solution could be transferred to reaction on the resin. Initially, thiazole was prepared in the liquid phase using Robinson-Gabriel cyclisation, to simulate the method of preparing thiazoles using an automated method.

The solid-phase peptide synthesis (SPPS) reaction using Automated Microwave Peptide Synthesiser (**Liberty Blue**) started with preloaded Fmoc-Val-Wang resin LL **4.40**. The first step removed the Fmoc group from the N-terminal of the resin (cleavage of the N-protecting group) using 20% piperidine-DMF, to give compound **4.41**. The deprotected resin was then washed with DMF and coupled with Fmoc-FGly(OEt)₂-OH **4.42** by activation of a carboxyl group using OxymaPure/DIC as a coupling reagent to give compound **4.43**. Next, repeat of the Fmoc deprotection step produced compound **4.44** which was then coupled with preactivated thiobenzotriazole **4.9b** as a key step to give compound **4.45**. The last step was cleavage from the resin with cyclization under acidic conditions by formic acid/CH₃CN to produce desired product **4.46**. It was anticipated that the acidic conditions would both deprotect the acetal and thereby cyclize to the thiazole and cleave the peptide from the resin to give an automated route to a thiazole containing target.



Scheme 4.19: Automated Method for the Synthesis of Thiazole **4.46**.

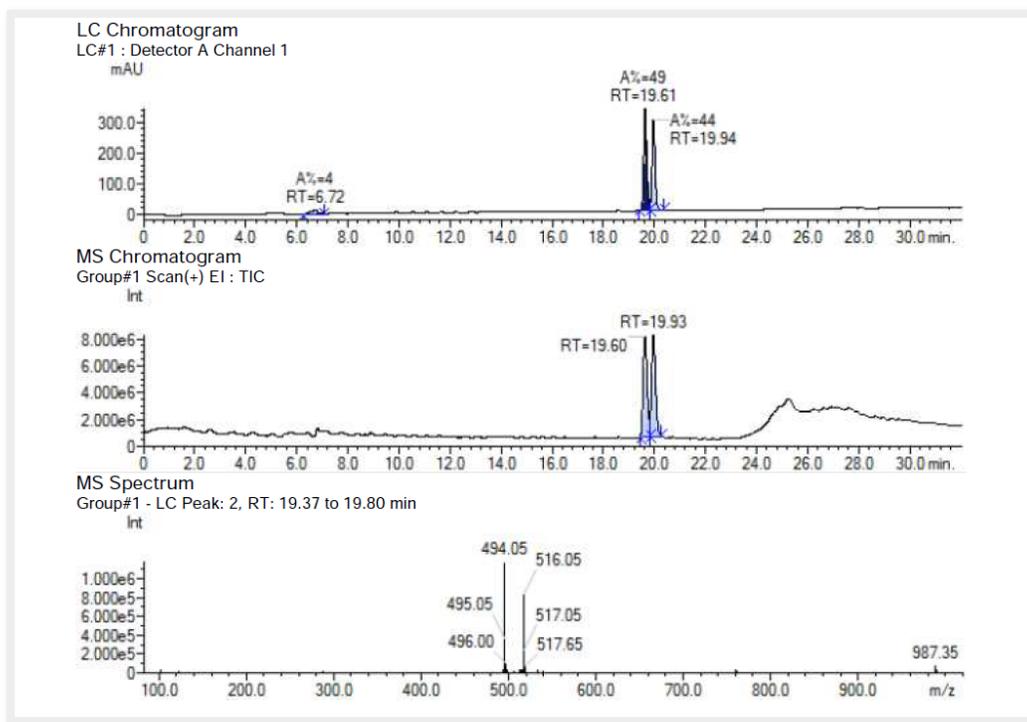


Figure 4.11: HPLC data analysis for Thiazole **4.46**.

Our investigation of the HPLC analysis of the thiazole **4.46** confirmed the low-resolution mass 494.05 consistent with successful thiazole formation and the existence of two stereoisomers of thiazole **4.46**.

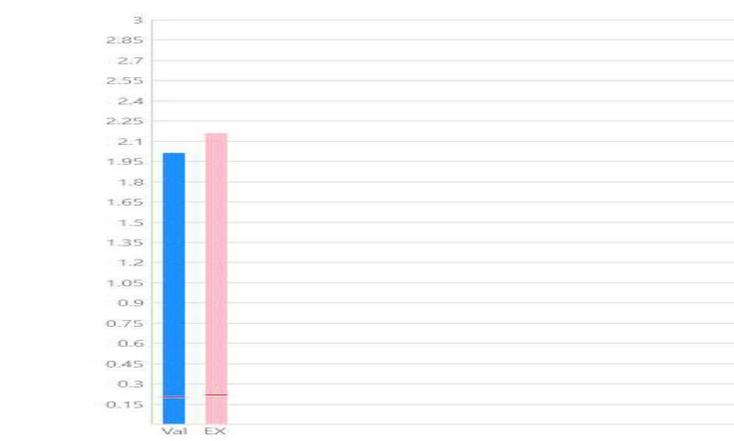


Figure 4.12: Deprotection kinetics for Fmoc-Val-Resin **4.40** and compound **4.43**.

values were calculated as the percentage of the theoretical resin loading by measuring absorbance at 300 nm

4.4 Conclusion

A new route towards chiral thiazoles was developed, although an efficient, rapid, automated and highly stereoselective route to synthesize thiazole with stereogenic center close to the ring is challenging. Thiazole **3.9** was successfully synthesized over five steps in an overall yield between 80 to 90% albeit the product was a racemic mixture. Using mixed carbonic anhydride methodology for peptide synthesis to make anilides, followed by direct thionation using P₄S₁₀ gave thioanilides **4.8**. Intramolecular mediated cyclisation of thioanilides **4.8** was investigated using nitrous acid generated in situ by protonation of NaNO₂ with AcOH to give the corresponding benzotriazoles **4.9**.

The amine to react these thioacylating agents with, compound **4.29**, was prepared by the reacting the appropriate alkenyl ester **4.28** with NBS in ethanol. The obtained bromide **4.29** was subsequently converted to its corresponding azide and ultimately afforded the corresponding amine **4.10b** upon treatment with Pd/C and H₂ gas in ethanol. After having the amines in hand, they were reacted with various benzotriazolyl thioacylated compounds **4.9a-e** to afford the targeted chiral thiazole derivatives **3.9** after acid mediated deprotection and Robinson-Gabriel cyclization. Although extremely efficient the challenge of accessing these targets in enantiopure form was not addressed and remains as a goal of future work.

The transfer of this technology to the solid phase has been studied with an automated method for the synthesis of thiazole **4.46** in racemic form started with preloaded Fmoc-Val-Wang resin LL **4.40**. Fmoc deprotection, activation and coupling with racemic Fmoc-FGly(OEt)₂-OH **4.42** gave dipeptide **4.43**; then it was deprotected at the *N*-terminus, coupled with preactivated thiobenzotriazole **4.9b** as a key step to give compound **4.45**. The last step was cleavage the resin with cyclization under acidic conditions by formic acid/CH₃CN to produce desired product **4.46**. Although this appeared to deliver the product, given the solution phase results, the treatment with acid had not only cleaved the product

from the resin but resulted in epimerization. Despite this, the technology and approach had been successful in accessing the target but would need further development to establish an enantiocontrolled method.

Chapter 5: Results and Discussions

5.1. A novel protocol for the synthesis of dihydropyrazinones via acid catalysed cyclisation reaction.

Piperazines, piperazinones, and their derivatives are common pharmacophores found in a wide range of medicines compounds. The derivatives of piperazines formed by a 6-membered aliphatic heterocyclic system with two nitrogen atoms in the 1,4 position, called piperazines (**5.1**), and their variants 2-ketopiperazines (**5.2**) and 3,4-dihydropyrazin-2-ones (**5.3**) (**Figure 5.1**).⁹¹ In addition to their presence in natural products, the pyrazinone and dihydropyrazinone moieties have also been incorporated into several synthetic scaffolds with significant biological activities.

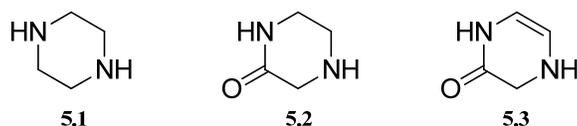


Figure 5.1: Heterocyclic system with two nitrogen atoms in the 1,4 position.

Such scaffolds are widely distributed in nature and can be found in various secondary metabolites such as schischkiniin⁹² (**5.4**), hamacanthin⁹³ (**5.5**) and aureusimine A⁹⁴ (**5.6**). Other relevant bioactive structures with a fused dihydropyrazin-2-one core are antihelmintics 2-azaemetine⁹⁵ and praziquantel⁹⁵. Additionally, pyrazin-2-ones dragmacidin⁹⁶ family (**5.7**) and bamaquimast⁹⁷ (**5.8**) display anti-inflammatory⁹⁶ and anti-allergic properties, respectively.

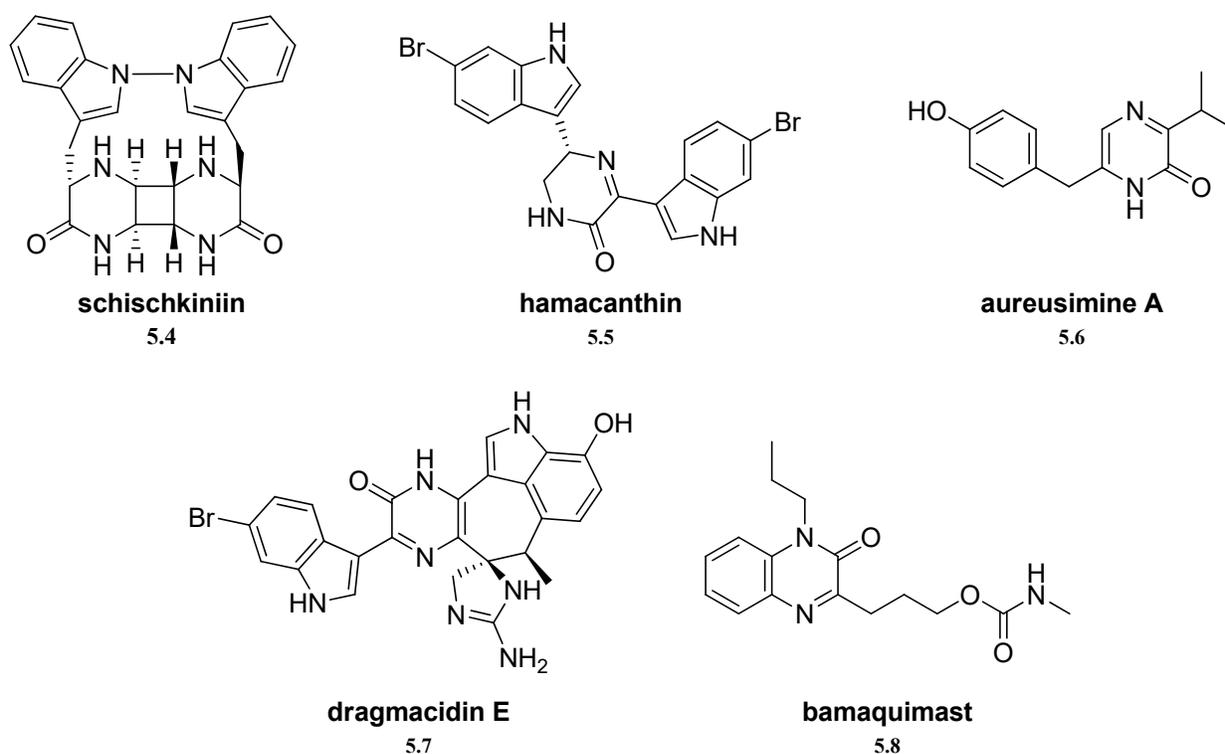
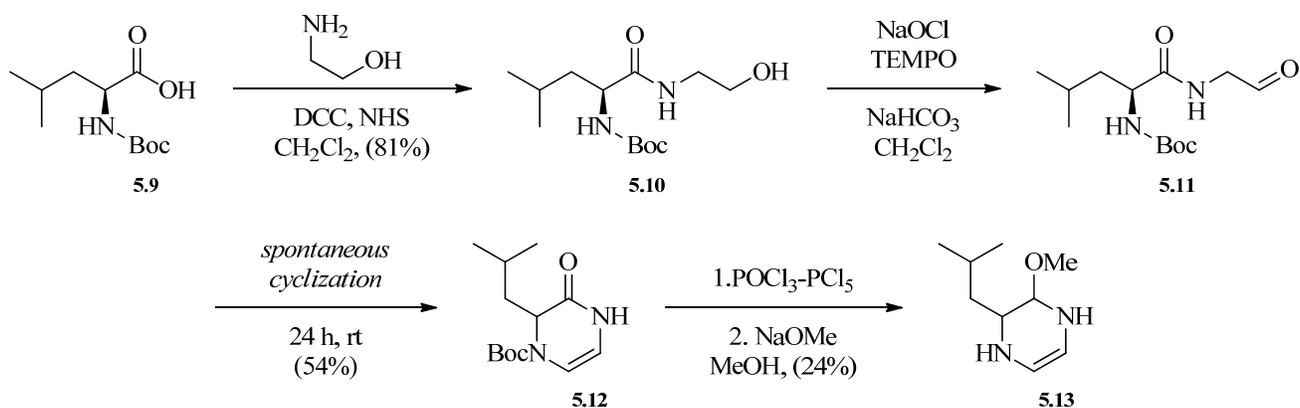


Figure 5.2: The structure of dihydropyrazin-2-one -containing agents

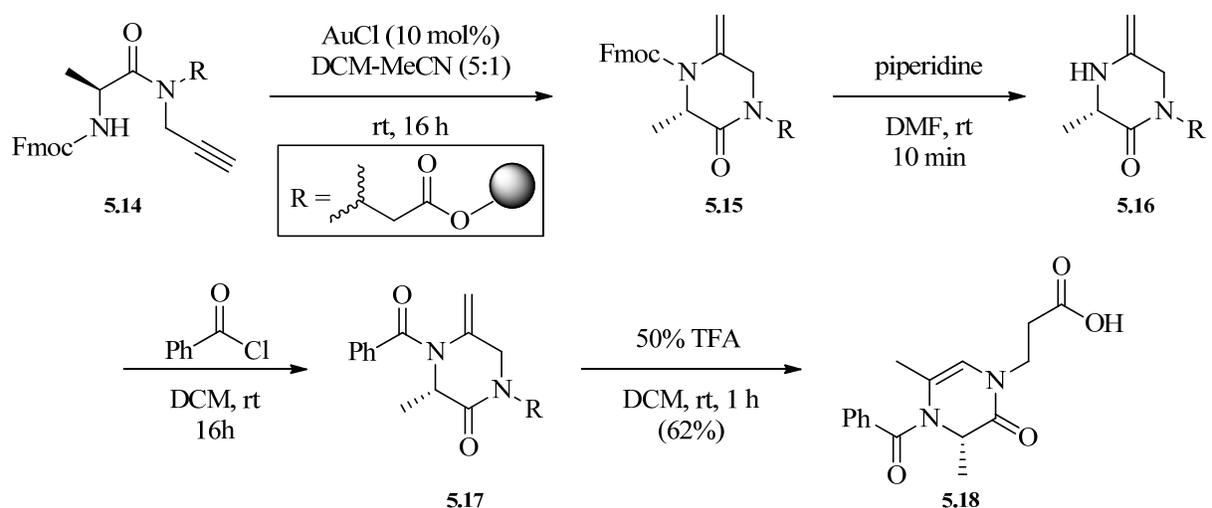
a number of synthetic pathways have been developed and recorded for their easy synthesis in a variety of structural forms.^{98–101}

Babin and colleagues have reported a novel reaction for the synthesis of substituted methoxypyrazines (**Scheme 5.1**),⁹⁹ which were analogs of natural products found in wine. First, a Boc-protected amino acid was condensed with 2-aminoethanol, yielding the amide intermediate. Next, the primary alcohol was oxidized to an aldehyde using TEMPO and household bleach. After leaving the resulting aldehyde to rest at room temperature for 24 hours, spontaneous cyclization furnished the Boc-protected dihydropyrazinone in 54% yield. Further deprotection and chlorination using phosphorous oxychloride with phosphorous pentachloride, followed by treatment with sodium methoxide gave IBMP (**5.13**) (24% yield). This compound is believed to contribute to the flavor profile of wine.⁹⁹

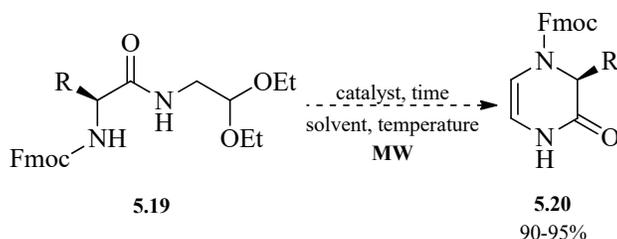


Scheme 5.1: Synthesis of the wine-derived compound IBMP.⁹⁹

Pribylka and Krechnak have described a new gold-catalyzed reaction (**Scheme 2**) for the synthesis of 3,4-dihydropyrazin-2(1*H*)-ones from *N*-propargyl peptides.¹⁰² The peptides were prepared from Wang resin-bound, Fmoc-protected amino acids and propargyl alcohol. Stirring the *N*-propargyl peptide with catalytic AuCl (10 mol%) in a 5:1 mixture of methylene chloride and acetonitrile at room temperature for 16 hours induced cyclization into the fmoc-protected piperazin-2-one intermediate. Next, *N*-acylation was accomplished by deprotecting with piperidine followed by treatment with benzoyl chloride at room temperature. Finally, 50% TFA was used to oxidize the intermediate into a 3,4-dihydropyrazin-2(1*H*)-one and released the product from the Wang resin. The final compound was isolated in 62% yield.¹⁰²

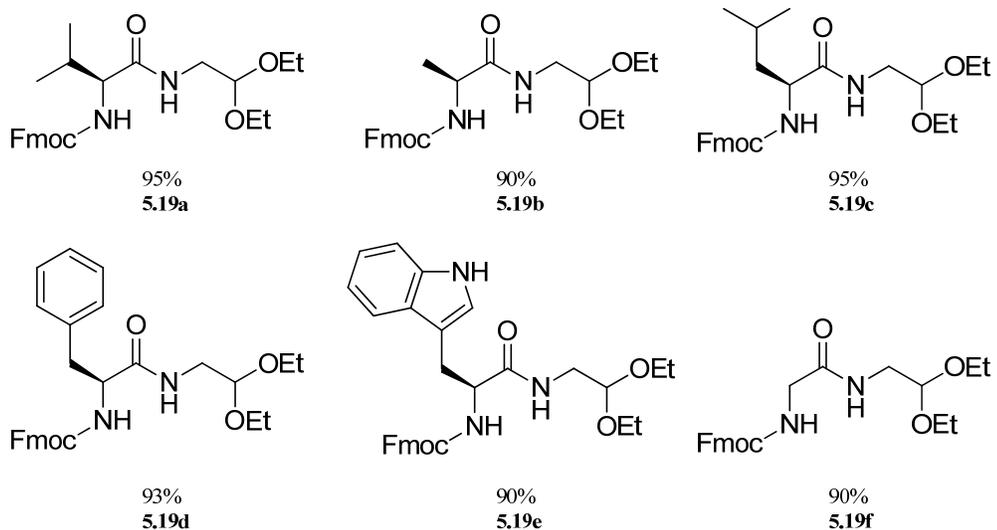
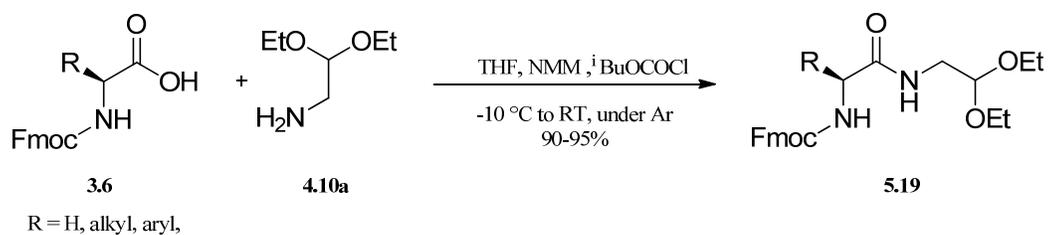


A new protocol for the synthesis of dihydropyrazinones using acid catalysed amide cyclisation reactions has been established.^{102, 103} Our approach to access structurally rich dihydropyrazinones was to subject amides **5.19** to acid catalysed dehydration reactions under microwave irradiation gave **5.20**.



5.1.1 Preparation of amide Cyclization Precursors

The synthesis of the uncommon dihydropyrazinone ring was accomplished by a few steps, starting with the corresponding N-Fmoc-L-amino acid, N-Methylmorpholine and isobutyl chloroformate were added to produce the mixed anhydride,⁷⁶ and then 2,2-diethoxy-ethanamine was added to complete the synthesis of the intermediate amide, which occurs after a nucleophilic attack of the nitrogen atom to the carbon atom of a molecule as shown below (**Scheme 5.4**).



Scheme 5.4: Mixed anhydride coupling reaction between amino acid and 2,2-diethoxy-ethanamine.

A range of substituents were tested in the aforementioned reaction. It was noticed that the amino acids containing bulkier alkyl/ aryl groups proved to be effective substrates as they provided the corresponding amide in excellent yields 90-95 %.

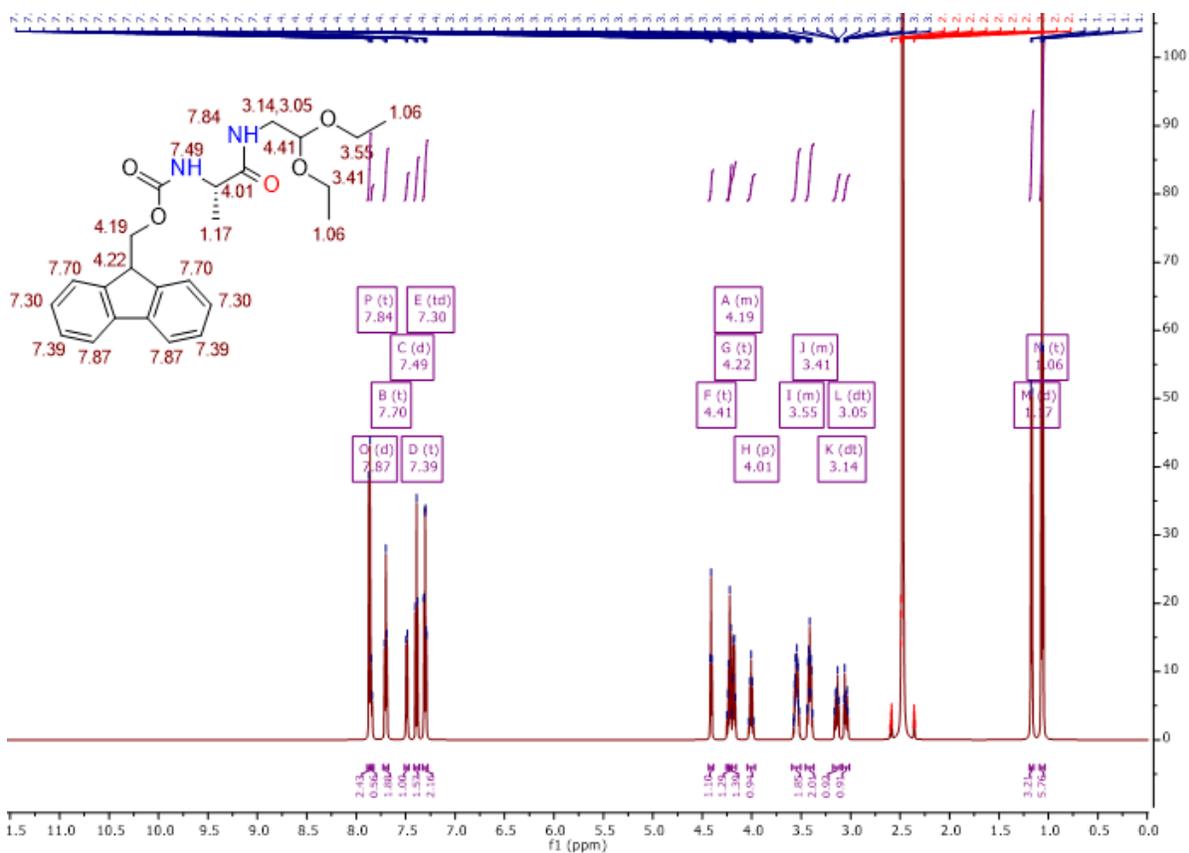
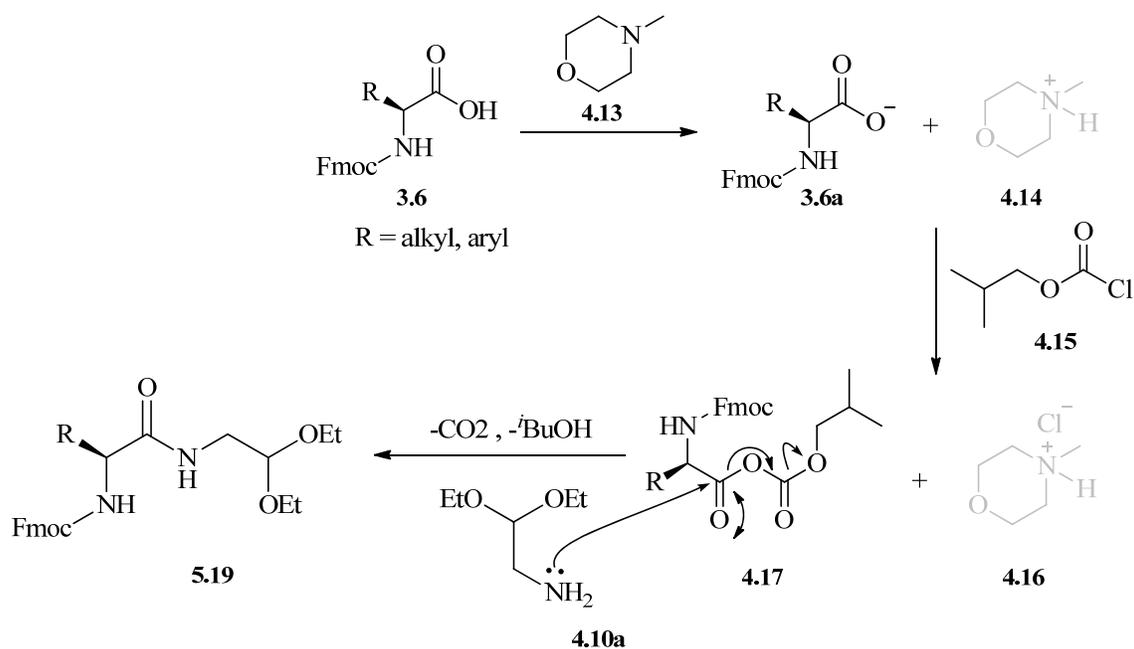


Figure 5.3: ¹H NMR spectrum of 1-(*N*-Fmoc-L-alaninamido)-2,2-diethoxyethane **5.19b**.

Structure elucidation was confirmed by analysis of ¹H NMR spectroscopic, mass spectrometric and melting point data. For example, amide **5.19b** analysis revealed the new resonance of the proton from the amide NH appeared at δ 7.84 ppm and from the diethoxy groups at δ 3.55 and 3.41 ppm. Mass spectrometric data confirmed the mass and identity of the product as the sodium adduct [M+Na]⁺ was observed at *m/z* 449.2037, consistent with successful amide formation. The melting point at 137-138 °C was relatively sharp.

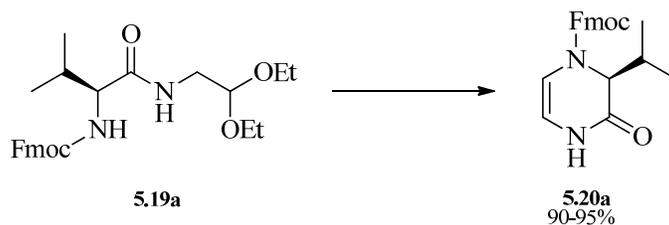
A general mechanism of the above reaction is outlined as follows (**Scheme 5.5**).



Scheme 5.5: A general mechanism for the synthesis of intermediate amide by NMM, IBCF.

5.1.2 Optimization of the cyclisation reaction conditions

Having synthesized amide derivatives **5.19a-f**, they were subjected to cyclization catalyzed by different acids at variable temperatures in different solvents. The results of these investigations are summarized in (Table 5.1).



Scheme 5.6: optimized conditions for cyclization

Entry	solvent	Reagent	Time	Temp. (°C)	Yield (%)
1	Toluene	Amberlyst 15	24h	25	90
2	MeCN	Formic acid ^a	35 min ^c	120	95
3	MeCN	Formic acid ^d	24 h	25	93
4	CH ₂ Cl ₂	PPh ₃ / I ₂ / Et ₃ N	24 h	25	No reaction
5	MeCN	H ₃ PO ₄ ^b	30 min ^c	120	90

a) acetonitrile containing 30% (v/v) of formic acid
b) acetonitrile containing 30% (v/v) of phosphoric acid
c) by microwave irradiation

Table 5.1: The *N*-protected amide **5.19** was subjected to different acidic catalysts

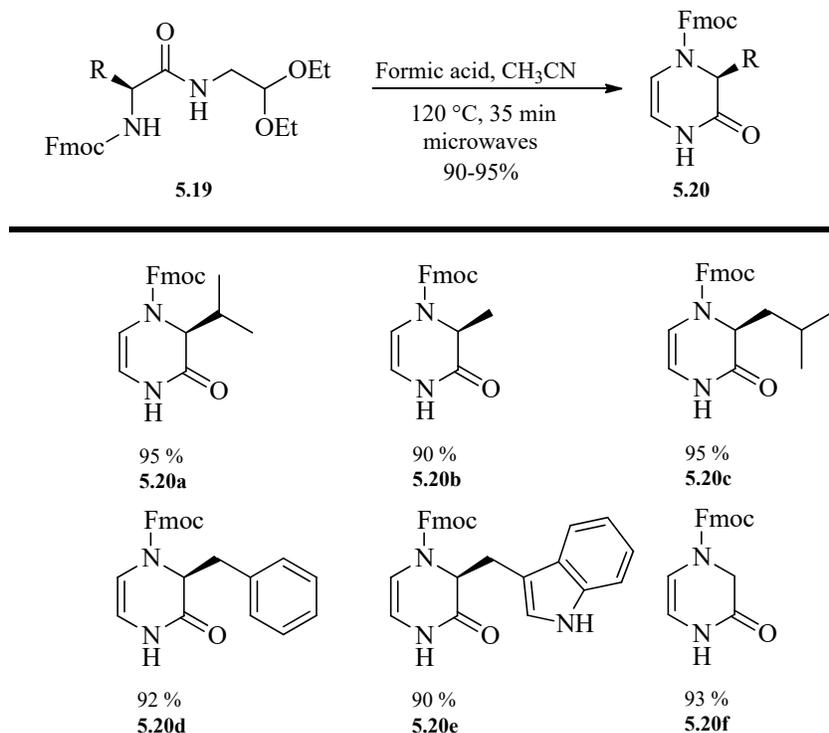
As a starting point for optimization, amide **5.19a** was selected as the model substrate. Assuming that in the presence of acid like formic acid or phosphoric acid at optimal temperatures, the amide **5.19a** will undergo smooth acetal deprotection, form the oxonium ion and cyclize with elimination of ethanol.

The reaction was performed in solvents like DCM, MeCN, and toluene at 25 °C for 24 hours and investigated using PPh₃/ I₂ / Et₃N, formic acid and Amberlyst-15, similar conditions for the the study of thiazole formation. The reaction was successful when Bronsted acids were used (**Table 5.1, entries 1 and 3**) at 25 °C for 24 hours but failed to proceed when the reaction was performed with PPh₃/ I₂ / Et₃N ³⁸ conditions which have been used to produce oxazoles from ketone precursors (**Table 5.1, entry 4**).

Next, we decided to execute the reaction under microwave irradiation to reduce the time and compare the results. To our delight, the reaction worked exceptionally well and gave 95% of the targeted dihydropyrazinones in 35 minutes when mediated by formic acid in acetonitrile solvent (**table 5.1, entry 2**). Keeping this in view, we decided to change the solvent and the acid catalyst; gratifying 90%

yield of the product was obtained when phosphoric acid mediated the reaction in acetonitrile solvents (table 5.1, entry 5).

Our optimization experiment found that the reaction works very well at room temperature and under microwave irradiation at 120 °C in the presence of acids such as formic acid and phosphoric acid. Since the reaction performed much better under microwave irradiation and completed in a shorter period of time these conditions were chosen to investigate the scope of the process. For all the transformations, formic acid was used as the acid catalyst, acetonitrile as the standard solvent, and 120 °C as the standard temperature under microwave irradiation for 35 min (hold time). The corresponding dihydropyrazinones and their yields are given as follows (Scheme 5.7).



Scheme 5.7: dihydropyrazinones prepared by formic acid mediated cyclization.

Structure elucidation was confirmed by analysis of ^1H and ^{13}C NMR spectroscopic and mass spectrometric data. For example, thiazole **5.20a** where the resonance frequency of the proton from the diethoxy group in starting materials disappeared and new resonances from the dihydropyrazinone ring appeared at δ 6.17 and 5.61 ppm. This was supported by data from the ^{13}C NMR spectrum where the resonant frequency of the anomeric carbon from the acetal group disappeared and a new resonance appeared from the dihydropyrazinone ring (3-C) and (4-C) at δ 109.3 and 108.3 ppm. Mass spectrometric data confirmed the mass and identity of the product as the sodium adduct $[\text{M}+\text{Na}]^+$ was observed at m/z 385.1539, consistent with successful dihydropyrazinone formation.

5.2 Conclusion

A new synthetic route to substituted dihydropyrazinone derivatives was developed, which was successfully synthesized in two steps with an overall yield of 90 to 95%. Using mixed carbonic anhydride methodology for peptide synthesis, starting with the corresponding *N*-Fmoc-L-amino acid with aminoacetal to make amides **5.19**, were the amides subject to formic acid in acetonitrile, and after acid mediated deprotection, cyclisation through an oxonium ion intermediate to afford dihydropyrazinones **5.20**. The reaction has been used for library synthesis and gives high quality products. It is possible to introduce an additional site of diversity into this dihydropyrazinones ring by using the more complex aminoacetal derivatives.

In conclusion, these studies have provided new methods for synthesis of thiazole-containing amino acid and dihydropyrazinone derivatives. It is anticipated that extending these studies into biologically-relevant scaffolds and other heterocyclic motifs will expand the utility of this work and, it is hoped, will ensure that the discoveries made as part of this thesis will find widespread application in the future.

Chapter 6: Experimental

6.1. Materials and Methods.

All reactions were performed in a fume hood under air unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 600 MHz spectrometer; chemical shifts are reported in ppm, with TMS as an internal standard; multiplicities are described using standard conventions (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, td = triplet of doublets, m = multiplet); coupling constants are recorded in Hz. LCMS analyses were performed on a 5 μm C18 110 Å column and compound purity was determined using a 30-minute gradient elution using water-acetonitrile with 0.1% formic acid (5 min at 5%, 5%-95% over 20 min, 5 min at 95%) with the UV detector at λ 254 nm. Infra-red (IR) spectra were recorded in the range 4000-600 cm^{-1} using a Perkinelmer 65 series FT IR spectrometer. High resolution mass spectrometry (HRMS) by electrospray ionization (ESI) was performed by Dr. Alaa Abdul-Sada (University of Sussex). TLC visualization was accomplished under UV light and with the aid of an aqueous potassium permanganate stain. Yields refer to isolated yields after purification by flash column chromatography or trituration; where noted, some products underwent additional recrystallization to remove trace impurities. Melting points using MPA100 automated melting point where the system automatically determining the melting points and melting ranges of chemical substances.

6.1.1. General method for synthesis of thiazole 3.9. (A)

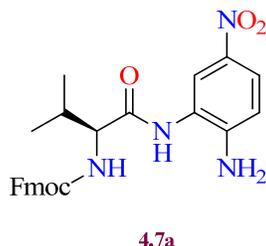
To a solution of acetonitrile containing 30% (v/v) of formic acid was added thioamide **4.11**. The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins , then cooled in a stream of compressed air, was added EtOAc, and the solution was washed successively with aqueous NaHCO_3 solution (5%) and brine, dried (MgSO_4) and evaporated in vacuo, the residue was purified by column chromatographed (hexane/EtOAc) to gave thiazole (**3.9**).

6.1.2. General method for synthesis of thiazole 3.9. (B)

To a solution of acetonitrile was added compound **3.8** (1 eq.) and ethyl bromopyruvate **1.21** (1e.q) The mixture was irradiated at 70 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 3 mints , then cooled in a stream of compressed air, was added EtOAc, and the solution was washed successively with aqueous NaHCO₃ solution (5%)and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatographed (hexane/EtOAc) to gave thiazole (**3.9**).

6.2 Experimental Procedures.

6.2.1. 1-(α -*N*-Fmoc-L-valinamido)-2-amino-5-nitrobenzene (4.7a)



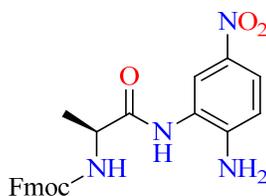
N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-valine (3.39 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. 4-Nitro-1,2-phenylenediamine (1.53 g, 10 mmol) was added, and the mixture was stirred at 0 °C for 2 h warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (250 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (4.7a) (3.8 g, 80%) as a colourless solid, mp 170-172 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3300 (N-H), 1689 (C=O), 1650 (C=O), 1524 (N-O), 1336 (C-N), 740 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 9.40 (1H, s, NH), 8.21 (1H, d, J = 2.5 Hz, 6-H), 7.87 (2H, d, J = 7.5 Hz, 4',5'-H), 7.84 (1H, dd, J = 9.0, 2.5 Hz, 4-H), 7.73 (2H, d, J = 7.5 Hz, 1',8'-H), 7.70 (1H, d, J = 8.0 Hz, NH'), 7.39 (2H, t, J = 7.5 Hz, 3',6'-H), 7.30 (2H, t, J = 7.5 Hz, 2',7'-H), 6.75 (1H, d, J = 9.0 Hz, 3-H), 6.41 (2H, s, NH₂), 4.27 (2H, m, CH₂), 4.22 (1H, t, J = 7.5 Hz, 9'-H), 4.00 (1H, t, J = 8.0 Hz, CH), 2.11 – 2.02 (1H, m, MeCHMe), 0.94 (3H, d, J = 6.0 Hz, MeCHMe), 0.93 (3H, d, J = 6.0 Hz, MeCHMe).

^{13}C -NMR (151 MHz, $\text{DMSO-}d_6$) δ ppm 171.5 (CONH), 157.0 (CO_2), 149.4 (2-C), 144.3 (C), 144.2 (C), 141.2 (5-C), 136.0 (1-C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 123.7 (4-CH), 121.6 (3-CH), 120.5 (1',8'-CH), 114.1 (6-CH), 66.2 (CH_2), 61.4 (C), 47.1 (9'-CH), 30.3 (MeCHMe), 19.1 (MeCHMe), 19.1 (MeCHMe).

HRMS-ESI (m/z) Calculated for $\text{C}_{26}\text{H}_{26}\text{N}_4\text{NaO}_5$ [$\text{M}+\text{Na}$] $^+$: 497.1795, found: 497.1797.

6.2.2. 1-(α -N-Fmoc-L-alaninamido)-2-amino-5-nitrobenzene (4.7b)



4.7b

N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-alanine (3.11 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at $-10\text{ }^\circ\text{C}$. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. 4-Nitro-1,2-phenylenediamine (1.53 g, 10 mmol) was added, and the mixture was stirred at $0\text{ }^\circ\text{C}$ for 2 h warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (250 mL), and the solution was washed successively with aqueous NaHCO_3 solution (5%) and brine, dried (MgSO_4) and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (4.7b) (3.35 g, 75%) as a pale yellow solid, mp $163\text{-}164\text{ }^\circ\text{C}$; IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3350 (N-H), 1681 (C=O), 1669 (C=O), 1529 (N-O), 1327 (C-N), 736 (N-H).

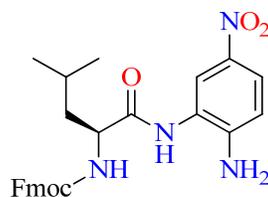
^1H -NMR (600 MHz, $\text{DMSO-}d_6$) δ ppm 9.33 (1H, s, NH), 8.15 (1H, d, $J = 2.5$ Hz, 6-H), 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.84 (1H, dd, $J = 9.0, 2.5$ Hz, 4-H), 7.72 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.70 (1H, d, $J = 7.0$ Hz, NH), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.31 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.74 (1H, d, $J = 9.0$

Hz, 3-H), 6.40 (2H, s, NH₂), 4.29 (2H, d, *J* = 7.5 Hz, CH₂), 4.21 (1H, t, *J* = 7.5 Hz, 9'-H), 4.20 (1H, q, *J* = 7.0 Hz, CHMe), 1.31 (3H, d, *J* = 7.0 Hz, CHMe).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 172.6 (CONH), 156.5 (CO₂), 149.9 (2-C), 144.3 (C), 144.2 (C), 141.2 (5-C), 135.9 (1-C), 128.0 (3',6'-CH), 127.5 (4',5'-CH), 125.7 (2',7'-CH), 123.6 (4-CH), 121.6 (3-CH), 120.6 (1',8'-CH), 114.0 (6-CH), 66.1 (CH₂), 51.0 (CHMe), 47.1(9'-CH), 18.1(Me).

HRMS-ESI (*m/z*) Calculated for C₂₄ H₂₂ N₄ Na O₅ [M+Na]⁺ : 469.1482, found: 469.1482.

6.2.3.1-(α -*N*-Fmoc-L-leucinamido)-2-amino-5-nitrobenzene (4.7c)



4.7c

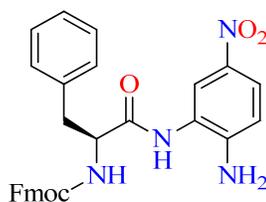
N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-leucine (3.53 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. 4-Nitro-1,2-phenylenediamine (1.53 g, 10 mmol) was added, and the mixture was stirred at 0 °C for 2 h warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (250 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (4.7c) (4.64 g, 95%) as a yellow solid, mp 164-165 °C; IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3400 (N-H), 1682 (C=O), 1662 (C=O), 1535 (N-O), 1298 (C-N), 740 (N-H).

$^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ ppm 9.38 (1H, s, NH), 8.14 (1H, d, $J = 2.5$ Hz, 6-H), 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.84 (1H, dd, $J = 9.0, 2.5$ Hz, 4-H), 7.71 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.70 (1H, d, $J = 7.0$ Hz, NH), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.74 (1H, d, $J = 9.1$ Hz, 3-H), 6.38 (2H, s, NH_2), 4.34 – 4.24 (2H, m, CO_2CH_2), 4.21 (1H, t, $J = 7.5$ Hz, 9'-H), 4.23 – 4.17 (1H, m, CHCH_2), 1.72 – 1.62 (1H, m), 1.61 – 1.50 (2H, m), 0.91 (3H, d, $J = 6.5$ Hz, MeCHMe), 0.89 (3H, d, $J = 6.5$ Hz, MeCHMe).

$^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ ppm 172.5 (CONH), 156.7 (CO_2), 149.8 (2-CH), 144.3 (C), 144.2 (C), 141.2 (5-C), 136.0 (1-C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.7 (2',7'-CH), 123.6 (4-CH), 121.6 (3-CH), 120.5 (1',8'-CH), 114.1 (6-CH), 66.1 (CH_2), 54.0 (CHCH_2), 47.1 (9'-CH), 40.6 (CHCH_2), 24.7 (MeCHMe), 22.5 (MeCHMe).

HRMS-ESI (m/z) Calculated for $\text{C}_{27}\text{H}_{28}\text{N}_4\text{NaO}_5$ [$\text{M}+\text{Na}$] $^+$: 511.1952, found: 511.1943.

6.2.4. 1-(α -*N*-Fmoc-L-phenylalaninamido)-2-amino-5-nitrobenzene (4.7d)



4.7d

N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-phenylalanine (3.87 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. 4-Nitro-1,2-phenylenediamine (1.53 g, 10 mmol) was added, and the mixture was stirred at 0 °C for 2 h warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (250 mL), and the solution was washed successively with

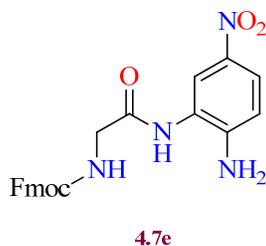
aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (**4.7d**) (4.7 g, 90%) as a yellow solid, mp 176-177 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3400 (N-H), 1684 (C=O), 1663 (C=O), 1511 (N-O), 1291 (C-N), 742 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 9.51 (1H, s, NH), 8.08 (1H, s, NH), 7.86 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.85 – 7.81 (2H, m, 6-H and, 4-H), 7.65 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.38 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.33 (2H, d, $J = 7.5$ Hz, 2',7'-H), 7.31 – 7.24 (4H, m, PhH), 7.19 (1H, t, $J = 7.5$ Hz, PhH), 6.73 (1H, d, $J = 9.0$ Hz, 3-H), 6.39 (2H, s, NH₂), 4.46 – 4.40 (1H, m, CHCH₂Ph), 4.21 (2H, d, $J = 7.5$ Hz, CO₂CH₂), 4.15 (1H, t, $J = 7.5$ Hz, 9'-H), 3.09 (1H, dd, $J = 13.6, 5.4$ Hz, CHCHHPh), 2.92 (1H, dd, $J = 13.6, 5.4$ Hz, CHCHHPh).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 171.5 (CONH), 156.5 (CO₂), 149.8 (2-C), 144.2(C), 144.1(C), 141.1 (5-C), 138.2(Ph), 135.8 (1-C), 129.8 (Ph), 128.5 (Ph), 128.0 (3',6'-CH), 127.5 (4',5'-CH), 126.8 (Ph), 125.7 (2',7'-CH), 123.6 (4-CH), 121.8 (3-CH), 120.5 (1',8'-CH), 114.0 (6-CH), 66.1 (CH₂), 57.1(CHCH₂Ph), 47.0 (9'-CH), 37.6 (CHCH₂Ph).

HRMS-ESI (m/z) Calculated for C₃₀ H₂₆ N₄ Na O₅ [M+Na]⁺ : 545.1795, found: 545.1798.

6.2.5. 1-(α -N-Fmoc-glycinamido)-2-amino-5-nitrobenzene (**4.7e**)



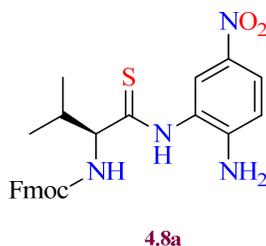
N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-glycine (2.97 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. 4-Nitro-1,2-phenylenediamine (1.53 g, 10 mmol) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (250 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (**4.7e**) (3.72 g, 86%) as a yellow solid, mp 130-131.

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 9.28 (1H, s, NH), 8.15 (1H, d, *J* = 2.7 Hz, 6-H), 7.88 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.85 (1H, dd, *J* = 9.1, 2.7 Hz, 4-H), 7.72 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.61 (1H, t, *J* = 6.0 Hz, NH), 7.40 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.32 (2H, t, *J* = 7.5 Hz, 2',7'-H), 6.74 (1H, d, *J* = 9.1 Hz, 3-H), 6.48 (2H, s, NH₂), 4.31 (2H, d, *J* = 7.0 Hz, CH₂), 4.23 (1H, t, *J* = 7.0 Hz, 9'-H), 3.85 (2H, d, *J* = 6.0 Hz, NHCH₂).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 169.2 (CONH), 157.1 (CO₂), 149.9 (2-C), 144.3 (C), 144.1 (C), 141.2 (5-C), 135.9 (1-C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.7 (2',7'-CH), 123.5 (4-CH), 121.5 (3-CH), 120.6 (1',8'-CH), 114.0 (6-CH), 66.2 (CH₂), 47.1 (9'-CH), 44.3 (NHCH₂).

HRMS-ESI (*m/z*) Calculated for C₂₃ H₂₀ N₄ Na O₅ [M+Na]⁺ : 455.1326, found: 455.1327.

6.2.6.1-(α -N-Fmoc-L-valinthioamido)-2-amino-5-nitrobenzene (**4.8a**)



Under an atmosphere of argon P₄S₁₀ (1.1 g, 2.5 mmol) was mixed with Na₂CO₃ (0.27 g, 2.5 mmol) in dry THF (100 mL). The mixture was stirred for 1 h at 25 °C and then cooled to 0 °C to this clear solution was added anilide **4.7a** (2.37 g, 5 mmol) and the reaction mixture was stirred at 0 °C for 30 min and at room temperature for 4 h. The mixture was filtered through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc/heptane (2/1, 75 mL) and washed with aqueous NaHCO₃ solution (5%) and aqueous layers were back extracted with EtOAc/heptane (75 mL). The combined organic layers were washed with brine, dried (MgSO₄), and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (**4.8a**) (2.04 g, 83%) as a yellow solid, mp 107-108 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3300 (N-H), 1694 (C=O), 1513 (N-O), 1311 (C-N), 740 (N-H).

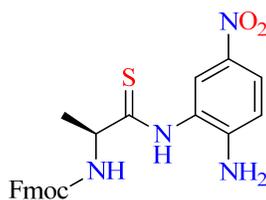
¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 11.37 (1H, s, NH), 8.00 (1H, d, $J = 7.0$ Hz, NH'), 7.94 (1H, dd, $J = 9.1, 2.6$ Hz, 4-H), 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.80 (1H, d, $J = 2.6$ Hz, 6-H), 7.74 (1H, d, $J = 7.5$ Hz, 1',8'-H), 7.70 (1H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.77 (1H, d, $J = 9.1$ Hz, 3-H), 6.33 (2H, s, NH₂), 4.33 – 4.28 (1H, m), 4.24 – 4.15 (2H,

m), 4.10 (1H, dd, $J = 9.0, 7.0$ Hz, CH), 2.13 (1H, h, $J = 6.8$ Hz, MeCHMe), 1.03 (3H, d, $J = 6.8$ Hz, MeCHMe), 0.97 (3H, d, $J = 6.8$ Hz, MeCHMe).

^{13}C -NMR (151 MHz, DMSO- d_6) δ ppm 207.9 (CSNH), 157.2 (CO₂), 151.1(2-C), 144.3 (C), 144.1(C), 141.1 (5-C), 135.5 (1-C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 124.9 (4-CH), 122.7 (3-CH), 120.6 (1',8'-CH), 114.4 (6-CH), 68.0 (C), 66.4 (CH₂), 47.0 (9'-CH), 31.8 (MeCHMe), 19.7(MeCHMe).

HRMS-ESI (m/z) Calculated for C₂₆ H₂₆ N₄ Na O₄ S [M+Na]⁺ : 513.1567, found: 513.1564.

6.2.7. 1-(α -N-Fmoc-L-alaninthioamido)-2-amino-5-nitrobenzene (**4.8b**)



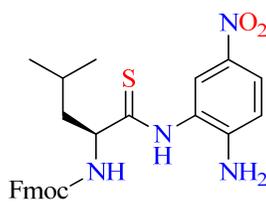
4.8b

Under an atmosphere of argon P₄S₁₀ (1.1 g, 2.5 mmol) was mixed with Na₂CO₃ (0.27 g, 2.5 mmol) in dry THF (100 mL). The mixture was stirred for 1 h at 25 °C and then cooled to 0 °C to this clear solution was added anilide **4.7b** (2.23 g, 5 mmol) and the reaction mixture was stirred at 0 °C for 30 min and at room temperature for 4 h. The mixture was filtered through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc/ heptane (2/1, 75 mL) and washed with aqueous NaHCO₃ solution (5%) and aqueous layers were back extracted with EtOAc/heptane (75 mL). The combined organic layers were washed with brine, dried (MgSO₄), and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (**4.8b**) (2.04 g, 88%) as a yellow solid, mp 104-105 °C; IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3350 (N-H), 1697 (C=O), 1515 (N-O), 1315 (C-N), 737 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 11.22 (1H, s, NH), 7.93 (1H, dd, *J* = 9.1, 2.7 Hz, 4-H), 7.91 (1H, d, *J* = 5.8 Hz, NH'), 7.87 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.86 (1H, d, *J* = 2.7 Hz, 6-H), 7.72 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 6.76 (1H, d, *J* = 9.1 Hz, 3-H), 6.36 (2H, s, NH₂), 4.51 (1H, p, *J* = 7.0 Hz, CHMe), 4.34 – 4.18 (3H, m, CO₂CH₂ and, 9'-H), 1.43 (3H, d, *J* = 7.0 Hz, CHMe).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 208.7(CSNH), 156.9 (CO₂), 151.1 (2-C), 144.3 (C), 144.2 (C), 141.1 (5-C), 135.8 (1-C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.7 (2',7'-CH), 125.1 (4-CH), 122.9 (3-CH), 120.5 (1',8'-CH), 114.4 (6-CH), 66.3 (CH₂), 60.1 (CHMe), 47.1 (9'-CH), 22.5 (Me).
HRMS-ESI (*m/z*) Calculated for C₂₄ H₂₂ N₄ Na O₄ S [M+Na]⁺ : 485.1254, found: 485.1250.

6.2.8. 1-(α -*N*-Fmoc-L-leucinthioamido)-2-amino-5-nitrobenzene (4.8c)



4.8c

Under an atmosphere of argon P₄S₁₀ (1.1 g, 2.5 mmol) was mixed with Na₂CO₃ (0.27 g, 2.5 mmol) in dry THF (100 mL). The mixture was stirred for 1 h at 25 °C and then cooled to 0 °C to this clear solution was added anilide **4.7c** (2.44 g, 5 mmol) and the reaction mixture was stirred at 0 °C for 30 min and at room temperature for 4 h. The mixture was filtered through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc/ heptane (2/1, 75 mL) and washed with aqueous NaHCO₃ solution (5%) and aqueous layers were back extracted with EtOAc/heptane (75 mL). The combined organic layers were washed with brine, dried (MgSO₄), and evaporated in vacuo.

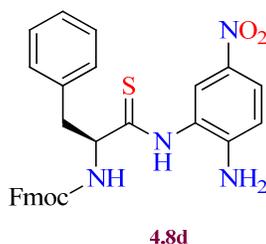
Purification by crystallization (EtOAc/hexane) gave the *title compound* (**4.8c**) (2.02 g, 80%) as a yellow solid, mp 106-107 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3400 (N-H), 1693 (C=O), 1513 (N-O), 1306 (C-N), 738 (N-H).

$^1\text{H-NMR}$ (600 MHz, DMSO- d_6) δ ppm 11.33 (1H, s, NH), 7.93 (1H, dd, $J = 9.1, 2.7$ Hz, 4-H), 7.91 (1H, d, $J = 7.6$ Hz, NH'), 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.83 (1H, d, $J = 2.7$ Hz, 6-H), 7.71 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.29 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.76 (1H, d, $J = 9.1$ Hz, 3-H), 6.33 (2H, s, NH₂), 4.48 (1H, q, $J = 7.0$ Hz, CHCH₂), 4.33 – 4.17 (3H, m, 9'-H and, CO₂CH₂), 1.77 – 1.64 (3H, m), 0.94 (3H, d, $J = 6.3$ Hz, MeCHMe), 0.91 (3H, d, $J = 6.3$ Hz, MeCHMe).

$^{13}\text{C-NMR}$ (151 MHz, DMSO- d_6) δ ppm 208.7 (CSNH), 156.9 (CO₂), 151.11 (2-CH), 144.3 (C), 144.0 (C), 141.1(5-CH), 135.8 (1-CH), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.7 (2',7'-CH), 125.1(4-CH), 122.9 (3-CH), 120.5 (1',8'-CH), 114.4 (6-CH), 66.3 (CH₂), 60.1 (CHCH₂), 47.1(9'-CH), 43.3 (CHCH₂), 24.8 (MeCHMe), 23.3 (MeCHMe), 22.4 (MeCHMe).

HRMS-ESI (m/z) Calculated for C₂₇ H₂₈ N₄ Na O₄ S [M+Na]⁺ : 527.1723, found: 527.1736.

6.2.9. 1-(α -N-Fmoc-L-phenylalaninthioamido)-2-amino-5-nitrobenzene (**4.8d**)



Under an atmosphere of argon P₄S₁₀ (1.1 g, 2.5 mmol) was mixed with Na₂CO₃ (0.27 g, 2.5 mmol) in dry THF (100 mL). The mixture was stirred for 1 h at 25 °C and then cooled to 0 °C to this clear solution was added anilide **4.7d** (2.61 g, 5 mmol) and the reaction mixture was stirred at 0 °C for 30

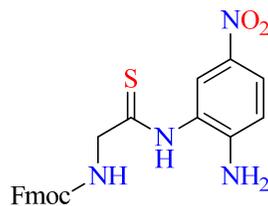
min and at room temperature for 4 h. The mixture was filtered through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc/ heptane (2/1, 75 mL) and washed with aqueous NaHCO₃ solution (5%) and aqueous layers were back extracted with EtOAc/heptane (75 mL). The combined organic layers were washed with brine, dried (MgSO₄), and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (**4.8d**) (2.56 g, 95%) as a yellow solid, mp 120-121 °C.

¹H NMR (600 MHz, DMSO-*d*₆) δ ppm 11.22 (1H, s, NH), 8.08 (1H, d, *J* = 7.0 Hz, NH), 7.90 (1H, dd, *J* = 9.1, 2.7 Hz, 6-H), 7.86 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.68 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.56 (1H, d, *J* = 2.7 Hz, 4-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.36 (2H, d, *J* = 7.5 Hz, 2',7'-H), 7.33 – 7.20 (5H, m, PhH), 6.73 (1H, d, *J* = 9.1 Hz, 3-H), 6.15 (2H, s, NH₂), 4.68 – 4.61 (1H, m, CHCH₂Ph), 4.30 – 4.25 (1H, m, CO₂CH₂), 4.20 – 4.16 (2H, m, CO₂CH₂ and, 9'-H), 3.14 (1H, dd, *J* = 13.4, 5.4 Hz, CHCHHPh), 3.06 (1H, dd, *J* = 13.4, 5.4 Hz, CHCHHPh).

¹³C NMR (151 MHz, DMSO-*d*₆) δ ppm 207.2 (CSNH), 156.7 (CO₂), 151.0 (2-C), 144.2 (C), 144.0 (C), 141.1 (5-C), 137.8 (Ph), 135.7 (1-C), 130.0 (Ph), 128.5 (Ph), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 127.0 (Ph), 125.7 (2',7'-CH), 125.0 (4-CH), 122.6 (3-CH), 120.5 (1',8'-CH), 114.3 (6-CH), 66.3 (CH₂), 63.0 (CHCH₂), 47.0 (9'-CH) 37.6 (CHCH₂).

HRMS-ESI (*m/z*) Calculated for C₃₀ H₂₆ N₄ O₄ S [M+H]⁺: 539.1748, found: 539.1718.

6.2.10. 1-(α -N-Fmoc-L-glycinthioamido)-2-amino-5-nitrobenzene (**4.8e**)



4.8e

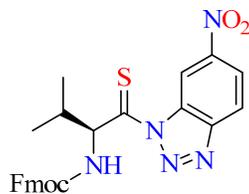
Under an atmosphere of argon P₄S₁₀ (1.1 g, 2.5 mmol) was mixed with Na₂CO₃ (0.27 g, 2.5 mmol) in dry THF (100 mL). The mixture was stirred for 1 h at 25 °C and then cooled to 0 °C to this clear solution was added anilide **4.7e** (2.16 g, 5 mmol) and the reaction mixture was stirred at 0 °C for 30 min and at room temperature for 4 h. The mixture was filtered through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc/ heptane (2/1, 75 mL) and washed with aqueous NaHCO₃ solution (5%) and aqueous layers were back extracted with EtOAc/heptane (75 mL). The combined organic layers were washed with brine, dried (MgSO₄), and evaporated in vacuo. Purification by crystallization (EtOAc/hexane) gave the *title compound* (**4.8e**) (1.93 g, 86%) as a yellow solid, mp 162-163 °C.

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 11.02 (1H, s, NH), 7.93 (1H, dd, $J = 9.2, 2.5$ Hz, 4-H), 7.87 (3H, d, $J = 7.5$ Hz, 4',5'-H, 6-H), 7.75 (1H, t, $J = 6.0$ Hz, NH), 7.72 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.31 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.76 (1H, d, $J = 9.2$ Hz, 3-H), 6.51 (2H, s, NH₂), 4.31 (2H, d, $J = 7.0$ Hz, CH₂), 4.23 (1H, t, $J = 7.0$ Hz, 9'-H), 4.16 (2H, d, $J = 6.0$ Hz, NHCH₂).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 203.2 (CSNH), 157.0 (CO₂), 151.2 (2-C), 144.3 (C), 144.2 (C), 141.2 (5-C), 135.6 (4-CH), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.7 (2',7'-CH), 123.5 (4-CH), 122.8 (3-CH), 120.6 (1',8'-CH), 114.4 (6-CH), 66.3 (CH₂), 51.9 (NHCH₂), 47.1 (9'-CH).

HRMS-ESI (m/z) Calculated for C₂₃ H₂₀ N₄ Na O₄ S [M+H]⁺: 449.1278, found: 449.1387.

6.2.11. 1-(*N*-Fmoc-L-thiovalinoyl)-6-nitrobenzotriazole (**4.9a**)



4.9a

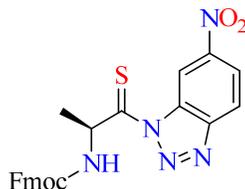
Thioanilide **4.8a** (0.98 g, 2 mmol) was dissolved in glacial acetic acid (12 mL) by gentle warming at 40 °C and then cooled to 0 °C, NaNO₂ (0.21 g, 3 mmol) was dissolved in water (1 mL) and added dropwise over 5 min with stirring. After 45 min, ice water (100 mL) was added, and the precipitated product was filtered and washed with water (20 mL), The solid residue was left to dry overnight then was dried in vacuo to afford *title compound* (**4.9a**) (0.81 g, 81%) as an orange amorphous solids, mp 80-81 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 1714 (C=O), 1534 (N-O), 1345 (C-N), 740 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 9.69 (1H, s, 7-H), 8.46 (1H, d, $J = 9.0$ Hz, 5-H), 8.32 (1H, d, $J = 9.0$ Hz, 4-H), 7.77 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.60 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.40 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.31 (2H, dt, $J = 7.5$ Hz, 2',7'-H), 6.20 (1H, dd, $J = 10.0, 5.4$ Hz, NHCH), 5.70 (1H, d, $J = 10.0$ Hz, NH), 4.49 (1H, q, $J = 7.0$ Hz, CO₂CH₂), 4.40 (1H, q, $J = 7.0$ Hz, CO₂CH₂), 4.23 (1H, t, $J = 7.0$ Hz, 9'-H), 2.34 (1H, h, $J = 6.8$ Hz, MeCHMe), 1.09 (3H, d, $J = 6.8$ Hz, MeCHMe), 1.00 (3H, d, $J = 6.8$ Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 209.1(C=S), 155.0 (CO₂), 149.7 (9-C), 149.1(C), 143.6 (C), 141.3 (6-C), 131.7, (8-C) 127.7 (3',6'-CH), 127.1 (4',5'-CH), 125.0 (2',7'-CH), 122.3 (5-CH), 121.6 (4-CH), 120.0 (1',8'-CH), 112.7 (7-CH), 67.0 (C), 66.0 (CH₂), 47.2 (9'-CH), 34.3 (MeCHMe), 20.2(MeCHMe), 17.0 (MeCHMe).

HRMS-ESI (m/z) Calculated for C₂₆ H₂₃ N₅ Na O₄ S [M+Na]⁺ : 524.1363, found: 524.1365.

6.2.12. 1-(*N*-Fmoc-L-thioalaninoyl)-6-nitrobenzotriazole (**4.9b**)



4.9b

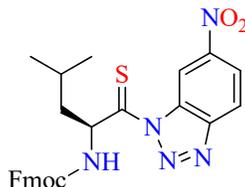
Thioanilide **4.8b** (0.92 g, 2 mmol) was dissolved in glacial acetic acid (12 mL) by gentle warming at 40 °C and then cooled to 0 °C, NaNO₂ (0.21 g, 3 mmol) was dissolved in water (1 mL) and added dropwise over 5 min with stirring. After 45 min, ice water (100 mL) was added, and the precipitated product was filtered and washed with water (20 mL), The solid residue was left to dry overnight then was dried in vacuo to afford *title compound* (**4.9b**) (0.90 g, 95%) as an orange amorphous solids, mp 78-79 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 1701 (C=O), 1528 (N-O), 1345 (C-N), 737 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 9.66 (1H, s, 7-H), 8.45 (1H, d, $J = 9.0$ Hz, 5-H), 8.31 (1H, d, $J = 9.0$ Hz, 4-H), 7.76 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.61 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.41 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.32 (2H, dt, $J = 7.5$ Hz, 2',7'-H), 6.26 (1H, p, $J = 7.0$ Hz, CHMe), 5.72 (1H, d, $J = 9.0$ Hz, NH), 4.53 (1H, q, $J = 7.0$ Hz, CO₂CH₂), 4.36 (1H, q, $J = 7.0$ Hz, CO₂CH₂), 4.23 (1H, t, $J = 7.0$ Hz, 9'-H), 1.69 (3H, d, $J = 7.0$ Hz, CHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 210.1 (C=S), 155.4 (CO₂), 149.6 (9-C), 149.0 (C), 143.7 (C), 141.3 (6-C), 131.7 (8-C), 127.8 (3',6'-CH), 127.1 (4',5'-CH), 125.0 (2',7'-CH), 122.3 (5-CH), 121.5 (4-CH), 120.0 (1',8'-CH), 112.9 (7-CH), 67.1 (CH₂), 57.4 (CHMe), 47.2 (9'-CH), 22.6 (CHMe).

HRMS-ESI (m/z) Calculated for C₂₄ H₁₉ N₅ Na O₄ S [M+Na]⁺ : 496.1050, found: 496.1056.

6.2.13. 1-(*N*-Fmoc-L-thioleucinoyl)-6-nitrobenzotriazole (4.9c)



4.9c

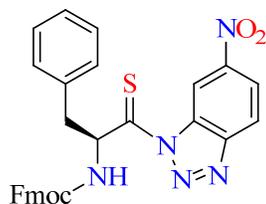
Thioanilide **4.8c** (1.01 g, 2 mmol) was dissolved in glacial acetic acid (12 mL) by gentle warming at 40 °C and then cooled to 0 °C, NaNO₂ (0.21 g, 3 mmol) was dissolved in water (1 mL) and added dropwise over 5 min with stirring. After 45 min, ice water (100 mL) was added, and the precipitated product was filtered and washed with water (20 mL), The solid residue was left to dry overnight then was dried in vacuo to afford *title compound* (**4.9c**) (0.82 g, 80%) as an orange amorphous solids, mp 79-80 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 1702 (C=O), 1528 (N-O), 1345 (C-N), 737 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 9.67 (1H, s, 7-H), 8.45 (1H, d, $J = 8.9$ Hz, 5-H), 8.31 (1H, d, $J = 8.9$ Hz, 4-H), 7.77 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.60 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.41 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.33 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.31 (1H, ddd, $J = 9.6, 3.1, 3.1$ Hz, NHCHCH₂), 5.56 (1H, d, $J = 9.6$ Hz, NH), 4.53 (1H, q, $J = 7.0$ Hz, CO₂CH₂), 4.40 (1H, q, $J = 7.0$ Hz, CO₂CH₂), 4.23 (1H, t, $J = 7.0$ Hz, 9'-H), 1.90 – 1.64 (3H, m, MeCHMe and, NHCHCH₂), 1.12 (d, $J = 6.5$ Hz, MeCHMe), 0.96 (d, $J = 6.5$ Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 210.5 (C=S), 155.9 (CO₂), 149.6 (9-C), 149.1(C),143.6 (C), 141.4 (6-C),131.7 (8-C), 127.8 (3',6'-CH), 127.1 (4',5'-CH), 125.0 (2',7'-CH), 122.2 (5-CH), 121.5 (4-CH), 120.0 (1',8'-CH), 112.9 (7-CH), 66.92 (CH₂), 60.5 (NHCHCH₂), 47.2 (9'-CH), 45.9 (NHCHCH₂), 25.7(MeCHMe), 23.3 (MeCHMe), 21.3 (MeCHMe).

HRMS-ESI (m/z) Calculated for C₂₇ H₂₅ N₅ Na O₄ S [M+Na]⁺: 538.1519, found: 538.1521.

6.2.14. 1-(*N*-Fmoc-L-thiophenylalaninoyl)-6-nitrobenzotriazole (**4.9d**)



4.9d

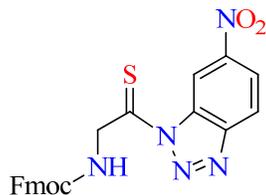
Thioanilide **4.8d** (1.08 g, 2 mmol) was dissolved in glacial acetic acid (12 mL) by gentle warming at 40 °C and then cooled to 0 °C, NaNO₂ (0.21 g, 3 mmol) was dissolved in water (1 mL) and added dropwise over 5 min with stirring. After 45 min, ice water (100 mL) was added, and the precipitated product was filtered and washed with water (20 mL), The solid residue was left to dry overnight then was dried in vacuo to afford *title compound* (**4.9d**) (0.91 g, 83%) as an orange amorphous solids, mp 126-127 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 1694 (C=O), 1535 (N-O), 1345 (C-N), 737 (N-H).

¹H NMR (600 MHz, Chloroform-*d*) δ ppm 9.63 (1H, s, 7-H), 8.46 (1H, dd, $J = 9.0, 2.1$ Hz, 5-H), 8.31 (1H, d, $J = 9.0$ Hz, 4-H), 7.77 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.54 (2H, t, $J = 7.5$ Hz, 1',8'-H), 7.40 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.29 (2H, t, $J = 7.5$ Hz, 2',7'-H), 7.25 – 7.14 (5H, m, PhH), 6.58 (1H, td, $J = 9.0, 5.4$ Hz, NHCHCH₂), 5.70 (1H, d, $J = 9.0$ Hz, NH), 4.42 (1H, q, $J = 7.0$ Hz, CO₂CH₂), 4.34 (1H, q, $J = 7.0$ Hz, CO₂CH₂), 4.18 (1H, t, $J = 7.0$ Hz, 9'-H), 3.42 (1H, dd, $J = 13.8, 5.4$ Hz, CH₂Ph), 3.11 (1H, dd, $J = 13.8, 5.4$ Hz, CH₂Ph).

¹³C NMR (151 MHz, DMSO-*d*₆) δ ppm 207.9 (C=S), 155.7 (CO₂), 149.6 (9-C), 149.0 (C), 143.6 (C), 141.3 (6-C), 135.1(Ph), 131.7 (8-C), 129.3 (Ph), 128.6 (Ph), 127.8 (3',6'-CH), 127.4 (Ph), 127.1 (4',5'-CH), 125.0 (2',7'-CH), 122.3 (5-CH), 121.6 (4-CH), 120.0 (1',8'-CH), 112.6 (7-CH), 67.1 (CH₂), 62.3 (NHCHCH₂), 47.1 (9'-CH), 42.7 (CH₂Ph).

HRMS-ESI (m/z) Calculated for C₃₀ H₂₃ N₅ Na O₄ S [M+Na]⁺ : 572.1368, found: 572.1365.

6.2.15. 1-(*N*-Fmoc-L-thioglycinoyl)-6-nitrobenzotriazole (**4.9e**)



4.9e

Thioanilide **4.8e** (0.90 g, 2 mmol) was dissolved in glacial acetic acid (12 mL) by gentle warming at 40 °C and then cooled to 0 °C, NaNO₂ (0.21 g, 3 mmol) was dissolved in water (1 mL) and added dropwise over 5 min with stirring. After 45 min, ice water (100 mL) was added, and the precipitated product was filtered and washed with water (20 mL), The solid residue was left to dry overnight then was dried in vacuo to afford *title compound* (**4.9e**) (0.73 g, 80%) as an orange amorphous solids.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 9.64 (1H, s, 7-H), 8.46 (1H, d, *J* = 9.0 Hz, 5-H), 8.32 (1H, d, *J* = 9.0 Hz, 4-H), 7.78 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.65 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.42 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.34 (2H, t, *J* = 7.5 Hz, 2',7'-H), 5.67 (1H, t, *J* = 6.5 Hz, NH), 5.21 (2H, d, *J* = 6.5 Hz, NHCH₂), 4.50 (2H, d, *J* = 7.0 Hz, CO₂CH₂), 4.28 (1H, t, *J* = 7.0 Hz, 9'-H).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 203.0 (C=S), 156.3 (CO₂), 149.6 (9-C), 148.8 (C), 143.7 (C), 141.4 (6-C), 131.8 (8-C), 127.8 (3',6'-CH), 127.1 (4',5'-CH), 125.1 (2',7'-CH), 122.2 (5-CH), 121.6 (4-CH), 120.0 (1',8'-CH), 112.2 (7-CH), 67.3 (CO₂CH₂), 52.9 (NHCH₂), 47.2 (9'-CH).

HRMS-ESI (*m/z*) Calculated for C₂₃ H₁₇ N₅ Na O₄ S [M+Na]⁺: 482.0893, found: 482.0897.

6.2.16. Ethyl 2-bromo-3,3-diethoxypropanoate (4.29)



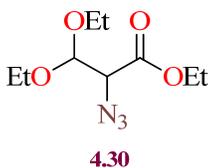
To a solution of Ethyl 3-ethoxyacrylate **4.28** (1.44 g, 10 mmol) in EtOH (100 mL) was added N-bromosuccinimide (1.77g, 10 mmol) and the mixture was stirred at RT overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in diethyl ether (150 mL), and the solution was washed successively with H₂O, and brine, dried over (MgSO₄) and evaporated in vacuo to gave *title compound* (**4.29**) (2.66 g, 99%) as a colourless oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 4.84 (1H, d, *J* = 8.3 Hz, EtOCHOEt), 4.21 (1H, q, *J* = 7.1 Hz, CO₂CH₂Me), 4.19 (1H, d, *J* = 8.3 Hz, BrCH), 3.68 (2H, m, OCH₂Me), 3.57 (2H, m, OCH₂Me), 1.27 (3H, t, *J* = 7.1 Hz, CO₂CH₂Me), 1.23 (3H, t, *J* = 7.1 Hz, OCH₂Me), 1.14 (3H, t, *J* = 7.1 Hz, OCH₂Me).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 167.8 (CO₂), 101.9 (EtOCHOEt), 64.1 (CO₂CH₂Me), 62.7 (OCH₂Me), 62.1 (OCH₂Me), 44.9 (BrCH), 15.1 (OCH₂Me), 15.0 (OCH₂Me), 13.9 (CO₂CH₂Me).

HRMS-ESI (*m/z*) Calculated for ⁷⁹Br C₉ H₁₇ Na O₄ [M+Na]⁺ : 291.0202, found: 291.0204.

6.2.17. Ethyl 2-azido-3,3-diethoxypropanoate (4.30)



To a solution of NaN₃ 1.5 e.q (0.487 g, 7.5 mmol) in 50 mL of DMSO at 60 °C, was added ethyl 2-bromo-3,3-diethoxypropanoate **4.29** (1.34 g, 5 mmol) dropwise and the mixture was stirred overnight

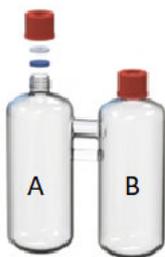
at 60 °C. The residue was dissolved in diethyl ether (100 mL), and the solution was washed successively with H₂O, and brine, dried over (MgSO₄) and evaporated in vacuo to gave *title compound* (**4.30**) (0.947 g, 82%) as a yellow oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 4.80 (1H, d, *J* = 5.7 Hz, EtOCHOEt), 4.24 (1H, q, *J* = 7.0 Hz, CO₂CH₂Me), 3.88 (1H, d, *J* = 5.7 Hz, N₃CH), 3.75 (2H, m, OCH₂Me), 3.59 (2H, m, OCH₂Me), 1.30 (3H, t, *J* = 7.0 Hz, CO₂CH₂Me), 1.23 (3H, t, *J* = 7.0 Hz, OCH₂Me), 1.19 (3H, t, *J* = 7.0 Hz, OCH₂Me).

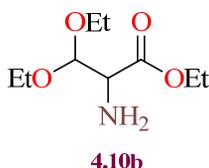
¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 167.7 (CO₂), 101.9 (EtOCHOEt), 63.7 (CO₂CH₂Me), 61.9 (OCH₂Me), 61.8 (OCH₂Me), 40.0 (N₃CH), 15.2 (OCH₂Me), 15.1 (OCH₂Me), 14.1 (CO₂CH₂Me).

HRMS-ESI (*m/z*) Calculated for C₉ H₁₇ Na N₃ O₄ [M+Na]⁺ : 254.1111, found: 254.1117.

6.2.18. Ethyl 2-amino-3,3-diethoxypropanoate (**4.10b**)



COWARE-Two-chamber glass



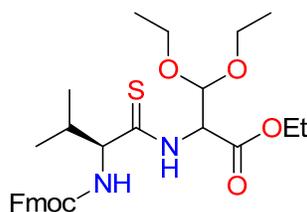
To a solution of ethyl 2-azido-3,3-diethoxypropanoate **4.30** (0.924 g, 4 mmol) in 30 mL of EtOH (chamber A) was added Pd/C (5% mmol, 0.0212 g) ; in the other side (chamber B) was added excess of Zn. The system was placed under N₂ for 10 min then to chamber B was added 7M. HCl 14 mL to produces hydrogen gas and, the mixture was stirred overnight at RT. The mixture was filtered, and the filtrate was evaporated to dryness in vacuo to gave the *title compound* (**4.10b**) (0.820 g, 100%) as a red oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 4.65 (1H, d, *J* = 4.3 Hz, EtOCHOEt), 4.21 (1H, q, *J* = 7.0 Hz, CO₂CH₂Me), 3.73 (2H, m, OCH₂Me), 3.68 (1H, d, *J* = 4.3 Hz, NH₂CH), 3.55 (2H, m, OCH₂Me), 2.49 (2H, s, NH₂CH), 1.28 (3H, t, *J* = 7.0 Hz, CO₂CH₂Me), 1.21 (3H, t, *J* = 7.0 Hz, OCH₂Me), 1.18 (3H, t, *J* = 7.0 Hz, OCH₂Me).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 171.4 (CO₂), 102.7 (EtOCHOEt), 63.9 (OCH₂Me), 63.8 (OCH₂Me), 61.3 (CO₂CH₂Me), 57.5 (NH₂CH), 15.2 (OCH₂Me), 15.1 (OCH₂Me), 14.2 (CO₂CH₂Me).

HRMS-ESI (*m/z*) Calculated for C₉ H₂₀ N O₄ [M+H]⁺ : 206.1387, found: 206.1380.

6.2.19. Ethyl 2-(*N*-Fmoc-L-valinthioamido)-3,3-diethoxypropanoate (**4.11a**)



4.11a

To a cooled solution of benzotriazoles **4.9a** (0.50 g, 1.0 mmol) in THF (15 mL) at 0 °C was added dropwise a solution of Ethyl 2-amino-3,3-diethoxypropanoate **4.10b** (0.205 g, 1.0 mmol) in THF (5 mL) over a period of 15 min. After stirring for 1 h the solvent was evaporated. The residue was dissolved in EtOAc (25 mL) and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by column chromatography eluting with hexane-EtOAc (1/1) gave the *title compound* (**4.11a**) (0.532 g, 98%) as a brown oil.

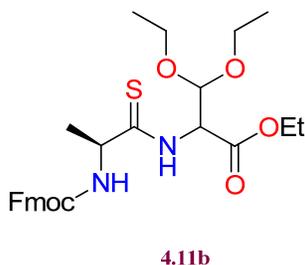
¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.07 (1H, d, *J* = 7.5 Hz, NH) 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.59 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.38 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 5.69 (1H, d, *J* = 9.0 Hz, NH), 5.39 (1H, d, *J* = 3.0 Hz, EtOCHOEt), 4.87 (1H, t, *J* = 3.0 Hz,

NHCHCO₂Et), 4.37 (2H, q, $J = 7.5$ Hz, CO₂CH₂), 4.34 (1H, m, NHCH), 4.22 (2H, q, $J = 7.0$ Hz, CO₂CH₂Me), 4.21 (1H, t, $J = 7.5$ Hz, 9'-H) 3.79 – 3.49 (4H, m, CH(OCH₂Me)₂), 2.23 (1H, m, MeCHMe), 1.24 (3H, t, $J = 7.0$ Hz, CO₂CH₂Me), 1.17 (3H, t, $J = 7.0$ Hz, OCH₂Me), 1.14 (3H, t, $J = 7.0$ Hz, OCH₂Me), 1.00 (3H, d, $J = 6.8$ Hz, MeCHMe), 0.97 (3H, d, $J = 6.8$ Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 205.1 (CSNH), 167.4 (CO₂CH₂Me), 155.9 (CO₂), 143.8 (C), 141.3 (C), 127.7 (3',6'-CH), 127.0 (4',5'-CH), 125.2 (2',7'-CH), 119.9 (1',8'-CH), 100.5 (EtOCHOEt), 67.1 (CH₂), 66.5 (NHCHCS), 64.0 (OCH₂Me), 63.7 (OCH₂Me), 61.7 (CO₂CH₂Me), 58.4 (NHCHCO₂), 47.1 (9'-CH), 34.0 (MeCHMe), 19.4 (MeCHMe), 17.9 (MeCHMe), 15.0 (OCH₂Me), 14.9 (OCH₂Me), 14.1(CO₂CH₂Me).

HRMS-ESI (m/z) Calculated for C₂₉ H₃₈ N₂ Na O₆ S [M+Na]⁺ : 565.2343, found: 565.2338.

6.2.20. Ethyl 2-(*N*-Fmoc-L-alaninthioamido)-3,3-diethoxypropanoate (**4.11b**)



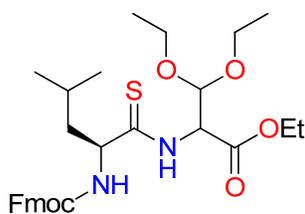
To a cooled solution of benzotriazoles **4.9b** (0.473 g, 1.0 mmol) in THF (15 mL) at 0 °C was added dropwise a solution of Ethyl 2-amino-3,3-diethoxypropanoate **4.10b** (0.205 g, 1.0 mmol) in THF (5 mL) over a period of 15 min. After stirring for 1 h the solvent was evaporated. The residue was dissolved in EtOAc (25 mL) and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by column chromatography eluting with hexane-EtOAc (1/1) gave the *title compound* (**4.11b**) (0.488 g, 95%) as a brown oil.

$^1\text{H-NMR}$ (600 MHz, Chloroform-*d*) δ ppm 8.09 (1H, d, $J = 8.0$ Hz, NH) 7.75 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.59 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 5.71 (1H, br s, NH), 5.34 (1H, d, $J = 3.0$ Hz, EtOCHOEt), 4.87 (1H, d, $J = 3.0$ Hz, NHCHCO₂Et), 4.62 (1H, q, $J = 6.8$ Hz, NHCHMe), 4.36 (2H, q, $J = 7.5$ Hz, CO₂CH₂), 4.22 (2H, q, $J = 7.0$ Hz, CO₂CH₂Me), 4.20 (1H, t, $J = 7.5$ Hz, 9'-H), 3.78 – 3.49 (4H, m, CH(OCH₂Me)₂), 1.51 (3H, d, $J = 6.8$ Hz, NHCHMe), 1.24 (3H, t, $J = 7.0$ Hz, CO₂CH₂Me), 1.18 (3H, t, $J = 7.0$ Hz, OCH₂Me), 1.16 (3H, t, $J = 7.0$ Hz, OCH₂Me).

$^{13}\text{C-NMR}$ (151 MHz, Chloroform-*d*) δ ppm 206.3 (CSNH), 167.4 (CO₂CH₂Me), 155.5 (CO₂), 143.8 (C), 141.2 (C), 127.7 (3',6'-CH), 127.0 (4',5'-CH), 125.2 (2',7'-CH), 120.0 (1',8'-CH), 100.4 (EtOCHOEt), 67.2 (CH₂), 64.1 (OCH₂Me), 63.7 (OCH₂Me), 61.8 (CO₂CH₂Me), 60.1(CHMe), 58.4 (NHCHCO₂), 47.1 (9'-CH), 22.4 (CHMe), 15.0 (OCH₂Me), 14.9 (OCH₂Me), 14.1(CO₂CH₂Me).

HRMS-ESI (m/z) Calculated for C₂₇ H₃₄ N₂ Na O₆ S [M+Na]⁺ : 537.2030, found: 537.2035.

6.2.21. Ethyl 2-(*N*-Fmoc-L-leucinthioamido)-3,3-diethoxypropanoate (**4.11c**)



4.11c

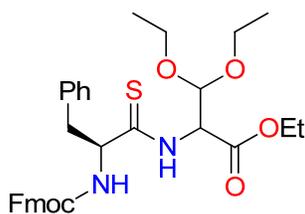
To a cooled solution of benzotriazoles **4.9c** (0.515 g, 1.0 mmol) in THF (15 mL) at 0 °C was added dropwise a solution of ethyl 2-amino-3,3-diethoxypropanoate **4.10b** (0.205 g, 1.0 mmol) in THF (5 mL) over a period of 15 min. After stirring for 1 h the solvent was evaporated. The residue was dissolved in EtOAc (25 mL) and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by column chromatography eluting with hexane-EtOAc (1/1) gave the *title compound* (**4.11c**) (0.545 g, 98%) as a brown oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.12 (1H, d, *J* = 8.0 Hz, NH) 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.59 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.38 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 5.60 (1H, *J* = 9.0 Hz, NH), 5.34 (1H, d, *J* = 3.0 Hz, EtOCHOEt), 4.87 (1H, d, *J* = 3.0 Hz, NHCHCO₂Et), 4.55 (1H, t, *J* = 9.0 Hz, NHCHCH₂), 4.36 (2H, q, *J* = 7.5 Hz, CO₂CH₂), 4.22 (2H, q, *J* = 7.0 Hz, CO₂CH₂Me), 4.20 (1H, t, *J* = 7.5 Hz, 9'-H), 3.78 – 3.48 (4H, m, CH(OCH₂Me)₂), 1.80 – 1.62 (3H, m, MeCHMe, NHCHCH₂), 1.24 (3H, t, *J* = 7.0 Hz, CO₂CH₂Me), 1.17 (3H, t, *J* = 7.0 Hz, OCH₂Me), 1.14 (3H, t, *J* = 7.0 Hz, OCH₂Me) 0.97 (3H, d, *J* = 6.0 Hz, MeCHMe), 0.96 (3H, d, *J* = 6.0 Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 206.5 (CSNH), 167.4 (CO₂CH₂Me), 155.7 (CO₂), 143.8 (C), 141.2 (C), 127.7 (3',6'-CH), 127.0 (4',5'-CH), 125.2 (2',7'-CH), 120.0 (1',8'-CH), 100.4 (EtOCHOEt), 67.2 (CH₂), 64.1 (OCH₂Me), 63.7 (OCH₂Me), 61.8 (CO₂CH₂Me), 59.6 (NHCHCH₂), 58.4 (NHCHCO₂), 47.1 (9'-CH), 45.3 (NHCHCH₂), 24.7 (MeCHMe), 22.7 (MeCHMe), 22.2 (MeCHMe), 15.0 (OCH₂Me), 14.9 (OCH₂Me), 14.1(CO₂CH₂Me).

HRMS-ESI (*m/z*) Calculated for C₃₀ H₄₀ N₂ Na O₆ S [M+Na]⁺ : 579.2499, found: 579.2482.

6.2.22. Ethyl 2-(*N*-Fmoc-L-phenylalaninthioamido)-3,3-diethoxypropanoate (**4.11d**)



4.11d

To a cooled solution of benzotriazoles **4.9d** (0.549 g, 1.0 mmol) in THF (15 mL) at 0 °C was added dropwise a solution of ethyl 2-amino-3,3-diethoxypropanoate **4.11b** (0.205 g, 1.0 mmol) in THF (5 mL) over a period of 15 min. After stirring for 1 h the solvent was evaporated. The residue was dissolved in EtOAc (25 mL) and the solution was washed successively with aqueous NaHCO₃ solution

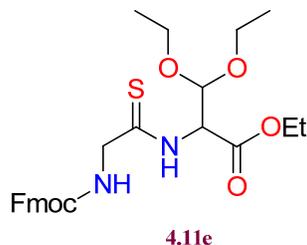
(5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by column chromatography eluting with hexane-EtOAc (1/1) gave the *title compound* (**4.11d**) (0.489 g, 83%) as a brown oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.97 (1H, d, *J* = 8.0 Hz, NH) 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.53 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.38 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 - 7.17 (7H, m, 2',7'-H, Ph), 5.63 (1H, br s, NH), 5.24 (1H, d, *J* = 3.0 Hz, EtOCHOEt), 4.80 (1H, d, *J* = 3.0 Hz, NHCHCO₂Et), 4.34 (2H, q, *J* = 7.5 Hz, CO₂CH₂), 4.28 (1H, m, NHCHCH₂), 4.20 (1H, t, *J* = 7.5 Hz, 9'-H), 4.19 (2H, q, *J* = 7.0 Hz, CO₂CH₂Me), 3.74 – 3.47 (4H, m, CH(OCH₂Me)₂), 3.28 (1H, d, *J* = 6.6 Hz, NHCHCH₂), 3.21 (1H, d, *J* = 6.6 Hz, NHCHCH₂), 1.25 (3H, t, *J* = 7.0 Hz, CO₂CH₂Me), 1.15 (3H, t, *J* = 7.0 Hz, OCH₂Me), 1.14 (3H, t, *J* = 7.0 Hz, OCH₂Me).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 203.3 (CSNH), 168.1 (CO₂CH₂Me), 155.6 (CO₂), 143.7 (C), 141.2 (C), 136.3 (Ph), 129.5 (Ph), 129.1 (Ph), 128.6 (Ph), 127.7 (3',6'-CH), 127.0 (4',5'-CH), 125.1 (2',7'-CH), 119.9 (1',8'-CH), 100.8 (EtOCHOEt), 67.2 (CH₂), 63.6 (OCH₂Me), 63.5 (OCH₂Me), 62.8 (NHCHCH₂), 61.9 (CO₂CH₂Me), 58.4 (NHCHCO₂), 47.1 (9'-CH), 42.1 (CHCH₂Ph), 15.1 (OCH₂Me), 14.9 (OCH₂Me), 14.1(CO₂CH₂Me).

HRMS-ESI (*m/z*) Calculated for C₃₃ H₃₈ N₂ Na O₆ S [M+Na]⁺ : 613.2343, found: 613.2350.

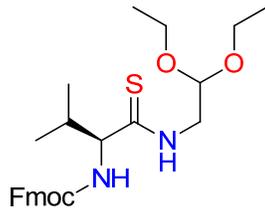
6.2.23. Ethyl 2-(*N*-Fmoc-glycinthioamido)-3,3-diethoxypropanoate (**4.11e**)



To a cooled solution of benzotriazoles **4.9e** (0.459 g, 1.0 mmol) in THF (15 mL) at 0 °C was added dropwise a solution of ethyl 2-amino-3,3-diethoxypropanoate **4.10b** (0.205 g, 1.0 mmol) in THF (5 mL) over a period of 15 min. After stirring for 1 h the solvent was evaporated. The residue was dissolved in EtOAc (25 mL) and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by column chromatography eluting with hexane-EtOAc (1/1) gave the *title compound* (**4.11e**) (0.400 g, 80%) as a brown oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.31 (1H, d, *J* = 8.0 Hz, NH) 7.76 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.60 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.40 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 5.63 (1H, br s, NH), 5.38 (1H, d, *J* = 3.0 Hz, EtOCHOEt), 4.87 (1H, d, *J* = 3.0 Hz, NHCHCO₂Et), 4.45 (2H, q, *J* = 7.1 Hz, CO₂CH₂Me), 4.41 (2H, d, *J* = 7.5 Hz, CO₂CH₂CH), 4.31 (2H, d, *J* = 6.0 Hz, NHCH₂), 4.25 (1H, t, *J* = 7.5 Hz, 9'-H), 3.78 – 3.48 (4H, m, CH(OCH₂Me)₂), 1.25 (3H, t, *J* = 7.0 Hz, CO₂CH₂Me), 1.15 (3H, t, *J* = 7.0 Hz, OCH₂Me), 1.14 (3H, t, *J* = 7.0 Hz, OCH₂Me).

6.2.24. 1-(*N*-Fmoc-L-valinthioamido)-2,2-diethoxyethane (**4.11f**)



4.11f

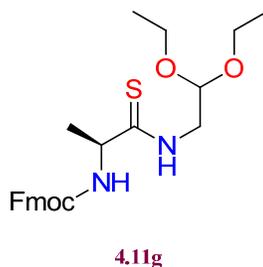
To a cooled solution of thioacylating reagent **4.9a** (0.25 g, 0.5 mmol) in 10 mL of THF at 0 °C was added dropwise a solution of 2,2-Diethoxyethylamine **4.10a** (0.066 g, 0.5 mmol) in 5 mL of THF over a period of 15 min. After 1 h the solvent was evaporated and the residue was dissolved in EtOAc (20 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by column chromatography eluting with hexane-EtOAc (1/1) to gave *title compound* (**4.11f**) (0.230 g, 98%) as a brown oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.94 (1H, br s, NH), 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.59 (2H, dd, *J* = 7.5 Hz, 1',8'-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 5.68 (1H, d, *J* = 9.1 Hz, NH), 4.66 (1H, t, *J* = 5.2 Hz, EtOCHOEt), 4.39 (2H, d, *J* = 7.0 Hz, CO₂CH₂), 4.34 (1H, t, *J* = 9.1 Hz, NHCH), 4.21 (1H, t, *J* = 7.0 Hz, 9'-H), 3.92 (1H, m, CHCH₂NH), 3.71 (1H, m, CHCH₂NH), 3.67 (2H, m, OCH₂Me), 3.53 (2H, m, OCH₂Me), 2.19 (1H, m, MeCHMe), 1.18 (6H, t, *J* = 7.0 Hz, (OCH₂Me)₂) 0.97 (3H, d, *J* = 6.7 Hz, MeCHMe), 0.92 (3H, d, *J* = 6.7 Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 204.3 (CSNH), 156.2 (CO₂), 143.8 (C), 143.7(C), 141.3 (C), 127.7 (3',6'-CH), 127.1 (4',5'-CH), 125.2 (2',7'-CH), 120.0 (1',8'-CH), 99.1 (EtOCHOEt), 67.2 (NHCH), 67.1 (CH₂), 63.1 (OCH₂Me), 63.0 (OCH₂Me), 47.7 (CHCH₂NH), 47.1 (9'-CH), 33.6 (MeCHMe), 19.5 (MeCHMe), 18.3 (MeCHMe), 15.3 (OCH₂Me), 15.2 (OCH₂Me).

HRMS-ESI (*m/z*) Calculated for C₂₆ H₃₄ N₂ Na O₄ S [M+Na]⁺ : 493.2131, found: 493.2141.

6.2.25. 1-(*N*-Fmoc-L-alaninthioamido)-2,2-diethoxyethane (**4.11g**)



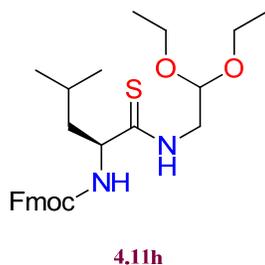
To a cooled solution of thioacylating reagent **4.9b** (0.24 g, 0.5 mmol) in 10 mL of THF at 0 °C was added dropwise a solution of 2,2-Diethoxyethylamine **4.10a** (0.066 g, 0.5 mmol) in 5 mL of THF over a period of 15 min. After 1 h the solvent was evaporated and the residue was dissolved in EtOAc (20 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by column chromatography eluting with hexane-EtOAc (1/1) to gave *title compound* (**4.11g**) (0.210 g, 95%) as a brown oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.97 (1H, br s, NH), 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.58 (2H, dd, *J* = 7.5 Hz, 1',8'-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.31 (2H, t, *J* = 7.5 Hz, 2',7'-H), 5.65 (1H, br s, NH), 4.66 (1H, t, *J* = 5.3 Hz, EtOCHOEt), 4.51 (1H, m, CHMe), 4.36 (2H, d, *J* = 7.0 Hz, CO₂CH₂), 4.20 (1H, t, *J* = 7.0 Hz, 9'-H), 3.85 (1H, t, *J* = 5.3 Hz, CHCH₂NH), 3.82 (1H, t, *J* = 5.3 Hz, CHCH₂NH), 3.67 (2H, q, *J* = 7.0 Hz, OCH₂Me), 3.53 (2H, q, *J* = 7.0 Hz, OCH₂Me), 1.48 (3H, d, *J* = 7.0 Hz, CHMe), 1.19 (6H, t, *J* = 7.0 Hz, (OCH₂Me)₂).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 205.0 (CSNH), 155.6 (CO₂), 143.7(C), 141.3 (C), 127.7 (3',6'-CH), 127.1 (4',5'-CH), 125.2 (2',7'-CH), 120.0 (1',8'-CH), 99.1 (EtOCHOEt), 67.1 (CH₂), 64.2 (NHCH), 63.1 (OCH₂Me), 63.0 (OCH₂Me), 49.7 (CHCH₂NH), 47.1 (9'-CH), 22.1 (CHMe), 15.3 (OCH₂Me), 15.2 (OCH₂Me).

HRMS-ESI (*m/z*) Calculated for C₂₄ H₃₀ N₂ Na O₄ S [M+Na]⁺ : 465.1818, found: 465.1827.

6.2.26. 1-(*N*-Fmoc-L-leucinthioamido)-2,2-diethoxyethane (**4.11h**)



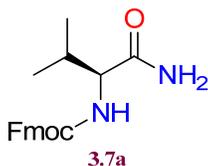
To a cooled solution of thioacylating reagent **4.9c** (0.26 g, 0.5 mmol) in 10 mL of THF at 0 °C was added dropwise a solution of 2,2-Diethoxyethylamine **4.10a** (0.066 g, 0.5 mmol) in 5 mL of THF over a period of 15 min. After 1 h the solvent was evaporated and the residue was dissolved in EtOAc (20 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo. Purification by column chromatography eluting with hexane-EtOAc (1/1) to gave *title compound* (**4.11h**) (0.237 g, 98%) as a brown oil.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.92 (1H, br s, NH), 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.58 (2H, dd, *J* = 7.5 Hz, 1',8'-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 5.50 (1H, d, *J* = 8.8 Hz, NH), 4.65 (1H, t, *J* = 5.6 Hz, EtOCHOEt), 4.39 (3H, m, CO₂CH₂, NHCHCH₂), 4.20 (1H, t, *J* = 7.0 Hz, 9'-H), 3.87 (1H, m, CHCH₂NH), 3.72 (1H, m, CHCH₂NH), 3.67 (2H, q, *J* = 7.0 Hz, OCH₂Me), 3.52 (2H, q, *J* = 7.0 Hz, OCH₂Me), 1.75 – 1.60 (3H, m, MeCHMe, NHCHCH₂), 1.19 (6H, q, *J* = 7.0 Hz, (OCH₂Me)₂), 0.94 (6H, d, *J* = 6.0 Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 205.3 (CSNH), 155.6 (CO₂), 143.7(C), 141.3 (C), 127.7 (3',6'-CH), 127.1 (4',5'-CH), 125.2 (2',7'-CH), 120.0 (1',8'-CH), 99.1 (EtOCHOEt), 67.3 (NHCHCH₂), 67.1 (CH₂), 63.1 (OCH₂Me), 63.0 (OCH₂Me), 47.8 (CHCH₂NH), 47.1 (9'-CH), 45.0 (NHCHCH₂), 24.8 (MeCHMe), 23.0 (MeCHMe), 22.8 (MeCHMe), 15.3 (OCH₂Me), 15.2 (OCH₂Me).

HRMS-ESI (*m/z*) Calculated for C₂₇ H₃₆ N₂ Na O₄ S [M+Na]⁺ : 507.2288, found: 507.2311

6.2.28. *N*-Fmoc-L-valinamide (**3.7a**)



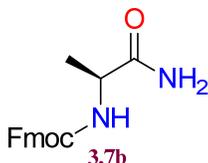
N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-valine (3.39 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. Ammonia solution 7 N in methanol (4.3 mL) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in DCM (150 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**3.7a**) (3.28 g, 97%) as a colourless solid, mp (Lit,⁷⁷ 195-196 °C); IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3280 (N-H), 2960, 1723 (C=O), 1686 (C=O), 1550 (C-C), 1259, 1217 (C-N), 727 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.73 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.32 (1H, s, *HNH*), 7.30 (1H, d, $J = 9.0$ Hz, *NHCH*), 7.29 (2H, t, $J = 7.5$ Hz, 2',7'-H), 7.02 (1H, s, *HNH*), 4.26 (1H, d, $J = 7.0$ Hz, CO₂CH₂), 4.20 (1H, t, $J = 7.0$ Hz, 9'-H), 4.19 (1H, d, $J = 7.0$ Hz, CO₂CH₂), 3.77 (1H, dd, $J = 9.0, 6.9$ Hz, *NHCH*), 1.93 (1H, h, $J = 6.9$ Hz, MeCHMe), 0.84 (3H, d, $J = 6.9$ Hz, MeCHMe), 0.83 (3H, d, $J = 6.9$ Hz, MeCHMe).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 173.6 (CONH₂), 156.6 (CO₂CH₂CH), 144.3 (C), 141.1 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.5 (1',8'-CH), 66.1 (CH₂), 60.5 (*NHCH*), 47.1 (9'-CH), 30.6 (MeCHMe), 19.8 (MeCHMe), 18.6 (MeCHMe).

HRMS-ESI (m/z) Calculated for C₂₀ H₂₂ N₂ O₃ Na [*M*+Na]: 361.1523, found: 361.1528.

6.2.29. *N*-Fmoc-L-alaninamide (**3.7b**)



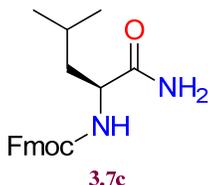
N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-alanine (3.11 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. Ammonia solution 7 N in methanol (4.3 mL) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in DCM (150 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**3.7b**) (2.73 g, 88%) as a colourless solid, mp 165-166 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3315 (N-H), 1686 (C=O), 1659 (C=O), 1531 (C-C), 1251 (C-N), 735 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.71 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (3H, t, $J = 7.5$ Hz, 3',6'-H, NHCH), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 7.26 (1H, s, HNH), 6.94 (1H, s, HNH), 4.23 (2H, d, $J = 7.0$ Hz, CO₂CH₂), 4.20 (1H, t, $J = 7.0$ Hz, 9'-H), 3.94 (1H, p, $J = 7.3$ Hz, NHCHMe), 1.18 (3H, d, $J = 7.3$ Hz, CHMe).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 174.9 (CONH₂), 156.1 (CO₂CH₂CH), 144.3 (C), 141.2 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.6 (1',8'-CH), 66.0 (CH₂), 50.3 (NHCH), 47.1 (9'-CH), 18.7 (CHMe).

HRMS-ESI (m/z) Calculated for C₁₈ H₁₈ N₂ O₃ Na [M+Na]: 333.1210, found: 333.1198.

6.2.30. *N*-Fmoc-L-leucinamide(3.7c)



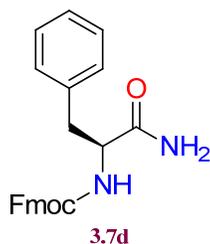
N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-leucine (3.53 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. Ammonia solution 7 N in methanol (4.3 mL) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in DCM (150 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**3.7c**) (3.27 g, 93%) as a colourless solid, mp 174-176 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3319 (N-H), 3194, 1684 (C=O), 1643 (C=O), 1526 (C-C), 1248 (C-N), 733 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.70 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.38 (1H, d, $J = 8.6$ Hz, NHCH), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 7.28 (1H, s, HNH), 6.94 (1H, s, HNH), 4.26 (1H, d, $J = 7.0$ Hz, CO₂CH₂), 4.22 (1H, t, $J = 7.0$ Hz, 9'-H), 4.19 (1H, d, $J = 7.0$ Hz, CO₂CH₂), 3.93 (1H, m, NHCHCH₂), 1.57 (1H, m, MeCHMe), 1.45 (1H, m, NHCHCH₂), 1.37 (1H, m, NHCHCH₂), 0.85 (3H, d, $J = 6.5$ Hz, MeCHMe), 0.82 (3H, d, $J = 6.5$ Hz, MeCHMe).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 174.9 (CONH₂), 156.4 (CO₂CH₂CH), 144.3 (C), 141.2 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.7 (1',8'-CH), 66.0 (CH₂), 53.5 (NHCH), 47.1 (9'-CH), 41.2 (NHCHCH₂), 24.7 (MeCHMe), 23.5 (MeCHMe), 21.8 (MeCHMe).

HRMS-ESI (m/z) Calculated for C₂₁ H₂₄ N₂ O₃ Na [M+Na]: 375.1679, found: 375.1662.

6.2.31. *N*-Fmoc-L-phenylalaninamide (3.7d)



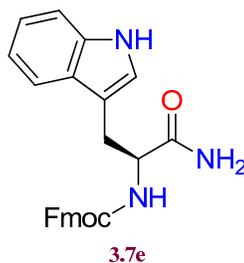
N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-phenylalanine (3.87 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. Ammonia solution 7 N in methanol (4.3 mL) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in DCM (150 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**3.7d**) (3.66 g, 95%) as a colourless solid, mp 189-190 °C; IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3328 (N-H), 3225, 1680 (C=O), 1644 (C=O), 1534 (C-C), 1405, 1253 (C-N), 736 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 7.85 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.61 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.54 (1H, d, $J = 8.8$ Hz, NHCH), 7.47 (1H, s, HNH), 7.38 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.29 (2H, t, $J = 7.5$ Hz, 2',7'-H), 7.28 (4H, m, PhH), 7.15 (1H, t, $J = 7.5$ Hz, PhH), 7.06 (1H, s, HNH), 4.26 (4H, m, 9'-H, CO₂CH₂, NHCHCH₂), 2.97 (1H, dd, $J = 13.6, 4.2$ Hz, CHCHHPh), 2.76 (1H, dd, $J = 13.6, 4.2$ Hz, CHCHHPh).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 173.9 (CONH₂), 156.2 (CO₂CH₂CH), 144.2 (C), 141.1 (C), 138.8 (Ph), 129.7 (Ph), 128.5 (Ph), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 126.6 (Ph), 125.8 (2',7'-CH), 120.5 (1',8'-CH), 66.0 (CH₂), 56.5 (NHCH), 47.0 (9'-CH), 37.9 (NHCHCH₂).

HRMS-ESI (m/z) Calculated for C₂₄ H₂₃ N₂ O₃ [M+H]: 387.1703, found: 387.1704.

6.2.32. *N*-Fmoc-L-tryptophanamide (3.7e)



N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-tryptophan (4.26 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. Ammonia solution 7 N in methanol (4.3 mL) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in DCM (150 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (3.7e) (4.1 g, 96%) as a light orange solid, mp 145-146 °C; IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3306 (N-H), 1665 (C=O), 1654 (C=O), 1529 (C-C), 1249 (C-N), 734 (N-H).

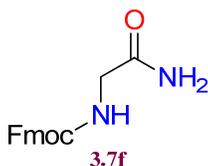
¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 10.81 (1H, d, $J = 2.4$ Hz, NH), 7.84 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.64 (1H, d, $J = 8.1$ Hz, 4-H), 7.60 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.46 (1H, s, HNH), 7.45 (1H, d, $J = 8.5$ Hz, NHCH), 7.38 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (1H, d, $J = 8.1$ Hz, 7-H), 7.28 (2H, t, $J = 7.5$ Hz, 2',7'-H), 7.15 (1H, d, $J = 2.4$ Hz, 2-H), 7.05 (1H, s, HNH), 7.02 (1H, t, $J = 7.5$ Hz, 6-H), 6.95 (1H, t, $J = 7.5$ Hz, 5-H), 4.19 (1H, ddd, $J = 9.8, 8.5, 4.5$ Hz, NHCHCH₂), 4.13 (3H, m, 9'-H, CO₂CH₂), 3.09 (1H, dd, $J = 14.5, 4.5$ Hz, CHCH₂), 2.91 (1H, dd, $J = 14.5, 9.8$ Hz, CHCH₂).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 174.3 (CONH₂), 156.2 (CO₂CH₂CH), 144.2 (C), 141.1 (C), 136.5 (C), 128.1 (3',6'-CH), 127.7 (C), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 124.1 (2-CH), 121.3 (6-CH),

120.5 (1',8'-CH), 119.0 (5-CH), 118.6 (4-CH), 111.7 (7-CH), 110.3 (3-CH), 66.0 (CO₂CH₂CH), 55.7 (NHCH), 47.0 (9'-CH), 31.5 (NHCHCH₂).

HRMS-ESI (*m/z*) Calculated for C₂₆ H₂₄ N₃ O₃ [M+H]: 426.1812, found: 426.1848.

6.2.33. *N*-Fmoc-glycinamide (3.7f)



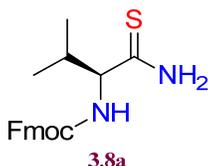
N-Methylmorpholine (2.2 mL, 20 mmol) was added to a stirred solution of Fmoc-L-glycine (2.96 g, 10 mmol) in dry THF (100 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (1.3 mL, 10 mmol) was added dropwise and the mixture was stirred for 10 min. Ammonia solution 7 N in methanol (4.3 mL) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in DCM (150 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (3.7f) (2.65 g, 90%) as a colourless solid, mp 160-161 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3346 (N-H), 3177, 1686 (C=O), 1659 (C=O), 1538 (C-C), 1256 (C-N), 736 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 7.87 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.69 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.44 (1H, t, *J* = 6.2 Hz, NHCH₂), 7.39 (3H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 7.25 (1H, s, HNH), 6.99 (1H, s, HNH), 4.25 (2H, d, *J* = 7.0 Hz, CO₂CH₂), 4.20 (1H, t, *J* = 7.0 Hz, 9'-H), 3.51 (1H, d, *J* = 6.2 Hz, NHCH₂).

^{13}C -NMR (151 MHz, $\text{DMSO-}d_6$) δ ppm 171.5 (CONH_2), 156.9 ($\text{CO}_2\text{CH}_2\text{CH}$), 144.3 (C), 141.2 (C), 128.1 ($3',6'\text{-CH}$), 127.5 ($4',5'\text{-CH}$), 125.7 ($2',7'\text{-CH}$), 120.6 ($1',8'\text{-CH}$), 66.1 (CH_2), 47.1 ($9'\text{-CH}$), 43.6 (NHCH_2).

HRMS-ESI (m/z) Calculated for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_3\text{Na}$ [$\text{M}+\text{Na}$]: 319.1053, found: 319.1042.

6.2.34. *N*-Fmoc-L-valinthioamide (**3.8a**)



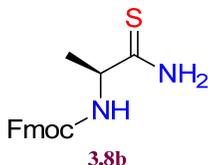
Under an atmosphere of argon Lawesson's reagent (2.0 g, 3.5 mmol) was mixed with Na_2CO_3 (0.37 g, 3.5 mmol) in dry THF (50 mL), to this clear solution was added Fmoc-Val- NH_2 (1.69 g, 5.0 mmol). The mixture was stirred overnight at 25 °C, to this mixture was added Et_2O (100 mL), the solution was washed successively with aqueous NaHCO_3 solution (5%) and brine, dried (MgSO_4) and evaporated in vacuo to give *title compound* (**3.8a**) (1.63 g, 92%) as a colourless solid, IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3280 (N-H), 2955, 1687 (C=O), 1449 (C-C), 1257 (C-N), 1219, 737 (N-H).

^1H -NMR (600 MHz, $\text{DMSO-}d_6$) δ ppm 9.63 (1H, s, *H*NH), 9.22 (1H, s, HNH), 7.87 (2H, d, $J = 7.5$ Hz, $4',5'\text{-H}$), 7.72 (2H, d, $J = 7.5$ Hz, $1',8'\text{-H}$), 7.39 (2H, t, $J = 7.5$ Hz, $3',6'\text{-H}$), 7.30 (1H, d, $J = 9.0$ Hz, *NHCH*), 7.29 (2H, t, $J = 7.5$ Hz, $2',7'\text{-H}$), 4.23 (1H, d, $J = 7.0$ Hz, CO_2CH_2), 4.20 (1H, t, $J = 7.0$ Hz, $9'\text{-H}$), 4.19 (1H, d, $J = 7.0$ Hz, CO_2CH_2), 4.0 (1H, dd, $J = 9.0, 6.9$ Hz, *NHCH*), 2.02 (1H, h, $J = 6.9$ Hz, *MeCHMe*), 0.86 (3H, d, $J = 6.9$ Hz, *MeCHMe*), 0.83 (3H, d, $J = 6.9$ Hz, *MeCHMe*).

^{13}C -NMR (151 MHz, $\text{DMSO-}d_6$) δ ppm 208.0 (CSNH_2), 156.2 ($\text{CO}_2\text{CH}_2\text{CH}$), 144.3 (C), 141.1 (C), 128.1 ($3',6'\text{-CH}$), 127.5 ($4',5'\text{-CH}$), 125.8 ($2',7'\text{-CH}$), 120.5 ($1',8'\text{-CH}$), 66.2 (CH_2), 60.2 (*NHCH*), 47.1 ($9'\text{-CH}$), 32.2 (*MeCHMe*), 19.9 (*MeCHMe*), 18.8 (*MeCHMe*).

HRMS-ESI (m/z) Calculated for $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_2\text{S}$ [$\text{M}+\text{H}$]: 355.1475, found: 355.1489.

6.2.35. *N*-Fmoc-L-alaninthioamide (**3.8b**)



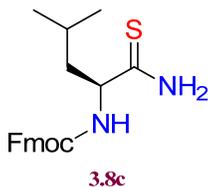
Under an atmosphere of argon Lawesson's reagent (2.0 g, 3.5 mmol) was mixed with Na₂CO₃ (0.37 g, 3.5 mmol) in dry THF (50 mL), to this clear solution was added Fmoc-Ala-NH₂ (1.55 g, 5.0 mmol). The mixture was stirred overnight at 25 °C, to this mixture was added Et₂O (100 mL), the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**3.8b**) (1.3 g, 80%) as a colourless solid, mp 157-158 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3307 (N-H), 1687 (C=O), 1661, 1525 (C-C), 1250 (C-N), 735 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 9.58 (1H, s, HNH), 9.13 (1H, s, HNH), 7.87 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.71 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.49 (1H, d, *J* = 7.0 Hz, NHCH), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 4.20 (3H, m, 9'-H, CO₂CH₂), 3.94 (1H, p, *J* = 7.0 Hz, NHCHMe), 1.27 (3H, d, *J* = 7.0 Hz, CHMe).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 206.1 (CSNH₂), 156.0 (CO₂CH₂CH), 144.3 (C), 141.2 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.6 (1',8'-CH), 66.0 (CH₂), 50.5 (NHCH), 47.1 (9'-CH), 18.9 (CHMe).

HRMS-ESI (*m/z*) Calculated for C₁₈ H₁₉ N₂ O₂ S [M+H]: 327.1162, found: 327.1143.

6.2.36. *N*-Fmoc-L-leucinthioamide (**3.8c**)



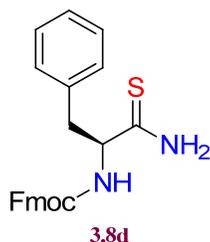
Under an atmosphere of argon Lawesson's reagent (2.0 g, 3.5 mmol) was mixed with Na₂CO₃ (0.37 g, 3.5 mmol) in dry THF (50 mL), to this clear solution was added Fmoc-Leu-NH₂ (1.76 g, 5.0 mmol). The mixture was stirred overnight at 25 °C, to this mixture was added Et₂O (100 mL), the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**3.8c**) (1.65 g, 90%) as a colourless solid, mp 117-118 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3320 (N-H), 2943, 1686 (C=O), 1643, 1526 (C-C), 1248 (C-N), 733 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 9.57 (1H, s, *HNH*), 9.14 (1H, s, *HNH*), 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.71 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.43 (1H, d, $J = 8.5$ Hz, *NHCH*), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.29 (2H, t, $J = 7.5$ Hz, 2',7'-H), 4.25 (3H, m, $J = 7.0$ Hz, CO₂CH₂, *NHCHCH*₂), 4.18 (1H, d, $J = 7.0$ Hz, 9'-H), 1.59 (1H, m, *MeCHMe*), 1.53 (1H, m, *NHCHCH*₂), 1.45 (1H, m, *NHCHCH*₂), 0.86 (3H, d, $J = 6.5$ Hz, *MeCHMe*), 0.83 (3H, d, $J = 6.5$ Hz, *MeCHMe*).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 209.6 (CSNH₂), 156.0 (CO₂CH₂CH), 144.3 (C), 141.2 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.5 (1',8'-CH), 66.0 (CO₂CH₂CH), 59.4 (*NHCH*), 47.1 (9'-CH), 43.8 (*NHCHCH*₂), 24.8 (*MeCHMe*), 23.5 (*MeCHMe*), 21.9 (*MeCHMe*).

HRMS-ESI (m/z) Calculated for C₂₁ H₂₄ N₂ O₂ Na S [M+Na]: 391.1451, found: 391.1465.

6.2.37. *N*-Fmoc-L-phenylalaninthioamide (**3.8d**)



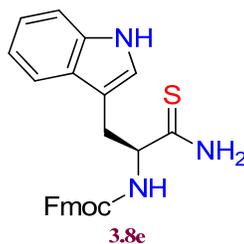
Under an atmosphere of argon Lawesson's reagent (2.0 g, 3.5 mmol) was mixed with Na₂CO₃ (0.37 g, 3.5 mmol) in dry THF (50 mL), to this clear solution was added Fmoc-Phe-NH₂ (1.93 g, 5.0 mmol). The mixture was stirred overnight at 25 °C, to this mixture was added Et₂O (100 mL), the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**3.8d**) (1.8 g, 90%) as a colourless solid, mp 158-160 °C; IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3323 (N-H), 3159, 1680 (C=O), 1627, 1530 (C-C), 1431, 1245 (C-N), 731 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 9.65 (1H, s, *HNH*), 9.30 (1H, s, *HNH*), 7.85 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.64 (1H, d, $J = 9.0$ Hz, *NHCH*), 7.63 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.37 (1H, d, $J = 7.5$ Hz, PhH), 7.32 (1H, d, $J = 7.5$ Hz, PhH), 7.28 (4H, m, 2',7'-H, PhH), 7.17 (1H, t, $J = 7.5$ Hz, PhH), 4.46 (1H, td, $J = 9.0, 4.1$ Hz, *NHCHCH*₂), 4.11 (3H, m, 9'-H, CO₂CH₂), 2.98 (1H, dd, $J = 13.6, 4.1$ Hz, *CHCHHPh*), 2.84 (1H, dd, $J = 13.6, 4.1$ Hz, *CHCHHPh*).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 208.3 (CSNH₂), 156.0 (CO₂CH₂CH), 144.2 (C), 141.1 (C), 138.5 (Ph), 129.8 (Ph), 128.5 (Ph), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 126.8 (Ph), 125.8 (2',7'-CH), 120.5 (1',8'-CH), 66.1 (CO₂CH₂CH), 62.4 (*NHCH*), 47.0 (9'-CH), 40.6 (*NHCHCH*₂).

HRMS-ESI (m/z) Calculated for C₂₄ H₂₃ N₂ O₂ S [M+H]: 403.1475, found: 403.1472.

6.2.38. *N*-Fmoc-L-tryptophanthioamide (3.8e)



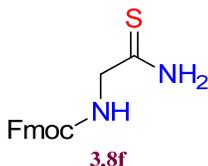
Under an atmosphere of argon Lawesson's reagent (2.0 g, 3.5 mmol) was mixed with Na₂CO₃ (0.37 g, 3.5 mmol) in dry THF (50 mL), to this clear solution was added Fmoc-Trp-NH₂ (2.12 g, 5.0 mmol). The mixture was stirred overnight at 25 °C, to this mixture was added Et₂O (100 mL), the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (3.8e) (2.1 g, 95%) as an off- colourless solid, mp 144-145 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3327 (N-H), 3190, 1718, 1690 (C=O), 1521 (C-C), 1426, 1246 (C-N), 736 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 10.83 (1H, d, $J = 2.5$ Hz, 1-NH), 9.67 (1H, s, HNH), 9.27 (1H, s, HNH), 7.85 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.67 (1H, d, $J = 8.5$ Hz, 4-H), 7.62 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.51 (1H, d, $J = 8.5$ Hz, NHCH), 7.37 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (1H, d, $J = 8.1$ Hz, 7-H), 7.28 (2H, t, $J = 7.5$ Hz, 2',7'-H), 7.22 (1H, d, $J = 2.5$ Hz, 2-H), 7.04 (1H, t, $J = 7.5$ Hz, 6-H), 6.96 (1H, t, $J = 7.5$ Hz, 5-H), 4.52 (1H, ddd, $J = 9.9, 8.5, 4.2$ Hz, NHCHCH₂), 4.11 (3H, m, 9'-H, CO₂CH₂), 3.18 (1H, dd, $J = 14.5, 4.2$ Hz, CHCH₂), 2.99 (1H, dd, $J = 14.5, 9.9$ Hz, CHCH₂).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 208.8 (CSNH₂), 156.0 (CO₂CH₂CH), 144.2 (C), 141.1 (C), 136.5 (C), 128.1 (3',6'-CH), 127.7 (C), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 124.5 (2-CH), 121.3 (6-CH), 120.5 (1',8'-CH), 119.0 (5-CH), 118.7 (4-CH), 111.7 (7-CH), 110.6 (3-CH), 66.1 (CO₂CH₂CH), 62.0 (NHCH), 47.0 (9'-CH), 31.0 (NHCHCH₂).

HRMS-ESI (m/z) Calculated for C₂₆ H₂₄ N₃ O₂ S [M+H]: 442.1584, found: 442.1609.

6.2.39. *N*- Fmoc-glycithioamide (3.8f)



Under an atmosphere of argon Lawesson's reagent (2.0 g, 3.5 mmol) was mixed with Na₂CO₃ (0.37 g, 3.5 mmol) in dry THF (50 mL), to this clear solution was added Fmoc-Gly-NH₂ (1.48 g, 5.0 mmol). The mixture was stirred overnight at 25 °C, to this mixture was added Et₂O (100 mL), the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**3.8f**) (1.48 g, 95%) as an off- colourless solid, mp 167-168 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3292 (N-H), 3143, 1681 (C=O), 1523 (C-C), 1444, 1253 (C-N), 731 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 9.69 (1H, s, HNH), 9.05 (1H, s, HNH), 7.87 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.70 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.64 (1H, t, *J* = 6.2 Hz, NHCH₂), 7.39 (3H, t, *J* = 7.5 Hz, 3',6'-H), 7.31 (2H, t, *J* = 7.5 Hz, 2',7'-H), 4.27 (2H, d, *J* = 7.0 Hz, CO₂CH₂), 4.21 (1H, t, *J* = 7.0 Hz, 9'-H), 3.85 (1H, d, *J* = 6.2 Hz, NHCH₂).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 204.2 (CSNH₂), 156.8 (CO₂CH₂CH), 144.3 (C), 141.2 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.6 (1',8'-CH), 66.2 (CO₂CH₂CH), 51.1 (NHCH₂), 47.1 (9'-CH).

HRMS-ESI (*m/z*) Calculated for C₁₇ H₁₇ N₂ O₂ S [M+H]: 313.1005, found: 313.1004.

6.2.40. (RS) Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)-2-methylpropyl]thiazole-4-carboxylate (3.9a)



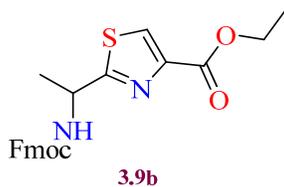
To a solution of acetonitrile (5 mL) containing 30% (v/v) of formic acid was added thioamide **4.11a** (0.271 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9a**) (0.215 g, 96%) as a light Brown gummy, IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3300 (N-H), 1726 (C=O), 1688 (C=O), 1539 (C-C), 1222 (C-N), 725 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.07 (1H, s, 5-H), 7.75 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.59 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 5.63 (1H, d, $J = 9.0$ Hz, NH), 4.92 (1H, dd, $J = 9.0, 6.5$ Hz, NHCH), 4.44 (1H, d, $J = 7.0$ Hz, CO₂CH₂CH), 4.42 (2H, q, $J = 7.0$ Hz, CO₂CH₂Me), 4.40 (1H, d, $J = 7.0$ Hz, CO₂CH₂CH), 4.22 (1H, t, $J = 7.0$ Hz, 9'-H), 2.42 (1H, m, CH), 1.39 (3H, t, $J = 7.0$ Hz, CO₂CH₂Me), 0.94 (3H, d, $J = 6.5$ Hz, MeCHMe), 0.93 (3H, d, $J = 6.5$ Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 172.2 (2-C), 161.2 (CO₂CH₂Me), 156.0 (CO₂CH₂CH), 147.4 (4-C), 143.8 (C), 141.3 (C), 127.7 (3',6'-CH), 127.1 (4',5'-CH), 126.9 (5-CH), 125.0 (2',7'-CH), 120.0 (1',8'-CH), 66.9 (CO₂CH₂CH), 61.5 (CH₂), 58.6 (NHCH), 47.2 (9'-CH), 33.4 (MeCHMe), 19.4 (MeCHMe), 17.7 (MeCHMe), 14.3 (CO₂CH₂Me).

HRMS-ESI (m/z) Calculated for C₂₅ H₂₇ N₂ O₄ S [M+H]: 451.1692, found: 451.1684.

6.2.41. (RS)-Ethyl 2-[1-(fluorenylmethoxycarbonylamino)ethyl]thiazole-4-carboxylate (**3.9b**)



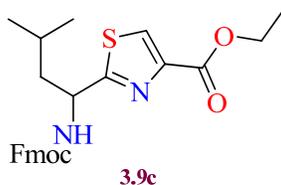
To a solution of acetonitrile (5 mL) containing 30% (v/v) of formic acid was added thioamide **4.11b** (0.257 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9b**) (0.206 g, 96%) as a white gummy, IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3350 (N-H), 1713 (C=O), 1689 (C=O), 1525 (C-C), 1245 (C-N), 733 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.07 (1H, s, 5-H), 7.75 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.58 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.38 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 5.56 (1H, d, $J = 8.0$ Hz, NH), 5.18 (1H, dd, $J = 8.0, 6.8$ Hz, NHCHMe), 4.47 (1H, d, $J = 7.0$ Hz, CO₂CH₂CH), 4.43 (1H, d, $J = 7.0$ Hz, CO₂CH₂CH), 4.41 (2H, q, $J = 7.0$ Hz, CO₂CH₂Me), 4.22 (1H, t, $J = 7.0$ Hz, 9'-H), 1.65 (3H, d, $J = 6.8$ Hz, CHMe), 1.39 (3H, t, $J = 7.0$ Hz, CO₂CH₂Me).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 173.7 (2-C), 161.2 (CO₂CH₂Me), 155.5 (CO₂CH₂CH), 147.2 (4-C), 143.7 (C), 141.3 (C), 127.7 (3',6'-CH), 127.2 (5-CH), 127.1 (4',5'-CH), 125.0 (2',7'-CH), 120.0 (1',8'-CH), 66.9 (CH₂CH), 61.5 (CH₂Me), 49.2 (NHCHMe), 47.2 (9'-CH), 21.8 (CHMe), 14.3 (CO₂CH₂Me).

HRMS-ESI (m/z) Calculated for C₂₃ H₂₃ N₂ O₄ S [M+H]: 423.1379, found: 423.1392.

6.2.42. (RS)-Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)-3-methylbutyl]thiazole-4-carboxylate (3.9c)



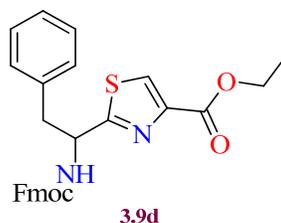
To a solution of acetonitrile (5 mL) containing 30% (v/v) of formic acid was added thioamide **4.11c** (0.278 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9c**) (0.225 g, 98%) as a light Brown gummy, IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3266 (N-H), 2965, 1723 (C=O), 1686 (C=O), 1522 (C-C), 1218 (C-N), 726 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.05 (1H, s, 5-H), 7.75 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.57 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 5.39 (1H, d, $J = 9.0$ Hz, NH), 5.12 (1H, td, $J = 9.0, 5.4$ Hz, NHCHCH₂), 4.44 (1H, d, $J = 7.0$ Hz, CO₂CH₂CH), 4.42 (2H, q, $J = 7.0$ Hz, CO₂CH₂Me), 4.40 (1H, d, $J = 7.0$ Hz, CO₂CH₂CH), 4.20 (1H, t, $J = 7.0$ Hz, 9'-H), 1.97 – 1.89 (1H, m, NHCHCH₂), 1.83 – 1.75 (1H, m, NHCHCH₂), 1.65 (1H, hept, $J = 6.5$ Hz, MeCHMe), 1.39 (3H, t, $J = 7.0$ Hz, CO₂CH₂Me), 0.96 (3H, d, $J = 6.5$ Hz, MeCHMe), 0.95 (3H, d, $J = 6.5$ Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 173.5 (2-C), 161.3 (CO₂CH₂Me), 155.7 (CO₂CH₂CH), 147.4 (4-C), 143.8 (C), 141.3 (C), 127.7 (3',6'-CH), 127.1 (4',5'-CH), 127.0 (5-CH), 125.0 (2',7'-CH), 120.0 (1',8'-CH), 66.8 (CO₂CH₂CH), 61.5 (CO₂CH₂Me), 51.6 (NHCH), 47.2 (9'-CH), 44.4 (NHCHCH₂), 31.0 (MeCHMe), 24.8 (MeCHMe), 22.9 (MeCHMe), 14.4 (CO₂CH₂Me).

HRMS-ESI (m/z) Calculated for $C_{25}H_{27}N_2O_4S$ [M+H]: 465.1848, found: 465.1827.

6.2.43. (RS)-Ethyl 2-[1-(fluorenylmethoxycarbonylamino)-2-phenylethyl]thiazole-4-carboxylate (3.9d)



To a solution of acetonitrile (5 mL) containing 30% (v/v) of formic acid was added thioamide **4.11d** (0.295 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous $NaHCO_3$ solution (5%) and brine, dried ($MgSO_4$) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9d**) (0.219 g, 88%) as a yellow gummy, IR (neat) ν_{max}/cm^{-1} : 3310 (N-H), 1714 (C=O), 1692 (C=O), 1526 (C-C), 1232 (C-N), 739 (N-H).

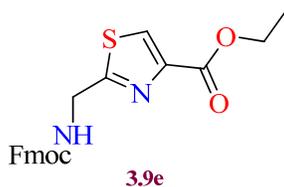
1H -NMR (600 MHz, Chloroform- d) δ ppm 8.03 (1H, s, 5-H), 7.75 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.51 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.38 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.32 – 7.19 (5H, m, 2',7'-H and, PhH), 7.09 (2H, d, $J = 6.6$ Hz, PhH), 5.55 (1H, d, $J = 8.0$ Hz, NH), 5.34 (1H, q, $J = 8.0$ Hz, $NHCH_2$), 4.43 (2H, q, $J = 7.0$ Hz, CO_2CH_2Me), 4.39 (1H, d, $J = 7.0$ Hz, CO_2CH_2CH) 4.36 (1H, d, $J = 7.0$ Hz, CO_2CH_2CH), 4.17 (1H, t, $J = 7.0$ Hz, 9'-H), 3.34 (1H, d, $J = 8.0$ Hz, $CHCHPh$), 3.32 (1H, d, $J = 8.0$ Hz, $CHCHPh$), 1.41 (3H, t, $J = 7.0$ Hz, CO_2CH_2Me).

^{13}C -NMR (151 MHz, Chloroform- d) δ ppm 172.0 (2-C), 161.3 (CO_2CH_2Me), 155.5 (CO_2CH_2CH), 147.4 (4-C), 143.8 (C), 141.3 (C), 136.0 (Ph), 129.4 (Ph), 128.7 (Ph), 127.7 (3',6'-CH), 127.3 (Ph),

127.1(4',5'-CH), 127.0 (5-CH), 125.0 (2',7'-CH), 120.0 (1',8'-CH), 67.0 (CO₂CH₂CH), 61.5 (CO₂CH₂Me), 54.3 (NHCHCH₂), 47.1 (9'-CH), 41.5 (NHCHCH₂), 14.4 (CO₂CH₂Me).

HRMS-ESI (*m/z*) Calculated for C₂₉ H₂₇ N₂ O₄ S [M+H]: 499.1962, found: 499.1975.

6.2.44. Ethyl 2-[1-(fluorenylmethyloxycarbonylamino)methyl]thiazole-4-carboxylate (**3.9e**)



To a solution of acetonitrile (5 mL) containing 30% (v/v) of formic acid was added thioamide **4.11e** (0.250 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9e**) (0.163 g, 80%) as a white gummy, IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3323 (N-H), 1713 (C=O), 1449 (C-C), 1213 (C-N), 729 (N-H).

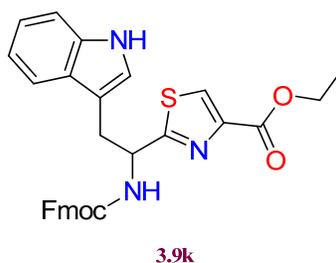
¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.12 (1H, s, 5-H), 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.57 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 5.60 (1H, t, *J* = 6.5 Hz, NH), 4.71 (1H, d, *J* = 6.5 Hz, NHCH₂), 4.46 (2H, d, *J* = 7.0 Hz, CO₂CH₂CH), 4.42 (2H, q, *J* = 7.0 Hz, CO₂CH₂Me), 4.22 (1H, t, *J* = 7.0 Hz, 9'-H), 1.39 (3H, t, *J* = 7.0 Hz, CO₂CH₂Me).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 171.2 (2-C), 161.2 (CO₂CH₂Me), 156.2 (CO₂CH₂CH), 147.0 (4-C), 143.5 (C), 141.3 (C), 128.0 (5-CH), 127.8 (3',6'-CH), 127.1 (4',5'-CH), 125.0 (2',7'-CH),

120.0 (1',8'-CH), 67.1 (CO₂CH₂CH), 61.5 (CO₂CH₂Me), 47.1 (9'-CH), 42.5 (NHCH₂), 14.3 (CO₂CH₂Me).

HRMS-ESI (*m/z*) Calculated for C₂₂ H₂₁ N₂ O₄ S [M+H]: 409.1222, found: 409.1205.

6.2.45. (RS)-Ethyl 2-[1-(fluorenylmethoxycarbonylamino)-2-(3-indolyl)ethyl]thiazole-4-carboxylate (3.9k)



To a solution of acetonitrile (5 mL) was added compound **3.8e** (0.22 g, 0.5 mmol) and EBPY **1.21** (0.097 g, 0.5 mmol). The mixture was irradiated at 70 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 3 minutes, then cooled in a stream of compressed air, was added EtOAc (20 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9k**) (0.255 g, 95%) as an off-white gummy, IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3360 (N-H), 1710 (C=O), 1503 (C-C), 1211 (C-N), 736 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.04 (1H, d, *J* = 2.5 Hz, 1-NH), 8.00 (1H, s, 5-H), 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.53 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.50 (1H, d, *J* = 7.5 Hz, 4-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.35 (1H, d, *J* = 8.1 Hz, 7-H), 7.28 (2H, t, *J* = 7.5 Hz, 2',7'-H), 7.19 (1H, t, *J* = 7.5 Hz, 6-H), 7.11 (1H, t, *J* = 7.5 Hz, 5-H), 6.85 (1H, d, *J* = 2.5 Hz, 2-H), 5.69 (1H, d, *J* = 8.0 Hz, NH), 5.46 (1H, q, *J* = 8.0 Hz, NHCH₂), 4.44 (2H, q, *J* = 7.0 Hz, CO₂CH₂Me), 4.39 (2H, d, *J* = 7.0 Hz,

CO₂CH₂CH), 4.18 (1H, t, *J* = 7.0 Hz, 9'-H), 3.57 (1H, d, *J* = 8.0 Hz, NHCHCH₂), 3.47 (1H, d, *J* = 8.0 Hz, NHCHCH₂), 1.42 (3H, t, *J* = 7.0 Hz, CO₂CH₂Me).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 173.0 (2-CH), 161.4 (CO₂CH₂Me), 155.6 (CO₂CH₂CH), 147.4 (4-CH), 143.7 (C), 141.3 (C), 136.0 (C), 127.7 (3',6'-CH), 127.4 (C), 127.1 (4',5'-CH), 127.0 (5-CH), 125.0 (2',7'-CH), 123.3 (2-CH''), 122.4 (6-CH''), 120.0 (1',8'-CH), 119.9 (5-CH''), 118.6 (4-CH''), 111.2 (7-CH''), 109.1 (3-CH''), 67.0 (CO₂CH₂CH), 61.5 (CO₂CH₂Me), 53.9 (NHCHCH₂), 47.1 (9'-CH), 31.5 (NHCHCH₂), 14.4 (CO₂CH₂Me).

HRMS-ESI (*m/z*) Calculated for C₃₁ H₂₈ N₃ O₄ S [M+H]: 538.1795, found: 538.1849.

6.2.46. (*RS*)-2-[1-(Fluorenylmethyloxycarbonylamino)-2-methylpropyl]thiazole (**3.9f**)



To a solution of acetonitrile (5 mL) containing 30% (v/v) of formic acid was added thioamide **4.11f** (0.235 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9f**) (0.170 g, 90%) as a light Brown gummy.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.79 (1H, d, *J* = 3.3 Hz, 4-H), 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.60 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.30 (2H, t, *J* = 7.5 Hz, 2',7'-H), 7.29 (1H, d, *J* = 3.2 Hz, 5-H), 5.85 (1H, d, *J* = 9.0 Hz, NH), 4.96 (1H, dd, *J* = 9.0, 6.8 Hz, NHCH), 4.42 (2H, d, *J* = 7.0 Hz, CO₂CH₂CH), 4.22 (t, *J* = 7.0 Hz, 1H), 2.39 (1H, h, *J* = 6.8 Hz, MeCHMe), 0.97 (3H, d, *J* = 6.8 Hz, MeCHMe), 0.94 (3H, d, *J* = 6.8 Hz, MeCHMe).

^{13}C -NMR (151 MHz, Chloroform-*d*) δ ppm 171.6 (2-C), 156.1 ($\text{CO}_2\text{CH}_2\text{CH}$), 143.8 (C), 141.6 (5-CH), 141.3 (C), 127.7 (3',6'-CH), 127.1 (4',5'-CH), 125.0 (2',7'-CH), 120.0 (1',8'-CH), 118.9 (4-CH), 67.0 ($\text{CO}_2\text{CH}_2\text{CH}$), 58.2 (NHCH), 47.2 (9'-CH), 33.7 (MeCHMe), 19.3 (MeCHMe), 18.0 (MeCHMe).

HRMS-ESI (*m/z*) Calculated for $\text{C}_{22}\text{H}_{23}\text{N}_2\text{O}_2\text{S}$ [M+H]: 379.1475, found: 379.1479.

6.2.47. (RS)-2-[1-(Fluorenylmethyloxycarbonylamino)ethyl]thiazole (3.9g)



To a solution of acetonitrile (5 mL) containing 30% (*v/v*) of formic acid was added thioamide **4.11g** (0.221 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous NaHCO_3 solution (5%) and brine, dried (MgSO_4) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9g**) (0.166 g, 95%) as a white gummy.

^1H -NMR (600 MHz, Chloroform-*d*) δ ppm 7.76 (3H, d, $J = 7.5$ Hz, 4',5'-H, 4-H), 7.60 (2H, t, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.31 (3H, t, $J = 7.5$ Hz, 2',7'-H, 5-H), 5.70 (1H, d, $J = 8.0$ Hz, NH), 5.23 (1H, m, NHCHMe), 4.44 (1H, d, $J = 7.0$ Hz, $\text{CO}_2\text{CH}_2\text{CH}$), 4.40 (1H, d, $J = 7.0$ Hz, $\text{CO}_2\text{CH}_2\text{CH}$), 4.22 (1H, t, $J = 7.0$ Hz, 9'-H), 1.67 (3H, d, $J = 6.8$ Hz, CHMe).

^{13}C -NMR (151 MHz, Chloroform-*d*) δ ppm 173.1 (2-C), 155.6 ($\text{CO}_2\text{CH}_2\text{CH}$), 143.8 (C), 141.7 (5-CH), 141.3 (C), 127.7 (3',6'-CH), 127.1 (4',5'-CH), 125.1 (2',7'-CH), 120.0 (1',8'-CH), 119.2 (4-CH), 66.9 ($\text{CO}_2\text{CH}_2\text{CH}$), 48.8 (NHCHMe), 47.1 (9'-CH), 22.0 (CHMe).

HRMS-ESI (*m/z*) Calculated for $\text{C}_{20}\text{H}_{19}\text{N}_2\text{O}_2\text{S}$ [M+H]: 351.1162, found: 351.1155.

6.2.48. (RS)-2-[1-(Fluorenylmethyloxycarbonylamino)-3-methylbutyl]thiazole (3.9h)



To a solution of acetonitrile (5 mL) containing 30% (v/v) of formic acid was added thioamide **4.11h** (0.242 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9h**) (0.186 g, 95%) as a light Brown gummy.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.73 (1H, d, *J* = 3.3 Hz, 4-H), 7.58 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.29 (2H, t, *J* = 7.5 Hz, 2',7'-H), 7.24 (1H, d, *J* = 3.3 Hz, 5-H), 5.53 (1H, d, *J* = 9.0 Hz, NH), 5.16 (1H, td, *J* = 9.0, 5.7 Hz, NHCHCH₂), 4.43 (2H, d, *J* = 7.0 Hz, CO₂CH₂CH), 4.21 (1H, t, *J* = 7.0 Hz, 9'-H), 1.89 (1H, m, NHCHCH₂), 1.78 (1H, m, NHCHCH₂), 1.67 (1H, m, MeCHMe), 0.97 (3H, d, *J* = 6.5 Hz, MeCHMe), 0.96 (3H, d, *J* = 6.5 Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 172.6 (2-C), 155.8 (CO₂CH₂CH), 143.9, 143.7 (C), 142.4 (5-CH), 141.3 (C), 127.7 (3',6'-CH), 127.1 (4',5'-CH), 125.1 (2',7'-CH), 120.0 (1',8'-CH), 118.7 (4-CH), 66.8 (CO₂CH₂CH), 51.3 (NHCH), 47.2 (9'-CH), 44.9 (NHCHCH₂), 24.8 (MeCHMe), 22.8 (MeCHMe), 22.0 (MeCHMe).

HRMS-ESI (*m/z*) Calculated for C₂₃ H₂₅ N₂ O₂ S [M+H]: 393.1631, found: 393.1624.

6.2.49. (RS)-2-[1-(Fluorenylmethoxycarbonylamino)-2-phenylethyl]thiazole (3.9i)



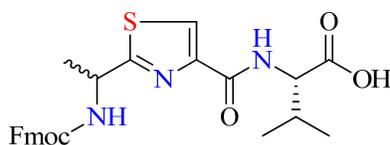
To a solution of acetonitrile (5 mL) containing 30% (v/v) of formic acid was added thioamide **4.11i** (0.259 g, 0.5 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 30 mins, then cooled in a stream of compressed air, was added EtOAc (25 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**3.9i**) (0.181 g, 85%) as a yellow gummy.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.78 (1H, d, *J* = 3.3 Hz, 4-H), 7.75 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.55 (1H, d, *J* = 7.5 Hz, 1'-H), 7.52 (1H, d, *J* = 7.5 Hz, 8'-H) 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.29 (2H, t, *J* = 7.5 Hz, 2',7'-H), 7.28 (1H, d, *J* = 3.3 Hz, 5-H), 7.23 (3H, m, Ph), 7.07 (2H, d, *J* = 7.0 Hz, Ph), 5.71 (1H, d, *J* = 8.0 Hz, NH), 5.39 (1H, q, *J* = 7.5 Hz, NHCHCH₂), 4.40 (1H, d, *J* = 7.0 Hz, CO₂CH₂CH) 4.34 (1H, d, *J* = 7.0 Hz, CO₂CH₂CH), 4.19 (1H, t, *J* = 7.0 Hz, 9'-H), 3.32 (1H, d, *J* = 6.0 Hz, NHCHCH₂).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 172.0 (2-C), 155.6 (CO₂CH₂CH), 143.7 (C), 141.3 (C), 140.6 (5-CH), 135.9 (Ph), 129.8 (Ph), 129.3 (Ph), 128.7 (Ph), 127.7 (3',6'-CH), 127.2 (Ph), 127.0 (4',5'-CH), 125.1 (2',7'-CH), 120.0 (1',8'-CH), 119.62 (4-CH), 67.0 (CO₂CH₂CH), 53.8 (NHCHCH₂), 47.1 (9'-CH), 41.7 (NHCHCH₂).

HRMS-ESI (*m/z*) Calculated for C₂₆ H₂₃ N₂ O₂ S [M+H]: 427.1475, found: 427.1475.

6.2.50. {2-[(1*RS*)-1-(Fluorenylmethyloxycarbonylamino)ethyl]thiazol-4-yl}carbonyl-L-valine (4.46).



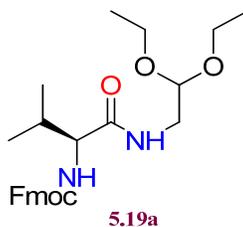
Automated method starts with 0.1 mmol of Fmoc-Val-Wang resin (100-200 mesh).

[The method was mentioned in chapter 4 in the section 4.3.5.]

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.02 (s, 1H), 7.86 (d, $J = 7.5$ Hz, 2H), 7.70 (s, 1H NH), 7.69 (d, $J = 7.5$ Hz, 2H), 7.44 (t, $J = 7.5$ Hz, 2H), 7.36 (t, $J = 7.5$ Hz, 2H), 6.49 (d, $J = 7.7$ Hz, 1H NH), 5.04 (q, $J = 7.0$ Hz 1H), 4.50 – 4.42 (m, 2H), 4.36 (t, $J = 8.7$ Hz, 1H), 4.26 (t, $J = 7.0$ Hz 1H), 2.27 (h, $J = 6.7$ Hz, 1H), 1.59 (d, $J = 7.0$ Hz, 3H), 0.99 (d, $J = 6.7$ Hz, 3H), 0.97 (d, $J = 6.7$ Hz, 3H).

HRMS-ESI (m/z) Calculated for C₂₆ H₂₉ N₃ O₅ S [$M+H^2$]: 495.1822, found: 495.1808.

6.2.51. 1-(*N*-Fmoc-L-valinamido)-2,2-diethoxyethane (5.19a)



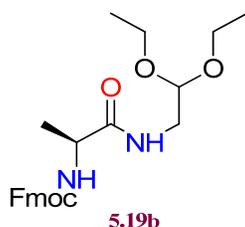
N-Methylmorpholine (1.1 mL, 10 mmol) was added to a stirred solution of Fmoc-L-val-OH (1.69 g, 5 mmol) in dry THF (50 mL) and the mixture was stirred at -10 °C Isobutyl chloroformate (0.75 mL, 5 mmol) was added dropwise and the mixture was stirred for 10 min. 2,2-diethoxy-ethanamine (0.67 g, 5 mmol) was added, and the mixture was stirred at 0 °C for 2 h warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in Ethyl acetate (70 mL), and the solution was washed successively with aqueous NaHCO₃

solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**5.19a**) (2.16 g, 95%) as a colourless solid, mp 137-138 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3295 (N-H), 2977, 1694 (C=O), 1650, 1536 (C-C), 1297, 1249 (C-N), 741 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 7.96 (1H, t, $J = 6.0$ Hz, *NHCH*₂), 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.72 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.40 (1H, d, $J = 9.0$ Hz, *NHCH*), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 4.43 (1H, t, $J = 5.5$ Hz, EtOCHOEt), 4.21 (3H, m, CO₂CH₂, 9'-H), 3.80 (1H, dd, $J = 9.0, 7.0$ Hz, *NHCH*), 3.55 (2H, m, OCH₂Me), 3.40 (2H, m, OCH₂Me), 3.18 (1H, t, $J = 6.0$ Hz, CHCH₂NH), 3.04 (1H, t, $J = 5.5$ Hz, CHCH₂NH), 1.98 (1H, h, $J = 7.0$ Hz, MeCHMe), 1.06 (6H, t, $J = 7.0$ Hz, (OCH₂Me)₂) 0.84 (3H, d, $J = 7.0$ Hz, MeCHMe), 0.82 (3H, d, $J = 7.0$ Hz, MeCHMe).
¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 171.8 (CONH), 156.5 (CO₂), 144.2 (C), 141.1 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.5 (1',8'-CH), 100.4 (EtOCHOEt), 66.1 (CH₂), 61.8 (OCH₂Me)₂, 60.7 (*NHCH*), 47.1 (9'-CH), 41.7 (CHCH₂NH), 30.7 (MeCHMe), 19.6 (MeCHMe), 18.8 (MeCHMe), 15.6 (OCH₂Me)₂.

HRMS-ESI (m/z) Calculated for C₂₆ H₃₄ N₂ O₅ Na [M+Na]⁺: 477.2360, found: 477.2342.

6.2.52. 1-(*N*-Fmoc-L-alaninamido)-2,2-diethoxyethane (**5.19b**)



N-Methylmorpholine (1.1 mL, 10 mmol) was added to a stirred solution of Fmoc-L-Ala-OH (1.55 g, 5 mmol) in dry THF (50 mL) and the mixture was stirred at -10 °C Isobutyl chloroformate (0.75 mL, 5 mmol) was added dropwise and the mixture was stirred for 10 min. 2,2-diethoxy-ethanamine (0.67

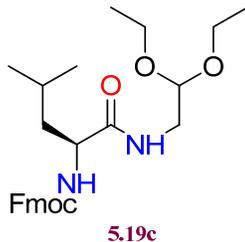
g, 5 mmol) was added, and the mixture was stirred at 0 °C for 2 h warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in Ethyl acetate (70 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to gave *title compound* (**5.19b**) (1.92 g, 90%) as a colourless solid, mp 139-140 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3304 (N-H), 2977, 1693 (C=O), 1651, 1533 (C-C), 1261, 1235 (C-N), 737 (N-H).

¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.85 (1H, t, $J = 6.0$ Hz, NHCH₂), 7.70 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.49 (1H, d, $J = 8.0$ Hz, NHCH), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 4.41 (1H, t, $J = 5.5$ Hz, EtOCHOEt), 4.20 (3H, m, CO₂CH₂ and, 9'-H), 4.01 (1H, p, $J = 7.0$ Hz, NHCHMe), 3.55 (2H, m, OCH₂Me), 3.41 (2H, m, OCH₂Me), 3.14 (1H, t, $J = 6.0$ Hz, CHCH₂NH), 3.05 (1H, t, $J = 5.5$ Hz, CHCH₂NH), 1.17 (1H, d, $J = 7.0$ Hz, NHCHMe), 1.06 (6H, t, $J = 7.0$ Hz, (OCH₂Me)₂).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 173.2 (CONH), 156.0 (CO₂), 144.3 (C), 141.2 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.6 (1',8'-CH), 100.5 (EtOCHOEt), 66.0 (CO₂CH₂), 62.0 (OCH₂Me)₂, 50.4 (NHCHMe), 47.1 (9'-CH), 41.9 (CHCH₂NH), 18.7 (NHCHMe), 15.7 (OCH₂Me)₂.

HRMS-ESI (m/z) Calculated for C₂₄ H₃₀ N₂ O₅ Na [M+Na]⁺: 449.2047, found: 449.2037.

6.2.53. 1-(*N*-Fmoc-L-leucinamido)-2,2-diethoxyethane (**5.19c**)



N-Methylmorpholine (1.1 mL, 10 mmol) was added to a stirred solution of Fmoc-L-leu-OH (1.76 g, 5 mmol) in dry THF (50 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (0.75 mL, 5 mmol) was added dropwise and the mixture was stirred for 10 min. 2,2-diethoxy-ethanamine (0.67 g, 5 mmol) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in Ethyl acetate (70 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**5.19c**) (2.22 g, 95%) as a colourless solid, mp 137-138 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3297 (N-H), 2958, 1691 (C=O), 1655, 1536 (C-C), 1279, 1242 (C-N), 736 (N-H).

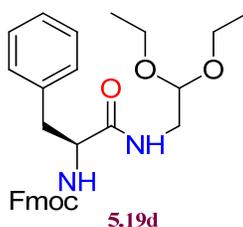
¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 7.90 (1H, t, $J = 6.0$ Hz, NHCH₂), 7.87 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.70 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.47 (1H, d, $J = 8.5$ Hz, NHCH), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.29 (2H, t, $J = 7.5$ Hz, 2',7'-H), 4.42 (1H, t, $J = 5.5$ Hz, EtOCHOEt), 4.21 (3H, m, CO₂CH₂, 9'-H), 3.99 (1H, ddd, $J = 10.0, 8.5, 5.2$ Hz, NHCHCH₂), 3.54 (2H, m, OCH₂Me), 3.40 (2H, m, OCH₂Me), 3.15 (1H, t, $J = 6.0$ Hz, CHCH₂NH), 3.03 (1H, t, $J = 5.5$ Hz, CHCH₂NH), 1.57 (1H, m, MeCHMe), 1.44 (1H, m, NHCHCH₂), 1.36 (1H, m, NHCHCH₂), 1.06 (6H, t, $J = 7.0$ Hz, (OCH₂Me)₂), 0.85 (3H, d, $J = 7.0$ Hz, MeCHMe), 0.81 (3H, d, $J = 7.0$ Hz, MeCHMe).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 173.0 (CONH), 156.3 (CO₂), 144.3 (C), 141.1 (C), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 120.5 (1',8'-CH), 100.4 (EtOCHOEt), 66.0 (CO₂CH₂), 62.0

(OCH₂Me)₂), 53.5 (NHCH), 47.1 (9'-CH), 41.9 (CHCH₂NH), 41.1 (CHCH₂CH), 24.6 (MeCHMe), 23.4 (MeCHMe), 22.0 (MeCHMe), 15.7 (OCH₂Me)₂).

HRMS-ESI (*m/z*) Calculated for C₂₇ H₃₆ N₂ O₅ Na [M+Na]⁺: 491.2516, found: 491.2504.

6.2.54. 1-(*N*-Fmoc-L-phenylalaninamido)-2,2-diethoxyethane (**5.19d**)



N-Methylmorpholine (1.1 mL, 10 mmol) was added to a stirred solution of Fmoc-L-phe-OH (1.93 g, 5 mmol) in dry THF (50 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (0.75 mL, 5 mmol) was added dropwise and the mixture was stirred for 10 min. 2,2-diethoxy-ethanamine (0.67 g, 5 mmol) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in Ethyl acetate (70 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**5.19d**) (2.32 g, 93%) as a colourless solid, mp 176-177 °C; IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3289 (N-H), 2977, 1690 (C=O), 1651, 1539 (C-C), 1258 (C-N), 738 (N-H).

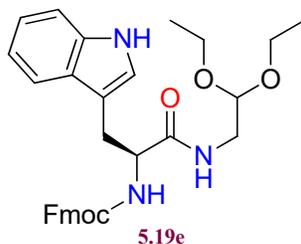
¹H-NMR (600 MHz, DMSO-*d*₆) δ ppm 8.06 (1H, t, *J* = 6.0 Hz, NHCH₂), 7.85 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.62 (1H, d, *J* = 9.0 Hz, NHCH), 7.59 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.38 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.29 (2H, t, *J* = 7.5 Hz, 2',7'-H), 7.24 (4H, m, Ph-H), 7.15 (1H, t, *J* = 7.5 Hz, Ph-H), 4.41 (1H, t, *J* =

5.5 Hz, EtOCHOEt), 4.22 (1H, td, $J = 9.6, 9.0, 4.2$ Hz, NHCHCH₂), 4.10 (3H, m, CO₂CH₂, 9'-H), 3.55 (2H, m, OCH₂Me), 3.41 (2H, m, OCH₂Me), 3.16 (1H, t, $J = 6.0$ Hz, CHCH₂NH), 3.09 (1H, t, $J = 5.5$ Hz, CHCH₂NH), 2.92 (1H, dd, $J = 13.7, 4.2$ Hz, NHCHCH₂), 2.75 (1H, dd, $J = 13.7, 10.6$ Hz, NHCHCH₂), 1.07 (6H, t, $J = 7.0$ Hz, (OCH₂Me)₂).

¹³C-NMR (151 MHz, DMSO-*d*₆) δ ppm 172.2 (CONH), 156.2 (CO₂), 144.3 (C), 141.1 (C), 138.6 (Ph), 129.7 (Ph), 128.5 (Ph), 128.1 (3',6'-CH), 127.5 (4',5'-CH), 126.7 (Ph), 125.8 (2',7'-CH), 120.5 (1',8'-CH), 100.6 (EtOCHOEt), 66.0 (CO₂CH₂), 62.0 (OCH₂Me)₂, 56.6 (NHCH), 47.1 (9'-CH), 42.0 (CHCH₂NH), 38.0 (CHCH₂), 15.7 (OCH₂Me)₂.

HRMS-ESI (*m/z*) Calculated for C₃₀ H₃₄ N₂ O₅ Na [M+Na]⁺: 525.2360, found: 525.2370.

6.2.55. 1-(*N*-Fmoc-L-tryptophanamido)-2,2-diethoxyethane (5.19e)



N-Methylmorpholine (1.1 mL, 10 mmol) was added to a stirred solution of Fmoc-L-trp-OH (2.13 g, 5 mmol) in dry THF (50 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (0.75 mL, 5 mmol) was added dropwise and the mixture was stirred for 10 min. 2,2-diethoxy-ethanamine (0.67 g, 5 mmol) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in Ethyl acetate (70 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (5.19e) (2.43

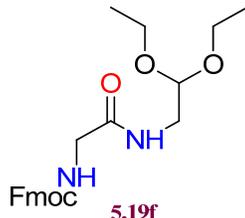
g, 90%) as a colourless, mp 187-188 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3394, 3295 (N-H), 2977, 1687 (C=O), 1648, 1538 (C-C), 1285, 1246(C-N), 738 (N-H).

$^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ ppm 10.80 (1H, d, $J = 2.4$ Hz, NH-1), 8.03 (1H, t, $J = 6.0$ Hz, NHCH₂), 7.85 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.63 (1H, d, $J = 9.0$ Hz, 4-H), 7.60 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.53 (1H, d, $J = 9.0$ Hz, NHCH), 7.38 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (1H, d, $J = 8.1$ Hz, 7-H), 7.28 (2H, t, $J = 7.5$ Hz, 2',7'-H), 7.15 (1H, d, $J = 2.4$ Hz, 2-H), 7.03 (1H, t, $J = 7.5$ Hz, 6-H), 6.95 (1H, t, $J = 7.5$ Hz, 5-H), 4.37 (1H, t, $J = 5.5$ Hz, EtOCHOEt), 4.25 (1H, td, $J = 9.0, 4.2$ Hz, NHCHCH₂), 4.11 (3H, m, CO₂CH₂, 9'-H), 3.53 (2H, m, OCH₂Me), 3.38 (2H, m, OCH₂Me), 3.17 (1H, t, $J = 6.0$ Hz, CHCH₂NH), 3.07 (1H, t, $J = 5.5$ Hz, CHCH₂NH), 3.03 (1H, dd, $J = 14.5, 4.5$ Hz, NHCHCH₂), 2.91 (1H, dd, $J = 14.5, 9.6$ Hz, NHCHCH₂), 1.07 (3H, t, $J = 7.0$ Hz, (OCH₂Me), 1.04 (3H, t, $J = 7.0$ Hz, (OCH₂Me).

$^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$) δ ppm 172.2 (CONH), 156.2 (CO₂), 144.3 (C), 141.1 (C), 138.5 (C), 128.1 (3',6'-CH), 127.7 (C), 127.5 (4',5'-CH), 125.8 (2',7'-CH), 124.3 (2-CH), 121.3 (6-CH), 120.5 (1',8'-CH), 119.0 (5-CH), 118.6 (4-CH), 111.7 (7-CH), 110.7 (3-CH), 100.6 (EtOCHOEt), 66.1 (CO₂CH₂), 62.1 (OCH₂Me)₂, 55.9 (NHCH), 47.1 (9'-CH), 42.1 (CHCH₂NH), 28.4 (NHCHCH₂), 15.7 (OCH₂Me)₂.

HRMS-ESI (m/z) Calculated for C₃₂ H₃₅ N₃ O₅ Na [M+Na]⁺: 564.2469, found: 564.2478.

6.2.56. 1-(*N*-Fmoc-glycinamido)-2,2-diethoxyethane (**5.19f**)



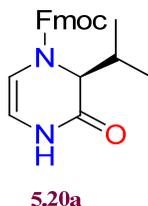
N-Methylmorpholine (1.1 mL, 10 mmol) was added to a stirred solution of Fmoc-L-gly-OH (1.48 g, 5 mmol) in dry THF (50 mL) and the mixture was stirred at -10 °C. Isobutyl chloroformate (0.75 mL, 5 mmol) was added dropwise and the mixture was stirred for 10 min. 2,2-diethoxy-ethanamine (0.67 g, 5 mmol) was added, and the mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. The residue was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in Ethyl acetate (70 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo to give *title compound* (**5.19f**) (1.85 g, 90%) as a colourless solid, mp 94-95 °C; IR (neat) $\nu_{\max}/\text{cm}^{-1}$: 3300 (N-H), 2977, 1712 (C=O), 1655, 1513 (C-C), 1240, 1223 (C-N), 731 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.76 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.58 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.39 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.30 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.14 (1H, s, NH), 5.47 (1H, d, $J = 9.9$ Hz, NHCH), 4.48 (1H, t, $J = 5.0$ Hz, EtOCHOEt), 4.41 (2H, d, $J = 7.0$ Hz, CO₂CH₂), 4.21 (1H, t, $J = 7.0$ Hz, 9'-H), 3.87 (2H, d, $J = 5.6$ Hz, CHCH₂NH), 3.68 (2H, m, OCH₂Me), 3.52 (2H, m, OCH₂Me), 3.41 (2H, t, $J = 5.5$ Hz, NHCH₂), 1.19 (6H, t, $J = 7.0$ Hz, (OCH₂Me)₂).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 167.0 (CONH), 154.8 (CO₂), 143.5 (C), 141.3 (C), 127.9 (3',6'-CH), 127.2 (4',5'-CH), 124.8 (2',7'-CH), 120.1 (1',8'-CH), 108.5 (EtOCHOEt), 67.7 (CO₂CH₂), 63.0 (OCH₂Me)₂, 47.1 (9'-CH), 46.0 (NHCH₂), 43.7 (CHCH₂NH), 14.9 (OCH₂Me)₂.

HRMS-ESI (m/z) Calculated for C₂₃ H₂₈ N₂ O₅ Na [M+Na]⁺: 435.1890, found: 435.1909.

6.2.57. (3S)-N⁴-Fmoc-3-(2-propyl)-3,4-dihydropyrazin-2-one (5.20a)



To a solution of acetonitrile (8 mL) containing 30% (v/v) of formic acid was added amide **5.19a** (0.454 g, 1.0 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 35 mins, then cooled in a stream of compressed air, was added EtOAc (30 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**5.20a**) (0.344 g, 95%) as a white gummy.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.82 (1H, s, *NH*), 7.76 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.56 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.40 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.31 (2H, t, *J* = 7.5 Hz, 2',7'-H), 6.17 (1H, d, *J* = 5.5 Hz, *CHCHNH*), 5.62 (1H, dd, *J* = 5.5, 4.5 Hz, *CHCHNH*), 4.56 (1H, d, *J* = 7.0 Hz, CO₂CH₂CH), 4.52 (1H, d, *J* = 8.0 Hz, CH), 4.48 (1H, d, *J* = 7.0 Hz, CO₂CH₂CH), 4.26 (1H, t, *J* = 7.0 Hz, 9'-CH), 2.02 (1H, h, *J* = 6.8 Hz, MeCHMe), 1.01 (3H, d, *J* = 6.8 Hz, MeCHMe), 0.91 (3H, d, *J* = 6.8 Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 166.3 (CO), 153.3 (CO₂CH₂CH), 143.6 (C), , 141.3 (C), 127.8 (3',6'-CH), 127.2 (4',5'-CH), 124.6 (2',7'-CH), 120.1 (1',8'-CH), 109.3 (*CHCHNH*), 108.3 (*CHCHNH*), 68.0 (CO₂CH₂CH), 61.9 (CH), 47.1 (9'-CH), 30.3 (MeCHMe), 19.2 (MeCHMe), 18.5 (MeCHMe).

HRMS-ESI (*m/z*) Calculated for C₂₂ H₂₂ N₂ O₃ [M+Na]⁺: 385.1523, found: 385.1539.

6.2.58. (3S)-N⁴-Fmoc-3-methyl-3,4-dihydropyrazin-2-one (5.20b)



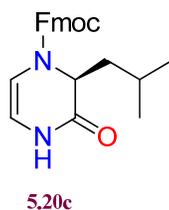
To a solution of acetonitrile (8 mL) containing 30% (v/v) of formic acid was added amide **5.19b** (0.426 g, 1.0 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 35 mins, then cooled in a stream of compressed air, was added EtOAc (30 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**5.20b**) (0.300 g, 90%) as a white gummy.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.77 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.56 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.50 (1H, s, *NH*), 7.40 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.32 (2H, t, *J* = 7.5 Hz, 2',7'-H), 6.12 (1H, d, *J* = 5.5 Hz, *CHCHNH*), 5.61 (1H, t, *J* = 5.5 Hz, *CHCHNH*), 4.84 (1H, q, *J* = 7.0 Hz, *CHMe*), 4.54 (1H, m, CO₂CH₂CH), 4.26 (1H, t, *J* = 7.0 Hz, 9'-CH), 1.31 (3H, d, *J* = 7.0 Hz, *CHMe*).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 168.1 (*CO*), 152.4(CO₂CH₂CH), 143.6 (C), 141.3 (C), 127.8 (3',6'-CH), 127.2 (4',5'-CH), 124.6 (2',7'-CH), 120.1 (1',8'-CH), 108.1 (*CHCHNH*), 107.2 (*CHCHNH*), 68.0 (CH₂CH), 52.7 (*CHMe*), 47.1 (9'-CH), 15.5 (*CHMe*).

HRMS-ESI (*m/z*) Calculated for C₂₀ H₁₈ N₂ O₃ Na [M+Na]⁺: 357.1210, found: 357.1201.

6.2.59. (3S)-N⁴-Fmoc-3-(1-[2-methyl]propyl)-3,4-dihydropyrazin-2-one (5.20c)



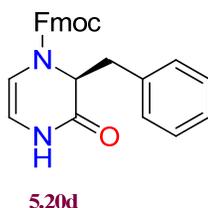
To a solution of acetonitrile (8 mL) containing 30% (v/v) of formic acid was added amide **5.19c** (0.468 g, 1.0 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 35 mins, then cooled in a stream of compressed air, was added EtOAc (30 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**5.20c**) (0.357 g, 95%) as a white gummy, IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 2959 (N-H), 1674 (C=O), 1400 (C-C), 1315, 1245 (C-N), 727 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.19 (1H, s, NH), 7.76 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.57 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.40 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.31 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.10 (1H, d, $J = 5.8$ Hz, CHCHNH), 5.66 (1H, dd, $J = 5.8, 4.5$ Hz, CHCHNH), 4.55 (1H, d, $J = 7.0$ Hz, CO₂CH₂CH), 4.53 (1H, t, $J = 7.5$ Hz, CHCH₂), 4.48 (1H, d, $J = 7.0$ Hz, CO₂CH₂CH), 4.25 (1H, t, $J = 7.0$ Hz, 9'-CH), 1.49 (3H, m, CHCH₂, MeCHMe), 0.97 (3H, d, $J = 6.5$ Hz, MeCHMe), 0.95 (3H, d, $J = 6.5$ Hz, MeCHMe).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 168.5 (CO), 152.9 (CO₂CH₂CH), 143.6 (C), 141.3 (C), 127.9 (3',6'-CH), 127.1 (4',5'-CH), 125.0 (2',7'-CH), 120.1 (1',8'-CH), 109.1 (CHCHNH), 108.0 (CHCHNH), 68.0 (CO₂CH₂CH), 54.7 (CHCH₂), 47.1 (9'-CH), 38.7 (CHCH₂), 24.2 (MeCHMe), 22.7 (MeCHMe), 21.1 (MeCHMe).

HRMS-ESI (m/z) Calculated for C₂₃ H₂₄ N₂ O₃ Na [M+Na]⁺: 399.1679, found: 399.1676.

6.2.60. (3S)-N⁴-Fmoc-3-(phenylmethyl)-3,4-dihydropyrazin-2-one (5.20d)



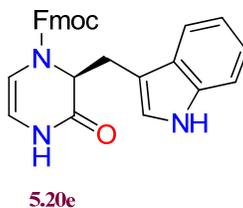
To a solution of acetonitrile (8 mL) containing 30% (v/v) of formic acid was added amide **5.19d** (0.502 g, 1.0 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 35 mins, then cooled in a stream of compressed air, was added EtOAc (30 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**5.20d**) (0.377 g, 92%) as a white gummy.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.73 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.52 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.45 (1H, s, *NH*), 7.39 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.31 (2H, t, *J* = 7.5 Hz, 2',7'-H), 7.18 (3H, m, Ph-H), 7.02 (2H, d, *J* = 7.5 Hz, Ph-H), 6.33 (1H, d, *J* = 5.8 Hz, *CHCHNH*), 5.58 (1H, dd, *J* = 5.8, 4.5 Hz, *CHCHNH*), 5.06 (1H, t, *J* = 6.5 Hz, *CHCH*₂), 4.24 (1H, d, *J* = 7.0 Hz, CO₂*CH*₂CH), 4.14 (1H, t, *J* = 7.0 Hz, 9'-CH), 4.04 (1H, d, *J* = 7.0 Hz, CO₂*CH*₂CH), 2.84 (1H, dd, *J* = 13.6, 8.6 Hz, *CHCHH*), 2.77 (1H, dd, *J* = 13.6, 4.8 Hz, *CHCHH*).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 166.5 (*CO*), 153.1 (CO₂CH₂CH), 143.6 (C), 141.3 (C), 135.8 (Ph), 129.7 (Ph), 128.4 (Ph), 127.9 (3',6'-CH), 127.1 (4',5'-CH), 126.9 (Ph), 125.0 (2',7'-CH), 120.1 (1',8'-CH), 108.5 (*CHCHNH*), 107.9 (*CHCHNH*), 68.0 (CO₂CH₂), 58.9 (*CHCH*₂), 47.1 (9'-CH), 36.2 (*CHCH*₂).

HRMS-ESI (*m/z*) Calculated for C₂₆ H₂₃ N₂ O₃ [M+H]⁺: 311.1703, found: 311.1697.

6.2.61. (3*S*)-*N*⁴-Fmoc-3-(indol-3-ylmethyl)-3,4-dihydropyrazin-2-one (5.20e)



To a solution of acetonitrile (8 mL) containing 30% (v/v) of formic acid was added amide **5.19e** (0.541 g, 1.0 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 35 mins, then cooled in a stream of compressed air, was added EtOAc (30 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**5.20e**) (0.404 g, 90%) as an off-white gummy.

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 8.13 (1H, s, NH-1), 7.77 (2H, d, *J* = 7.5 Hz, 4',5'-H), 7.64 (1H, d, *J* = 7.5 Hz, 4-H), 7.54 (2H, d, *J* = 7.5 Hz, 1',8'-H), 7.43 (1H, d, *J* = 8.0 Hz, 7-H), 7.40 (2H, t, *J* = 7.5 Hz, 3',6'-H), 7.39 (1H, s, NH), 7.31 (2H, t, *J* = 7.5 Hz, 2',7'-H), 7.17 (1H, t, *J* = 7.5 Hz, 6-H), 7.10 (1H, t, *J* = 7.5 Hz, 5-H), 6.49 (1H, s, 2-H), 5.49 (1H, d, *J* = 4.0 Hz, CHCHNH), 4.99 (1H, d, *J* = 4.0 Hz, CHCHNH), 4.82 (1H, d, *J* = 5.5 Hz, CHCH₂), 4.55 (1H, d, *J* = 6.2 Hz, CO₂CH₂CH), 4.51 (1H, d, *J* = 6.2 Hz, CO₂CH₂CH), 4.23 (1H, t, *J* = 6.2 Hz, 9'-CH), 3.03 (1H, d, *J* = 5.8 Hz, CHCHH), 2.94 (1H, d, *J* = 5.8 Hz, CHCHH).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 164.2 (CO), 154.0 (CO₂CH₂CH), 143.4 (C), 141.4 (C), 136.0 (C), 127.9 (3',6'-CH), 127.7 (C), 127.2 (4',5'-CH), 124.8 (2',7'-CH), 122.7 (2-CH), 120.2 (1',8'-CH), 120.0 (6-CH), 119.8 (5-CH), 118.6 (4-CH), 111.3 (7-CH), 108.9 (3-CH), 66.8 (CH₂), 53.7 (CHCH₂), 47.2 (9'-CH), 25.5 (CHCH₂).

HRMS-ESI (*m/z*) Calculated for C₂₈ H₂₃ N₃ O₃ Na [M+Na]⁺: 472.1632, found: 472.1614.

6.2.62. *N*⁴-Fmoc-3,4-dihydropyrazin-2-one (5.20f)



To a solution of acetonitrile (8 mL) containing 30% (v/v) of formic acid was added amide **5.19f** (0.412 g, 1.0 mmol). The mixture was irradiated at 120 °C (initial power 200 W; maximum pressure 200 psi) in a sealed-vessel for 35 mins, then cooled in a stream of compressed air, was added EtOAc (30 mL), and the solution was washed successively with aqueous NaHCO₃ solution (5%) and brine, dried (MgSO₄) and evaporated in vacuo, the residue was purified by column chromatography (hexane/EtOAc) to give *title compound* (**5.20f**) (0.297 g, 93%) as a white gummy, IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3000 (N-H), 1678 (C=O), 1401 (C-C), 1315, 1240 (C-N), 726 (N-H).

¹H-NMR (600 MHz, Chloroform-*d*) δ ppm 7.77 (2H, d, $J = 7.5$ Hz, 4',5'-H), 7.68 (1H, s, NH), 7.56 (2H, d, $J = 7.5$ Hz, 1',8'-H), 7.41 (2H, t, $J = 7.5$ Hz, 3',6'-H), 7.32 (2H, t, $J = 7.5$ Hz, 2',7'-H), 6.25 (1H, d, $J = 6.0$ Hz, CHCHNH), 5.59 (1H, t, $J = 5.5$ Hz, CHCHNH), 4.53 (2H, dd, $J = 7.0$ Hz, CO₂CH₂CH), 4.30 (2H, s, COCH₂), 4.27 (1H, t, $J = 7.0$ Hz, 9'-CH).

¹³C-NMR (151 MHz, Chloroform-*d*) δ ppm 165.1 (CO), 153.0 (CO₂CH₂CH), 143.4 (C), 141.3 (C), 127.9 (3',6'-CH), 127.2 (4',5'-CH), 124.9 (2',7'-CH), 120.1 (1',8'-CH), 108.8 (CHCHNH), 107.5 (CHCHNH), 68.5 (CH₂), 47.0 (9'-CH), 46.7 (COCH₂).

HRMS-ESI (m/z) Calculated for C₁₉ H₁₇ N₂ O₃ [M+H]: 321.1234, found: 321.1231.

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