Synthetic Approaches towards Heterocyclic Natural Products

Xin Xiong

Thesis submitted for the degree of Doctor of Philosophy

CARDIFF UNIVERSITY

August 2005

UMI Number: U487588

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

UMI U487588
Published by ProQuest LLC 2013. Copyright in the Dissertation held by the Author.
Microform Edition © ProQuest LLC.
All rights reserved. This work is protected against
unauthorized copying under Title 17, United States Code.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

ABSTRACT

A variety of tri- and tetrasubstituted pyridines are prepared by new one-pot processes, developed by modification and improvement of the traditional Bohlmann-Rahtz reaction. The use of zinc(II) bromide catalysis, microwave irradiation and conductive heating in a sealed tube is described to facilitate rapid Michael addition-cyclodehydration of an enamine and alkynone in a single synthetic step and with total control of regiochemistry. Expanding the Bohlmann-Rahtz heteroannulation reaction leads to the development of a mild one-pot three-component synthesis of pyridines, a tandem oxidation-heteroannulation process for the one-pot synthesis of pyridines from propargylic alcohols, a highly facile combined three-component tandem oxidation-heteroannulation process and a novel tetrasubstituted bromopyridine synthesis mediated by *N*-bromosuccinimide.

The synthesis of dimethyl sulfomycinamate (**13**), the acidic methanolysis product of the sulfomycin family of thiopeptide antibiotics, from methyl 4-(trimethylsilyl)-2-oxobut-3-ynoate (**216**) is achieved in 13 steps by the Bohlmann-Rahtz heteroannulation of 1-(oxazol-4-yl)enamine **252** or in 12 steps and 9% overall yield by three-component cyclocondensation with *N*-[3-oxo-3-(oxazol-4-yl)propanoyl]serine **251** and ammonia in methanol, in a 2,3,6-trisubstituted pyridine synthesis that proceeds with total regiocontrol.

Bohlmann-Rahtz reaction of a chiral non-racemic enamine **312** and thiazolylpropynone **114** gives a terminal-protected pyridine-containing γ-amino acid **311** in high optical purity in a sequential one pot multicomponent reaction that proceeds with total control of regiochemistry and with minimal racemisation. Further elaboration has established the first synthesis of the γ-lactam acidic hydrolysate of the macrocyclic thiopeptide antibiotic cyclothiazomycin, a selective renin inhibitor, in only four steps and 30% overall yield and has confirmed its structure.

ACKNOWLEDGMENTS

First of all, I would like to thank Dr. Mark Bagley for allowing me to join his research group and for looking after me during my time at Cardiff. I am greatly indebted to him for his tremendous contribution to my development as an organic chemist. Thanks for his help, support, continued guidance, understanding and providing such an extremely positive and encouraging work environment over the last a few years. It has been a privilege to work under his tutelage. I am especially proud to have been a member in the Bagley group.

I would also like to thank Professor David Knight for the support he has given me. He was a fantastic mentor that has inspired me throughout the course of my Ph.D. In addition, I am indebted to Professor Thomas Wirth for being kindly invited to dinner in his house every Christmas. I also enjoyed attending Professor Wirth's "Coffee Lecture" every Wednesday morning in my first year of Ph.D. I am grateful as well for encouragement, advice, knowledge of chemistry and friendship received from Dr. Nick Tomkinson, who is one of the nicest persons I have ever met.

At this point I would like to thank my current advisor Dr Darren Dixon, in whose research group I have been working as a research assistant. Darren is such a wonderful person that it is not possible at all to finish this thesis without his strong support and full understanding.

I would like to acknowledge my family, especially my wife Yan. She has been the best part of my life, and I cannot imagine what these years guested in the UK would have been like without her.

I also must thank the members of the Bagley group, Dave (the postdoc), James, Christian, Krishna for all their support and all other members of Lab 1.119 with which I have spent the last few years working with. Individual thanks go to Robert Jenkins, Gary Coleman, Robin Hicks and finally a thank you to all of my friends that I have met here at Cardiff over the last four years.

ABBRVIATIONS

aatRNA	aminoacyl-Ribose Nucleic Acid
Ac	Acetyl
aq.	Aqueous
Ar	Unspecified aryl substituent
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
Bu	Butyl
BuLi	Butyllithium
BOP	Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
cat.	Catalytic
Cbz (Z)	Benzyloxycarbonyl
CID	Collisionally induced dissociation
conc.	Concentrated
COSY	Correlation Spectroscopy
Cys	Cysteine
Δ	Heat
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
d.e.	Diastereomeric excess
DEAD	Diethylazodicarboxylate
DIBAL	Di-*i*-butylaluminium hydride
DIPEA	Di*iso*propylethylamine
DMAP	4-Dimethylamino pyridine
DME	1,2-Dimethoxyethane
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide

b.p.	Boiling point
DPPA	Diphenylphosphoryl azide
dppp	1,3-*bis*(Diphenylphosphino)propane
EDCI	1-(3-Dimethylaminopropyl)3-ethylcarbodiimide hydrochloride
e.e.	Enantiomeric excess
Ef-G	Elongation factor - G
Ef-Tu	Elongation factor -Tu
ESCA	Electron Spectroscopy Chemical Analysis
Et	Ethyl
equiv. or eq.	Equivalent
Fu	Furyl
g	Grams
GC-MS	Gas Chromatography-Mass Spectrometry
GDP	Guanidine diphosphate
Gly	Glycine
GTP	Guanidine triphosphate
GTPase	Guanidine triphosphate hydrolysis enzyme
HAC	Hydrogen-bond Acceptor
HATU	O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate
HDO	Hydrogen-bond Donator
HMBC	Hetronuclear Multiple-Bond Correlation
HMQC	Hetronuclear Multiple-Quantum Correlation
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxy-1*H*-benzotriazole
HPLC	High Pressure (Performance) Liquid Chromatography
HR-FAB-MS	High Resolution Fast Atom Bombardment Mass Spectrometry
HR-MALDI-TOF-MS	High Resolution Time of Flight Mass Spectrometry
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single-Quantum Correlation
i	*iso*

IBX	*o*-Iodoxybenzoic acid
IC_{50}	Inhibitory concentration to reduce uptake by 50%
in situ	*Latin* in the normal, nature, original, or appropriate position.
in vitro	*Latin* occurring or made to occur outside an organism
in vivo	*Latin* occurring or made to occur within a living organism
IPA	Isopropyl alcohol
IR	Infra Red
J	Coupling constant (in Hz)
L11BD	L11 Binding Domain
LDA	Lithium Di*iso*propylamine
LRMS	Low Resolution Mass Spectrometry
M	Molar
m-CPBA	*meta*-Chloroperbenzoic acid
MCR	Multiple Component Reaction
MDR	Multi-Drug Resistance
Me	Methyl
min	Minutes
MOM	Methoxymethyl
m.p.	Melting point
mRNA	messenger-Ribose Nucleic Acid
MRSA	Methicillin Resistant Staphylococcus Aureus
MS	Mass Spectrometry
MsCl	Mesyl chloride
MWT	Molecular Weight
n	*normal*
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
NMM	*N*-Methyl morpholine
NMR	Nuclear Magnetic Resonance
nOe	nuclear Overhouser effect
NOSEY	nuclear Overhouser effect Spectroscopy

p	*para*
P	Protecting group
Pac	Phenylacyl
PCC	Pyridinium chlorochromate
Ph	Phenyl
P_i	Inorganic phosphate
PP_i	Inorganic diphosphate
PTS	*para*-Toluene sulfonic acid
Py	Pyridyl
R	Specified substituent
rmsd	root mean square deviation
rRNA	ribosomal-Ribose Nucleic Acid
ROESY	Rotating-frame Overhauser Enhancement
rt	room temperature
Ser	Serine
SEM	2-(Trimethylsilyl)ethoxymethyl
t	time
TBAF	Tetra *n*-butylammonium Fluoride
TBDMS-Cl	*tert*-Butyldimethylsilyl chloride
TBDPS-Cl	*tert*-Butyldiphenylsilyl chloride
TBS	*tert*-Butyldimethylsilyl
tert	*tertiary*
Tf	Trifluoromethanesulfonyl
TFA	Triflouroacetic acid
TFAA	Trifluorocacetic anhydride
THF	Tetrahydrofuran
Thr	Threonine
TIPS-Cl	Tri*iso*propylsilyl chloride
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
TPS	Triphenylsilyl

tRNA	transfer-Ribose Nucleic Acid
quant.	Quantitative
UV	Ultraviolet
X or Y	Unspecified substituent
Xc	Chiral auxiliary

CONTENTS

Chapter One

Introduction

1. The Thiopeptide Antibiotics

1.1 Introduction

The crisis currently facing antibacterial chemotherapy threatens to return our treatment of bacterial infections to the so-called 'dark age' of a pre-antibiotic era with the alarming emergence of bacterial strains resistant to conventional treatments.[1] In the face of this medical crisis, many resources have been committed to improving the potency of existing antibiotic classes, discovering new antibacterial agents with novel modes of action and understanding the mechanisms of resistance that are adopted by different bacterial pathogens to overcome antibacterial action. A discussion of the resistance mechanisms used by antibiotic-producing organisms has been the subject of a number of excellent reviews.[2,3] Antibiotic producers adopt different self defence mechanisms in order to avoid their own suicide, protecting themselves against extra-cellular drugs by inactivating their antibiotic products, modifying the antibiotic target sites, such as enzymes or ribosomes, or by blocking the entrance of active compounds into the cell. Characterising the strategies used by either the producers or related bacterial strains to avoid intoxication requires a detailed understanding of how each antibiotic class functions, as well as knowledge of the biosynthetic machinery operating in the organism, in order to predict mechanisms of multi-drug resistance (MDR) prior to their clinical emergence in the design of new or strategically-modified treatments.

Apart from classical antibiotics such as the sulfonamides, penicillins and cephalosporins amongst others, there is a new class of antibacterial agent known as the thiopeptide antibiotics. They are naturally occurring, sulfur containing, highly modified macrocyclic peptides, nearly all of which inhibit protein synthesis in bacteria. These complex natural products, grouped as thiazolyl peptides for Bérdy's classification of antibiotics according to chemical structure,[4] share a number of common structural features: a tri- or tetrasubstituted nitrogen heterocycle clustered in a central polyazole domain that is part of a macrocyclic framework consisting of modified heterocyclic residues, including thiazoles, oxazoles and indoles, and dehydroamino acids. These biologically active substances are secondary

metabolites produced by actinomycetes, Gram-positive mycelial sporulating bacteria, largely of the genus *Streptomyces* that can be subdivided into 29 different antibiotic families. In spite of their chemical and taxonomical diversity, many of them broadly seem to share a similar biological profile, displaying almost no activity against Gram-negative bacteria, whereas against Gram-positive bacteria they are highly active inhibitors of protein synthesis and are, in many cases, effective against methicillin resistant *Staphylococcus aureus* (MRSA), a bacterial strain that is resistant to most conventional treatments.

Since the isolation of micrococcin, the first member of thiopeptide family, in 1948, it has been a rapid expanding group of antibiotics, which contains well over 76 structurally distinct entities up to the present. Various fascinating biological activities of the thiopeptide antibiotics reported so far make them a promising candidate of a new generation of antibacterials. More importantly, due to the novel mode of action studied in detail for thiostrepton (**1**), the parent of thiopeptides, this antibiotic class has great potential to play a key part in fighting against the forthcoming emergence of bacterial resistance, hence attracts great attention from researchers in relevant disciplines all over the world.

1.2 Classification

The individual chemical identity of structurally distinct compounds isolated from natural sources and classified as thiopeptide or thiazolylpeptide antibiotics is relatively diverse. The identification of a plethora of unique structural motifs and unusual functional groups assembled in a macrocyclic array has provided us with a series of tantalising heterocyclic chemical targets that a number of international synthetic groups have found impossible to resist. Of a more intriguing nature, in spite of many similarities in biosynthetic origin, these secondary metabolites have been isolated from a number of different strains of actinomycetes, predominantly soil bacteria but also from marine sources, and elicit a wide range of different biological responses. The complexity of the many mechanisms employed by pathogens to avoid intoxication, and used by these metabolites in order to inhibit

bacterial protein synthesis, are only now coming to light, facilitated by advances in crystallography, NMR spectroscopy and our understanding of *Streptomyces* transcriptional mechanisms and the dynamic function of the bacterial ribosome. Although further research may reveal that many of these processes are interrelated, at present most can be attributed to certain regions or motifs in metabolite structure particular to certain families or groups of families related by functional group commonality. For this reason, classifying thiopeptide antibiotics according to structure, in particular in the nature of the central heterocyclic domain, also categorises their biological properties and is useful for highlighting structural relationships between the 29 different antibiotic families identified to date. Examining the structure of individual thiopeptides reveals that there are essentially five distinct classes of these natural products, assigned according to the oxidation state of the central heterocyclic domain. Each class can be further subdivided into families that group cyclic peptides with a high degree of structural homology or in some cases that were isolated from the same antibiotic producing organism (**Table 1-1**). It was Hensens who first suggested grouping thiopeptide antibiotics according to the structure and oxidation state of the central heterocyclic domain, in order to distinguish between different constituents of the thiopeptins (**5**).[5] We have extended Hensens' classification system to describe the five distinct heterocyclic domains. Thus, the parent of the thiopeptide antibiotics, thiostrepton (**1**), as well as the siomycins (**4**), are classified as '*b*' series thiopeptides, whereas some of the thiopeptin factors (**5**) and Sch 18640 (**6**) possess the fully reduced '*a*' series central domain, tetrasubstituted dehydropiperidine or saturated piperidine heterocycles, respectively. Clearly related to the series *a* and *b* thiopeptides, the '*c*' series which to date only consists of a single antibiotic, Sch 40832 (**7**), has an unusual imidazopiperidine core of unique structure. With increasing unsaturation, in line with Hensens' classification, by far the most prolific thiopeptide class is the series *d* antibiotics, possessing a 2,3,6-trisubstituted pyridine domain, which is shared by 19 different families including the first thiopeptide to be isolated and identified, micrococcin. Finally series *e* thiopeptides, such as nosiheptide (**41**), exhibit a structurally related central motif, oxidised in comparison with their series *d* counterparts, containing a tetrasubstituted hydroxypyridine.

Table 1-1 Thiopeptide antibiotics in series *a* to *e* according to their central heterocyclic domain

Series *a* and *b*	Series *c*	Series *d*	Series *e*
Bryamycin (A-8506)†	Sch 40832	A10255	Glycothiohexide α
Sch 18640 (68-1147)		Amythiamicin	MJ347-81F4
Siomycin		Berninamycin	Multhiomycin‡
Thiactin†		Cyclothiazomycin	Nocathiacin
Thiopeptin		GE2270	Nosiheptide
Thiostrepton		GE37468	S-54832
		Geninthiocin	
		Methylsulfomycin	
		Micrococcin	
		Promoinducin	
		Promothiocin	
		QN3323	
		Radamycin	
		Sulfomycin	
		Thioactin	
		Thiocillin	
		Thiotipin	
		Thioxamycin	
		YM-266183&4	

† Shown to be identical to thiostrepton; ‡ Shown to be identical to nosiheptide

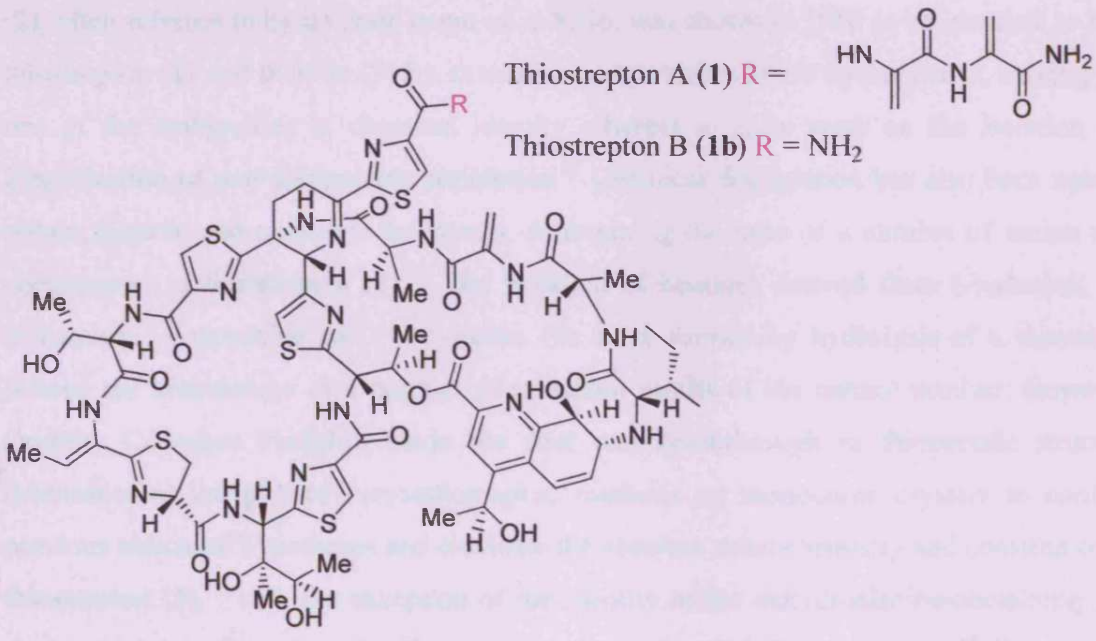
1.3 Isolation and structure elucidation

1.3.1 Piperidines and Dehydropiperidines

All of the series *a* and *b* thiopeptide antibiotics, with a high degree of structural homology, can be identified by their piperidine or dehydropiperidine central heterocyclic domain and bis-macrocyclic peptide backbone, containing a quinaldic acid, thiazoline, dehydroalanine

and dehydrodemethylvaline residues as well as a number of thiazole heterocycles. There are four families and fifteen structurally distinct entities within these two series, although the structural differences are only very minor. Due to their complex nature, extensive chemical degradation has been used to determine structure as well as multiple NMR spectroscopic techniques and in some cases X-ray crystallographic data.

Figure 1-1 Structure of thiostrepton A (1) and B (1b)



1

1.3.1.1 Thiostrepton

Thiostrepton (**1**) ($C_{72}H_{85}N_{19}O_{18}S_5$), sometimes called thiostrepton A or A_1, is often referred to as the parent compound of the thiopeptide antibiotics (**Figure 1-1**). First isolated from *Streptomyces azureus* in 1954[6-8] this secondary metabolite was found to be effective against Gram-positive bacteria with activity comparable to that of the penicillins.[9] However, in spite of a very promising biological profile, thiostrepton (**1**) has not been developed for clinical use as resistance by the bacterium develops before a therapeutic dose can be reached,

primarily as a consequence of its low aqueous solubility, a problem inherent with most of the thiopeptide antibiotics.

Early developments in the identification of new thiopeptide antibiotics paint a very confusing picture, exemplified by thiostrepton's story. Following isolation of this natural product in 1954, a new thiopeptide metabolite called bryamycin (**2**) was isolated in 1955 from *Streptomyces hawaiiensis*, a strain of soil bacteria discovered in Hawaii.[10] Bryamycin (**2**), often referred to by its trade name of A-8506, was shown in 1963 to be identical to both thiostrepton (**1**) and thiactin (**3**) by extensive comparison of their hydrolysates, solving just one of the ambiguities in chemical identity inherent in early work on the isolation and identification of new thiopeptide antibiotics.[11] Chemical degradation has also been used to obtain thiazole and quinoline fragments, determining the ratio of a number of amino acid components of thiostrepton (**1**).[12] The isolation of residues derived from (-)-alanine, (-)-isoleucine, (-)-threonine and (+)-cysteine, the latter formed by hydrolysis of a thiazoline, infered the architecture of a number of structural motifs of the natural product. However, Dorothy Crowfoot Hodgkin made the first real breakthrough in thiopeptide structure determination using X-ray crystallographic methods on monoclinic crystals to confirm previous structural hypotheses and elucidate the absolute stereochemistry and constitution of thiostrepton (**1**),[13] with the exception of the identity of the dehydroalanine-containing side chain which was first solved by Tori *et al.* on the basis of NMR experiments.[14] Thiostrepton (**1**), later also isolated from *Streptomyces laurentii*,[15] has been subjected to detailed ^{1}H and ^{13}C NMR spectroscopic analyses by Hensens and Albers-Schönberg,[16] with reinvestigation in 1989, by Floss *et al.*, who used 2D NMR spectroscopic techniques on both unlabelled and biosynthetically multiple ^{13}C-labelled samples to confirm the 1970 structural assignment.[17] Furthermore, a recent investigation by Hunter *et al.* solved the structure of a tetragonal crystal form of thiostrepton (**1**), using the anomalous dispersive signal from sulfur collected at the Cu *Kα* wavelength, and placed the coordinates in the public domain.[18] The closely related thiopeptide, known as thiostrepton B (or A_2) (**1b**) ($C_{66}H_{79}N_{17}O_{16}S_5$), isolated from *Streptomyces azureus* as a minor component with thiostrepton (**1**), has received much less

attention. Analysis by ^{13}C NMR spectroscopic methods elucidated the structure, which indicates that thiostrepton B (**1b**) might be an artifact from thiostrepton (**1**) (**Figure 1-1**).[19]

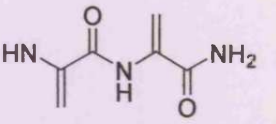
1.3.1.2 Other series *a* and *b* thiopeptides

Isolation and structure elucidation information of the rest families in series *a* and *b* of thiopeptide antibiotics, including the siomycins (**4**), the thiopeptins (**5**) and Sch 18640 (**6**), is summarized in **Table 1-2.**

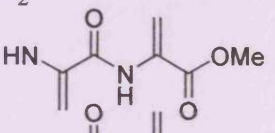
Table 1-2 Isolation and structure elucidation information of the thiopeptide antibiotics 4, 5 and 6

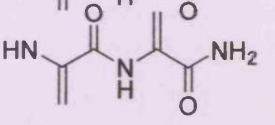
Thiopeptide antibiotics Series *a* and *b*	Structure	Producer	Main techniques of structure elucidation
The siomycins (**4**)	**Figure 1-2**	*Streptomyces sioyaensis*[20]	Chemical degradation[21] ^{1}H, ^{13}C NMR[14,19,22]
The thiopeptins (**5**)	**Figure 1-3**	*Streptomyces tateyamensis*[23]	Chemical degradation[25,26] ^{1}H, ^{13}C NMR[5,19,24]
Sch 18640 (68-1147) (**6**)	**Figure 1-4**	*Micromonospora arborensis*[27]	Chemical degradation ^{1}H, ^{13}C NMR, Plasma desorption mass, amino acid analysis[27]

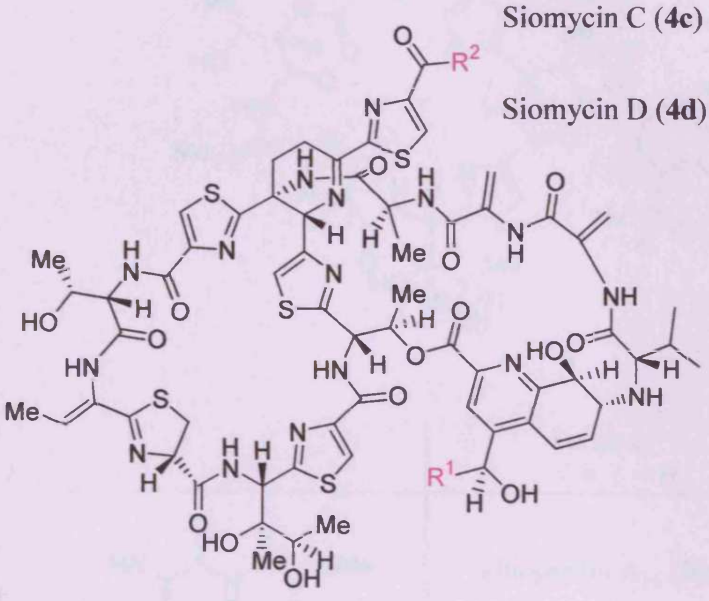
Figure 1-2 Structure of the siomycins (4)

Siomycin A (**4a**) R^1 = Me, R^2 = 

Siomycin B (**4b**) R^1 = Me, R^2 = NH_2

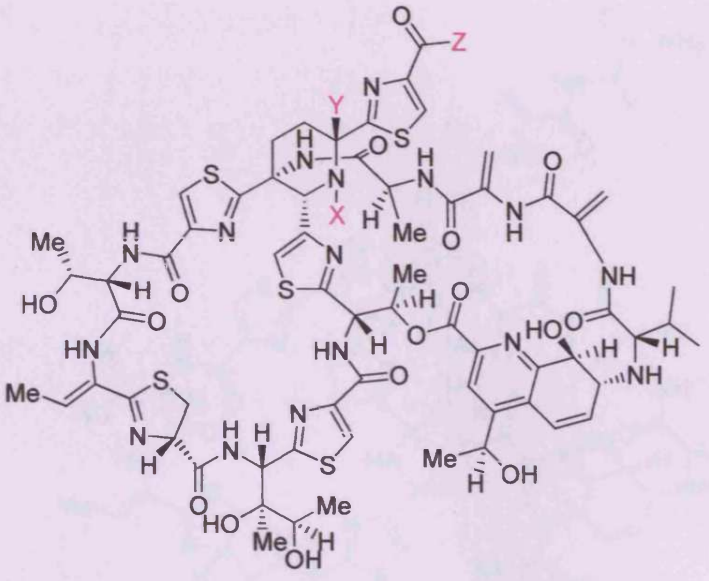
Siomycin C (**4c**) R^1 = Me, R^2 = 

Siomycin D (**4d**) R^1 = H, R^2 = 



4

Figure 1-3 Structure of the thiopeptins (5)



5

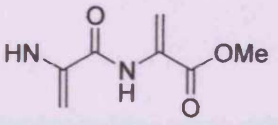
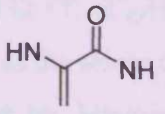
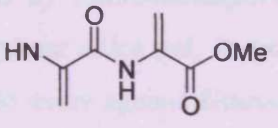
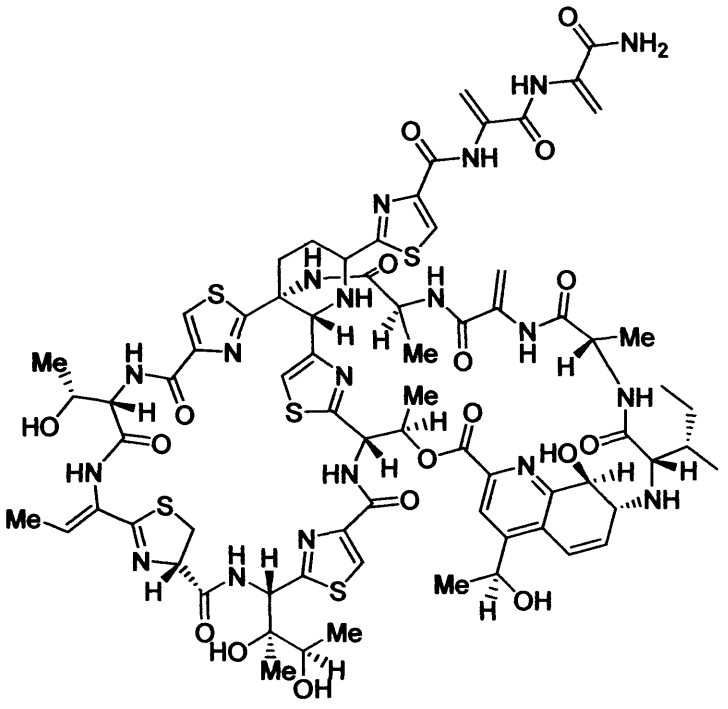
Z	Series *a*, X = Y = H	Series *b*, X,Y = π bond
	Thiopeptin A_{1a} (**5a**)	Thiopeptin A_{1b} (**5b**)
NH_2	Thiopeptin A_{3a} (**5c**)	Thiopeptin A_{3b} (**5d**)
	Thiopeptin A_{4a} (**5e**)	Thiopeptin A_{4b} (**5f**)
	Thiopeptin B_a (**5g**)	Thiopeptin B_b (**5h**)

Figure 1-4 Structure of Sch 18640 (6)



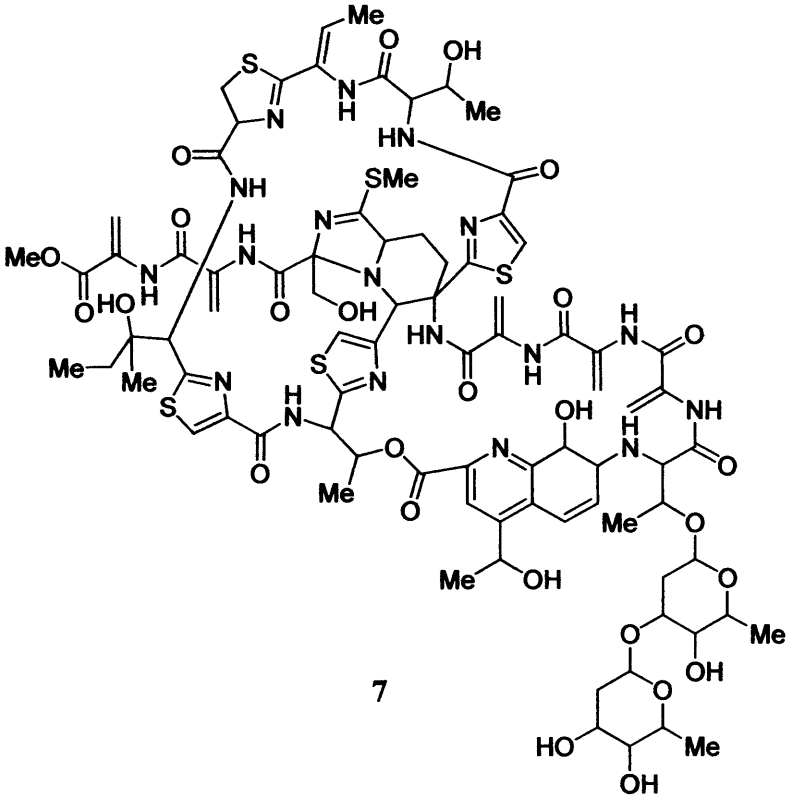
6

1.3.2 Dihydroimidazopiperidines

Sch 40832 (7) ($C_{84}H_{104}N_{18}O_{26}S_5$) is the only example of a series *c* thiopeptide and was isolated as the minor component from the antibiotic complex, referred to as 13-384 complex, produced by *Micromonosporia carbonecea* var. *africana* (ATCC 39149).[28] Purified on reverse phase silica gel, its potent *in vitro* activity was determined using a disc-diffusion agar plate assay against Gram-positive bacteria. Analysis by IR spectroscopy confirmed the presence of NH, OH and amide functional groups, amino acid analysis provided one mole of cysteine, four moles of threonine and one mole of lysine, FAB mass spectrometry determined the molecular weight and molecular composition and NMR spectroscopic experiments, using a combination of COSY, HMBC and ^{13}C techniques, elucidated the connectivity of Sch 40832 (7) (**Figure 1-5**) and established its distinctiveness from both thiostrepton (**1**) and Sch 18640 (**6**). This thiopeptide possesses a unique and unusual structure, with a central domain consisting of a fully saturated piperidine heterocycle fused

to an imidazoline ring derived from a modified thiazoline. In addition to the dihydroimidazo[1,5-*a*]piperidine, Sch 40832 (7) contains a disaccharide moiety attached to a threonine side chain, delineated in a Hartman-Hahn (HOHAHA) experiment and tentatively assigned as β-D chromose A and B, as well as a deoxythiostreptine residue in the peptide backbone.

Figure 1-5 Structure of Sch 40832 (7)



7

1.3.3 Trisubstituted pyridines

Series *d* thiopeptides differ strikingly from the piperidine or dehydropiperidine series *a* and *b* natural products. Predominantly they contain only one macrocyclic peptide loop, centred around a 2,3,6-trisubstituted pyridine heteroaromatic domain clustered with thiazole and/or oxazole heterocycles with a peptide side chain consisting of heterocyclic or dehydrated

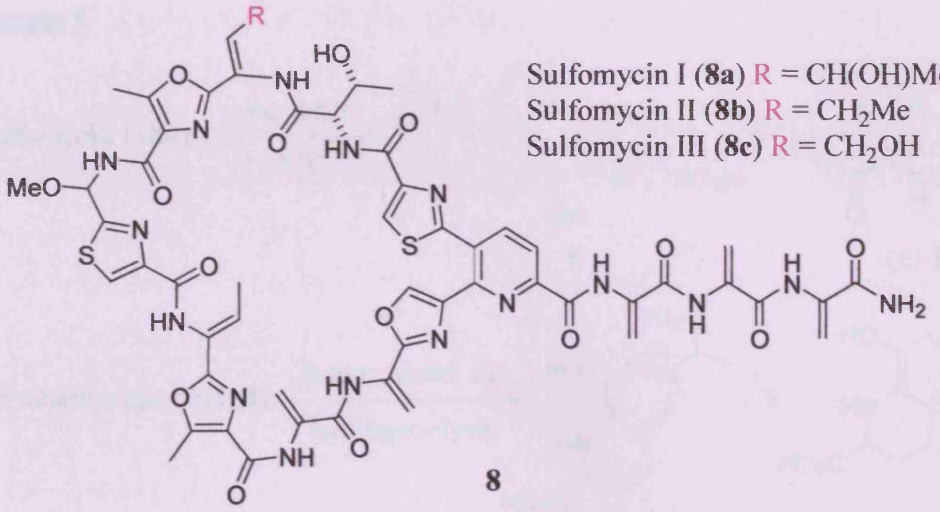
amino acid residues attached at the pyridine 6-position. Numerically series *d* thiopeptides are the dominant class of thiopeptide antibiotics, with 19 families and over 49 distinct entities, which show diversity in structure as well as biological activity.

The sulfomycins (**8**) and cyclothiazomycin (**18**), the synthetic targets of this research project, along with the micrococcins (**21**), which is recorded as the first example of a thiopeptide antibiotic and also known for the dramatic campaign to determine the structure and stereochemistry, will be given emphasis here, whereas the remainders will be outlined briefly in **Table 1-3**.

1.3.3.1 The sulfomycins

The sulfomycins (**8**), first isolated in 1969, comprise a family of three cyclic peptides, consisting of sulfomycin I (**8a**) ($C_{54}H_{52}N_{16}O_{16}S_2$), II (**8b**) ($C_{54}H_{52}N_{16}O_{15}S_2$) and III (**8c**) ($C_{53}H_{50}N_{16}O_{16}S_2$) (**Figure 1-6**). Sulfomycin I (**8a**) was obtained from *Streptomyces viridochromogenes* subsp. *sulfomycini* ATCC 29776 and exhibits strong inhibitory activity against Gram-positive bacteria,[29] whereas all three have been isolated from *Streptomyces viridochromogenes* MCRL-0368. The structure of sulfomycin I (**8a**) was determined using a combination of ^{1}H and ^{13}C NMR spectroscopic techniques, FAB mass spectrometric analysis[30] and chemical evidence.

Figure 1-6 Structure of the sulfomycins (8)

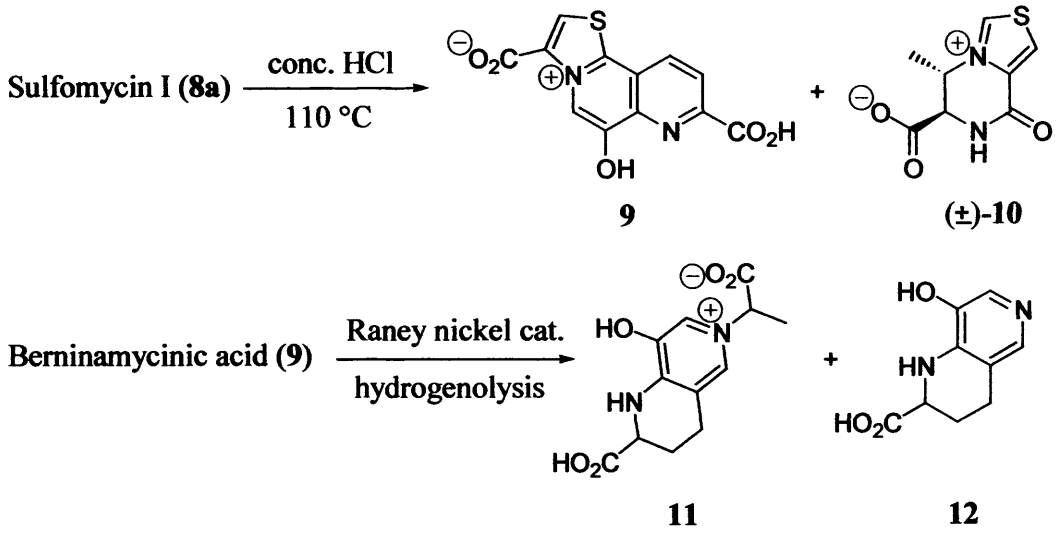


Sulfomycin I (**8a**) R = CH(OH)Me
Sulfomycin II (**8b**) R = CH_2Me
Sulfomycin III (**8c**) R = CH_2OH

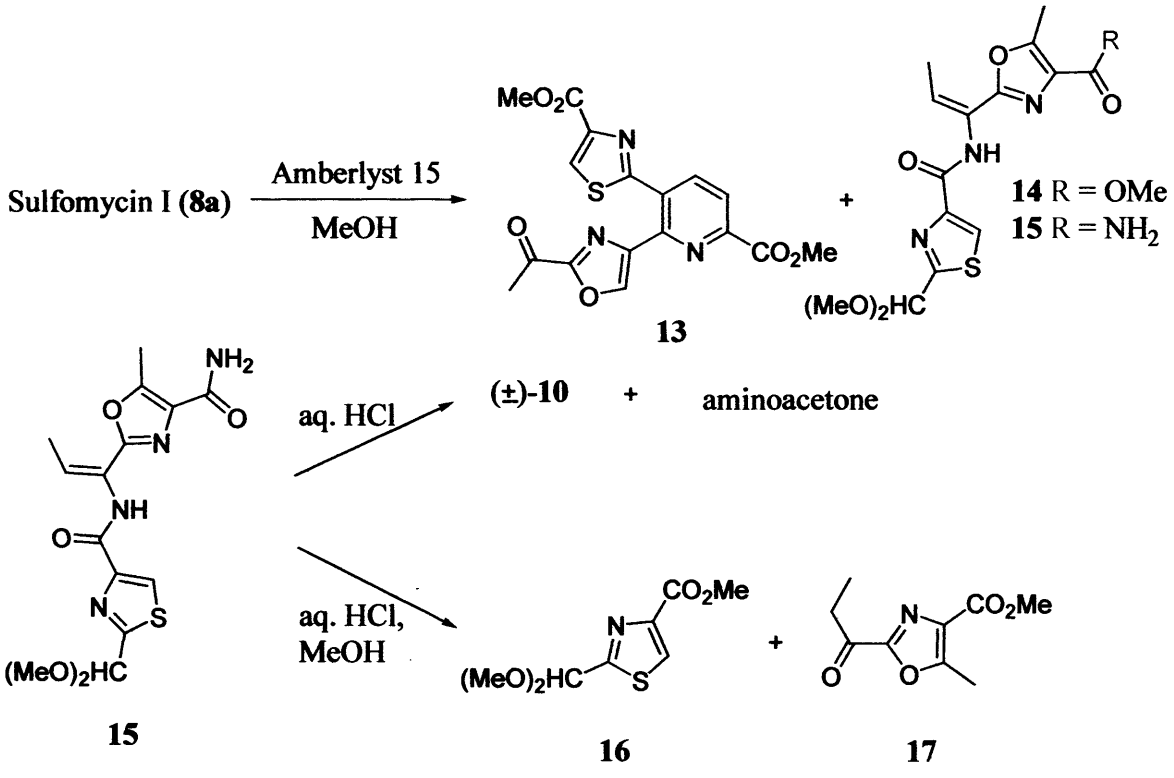
8

Chemical degradation of sulfomycin I (**8a**) was extensively studied by Abe *et al.* and provided a number of different fragments, which gave support to the assignment of the total structure of the sulfomycins (**8**). Acidic hydrolysis of **8a** was carried out in concentrated HCl in a sealed tube and maintained at 110 °C for 6 hours.[31] A known compound berninamycinic acid (**9**)[32] and a new degradation product named sulfomycinine (**10**)[33] were isolated both as their hydrochlorides. Pyruvic acid, threonine and aminoacetone were obtained from the filtrate of this reaction. Hydrogenolysis of **9** with Raney nickel catalyst in alkaline aqueous solution gave two novel products, **11** and **12** (**Scheme 1-1**). Acidic methanolysis of sulfomycin I (**8a**) provided a number of different fragments including dimethyl sulfomycinamate (**13**),[34] the structure of which has been confirmed by X-ray crystallographic studies and chemical synthesis.[35,36] The other two major components from acidic methanolysis of **8a** were methyl sulfomycinate (**14**) and sulfomycinic amide (**15**), which were assigned to be the methyl ester and amide of the same carboxylic acid, respectively. It was suggested that **15** was the initial degradation product but was converted to **14** during the course of prolonged reaction. Sulfomycinic amide (**15**) was submitted to acidic hydrolysis and methanolysis to give sulfomycinine (**10**) and aminoacetone or compounds **16** and **17**, respectively (**Scheme 1-2**).

Scheme 1-1 Acidic hydrolysis of sulfomycin I (8a) and further degradation of hydrolysate 9



Scheme 1-2 Acidic methanolysis of sulfomycin I (8a) and further degradation of hydrolysate 15

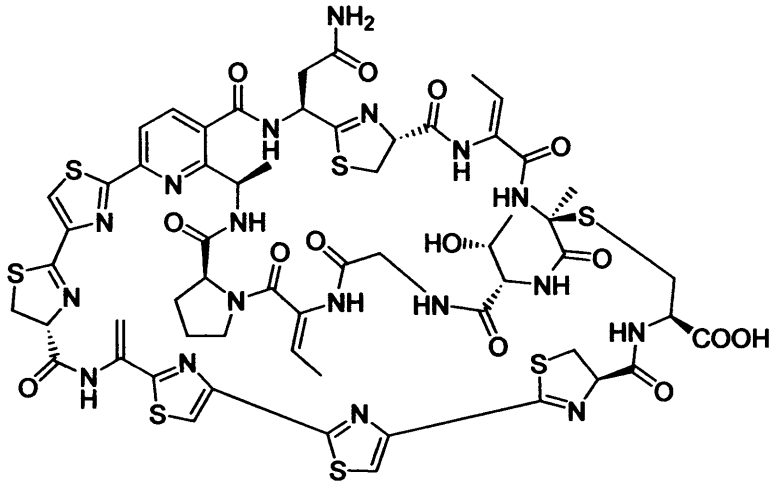


Sulfomycin II (**8b**) and III (**8c**), isolated from subspecies MCRL-0368, have closely related structures that vary only in the nature of the side chain (R) located on a 2-(2-aminoalkenyl)oxazole residue in the peptide backbone.[37] These cyclic peptides share a common oxazole–thiazole–pyridine type *d* central heterocyclic domain and, as well as a prevalence of dehydroamino acids, contain an unusual alkoxythiazolylmethyl amide unit characteristic of this thiopeptide family. All of the sulfomycins (**8**) strongly inhibit the growth of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, but are not active against Gram-negative bacteria.

1.3.3.2 Cyclothiazomycin

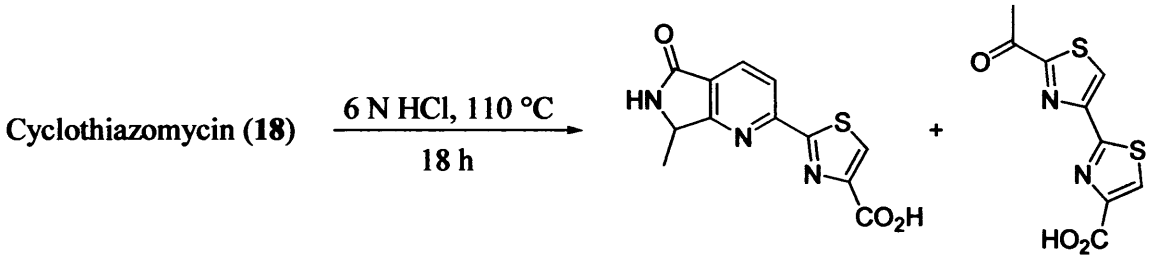
Cyclothiazomycin (**18**) ($C_{59}H_{64}N_{18}O_{14}S_7$) is an unusual series *d* thiazolylpeptide that possesses a number of unique structural features. Isolated from the fermentation broth of *Streptomyces* sp. NR0516 from a soil sample collected at Kanagawa, Japan, and purified first by column chromatography and then by preparative HPLC,[38] initial structure determination, using high-resolution FAB mass spectrometry, elemental analysis and ^{13}C and ^{1}H NMR spectroscopic data, was supported by chemical degradation studies, acidic hydrolysis in 6 N hydrochloric acid generating an unusual pyridine-containing γ-amino acid as lactam **19**, the identity of which has been verified by synthesis,[39] and saramycetic acid I (**20**) (**Scheme 1-3**).[40] NOESY experimental data elucidated both the structure and stereochemistry of cyclothiazomycin (**18**), the latter supported by amino acid analyses, and showed this unique series *d* thiazolylpeptide lacked the characteristic 2- and 3-azole substituents on the central domain, containing instead an alanine-derived heterocyclic residue of (*R*)-configuration, quaternary sulfide and two macrocyclic peptide loops (**Figure 1-6**).[41] Although no antibacterial data has been associated with cyclothiazomycin (**18**), this thiopeptide is still very worthy of note as a novel and selective inhibitor of human plasma renin, with an IC_{50} of 1.7 μM.[105]

Figure 1-6 Structure of cyclothiazomycin (18)



18

Scheme 1-3 Acidic hydrolysis of cyclothiazomycin (18)



19 **20**

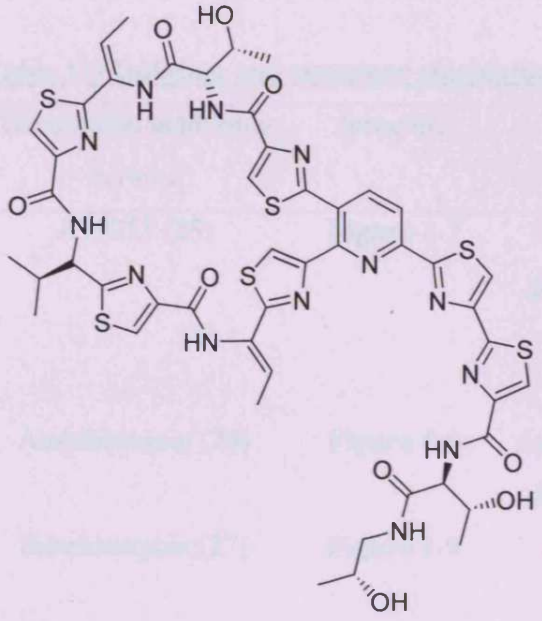
1.3.3.3 The micrococcins

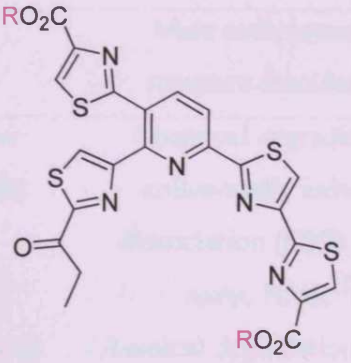
First discovered in 1948 from a strain of *Micrococcus* found in sewage from the city of Oxford and reported in "The British Journal of Experimental Pathology",[42] the isolate[43] that was to be named micrococcin (**21**)[44] is recorded as the first example of a thiopeptide antibiotic, although no work to elucidate the chemical structure of that material was ever reported. An antibiotic with therapeutic activity later obtained from the *B. pumilus* group of spore-bearing bacillus, isolated from soil collected in East Africa,[45] demonstrated a considerable degree of identity or near-identity with the antibiotic isolated from

Micrococcus, perhaps suggesting two members of a closely related family, on the basis of their similar properties and behaviour, and so the new complex was named micrococcin P (**21**), in spite of the taxonomic implications. This antibiotic actually consists of two distinct components, present in the complex in *ca.* a 7:1 ratio and designated micrococcin P_1 and micrococcin P_2, respectively (alternatively written as micrococcin P1 and P2). Gratifyingly, with difficulties experienced in obtaining the complex from these original sources, micrococcin P_1 has recently been obtained from foodborne *Staphylococcus equorum* WS2733 isolated from French Raclette cheese,[46] demonstrating how thiopeptide antibiotics can often be discovered from seemingly unrelated sources. Work that spanned the 50 year period following initial isolation to elucidate the structure of micrococcin P_1 tells a remarkable story, amazingly still unresolved, that, in spite of considerable spectroscopic advances and synthetic achievements, bears witness to a number of oversights and the propagation of unsubstantiated hypotheses, accepted erroneously as the truth. With a lack of structural data, the major isolated component, micrococcin P_1 (also referred to as MP1), was characterised initially purely by examination of its acid hydrolysates which enabled a provisional molecular weight estimate to be made.[47] Analysis of the acid-soluble fraction gave a laevorotatory hydrochloride that was identified conclusively as L-threonine•HCl by IR spectroscopy, thus confirming the identity and stereochemistry of one of the residues of the natural product. Further analysis of the acid-insoluble fraction established that micrococcin P_1 must contain an extended chromophoric system,[48] by isolating two derivatives of the central heterocyclic domain identified as micrococcinic acid (**22**) and methyl micrococcinate (**23**), and separated an aminoalcohol from the acid-soluble hydrolysate of the peptide side chain. Although this residue was assigned originally as alaninol (2-aminopropan-1-ol),[49] it was later identified as D-(*R*)-isoalaninol (2-hydroxypropylamine) from ^{13}C NMR spectroscopic data of the natural product.[50] This study culminated in the Walker-Lukacs structure **24** of micrococcin P_1 which, although a considerable advance, had assembled the order of the individual residues without evidence but rather based upon an assumed structural homology with two other thiopeptides, thiostrepton (**1**) and nosiheptide (**41**). Prompted by the publication of the Walker-Lukacs structure, Bycroft and Gowland separated micrococcin P_1 ($C_{48}H_{49}N_{13}O_9S_6$) and micrococcin P_2 ($C_{48}H_{47}N_{13}O_9S_6$) and carried out their own NMR spectroscopic studies and analyses of

the acid hydrolysates of both micrococcin P_1 and its sodium borohydride derivative.[51] In contrast to Walker's findings, only one mole of threonine was produced in each of these hydrolysis experiments, leading to the proposal of the alternative Bycroft-Gowland structure for both micrococcin P_1 (**21a**) and P_2 (**21b**), that accounted for their hydrolytic behaviour (**Figure 1-7**). The proposed structure was accepted for over twenty years but was finally shown to be erroneous when, in 1999, Ciufolini completed his landmark total synthesis of MP1 (**21a**) and demonstrated that this architecture did not correspond to that of the natural product.[52] This confusing situation has been compounded further by Shin's synthesis of two epimeric substances of the Walker-Lukacs and Bycroft-Gowland structures, described as micrococcin P[53] and micrococcin P_1 (**21c**),[54] respectively, containing in each case an (*S*)-isoalaninol residue in the peptide side chain in place of (*R*)-isoalaninol, established unequivocally by a combination of degradation studies[49] and NMR spectroscopy.[50] Ciufolini later drew together all of these findings and validated the 1978 Bycroft-Gowland hypothesis with extensive NMR studies, confirming that the order of residues in micrococcin P_1 was in accord with their original proposal.[55] It would appear, on the basis of these results, that the difference between synthetic MP1 (**21a**) and the natural material, as examined by Bycroft and Gowland in 1978, is purely stereochemical and therefore must have its origin in either the configuration of the L-threonine-derived thiazole that forms part of the central heterocyclic domain or in the (*R*)-valine-derived thiazole in the peptide macrocycle, both of which were proposed in the absence of reliable experimental evidence. The stereochemical assignment of the latter was derived originally from chemical degradation studies, but the hydrochloride salt of the key hydrolysate, isolated from the natural product, was identified as both (+)- and (-)-2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid in separate studies,[47] with a specific rotation that varied for the latter between 0 and –20.8° leading to a conclusion that the stereochemistry of the valine-derived residue 'is *probably* of the D-configuration'.[56] Considering these instrumental limitations and Ciufolini's findings, it would appear that the most probable structure for MP1 (**21d**) and MP2 (**21e**) is at variance in the stereochemical assignment of this unit, but it is hoped this long standing mystery will soon be solved by chemical synthesis.

Figure 1-6 Proposed structures for the micrococcins (21) and their chemical derivatives

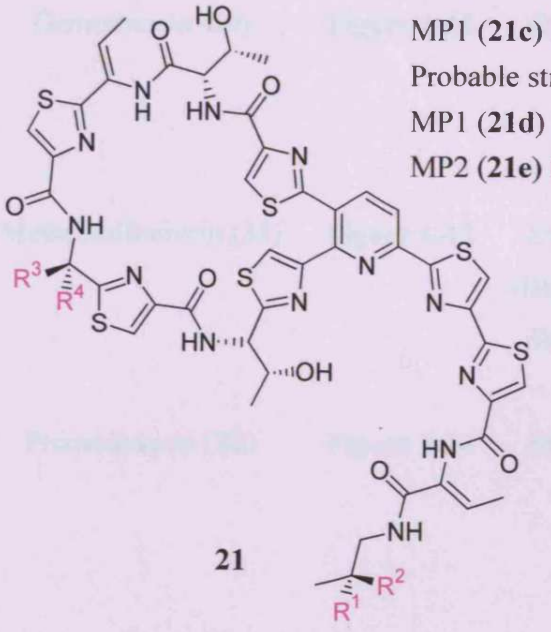




R = H, micrococcinic acid (**22**)
R = Me, methyl micrococcinate (**23**)

Walker-Lukacs structure of MP1 (**24**)

Bycroft-Gowland structure of:
MP1 (**21a**) R^1 = OH, R^2 = H, R^3 = $CHMe_2$, R^4 = H;
MP2 (**21b**) R^1,R^2 = O (π-bond), R^3 = $CHMe_2$, R^4 = H;
Shin's totally synthetic epimer of:
MP1 (**21c**) R^1 = H, R^2 = OH, R^3 = $CHMe_2$, R^4 = H;
Probable structure of:
MP1 (**21d**) R^1 = OH, R^2 = H, R^3 = H, R^4 = $CHMe_2$;
MP2 (**21e**) R^1,R^2 = O (π-bond), R^3 = H, R^4 = $CHMe_2$



21

1.3.3.4 Other series *d* thiopeptides

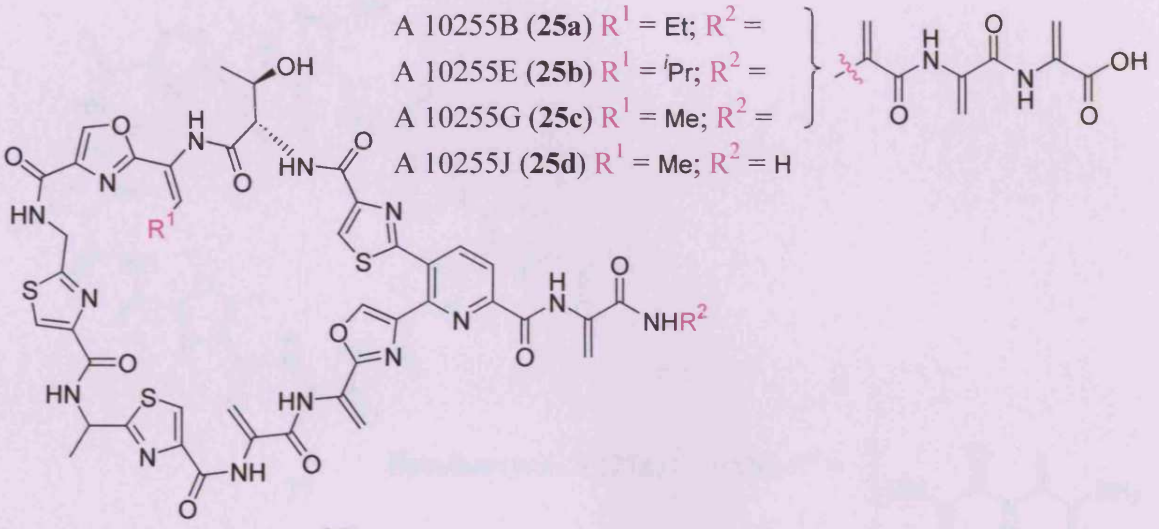
Table 1-3 Isolation and structure elucidation information of the thiopeptide antibiotics

Thiopeptide antibiotics Series *d*	Structure	Producer	Main techniques of structure elucidation
A10255 (**25**)	**Figure 1-7**	*Streptomyces gardneri* NRRL 15537[57]	Chemical degradation, collisionally induced dissociation (CID), FAB mass, NMR[17]
Amythiamicin (**26**)	**Figure 1-8**	*Amycolatopsis* sp. MI481-42F4[58]	Chemical degradation, NMR, FAB mass[59,60]
Berninamycin (**27**)	**Figure 1-9**	*Streptomyces bernensis*[30]	Chemical degradation, NMR,[32,61] X-ray diffraction,[62] chemical synthesis[63]
GE37468 (**28**)	**Figure 1-10**	*Streptomyces* sp. ATCC 55365[64]	Chemical degradation, ^{1}H and ^{13}C NMR, FAB mass[65]
GE2270 (**29**)	**Figure 1-11**	*Planobispora rosea* ATCC 53773[66]	Chemical degradation,[69,70] UV, IR, NMR,[67] mass,[68]
Geninthiocin (**30**)	**Figure 1-12**	*Streptomyces* sp. DD84[71]	UV, IR, COSY, HMQC, HMBC, nOe, ^{1}H and ^{13}C NMR, chiral-TLC, high-resolution FAB mass[71]
Methylsulfomycin (**31**)	**Figure 1-13**	*Streptomyces* sp. HIL Y-9420704[72,73] *Streptomyces* sp. RSP9[74]	Mass, chemical analysis, COSY, nOe, ^{1}H and ^{13}C NMR[73]
Promoinducin (**32**)	**Figure 1-14**	*Streptomyces* sp. SF2741[75]	COSY, NOESY, HSQC, ^{1}H and ^{13}C NMR, ^{13}C-decoupled HMBC, chiral-TLC, high-resolution FAB mass[75]

(**Table 1-3** continued)

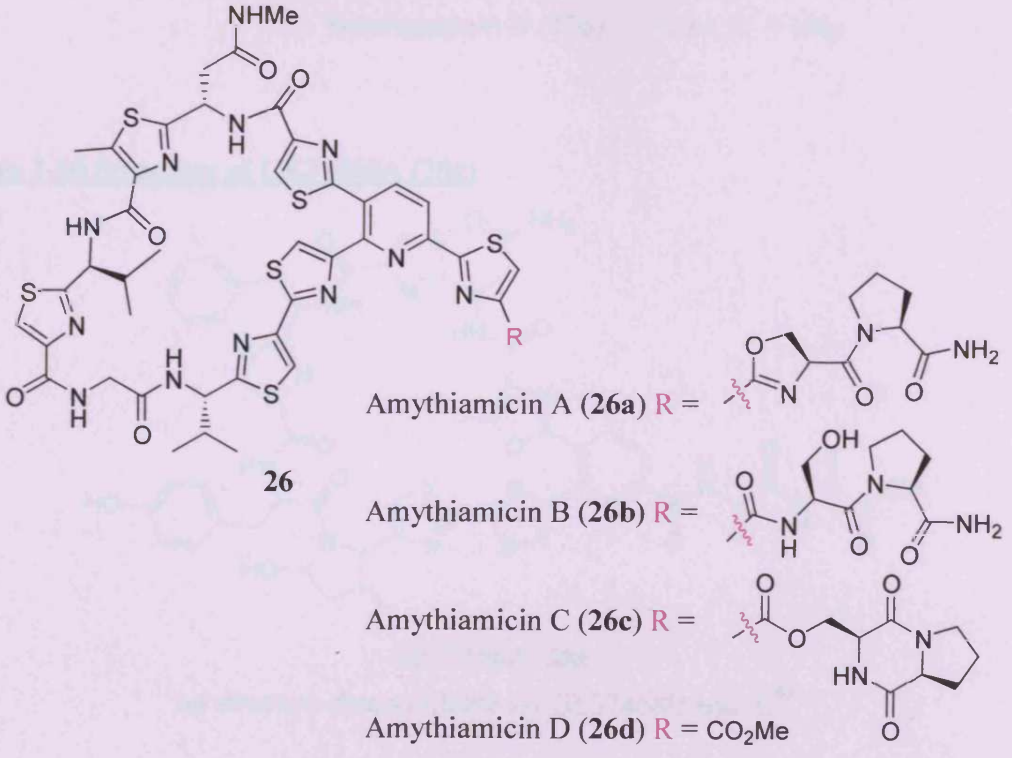
Thiopeptide antibiotics Series *d*	Structure	Producer	Main techniques of structure elucidation
Promothiocin (**33**)	**Figure 1-15**	*Streptomyces* sp. SF2741[76]	IR, high-resolution FAB mass, 2D NMR, amino acid analyses,[76] chemical synthesis[77,78]
QN3323 (**34**)	**Figure 1-16**	*Bacillus* genus[79]	^{1}H and ^{13}C NMR[79]
Radamycin (**35**)	**Figure 1-17**	*Streptomyces* sp. RSP9[74]	IR, high-resolution FAB mass, 1D and 2D NMR[80]
Thioactin (**36**)	**Figure 1-18**	*Streptomyces* sp. DP94[81]	^{1}H and ^{13}C NMR[81]
Thiocillin (**37**)	**Figure 1-19**	*Bacillus cereus* G-15, *Bacillus badius* AR-91[82]	Chemical degradation, ^{1}H and ^{13}C NMR[83]
Thiotipin (**38**)	**Figure 1-14**	*Streptomyces* sp. DT31[84]	high-resolution FAB mass, chiral-TLC, COSY, HMQC, nOe, ^{1}H and ^{13}C NMR[84]
Thioxamycin (**39**)	**Figure 1-18**	*Streptomyces* sp. DP94[81] *Streptomyces* sp. PA-46025[85]	^{1}H and ^{13}C NMR[81]
YM-266183-4 (**40**)	**Figure 1-19**	*B. cereus* QN3323 (from the marine sponge *Halichonadria japonica*)[86]	high-resolution MALDITOF mass, NMR[87]

Figure 1-7 Structure of the A10255 factors (25)



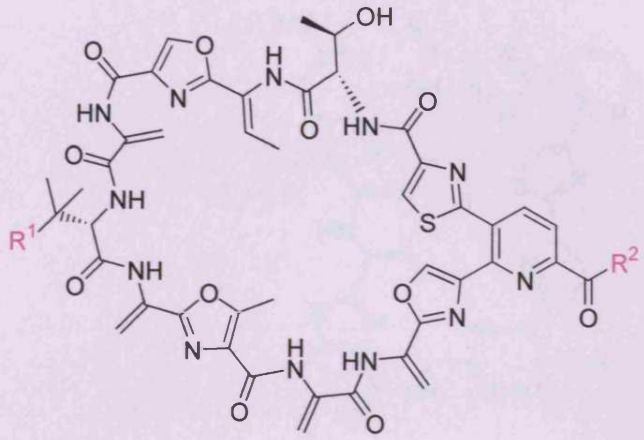
25

Figure 1-8 Structure of amythiamicin A–D (26)



26

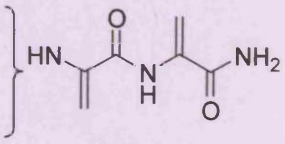
Figure 1-9 Structure of the berninamycins (27)



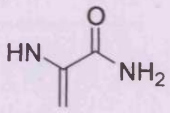
27

Berninamycin A (**27a**) R^1 = OH, R^2 =

Berninamycin B (**27b**) R^1 = H, R^2 =

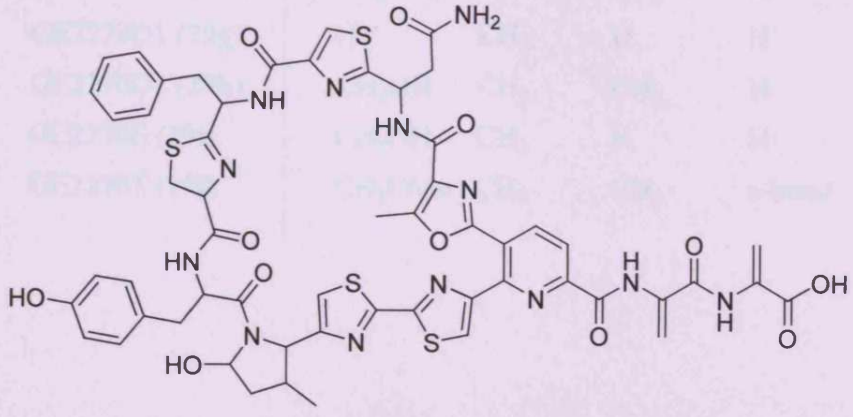


Berninamycin C (**27c**) R^1 = OH, R^2 =



Berninamycin D (**27d**) R^1 = OH, R^2 = NH_2

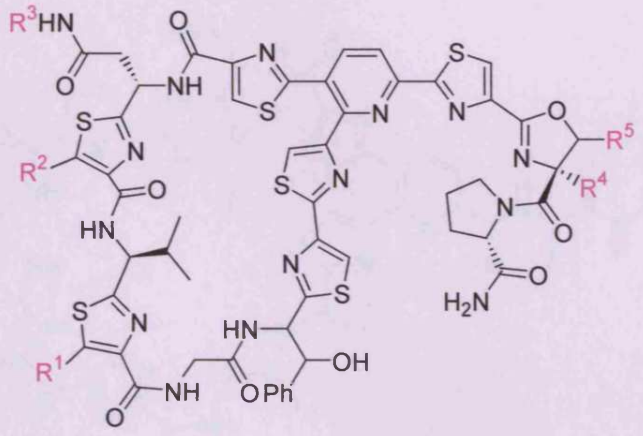
Figure 1-10 Structure of GE37468A (28a)



GE37468A (**28a**)

no structure data available for GE37468B and -C[88]

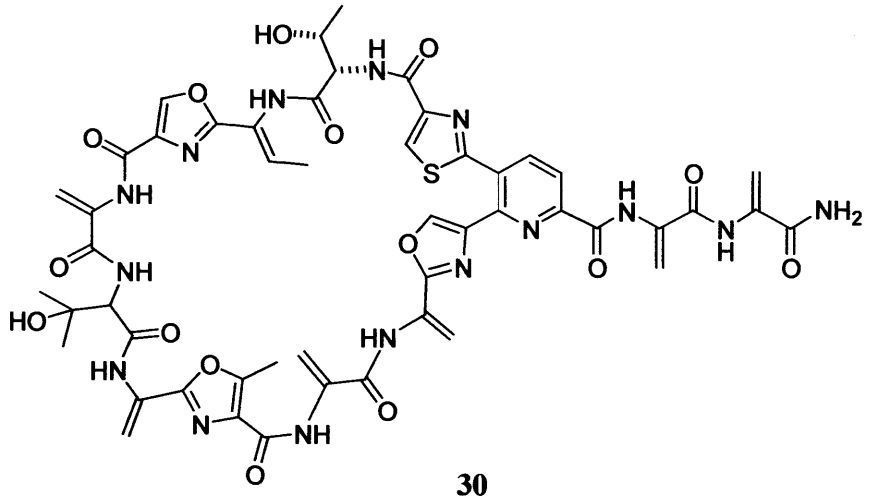
Figure 1-11 Structure of the GE2270 factors (29)



29

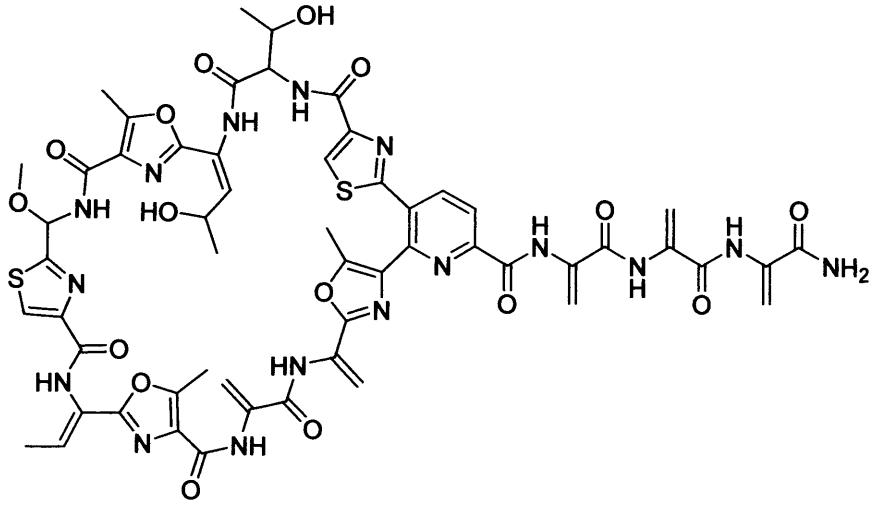
Factor	R^1	R^2	R^3	R^4, R^5
GE2270A (**29a**)	CH_2OMe	CH_3	CH_3	H
GE2270B1 (**29b**)	CH_2OMe	CH_3	H	H
GE2270B2 (**29c**)	CH_3	CH_3	CH_3	H
GE2270C1 (**29d**)	H	CH_3	CH_3	H
GE2270C2a (**29e**)	CH_2OMe	CH_2OH	CH_3	H
GE2270C2b (**29f**)	CH_2OMe	H	CH_3	H
GE2270D1 (**29g**)	H	CH_3	H	H
GE2270D2 (**29h**)	CH_2OH	CH_3	CH_3	H
GE2270E (**29i**)	CH_2OH	CH_3	H	H
GE2270T (**29j**)	CH_2OMe	CH_3	CH_3	π-bond

Figure 1-12 Structure of geninthiocin (30)



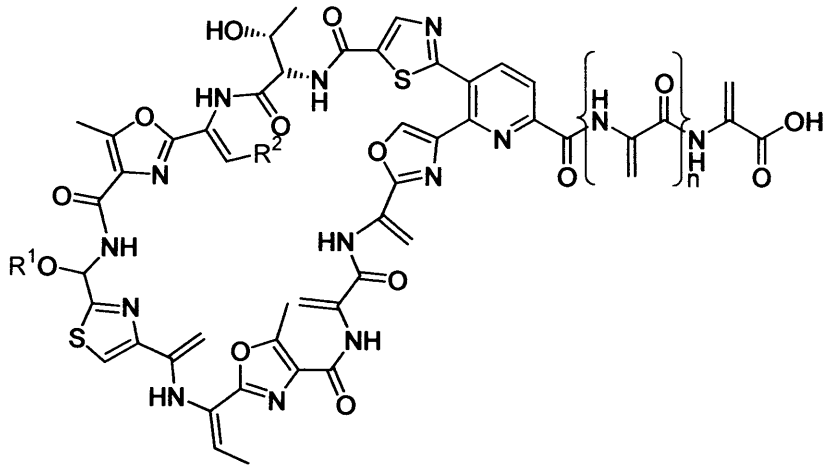
30

Figure 1-13 Structure of methylsulfomycin (31)



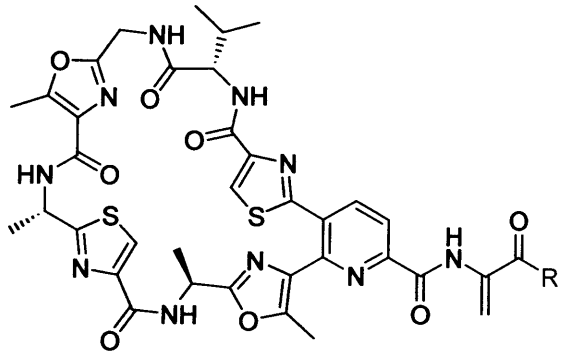
31

Figure 1-14 Structure of promoinducin (32) and thiotipin (38)



Promoinducin (**32**) R^1 = Me, R^2 = CH(OH)Me, n = 3;
Thiotipin (**38**): R^1 = H, R^2 = Me, n = 2

Figure 1-15 Structure of the promothiocins (33)



33

Promothiocin A (**33a**) R = NH_2

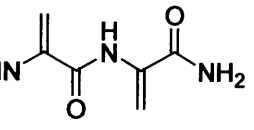
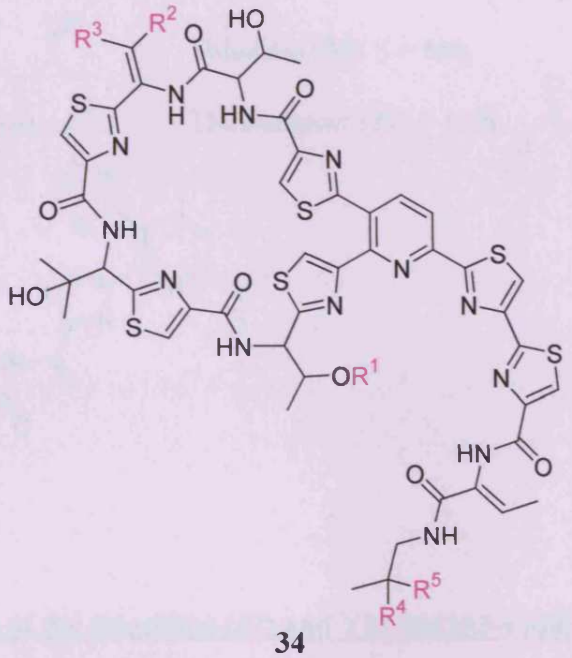
Promothiocin B (**33b**) R = 

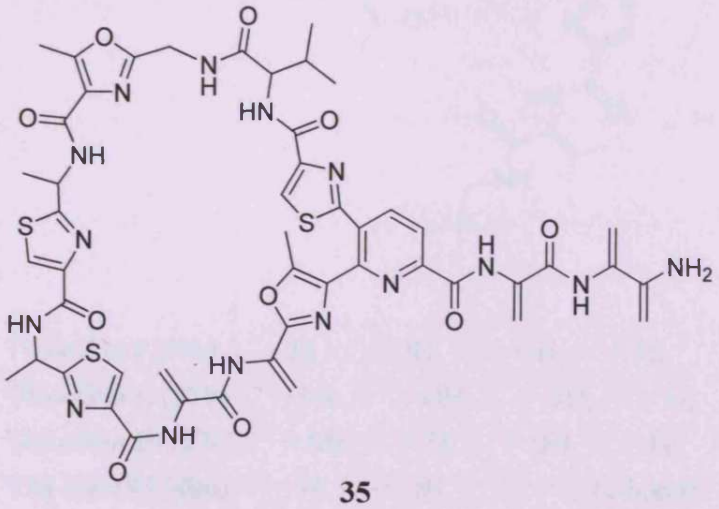
Figure 1-16 Structure of the QN3323 factors (34)



34

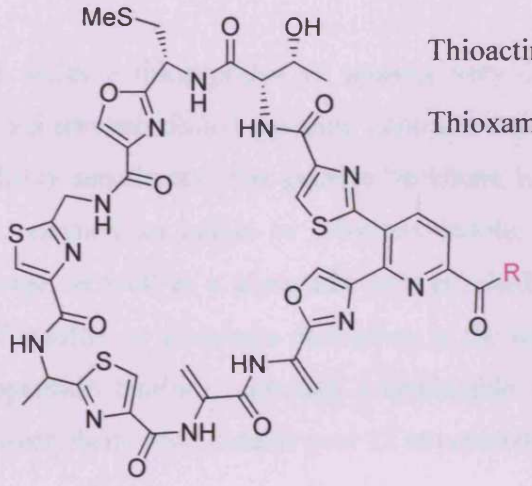
QN3323A (**34a**) R^1 = H, R^2 = Me, R^3 = H, R^4/R^5 = O (π-bond);
QN3323B (**34b**) R^1 = H, R^2 = Me, R^3 = Me, R^4/R^5 = O (π-bond);
QN3323Y[1] (**34c**) R^1 = Me, R^2 = H, R^3 = H, R^4 = OH, R^5 = H

Figure 1-17 Structure of radamycin (35)



35

Figure 1-18 Structure of thioactin (36) and thioxamycin (39)



Thioactin (**36**) R = NH_2

Thioxamycin (**39**) R =

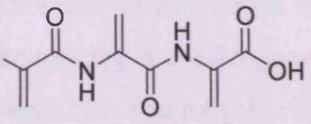
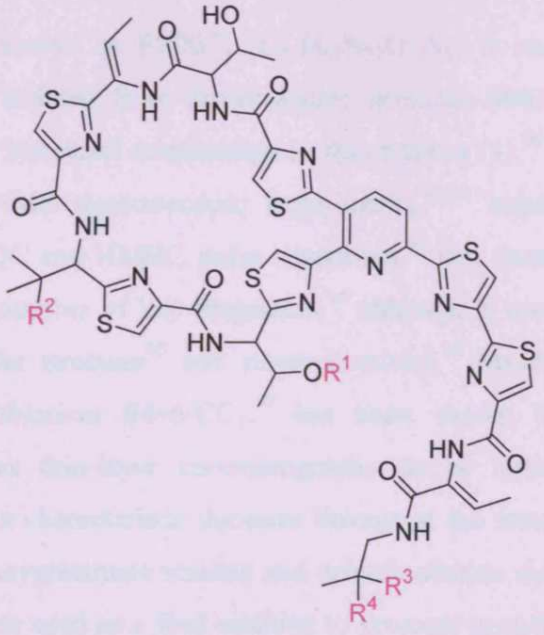


Figure 1-19 Structure of the thiocillins (37) and YM-266183-4 (40)



Thiocillin I (**37a**) R^1 = H, R^2 = OH, R^3 = OH, R^4 = H;

Thiocillin II (**37b**) R^1 = Me, R^2 = OH, R^3 = OH, R^4 = H;

Thiocillin III (**37c**) R^1 = Me, R^2 = H, R^3 = OH, R^4 = H;

YM-266183 (**40a**) R^1 = H, R^2 = OH, R^3,R^4 = O (π-bond);

YM-266184 (**40b**) R^1 = Me, R^2 = OH, R^3,R^4 = O (π-bond)

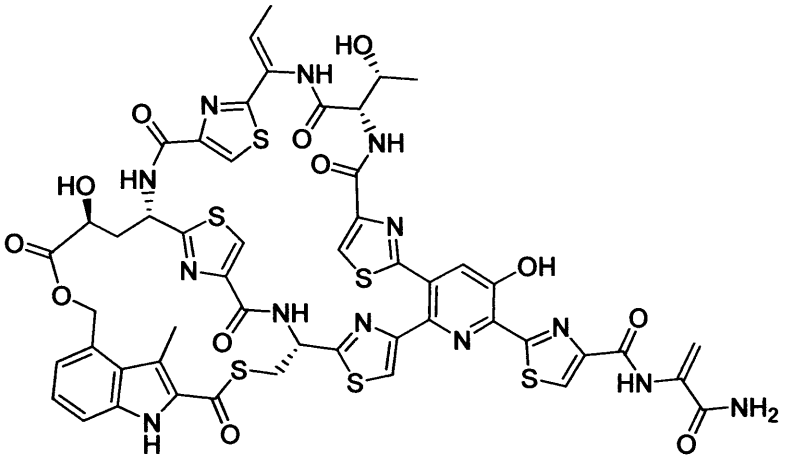
1.3.4 Hydroxypyridines

The series *e* thiopeptides all possess very closely related structures, characterised by a 2,3,5,6-tetrasubstituted pyridine central heterocyclic domain containing a 5-alkoxy or 5-hydroxy substituent. The peptide backbone is divided into at least two macrocyclic loops and contains an indole or 1-hydroxyindole, connected in some cases by an *S*-thioester linkage, as well as a glycosidic unit attached through the anomeric position to a γ-amino acid residue, or glutamate derivative, in the macrocycle. This series consists of at least five thiopeptide families, although considerable structural homology or near identity exists between them, and contains over 12 structurally distinct compounds.

1.3.4.1 Nosiheptide

Nosiheptide (**41**) (also known as RP9671, $C_{51}H_{43}N_{13}O_{12}S_6$) is one of the oldest known thiopeptide antibiotics, isolated from *Streptomyces actuosus* 40037 (NRRL 2954).[89] Its general formula, and its structural relationship to thiostrepton (**1**),[90] was first suggested by combustion analyses, NMR spectroscopic experiments,[91,92] improved subsequently by modification of the HSQC and HMBC pulse sequences,[93] and chemical hydrolysis, which isolated and analysed a number of key fragments,[94] although it was X-ray crystallography that finally elucidated the structure[95] and stereochemistry.[96] Multhiomycin (**42**), isolated from *Streptomyces antibioticus* 8446-CC_1,[97] has been shown by ^{13}C NMR and IR spectroscopy as well as thin-layer chromatography to be structurally identical with nosiheptide (**41**),[98] with a characteristic thioester linkage at the macrocyclic bridgehead, 3-methylindole unit, hydroxyglutamate residue and dehydroalanine side chain (**Figure 1-20**). Nosiheptide (**41**) has been used as a feed additive to promote growth in pigs and poultry,[99] and can be monitored in meat and egg samples by liquid chromatography with fluorescence detection.[100] This antibiotic is very active *in vitro* against Gram-positive bacteria (MIC 0.9 ng/ml against *S. aureus* ATCC 6538 P) but inactive *in vivo* in experimentally infected mice,[89] and selectively inhibits protein synthesis in whole cells of *Bacillus subtilis* and in *Escherichia coli* lamelloplast by binding directly to the ribosomes.[101]

Figure 1-20 Structure of nosiheptide (41)



41

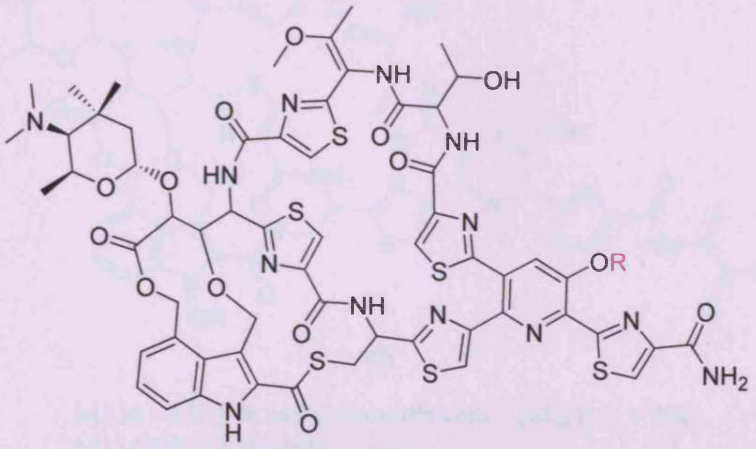
1.3.4.2 Other series *e* thiopeptides

Isolation and structure elucidation of the remaining series *e* thiopeptide antibiotics, including glycothiohexide α (**43**), MJ347-81F4 (**45**), nocathiacin (**46**) and S-54832 (**44**), is outlined in **Table 1-4.**

Table 1-2 Isolation and structure elucidation of thiopeptide antibiotics 43–46

Thiopeptide antibiotics Series *e*	Structure	Producer	Main techniques of structure elucidation
Glycothiohexide α (**43**)	**Figure 1-21**	*Sebekia benihana* (NRRL 21083)[102,103]	2D NMR, IR, high-resolution FAB mass[104]
S-54832 (**44**)	**Figure 1-22**	*Micromonospora globosa*[105]	Amino acid analyses, UV, IR[105]
MJ347-81F4 (**45**)	**Figure 1-23**	*Amycolatopsis* sp. MJ347-81F4[106]	Chemical degradation spectroscopic analyses[106]
Nocathiacin (**46**)	**Figure 1-24**	*Nocardia* ATCC-202099 or *Amicolaptosis* sp.[107]	2D NMR on both ^{13}C- and ^{15}N-labeled and unlabeled samples, metal chelate chiral capillary electrophoresis[107]

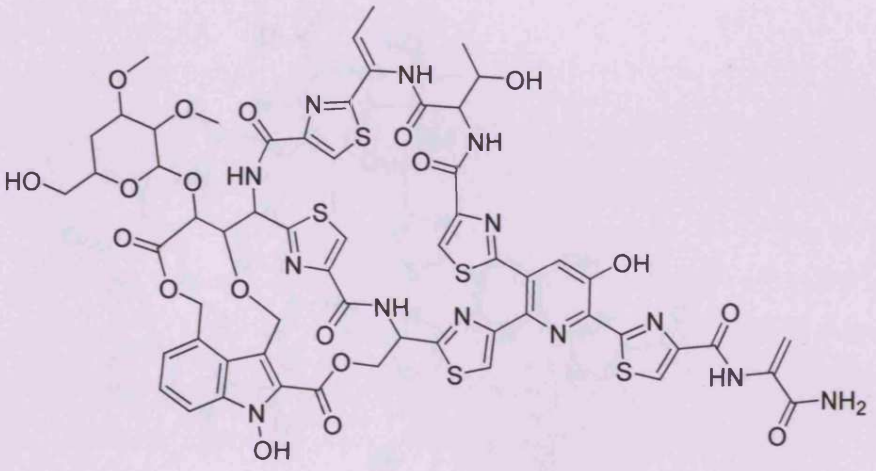
Figure 1-21 Structure of glycothiohexide α (43)



43

Glycothiohexide α (**43a**) R = H;
O-Methyl-glycothiohexide α (**43b**) R = Me

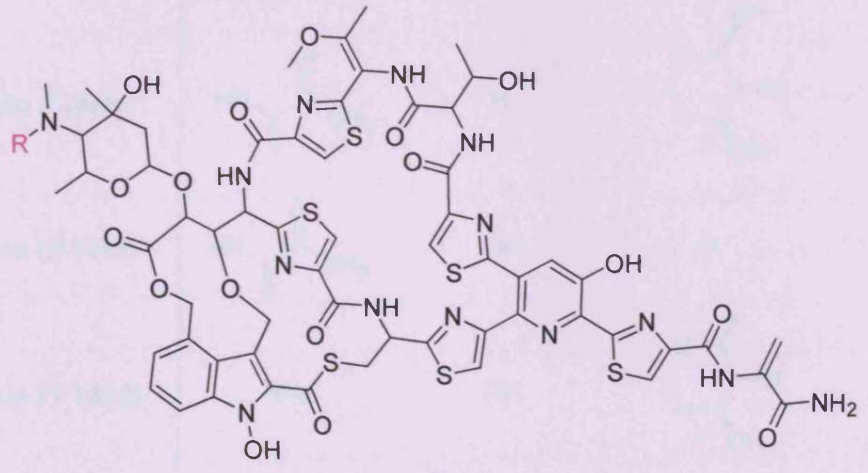
Figure 1-22 Structure of S 54832 A-I



S 54832 A-I (**44a**)

no conclusive structure data for S 54832 A-II and A III

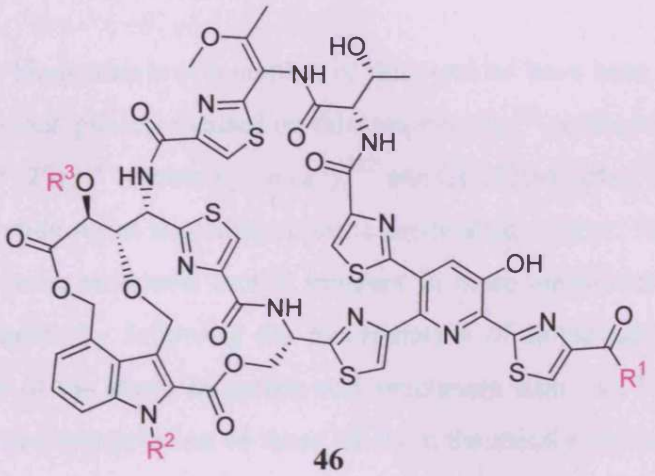
Figure 1-23 Structure of MJ347-81F4 thiopeptides (45)



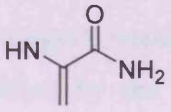
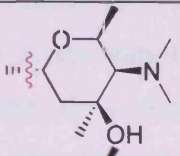
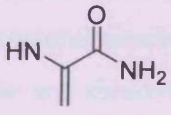
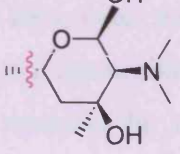
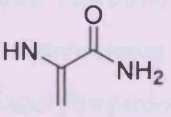
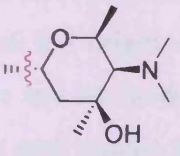
45

MJ347-81F4 A (**45a**) [nocathiacin I (**46a**)] R = Me
MJ347-81F4 B (**45b**) R = H

Figure 1-24 Structure of nocathiacins I–IV (46)



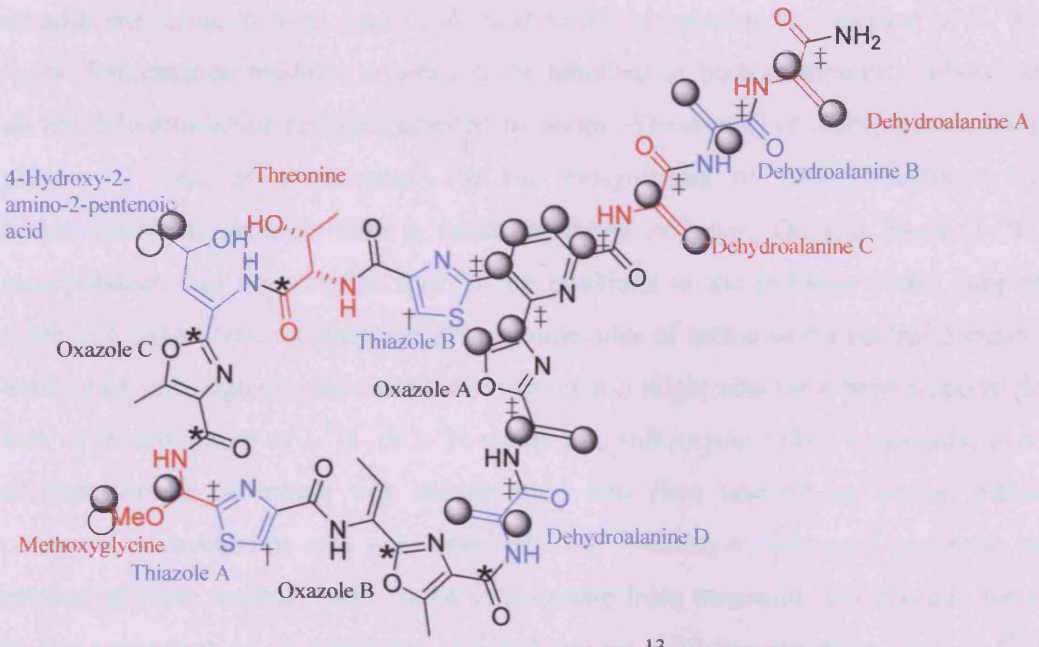
46

	R^1	R^2	R^3
Nocathiacin I (**46a**) [MJ347-81F4 A (**45a**)]		OH	
Nocathiacin II (**46b**)		H	
Nocathiacin III (**46c**)		OH	H
Nocathiacin IV (**46d**)	NH_2	OH	

1.4 Biosynthesis

Studies into the biosynthesis of a number of thiopeptides have been reported since the early 1990's. These investigations focused on thiostrepton (**1**),[108] nosiheptide (**41**),[109] sulfomycin I (**8a**),[110] A10255 (**25**),[111] berninamycin (**27**),[112] and GE2270A (**29a**),[113] the families from four different thiopeptide series according to our classification system. The origin of many of the unusual heterocyclic structural motifs inherent in these antibiotics was determined using feeding experiments by following the incorporation of isotopically labelled amino acids. Replacing some of the atoms of amino acid precursors with ^{13}C, ^{14}C, deuterium or tritium and examining the incorporation of these labels in the metabolite has been used not only to indicate the biosynthetic pathways operating in the producing organism, but also to validate stereochemical hypotheses, confirm structural identity and suggest biomimetic routes for their chemical synthesis, the latter of which has been put to good use in separate studies by Nicolaou[114] and Moody[115] for the laboratory synthesis of the central heterocyclic domain of series *a/b* and *d* thiopeptide targets, respectively. The various components all originate from amino acids, heavily modified by the organism to elaborate the complex heterocyclic structural arrays, although notable biosynthetic differences have been found in different bacterial strains. Distinct structural similarities exist between the thiopeptide antibiotics and a number of other oxazoline and thiazoline peptide natural products, for which the nature and function of the molecular machinery responsible for the biosynthesis of heterocyclic components from amino acid precursors is well described,[116] and so one might expect that the biogenesis of cyclic thiazolylpeptides is well understood also. Indeed, as a result of a number of key biosynthetic studies, much is known about the origin of many of the components, but the mechanisms of these multistep processes and the mode of assembly of the various modified components, poorly characterised in the past, remain salient points for discussion that are only now being unravelled. The reported biosynthesis of sulfomycin I (**8a**), one of the synthetic targets in our research plan, will be described in detail below.

Figure 1-25 ^{13}C incorporation into sulfomycin I (8a) using isotopically labelled cysteine, glycine, methionine, threonine, and serine



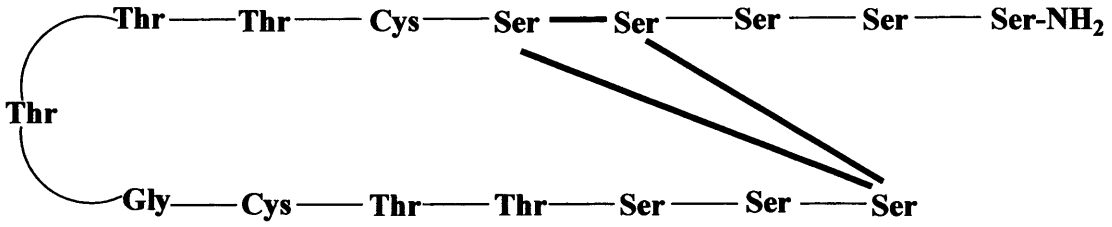
1.4.1 Biosynthesis of sulfomycin I

Isotopic labelling studies on the biogenesis of sulfomycin I (**8a**) by *Streptomyces arginensis* demonstrated a number of unusual features not observed in the biosynthesis of other thiopeptides.[110] In accordance with related studies, fortifying the fermentation medium of *S. arginensis* with ^{13}C- or ^{2}H-labelled threonine, serine, glycine or methionine led to the following results and indications. Incorporation of 3-^{13}C-cysteine was observed into thiazoles A and B, which suggests that thiazoles A and B were derived from the condensation of cysteine with the carbonyl of an adjacent amino acid residue, in each case a modified serine moiety. All dehydroalanine residues within the molecule originate from

dehydration of serine as demonstrated by the incorporation of 1-^{13}C-serine into the amide carbon of dehydroalanine A, B, C, D and the one, in which the carboxyl has condensed with an adjacent serine to form oxazole A, next to dehydroalanine D. Addition of 2-^{13}C-glycine to the fermentation medium resulted in the labelling of both methylenic carbons present in all the dehydroalanine residues labelled by serine. These sites of incorporation indicate that glycine is used as a precursor for the biosynthesis of serine, mediated by serine hydroxymethyltransferase with a tetrahydrofolate cofactor. On this basis, 2-^{13}C-glycine incorporation was observed at four of the positions in the pyridine motif, supporting the route of a tail-to-tail condensation of two molecules of serine to the central domain of these antibiotics, although glycine-serine interconversion might also have been responsible for the lack of incorporation of 3-^{2}H- or 3-^{3}H-serine into sulfomycin I (**8a**). Unusually, in a number of experiments, threonine was incorporated into sites labelled by serine, although the converse incorporation was not observed. The 2-amino-4-hydroxy-2-pentenoic acid and methoxyglycine residues were found to originate from threonine and glycine, respectively, by the appendage of an additional one-carbon unit, with incorporation from [2-^{13}C]glycine or methionine-*methyl*-^{13}C in both cases. The label from 1-^{13}C-threonine was incorporated into the amide carbon of an unmodified threonine residue in the macrocycle. Oxazole B and C were also labelled by threonine, which gives a clue that they are both formed by the dimerization of two threonines, one modified to an unusual 2-amino-4-hydroxy-2-pentenoic acid moiety in the case of oxazole C.

Thus, it would appear from the above findings that the macrocyclic peptide sulfomycin I (**8a**) may be made from 17 amino acids (with a large number of serine residues adjacent to one another to generate the linear dehydroalanine side chain, part of the pyridine core and a portion of the macrocycle) (**Diagram 1-1**).

Diagram 1-1. A postulated linear peptide for the biosynthesis of sulfomycin I (8a)



1.5 Biological activities

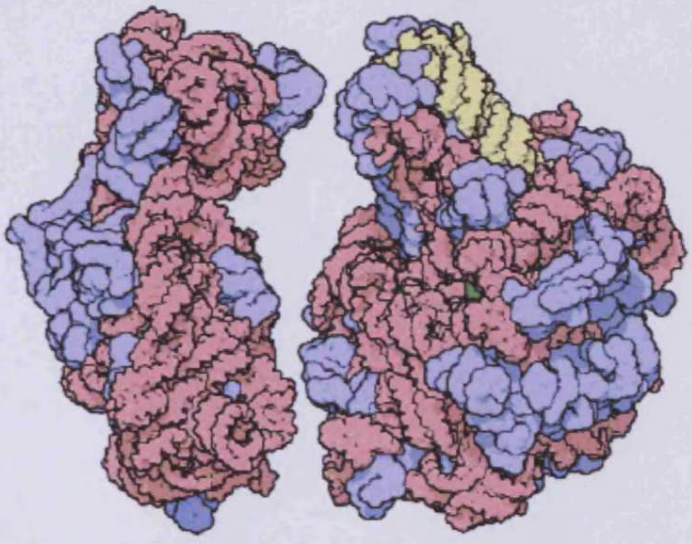
The thiopeptide antibiotics largely inhibit the growth of Gram-positive bacteria, although the activity of some of these metabolites as antifungal or anticancer agents, against Gram-negative bacteria, as renin inhibitors or against *Plasmodium falciparum*, the malaria parasite, has also been reported. In spite of considerable structural homology the site and mode of action for these antibiotics actually varies in different thiopeptide families and can be categorised, broadly, into two classes: those that bind to a region of the 23S ribosomal RNA (rRNA) known as the L11 binding domain (L11BD) and those that bind to a protein (Ef-Tu) complex involved in the elongation cycle.

1.5.1 Ribosomal inhibitors

The antibacterial activity of the thiopeptide antibiotics *in vitro* is comparable to that of the penicillins, with little or no adverse toxicological effects in mammalian cells, disrupting protein synthesis in the bacterial cell's protein factory, the ribosome. Prokaryotic and eukaryotic ribosomes interpret the information in messenger RNA (mRNA) and use it to assemble the corresponding sequence of amino acids in a protein.[117] Although bacterial and mammalian ribosomes do exhibit many structural similarities, they differ considerably in size, the latter being about 30% larger and containing so-called expansion sequences in the rRNA as well as a number of additional ribosomal proteins. The job of the ribosome is translation, that is to read each codon of the mRNA in turn and match it with the anticodon of the corresponding transfer RNA (tRNA) bound amino acid, assembled by the respective

synthetase, and thus build up the protein, residue by residue, that it encodes. All ribosomes are composed of two subunits of unequal size; the bacterial ribosome, with a relative sedimentary rate of 70S, consisting of a large 50S and a small 30S subunit. These two subunits are associated through non-covalent interactions and organise to give a ribonucleoprotein particle 2.6-2.8 MDa in size, with a diameter of 200-250 Å, that functions as a platform for bacterial protein synthesis. In the eubacteria *Escherichia coli* each subunit consists of proteins and rRNA fragments, the small 30S subunit containing 21 proteins (S1-S21) and a 16S rRNA strand whereas the 50S subunit comprises 34 proteins (L1-L34) and two strands of rRNA, the 23S and 5S (**Figure 1-26**).

Figure 1-26 Composition of the 70S bacterial ribosome[118,119]



Color key: Left: small 30S subunit; proteins S1-S21 (blue) and 16S rRNA strand (pink); Right: large 50S subunit; proteins L1-L34 (blue), 23S rRNA (pink) and 5S rRNA (yellow). (Credit to David S. Goodsell of The Scripps Research Institute.)

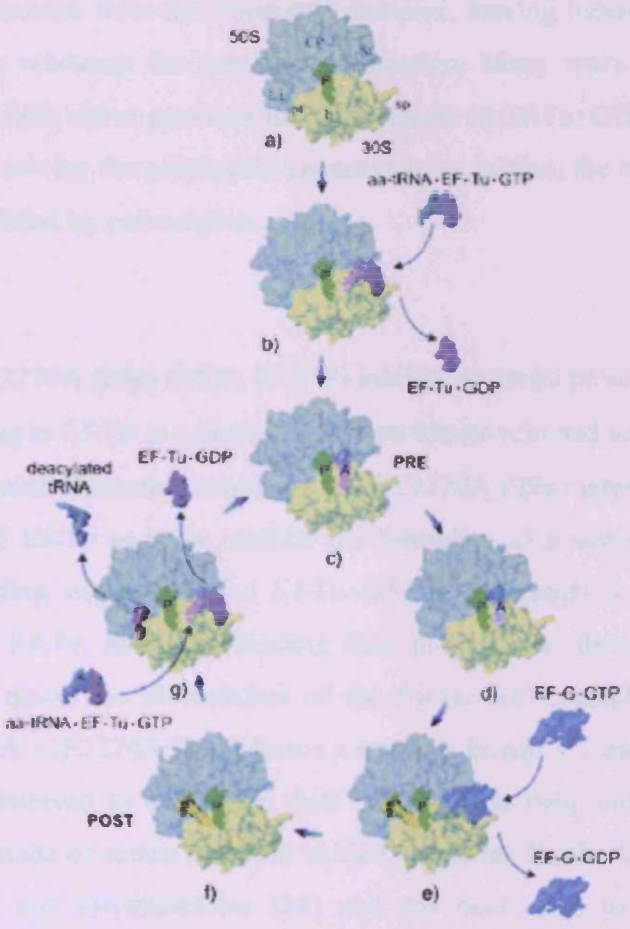
The sites on the ribosome involved in the sequential construction of the nascent protein from the individual amino acid components are denoted as the A site, where aminoacyl-tRNA (aa-tRNA) containing the next amino acid residue docks on instruction from the codon of its corresponding mRNA, the P site, where the growing peptide chain waits in readiness to form the next peptide bond, and the E site, that receives the tRNA for its exit at the end of the sequence (**Scheme 1-4**). Once bacterial protein synthesis has been initiated by

interaction of the 3' end of the 16S rRNA in the 30S subunit with a complementary sequence on mRNA,[120] the initiator tRNA binds directly to the P site (**Scheme 1-4a**) and each aa-tRNA in accordance with its corresponding codon is delivered to the A site as a ternary complex (**Scheme 1-4b**) formed by combination with the elongation factor Tu (Ef-Tu) and GTP. GTP hydrolysis causes a conformational change in the ternary complex that releases Ef-Tu·GDP from the ribosome to be recycled back to Ef-Tu·GTP, leaving the aa-tRNA bound in the A site (**Scheme 1-4c**). The next peptide bond is then formed on the large ribosomal subunit by the transfer of the peptide to the A site, generating a peptidyl-tRNA whilst leaving behind its own tRNA in the P site (**Scheme 1-4d**). Translocation of the peptidyl-tRNA from the A site back to the P site is then mediated by a different elongation factor, Ef-G (**Scheme 1-4e**), which vacates the A site and moves the deacylated tRNA to the E site ready for exit (**Scheme 1-4f**). When the next aa-tRNA-containing ternary complex binds (**Scheme 1-4g**), the tRNA docked in the E site is released and the protein elongation cycle repeats itself (**Scheme 1-4c**) until protein termination factors liberate the finished peptide and dissociates the ribosome.

Many thiopeptide antibiotics interfere with bacterial protein synthesis on the ribosome, although the precise inhibitory mechanism operating for many of these agents has not been established. The most closely studied mode of action of all of the thiopeptides is that of thiostrepton (**1**), which has been applied in rational structure-based drug design in an attempt to address problems with this antibiotic's low solubility and poor bioavailability.[121] *In vivo* thiostrepton (**1**) inhibits the binding of the aminoacyl-tRNA-containing ternary complex to the ribosomal A site.[122] The energy for protein translation is provided by the action of the elongation factors Ef-Tu and Ef-G, these hydrolysis reactions taking place on the large ribosomal subunit at a GTPase centre located on a double hairpin structure within domain II of 23S rRNA,[123] where ribosomal protein L11 and the pentameric complex L10•$(L12)_4$ assemble cooperatively, and on a ribotoxin hairpin loop within domain VI of 23S rRNA.[124] The action of thiostrepton (**1**) inhibits peptide elongation probably by impeding a conformational change within protein L11, when bound in a region of the 23S rRNA known as the L11 binding domain (L11BD).[125] The RNA-binding domain of protein L11 recognises

an rRNA tertiary structure that is stabilised by thiostrepton (**1**),[126] the antibiotic preventing one or more conformational transitions critical for stimulating the GTPase action of the elongation factors,[127] necessary to drive the directional movement of transfer and messenger RNA on the ribosome.[128]

Scheme 1-4 Overview of Protein Translation[117]



The small 30S subunit is depicted in yellow, the 50S subunit is depicted in blue. A, P and E denotes sites on the ribosome that can be occupied by tRNA. The A site is where aa-tRNA binds, the P site is where peptidyl-tRNA binds before peptide bond formation and the E site is the exit site for deacylated tRNA. Translation cycle consists of: (a) initiator tRNA binds in the P site; (b) aa-tRNA·Ef-Tu·GTP is delivered to the A site; (c) aa-tRNA is bound in the A site; (d) peptide is transferred to the A site with formation of next peptide bond; (e) translocation of peptidyl-tRNA from the A site back to the P site is mediated by Ef-G·GTP; (f) deacylated tRNA waits in the E-site; (g) aa-tRNA·Ef-Tu·GTP is delivered to the A site releasing the deacylated tRNA from the E site. (Copyright 2003 Wiley-VCH Verlag GmbH & Co. KgaA, Weinheim.)

1.5.2 Inhibition of Elongation Factor Tu

Elongation factor Tu (Ef-Tu) is the most abundant protein of the bacterial cell and participates in peptide elongation by mediating the recognition and delivery of noninitiator aminoacyl-tRNA, as a ternary complex with GTP, to the mRNA codon in the acceptor site of the bacterial ribosome. When its GTPase action is triggered, Ef-Tu·GDP, which does not bind aa-tRNA, dissociates from the ribosomal complex, leaving behind the aa-tRNA in the ribosomal A site in readiness for peptide translocation. Many types of antibiotics act by binding to Ef-Tu, which either prevents the dissociation of Ef-Tu·GDP from the ribosomal complex, as is the case for the polyketide kirromycin, or inhibits the binding of aa-tRNA to Ef-Tu•GTP, exemplified by pulvomycin.

The thiopeptide GE2270A (**29a**) (MDL 62,879) inhibits bacterial protein synthesis at an IC_{50} of 0.4 μM by binding to Ef-Tu at a distinct site from kirromycin and so differs in its mode of action from thiopeptide ribosomal inhibitors.[129] GE2270A (**29a**) interacts directly with the GTP-bound form of Ef-Tu and this inhibits the formation of a stable ternary complex by preventing the binding of aa-tRNA to Ef-Tu·GTP.[130] Although it does not change the GTPase activity of Ef-Tu, antibiotic binding does prevent the ribosomal catalysis of this process and slows down the dissociation of the Ef-Tu·GTP complex to even a greater degree than aa-tRNA. GE2270A (**29a**) forms a strongly bound 1:1 molar complex with Ef-Tu, which can be observed by a mobility shift in an electric field, only reversed by protein denaturation. This mode of action has been shown to operate for the GE2270 complex (**29**), GE37468 (**28**) and the amythiamicins (**26**) and has been used to develop a screening programme for thiopeptide antibiotics with similar binding properties, by selecting activities antagonised by exogenous Ef-Tu.[131,132]

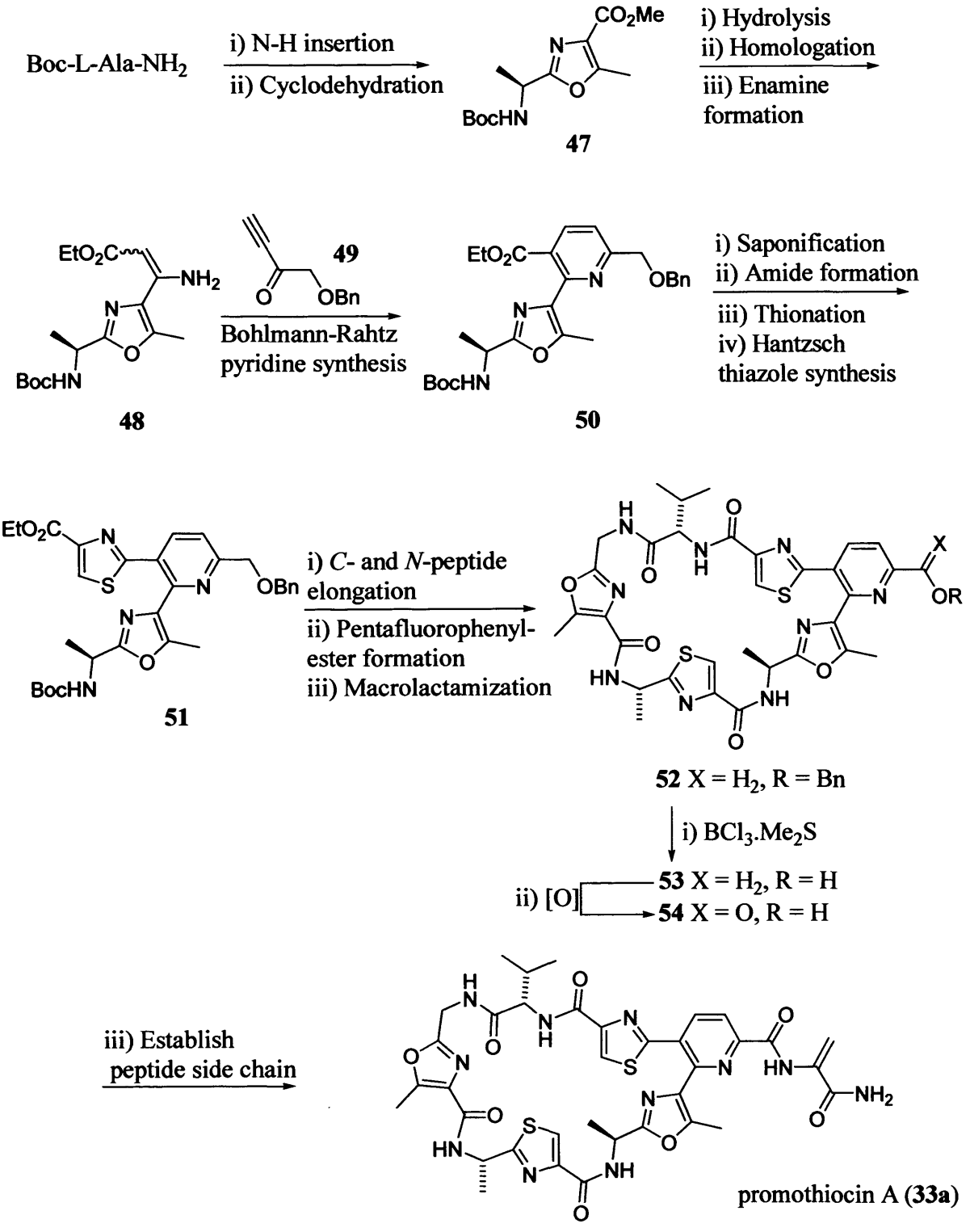
1.6 Relevant total synthesis

In recent years significant advances towards the synthesis of many of the thiopeptide antibiotics and their unusual heterocyclic or heavily modified constituent components have been made. The structural complexity of many of these antibiotics means that efforts towards their total synthesis rarely result in success. In spite of, or perhaps because of, the significant challenge, considerable efforts have been directed towards a number of thiopeptide families in recent years, culminating in the total synthesis of promothiocin A (**33a**) and more recently amythiamicin D (**26d**) and thiostrepton (**1**). Considerable progress has been made towards the acidic hydrolysates of many thioeptide antibiotics, including dimethyl sulfomycinamate (**13**),[35,36] berninamycinic acid (**9**)[63] and micrococcinic acid (**22**),[133] as well as useful building blocks for the synthesis of heterocyclic components of, amongst others, thiostrepton (**1**),[134] nosiheptide (**41**),[135-137] glycothiohexide α (**43**),[138] the promothiocins (**33**),[139] sulfomycins (**8**),[36,140] amythiamicins (**26**),[141] berninamycins (**27**),[142] cyclothiazomycin (**18**),[39,143,144] A10255 (**25**),[145] GE2270A (**29a**)[146] and the micrococcins (**21**),[147] the latter being perhaps the most unusual thiopeptide targets that have witnessed three separate total syntheses, none of which have yielded a natural product.[52-54] However, with significant advances made in this decade towards the expedient preparation of some of the more complex members of this antibiotic family, it can be presumed with some degree of certainty that major synthetic achievements will be made in this area in the near future.

1.6.1 Total synthesis of promothiocin A

The first chemical synthesis of a thiopeptide natural product was the preparation of promothiocin A (**33a**) by Moody.[77,78] This landmark synthesis featured the little-used Bohlmann-Rahtz heteroannulation reaction to establish the central oxazole–thiazole–pyridine domain **51**, by saponification, amide formation, thionation and Hantzsch thiazole synthesis. The Bohlmann-Rahtz synthesis of pyridine **50** from ethyl 3-amino-3-(4-oxazolyl)propenoate **48**, prepared from (*S*)-*N*-*tert*-butoxycarbonylalaninamide by dirhodium(II)-catalysed carbenoid insertion into the amide N–H followed by cyclodehydration with triphenylphosphine-iodine under basic conditions to give the oxazole intermediate **47**, saponification, homologation using the magnesium enolate of ethyl potassium malonate and enamine formation, and 1-benzyloxybut-3-yn-2-one (**49**), obtained by Grignard addition and propargylic oxidation, proceeds in two steps by initial Michael addition at 50 °C and subsequent double bond isomerization-cyclisation at 140 °C in the absence of solvent (**Scheme 1-5**). The elongation of the linear peptide by *N*- and *C*-terminus functionalization, followed by macrolactamisation under basic conditions via the pentafluorophenylester gave macrocycle **52**. Benzyl ether cleavage with boron trichloride dimethyl sulfide complex gave alcohol **53**, which was oxidised to carboxylic acid **54** using *o*-iodoxybenzoic acid (IBX) in DMSO followed by treatment with sodium chlorite, and then coupled with an *O*-protected serinamide derivative using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI). Protodesilylation and dehydration by mesylate formation followed by treatment with triethylamine, installed the dehydroalanine side chain, established the first total synthesis of one of the thiopeptide antibiotics and verified the (*S*)-stereochemistry of all three stereogenic centres in the metabolite isolated from *Streptomyces* sp. SF2741.

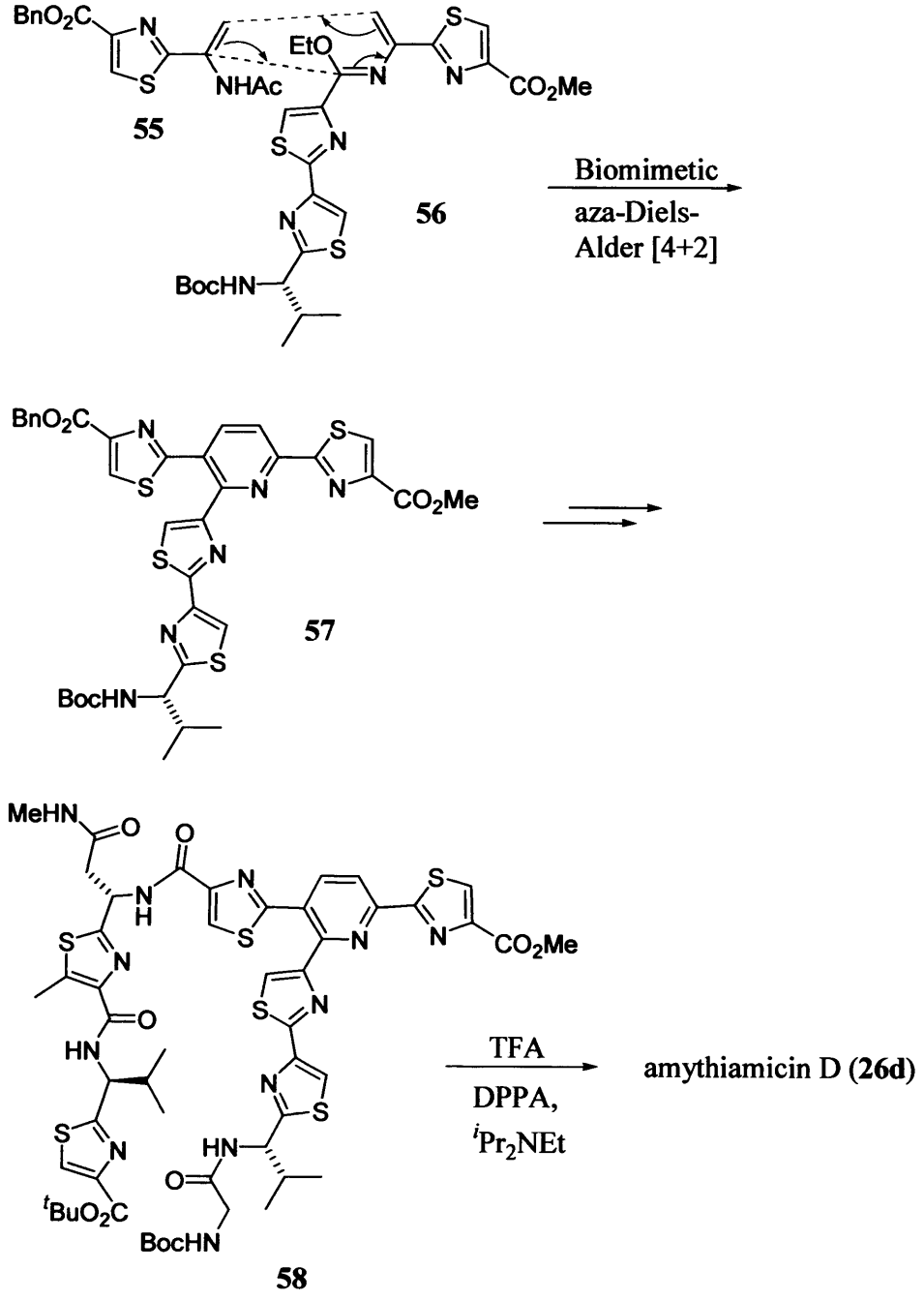
Scheme 1-5 Total Synthesis of Promothiocin A (33a)



1.6.2 Total synthesis of amythiamicin D

An alternative route to the series *d* domain by Moody used a biomimetic strategy for the synthesis of the 2,3,6-tris(thiazolyl)pyridine **57** of the amythiamicins (**26**), elaborated to amythiamicin D (**26d**) to establish the (*S*)-stereochemistry of the three stereogenic centres of the natural product (**Scheme 1-6**).[148] The formal aza-Diels-Alder cycloaddition of dehydroalanine dienophile **55** and 2-azadiene **56** proceeded in modest yield by microwave irradiation[115] in toluene at 120 °C for 12 hours. Elongation of the central pyridine domain **57** gave linear peptide **58**, which was cyclised by liberation of both *C*- and *N*-termini using trifluoroacetic acid (TFA) followed by treatment with diphenylphosphoryl azide (DPPA) and Hünig's base in DMF to give the macrocyclic natural product amythiamicin D (**26d**).

Scheme 1-6. Total synthesis of amythiamicin D (26d)



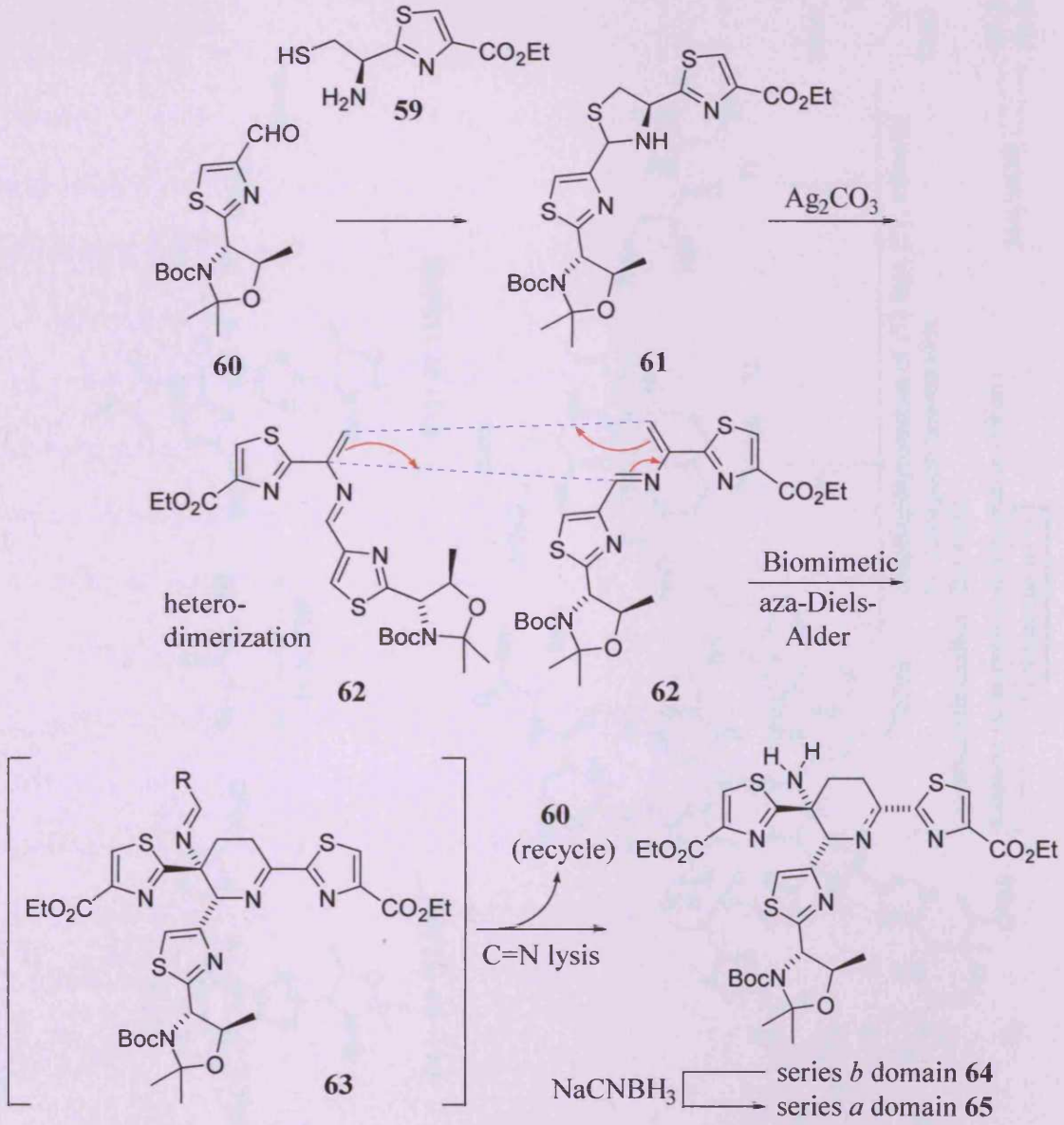
1.6.3 Total synthesis of thiostrepton

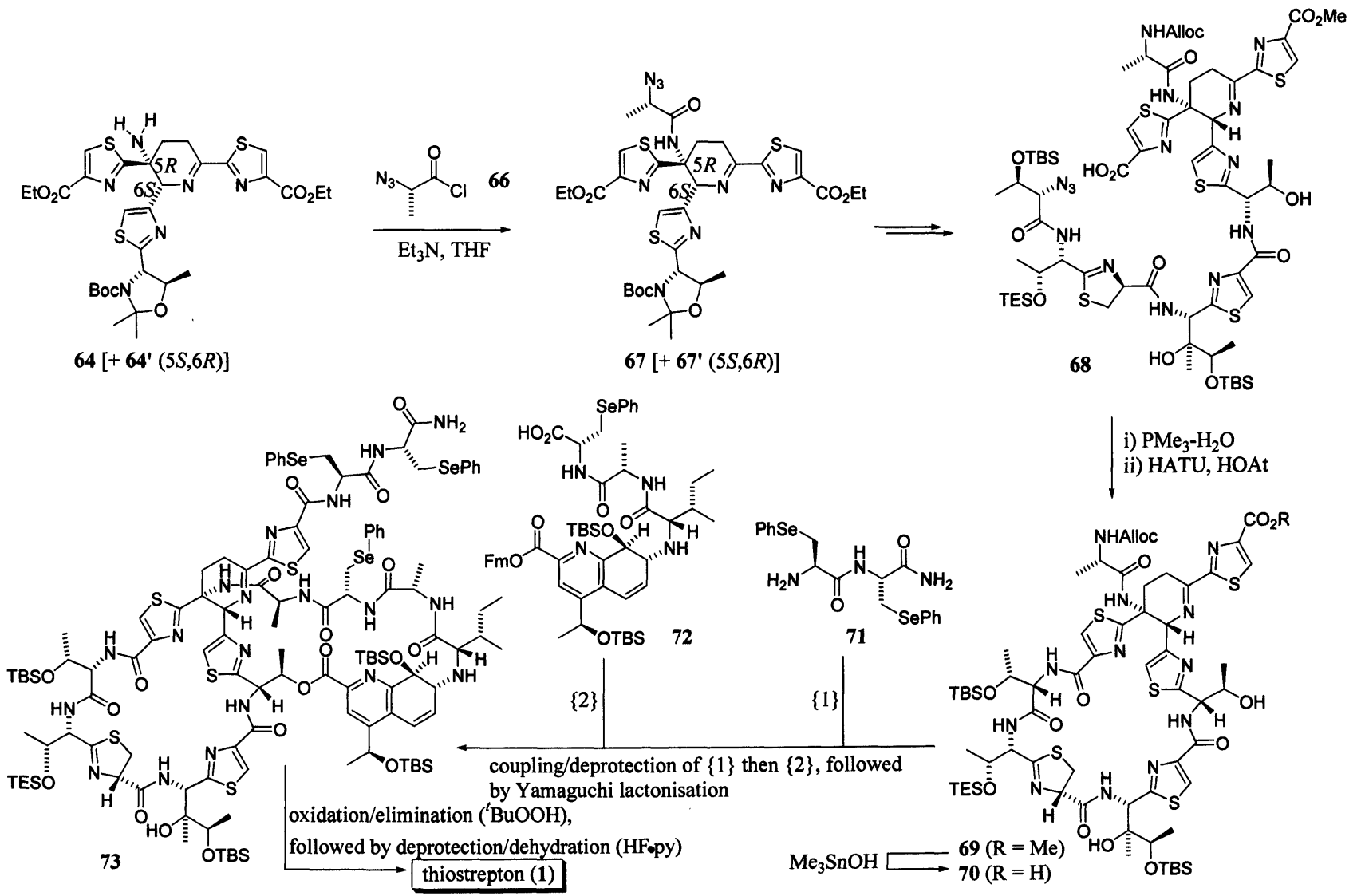
The Nicolaou group's total synthesis of thiostrepton (**1**) in 2004 provides another impressive example of biomimetic synthesis, which established the dehydropiperidine domain of thiostrepton (**1**) and this, with the stereoselective synthesis of the quinaldic acid-containing macrocycle[149] and construction of all requisite components,[150,151] led to the highly convergent total synthesis of the most complex member among the structurally known thiopeptide antibiotics.[152] The regioselective and *endo*-selective hetero-Diels-Alder dimerisation of 2-azadiene **62**, obtained from compound **61** *via* the rupture of the thiazolidine moiety mediated by silver carbonate, proceeded without facial selectivity to give dehydropiperidine **64** as a 1:1 mixture of diastereomers in a cascade sequence with *in situ* lysis of imine intermediate **63** and release of aldehyde **60** to be recycled by condensing with fragment **59** to dimerisation precursor **61** (**Scheme 1-7**).[114] Stereospecific reduction of cycloadduct **64** using sodium cyanoborohydride generated piperidine **65** and demonstrated that a biomimetic heterodimerisation approach can provide expedient access to the central domain of either series *a* (**65**) or *b* (**64**) thiopeptide antibiotics.

The application of this work in the total synthesis of thiostrepton (**1**) was realised by capturing the free amino group of the dehydropiperidine intermediate **64** with the acyl chloride of an azide derivative of alanine (**66**), to produce amide **67** exclusively, as a diastereomeric mixture (**Scheme 1-8**).[151] After peptide elaboration, closure of the thiazoline-containing macrocycle was successful for only one (**68**) of two monoacids, formed by the action of Me_3SnOH in 1,2-dichloroethane, to give the desired macrocycle **69** following reduction with PMe_3–H_2O and treatment with HATU–HOAt–$^{i}Pr_2NEt$. The construction of the two macrocyclic domains was effected by attachment of a phenylseleno-disubstituted peptide **71**, to serve as a precursor for the dehydroalanine subunits in the side chain, to acid **70**, followed by *N*-terminal deprotection, coupling with quinaldic acid linear peptide **72** and macrolactonisation under Yamaguchi conditions with 2,4,6-trichlorobenzoyl chloride.[152] The synthesis was completed by ^{t}BuOOH-mediated oxidation of all three phenylseleno groups in bis-macrocycle **73**, which brought about spontaneous selenoxide *syn* elimination,

followed by silyl ether deprotection with hydrogen fluoride•pyridine, with concomitant elimination to form the thiazoline-conjugated *Z* double bond. This landmark route gave synthetic thiostrepton (**1**) with identical physical properties to the natural product, constituting a highly convergent and stereoselective synthesis of a highly complex and sensitive thiopeptide antibiotic that may pave the way for related synthetic studies or biological examination of the thiazolyl peptides in the future.

Scheme 1-7 Biomimetic synthesis of series *a* or *b* piperidine domain





Scheme 1-8 Total synthesis of thiostrepton (1)

1.7 Summary and conclusions

Over half a century has past since the first isolation of micrococcin (**21**) from *Micrococcus* in 1948. A large number of thiopeptide natural products have been and continue to be isolated from various sources since then. Alongside this increase in diversity, analytical methods in particular X-ray crystallography and NMR techniques well suited to these macrocyclic natural products have evolved to elucidate thiopeptide structure and stereochemistry with much greater certainty, removing many of the structural ambiguities inherent in earlier work in the area. Considerable advances have been made in our understanding of the dynamic function of the bacterial ribosome, the mode of action and site of binding of thiopeptide ribosomal inhibitors, the inhibition of organellar protein synthesis by these agents in *Plasmodium falciparum* and the manner in which these metabolites are assembled in the organism and it is suspected that many more revelations in these areas will be forthcoming. Furthermore significant progress in the chemical synthesis of complex molecular architectures found in this family of antibiotics has been made, with the total synthesis of promothiocin A (**33a**), amythiamicin D (**26d**) and the stunningly complex thiostrepton (**1**), making it appear likely that further success will be enjoyed in the synthesis of similarly challenging metabolites and derivatives thereof in the future, to optimise their biological function in the computer-assisted design of analogue structures. With bacterial evolution threatening to overthrow our current antibiotic regime, we can be certain that the thiopeptide antibiotics will continue to enjoy increasing attention from a wide variety of scientific consortia, whose work will continue to surprise us with its innovation, tenacity, ambition and strategic relevance.

Chapter Two

Results and Discussion

2. New Methodology for the Synthesis of Substituted Pyridines

2.1 Introduction

In order to establish a solid foundation for the total synthesis of the natural products in series *d* and *e* of thiopeptide antibiotic families, which are the great majority and share a common pyridine central domain, the decision was made to develop novel methodology for the construction of polysubstituted pyridines based on the Bohlmann-Rahtz pyridine synthesis, which showed huge potential and synthetic utility as a key step in the total synthesis of promothiocin A (**33a**)[77,78] as described in section **1.6.1**.

2.2 Pyridine syntheses

There are very many ways of achieving the synthesis of a pyridine ring. In general, most syntheses rely upon one of two approaches: the condensation of carbonyl compounds or cycloaddition reactions. The Bohlmann-Rahtz pyridine synthesis, as well as the Hantzsch synthesis and condensation of 1,5-dicarbonyl compounds and ammonia, belong to the former category; on the other hand, preparation of pyridine rings by Diels-Alder reactions with azadienes is the most straightforward cycloaddition approach in the latter category.

2.2.1 Diels-Alder reactions with azadienes

A hetero-Diels-Alder route to pyridines involves reaction between an azadiene and an alkene or alkyne, followed by subsequent aromatization. This method has been rarely used in synthesis since its discovery, as the Diels-Alder reaction is disfavored on electronic, conformational, and thermodynamic grounds. However, the recently completed total synthesis of the thiopeptide amythiamicin D (**26d**) by Moody *et al.*, employed a biosynthesis-inspired Diels-Alder reaction to construct the central 2,3,6-trisubstituted pyridine core **57** (see sectin **1.6.2**).[148] In addition, Nicolaou and co-workers also described a related biomimetic hetero-Diels-Alder approach to the tetrahydropyridine domain in the

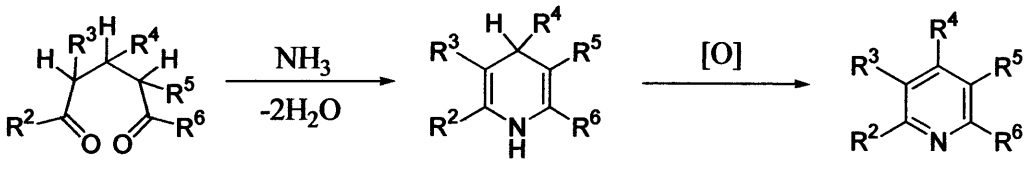
total synthesis of thiostrepton (**1**) (see section **1.6.3**).[151,152] These two elegant total syntheses highlighted the great potential of the hetero-Diels-Alder reactions in natural product and heterocyclic chemistry, especially in the area of thiopeptide antibiotics.

2.2.2 Condensation of 1,5-dicarbonyl compounds

Condensation of 1,5-diones with ammonia is the simplest approach to prepare pyridine rings. A typical process involves reactions between ammonia and 1,5-dicarbonyl compounds to give 1,4-dihydropyridines, which are subsequently dehydrogenated to pyridines (**Scheme 2-1**). 1,5-Diketones are accessible *via* a number of routes, classically by Michael addition of enolate to enone (or precursor Mannich base[153]) or by ozonolysis of a cyclopentene precursor, or more recently by reaction of silyl enol ethers with 3-methoxyallylic alcohols.[154] They react with ammonia, with loss of two mole equivalents of water to produce a cyclic bis-enamine, which is generally unstable but can be easily and efficiently dehydrogenated to the aromatic heterocycle.

This method has been used by Ciufolini, *et al.*, for the synthesis of the heterocyclic core of the Bycroft-Gowland structure of thiopeptide micrococcin P1 (**21a**). The reaction of an enone and a substituted ketone generated the 1,5-diketone, which was reacted with a source of ammonia to give the 1,4-dihydropyridine precursor. Finally, the oxidative step gave the trisubstituted pyridine central domain.[147a] The application of this pyridine ring formation in total synthesis proved the feasibility of this method to serve as an alternative way alongside others to construct polysubstituted pyridine core of series *d* and *e* thiopeptides.

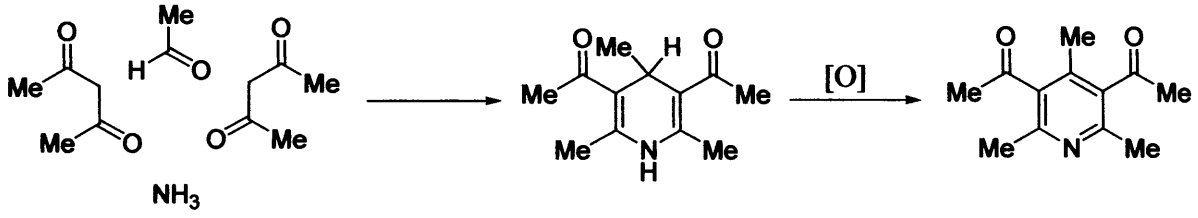
Scheme 2-1 Pyridine synthesis from 1,5-dicarbonyl compounds and ammonia



2.2.3 The Hantzsch synthesis

The classical Hantzsch pyridine synthesis, reported in 1882,[155] is the four-component reaction between two β-ketoesters, an aldehyde and ammonia. The initial reaction sets up a 1,4-dihydropyridine system, which is oxidised with nitric acid, or nitrous acid, to give the corresponding pyridine. The product from the classical Hantzsch synthesis is necessarily a symmetrically substituted 1,4-dihydropyridine since two mole equivalents of the one dicarbonyl component are utilised, the aldehyde carbonyl carbon becoming the pyridine C-4 (**Scheme 2-2**). Due to this feature, the synthetic applicability of Hantzsch pyridine synthesis is very limited in the area of thiopeptide antibiotics.

Scheme 2-2 Hantzsch pyridine synthesis



2.2.4 The Bohlmann-Rahtz pyridine synthesis

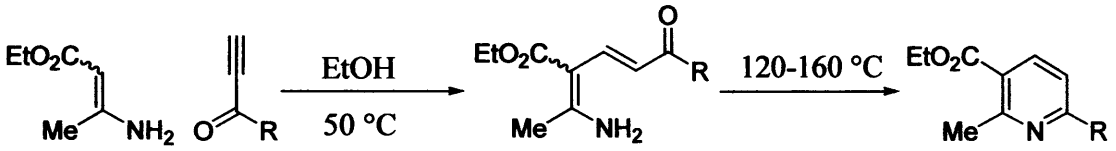
2.2.4.1 Introduction

Bohlmann and Rahtz first reported a new synthesis of trisubstituted pyridines from ethyl β-aminocrotonate and ethynyl ketones back in 1957.[156] However, in the subsequent forty years after its discovery, the Bohlmann-Rahtz pyridine synthesis found little use until a resurgence of interest in the late 1990s.

The traditional Bohlmann-Rahtz pyridine synthesis is a two-step process involving, for example, initial Michael addition of ethyl β-aminocrotonate and an ethynyl carbonyl compound in ethanol at 50 °C. The aminodienone intermediate is isolated and subsequently

heated to high temperatures to affect cyclodehydration to pyridine (**Scheme 2-3**). Although the product usually requires purification, yields are generally good. Therefore, the method offers considerable potential for application in heterocyclic chemistry.

Scheme 2-3 The traditional Bohlmann-Rahtz pyridine synthesis



2.2.4.2 New improvement of the traditional Bohlmann-Rahtz reaction

In 2001, Bagley *et al.* reported a series of new modifications of the Bohlmann-Rahtz pyridine synthesis.[157] A range of highly functionalized pyridines was prepared from enamino esters and alkynones in a single synthetic step by the use of acetic acid, Amberlyst 15 ion exchange resin, zinc(II) bromide or ytterbium(III) triflate as catalyst (**Scheme 2-4, Table 2-1**).[158] These developments for Bohlmann-Rahtz pyridine synthesis allow conjugate addition and subsequent cyclodehydration to be conducted in a single step without any need for isolation of intermediates and at a much lower reaction temperature, consequently, the utility of the Bohlmann-Rahtz reaction has been improved and the transformation of this technology to the solid phase has been facilitated.

Scheme 2-4 General scheme of one-step Bohlmann-Rahtz heteroannulation procedures

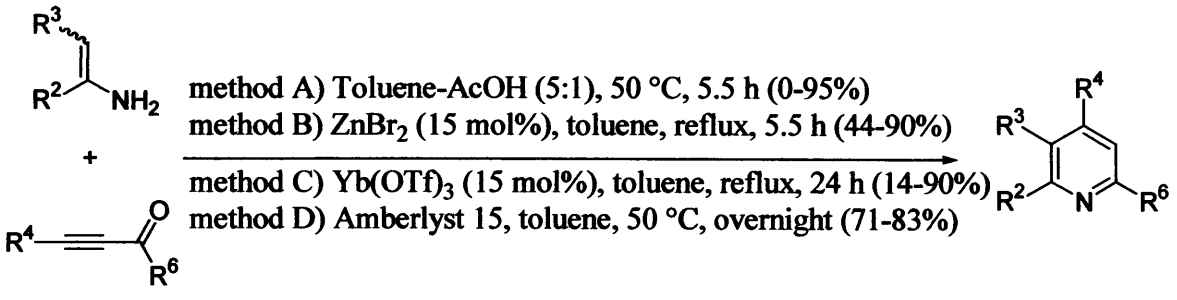


Table 2-1 The scope of one-step heteroannulation methods for the synthesis of pyridines

Entry/ Product	R^2	R^3	R^4	R^6	Method A/B/C/D Yield (%)
1	Me	EtO_2C	Me_3Si	Me	**79/90/90/—**
2	Me	EtO_2C	Ph	Me	**0/—/—/71**
3	Me	EtO_2C	Et	Me	**85/67/83/—**
4	Me	EtO_2C	Me_3Si	CO_2Et	**—/44**[a]**/33/—**
5	Me	EtO_2C	Ph	CO_2Et	**95/85/55/—**
6	Ph	EtO_2C	H	Me	**73/70/58/—**
7	Ph	EtO_2C	Ph	Me	**—/62/68/—**
8	Ph	EtO_2C	Et	Me	**65/72/32/—**
9	Ph	EtO_2C	Ph	CO_2Et	**—/65/44/—**
10	2-Furyl	EtO_2C	Me_3Si	Me	**80/—/—/73**[b]
11	2-Pyridyl	EtO_2C	Et	Me	**—/62/68/—**
12	Me	$^tBuO_2C$	H	Me	**0/—/—/83**
13	Me	$^tBuO_2C$	Ph	Me	**—/85/32/76**
14	Me	$^tBuO_2C$	Et	Me	**—/70/14/80**

[a] Protodesilylated pyridine (R^4 = H) was also produced. [b] Desilylation occurred during the course of the reaction.

Following the establishment of acid-catalysed one-step heteroannulation methods, a new one-pot three-component condensation reaction for the synthesis of 2,3,4,6-tetrasubstituted pyridines based on Bohlmann-Rahtz pyridine synthesis was developed by the same research group.[159] A range of different β-ketoesters and alkynones were heated at reflux in toluene (or benzene) under acidic conditions in the presence of an excess of ammonium acetate to generate the corresponding pyridine products in good to excellent yield and with total regiocontrol (**Scheme 2-5**, **Table 2-2**). The discovery of this new multiple-component condensation reaction provides a much more efficient route towards highly functionalised pyridines and has a larger range of substrate availability.

Scheme 2-5 Three-component heteroannulation of β-ketoester 74–77, alkynone 78–81 and ammonia under acidic conditions

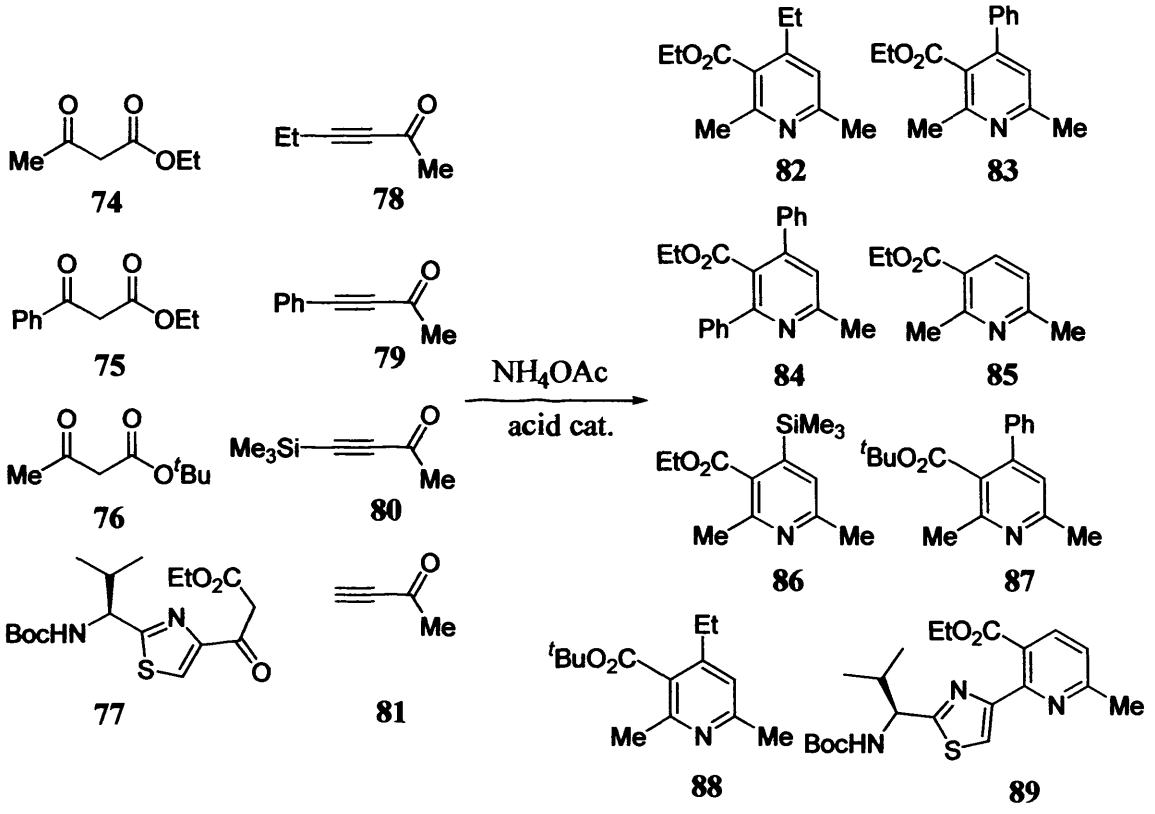


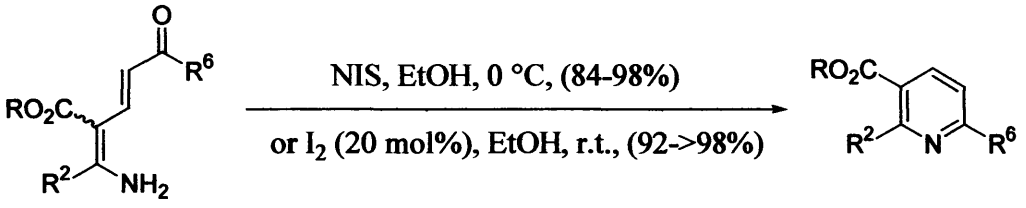
Table 2-2 The scope of the three-component heteroannulation reaction

Entry	β-ketoester	Alkynone/ Equivalents	Acid catalyst	Conditions	Product	Yield (%)[a]
1	**74**	**78**/2	$ZnBr_2$	Toluene/reflux	**82**	96
2	**74**	**79**/2	AcOH	Toluene/reflux	**83**	80
3	**75**	**79**/2	AcOH	Toluene/reflux	**84**	70
4	**75**	**79**/2	$ZnBr_2$	Toluene/reflux	**84**	88
5	**74**	**80**/2	$ZnBr_2$	Toluene/reflux	**85, 86** (44:56)	55
6	**74**	**80**/2	AcOH	Toluene/reflux	**85**	75
7	**76**	**78**/3	$ZnBr_2$	Toluene/reflux	**88**	49
8	**76**	**79**/2	Amberlyst 15	Toluene/reflux	**87**	53
9	**76**	**79**/3	Amberlyst 15	Toluene/reflux	**87**	60
10	**76**	**78**/3	Amberlyst 15	Toluene/reflux	**88**	55
11	**77**	**81**/3	AcOH	Toluene/reflux	**89**	68[b]
12	**77**	**81**/3	AcOH	Benzene/reflux	**89**	71[b]

[a] Yield of pure isolated product. [b] Formed in 78% *ee* (**entry 11**) or 98% *ee* (**entry 12**) by HPLC analysis [Chiralpak AD column, hexane-IPA (92:8)].

More recently, 2,3,6-trisubstituted pyridines have been generated by cyclodehydration of Bohlmann-Rahtz aminodienones in the presence of stoichiometric amount of *N*-iodosuccinimide[160] or catalytic amount (20 mol%) of iodine[161] in excellent yield with total regiocontrol (**Scheme 2-6**), and without the need for column chromatography in the later case. These remarkable new methods offer the mildest ever conditions for the synthesis of pyridines, and therefore have great potential utility in asymmetric synthesis and combinatorial chemistry.

Scheme 2-6 NIS or iodine-mediated cyclodehydration of Bohlmann-Rahtz aminodienone intermediates

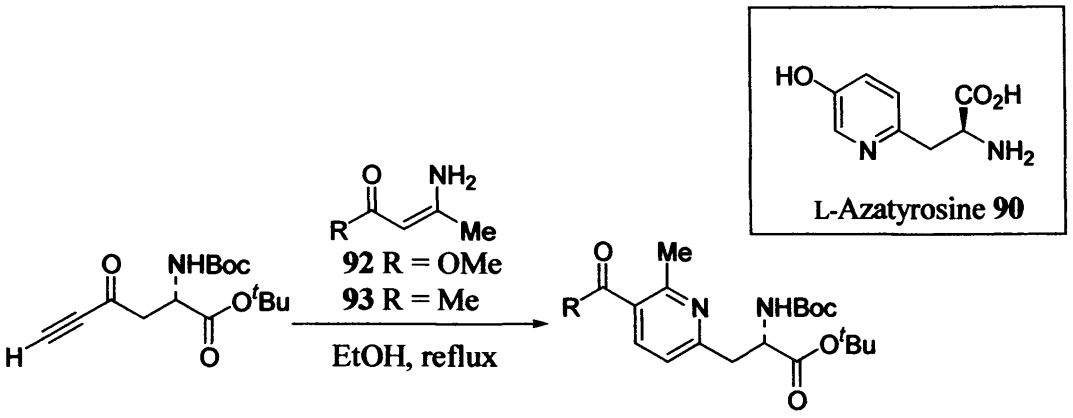


2.2.4.3 Synthetic applications

Moody and Bagley described the construction of the modified oxazole-thiazole-pyridine core **51** of the promothiocin antibiotics (**33**) in 1998 (see section **1.6.1**),[77,78,162] which pioneered a number of syntheses of polysubstituted pyridines and other related nitrogen heterocycles using the Bohlmann-Rahtz reaction.

In an attempt to access pyridine substituted amino acids related to L-azatyrosine **90**, which have been observed to display antibiotic activity, condensation of (*S*)-2-*tert*-butoxycarbonylamino-4-oxohex-5-ynoic acid *tert*-butyl ester **91** with either enamine **92** or **93** led to the generation of pyridines **94** or **95**,[163] respectively (**Scheme 2-7**).

Scheme 2-7 The synthesis of pyridine substituted α-amino acids by Bohlmann-Rahtz reaction

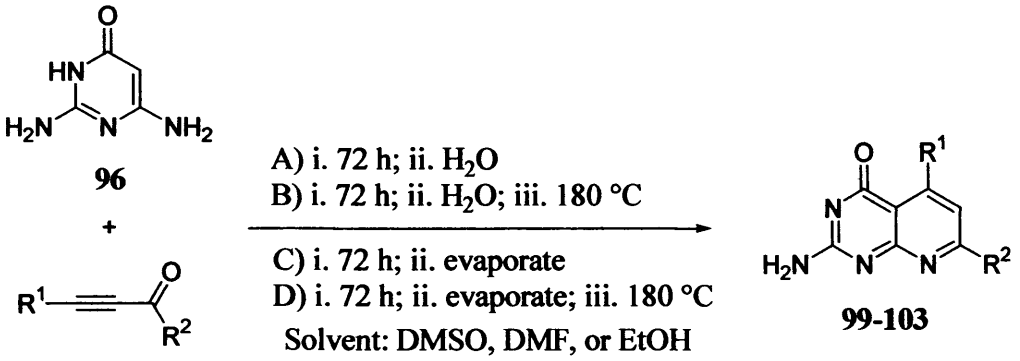


91

94 R = OMe 81%
95 R = Me 91%

A new synthesis of pyrido[2,3-*d*]pyrimidines was developed based on the Bohlmann-Rahtz reaction.[164] Michael addition and subsequent cyclodehydration of 2,6-diaminopyrimidin-4-one (**96**) and a range of alkynones in a number of different solvents at room temperature or 60 °C generated the corresponding pyrido[2,3-*d*]pyrimidines **99–103** in excellent yield (**Scheme 2-8**). This highly expedient approach would offer many advantages over existing methodology based upon condensation reactions and pyridine annelation reactions for the synthesis of pyrido[2,3-*d*]pyrimidine derivatives, employing readily-available alkynones as Michael acceptors and thus obviating the need for subsequent oxidation, to provide target heterocycles directly without need for purification and with total regiochemical control. Furthermore, following the development of the Lewis acid catalysed Bohlmann-Rahtz heteroannulation reaction by Bagley and co-workers,[157b] it was demonstrated that the synthesis of pyrido[2,3-*d*]pyrimidines was facilitated in a single synthetic step in excellent yield at a lower reaction temperature in the presence of a Lewis acid.[157b]

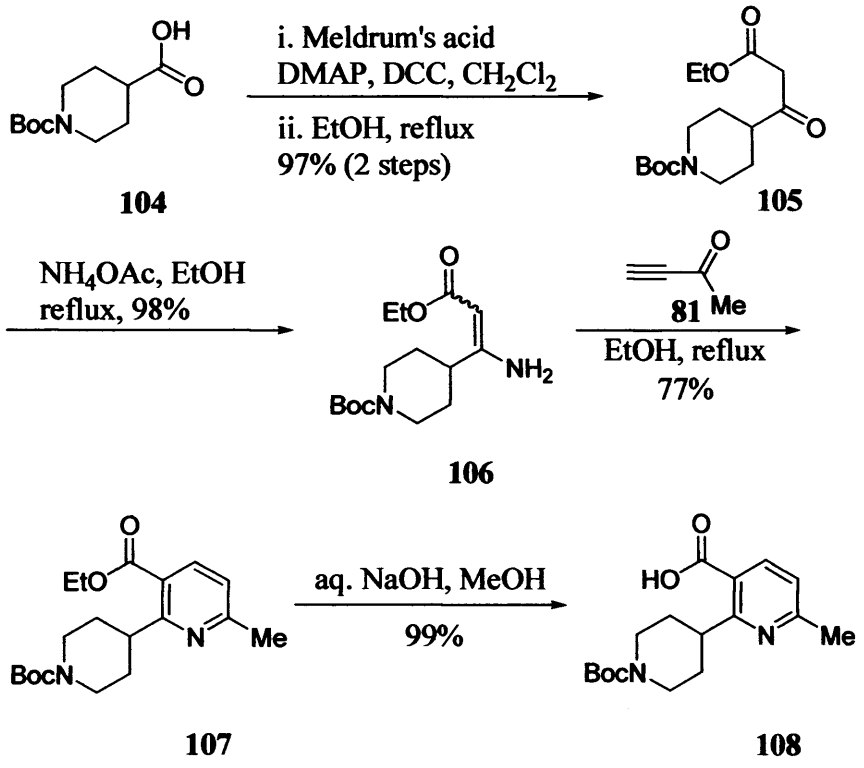
Scheme 2-8 Reaction of pyrimidinone 96 and alkynones 78–81, 97–98 in different solvents by method A, B, C or D



81 R^1 = H; R^2 = Me
80 R^1 = TMS; R^2 = Me
78 R^1 = Et; R^2 = Me
79 R^1 = Ph; R^2 = Me
97 R^1 = TMS; R^2 = CO_2Et
98 R^1 = Ph; R^2 = CO_2Et

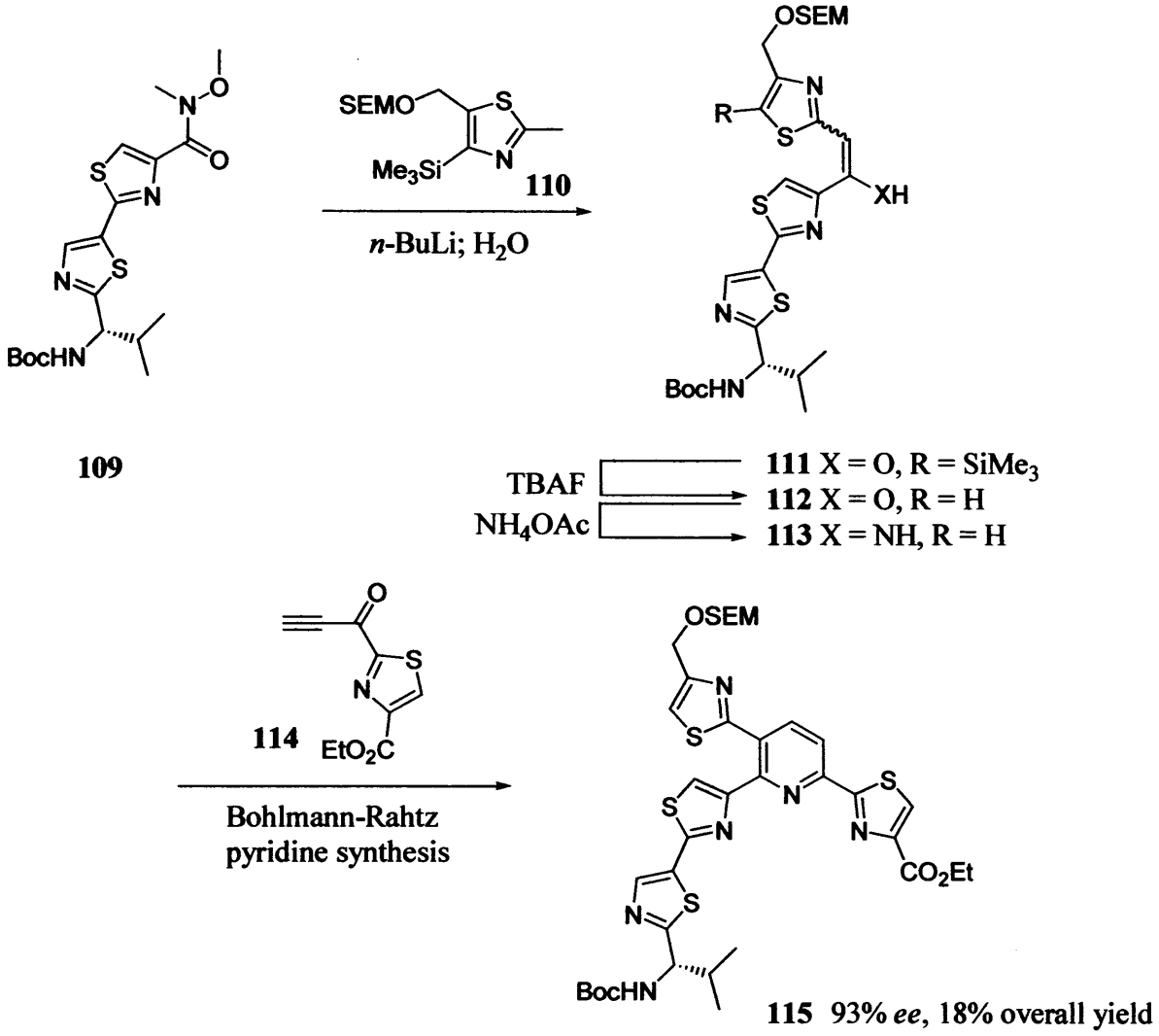
In an attempt to develop a new approach for the generation of combinatorial libraries of pyridine derivatives, Moody employed an optimised Bohlmann-Rahtz route to synthesize the pyridine scaffold **108** containing two points of diversity on a large scale of ca. 500 g (**Scheme 2-9**), which was subsequently used in library synthesis.[165]

Scheme 2-9 The Bohlmann-Rahtz synthesis of pyridine scaffold 108



Inspired by the efficient Bohlmann-Rahtz construction of the central pyridine domain in total synthesis of promothiocin A,[77,78,162] Bagley and co-workers presented another example of the use of this strategy for the preparation of the pyridine core of the amythiamicin antibiotics.[141] The orthogonaly protected core **115** of the thiopeptide antibiotic amythiamicins (**26**) was obtained in highly enantiomeric enriched form by Michael addition-cyclodehydration of enamine **113** and 1-(2-thiazolyl)propynone **114** (**Scheme 2-10**). The assembly of 2-(2-thiazolyl)enamine **113** initiated from the chiral *bis*-thiazole **109**, which was achieved from Boc-protected L-valine by double Hantzsch thiazole synthesis followed by alkaline hydrolysis and weinreb amide formation. Reaction of Weinreb amide **109** with the lithioderivative of 2-methylthiazole **110** gave Claisen condensation product **111**. Desilylation using tetrabutylammonium fluoride followed by microwave irradiation of thiazole **112** and ammonium acetate in toluene at 120 °C provided the desired enamine **113**.

Scheme 2-10 Bohlmann-Rahtz construction of the amythiamicin central heterocyclic domain 115



2.2.4.4 Conclusion

Following the discovery of the Bohlmann-Rahtz pyridine synthesis half a century ago the reaction became largely forgotten for many years, however a recent resurgence in interest has seen the scope of this methodology expanded significantly. A number of synthetic applications of the Bohlmann-Rahtz reaction have appeared in literature, which highlight the great value of this reaction in synthetic organic chemistry, especially in the total synthesis of

thiopeptide natural products. In spite of these developments, the synthetic potential of the Bohlmann-Rahtz reaction is still far from being fully explored. With increasing attention on this reaction, further improvements can be expected in the forthcoming years, which will be accompanied by more and more applications in natural product total synthesis.

2.3 Modification of the traditional Bohlmann-Rahtz pyridine synthesis

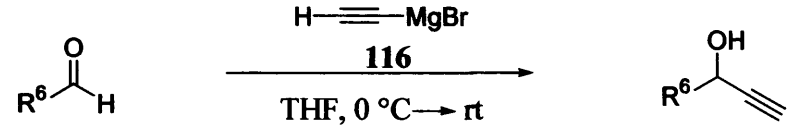
2.3.1 Preparation of enamines and alkynones–the Bohlmann-Rahtz precursors

Before carrying out modification of the traditional Bohlmann-Rahtz reaction, it was expected that enamines and alkynones, the two starting components of the Bohlmann-Rahtz pyridine synthesis, with various substituents would be needed for the purpose of investigating reaction scope and versatility. However, due to the poor commercial availability of enamines and alkynones, preparation of enamines and alkynones turned out to be a top priority.

2.3.1.1 Alkynone synthesis

Synthesis of alkynones was achieved in a two step process *via* the corresponding propargylic alcohols. Firstly, addition of ethynylmagnesium bromide **116** to aldehydes **117** and **118** resulted in quantitative formation of the corresponding propargylic alcohols **119** and **120** (**Scheme 2-11**), respectively. In the second step, mild oxidation of the propargylic alcohols **119–121** using IBX in DMSO afforded the desired alkynones **122–124** in excellent yield (**Scheme 2-12**).

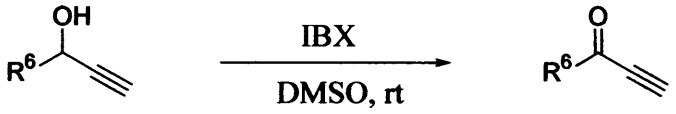
Scheme 2-11 Preparation of propargylic alcohols 119 and 120



117 $R^6 = 4'\text{-}C_6H_4Cl$
118 $R^6 = 4'\text{-}C_6H_4OMe$

119 $R^6 = 4'\text{-}C_6H_4Cl$ 99%
120 $R^6 = 4'\text{-}C_6H_4OMe$ 99%

Scheme 2-12 Preparation of alkynones 122–124



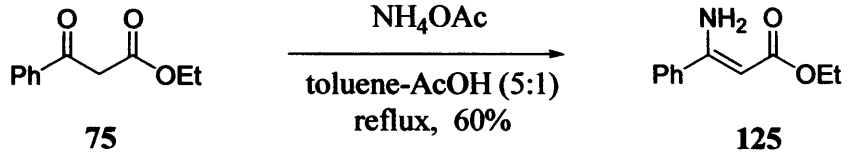
121 $R^6 = C_6H_5$
119 $R^6 = 4'\text{-}C_6H_4Cl$
120 $R^6 = 4'\text{-}C_6H_4OMe$

124 $R^6 = C_6H_5$ 99%
122 $R^6 = 4'\text{-}C_6H_4Cl$ 94%
123 $R^6 = 4'\text{-}C_6H_4OMe$ 98%

2.3.1.2 Enamine synthesis

Having synthesised a number of suitable alkynones, enamine **125** was generated from the corresponding β-ketoester by following the work of Moody, *et al.*,[77,78] who used this method to generate the Bohlmann-Rahtz precursor for the construction of the pyridine core of promothiocin antibiotics. Commercially available ethyl benzoylacetate (**75**) was converted into the corresponding enamine by treatment with ammonium acetate under acidic conditions. **75** was heated at reflux in toluene-glacial acetic acid (5:1) in the presence of a large excess of ammonium acetate to facilitate the generation of enamine **125** in 60% yield **(Scheme2-13).**

Scheme 2-13 Preparation of enamines 125



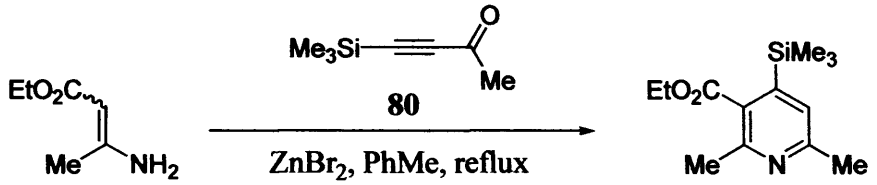
75 125

2.3.2 Lewis acid catalysed one-step Bohlmann-Rahtz reaction

In order to establish the optimum reaction conditions of the newly disclosed one-step Bohlmann-Rahtz heteroannulation catalysed by zinc(II) bromide,[157b] the relationship between the efficiency of the Lewis acid catalysis and the quantity of Lewis acid used was investigated. A solution of ethyl β-aminocrotonate (**126**) and 4-(trimethylsilyl)but-3-yn-2-one (**80**) in toluene was heated at reflux for 5 or 18 hours in the presence of 10–100 mol% of zinc(II) bromide (**Scheme 2-14**). The results are summarized in **Table 2-3**. It was found that, in all of the experiments, formation of pyridine **86** was observed. The yield of reaction varied considerably depending upon the quantity of zinc(II) bromide that was used and the reaction time. The best result was achieved when a mixture of ethyl β-aminocrotonate (**126**) and 4-(trimethylsilyl)but-3-yn-2-one (**80**) in toluene was heated at reflux for 5 hours with 15 mol% catalyst loading (**entry 2, Table 2-3**). On the other hand, varying the amount of zinc(II) bromide either above or under 15 mol% resulted in a drop in yield.

The product **86** was identified by ^{1}H NMR spectroscopic analysis that showed the presence of a down-field singlet proton resonance at 7.12 ppm corresponding to the 5-H aromatic methine proton and a methyl singlet at 0.26 ppm from the trimethylsilyl resonance. An ester carbonyl stretch at 1724 cm^{-1} was observed in the IR spectrum and mass spectrometry provided the parent MH^+ ion at 252 *m/z*. NOe, HMQC and HMBC NMR techniques were employed to confirm the spatial arrangement of the substituents on pyridine **86**.[166]

Scheme 2-14 The zinc(II) bromide catalysed Bohlmann-Rahtz reaction



126 **86**

Table 2-3 Optimisation of the zinc(II) bromide catalysed Bohlmann-Rahtz reaction

Entry	Catalyst (mol%)	Reaction time (h)	Yield (%)[a]
1	10	5	53
2	15	5	90
3	15	18	68
4	20	5	71
5	20	18	82
6	30	5	59
7	40	5	66
8	50	5	57
9	100	5	44

[a] Isolated yield of pyridine **86** after column chromatography

2.3.3 Microwave-assisted One-step Bohlmann-Rahtz reaction

2.3.3.1 Introduction

In recent years, the use of microwave dielectric heating in synthetic chemistry has emerged as a valuable alternative to conventional conductive heating methods.[167] By taking advantage of this efficient source of energy, compound libraries for lead generation and optimization can be assembled in a fraction of the time required by classical thermal methods.

Microwaves differ from traditional heat sources, in that reactants are directly heated without heating the reaction vessel. By conventional methods, reactants are slowly activated by an

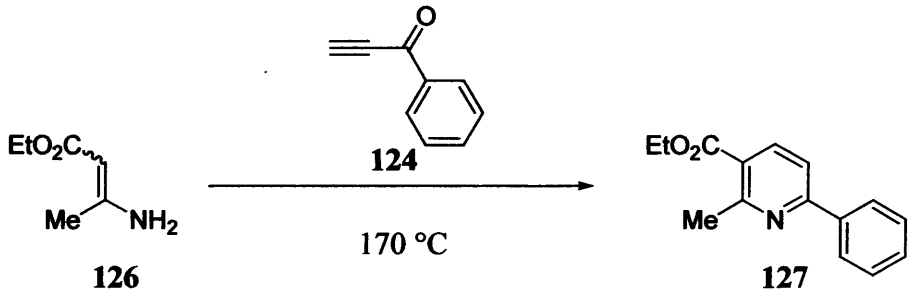
external heat source. Heat is transferred to the substance, conducting first through the walls of the vessel in order to reach the solvent and reactants. This is a slow and inefficient method for transferring energy into the reacting system. Microwave heating is much more efficient in terms of the energy used. The molecules in the reaction mixture absorb energy from microwave irradiation by either dipole rotation or ionic conduction, as the two fundamental mechanisms for transferring energy from microwaves to the substances being heated,[168] leading to a rapid rise in temperature.

In 1986, Gedey *et al.*, described the first example of the use of microwave irradiation in organic synthesis, which was performed using domestic microwaves.[169] Since then, the interest in microwave-assisted reactions has increased spectacularly. With many potential benefits and as a result of the advent of dedicated ovens for synthesis that focus microwaves in a monomodal cavity, the development of new reproducible microwave-assisted procedures has been a popular area of chemistry of late.[170]

2.3.3.2 Methodology development

Following the successful development of a one-pot Bohlmann-Rahtz heteroannulation method using Lewis acid catalysis, our attention turned to the use of microwave irradiation in synthesis of heterocycles,[171] in particular pyridine heterocycles.[172]

Scheme 2-15 One-step Bohlmann-Rahtz synthesis of pyridine 127



A solution of ethyl β-aminocrotonate (**126**) and an excess of 1-phenylprop-2-yn-1-one (**124**) (**Scheme 2-15**) was stirred in toluene or DMSO, solvents that have been used in Bohlmann-Rahtz reactions in previous studies within the group,[158] at 170 °C by irradiating initially at 150 or 160 Watts using a self-tunable microwave synthesiser (**Table 2-4**). The reaction conducted in toluene was found to be sluggish at best, providing pyridine **127** in 76% yield after 90 minutes following purification by column chromatography on silica (**entry 1, Table 2-4**). The use of a more polar solvent, DMSO that can couple more efficiently with the microwave radiation, resulted in a more rapid reaction, Michael addition and spontaneous cyclodehydration were complete after 20 minutes, to give pyridine **127** in 87% yield (**entry 2, Table 2-4**). Reactions conducted in toluene were accelerated dramatically by the presence of zinc(II) bromide (15 mol%) as a Lewis acid catalyst providing the product in 80% yield after 10 minutes at 170 °C (**entry 3, Table 2-4**). However, the optimum conditions for this transformation employed acetic acid as a Brønsted acid catalyst. After stirring for 10 minutes in a solution of toluene–acetic acid (5:1) at 170 °C (160 Watts), pyridine **127** was isolated in 98% yield following purification on silica; the highest ever yield reported for a Bohlmann-Rahtz reaction to date (**entry 4, Table 2-4**). Finally, in a bid to explore solventless reaction conditions, a mixture of enamine **126** and alkynone **124** was irradiated at 170 °C (150 Watts) for 20 minutes to give the product in 84% yield (**entry 5, Table 2-4**). Although this final experiment was not as efficient, the use of solventless reaction conditions does have some intrinsic ecological and chemical value.[173]

Table 2-4 Reaction under microwave-assisted conditions

Entry	Solvent	Reaction time (min)	Yield (%)[a]
1	Toluene	90	76
2	DMSO	20	87
3	Toluene–$ZnBr_2$ (15 mol%)	10	80
4	Toluene–acetic acid (5:1)	10	98
5	Neat	20	84

[a] Isolated yield of pyridine **127** after purification by column chromatography on silica

All of the microwave-assisted experiments facilitated both Michael addition and cyclodehydration in a single synthetic step and had generated the target pyridine **127** as a single regioisomer. Although the use of microwave irradiation had been successful, it was decided to investigate if traditional conductive heating methods could facilitate a similar one-pot transformation. The reaction of ethyl β-aminocrotonate (**126**) and 1-phenylprop-2-yn-1-one (**124**) (**Scheme 2-15**) was repeated in a range of different solvents in a sealed tube at 170 °C using an oil bath as an external heat source (**Table 2-5**) and the results were compared with the microwave-assisted reactions (**Table 2-4**). In almost all of the experiments, the microwave-assisted conditions gave the product in a higher yield, of particular relevance for the reaction conducted in toluene in the presence of 15 mol% of zinc(II) bromide (**entry 3, Table 2-4** and **2-5**), although in many instances comparable yields were obtained (**entries 2, 4** and **5, Table 2-4** and **2-5**). Only the solventless reaction (**entry 5, Table 2-4** and **2-5**) gave superior results in a Carius tube, making the microwave irradiation procedure the method of choice for a rapid one-pot entry to trisubstituted pyridines.

Table 2-5 Reaction in a sealed tube using conventional heating techniques

Entry	Solvent	Reaction time (min)	Yield (%)[a]
1	Toluene	90	54
2	DMSO	20	80
3	Toluene–$ZnBr_2$ (15 mol%)	10	33
4	Toluene–acetic acid (5:1)	10	95
5	Neat	20	93

[a] Isolated yield of pyridine **127** after purification by column chromatography on silica

Following the preliminary study of a microwave-assisted one-pot synthesis of pyridines, the heteroannulation of ethyl β-aminocrotonate (**126**) and 4-(trimethylsilyl)but-3-yn-2-one (**80**) was investigated under microwave-assisted conditions and using conventional heating techniques (**Scheme 2-16**). This alkynone has been shown to undergo spontaneous protodesilylation under traditional Bohlmann–Rahtz conditions,[158] giving trisubstituted pyridine **85** in preference to tetrasubstituted pyridine **86**, and so these experiments would explore the scope of our new one-pot reactions.

Scheme 2-16 One-pot heteroannulation of enamine 126 and butynone 80

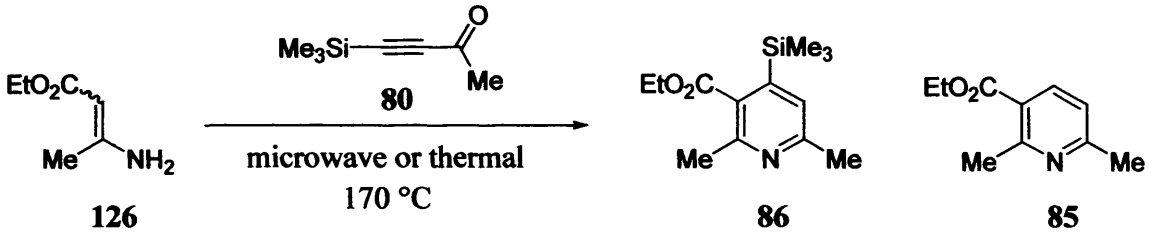


Table 2-6 Comparison of microwave-assisted conditions and conventional heating techniques for heteroannulation of enamine 130 and butynone 84

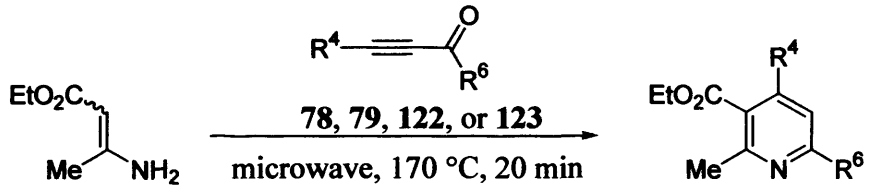
Entry	Solvent	Time (min)	Carius tube product (Yield%)[a]	Microwave product (Yield%)[a]
1	DMSO	90	**85** (21)	**85** (62)
2	DMSO	20	**85** (8)	**85** (69)
3	Toluene–$ZnBr_2$ (15 mol%)	10	**86** (27)	**86** (60)
4	Neat	10	None (0)	**86** (10)
5	Toluene–acetic acid (5:1)	20	**86, 85** [2.8:1][b] (29)	**86, 85** [2.8:1][b] (25)

[a] Isolated yield after purification by column chromatography on silica
[b] Ratio determined by ^1H NMR analysis of the crude mixture

When a mixture of ethyl β-aminocrotonate (**126**) and 4-(trimethylsilyl)but-3-yn-2-one (**80**) was irradiated at 170 °C in a range of different solvents (**Table 2-6**) pyridine **86** or **85** was formed, depending upon the solvent and catalyst employed. These transformations proved to be less efficient than the reactions of 1-phenylprop-2-yn-1-one (**124**) (**Table 2-4**) and the experiment conducted under solventless conditions (**entry 4, Table 2-6**) gave only a very meagre yield of pyridine **86**. However, in comparison with experiments conducted using conventional heating techniques, which were extremely inefficient with alkynone **80**, the microwave-assisted reactions were a success–the optimum experimental conditions involving irradiation of reagents in DMSO for 20 minutes to give trisubstituted pyridine **85** in 69% yield (**entry 2, Table 2-6**).

With the optimal microwave-assisted one-step pyridine synthesis reaction conditions in hand, we set out to establish the scope of this protocol. Ethyl β-aminocrotonate (**126**) was reacted with four other alkynyl ketones **78**, **79**, **122**, and **123** by irradiating a solution of the reactants in DMSO at 170 °C for 20 minutes (**Scheme 2-17**). In all of the experiments, a single regioisomeric pyridine was formed (**Table 2-7**). Although the efficiency of the reaction of ethyl β-aminocrotonate (**126**) and 4-phenylbut-3-yn-2-one (**79**) was low (**entry 1, Table 2-7**), this alkynone has been noted to be problematic in similar heteroannulation reactions.[157a] The other microwave-assisted reactions gave pyridine products **82**, **128**, and **129** in good yields after purification by column chromatography (**entries 2–4, Table 2-7**), illustrating that the one-pot microwave-assisted Bohlmann–Rahtz reaction represents a simple and highly-expedient route to tri- and tetrasubstituted pyridines.

Scheme 2-17 One-pot heteroannulation of enamine 126 and alkynones 78, 79, 122, and 123



126 **82, 83, 128, 129**

Table 2-7 Microwave-assisted synthesis of pyridines 82, 83, 128, and 129

Entry	Alkynone	R^4	R^6	Product	Yield (%)[a]
1	**79**	Ph	Me	**83**	24
2	**78**	Et	Me	**82**	94
3	**122**	H	4'-C_6H_4Cl	**128**	75
4	**123**	H	4'-C_6H_4OMe	**129**	66

[a] Isolated yield after purification by column chromatography on silica

2.3.4 Conclusion

Three new methods for the synthesis of tri- or tetrasubstituted pyridines were explored successfully by modification of the traditional Bohlmann-Rahtz pyridine synthesis using either Lewis acid catalysis or physical techniques.

In the case of Lewis acid catalysis with 10–100 mol% of zinc(II) bromide, ethyl β-aminocrotonate (**126**) reacted with 4-(trimethylsilyl)but-3-yn-2-one (**80**) to generate tetrasubstituted pyridine **86** as the only regioisomeric product in a single preparative step in 44–90% yield. Optimum yield was observed when the transformation was conducted in toluene at reflux in the presence of 15 mol% of zinc(II) bromide for 5 hours. Protodesilylation occured in none of these experiments, confirming that the trimethylsilyl moiety was stable under Lewis acid catalysed conditions.

Microwave irradition or conductive heating techniques in a sealed tube facilitated Michael addition of enamines to alkynones and subsequent spontaneous cyclodehydration of the aminodienone intermediates to generate tri- or tetrasubstituted pyridines in one pot in good to excellent yield with total control of regiochemistry. The reaction time of the Bohlmann-Rahtz reaction was reduced to as short as 10 minutes in both of the two new methods. The results of the comparative experiments indicated that the microwave-assisted Bohlmann-Rahtz pyridine synthesis was generally superior to synthesis in a Carius tube. The optimum conditions for the microwave-assisted one-pot synthesis of pyridines were established and typically involved irradiating the enamine and alkynone in DMSO at 170 °C for 20 minutes.

2.4 Expanding Bohlmann-Rahtz synthetic method

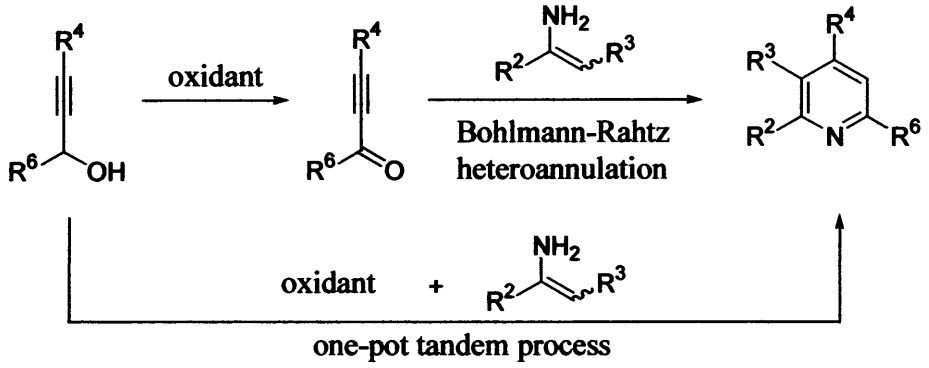
Our work on the modification of the traditional two-step Bohlmann-Rahtz heteroannulation reaction has led to the development of a number of new heteroannulation processes,[158,172] that proceed in good yield. The utility of this strategy for the preparation of nitrogen-containing aromatic heterocycles, especially polysubstituted pyridines, has been improved thereby. However, poor availability of the two Bohlmann-Rahtz starting substrates, enamines and alkynones, is a significant drawback of this reaction that prevents it reaching its full synthetic potential. To address the above shortcomings, new methods for one-pot synthesis of pyridines based on the Bohlmann-Rahtz reaction that use more readily available materials were investigated.

2.4.1 The *in situ* oxidation-Bohlmann-Rahtz reaction

2.4.1.1 Introduction

Facile one-pot transformations involving alcohol oxidation followed by *in situ* trapping of the resulting aldehyde have attracted considerable attention in recent years. Taylor has reported a number of tandem manganese dioxide mediated processes,[174] particularly *in situ* oxidation–Wittig methodology that avoids the need to isolate aldehyde intermediates that may be toxic, volatile or highly reactive. Similar procedures using a range of different oxidants, including *o*-iodoxybenzoic acid (IBX),[175] the Dess-Martin periodinane,[176] and barium manganate,[177] have been shown to be successful in a number of tandem processes for rapid access to different synthetic targets. In order to increase the scope of the Bohlmann-Rahtz heteroannulation reaction we set out to establish a whole new type of tandem process that was appropriate for the rapid synthesis of pyridines, thus preparing different heteroaromatic building blocks[178] from a single propargylic alcohol subset by *in situ* oxidation to alkynone and subsequent heteroannulation with the enamine in a single preparative step (**Scheme 2-18**).

Scheme 2-18 Tandem oxidation-heteroannulation of propargylic alcohols

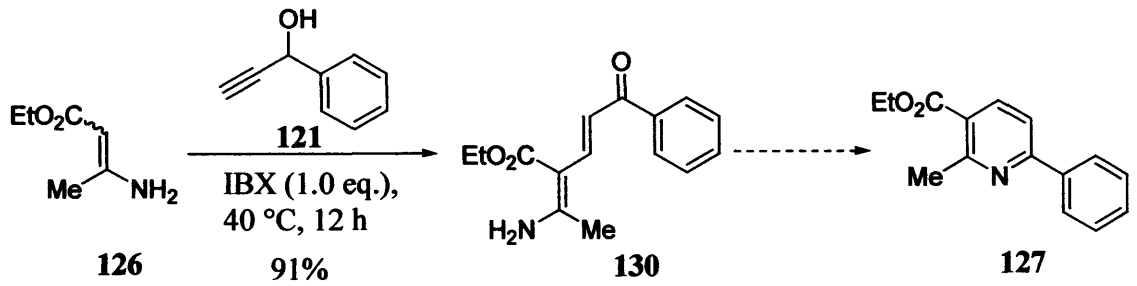


2.4.1.2 Initial studies

In order to examine if the tandem oxidation-heteroannulation of propargylic alcohols was viable as a new one-pot method for the synthesis of nitrogen-containing heterocycles, a mixture of 1-phenyl-2-propyn-1-ol (**121**), IBX and ethyl β-aminocrotonate (**126**) was heated to 40 °C in DMSO overnight. When equal equivalents of IBX and propargylic alcohol **121** were used in the reaction, aminoheptadienone **130** was isolated in 91% yield (**Scheme 2-19**). On the other hand, when any equivalents of IBX other than one with respect to alcohol **121** were used, either the intermediate **130** was isolated in poor yield or, in most cases, no desired intermediate **130** or pyridine product **127** was observed.

In view of the results from the preliminary study, the isolation of aminoheptadienone **130** in high yield looked promising for the development of new oxidation-Bohlmann-Rahtz tandem reactions. The reaction conditions shown in **Scheme 2-19** gave rise to the *in situ* oxidation of propargylic alcohol **121** to the corresponding ethynyl ketone **124** and the subsequent Michael addition of ethyl β-aminocrotonate **1** to form the Bohlmann-Rahtz intermediate **130**. Despite the fact that the initial work failed to promote the final cyclodehydration step to generate the expected pyridine **127**, our protocol indicated that modification of the reaction conditions would lead to the discovery of a tandem process for facile synthesis of polysubstituted pyridines.

Scheme 2-19 Test reaction of the new tandem process



2.4.1.3 A new one-pot tandem process

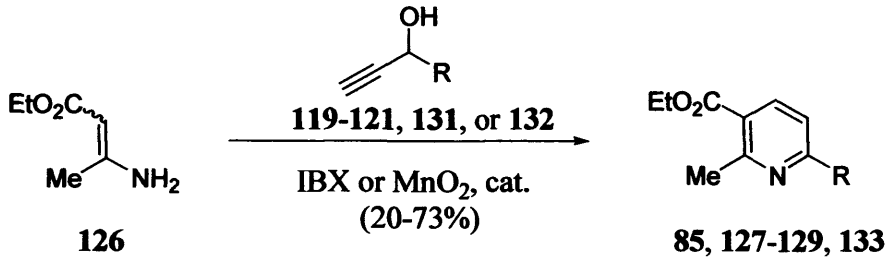
Propargylic alcohol **121** was reacted with ethyl β-aminocrotonate (**126**) in the presence of IBX or manganese dioxide (**Scheme 2-20**). Pyridine formation from 1-phenylprop-2-yn-1-one (**124**) traditionally requires very high temperatures and so these tandem processes were conducted in the presence of either a Brønsted or Lewis acid as a catalyst to facilitate cyclodehydration to the target heterocycles. A range of conditions were investigated (**Table 2-7**) and although *in situ* oxidation with MnO_2 and IBX gave comparable yields, the IBX mediated process was found to be superior for the synthesis of pyridine **127**. Optimum conditions involved heating enamine **126** and a one-fold excess of both the propargylic alcohol **121** and IBX in DMSO–acetic acid (5:1) at 65 °C overnight to give pyridine **127** in 70% isolated yield after purification on silica (**entry 2, Table 2-7**).

Table 2-7 Optimizing the synthesis of pyridine 127 by tandem oxidation-heteroannulation of 1-phenyl-2-propyn-1-ol (121)

Entry	Oxidant	**126**:**121**:Oxidant	Reaction conditions	Yield (%)[a]
1	IBX	1:1:1	DMSO–AcOH (5:1), 65 °C	54
2	**IBX**	**1:2:2**	**DMSO–AcOH (5:1), 65 °C**	**70**
3	IBX	1:2:2	DMSO–AcOH (5:1), 55 °C	69
4	IBX	1:2:4	DMSO–AcOH (5:1), 65 °C	24
5	MnO_2	1:2:10	PhMe–AcOH (5:1), 50 °C	45
6	MnO_2	1:2:10	$CHCl_3$–AcOH (5:1), reflux	23
7	MnO_2	1:2:10	PhMe, $ZnBr_2$ (20 mol%), reflux	64

[a] Isolated yield of pyridine **127** after purification by column chromatography on silica

Scheme 2-20 Test reaction of the new tandem process



126 85, 127-129, 133

With successful conditions established for the tandem process, a range of propargylic alcohols **119–121**, **131**, and **132** was submitted to *in situ* oxidation–heteroannulation with ethyl β-aminocrotonate (**126**) mediated by IBX in DMSO–acetic acid (5:1) at 65 °C (**Scheme 2-20**). It was found that the efficiency of the reaction was highly dependent upon the nature of propargylic alcohols, pyridine **85**, **127–129**, and **133** generated in between 20 and 73% yield (**Table 2-8**). Although the efficiency of the transformation was quite variable, in most instances it nonetheless compared quite favorably with traditional methods involving three separate preparative steps.

Table 2-8 One-pot synthesis of pyridines 85, 127–129, and 133 mediated by IBX

Entry	Propargylic alcohol	Product	R	Yield (%)[a]
1	**121**	**127**	Ph	70
2	**131**	**85**	Me	45
3	**132**	**133**	Et	20
4	**119**	**128**	4'-C_6H_4Cl	73
5	**120**	**129**	4'-C_6H_4OMe	53

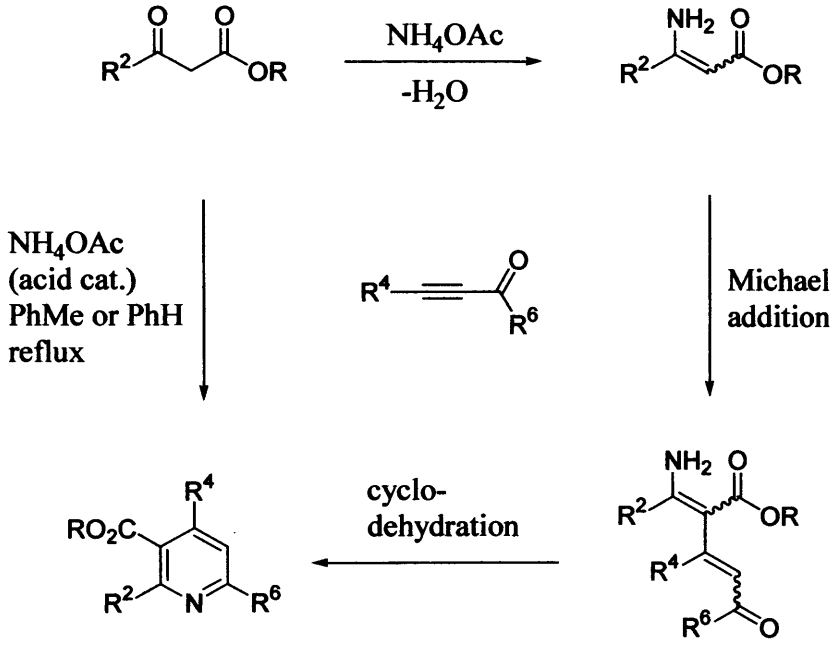
[a] Isolated yield after purification by column chromatography on silica

2.4.2 One-pot multicomponent Bohlmann-Rahtz reaction

A new tandem route for the construction of highly functionalized pyridines in one-pot from propargylic alcohols with greater general availability than the corresponding alkynones, the traditional Bohlmann-Rahtz precursor, had already been established. Then, our attention

turned to another weakness of the Bohlmann Rahtz reaction, the poor availability of enamine substrates, which also restricts the applicability of this reaction. Typically, enaminoesters are prepared from their corresponding β-ketoesters by reaction with ammonium acetate under acidic conditions (benzene–acetic acid) with azeotropic removal of water according to the procedure of Baraldi, *et al.*.[179] Thus, a one-pot method for the synthesis of pyridines from a β-ketoester, ammonia and an alkynone using a Brønsted or Lewis acid catalyst (see section **2.2.4.2**) was reported recently by our research group (**Scheme 2-21**),[159] which overcame the above-mentioned problems considerably. However, the relatively harsh conditions, high temperature and acid catalysis, involved in this newly disclosed three-component reaction reduces its synthetic utility, especially in the area of natural product synthesis. The complex structures and multiple functionality of many natural product targets demand mild and selective methods for their construction. Therefore, we set out to establish a new and mild multiple-component condensation reaction, which was applicable to the total synthesis of heterocyclic natural products.

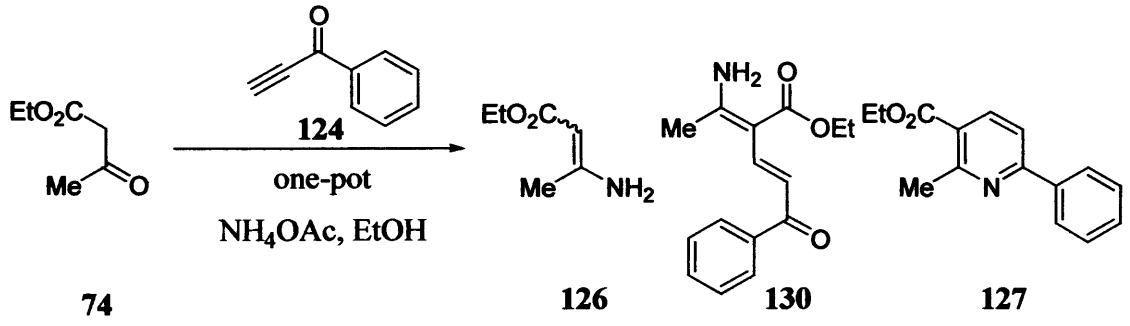
Scheme 2-21 Bohlmann-Rahtz and three-component pyridine synthesis



2.4.2.1 Initial optimisation study

In view of the facility of the Bohlmann-Rahtz pyridine synthesis in alcoholic solvents,[163,165] we first set out to explore a new three-component condensation process for the synthesis of pyridine heterocycles that avoided the use of an acid catalyst and high temperatures. To examine if a one-step three-component heteroannulation reaction was possible under these conditions, a mixture of ethyl acetoacetate (**74**), 1-phenylprop-2-yn-1-one (**124**), and ammonium acetate was stirred in ethanol under different reaction conditions (**Scheme 2-22**, **Table 2-9**). Reactions conducted at room temperature or at reflux with up to 4 equivalents of ammonium acetate did not go to completion, giving mixtures of pyridine **127**, aminodienone **130** and/or enamine **126** (**entry 1–3**, **Table 2-9**). However, when the reaction was performed at reflux with a large excess of ammonium acetate, pyridine **127** was isolated as the only reaction product in excellent yield, cyclodehydration occurring spontaneously under the reaction conditions without the use of an acid catalyst. The optimal system used an excess of ethyl acetoacetate (**74**), a large excess of ammonium acetate and heated the mixture at reflux for 24 hours (**entry 5**, **Table 2-9**).

Scheme 2-22 One-pot synthesis of pyridine 127 under mild conditions



74 **126** **130** **127**

Table 2-9 Optimizing the one-pot synthesis of pyridine 127

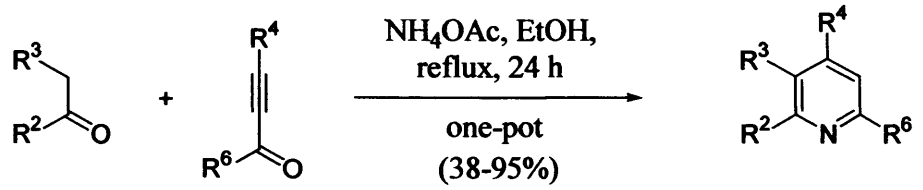
Entry	Ratio of **124/74**	NH_4OAc equivalents[a]	Temperature	Time (h)	Product (Yield%)[b]
1[d]	0.6	5	Room temperature	72	**130** (70) and **127** (23)
2[d]	1.0	2	Reflux	24	**126** and **127** [1:1][c]
3[d]	1.0	4	Reflux	24	**126** and **127** [1.4:1][c]
4[d]	1.0	10	Reflux	24	**127** (89)
5	0.6	10	Reflux	24	**127** (95)
6	1.7	10	Reflux	24	**127** (91)

[a] Equivalents of NH_4OAc with respect to β-ketoester **74**.
[b] Isolated yield after purification by column chromatography on silica.
[c] Ratio determined by ^1H NMR analysis of the crude mixture and yield not determined
[d] Experiments were carried out by Krishna Chapaneri.

2.4.2.2 The Scope of the reaction

In order to investigate the scope of this reaction, β-ketoesters **74** and **76** were reacted with alkynones **78–80**, and **122–124** in ethanol at reflux in the presence of one or ten equivalents of ammonium acetate (**Scheme 2-23**). In most experiments (**Table 2-10**), pyridines **82**, **83**, **85**, **88**, **127–129**, and **134** were generated in moderate to excellent yield (**entries 1–10**, **Table 2-10**, 38-95% yield) as the only regioisomeric product. When 4-(trimethylsilyl)but-3-yn-2-one (**80**) was used, only the protodesilylated pyridine **85** was obtained. Unfortunately, reactions carried out using a mixture of benzoylacetate **75** (R^2 = Ph), ammonium acetate and 1-arylprop-2-ynones **122–124** did not give the desired pyridines and instead produced the corresponding enamine, ethyl 3-amino-3-phenylpropenoate (**125**), and a number of side products with degradation of the alkynones. In spite of this, the reaction worked for a wide variety of substrates (**entries 1–10**, **Table 2-10**), and constitutes a mild method for the synthesis of polysubstituted pyridines.

Scheme 2-23 Examining the scope of a one-pot three-component reaction for the synthesis of pyridines 82, 83, 85, 88, 127–129, and 134



74, 76 **78-80, 122-124** **82, 83, 85, 88, 127-129, 134**

Table 2-10 The scope of a one-pot three-component reaction for the synthesis of pyridines 82, 83, 85, 88, 127-129, and 134

Entry	β-ketoester/ Equivalents[a]	Alkynone	R^2	R^3	R^4	R^6	Product (Yield%)[b]
1	**74**/0.1	**124**	Me	CO_2Et	H	Ph	**127** (95)
2	**74**/0.1	**122**	Me	CO_2Et	H	4'-C_6H_4Cl	**128** (84)
3	**74**/0.1	**123**	Me	CO_2Et	H	4'-C_6H_4OMe	**129** (90)
4[d]	**74**/0.1	**78**	Me	CO_2Et	Et	Me	**82** (38)
5[d]	**74**/1.0	**80**	Me	CO_2Et	TMS	Me	**85** (90)[c]
6[d]	**74**/0.1	**80**	Me	CO_2Et	TMS	Me	**85** (90)[c]
7[d]	**74**/1.0	**79**	Me	CO_2Et	Ph	Me	**83** (51)
8[d]	**76**/0.1	**124**	Me	$CO_2{}^tBu$	H	Ph	**134** (89)
9[d]	**76**/1.0	**78**	Me	$CO_2{}^tBu$	Et	Me	**88** (71)
10[d]	**76**/0.1	**78**	Me	$CO_2{}^tBu$	Et	Me	**88** (63)

[a] Equivalents of β-ketoester with respect to NH_4OAc
[b] Isolated yield after purification by column chromatography on silica.
[c] Only protodesilylated pyridine **85** (R^4 = H) was produced.
[d] Experiments were carried out by Krishna Chapaneri.

2.4.3 Combination of two approaches

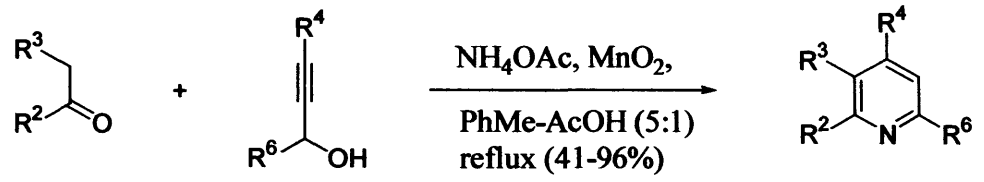
At this stage we were pleased that we had developed two efficient one-pot routes to highly functionalised pyridines, which addressed the availability of the two traditional Bohlmann-Rahtz precursors, enamines and alkynones. It was proposed ambitiously that a combination of the new tandem oxidation-heteroannulation process and the three-component pyridine synthesis[159] could lead to the discovery of an extremely facile procedure for one-pot

synthesis of pyridines starting from a β-ketoester and a propargylic alcohol. More importantly, it was postulated that the moderate yield of the tandem oxidation-Bohlmann-Rahtz reaction was a consequence of the oxidative degradation of enamine under the reaction conditions. Thus, generating enamine *in situ* by the condensation of a β-ketoester precursor and ammonia should reduce enamine degradation and increase the yield.

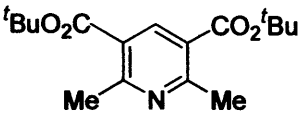
Fortunately, when a range of propargylic alcohols **119–121**, **131**, **132**, and **135**, β-ketoester **74–76**, and **136** and ammonium acetate was heated at reflux in toluene–acetic acid (5:1) in the presence of manganese dioxide (**Scheme 2-24**), pyridines **82**, **85**, **88**, **127–129**, **133**, **134**, and **137–141** were formed directly in reasonable to excellent yield (**Table 2-11**). It is worth noting that pyridine **128** and **129** were produced in 96% and 85% yield respectively (**entry 2** and **3, Table 2-11**) under the reaction conditions described in **Scheme 2-24**, a considerable improvement over the tandem oxidation-heteroannulation process (**entry 4** and **5, Table 2-8**) shown in **Scheme 2-20**. These findings gave support to our previous hypotheses.

To our surprise, a symmetrical pyridine **142** was obtained in 10% yield alongside the isolation of expected pyridine **88** in 60% yield (**entry 11, Table 2-8**). Pyridine **142**, hitherto unseen in the Bohlmann-Rahtz heteroannulation reaction, was identified by ^{1}H NMR spectroscopic analysis that showed the presence of a down-field singlet proton resonance at 8.46 ppm corresponding to the 4-H aromatic methine proton and two singlets, corresponding to the methyl groups at 2.73 ppm and *tert*-butyl groups at 1.54 ppm, respectively, which proved the symmetrical structure pattern of pyridine **142**. An ester carbonyl stretch at 1715 cm^{-1} was observed in the IR spectrum and mass spectrometry provided the parent MH^{+} ion at 308 *m/z*. Finally, comparison of characterization data with literature studies confirmed the structure undoubtedly.

Scheme 2-24 New three-component synthesis of pyridines from propargylic alcohols and β-ketoesters



74-76, 136 **119-121, 131, 132, 135** **82, 85, 88, 127-129, 133, 134, 137-141**



142

Table 2-11 The scope of a one-pot three-component reaction for the synthesis of pyridines 82, 85, 88, 127–129, 133, 134, and 137–141

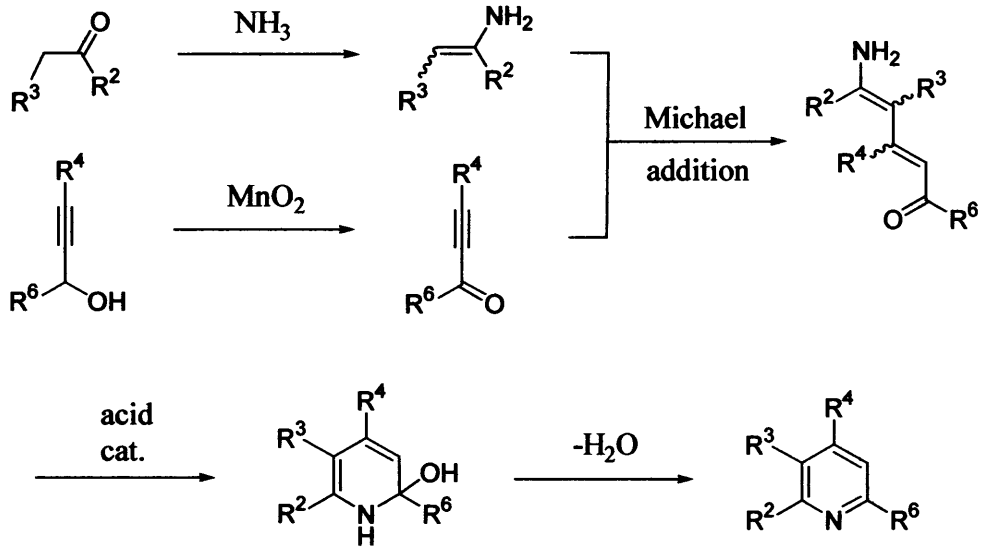
Entry	β-ketoester	Propargylic alcohol	R^2	R^3	R^4	R^6	Product (Yield%)[a]
1	**74**	**121**	Me	CO_2Et	H	Ph	**127** (42)
2	**74**	**119**	Me	CO_2Et	H	4'-C_6H_4Cl	**128** (96)
3	**74**	**120**	Me	CO_2Et	H	4'-C_6H_4OMe	**129** (85)
4	**74**	**131**	Me	CO_2Et	H	Me	**85** (66)
5	**74**	**132**	Me	CO_2Et	H	Et	**133** (49)
6	**74**	**135**	Me	CO_2Et	Et	Me	**82** (73)
7	**75**	**121**	Ph	CO_2Et	H	Ph	**137** (41)
8	**75**	**119**	Ph	CO_2Et	H	4'-C_6H_4Cl	**138** (63)
9	**75**	**120**	Ph	CO_2Et	H	4'-C_6H_4OMe	**139** (60)
10	**75**	**135**	Ph	CO_2Et	Et	Me	**140** (56)
11	**76**	**135**	Me	$CO_2{}^tBu$	Et	Me	**88** (60)[b]
12	**76**	**121**	Me	$CO_2{}^tBu$	H	Ph	**134** (73)
13	**136**	**121**	Ph	COSEt	H	Ph	**141** (58)

[a] Isolated yield after purification by column chromatography on silica.
[b] Symmetrical pyridine **142** was also isolated in 10% yield in this reaction.

This highly facile one-pot process presumably proceeds by *in situ* oxidation of propargylic alcohol and simultaneous formation of enamine, which is trapped by Michael addition to alkynone, double bond isomerization under the reaction conditions and subsequent cyclodehydration (**Scheme 2-25**), although the involvement of an alternative mechanistic

pathway that proceeds *via* Michael addition of the β-ketoester and alkynone prior to condensation with ammonia cannot be discounted.

Scheme 2-25 Proposed mechanistic course of the one-pot three-component synthesis of pyridines 6 from propargylic alcohols and β-ketoesters



2.4.4 Conclusion

Three novel methods for the one-pot synthesis of pyridines were developed successfully, including the *in situ* oxidation-heteroannulation of propargylic alcohols with either IBX or manganese dioxide, a new mild one-pot three-component condensation reaction and a practical combination of the above two approaches. These new developments extended the scope and versatility of the Bohlmann-Rahtz pyridine synthesis by providing a new one-pot tandem route to nitrogen-containing heteroaromatic building blocks, affecting up to four separate synthetic transformations in a single preparative step. Many of these heteroannulation reactions proceed in good yield and, for the synthesis of pyridines, with total regiocontrol from either enamine or β-ketoester precursors.

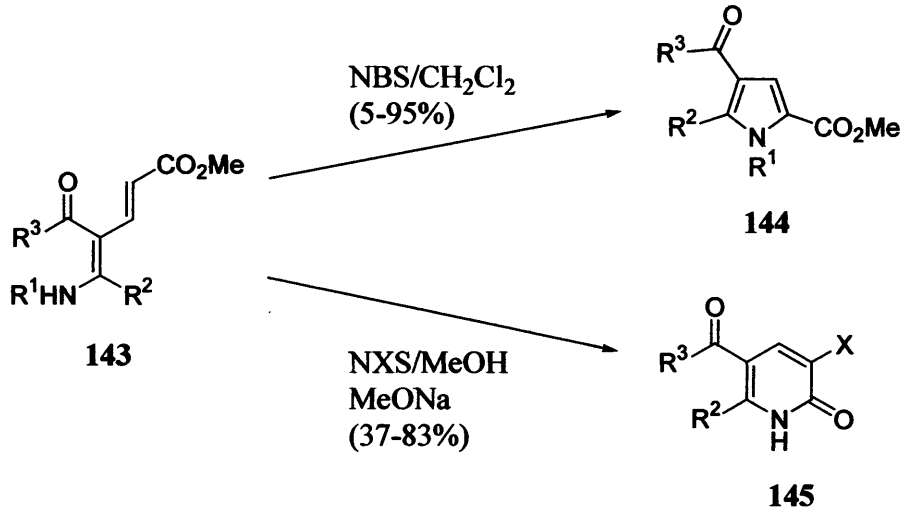
2.5 Synthesis of highly functionalised 5-bromopyridines

2.5.1 Introduction

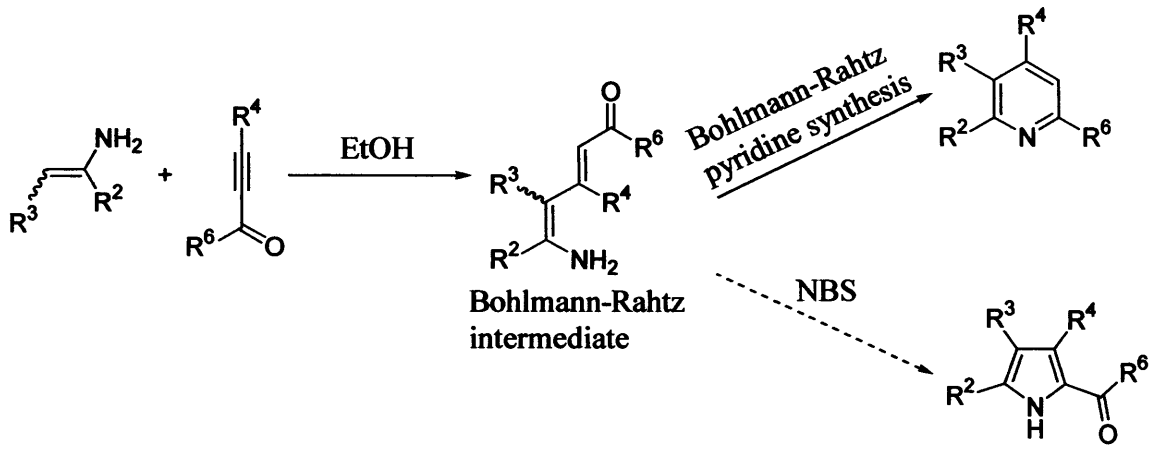
Our earlier work has described a series of new heteroannulation methods for the synthesis of 2,3,6-trisubstituted and 2,3,4,6-tetrasubstituted pyridines, which enlarged the scope of the Bohlmann-Rahtz reaction. We then focused on expanding the versatility of this reaction by way of introducing diversity in the target heterocycles accessible from these precursors and intermediates. Thus, we set out to investigate the bromocyclization of aminodienones isolated in the traditional two-step Bohlmann-Rahtz reaction.

Recently, Dechoux reported that when δ-dienaminoesters **143** were treated with *N*-bromosuccinimide (NBS) under neutral conditions, 1,2,3,5-tetrasubstituted pyrroles **144** were formed,[180] whereas under basic conditions treatment with *N*-halosuccinimides generated the corresponding 3-halo-2-1*H*-pyridones **145** in reasonable yield (**Scheme 2-26**).[181] Based upon this precedent, the NBS-mediated bromocyclization of Bohlmann–Rahtz intermediates, generated by a Michael addition, under neutral conditions was expected to provide a two-step route to pyrrole heterocycles (**Scheme 2-27**).

Scheme 2-26 Dechoux's synthesis of pyrroles 144 and pyridinones 145



Scheme 2-27 Proposed approach for pyrrole synthesis



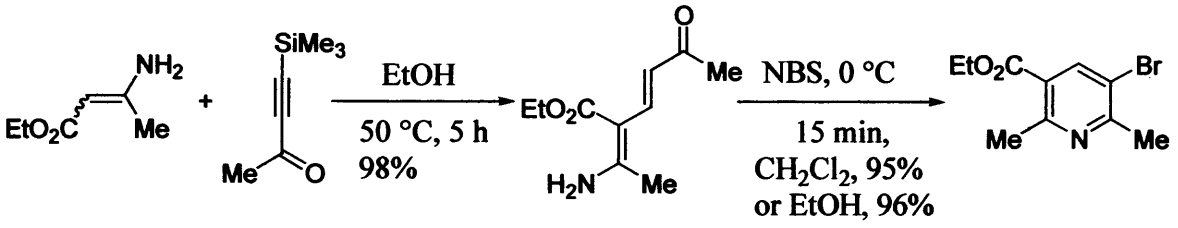
2.5.2 Method development with unexpected results

In order to examine the viability of the proposed bromocyclization, aminoheptadienone **130** was prepared according to the procedure reported previously by us.[158] Ethyl β-aminocrotonate (**126**) was heated in ethanol at 50 °C with an excess of 4-(trimethylsilyl)but-3-yn-2-one (**80**) for 5 hours to affect the conjugate addition with spontaneous protodesilylation, providing the corresponding dienone **130** in almost quantitative yield (98%). The bromocyclization of aminodienone **130** with NBS was investigated in CH_2Cl_2 at 0 °C according to the literature reported conditions.[180] However, surprisingly, no pyrrole could be isolated from the reaction mixture and instead facile bromination-cyclodehydration occurred to give the bromopyridine **151** in 95% yield. When the reaction was repeated in ethanol under the same reaction conditions, the bromopyridine **151** was isolated again in 96% yield (**Scheme 2-28**).

The product was identified by ^1H NMR spectroscopy showing the presence of the 4-H aromatic methine proton resonance at 8.23 ppm. IR spectroscopy indicated the presence of a single ester carbonyl stretch at 1725 cm^{-1} and C—Br stretch at 680 cm^{-1}. Analysis of the mass spectrum showed the appropriate isotope pattern for a mono-brominated compound,

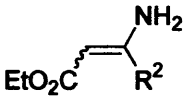
consisting of the lower and higher mass parent MH^+ isotope at 258 and 260 *m/z* respectively, with relative abundance in the ratio of approximately 1:1.

Scheme 2-28 Unexpected formation of bromopyridine 151

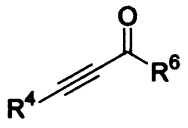


126 **80** **130** **151**

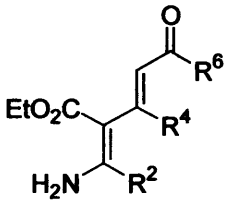
Scheme 2-29 Synthesis of 5-bromopyridines 151–156



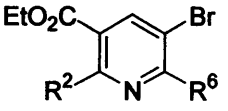
125 R^2 = Ph
126 R^2 = Me
+



EtOH, 50 °C (23-98%)



EtOH, NBS, 0 °C, 15 min, (83-98%)



124 R^4 = H, R^6 = Ph
123 R^4 = H, R^6 = 4-$MeOC_6H_4$
122 R^4 = H, R^6 = 4-ClC_6H_4
80 R^4 = $SiMe_3$, R^6 = Me

130 R^2 = Me, R^4 = H, R^6 = Me
146 R^2 = Me, R^4 = H, R^6 = 4-$MeOC_6H_4$
147 R^2 = Me, R^4 = H, R^6 = 4-ClC_6H_4
148 R^2 = Me, R^4 = H, R^6 = Ph
149 R^2 = Ph, R^4 = H, R^6 = Ph
150 R^2 = Ph, R^4 = H, R^6 = Me

151 R^2 = Me, R^6 = Me
152 R^2 = Me, R^6 = Ph
153 R^2 = Me, R^6 = 4-$MeOC_6H_4$
154 R^2 = Me, R^6 = 4-ClC_6H_4
155 R^2 = Ph, R^6 = Ph
156 R^2 = Ph, R^6 = Me

2.5.3 The Scope of the reaction

Having found interesting results from our initial experiments on the NBS-mediated bromocyclization of Bohlmann–Rahtz intermediates, the scope of this new bromination-heteroannulation procedure was investigated. A range of different aminodienones **130**, and **146–150** were prepared by standard methods and treated with NBS at 0 °C in ethanol for 15 minutes to give the 5-bromopyridines **151–156** in uniformly excellent yield (**Scheme 2-29**, **Table 2-11**).

Table 2-11 The scope of the new NBS-mediated synthesis of bromopyridines 151–156

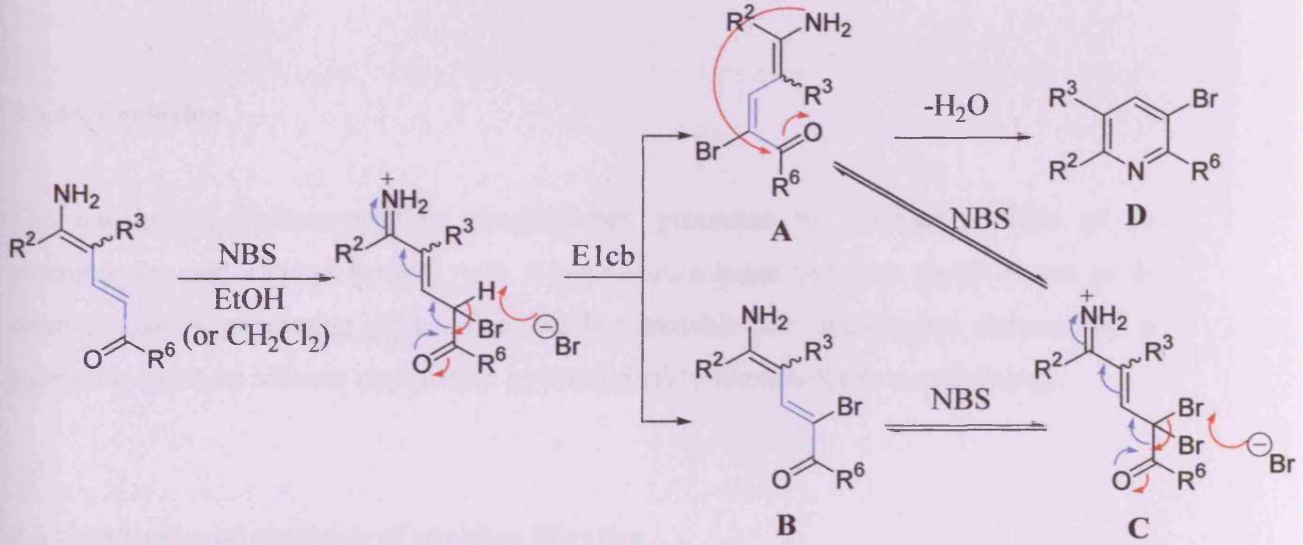
Entry	Enamine	Alkynone	Time[a] (h)	Dienone intermediate (Yield%)[b]	Product (Yield%)[b]
1	**126**	**124**	1	**130** (98)	**151** (96)
2	**126**	**123**	6	**146** (95)	**153** (88)
3	**126**	**122**	6	**147** (86)	**154** (89)
4	**126**	**80**	5	**148** (85)	**152** (92)
5	**125**	**124**	7	**149** (68)	**155** (83)[c]
6	**125**	**80**	3	**150** (23)	**156** (98)

[a] Reaction time of Michael addition.
[b] Isolated yield after purification by column chromatography on silica.
[c] Reaction was run at –10 °C to prevent formation of debrominated pyridine from spontaneous cyclodehydration of aminodienone **149**.

Considering the difficulty experienced in previous studies to facilitate the acid-catalyzed cyclodehydration of Bohlmann-Rahtz intermediates,[158] which proceeds under traditional uncatalyzed conditions often at temperatures in excess of 120 °C (and for certain substrates requires temperatures in the region of 200 °C),[156,164] this finding seemed startling since the reaction was complete after only 15 minutes at 0 °C. Specifically, we rationalized that the two possible geometrical isomers **A** and **B** from initial bromination of Bohlmann-Rahtz intermediates might readily engage in equilibrium *via* double brominated intermediate **C**. Spontaneous cyclodehydration of (4*E*)-hexadienone intermediate **A** under the reaction conditions pulled the equilibrium over towards pyridine **D**, which overcame the usually

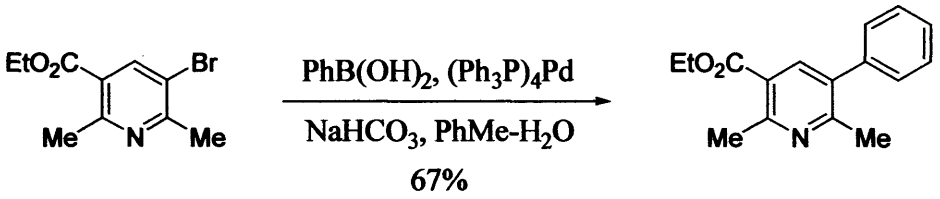
problematic double bond isomerization(**Scheme 2-30**). Cyclization to give the bromopyridine rather than the pyrrole product could be rationalized by considering the increased reactivity (and increased electrophilicity) of the ketone, compared to ester function, in the cyclodehydration (addition-elimination) mechanism.

Scheme 2-30 Proposed mechanistic course of bromocyclization



Finally, in an effort to demonstrate the applicability of the NBS bromocyclization methodology and access 2,3,5,6-tetrasubstituted pyridines with greater diversity in the nature of the 5-substituent, bromopyridine **151** was submitted to a Suzuki cross-coupling reaction with benzeneboronic acid. Heating a mixture of ethyl 5-bromo-2,6-dimethylpyridine-3-carboxylate **151**, benzeneboronic acid, tetrakis(triphenylphosphine)-palladium(0) and aqueous sodium carbonate solution to 80 °C for 4 hours in toluene gave 5-phenylpyridine **157** in 67% yield (**Scheme 2-31**).

Scheme 2-31 Suzuki coupling of 5-bromopyridine 151



151 **157**

2.5.4 Conclusion

The reaction of Bohlmann-Rahtz intermediates, generated by Michael addition of an enaminoester and ethynyl ketone, with *N*-bromosuccinimide provides facile access to 5-bromopyridines possessing latent functionality suitable for subsequent elaboration, a substitution pattern hitherto unavailable by traditional Bohlmann-Rahtz methodology.

2.6 Combinatorial synthesis of pyridine libraries

2.6.1 Introduction

The development of new methods for the synthesis of heterocyclic compound libraries, both in solution and on solid phase, is an ever-expanding area in combinatorial chemistry. The pyridine structural motif may be found in a large number of pharmaceutical agents with a diverse range of biological properties,[182] as a pharmacophore of considerable historical importance. Although there is a wide range of methods available for the synthesis of pyridines, very few of these procedures have been developed in combinatorial chemistry[183] and there is a great need for new, simple, and facile procedures that can incorporate a number of points of structural diversity and a variety of substitution patterns in the target pyridine library.

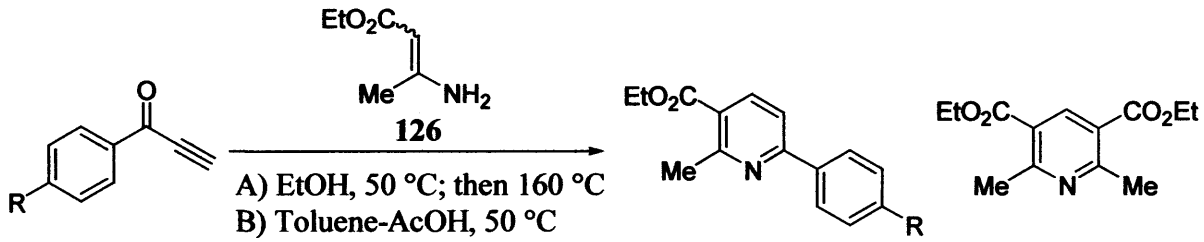
2.6.2 Combinatorial library synthesis

To examine the behavior of the different Bohlmann-Rahtz heteroannulation methods for the solution phase combinatorial synthesis of pyridine libraries, including the traditional method and the procedures developed in our laboratories,[158] ethyl β-aminocrotonate (**126**) was reacted with a mixture of three alkynones **122–124** (**Scheme 2-32**). These alkynones were predicted to have different reactivities in this process, and so this study would provide a valuable comparison between the available procedures. In three separate combinatorial reactions, ethyl β-aminocrotonate (**126**) was reacted with a mixture of 1-phenylprop-2-yn-1-one (**124**), 1-(4-chloro)phenylprop-2-yn-1-one (**122**), and 1-(4-methoxy)phenylprop-2-yn-1-one (**123**) using either traditional Bohlmann-Rahtz conditions (method A), stirring in acetic acid-toluene (method B), or the Lewis acidcatalyzed heteroannulation process (method C). The pyridine products **127–129** were isolated following an acid-base workup, which was found to be superior for these compounds according to the results from a previous investigation into the purification of pyridine libraries.[166] Analysis by ^{1}H NMR spectroscopy and comparison to the spectra of the pure pyridines isolated in previous studies determined the product ratios by integration of pyridine 4-H or 5-H resonances and library purity by reference to a known quantity of tetramethylsilane as an internal standard according to established methodology (this method of purity determination was further validated by integration of all impurities and, in all cases, was found to be reliable).

In comparing the heteroannulation procedures, the traditional Bohlmann-Rahtz reaction (method A) resulted in the formation of pyridines **127–129**, although it was alkynone **122** that seemed to behave poorly in the heteroannulation reaction leading to a product ratio (R) that varied between $1 < R < 3.7$. Using acid-catalyzed conditions, the overall yield was lowered and product ratios increased although in the zinc(II) bromide-catalyzed heteroannulation reaction a very high library purity was obtained. Curiously, these reactions also gave rise to a new pyridine product **158**, hitherto unseen in the Bohlmann-Rahtz heteroannulation reaction, and identified by ^{1}H and ^{13}C NMR spectroscopic analysis, high- and low-resolution mass spectrometry, and comparison of characterization data with

literature studies. Although surprising, this did serve to increase the diversity of the pyridine library and must have been generated by the hitherto unreported degradation of ethyl β-aminocrotonate under the reaction conditions (**Table 2-12**).

Scheme 2-32 Combinatorial synthesis of pyridines 127–129, and 158



124 R = H
122 R = Cl
123 R = OMe

127 R = Ph
128 R = ClC_6H_4
129 R = $MeOC_6H_4$

158

Table 2-12 Comparison of Bohlmann-Rahtz reaction for the combinatorial synthesis of pyridines 127–129, and 158

Entry	Method	Products	Overall yield (%)	Ratio	Library purity (%)
1	A	**127–129**	53	89:27:100	78
2	B	**127–129, 158**	43	89:10:100:5	74
3	C	**127–129, 158**	38	92:17:100:28	94

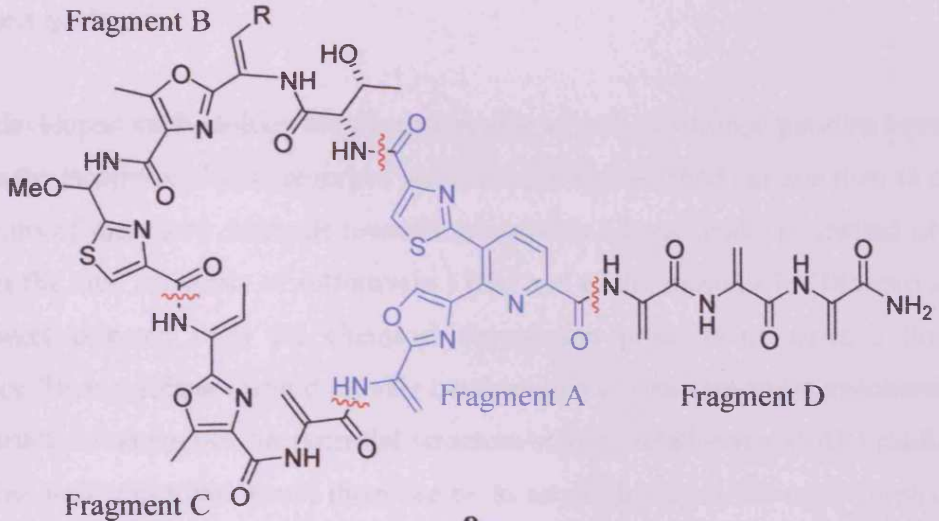
2.6.3 Conclusion

In all of the reactions investigated, a complete pyridine library was generated with high levels of purity using a simple acid-base extraction workup procedure. Product ratios varied depending upon the heteroannulation method used and alkynone structure. Thus, the Bohlmann-Rahtz heteroannulation reaction was successfully developed as a valuable tool for the combinatorial synthesis of pyridine libraries in solution.

Chapter Three

Results and Discussion

Figure 3-1 Shin's disconnection of sulfomycin I (8a)



8a

3.2.1.1 Kelly's work: total synthesis of dimethyl sulfomycinamate

In 1995, Kelly reported the first total synthesis of dimethyl sulfomycinamate (**13**) in 11 steps and 7% overall yield.[35] Palladium-catalyzed cross-coupling reactions were adopted to form the heterobiaryl bonds and attach the oxazole and thiazole rings onto the pyridine 2- and 3-positions, respectively. The total synthesis started from commercially available 5-hydroxy-2-methylpyridine (**159**); bromination using bromine in pyridine gave **160** regioselectively in 77% yield, and methylation of **160** by iodomethane and K_2CO_3 in acetone at reflux gave methyl ether **161** in 88% yield. Oxidation of **161** followed by esterification provided methyl ester **163** in 65% yield over two steps. Then bromopyridine **163** was mixed with $AlCl_3$ to give pyridinol **164** cleanly in 93% yield, which was converted to triflate **165** in 97% yield using Tf_2O and 2,6-lutidine. Stille coupling between bromotriflate **165** and 1 equivalent of vinylstannane **166** proceeded in a highly selective manner to afford **167** in 97% yield. Reaction of **167** with NBS and water in THF provided bromoketone **168** directly in 95% yield. Condensation of **168** with methacrylamide (**169**) gave oxazole **170**, which was submitted to OsO_4 and $NaIO_4$-mediated oxidation to furnish **171** in 85% yield. The whole sequence was concluded by the second palladium-catalyzed cross-coupling between triflate

3. Synthetic Studies towards The Series *d* Thiopeptide Natural Products

3.1 Project goals

Having developed methodology for the preparation of polysubstituted pyridine heterocycles based on the Bohlmann-Rahtz reaction, we subsequently switched our attention to synthetic applications of these new methods towards thiopeptide natural products. Instead of directly aiming at the total synthesis of sulfomycin I (**8a**) and cyclothiazomycin (**18**), two synthetic targets were selected from the chemical degradation products of series *d* thiopeptide antibiotics. Their syntheses would provide confirmation of structure and stereochemistry and access structural analogues for potential structure-activity relationship (SAR) studies in the future. The next milestone would therefore be to establish a route for rapid construction of the heterocyclic central domain of series *d* thiopeptides.

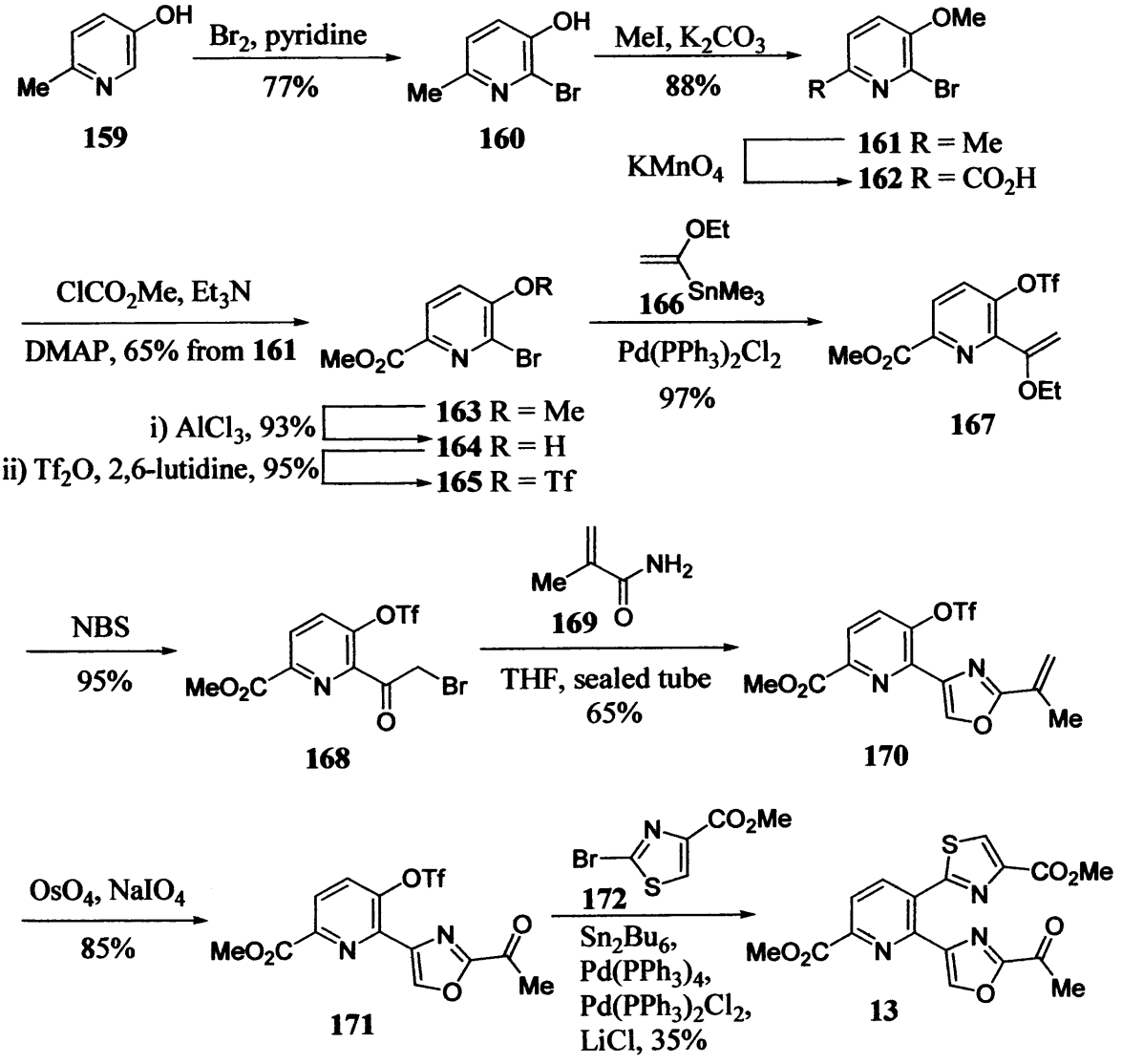
3.2 Progress towards total synthesis of the sulfomycins

3.2.1 Previous synthetic work towards the sulfomycins

With fascinating biological activities and a complex macrocyclic structure (see section **1.3.3.1**), the sulfomycins (**8**) have attracted considerable interest from organic chemists around the world since their first isolation from *Streptomyces viridochromogenes* by Egawa *et al.* in 1969.[29] Although there has been no total synthesis of the sulfomycins (**8**) published to date, syntheses of various fragments have been reported, including Kelly's synthesis of dimethyl sulfomycinamate (**13**),[35] the degradation product from acidic methanolysis of sulfomycin I (**8a**) (**Scheme 1-2**),[34] and Shin's syntheses of both the central heterocyclic domain (fragment A in **Figure 3-1**)[145] and the main tridehydropentapeptide skeleton (fragments B and C in **Figure 3-1**).[140]

171 and bromothiazole 172 to give dimethyl sulfomycinamate (13) in 35% yield (**Scheme 3-1**).

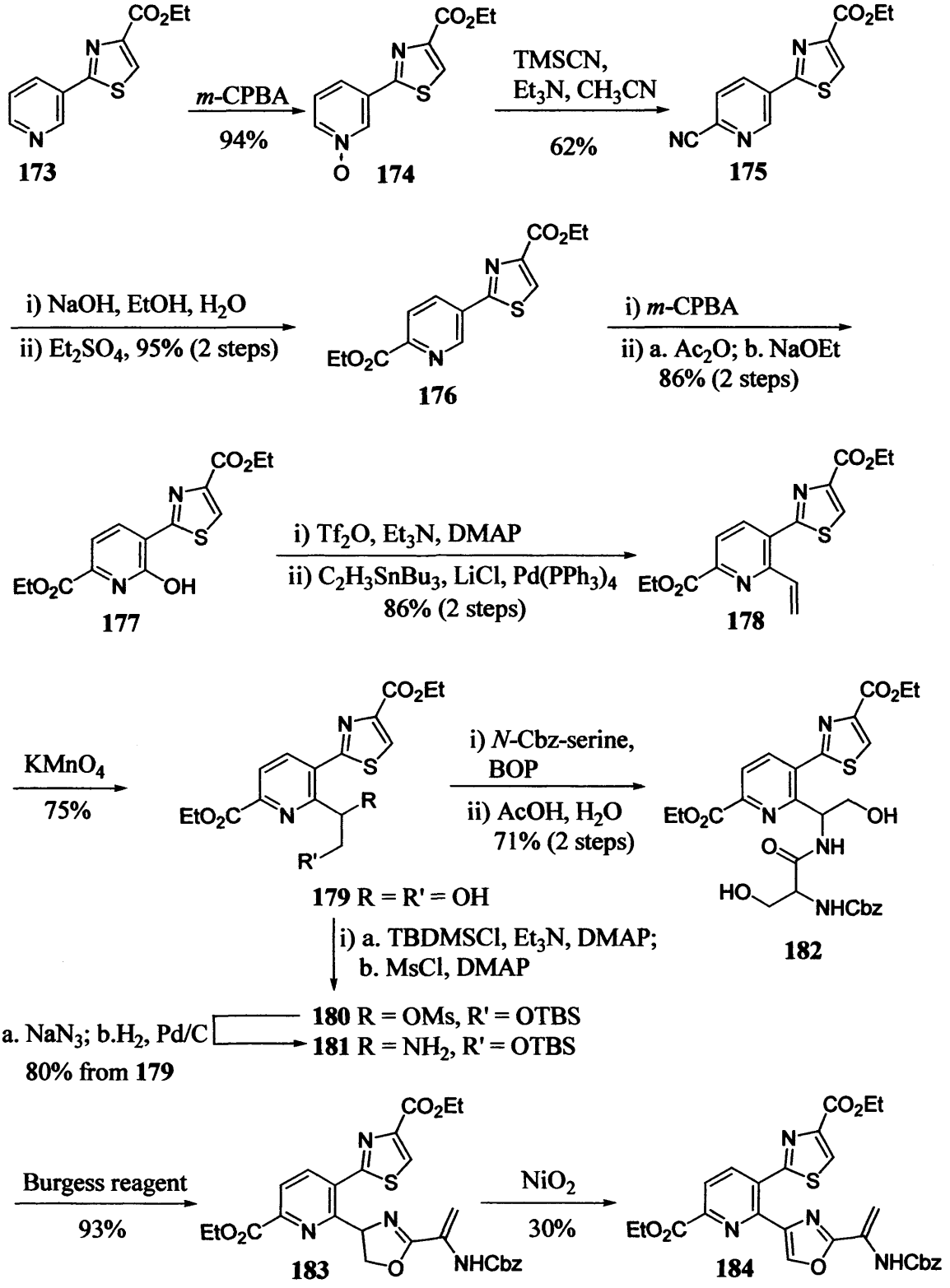
Scheme 3-1 Kelly's total synthesis of dimethyl sulfomycinamate (13)



3.2.1.2 Shin's work: syntheses of fragments of sulfomycin I

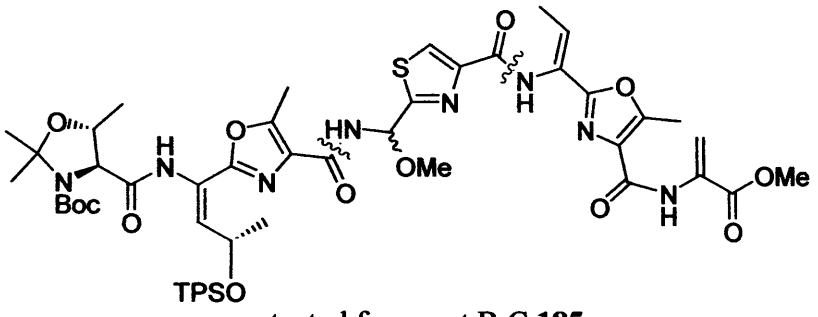
Shin and co-workers have made significant progress towards the total synthesis of sulfomycin I (**8a**). According to Shin's relevant reports, sulfomycin I (**8a**) was divided into four fragments A, B, C, and D by disconnection of four peptide bonds within the whole structure (**Figure 3-1**).[140] The synthesis of the central heterocyclic domain (fragment A) (**184**), which is the common structure shared by a number of thiopeptide families, such as A10255 (**25**) (**Figure 1-7**), the berninamycins (**27**) (**Figure 1-9**) and promoinducin (**32**) (**Figure 1-14**), was accomplished in 17 steps and 4.8% overall yield *via* stepwise introduction of groups into the 2- and 6-position of thiazolylpyridine **173**.[145] The oxidation of **173** with *m*-chloroperbenzoic acid gave the corresponding *N*-oxide **174**, and then treatment of **174** with trimethylsilyl cyanide provided 6-cyanopyridine **175** with reasonable regioselectivity in 62% yield. After conversion of the cyano group to an ethoxycarbonyl functionality by successive treatment with sodium hydroxide in aqueous methanol and diethyl sulfate, the 2-hydroxyl group was introduced into pyridine **176** using the same strategy as the previous introduction of 2-cyano substituent to afford hydroxypyridine **177**. Activation of the 6-position of pyridine **177** as the triflate allowed the following palladium-mediated coupling reaction with vinyltributyltin to give pyridine **178** in 86% yield. Dihydroxylation of the vinyl group of **178** with $KMnO_4$ gave the corresponding diol **179** in 75% yield. Pyridine **182** was obtained through a number of transformations from pyridine **179** including protection of the primary hydroxyl group, *O*-mesylation of the secondary hydroxyl group, azidation, reduction, condensation with *N*-benzyloxycarbonyl-L-serine, and deprotection. Amide **182** was then treated with Burgess reagent to afford oxazoline **183**, which was finally oxidized to the target molecule **184** in 30% yield (**Scheme 3-2**).

Scheme 3-2 Shin's synthesis of the central heterocyclic core 184 of sulfomycin I (8a)

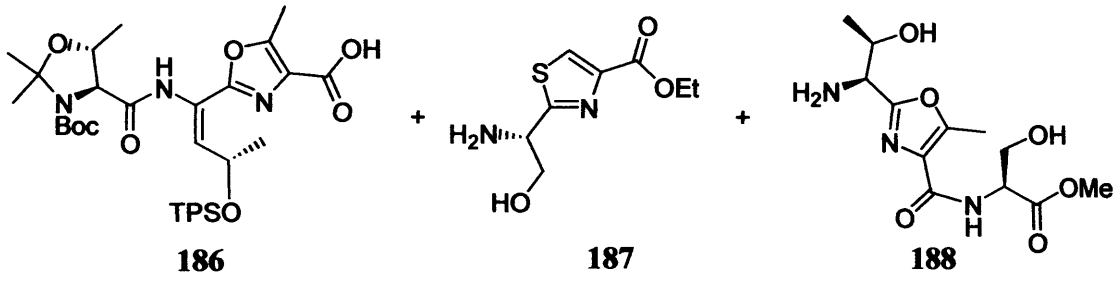


CARDIFF UNIVERSITY

Scheme 3-3 Shin's retrosynthetic analysis of protected fragment B-C 185



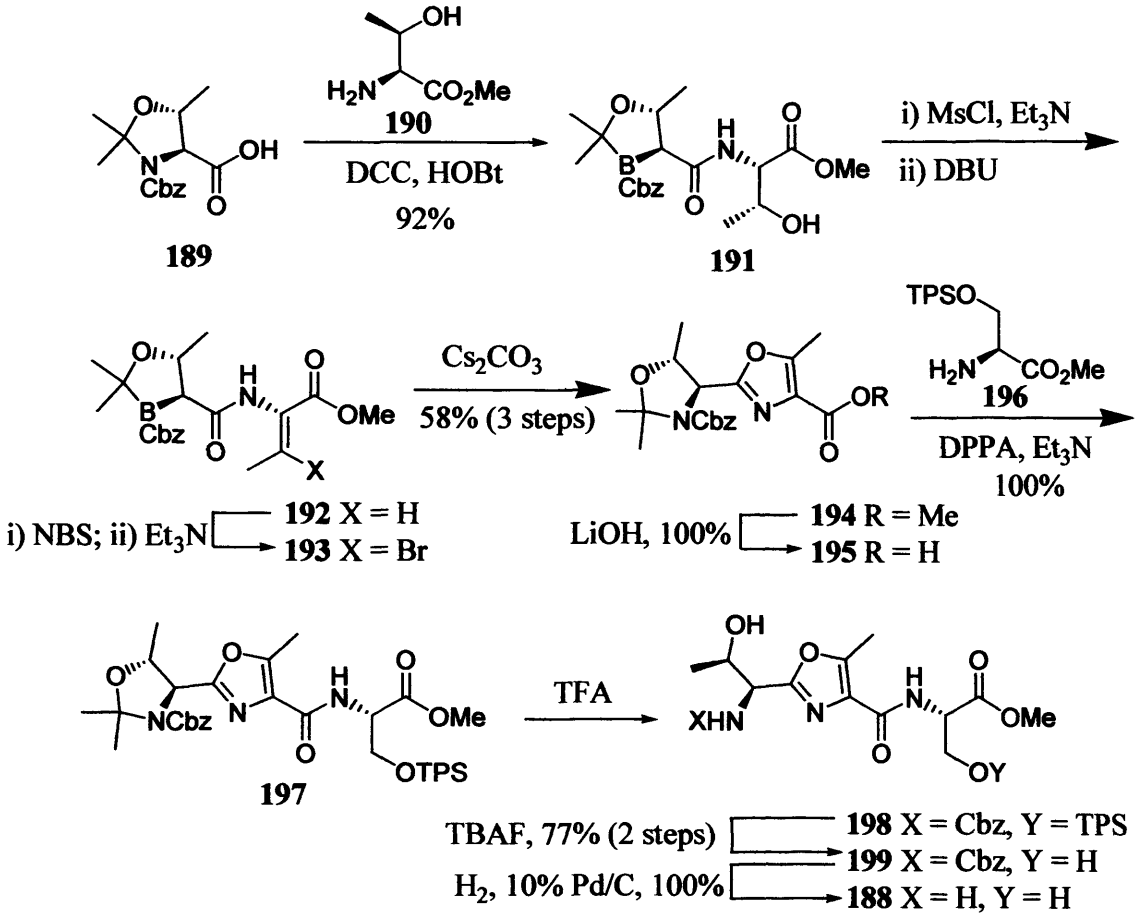
protected fragment B-C **185**



186 **187** **188**

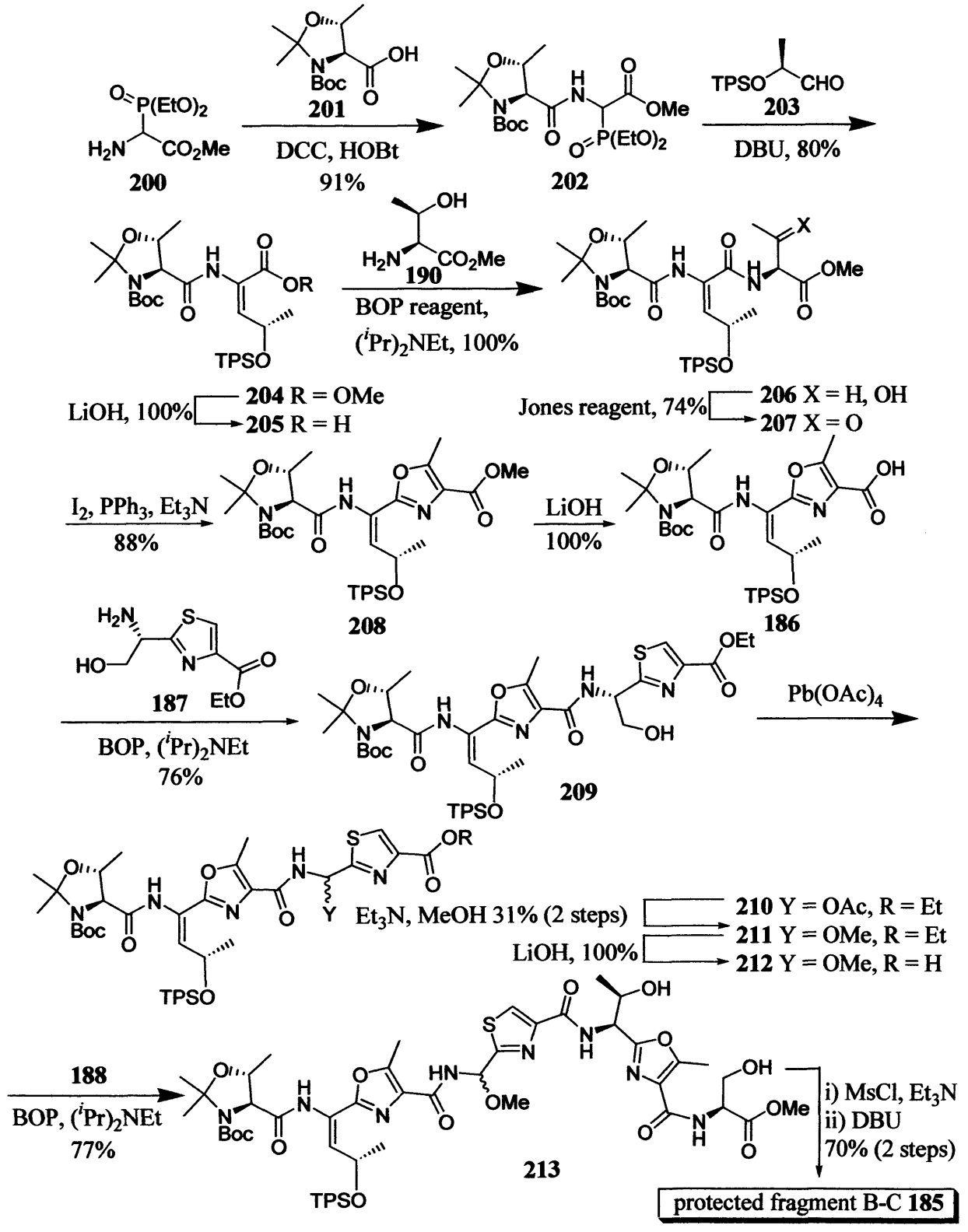
The main peptide skeleton (fragments B and C) was assembled in a highly convergent manner.[140] Retrosynthetic cleavage of the protected fragment B-C **185** at the two indicated peptide bonds led to three heterocyclic derivatives **186**, **187**, and **188** (**Scheme 3-3**), which were coupled together at the final stage of the synthetic process. The construction of oxazole **188** began from the formation of dipeptide **191** by coupling the threonine derivative **189** with **190**. At this point, *O*-mesylation of **191** was followed by DBU-mediated elimination to give alkene **192**, which was brominated using NBS and subsequently treated with Cs_2CO_3 to provide oxazole **194** in 58% overall yield. Ester hydrolysis of **194** gave the corresponding acid **195**, which was coupled with *O*-triphenylsilylserine methyl ester (**196**) using diphenyl phosphorazidate and Et_3N to afford peptide **197** in quantitative yield. Finally, three sequential deprotection reactions, effected by the use of trifluoroacetic acid, tetrabutylammonium fluoride, and palladium on carbon, respectively, provided the expected segment **188** in 77% yield from **197** (**Scheme 3-4**).

Scheme 3-4 Shin's synthesis of building block 188



The synthesis of another oxazole containing building block **186** commenced with condensation of phosphorylglycine **200** and *N*-Boc-*N*,*O*-isopropylidene-L-threonine (**201**) to give dipeptide **202** in 91% yield. Subsequently, Horner-Wadsworth-Emmons reaction between phosphonate **202** and aldehyde **203** afforded the expected product **204** in 80% yield. Alkaline hydrolysis of ester **204** using LiOH was followed by another peptide coupling between the produced acid **205** and L-threonine methyl ester **190** to provide β-hydroxyamide **206**, which was oxidized to the corresponding ketone **207** in 74% yield. Cyclodehydration of **207** mediated by I_2 and PPh_3 in the presence of Et_3N gave the desired oxazole **208** in 88% yield. Finally, the methyl ester of **208** was hydrolyzed quantitatively to give compound **186** with free carboxylic acid functionality ready for the following transformations (**Scheme 3-5**).

Scheme 3-5 Completion of Shin's synthesis of fragment B-C 185



With all necessary building blocks in hand, operations began to join these units into the target molecule **185**. Firstly, coupling of **186** with ethyl 2-(1-amino-2-hydroxyethyl)thiazole-4-carboxylate (**187**) gave a larger fragment **209** in 76% yield. Then, the hydroxymethylene group of peptide **209** was converted to acetate group using $Pb(OAc)_4$ to give the intermediate **210**, which was methoxylated using methanol in the presence of Et_3N to provide the desired product **211** as a mixture of two diastereoisomers in a 1:1 ratio. Ester hydrolysis of **211** gave the corresponding carboxylic acid **212** quantitatively, which was condensed with fragment **188** mediated by BOP reagent and $(^{i}Pr)_2NEt$ to afford **213** in 77% yield. Finally, the target compound **185**, derived from fragment B-C of sulfomycin I (**8a**), was achieved in 70% overall yield *via* double dehydration using standard mesylation-elimination procedure from **213** (**Scheme 3-5**).

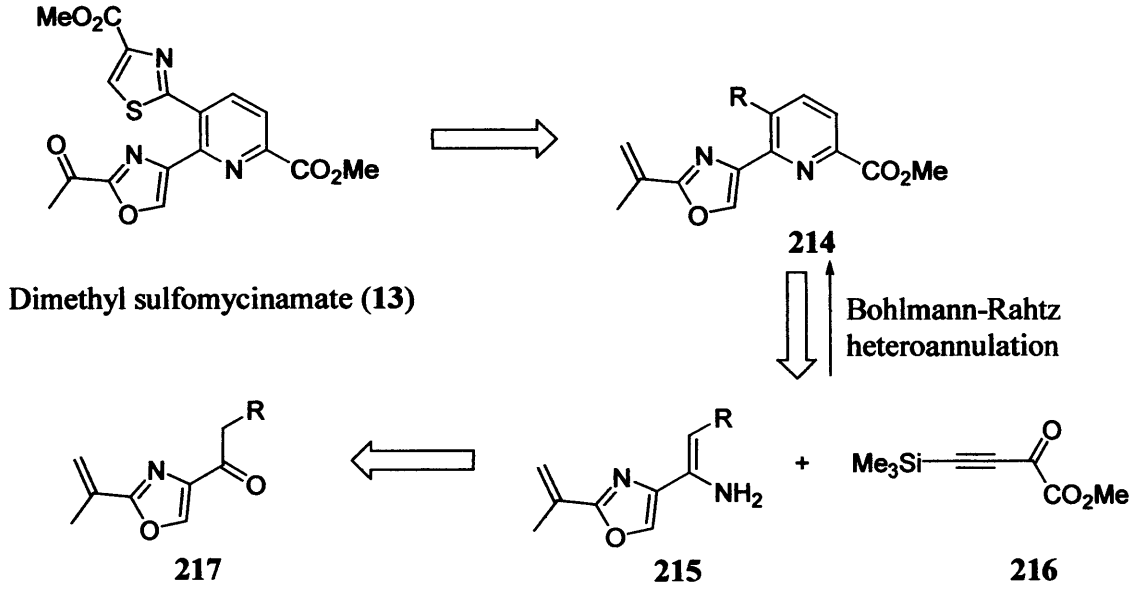
3.2.2 Total synthesis of dimethyl sulfomycinamate

The previous synthetic studies towards the sulfomycins (**8**) carried out by other research groups, especially the syntheses of the heterocyclic central domain using palladium-catalyzed cross-coupling strategy[35] and stepwise introduction of substituents into the pyridine based starting material,[145] reported by Kelly and Shin respectively, demonstrated some diversity in approach for the construction of the sulfomycins (**8**) central pyridine core. However, both of their approaches suffered from low versatility and efficiency and employed transition metal mediated processes. In order to find a facile and more environmentally-benign route to the pyridine domain of series *d* thiopeptide antibiotics, dimethyl sulfomycinamate (**13**) was selected as a viable target molecule to test the feasibility of our approach towards a series of structurally-related pyridine based thiopeptide natural products.

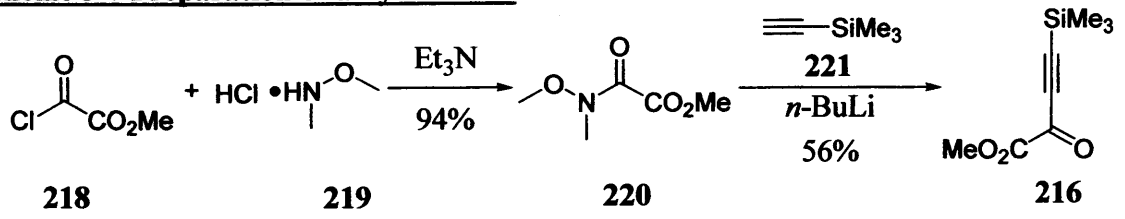
3.2.2.1 Retrosynthetic analysis

Our synthetic plan for accessing dimethyl sulfomycinamate (**13**) hoped to establish pyridine **214** by the Bohlmann-Rahtz reaction of methyl 2-oxo-4-(trimethylsilyl)but-3-ynoate (**216**) and a suitable enamine **215**, already bearing the thiazole substituent (-R) and prepared from oxazolyl ketone **217** by reaction with ammonia (**Scheme 3-6**).

Scheme 3-6 Dimethyl sulfomycinamate (13) disconnective scheme



Scheme 3-7 Preparation of alkynone 216



3.2.2.2 Preparation of alkynone

Based on the retrosynthetic analysis outlined in **Scheme 3-6**, the synthetic approach towards dimethyl sulfomycinamate (**13**) began with the preparation of methyl 2-oxo-4-(trimethylsilyl)but-3-ynoate (**216**), the required starting material for Bohlmann-Rahtz synthesis of the key pyridine intermediate **214**.

Alkynone **216** was readily prepared in two steps. Methyl oxalyl chloride (**218**) condensed with *N,O*-dimethylhydroxylamine hydrochloride (**219**) in the presence of Et_3N to give intermediate **220** in 94% isolated yield as a colourless oil. Subsequent reaction of the Weinreb amide **220** and lithiated trimethylsilylacetylene (**221**) provided the expected product **216** in 56% yield (**Scheme 3-7**).

3.2.2.3 Model studies

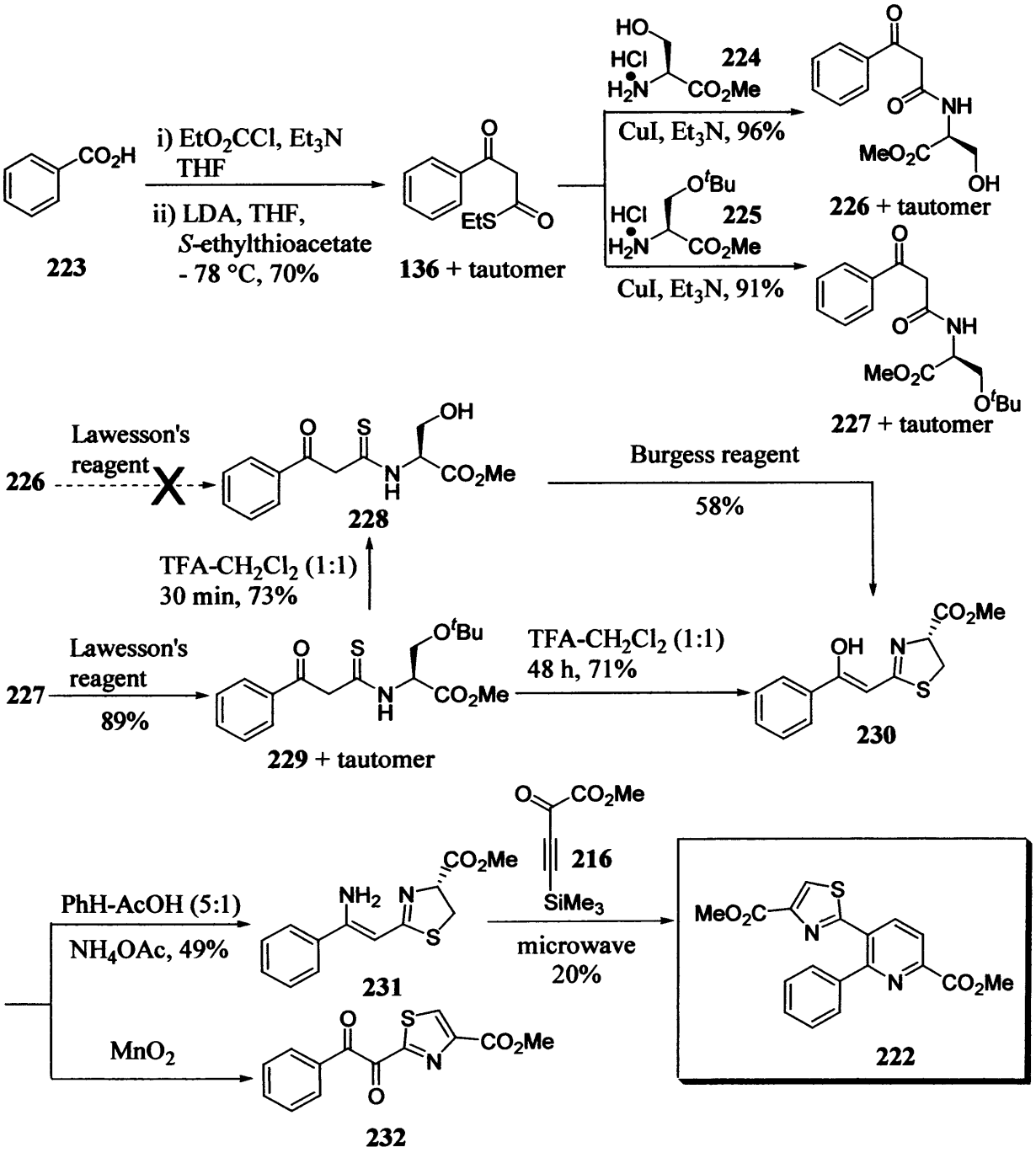
For convenience and to avoid unnecessary waste, we designed a model target compound **222** for initial investigation and started the synthetic exploration from readily available starting materials.

3.2.2.3.1 First approach

The model studies began with the synthesis of β-ketothioester **136** prepared as an isomeric mixture of ketone and enol form in equilibrium in 70% yield from commercially available benzoic acid (**223**) by homologation with *S*-ethyl thioacetate according to the procedure of Olsen.[184] Coupling of **136** with either L-serine methyl ester (**224**) or *O-tert*-butyl-L-serine methyl ester (**225**) in the presence of CuI then yielded β-ketoamide **226** or **227** in 96% or 91% yield, respectively. Thionation of **226** using Lawesson's reagent failed to give the

expected thioamide **228** presumably due to the unprotected hydroxyl group. On the other hand, using the *O*-protected version **227** gave the corresponding thioamide **229** in 89% yield.

Scheme 3-8 First approach towards the model compound 222



Treatment of **229** with TFA for 30 minutes generated not only **228** in 73% yield but also a side product in significant amount, the structure of which was confirmed to be the cyclysed thiazoline **230**, when the identical compound was obtained from Burgess reagent-mediated cyclization of β-hydroxythioamide **228**. Accordingly, resubmission of **229** to the same deprotection conditions for prolonged reaction time (48 hours) gave thiazoline **230** directly in 71% yield, eliminating one step from the whole synthetic sequence. An attempt to dehydrogenate thiazoline **230** with MnO_2 did not give the corresponding thiazole but provided the dicarbonyl compound **232**, the ^{13}C NMR of which contained two deshielded quaternary carbon signals at 190.7 and 185.0 ppm representing the two adjacent carbonyl groups. Thus, changing the plan to conduct the dehydrogenation at a later stage, enamine **231** was prepared from **230** using ammonium acetate in 49% yield. A number of modified Bohlmann-Rahtz reaction conditions failed to effect pyridine formation from enamine **231** and alkynone **216**, except the microwave irradiation conditions that provided the model compound **222** directly in 20% yield (**Scheme 3-8**).

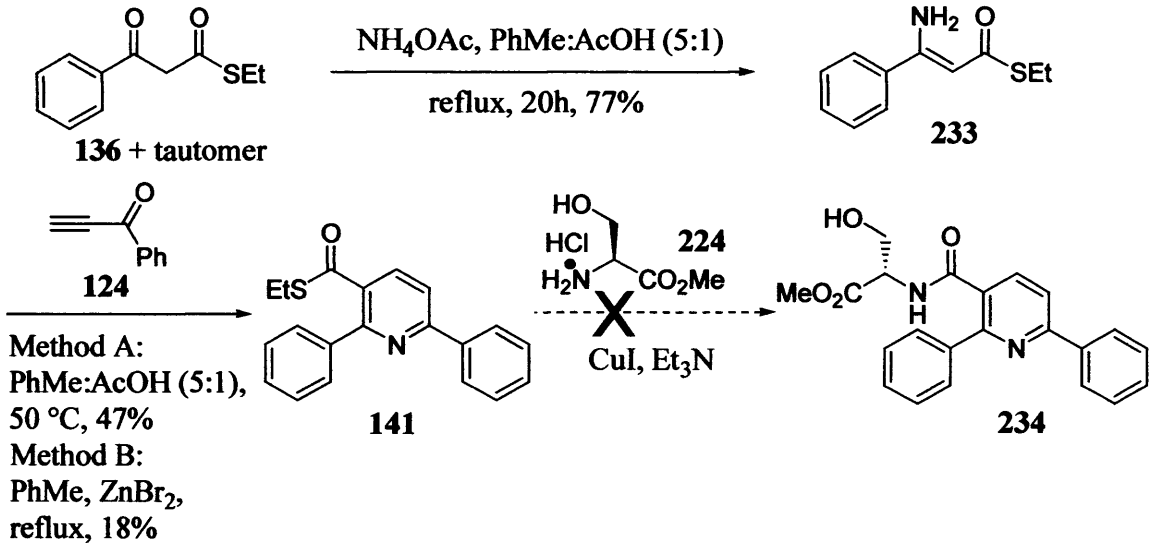
3.2.2.3.2 Revised approach

Although the initial approach towards model pyridine **222** looked positive (**Scheme 3-8**), low yields and problematic transformations meant, it was not ideal for the total synthesis of dimethyl sulfomycinamate (**13**), and so an alternative strategy for the preparation of 3-thiazolylpyridine **222** was considered. It was proposed that the pyridine skeleton could be formed at an early stage of the synthesis in order to avoid handling relatively sensitive intermediates, such as compound **230** and **231**, which lowered the overall yield dramatically.

With the above considerations in mind, a number of possible new routes for the synthesis of model compound **222** were investigated. Firstly, a model pyridine **141** with 3-ethylthio carbonyl substituent was prepared by either acetic acid-catalysed or zinc(II) bromide-catalysed Bohlmann-Rahtz reactions between alkynone **124** and enamine **233**, generated by treatment of β-ketothioester **136** with ammonium acetate. However, CuI-mediated coupling

of serine derivative **224** to pyridine **141** failed to provide the advanced pyridine **234** containing a serine residue that would later serve as the 3-thiazole ring precursor, and only unreacted starting materials were recovered instead (**Scheme 3-9**).

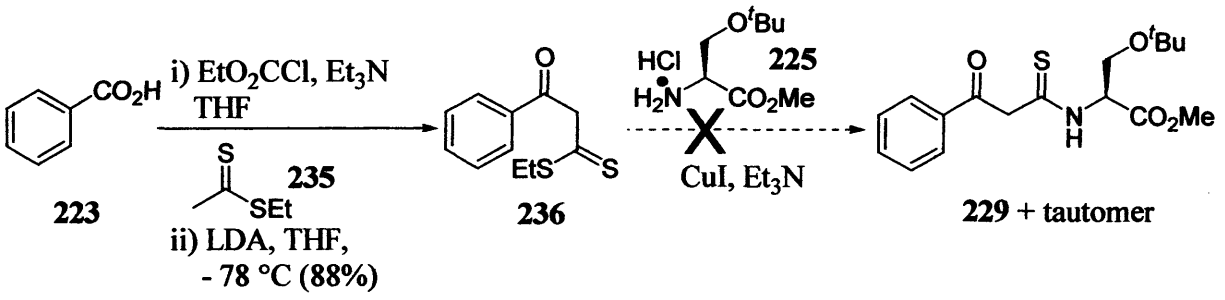
Scheme 3-9 synthetic efforts to pyridine 234



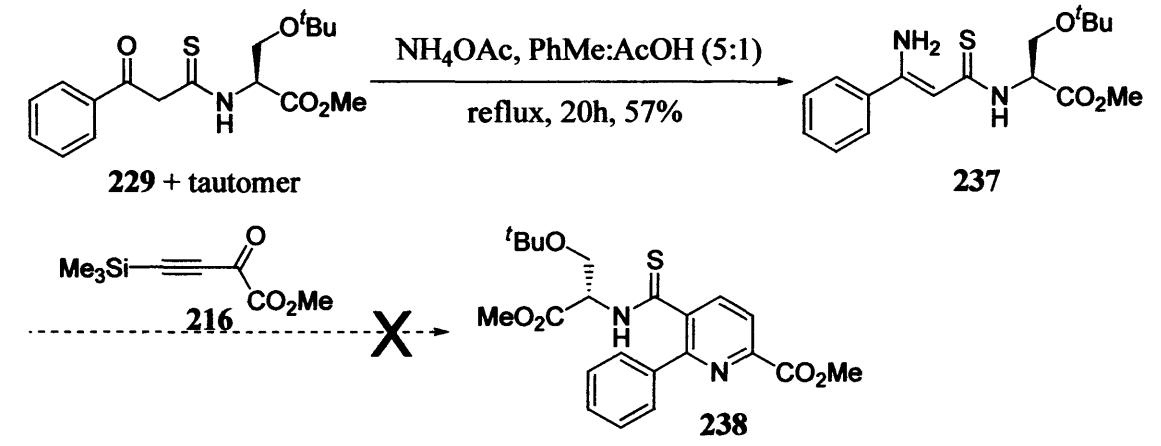
The next attempt was to construct pyridine **238** that had a *O-tert*-butyl serine residue in place for further elaboration to the target molecule **222**. In order to examine the viability of such a sequence starting from β-ketothioamide **229**, efforts were first directed towards the concise synthesis of key precursor **229**. As shown in **Scheme 3-10**, condensation of benzoic acid (**223**) with commercially available ethyl dithioacetate (**235**) using Olsen's procedure gave β-ketodithioester **236** in 88% yield. The subsequent CuI-mediated coupling of **236** with *O-tert*-butyl-L-serine methyl ester (**225**) would provide desired β-ketothioamide **229** in two steps from benzoic acid (**223**). Disappointingly, the reaction did not proceed and gave only unreacted starting materials, which meant the preparation of **229** required the thionation step in accordance with our original three-step process (**Scheme 3-8**). Even more discouragingly, although the required Bohlmann-Rahtz precursor, enamine **237**, was formed in reasonable yield by reaction of β-ketothioamide **229** with ammonium acetate in toluene–acetic acid, the

following heteroannulation failed to give pyridine **238** under a number of different reaction conditions (**Scheme 3-11**).

Scheme 3-10 synthetic efforts to thioamide 229

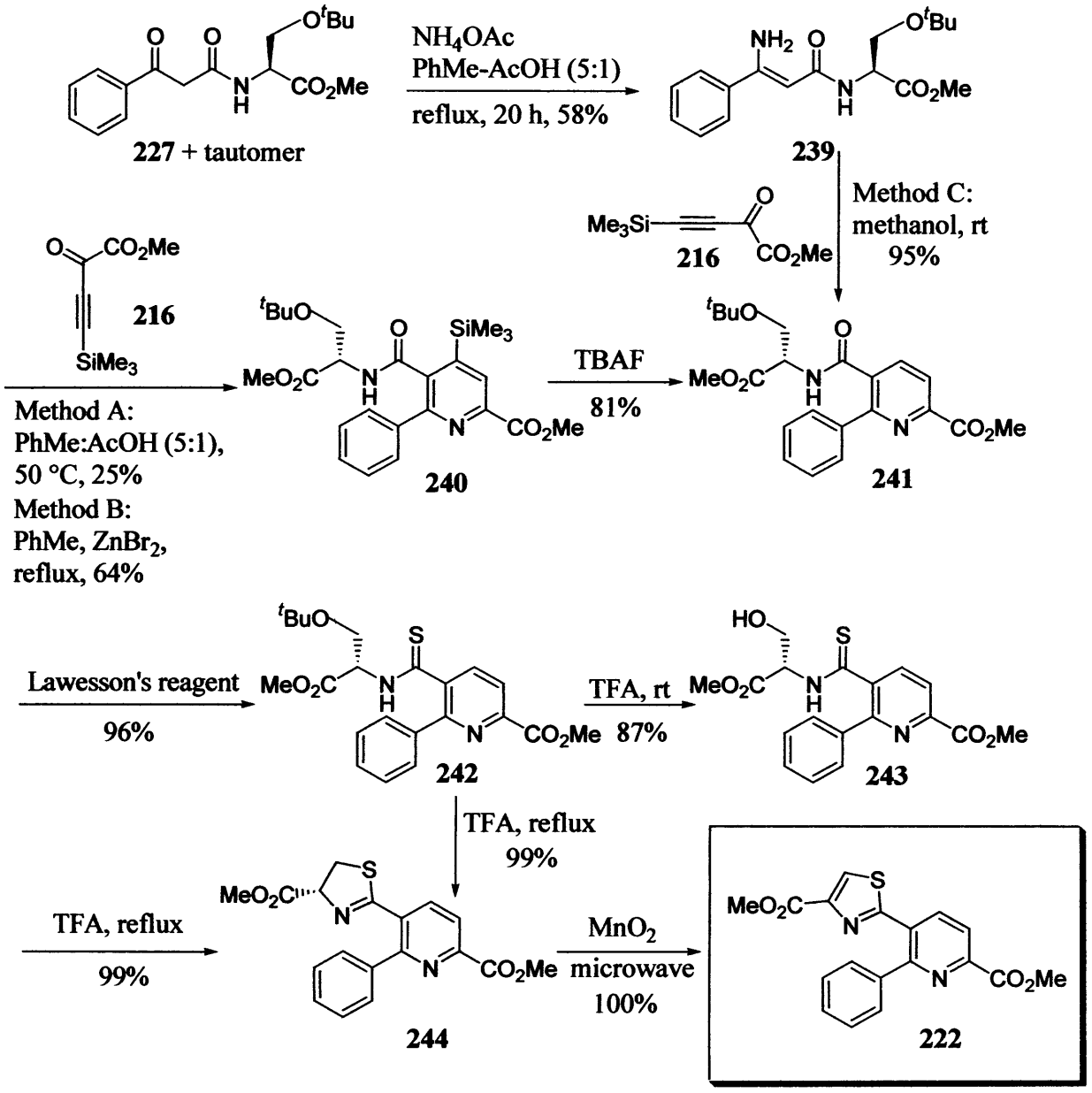


Scheme 3-11 synthetic efforts to pyridine 238



As the above-mentioned attempts at the preparation of pyridines **234** and **238** were unsuccessful, another possible pathway towards model compound **222** was investigated. A mixture of β-ketoamide **227** and its enol tautomer was converted into the corresponding enamine **239** using ammonium acetate in 58% yield. The subsequent key reaction of enamine **239** with alkynone **216** proved to be quite challenging after a number of modified Bohlmann-Rahtz methods failed to generate the desired pyridine. Only acetic acid and Lewis acid catalysed reaction conditions gave C-4 trimethylsilyl substituted pyridine **240** in 25% and 64% yield, respectively. Thionation of amide **240** using Lawesson's reagent was unsuccessful, presumably because of steric hindrance by the bulky TMS group. On the other hand, treatment of amide **241**, which was initially produced from pyridine **240** by protodesilylation using TBAF in 81% yield, with Lawesson's reagent gave the corresponding thioamide **242** in 96% yield. This result complicated our plan of global deprotection to remove the TMS group at the same time as the *tert*-butyl group and would lead to a longer synthesis than expected. However, stirring a solution of enamine **239** and alkynone **216** in methanol at room temperature facilitated Michael addition and spontaneous

Scheme 3-12 Second approach towards the model compound 222



cyclodehydration to give protodesilylated pyridine **241** directly in excellent yield. This surprisingly effective construction of the pyridine skeleton provided a solid foundation for a new synthetic approach towards 3-thiazolylpyridines. Subsequently, cleavage of *tert*-butyl ether of **242** was followed by spontaneous cyclization in one-pot in TFA at reflux to give thiazoline **244** quantitatively *via* intermediate **243**, that could be isolated by treatment of **242**

with TFA at room tempertature. Finally, thiazoline **244** was easily oxidized to model compound **222** using manganese dioxide under microwave irradiation in quantitative yield.

3.2.2.4 The total synthesis

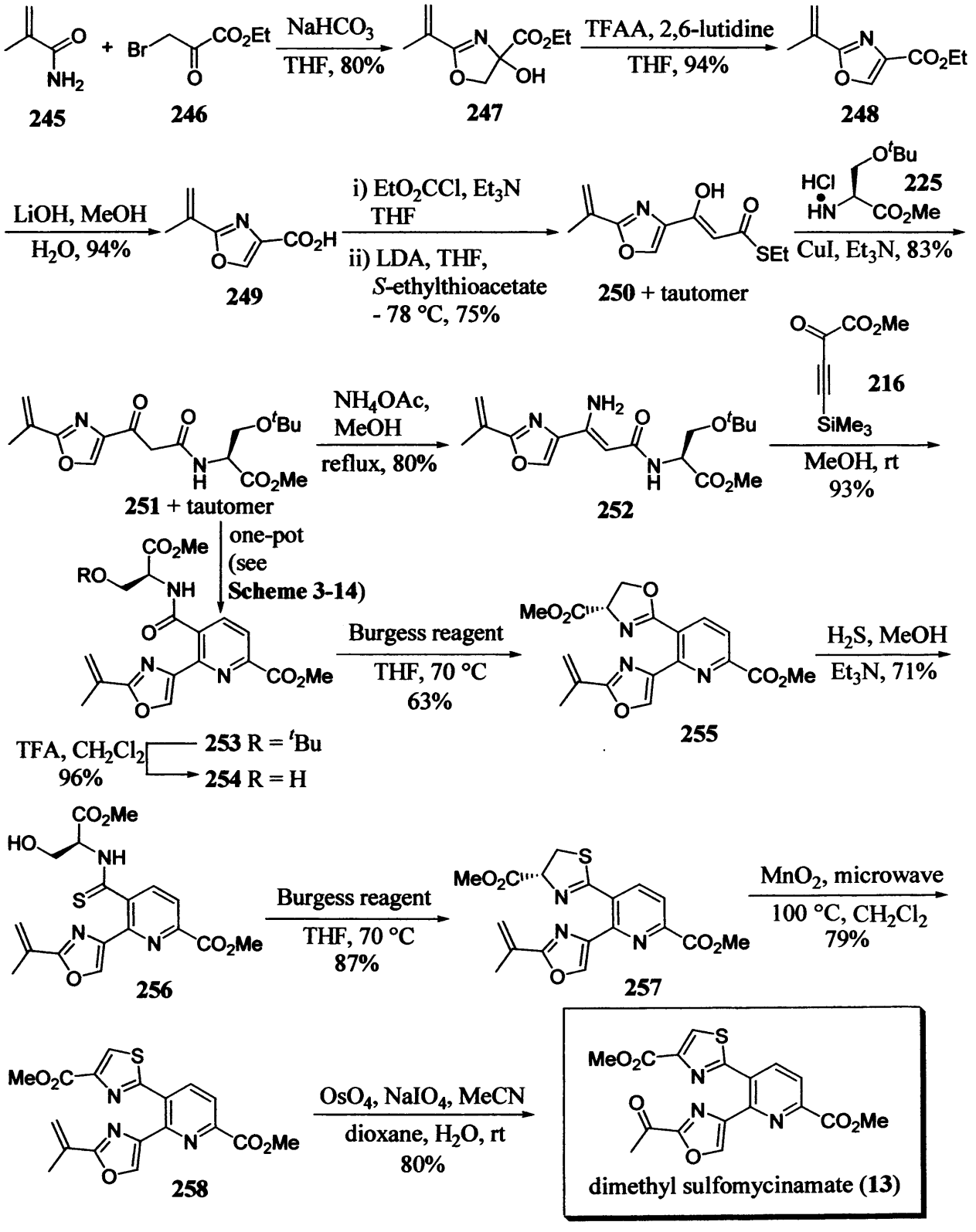
With success in the model study using the newly modified synthetic route, the task turned to extending the same strategy to the construction of dimethyl sulfomycinamate (**13**), the real target molecule. Starting from 2-methacrylamide (**245**), oxazole **248** was prepared in excellent yield *via* a two-step modified Hantzsch reaction with ethyl bromopyruvate (**246**), in which the condensation was performed under basic conditions with subsequent hydroxythiazoline dehydration using a mixture of TFAA and 2,6-lutidine. Then, the ethyl ester of **248** was hydrolysed with lithium hydroxide in methanol-water to produce the corresponding carboxylic acid **249** as a colorless solid in 94% yield (**Scheme 3-13**).

Having effected the synthesis of **249**, related to the corresponding model starting material benzoic acid (**223**), the critical phase of our synthetic plan towards dimethyl sulfomycinamate (**13**) could now be fully tested. Thus, as shown in **Scheme 3-13**, carboxylic acid **249** was treated with ethyl chloroformate under basic conditions and homologated by reaction with the lithium enolate of *S*-ethyl thioacetate to give *S*-ethyl 3-hydroxypropenoate **250** in equilibrium with its keto tautomer in 75% yield. Thiolate displacement with *O-tert*-butyl-L-serine methyl ester hydrochloride in dichloromethane in the presence of copper(I) iodide and triethylamine generated amide **251** as a mixture of tautomers that was heated at reflux overnight in methanol in the presence of ammonium acetate to give the Bohlmann-Rahtz precursor, enamine **252**, as a single tautomer in 80% yield.

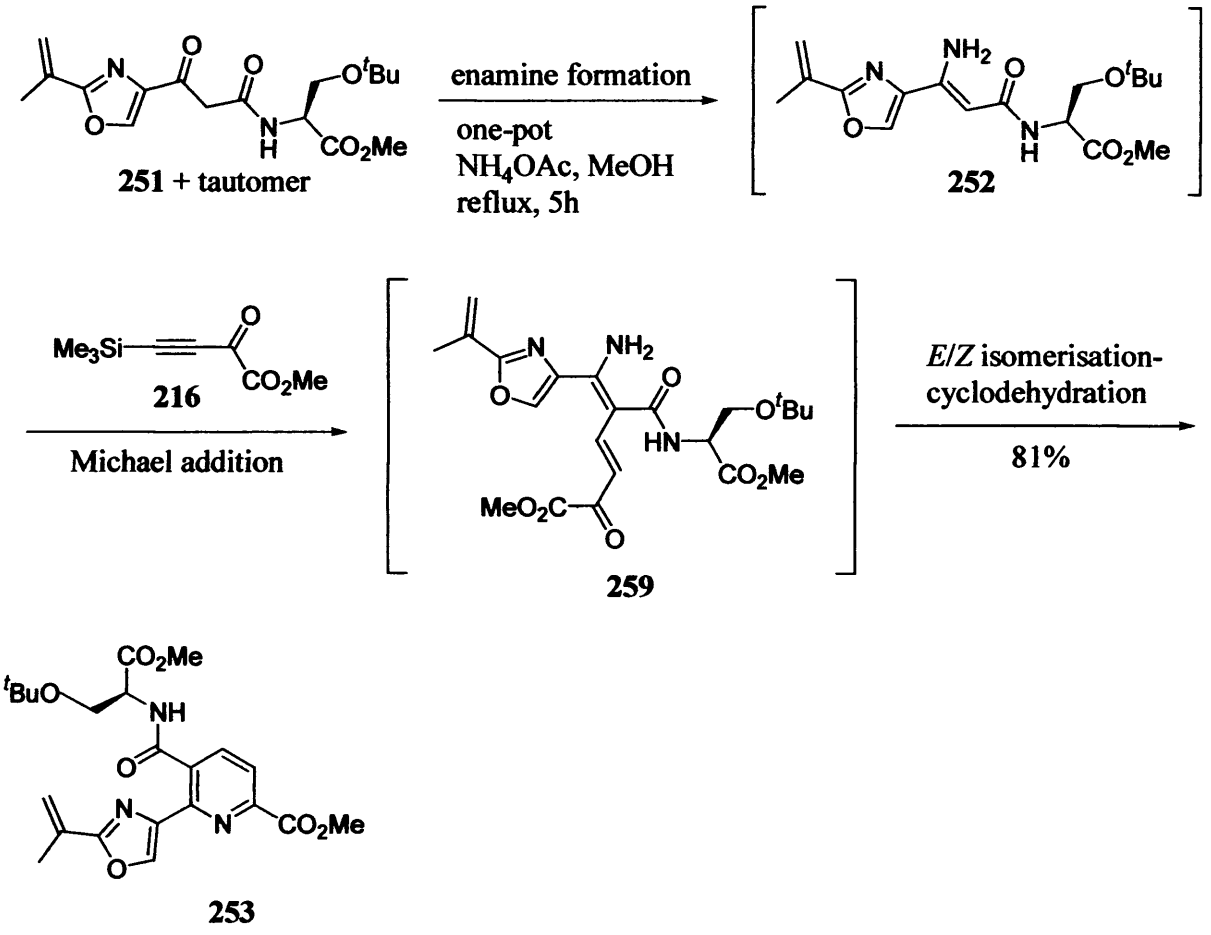
The key heteroannulation reaction was first tried for comparison using enamine **252** in a standard Bohlmann-Rahtz reaction on the basis of the model study. Stirring a solution of

enamine **252** and methyl oxobutynoate **216** in methanol at room temperature facilitated Michael addition and spontaneous cyclodehydration even under ambient conditions to give pyridine **253** in 93% yield, as a single regioisomer. We were delighted to see that these extremely mild Bohlmann-Rahtz reaction conditions were suitable to a more complex substrate like **252**. So far as can be seen, the model study has given superb guidance to the total synthesis of dimethyl sulfomycinamate (**13**). However, in spite of the failure of reactions of ethyl benzoylacetate (**75**) in the absence of an additional acid catalyst (see section **2.4.2.2**), the pyridine synthesis was improved overall by a one-pot 3-component process. Heating β-ketoamide **251**, used as a tautomeric mixture, 2-oxobutynoate **216** and a large excess of ammonium acetate at reflux in methanol for 5 hours gave pyridine **253** directly in 81% yield, presumably via enamine **252** in a one-pot Bohlmann-Rahtz heteroannulation reaction (**Scheme 3-14**). Since thionation of the carbonyl group in pyridine **253** using Lawesson's reagent failed to generate the expected thioamide functionality, an alternative way to introduce the thioamide moiety to **253** was adopted according to good literature precedent.[185] Deprotection under acidic conditions gave alcohol **254**; subsequent cyclization to the oxazoline **255** with Burgess reagent; thionation with hydrogen sulfide in pyridine-triethylamine gave thioamide **256** in 71% yield; cyclization with Burgess reagent led to thiazoline **257** in 87% yield. The microwave-assisted oxidation of **257** to thiazole **258** with manganese dioxide, in accordance with the model study, took the total synthesis just one transformation away from the target. Finally, the terminal alkene of **258** was oxidized in a two-step process with osmium tetroxide/sodium periodate in one-pot to provide dimethyl sulfomycinamate (**13**) (**Scheme 3-13**), mp 159–161 °C (lit.[34] mp 160.5–161.0 °C) whose spectroscopic properties were in agreement with literature data (**Appendix 1–12**).[34,35]

Scheme 3-13 Total synthesis of dimethyl sulfomycinamate (13)



Scheme 3-14 One-pot synthesis of pyridine 253



3.2.3 Conclusion

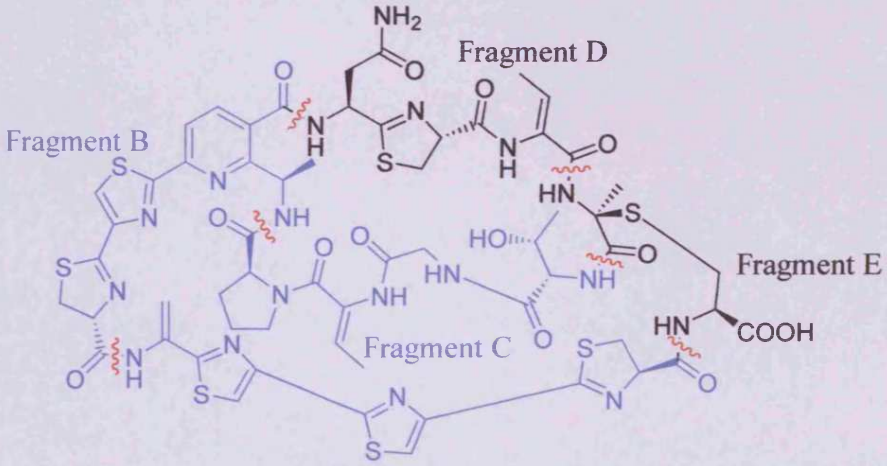
In conclusion, this 12-step total synthesis of dimethyl sulfomycinamate (**13**) in 9% overall yield demonstrates that the Bohlmann-Rahtz heteroannulation reaction is a facile and highly efficient route to pyridine-containing heterocyclic clusters. The approach complements the cross-coupling methodology of Kelly towards thiopeptide degradation products and synthetic studies of Shin towards the sulfomycins (**8**), and offers rapid access with high efficiency to the tris-heterocyclic core of the parent actinomycete metabolites.

3.3 Progress towards the total synthesis of cyclothiazomycin

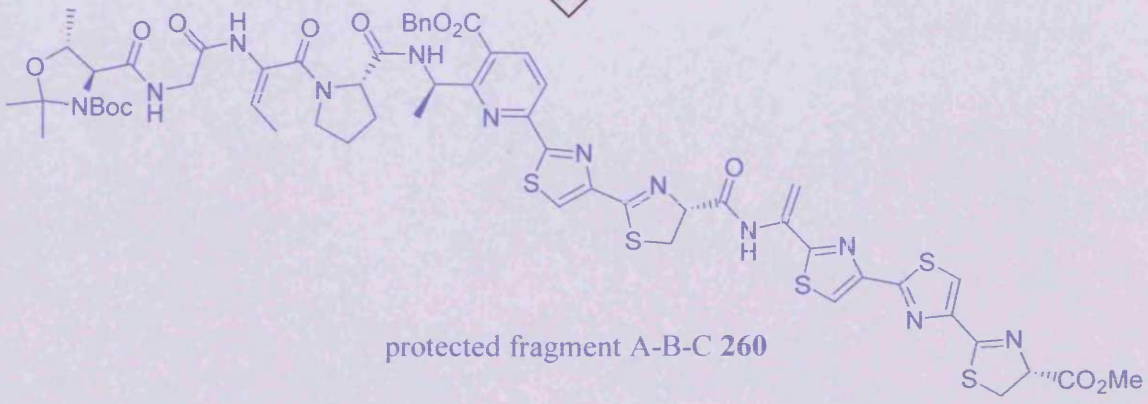
3.3.1 Previous synthetic work towards cyclothiazomycin

Cyclothiazomycin (**18**), another member of series *d* thiopeptide antibiotics, features a unique structure and impressive biological profile (see section **1.3.3.2**), which attracted our attention and prompted us to make synthetic efforts towards its challenging architecture. So far, no total synthesis of cyclothiazomycin (**18**) has yet been reported, whereas a few syntheses of fragments were published recently by Shin and co-workers.[143,144]

Scheme 3-15 Shin's disconnection of cyclothiazomycin (18)



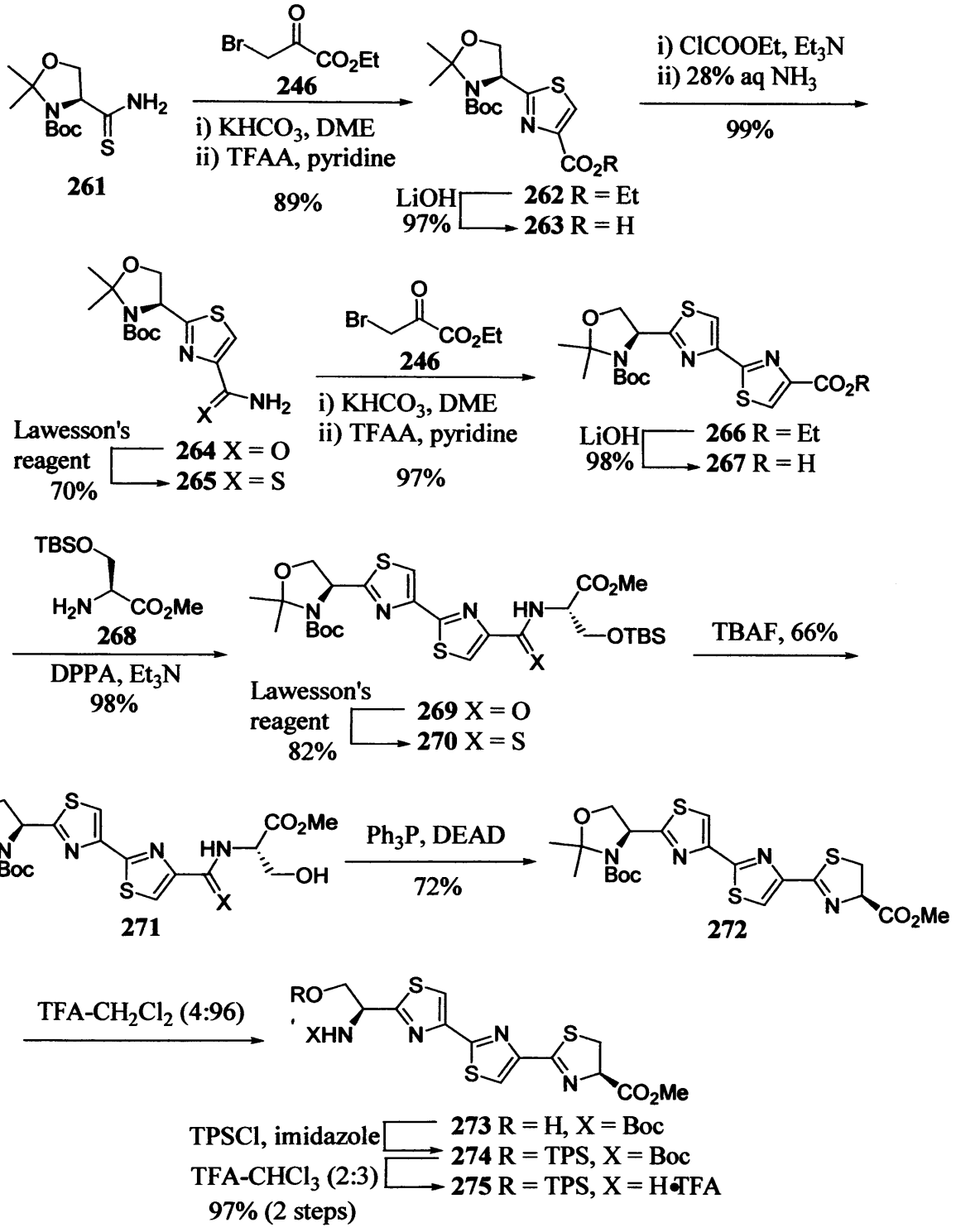
18



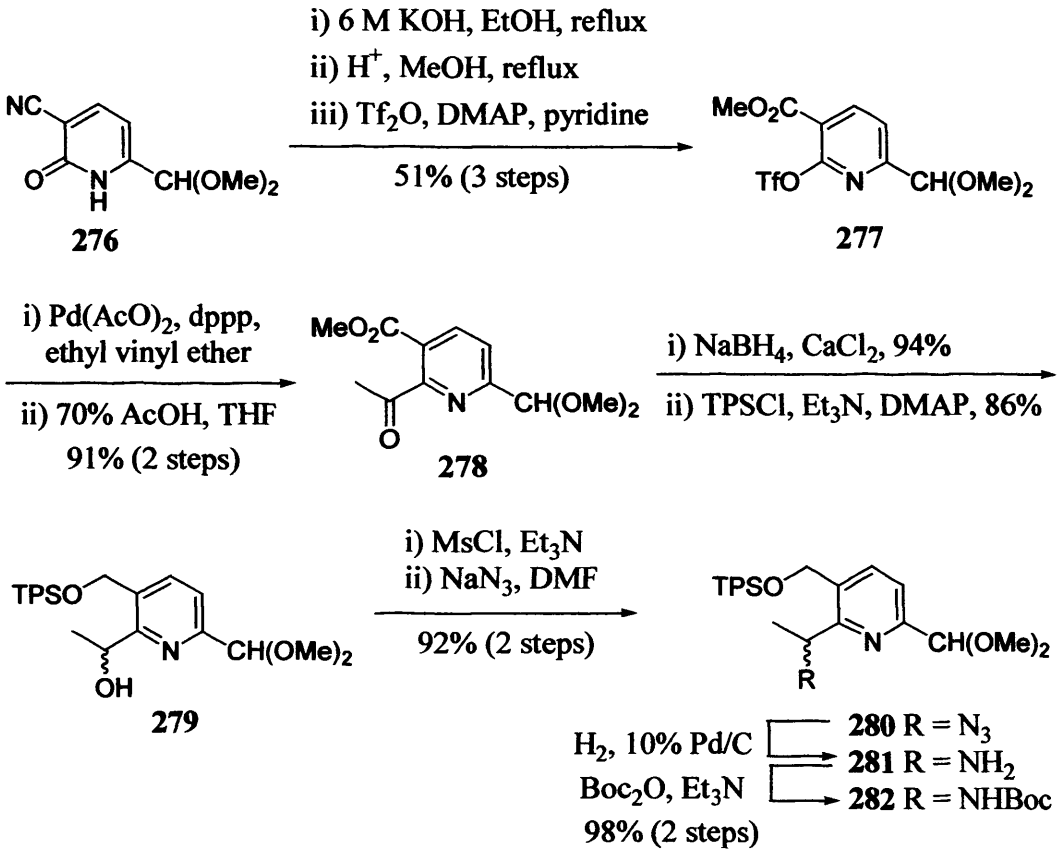
In accordance with Shin's reports,[143] cyclothiazomycin (**18**) was retrosynthetically disassembled into five fragments A, B, C, D, and E through cleavage of the peptide bonds indicated on structure **18** (**Scheme 3-15**). The protected fragment A-B-C (**260**) was constructed convergently by the mergence of fragments A, B, and C that were prepared separately from readily available building blocks.

Shin's synthesis of protected fragment A (**275**) began with repeated Hantzsch thiazole condensations of 3-*tert*-butyloxycarbonyl-2,2-dimethyloxazolidinone-4-thiocarboxamide (**261**) followed by ester hydrolysis to give the *bis*-thiazole-acid **267** in 57% yield over nine steps. Acid **267** was then coupled with *O-tert*-butyldimethylsilylserine methyl ester (**268**), thionated with Lawesson's reagent, deprotected with TBAF in tetrahydrofuran to generate β-hydroxythioamide **271** that reacted with triphenylphospine and DEAD to give thiazoline **272**. Treatment of **272** with dilute TFA removed the acetonide protecting group and allowed protection of the resulting primary alcohol using TBDPSCl in the presence of imidazole followed by removal of the *N-tert*-butyloxycarbonyl protecting group under strong acidic conditions, to afford desired compound **275** as a TFA salt in 32% yield over seven steps (**Scheme 3-16**).

Scheme 3-16 Shin's preparation of protected fragment A 275



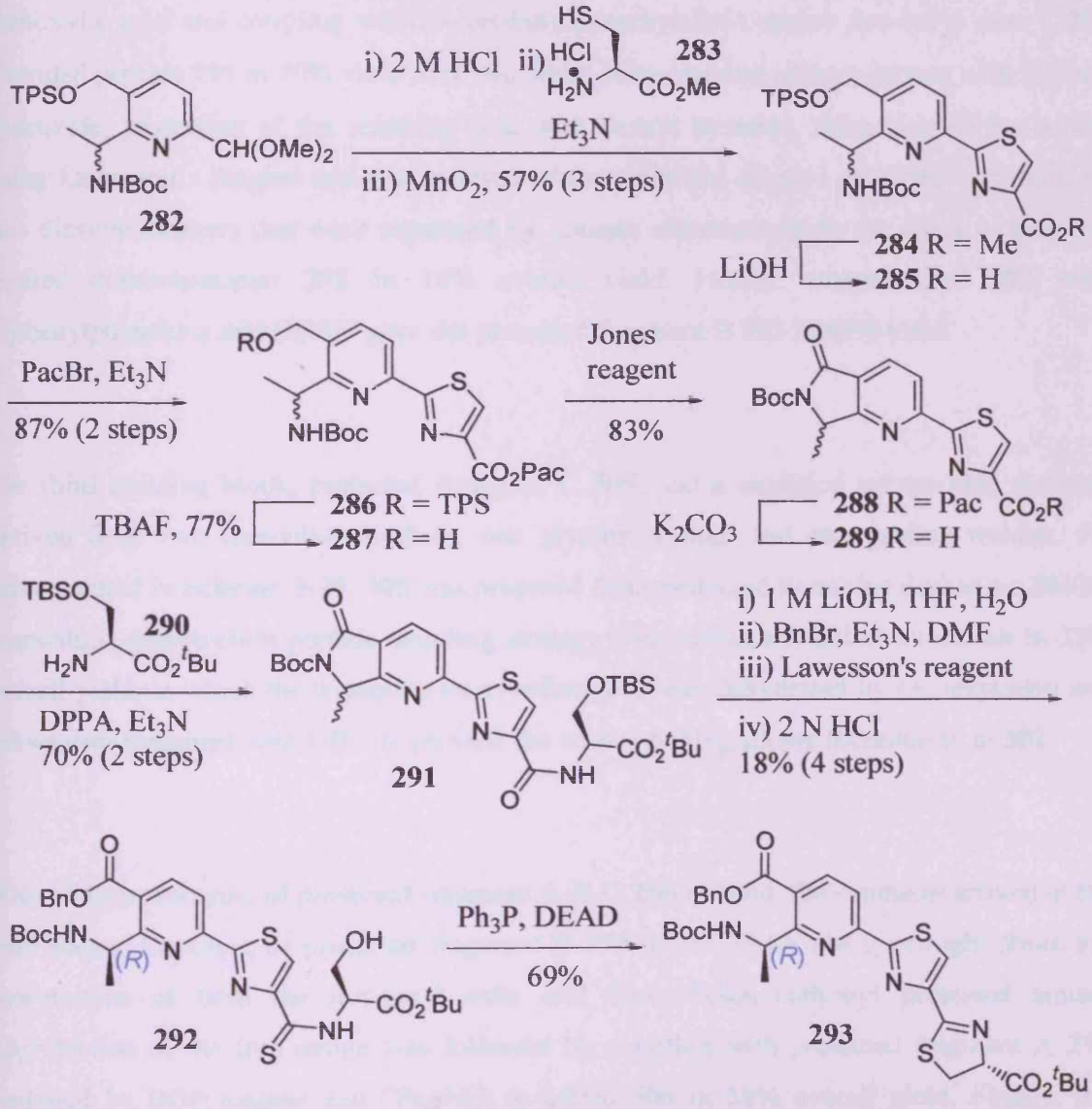
Scheme 3-17 Shin's preparation of advanced intermediate 282



The construction of protected fragment B **293** commenced with the preparation of 6-dimethoxymethyl-2-[1-(*N-tert*-butoxycarbonyl)aminoethyl]pyridine derivative **282** (**Scheme 3-17**). Treatment of pyridinone **276** with aqueous potassium hydroxide in ethanol was followed by esterification in an acidic methanolic solution and reaction with triflic anhydride to give pyridine **277** in 51% yield over three steps. Reaction of **277** with ethyl vinylether in the presence of palladium(II) acetate and 1,3-bis(diphenylphosphino)propane followed by treatment with 70% acetic acid gave ketone **278** in 91% yield over two steps. Reduction with sodium borohydride and regioselective protection of the primary alcohol with TBDPSCl provided secondary alcohol **279**, which was mesylated using methanesulfonyl chloride in the presence of Et_3N and then azidated with sodium azide in one-pot to afford the corresponding 2-(1-azidoethyl)pyridine derivative **280** in 79% yield

over four steps. Hydrogenation of the azide and subsequent *tert*-butyloxycarbonyl protection of the primary amine gave pyridine **282** in 98% yield over two steps.

Scheme 3-18 Shin's synthesis of protected fragment B 293



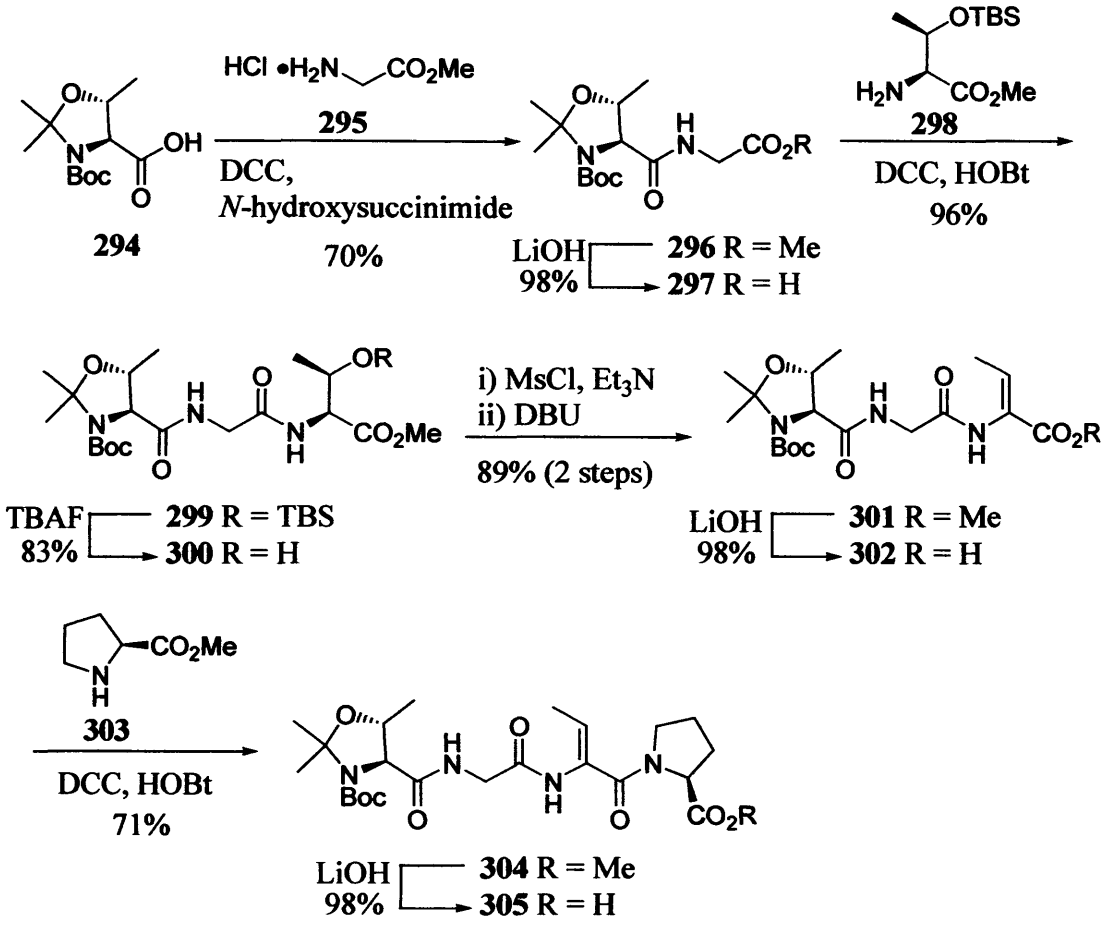
Having established the pyridine core **282**, the elaboration of protected fragment B **293** only required a handful of steps (**Scheme 3-18**). The 6-methoxymethyl group of **282** was

hydrolyzed using 2 M hydrochloric acid and reacted with L-cysteine derivative **283**, followed by oxidation to give 6-thiazolylpyridine **284** in 57% overall yield. Hydrolysis of the thiazole-4-methyl ester of pyridine **284** and conversion to the Pac ester gave pyridine **286** in 87% yield over two steps. Deprotection using TBAF and oxidation with Jones reagent afforded the γ-lactam derivative **288**. Cleavage of the Pac ester to the corresponding carboxylic acid and coupling with *O-tert*-butyldimethylsilyl-L-serine *tert*-butyl ester (**290**) provided peptide **291** in 70% yield over two steps. Ring opening of the γ-lactam with lithium hydroxide, protection of the resulting acid with benzyl bromide, thionation of the amide using Lawesson's reagent and deprotection of the protected alcohol provided a mixture of two diastereoisomers that were separated by column chromatography on silica to give the desired diastereoisomer **292** in 18% overall yield. Finally, treatment of **292** with triphenylphosphine and DEAD gave the protected fragment B **293** in 69% yield.

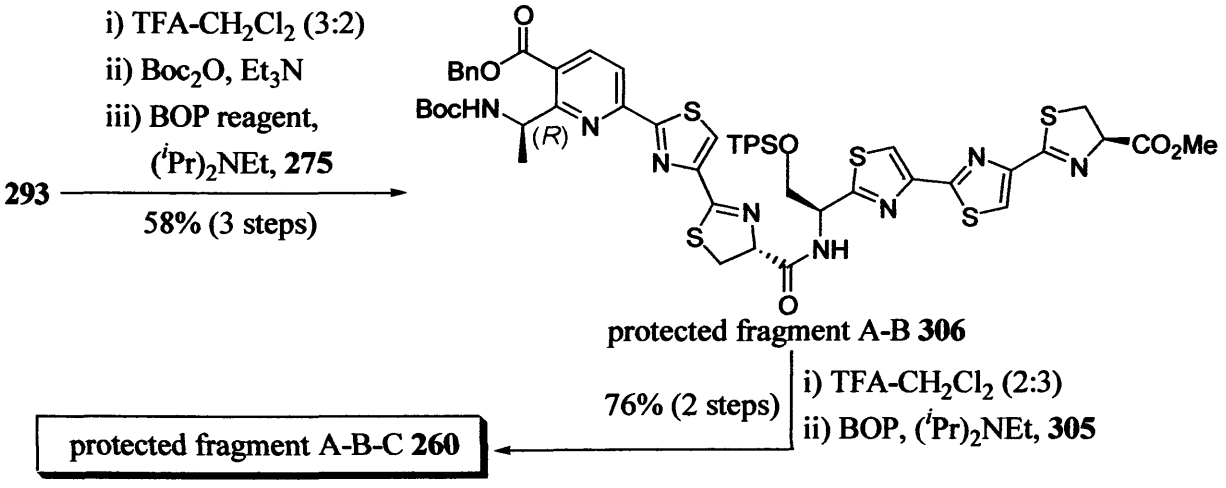
The third building block, protected fragment C **305**, had a modified tetrapeptide skeleton derived from two threonine residues, one glycine residue and one proline residue. As demonstrated in **Scheme 3-19**, **305** was prepared from protected threonine derivative **294** by sequentical deprotection-peptide coupling strategy with standard reaction conditions in 33% overall yield, in which the tripeptide intermediate **300** was dehydrated by *O*-mesylation and subsequent treatment with DBU to provide the corresponding alkene fuctionality in **301.**

With all three subunits of protected fragment A-B-C **260** in hand, the synthesis arrived at the final stages. Exposure of protected fragment B **293** to 3:2 TFA/CH_2Cl_2 brought about the deprotection of both the *tert*-butyl ester and *N-tert*-butoxycarbonyl protected amine. Reprotection of the free amine was followed by coupling with protected fragment A **275** mediated by BOP reagent and $(^{i}Pr)_2NEt$ to afford **306** in 58% overall yield. Finally, the target compound **260** was obtained in 76% overall yield *via* deprotection and subsequent peptide coupling with protected fragment C **305**.

Scheme 3-19 Shin's synthesis of protected fragment C 305

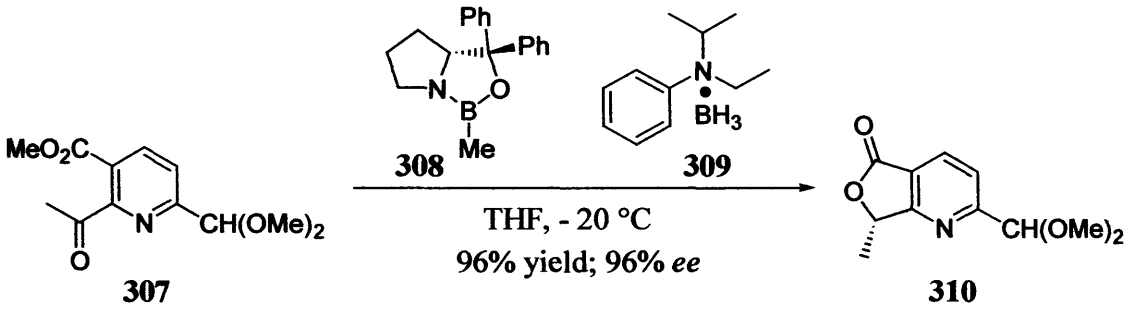


Scheme 3-20 Completion of Shin's synthesis of protected fragment A-B-C 260



More recently, Shin and co-workers disclosed an asymmetric synthesis of the main pyridine skeleton **310** of cycothiazomycin (**18**).[144] Stoichiometric reduction of the ketone group of methyl 2-acetyl-6-(dimethoxymethyl)nicotinate (**307**) with CBS reagent **308** and trihydro(*N*-ethyl-*N*-isopropylamine)boron (**309**) proceeded smoothly to afford γ-lactone **310** in 96% yield and 96% *ee* (**Scheme 3-21**).

Scheme 3-21 Shin's asymmetric synthesis of pyridine skeleton 313



3.3.2 Synthesis of the γ-lactam hydrolysate

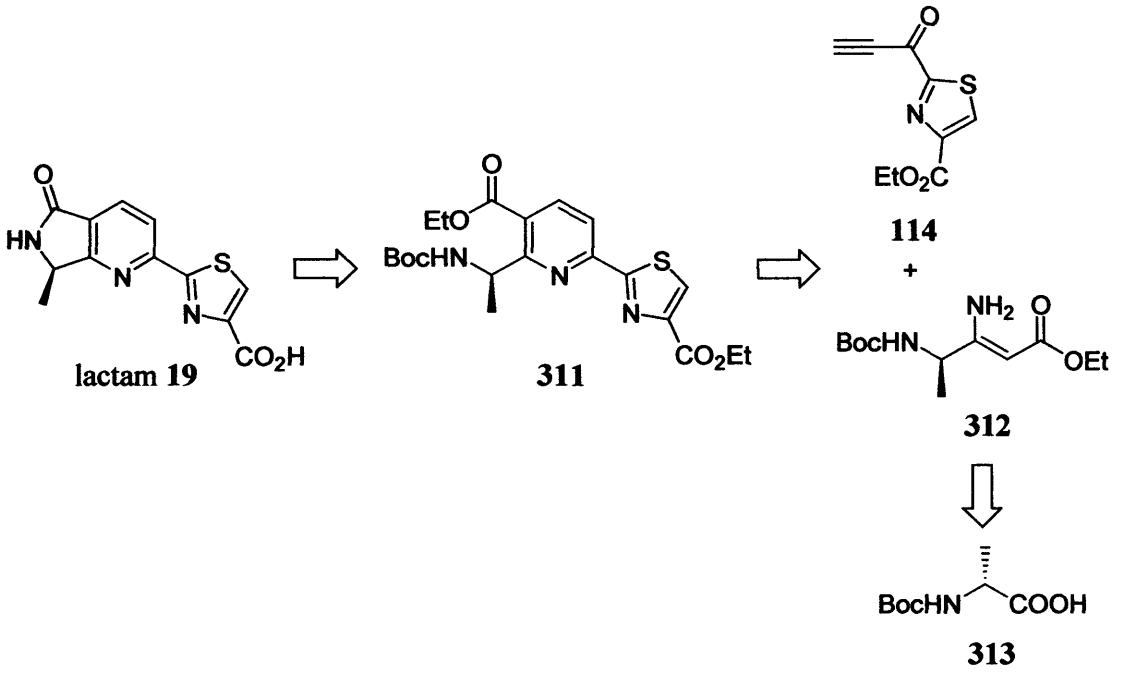
Due to the lack of effective methods for the asymmetric synthesis of (1-amino-1-ethyl)pyridine heterocyclic domain of cyclothiazomycin (**18**), we deemed the development of a highly expedient route towards such an unusual structural motif as the first task in connection with the total synthesis of cyclothiazomycin (**18**). Accordingly, γ-lactam **19**, a chemical degradation product isolated from the acid hydrolysate of cyclothiazomycin (**18**) (see **Scheme 1-3** in section **1.3.3.2**), was selected as a viable synthetic target to test the validity of our approach towards the cyclothiazomycin (**18**) γ-amino acid central heterocyclic domain.

3.3.2.1 Retrosynthetic analysis

Our approach, outlined retrosynthetically in **Scheme 3-22**, utilized a Bohlmann-Rahtz

reaction[156] combined with a chiral pool strategy. The degradation studies on cyclothiazomycin (**18**) (**Scheme 1-3**)[40] suggested that **19** could be produced directly by acidic hydrolysis of the protected cyclothiazomycin domain **311**. The pyridine domain **311** should be available by Bohlmann-Rahtz heteroannulation of the corresponding alkynone **114** and enamine **312** derived from alanine derivative **313**.

Scheme 3-22 Disconnection strategy of γ-lactam 19

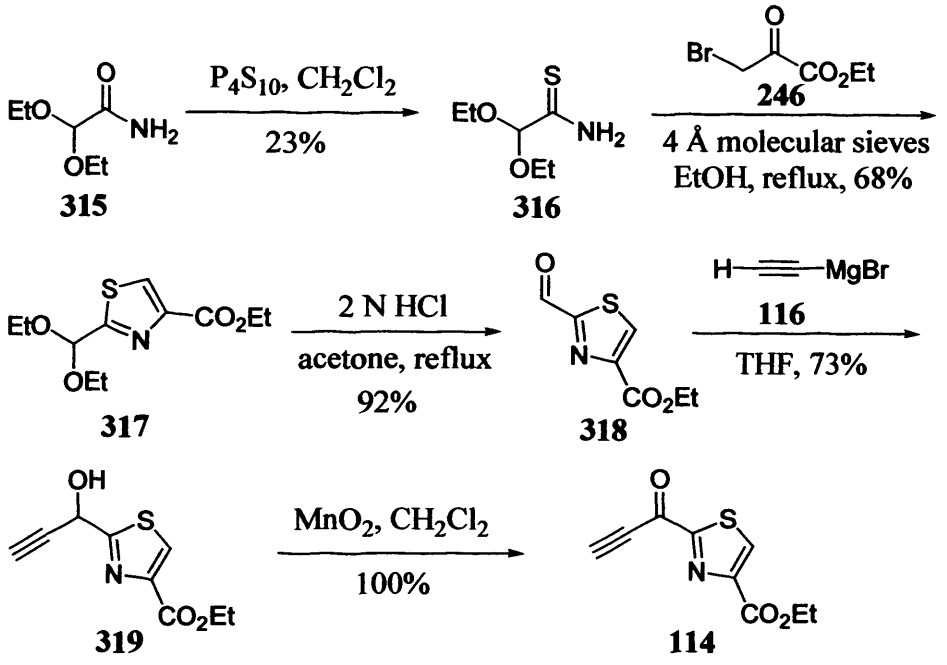


3.3.2.2 Preparation of alkynone

1-(2-thiazolyl)propyn-1-one **114**, which was used previously as a key component for the construction of an amythiamicin pyridine cluster **115** (**Scheme 2-10**) by our research group,[141] was prepared in five steps (**Scheme 3-23**) from 2,2-diethoxyacetamide (**315**) by thionation with phosphorus pentasulfide. Hantzsch thiazole synthesis with ethyl bromopyruvate (**246**) in ethanol in the presence of 4 Å molecular sieves, followed by acid catalysed acetal deprotection gave aldehyde **318**. Addition of ethynylmagnesium bromide

(116) in THF with aqueous work up and subsequent oxidation of propargylic alcohol **319** with manganese (IV) oxide gave propynone **114**.

Scheme 3-23 Synthesis of 1-(2-thiazolyl)propyn-1-one 114



3.3.2.3 Stereoselective synthesis of γ-lactam

Having realized a synthesis of alkynone **114**, attention could now be focus on generating its Bohlmann-Rahtz heteroannulation partner, enamine **312**. The critical challenge posed by this building block was finding a means to maintain its optical purity.

The synthesis began with *N*-Boc-D-alanine (**313**) (> 99% ee) (**Scheme 3-24**), mixed anhydride formation followed by homologation with magnesium ethyl malonate gave the desired (*R*)-β-ketoester **320** as a mixture of ketone and enol forms. In this synthetic step, a number of different homologation reaction conditions and methods were tried to avoid potential racemization at the α stereogenic centre. As summarised in **Table 3-1**, method B,

which utilised mixed anhydride methodology to activate the acid and then reacted with the lithium enolate of ethyl acetate, gave **320** in moderate yield without loss of optical purity as indicated by HPLC analysis on a chiral stationary phase (**entry 5, Table 3-1**).

Scheme 3-24 Asymmetric synthesis of (*R*)-β-ketoester 320

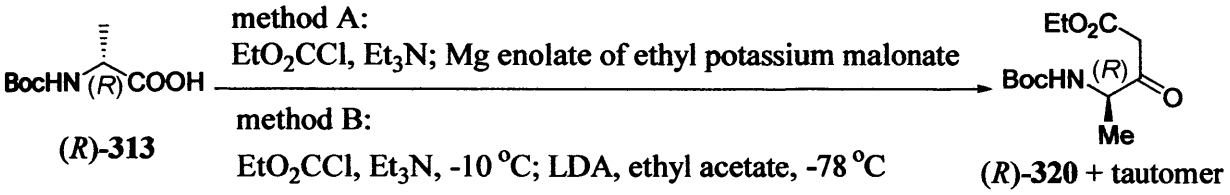


Table 3-1 Homologation of *N*-Boc-D-alanine (313)

Entry	Method	Reaction conditions[a]	Yield (%)[b]	Ee (%)[c]
1	A	0 °C; rt., 12 h	75	81
2	A	0 °C; rt., 4 h	63	84
3	A	-10 °C; -10 °C, 12 h	66	92
4	A	-78 °C; -10 °C, 12 h	28	90
5	**B**	**-10 °C; -78 °C, 20 min**	**50**	**>99**

[a] First entry indicates temperature at which mixed anhydride formation was carried out; second entry indicates temperature at which homologation was carried out; time indicates reaction duration.
[b] Isolated yield of (*R*)-β-ketoester **320** after column chromatography.
[c] Determined by chiral HPLC analysis [ChiralPak AD column, hexane-IPA (99:1)]

To this end, reacting (*R*)-β-ketoester **320** with ammonium acetate gave enamine **312**. However, during its formation or purification, this chiral intermediate racemised on exposure to heat (ethanol at reflux), Brønsted acids (5:1 toluene-acetic acid) or silica gel and could only be isolated in 70% yield and 92% *ee* by carrying out the reaction at room temperature in ethanol and using the crude material without purification (Scheme 3). The Bohlmann-Rahtz reaction of enamine **312** and propynone **114** under traditional heteroannulation conditions, Michael addition at 50 °C for 10 minutes followed by cyclodehydration at 135 °C (**entry 1, Table 3-2**), gave pyridine **311** as a single regioisomer in 73% yield albeit only in 14% *ee*. The microwave assisted reaction[172] resulted in

appreciable loss of material but did improve the optical purity somewhat (**entry 2, Table 3-2**). As predicted, the one-pot acid catalysed heteroannulation process[157a] was much more efficient but did little to prevent racemisation throughout the process (**entry 3, Table 3-2**), presumably as a consequence of the acid instability of hydroxydihydropyridine **322**. However, in combination with a Michael addition under traditional Bohlmann-Rahtz conditions, the acid catalysed cyclodehydration of the diaminodienone intermediate **321** at 60 °C caused a significant increase in the optical activity of pyridine **311** (**entry 4, Table 3-2**). Changing the cyclodehydrating agent from a Brønsted acid to *N*-iodosuccinimide (NIS), a reagent effective in this transformation at 0 °C,[160] further improved the stereoselectivity of the process (**entry 5, Table 3-2**), giving pyridine **311** in 92% *ee*. Although an excellent result, the optical purity of the product was still limited by the stereochemical instability of enamine **312** following isolation of this Bohlmann-Rahtz precursor.

In order to overcome the limiting racemisation of enamine **312**, a new sequential one-pot process was investigated for the stereoselective synthesis of the cyclothiazomycin domain. Ammonium acetate was added to a solution of β-ketoester **320** (>99% *ee*) in ethanol. After 4 hours at room temperature, thiazolylpropynone **114** was added and the mixture was stirred to complete the Michael addition. The mixture was then cooled to 0 °C and *N*-iodosuccinimide was added (**entry 6, Table 3-2**). Pleasingly, after column chromatography, *C*- and *N*-terminal protected amino acid **311** was isolated directly in 55% yield and 96% *ee* from this one-pot reaction, demonstrating a facile stereoselective route to pyridyl γ-amino acids from the corresponding β-ketoester that avoids isolation of chiral enamine intermediates.

Scheme 3-25 Asymmetric synthesis of cyclothiazomycin domain 311

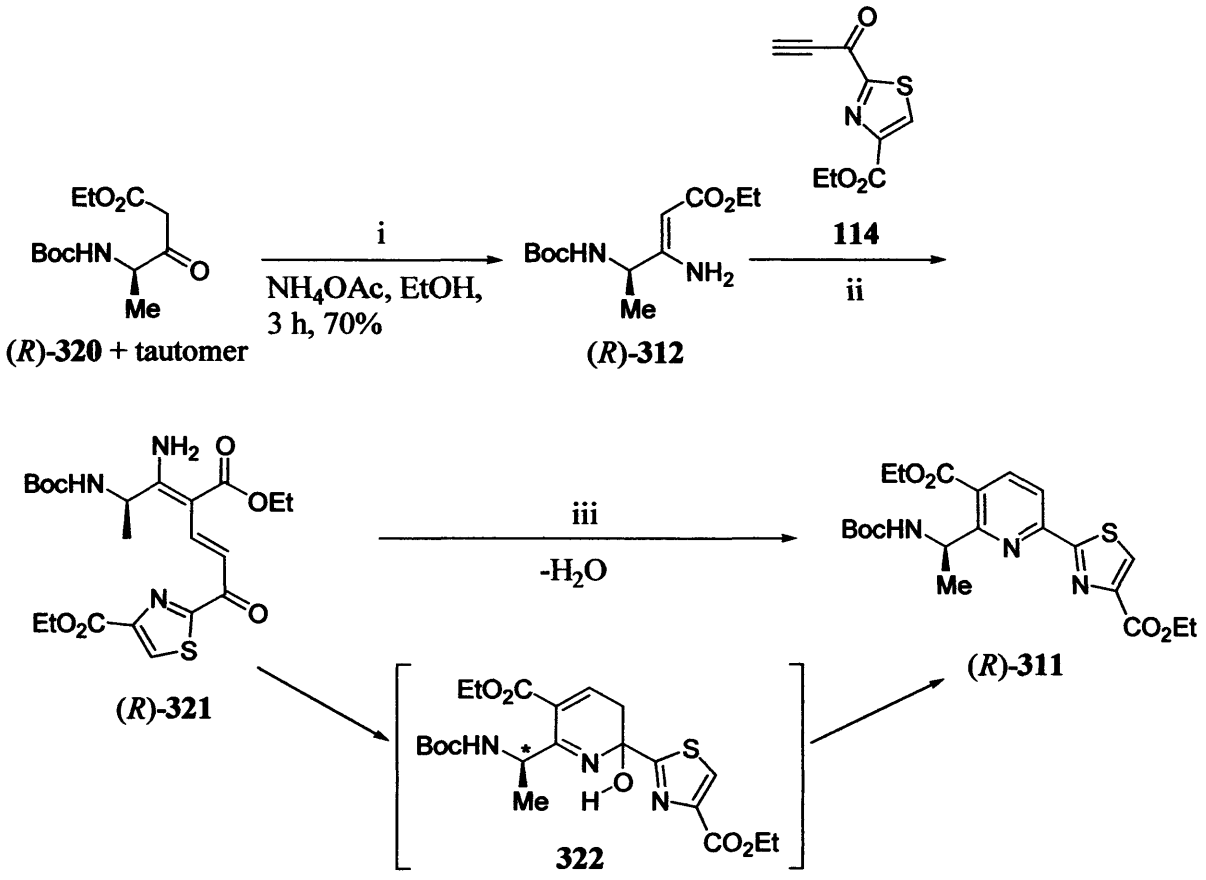


Table 3-2 Asymmetric synthesis of cyclothiazomycin domain 311

Entry	Reagents and conditions	Yield (%)[a]	Ee (%)[b]
1	ii) EtOH, 50 °C, 10 min; iii) neat, 135 °C, 4 h	73	14
2	ii) and iii) microwave, 170 °C, 20 min	10	33
3	ii) and iii) PhMe–AcOH, 60 °C, 90 min	73	47
4	ii) EtOH, 50 °C, 10 min; iii) PhMe–AcOH, 60 °C, 90 min	66	81
5	ii) EtOH, 50 °C, 10 min; iii) NIS, 0 °C, 15 min	71	92
6	i)–iii) NH_4OAc, EtOH, 4 h; **114**, 1 h; NIS, 0 °C, 15 min	55	96

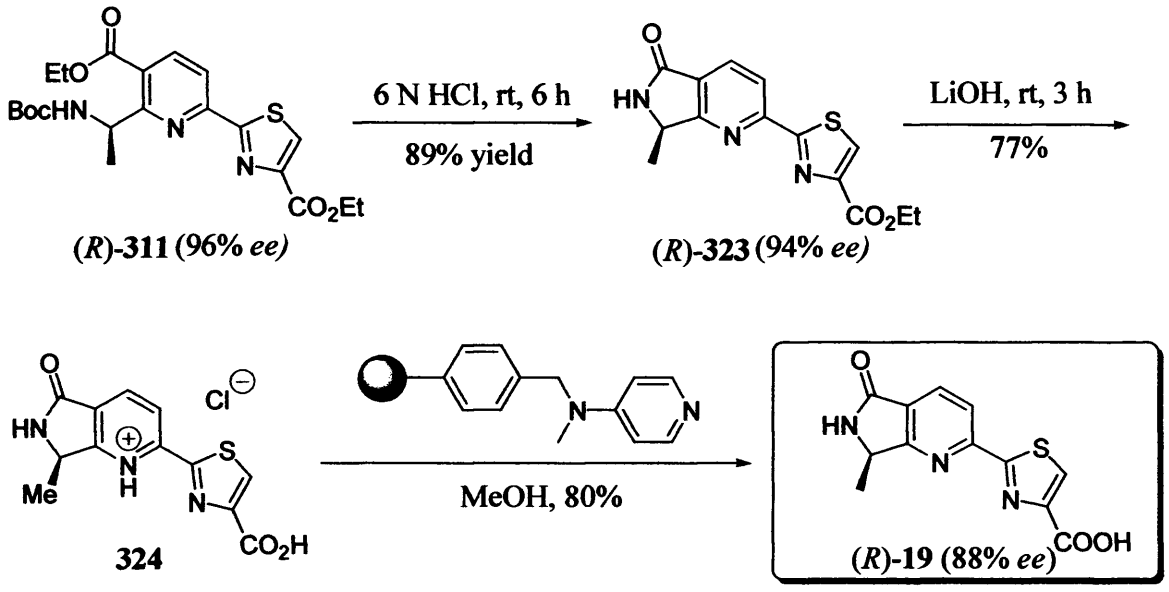
[a] Isolated yield of (*R*)-**311** after column chromatography.
[b] Determined by chiral HPLC analysis [ChiralPak AD column, hexane-IPA (95:5)]

From chiral non-racemic pyridine **311** it was anticipated that the total synthesis of the cyclothiazomycin lactam **19**, would be realised simply by hydrolysis in accordance with the degradation studies on the natural product (**Scheme 1-3**).[40] To our surprise, when pyridine **311** was heated to 110 °C in 6 N hydrochloric acid, a complex mixture of products was obtained. Under milder conditions, when the reaction was repeated at room temperature, the *N*- and *C*-terminal protecting groups were cleaved but the ethyl thiazole-4-carboxylate group remained untouched to give γ-lactam **323** in 94% *ee* (**Scheme 3-26**). Base catalysed hydrolysis at room temperature, with acidic work up, was expected to complete the synthesis of the cyclothiazomycin hydrolysate **19**, but the UV, ^{1}H and ^{13}C NMR spectroscopic data of the material isolated from the natural product[40] failed to match that of the colorless precipitate obtained from the reaction, which was identified as hydrochloride **324**. Even more surprising was the observation that elution on a solid phase extraction column, a procedure used to isolate hydrolysate **19** from the natural product,[i] returned only the pyridinium salt.[ii] However, when hydrochloride **324** was stirred with one equivalent of polymer-supported 4-(dimethylamino)pyridine (PS-DMAP) in dry methanol for 18 hours, filtered and evaporated, chiral non-racemic 2-(2-pyridyl)thiazole-4-carboxylic acid **19** was obtained in 80% yield and 88% *ee*, the spectroscopic characteristics and chromatographic behaviour of which were in agreement with reported literature data (**Appendix 13–19**).[40,i]

[i] We are grateful to Masahiro Aoki (Chugai Pharmaceutical Co., Ltd., Japan) and Prof. Haruo Seto (Institute of Applied Microbiology, The University of Tokyo, Japan) for further details. Hydrolysis of **18** (ref 40) in 6 N aq HCl at 110 °C under nitrogen gave a residue that was suspended in water and transferred to a solid phase extraction column (Sep-pak C18), gradient eluting with water and methanol. Purification by column chromatography on Sephadex LH20, eluting with methanol, gave hydrolysate **19** as a film, R_f 0.64 on silica (*n*BuOH-AcOH-H_2O 3:1:1).

[ii] Pyridinium hydrochloride **324** was obtained as a colorless solid, R_f 0.58 on silica (*n*BuOH-AcOH-H_2O 3:1:1). See Chapter 4 Experimental and Chapter 5 Appendix for more experimental details and spectroscopic data.

Scheme 3-26 Synthesis of the cyclothiazomycin hydrolysate, γ-lactam 19



3.3.3 Conclusion

In summary, the entire synthetic sequence towards the γ-lactam hydrolysate **19** of cyclothiazomycin (**18**) gives the *C*- and *N*-terminal protected heterocyclic domain **311** in 96% *ee* and 55% yield in only one step from (*R*)-β-ketoester **320** and generates γ-lactam **19** in 88% *ee* and 30% overall yield in only four steps. We have developed methodologies for the stereoselective synthesis of a chiral diaminoalkenoate, diaminodienone and the γ-lactam form of an heterocyclic amino acid, corresponding to Bohlmann-Rahtz enamine, dienamine intermediate and pyridine product, respectively, in high optical purity, good yield and very few synthetic steps from a chiral pool precursor with total regiocontrol. The utility of our facile approach, centered on a sequential one-pot three-component process, has been demonstrated in the first successful stereoselective synthesis of the cyclothiazomycin γ-lactam hydrolysate, which verifies its structure, with minimum racemisation and now is expected to be used in the total synthesis of cyclothiazomycin (**18**).

Chapter Four

Experimental

4. Experimental

4.1 General

Commercially available reagents were used as received without further purification; solvents were dried by standard procedures. Light petroleum refers to the fraction with b.p. 40–60 °C. Unless otherwise stated, reactions were performed under an atmosphere of dry nitrogen. Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF_{254} that were visualised under UV light (at 254 and/or 360 nm). Preparative thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF_{254}. Visualisation was achieved by UV light (at 254 and/or 360 nm) and/or potassium permanganate stain.

Fully characterised compounds were chromatographically homogeneous. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Infra-red spectra were recorded in the range 4000-600 cm^{-1} on a Perkin-Elmer 1600 series FT-IR spectrometer either as a KBr disk, nujol mull, in solution in dry dichloromethane or as a thin film between NaCl plates, as indicated. NMR spectra were recorded using a Bruker DPX 400 instrument operating at 400 MHz for ^{1}H spectra and 100 MHz for ^{13}C spectra (internal standard TMS); *J* values were recorded in Hz and multiplicities were expressed by the usual conventions. Low-resolution mass spectra were determined using a Fisons VG Platform II Quadrupole instrument using electrospray ionisation (ES) unless otherwise stated. APcI refers to atmospheric pressure chemical ionisation, CI refers to chemical ionisation (ammonia) and EI refers to electron ionisation. High-resolution mass spectra were obtained courtesy of the EPSRC Mass Spectrometry Service at University College of Wales, Swansea, UK using the ionisation methods specified. Calculated accurate masses of parent compounds denote the mass of the ion (mass of electron is 0.00055 Da) except where indicated. Microanalyses were recorded using a Perkin-Elmer 240C Elemental Analyzer in-house, or courtesy of Warwick Analytical Services Ltd at University of Warwick Science Park, UK using an Exeter 440 Elemental Analyser. Chiral compounds were analysed using a AA-1000

Polarimeter apparatus from Optical Activity LTD., using the sodium D line and at the indicated temperature, and are given in deg $cm^3g^{-1}dm^{-1}$ for [α] and 10^{-2} gcm^{-3} for *c*. HPLC was carried out using a HP-1100 HPLC machine equipped with a variable wavelength UV detector and variable flow pump. Microwave experiments were carried out in a CEM Discover™ microwave synthesiser at the temperature and initial power stated.

4.2 General Experimental procedures

General procedure for the synthesis of propargylic alcohols from aldehydes (GP01)

A solution of the aldehyde (5.0 mmol, 1 equiv.) in dry tetrahydrofuran (10 mL) was added dropwise over a period of 5 minutes to a stirred solution of ethynylmagnesium bromide (**116**) in tetrahydrofuran (0.5 M; 15 mL, 7.5 mmol, 1.5 equiv.) at 0 °C. The mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. Saturated aqueous ammonium chloride solution (2 mL) was added and the mixture was concentrated *in vacuo* and partitioned between diethyl ether (30 mL) and saturated aqueous ammonium chloride solution (30 mL). The ethereal layer was washed with brine (30 ml), dried (Na_2SO_4) and concentrated *in vacuo* to give the crude propargylic alcohol.

General procedure for the synthesis of alkynones from propargylic alcohols (GP02)

A solution of *o*-iodoxybenzoic acid (3.50 g, 12.5 mmol, 2.5 equiv.) in DMSO (90 mL) was stirred for 15 min at room temperature until homogeneous. A solution of the propargylic alcohol (5.0 mmol, 1 equiv.) in DMSO (5 mL) was added and the mixture was stirred for 5–24 h. Water (10 mL) was added and the mixture was stirred at room temperature for 10 min, cooled in ice and partitioned between water (80 mL) and diethyl ether (60 mL). The mixture was filtered through Celite® and the aqueous layer was further extracted with diethyl ether (50 mL). The organic extracts were combined, washed sequentially with water (3 x 50 mL), saturated aqueous sodium hydrogen carbonate solution (70 mL) and brine (70 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude alkynone.

General procedure for Michael addition of enamines and alkynones (GP03)

A solution of enamine (~1 mmol, 1 equiv.) and alkynone (1.5 equiv.) in ethanol (15 mL) was stirred at 50 °C for 1–7 h, cooled and evaporated *in vacuo* to give the crude aminodienone product.

General procedure for the traditional two-step Bohlmann-Rahtz reaction (GP04)

A solution of the enamine (~1 mmol, 1 equiv.) and alkynone (1.2–2.4 equiv.) in ethanol (5 ml) was stirred at 50 °C for 5 h, cooled and then evaporated *in vacuo* to give dienone intermediate. The residue was heated at 140–160 °C in a flask fitted with drying tube for 1–2 h and allowed to cool to give the crude pyridine product.

General procedure for the acetic acid catalysed one-step Bohlmann-Rahtz reaction (GP05)

A solution of the enamine (~1 mmol, 1 equiv.) and alkynone (1.2–2.4 equiv.) in toluene-glacial acetic acid (5:1) (5 mL) was stirred at 50 °C for 6 h. The mixture was partitioned between toluene (30 mL) and saturated aqueous sodium hydrogen carbonate solution (30 mL), the aqueous layer was further extracted with toluene (2 x 20 mL) and the combined organic extracts were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude pyridine product.

General procedure for the zinc(II) bromide catalysed one-step Bohlmann-Rahtz reaction (GP06)

A solution of the enamine (~1 mmol, 1 equiv.), alkynone (1.2–2.4 equiv.) and zinc(II) bromide (15–20 mol%) in toluene (6 mL) was heated at reflux for 6 h, allowed to cool and water (6 mL) added. The mixture was stirred for 20 min and extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were washed with brine (6 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude pyridine product.

General procedure for the one-step synthesis of pyridines in Carius tube (GP07)

A solution of the enamine (~2 mmol, 1 equiv.) and alkynone (1.2 equiv.) in DMSO (3 mL) was heated in a sealed Carius tube (25 mL) using a sand bath at 170 °C for 20 min and allowed to cool. The reaction mixture was poured into water (15 mL) and extracted with ethyl acetate (8 mL). The aqueous layer was further extracted with ethyl acetate (8 mL) and the organic extracts were combined, washed successively with saturated aqueous sodium

hydrogen carbonate solution (10 mL) and brine (10 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude pyridine product.

General procedure for the one-step synthesis of pyridines under microwave-assisted conditions (GP08)

A solution of the enamine (~2 mmol, 1 equiv.) and alkynone (0.5 equiv.) in DMSO (3 mL) was irradiated in a sealed pressure-rated reaction tube (10 mL) at 170 °C (initial power 150 W) for 20 min in a self tuning single mode CEM Discover™ Focused Synthesiser. The mixture was cooled rapidly to room temperature, by passing compressed air through the microwave cavity for 5 min, poured into water (15 mL) and extracted with ethyl acetate (8 mL). The aqueous layer was further extracted with ethyl acetate (8 mL) and the organic extracts were combined, washed successively with saturated aqueous sodium hydrogen carbonate solution (10 mL) and brine (10 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude pyridine product.

General procedure for the one-pot synthesis of pyridines from β-ketoesters in alcoholic solvent (GP09)

A solution of the β-ketoester (1.0 mmol, 1 equiv.), alkynone (0.6 mmol, 0.6 equiv.) and ammonium acetate (0.77 g, 10.0 mmol, 10 equiv.) in methanol or ethanol (10 mL) was stirred at reflux for 24 h, allowed to cool and evaporated *in vacuo*. The residue was partitioned between saturated aqueous sodium hydrogen carbonate solution (30 mL) and ethyl acetate (30 mL) and the aqueous layer was further extracted with ethyl acetate (20 mL). The combined organic extracts were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude pyridine product.

General procedure for the one-pot synthesis of pyridines from propargylic alcohols and enamines (GP10)

A solution of IBX (0.56 g, 2.0 mmol, 2 equiv.) in DMSO–glacial acetic acid (5:1) (18 mL) was stirred at 65 °C until homogeneous. A solution of the propargylic alcohol (2.0 mmol, 2 equiv.) and enamine (1.0 mmol, 1 equiv.) in DMSO (1 mL) was added and the resulting solution was stirred at 65 °C overnight. Water (10 mL) was added and the mixture was stirred for 10 min, allowed to cool, diluted with water (40 mL) and extracted with ethyl acetate (2 x 30 mL). The organic extracts were combined, washed sequentially with

saturated aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude pyridine product.

General procedure for the one-pot synthesis of pyridines from propargylic alcohols and *β*-ketoesters (GP11)

A solution of the *β*-ketoester (0.3 mmol, 1 equiv.), propargylic alcohol (0.6 mmol, 2 equiv.), ammonium acetate (0.46 g, 6.0 mmol, 20 equiv.) and activated manganese(IV) oxide (0.52 g, 6.0 mmol, 20 equiv.) in toluene–glacial acetic acid (5:1) (5 mL) was heated at reflux overnight. The mixture was allowed to cool, filtered through Celite®, partitioned between saturated aqueous sodium hydrogen carbonate solution (30 mL) and ethyl acetate (30 mL) and the aqueous layer was further extracted with ethyl acetate (20 mL). The combined organic layers were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude pyridine product.

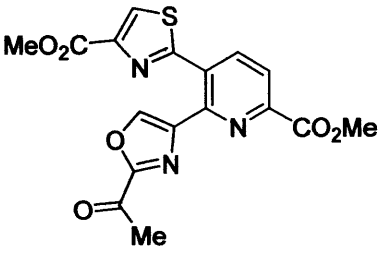
General procedure for the bromocyclization of aminodienones using NBS (GP12)

A solution of aminodienone (0.28 mmol, 1 equiv.) and *N*-bromosuccinimide (60 mg, 0.34 mmol, 1.2 equiv.) in ethanol (5 mL) was stirred at 0 °C for 15–60 min and evaporated *in vacuo* to give the crude bromopyridine product.

General procedure for copper(I) iodide promoted condensation of *α*-amino acids with *β*-keto thioesters (GP13)

A solution of *β*-keto thioester (2.0 mmol, 1 equiv.) in dry dichloromethane (5 mL) was added to a stirred solution of triethylamine (0.56 mL, 4.0 mmol, 2 equiv.) and *α*-amino acid (2.0 mmol, 1 equiv.) in dry dichloromethane (15 mL). Copper(I) iodide (0.76 g, 4.0 mmol, 2 equiv.) was added, the mixture was stirred at room temperature overnight. Dichloromethane (5 mL) and dilute hydrochloric acid (1 N; 5 mL) were added, and the mixture was filtered. The organic filtrate was washed sequentially with dilute hydrochloric acid (1 N; 10 mL), saturated aqueous sodium hydrogen carbonate solution (10 mL) and brine (10 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude *β*-keto amide product.

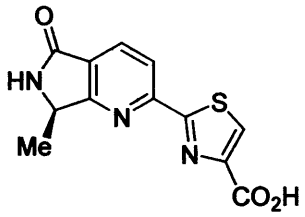
4.3 Experimental



Methyl 2-(2-acetyloxazol-4-yl)-3-[4-(methoxycarbonyl)thiazol-2-yl]pyridine-6-carboxylate (dimethyl sulfomycinamate) (13)

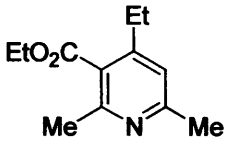
A solution[i] of osmium(VIII) tetroxide (1.2 mg, 4.7 μmol) in acetonitrile (60 μL) was added to a solution of methyl 2-[2-(2-propenyl)oxazol-4-yl]-3-[4-(methoxycarbonyl)thiazol-2-yl]pyridine-6-carboxylate (**258**) (16 mg, 0.04 mmol) in dioxane–water (1:1) (8 mL). Sodium periodate (17 mg, 0.08 mmol) was added and the mixture was stirred at room temperature overnight and extracted with dichloromethane (2 x 5 mL). The combined organic extracts were washed sequentially with aqueous sodium metabisulphite solution (8 mL), water (8 mL) and brine (8 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:2), gave the *title compound* (12 mg, 80%) as colourless crystals, mp 159.0–161.0 °C (from diethyl ether–hexane) (lit.[34] mp 160.5–161.0 °C) (lit.[35b] mp 157.3–160.2 °C): IR (KBr) 3150, 2954, 1728, 1702, 1573, 1534, 1477, 1435, 1373, 1338, 1316, 1219, 1128, 1096, 1005, 963, 869, 842, 768 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.33 (1 H, s, CH), 8.31 (1 H, s, CH), 8.16 (2 H, app s, 4,5-PyH), 3.98 (3 H, s, OMe), 3.91 (3 H, s, OMe), 2.41 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.6 (C), 164.7 (C), 164.2 (C), 161.6 (C), 157.0 (C), 148.8 (C), 148.0 (C), 147.2 (C), 142.7 (CH), 140.4 (CH), 140.3 (C), 130.7 (C), 129.7 (CH), 124.2 (CH), 53.3 (Me), 52.8 (Me), 26.6 (Me); MS (APcI) *m/z* (relative intensity) 388 (MH^+, 100%); HRMS calcd for $C_{17}H_{14}N_3O_6S$ (MH) 388.0595, found 388.0604.

[i] From a 0.079 M stock solution of osmium(VIII) tetroxide (250 mg, 0.98 mmol) in acetonitrile (12.5 mL).



(*R*)-2-(4-Carboxythiazol-2-yl)-7-methyl-5-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridine (19)

To a stirred solution of (*R*)-2-(4-carboxythiazol-2-yl)-7-methyl-5-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridinium chloride (**324**) (25 mg, 0.08 mmol) in dry methanol (50 mL) was added dry polymer-bound 4-(*N*-benzyl-*N*-methylamino)pyridine (32 mg, 0.08 mmol) and powdered 4Å molecular sieves (2 g). The reaction mixture was stirred at room temperature for 18 h and filtered through Celite®, washed with methanol (2 x 20 mL) and concentrated *in vacuo*. Recrystallization from methanol gave the *title compound*[i,ii] (18 mg, 80%) as a colourless solid, mp 185 °C dec (from methanol): $[\alpha]_D^{28}$ +22.7 (*c* 0.12, MeOH); UV λ_{max} (methanol) 230 nm (ε 12200), 321 nm (ε 14400); IR (KBr) 3385, 3227, 2925, 1701, 1601, 1485, 1413, 1372, 1289, 1017, 858, 787, 756, 724 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 9.05 (1 H, s, NH), 8.38 (1 H, d, *J* = 8.0, PyH), 8.25 (1 H, d, *J* = 8.0, PyH), 8.01 (1 H, s, SCH), 4.80 (1 H, q, *J* = 6.6, C*H*Me), 1.51 (3 H, d, *J* = 6.6, CH*Me*); ^{13}C NMR (100 MHz, d_6-DMSO) δ 168.6 (C), 167.1 (C), 165.9 (C), 164.6 (C), 160.4 (C), 153.7 (C), 133.3 (CH), 126.4 (C), 125.1 (CH), 119.7 (CH), 53.5 (CH), 19.1 (Me); MS (ES) *m/z* (relative intensity) 320 (M – H + $2Na^+$, 100%), 298 (M + Na^+, 18), 232 (32), 109 (28); HRMS calcd for $C_{12}H_8N_3O_3S$ (M – H) 274.0292, found 274.0290.



Ethyl 2,6-dimethyl-4-ethylpyridine-3-carboxylate (82)

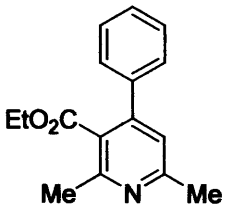
I. Preparation from hex-3-yn-2-one (78) and ethyl *β*-aminocrotonate (126). Hex-3-yn-2-one (**78**) (48 mg, 0.5 mmol) and ethyl *β*-aminocrotonate (**126**) (129 mg, 1.0 mmol) were

[i] HPLC: ee = 88%; ChiralPak OD-R, water–acetonitrile (95:5), λ_{max} = 321 nm, 0.5 mL min^{-1}, R_T = 18.9 min (*S*-isomer has R_T = 15.0 min).

[ii] Literature reported data (ref 40): FAB mass, positive 276 $(M+H)^+$, negative 274 $(M-H)^-$; ^1H-NMR (δ ppm DMSO-d_6) 1.45 (3H, d), 4.74 (1H, q), 8.01 (1H, s), 8.18 (1H, d, *J* = 8 Hz), 8.38 (1H, d, *J* = 8 Hz), 9.00 (1H, s, D_2O exchangeable); ^{13}C-NMR 168.2, 166.5, 165.6, 164.3, 159.8, 153.2, 132.7, 126.9, 124.6, 119.3, 53.0, 18.6; UV λ_{max} (methanol) 230 nm (ε 12200), 321 nm (ε 14400).

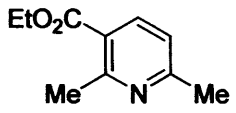
reacted according to general procedure **GP08**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (3:1), gave the *title compound* (97 mg, 94%) as a pale yellow oil.

II. Preparation from hex-3-yn-2-ol (135) and ethyl acetoacetate (74). Ethyl acetoacetate (**74**) (39 mg, 0.3 mmol) and hex-3-yn-2-ol (**135**) (59 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (3:1), gave the *title compound* (45 mg, 73%) as a pale yellow oil: IR (film) 2975, 2937, 1726, 1595, 1561, 1190, 1089 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.85 (1 H, s, PyH), 4.32 (2 H, q, J = 7.1, OCH_2Me), 2.50 (2 H, q, J = 7.6, CH_2Me), 2.44 (3 H, s, Me), 2.42 (3 H, s, Me), 1.30 (3 H, t, J = 7.1, OCH_2Me), 1.12 (3 H, t, J = 7.6, CH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5 (C), 158.9 (C), 154.7 (C), 151.1 (C), 126.7 (C), 120.7 (CH), 61.6 (CH_2), 26.6 (CH_2), 24.7 (Me), 23.2 (Me), 15.0 (Me), 14.5 (Me); MS (APcI) *m/z* (relative intensity) 208 (MH^+, 100%); HRMS calcd for $C_{12}H_{18}NO_2$ (MH) 208.1335, found 208.1337.



Ethyl 2,6-dimethyl-4-phenylpyridine-3-carboxylate (83)

Ethyl *β*-aminocrotonate (**126**) (0.26 g, 2.0 mmol) and 4-phenylbut-3-yn-2-one (**79**) (0.14 g, 1.0 mmol) were reacted according to general procedure **GP08**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (3:1), gave the *title compound* (0.06 g, 24%) as a pale yellow oil: IR (film) 2978, 2926, 1725, 1587, 1548, 1266, 1206, 1083, 870, 767, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (5 H, PhH), 6.94 (1 H, s, PyH), 4.01 (2 H, q, J = 7.1, OCH_2Me), 2.54 (3 H, s, Me), 2.50 (3 H, s, Me), 0.90 (3 H, t, J = 7.1, OCH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5 (C), 159.1 (C), 155.5 (C), 148.9 (C), 139.2 (C), 128.9 (CH), 128.8 (CH), 128.2 (CH), 126.1 (C), 121.6 (CH), 61.7 (CH_2), 24.9 (Me), 23.2 (Me), 14.0 (Me); MS (APcI) *m/z* (relative intensity) 256 (MH^+, 100%), 252 (2); HRMS calcd for $C_{16}H_{18}NO_2$ (MH) 256.1334, found 256.1337.



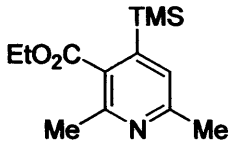
Ethyl 2,6-dimethylpyridine-3-carboxylate (85)

I. Preparation from but-3-yn-2-one (81) and ethyl β-aminocrotonate (126). Ethyl β-aminocrotonate (**126**) (129 mg, 1.0 mmol) and but-3-yn-2-one (**85**) (163 mg, 2.4 mmol) were reacted according to general procedure **GP05** or **GP06**. Purification by flash chromatography on silica, eluting with light petroleum–diethyl ether (3:1), gave the *title compound* (138 mg, 77%, for method **GP05**; 116 mg, 65%, for method **GP06**) as a pale yellow oil.

II. Preparation from 4-(trimethylsilyl)but-3-yn-2-one (80) and ethyl β-aminocrotonate (126). Ethyl β-aminocrotonate (**126**) (129 mg, 1.0 mmol) and 4-(trimethylsilyl)but-3-yn-2-one (**80**) were reacted according to general procedure **GP07** or **GP08**. Purification by flash chromatography on silica, eluting with light petroleum–diethyl ether (3:1), gave the *title compound* (38 mg, 21%, for method **GP07**; 62 mg, 69%, for method **GP08**) as a pale yellow oil.

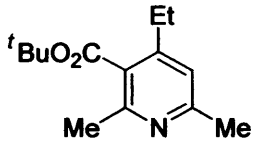
III. Preparation from but-3-yn-2-ol (131) and ethyl β-aminocrotonate (126). Ethyl β-aminocrotonate (**126**) (129 mg, 1.0 mmol) and but-3-yn-2-ol (**131**) (140 mg, 2.0 mmol) were reacted according to general procedure **GP10**. Purification by flash chromatography on silica, eluting with light petroleum–diethyl ether (3:1), gave the *title compound* (81 mg, 45%) as a pale yellow oil.

IV. Preparation from but-3-yn-2-ol (131) and ethyl acetoacetate (74). Ethyl acetoacetate (**74**) (39 mg, 0.3 mmol) and but-3-yn-2-ol (**131**) (42 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–diethyl ether (3:1), gave the *title compound* (36 mg, 66%) as a pale yellow oil: IR (film) 2983, 1724, 1593, 1446, 772 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (1 H, d, J = 8.0, PyH), 6.99 (1 H, d, J = 8.0, PyH), 4.29 (2 H, q, J = 7.1, OCH_2Me), 2.74 (3 H, s, Me), 2.50 (3 H, s, Me), 1.32 (3 H, t, J = 7.1, OCH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1 (C), 161.6 (C), 159.8 (C), 139.2 (CH), 123.1 (C), 120.8 (CH), 61.4 (CH_2), 25.2 (Me), 25.1 (Me), 14.7 (Me); MS (APcI) *m/z* (relative intensity) 180 (MH^+, 100%); HRMS calcd for $C_{10}H_{14}NO_2$ (MH) 180.1021, found 180.1024.



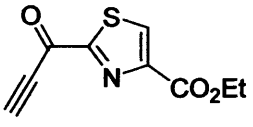
Ethyl 2,6-dimethyl-4-(trimethylsilyl)pyridine-3-carboxylate (86)

Ethyl *β*-aminocrotonate (**126**) (129 mg, 1.0 mmol) and 4-(trimethylsilyl)but-3-yn-2-one (**80**) (168 mg, 1.2 mmol) were reacted according to general procedure **GP06**. Purification by flash chromatography on silica, eluting with light petroleum–diethyl ether (3:1), gave the *title compound* (226 mg, 90%) as a pale yellow oil: IR (film) 2956, 2903, 1724, 1529, 1104, 1085, 1016 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.12 (1 H, s, PyH), 4.35 (2 H, q, J = 7.2, OCH_2Me), 2.54 (3 H, s, Me), 2.51 (3 H, s, Me), 1.37 (3 H, t, J = 7.2, OCH_2Me), 0.26 (9 H, s, $SiMe_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1 (C), 158.5 (C), 154.9 (C), 149.4 (C), 131.5 (C), 126.6 (CH), 62.1 (CH_2), 30.5 (Me), 24.1 (Me), 14.9 (Me), 0.0 (Me); MS (APcI) *m/z* (relative intensity) 252 (MH^+, 100%), 236 (2); HRMS calcd for $C_{13}H_{22}NO_2Si$ (MH) 252.1420, found 252.1420.



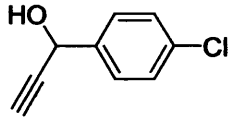
***tert*-Butyl 2,6-dimethyl-4-ethylpyridine-3-carboxylate (88)**

tert-Butyl acetoacetate (**76**) (47 mg, 0.3 mmol) and hex-3-yn-2-ol (**135**) (59 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (4:1), gave the *title compound* (42 mg, 60%) as a pale yellow oil: IR (film) 2974, 2932, 1721, 1549, 1562, 1162, 1092 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.83 (1 H, s, PyH), 2.54 (2 H, q, J = 7.6, CH_2Me), 2.45 (3 H, s, Me), 2.44 (3 H, s, Me), 1.53 (9 H, s, CMe_3), 1.14 (3 H, t, J = 7.6, CH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (C), 160.7 (C), 156.5 (C), 152.6 (C), 130.4 (C), 123.1 (CH), 85.0 (C), 30.8 (Me), 28.8 (CH_2), 27.1 (Me), 25.4 (Me), 17.4 (Me); MS (APcI) *m/z* (relative intensity) 236 (MH^+, 100%), 181 (5), 180 (60); HRMS calcd for $C_{14}H_{22}NO_2$ (MH) 236.1651, found 236.1650.



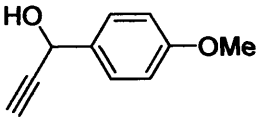
Ethyl 2-(propynoyl)thiazole-4-carboxylate (114)

Activated manganese(IV) oxide (69 mg, 0.71 mmol) was added to a solution of ethyl 2-(1-hydroxyprop-2-ynyl)thiazole-4-carboxylate (**319**) (28 mg, 0.14 mmol) in dichloromethane (2 mL). After stirring for 10 min at room temperature, a further aliquot of activated manganese(IV) oxide (69 mg, 0.71 mmol) was added and the mixture was stirred for a further 10 min. The resulting mixture was filtered through Celite®, washed with dichloromethane (1 mL) and evaporated without heat under a nitrogen atmosphere to give the *title compound* (28 mg, 100%) as a pale yellow solid that was used without further purification, mp 101–102 °C (from dichloromethane): IR (CH_2Cl_2) 3054, 2984, 2103, 1732, 1650, 1447, 1367, 1334, 1222, 1104, 1020, 969, 932 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.44 (1 H, s, 5-H), 4.40 (2 H, q, J = 7.1 CH_2Me), 3.66 (1 H, s, CCH), 1.40 (3 H, t, J = 7.1, CH_2*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5 (C), 166.5 (C), 161.0 (C), 149.0 (C), 134.0 (CH), 85.4 (CH), 79.3 (C), 62.5 (CH_2), 14.7 (Me); MS (APcI) *m/z* (relative intensity) 210 (MH^+, 100%); HRMS calcd for $C_9H_8NO_3S$ (MH) 210.0225, found 210.0224.



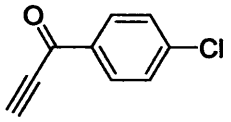
1-(4-Chlorophenyl)prop-2-yn-1-ol (119)

4-Chlorobenzaldehyde (**117**) (703 mg, 5.0 mmol) was reacted according to general procedure **GP01**. Purification by flash chromatography on silica, eluting with dichloromethane, gave the *title compound*[186] (825 mg, 99%) as a pale yellow oil: IR (film) 3297, 1597, 1490, 1406, 1258, 1192, 1092, 1015, 950, 835, 791 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 (2 H, d, J = 8.5, *o*-Ph*H*), 7.24 (2 H, d, J = 8.5, *m*-Ph*H*), 5.31 (1 H, d, J = 2.1, 1-H), 2.58 (1 H, d, J = 2.1, 3-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9 (C), 134.6 (C), 129.2 (CH), 128.6 (CH), 83.7 (C), 75.9 (CH), 63.7 (CH); MS (EI) *m/z* (relative intensity) 168 (M^+, 13%), 166 (M^+, 43), 149 (100), 131 (100), 113 (27), 103 (72), 77 (65), 63 (26), 53 (100); HRMS calcd for $C_9H_6{}^{35}ClO$ (M – H) 165.0106, found 165.0106.



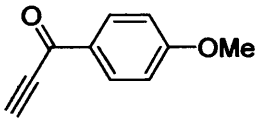
1-(4-Methoxyphenyl)prop-2-yn-1-ol (120)

p-Anisaldehyde (**118**) (681 mg, 5.0 mmol) was reacted according to general procedure **GP01**. Purification by flash chromatography on silica, eluting with dichloromethane, gave the *title compound*[187] (801 mg, 99%) as a pale yellow oil: IR (film) 3288, 1611, 1512, 1464, 1249, 1175, 1031, 948, 834, 767 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 (2 H, d, *J* = 8.7, *o*-Ph*H*), 6.80 (2 H, d, *J* = 8.7, *m*-Ph*H*), 5.30 (1 H, d, *J* = 2.1, 1-H), 3.70 (3 H, s, OMe), 2.57 (1 H, d, *J* = 2.1, 3-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.8 (C), 133.2 (C), 128.6 (CH), 114.3 (CH), 84.7 (C), 75.2 (CH), 63.8 (CH), 55.6 (Me); MS (EI) *m/z* (relative intensity) 162 (M^+, 81%), 145 (100), 131 (62), 109 (82), 77 (69), 63 (70), 53 (100); HRMS calcd for $C_{10}H_{10}O_2$ (M) 162.0681, found 162.0684.



1-(4-Chlorophenyl)prop-2-yn-1-one (122)

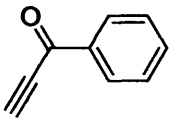
1-(4-Chlorophenyl)prop-2-yn-1-ol (**119**) (833 mg, 5.0 mmol) was reacted according to general procedure **GP02**. Purification by flash chromatography on silica, eluting with chloroform, gave the *title compound* (774 mg, 94%) as a light brown solid, mp 104–105 °C (from aqueous ethanol) (lit.[188] mp 68–69 °C): IR (KBr) 3221, 2099, 1637, 1587, 1401, 1259, 1173, 1093, 1010, 843, 751, 674 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (2 H, d, *J* = 8.6, *o*-Ph*H*), 7.42 (2 H, d, *J* = 8.6, *m*-Ph*H*), 3.40 (1 H, s, 3-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5 (C), 141.6 (C), 134.9 (C), 131.4 (CH), 129.5 (CH), 82.0 (CH), 80.3 (C); MS (APcI) *m/z* (relative intensity) 167 (MH^+, 36%), 165 (MH^+, 100), 139 (1), 109 (1), 97 (3); HRMS calcd for $C_9H_5{}^{35}ClO$ (M) 164.0029, found 164.0030; Anal. Calcd for C_9H_5ClO: C, 65.7; H, 3.1; N, 0.0. Found: C, 65.3; H, 3.1; N, 0.0.



1-(4-Methoxyphenyl)prop-2-yn-1-one (123)

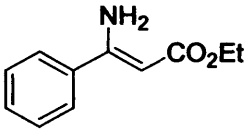
1-(4-Methoxyphenyl)prop-2-yn-1-ol (**120**) (811 mg, 5.0 mmol) was reacted according to general procedure **GP02**. Purification by flash chromatography on silica, eluting with chloroform, gave the *title compound* (784 mg, 98%) as a pale yellow solid, mp 84–85 °C (from aqueous ethanol) (lit.[189] mp 85–87 °C): IR (KBr) 3250, 2094, 1639, 1599, 1511, 1424, 1272, 1171, 1024, 840, 758, 686 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (2 H, d, *J* = 8.9,

o-Ph*H*), 6.90 (2 H, d, J = 8.9, *m*-Ph*H*), 3.83 (3 H, s, OMe), 3.31 (1 H, s, 3-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4 (C), 165.2 (C), 132.5 (CH), 129.9 (C), 114.4 (CH), 80.8 (C), 80.8 (CH), 56.0 (Me); MS (APcI) *m/z* (relative intensity) 161 (MH^+, 100%), 135 (1); HRMS calcd for $C_{10}H_8O_2$ (M) 160.0524, found 160.0523; Anal. Calcd for $C_{10}H_8O_2$: C, 75.0; H, 5.0; N, 0.0. Found: C, 74.4; H, 5.1; N, 0.0.



1-Phenylprop-2-yn-1-one (124)

1-Phenylprop-2-yn-1-ol (**121**) (1.60 g, 12.1 mmol) was reacted according to general procedure **GP02** to give the *title compound* (1.56 g, 99%) as a pale yellow solid, mp 49–50 °C (from methanol) (lit.[190] mp 47–48 °C): IR (KBr) 3232, 2094, 1644, 1596, 1579, 1452, 1315, 1265, 1176, 1007, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (2 H, m, *o*-Ph*H*), 7.57 (1 H, m, *p*-Ph*H*), 7.50 (2 H, m, *m*-Ph*H*), 3.38 (1 H, s, 3-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4 (C), 135.0 (C), 133.5 (CH), 128.6 (CH), 127.7 (CH), 80.1 (C), 79.2 (CH); MS (EI) *m/z* (relative intensity) 130 (M^+, 83%), 102 (100), 77 (37); HRMS calcd for C_9H_6O (M) 130.0419, found 130.0418.

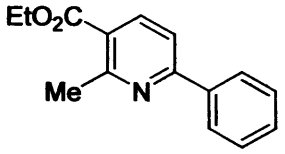


Ethyl 3-amino-3-phenylpropenoate (125)[i]

A solution of ethyl benzoylacetate (**75**) (5 mL, 29.0 mmol) and ammonium acetate (13.4 g, 0.17 mol) was heated at reflux in toluene–glacial acetic acid (5:1) (40 mL) for 20 h using a Dean and Stark trap. The mixture was partitioned between water (100 mL) and diethyl ether (60 mL), the aqueous layer was further extracted with diethyl ether (2 x 25 mL) and the combined organic layers were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (50 mL) and brine (25 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography in silica, eluting with light petroleum–ethyl acetate (3:1), gave the *title compound* (3.32 g, 60%) as a pale yellow oil: IR (film) 3440, 3326, 3066,

[i] All enaminoesters and enols prepared by us are as homogenous single geometrical isomer with structures as shown in the figures. The configuration of double bond was proposed based on the potential hydrogen bonding between the hydrogen on NH_2 or OH and the carbonyl oxygen of the ester group.

2979, 1663, 1556, 796, 772, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.50–6.50 (1 H, br s, N*H*H), 7.48 (2 H, m, *o*-Ph*H*), 7.35 (3 H, *m,p*-Ph*H*), 6.10–3.80 (1 H, br s, NH*H*), 4.90 (1 H, s, CH), 4.11 (2 H, q, $J = 7.1$, CH_2), 1.23 (3 H, t, $J = 7.1$, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9 (C), 160.9 (C), 138.1 (C), 130.7 (CH), 129.3 (CH), 126.6 (CH), 85.0 (CH), 59.4 (CH_2), 15.0 (Me); MS (APcI) *m/z* (relative intensity) 192 (MH^+, 100%), 186 (36), 103 (11); HRMS calcd for $C_{11}H_{14}NO_2$ (MH) 192.1025, found 192.1024.



Ethyl 2-methyl-6-phenylpyridine-3-carboxylate (127)

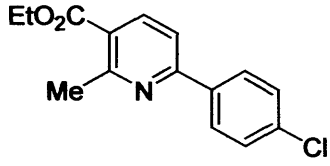
I. Preparation from 1-phenylprop-2-yn-1-one (124) and ethyl *β*-aminocrotonate (126). Ethyl *β*-aminocrotonate (**126**) (0.13 g, 1.0 mmol) and 1-phenylprop-2-yn-1-one (**124**) were reacted according to general procedure **GP04**, **GP05**, **GP06**, **GP07** or **GP08**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (0.19 g, 80%, for method **GP04**; 0.20 g, 85%, for method **GP05**; 0.21 g, 86%, for method **GP06**; 0.19 g, 80%, for method **GP07**; 0.11 g, 87%, for method **GP08**) as a pale yellow solid.

II. Preparation from 1-phenylprop-2-yn-1-one (124) and ethyl acetoacetate (74). 1-Phenylprop-2-yn-1-one (**124**) (80 mg, 0.6 mmol) and ethyl acetoacetate (**74**) (130 mg, 1.0 mmol) were reacted according to general procedure **GP09**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (138 mg, 95%) as a pale yellow solid.

III. Preparation from 1-phenylprop-2-yn-1-ol (121) and ethyl *β*-aminocrotonate (126). 1-Phenylprop-2-yn-1-ol (**121**) (260 mg, 2.0 mmol) and ethyl *β*-aminocrotonate (**126**) (130 mg, 1.0 mmol) were reacted according to general procedure **GP10**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (169 mg, 70%) as a pale yellow solid.

IV. Preparation from 1-phenylprop-2-yn-1-ol (121) and ethyl acetoacetate (74). 1-Phenylprop-2-yn-1-ol (**121**) (79 mg, 0.6 mmol) and ethyl acetoacetate (**74**) (39 mg, 0.3 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title*

compound (30 mg, 42%) as a pale yellow solid, mp 44–45 °C (from methanol) (lit.[156] mp 44 °C): IR (KBr) 2980, 2925, 2890, 1717, 1581, 1476, 1277, 1090, 1022 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (1 H, d, *J* = 8.2, PyH), 8.00 (2 H, m, *o*-Ph*H*), 7.55 (1 H, d, *J* = 8.2, PyH), 7.41 (3 H, *m,p*-Ph*H*), 4.33 (2 H, q, *J* = 7.1, OCH_2Me), 2.85 (3 H, s, Me), 1.35 (3 H, t, *J* = 7.1, OCH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7 (C), 159.1 (C), 158.1 (C), 138.5 (CH), 137.6 (C), 128.7 (CH), 128.0 (CH), 126.5 (CH), 122.8 (C), 116.5 (CH), 60.3 (CH_2), 24.6 (Me), 13.5 (Me); MS (APcI) *m/z* (relative intensity) 241 (M^+, 91%), 240 (69), 212 (32), 196 (100), 195 (98), 168 (43), 167 (40); HRMS calcd for $C_{15}H_{16}NO_2$ (MH) 242.1182, found 242.1182; Anal. Calcd for $C_{15}H_{15}NO_2$: C, 74.7; H, 6.3; N, 5.8. Found: C, 74.4; H, 6.5; N, 5.6.



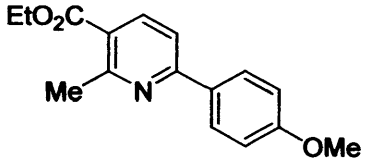
Ethyl 2-methyl-6-(4-chlorophenyl)pyridine-3-carboxylate (128)

I. Preparation from 1-(4-chlorophenyl)prop-2-yn-1-one (122) and ethyl β-aminocrotonate (126). Ethyl β-aminocrotonate (**126**) (65 mg, 0.5 mmol) and 1-(4-chlorophenyl)prop-2-yn-1-one (**122**) were reacted according to general procedure **GP06** or **GP08**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (91 mg, 66%, for method **GP06**; 52 mg, 75%, for method **GP08**) as a pale yellow solid.

II. Preparation from 1-(4-chlorophenyl)prop-2-yn-1-one (122) and ethyl acetoacetate (74). 1-(4-Chlorophenyl)prop-2-yn-1-one (**122**) (99 mg, 0.6 mmol) and ethyl acetoacetate (**74**) (130 mg, 1.0 mmol) were reacted according to general procedure **GP09**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (139 mg, 84%) as a pale yellow solid.

III. Preparation from 1-(4-chlorophenyl)prop-2-yn-1-ol (119) and ethyl β-aminocrotonate (126). 1-(4-Chlorophenyl)prop-2-yn-1-ol (**119**) (166 mg, 1.0 mmol) and ethyl β-aminocrotonate (**126**) (65 mg, 0.5 mmol) were reacted according to general procedure **GP10**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (100 mg, 73%) as a pale yellow solid.

IV. Preparation from 1-(4-chlorophenyl)prop-2-yn-1-ol (119) and ethyl acetoacetate (74). 1-(4-Chlorophenyl)prop-2-yn-1-ol (**119**) (100 mg, 0.6 mmol) and ethyl acetoacetate (**74**) (39 mg, 0.3 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (80 mg, 96%) as a pale yellow solid, mp 47–48 °C (from aqueous ethanol) (lit.[191] mp 47–48 °C): IR (KBr) 2982, 1719, 1585, 1456, 1270, 1093, 1012, 834, 784 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (1 H, d, J = 8.2, PyH), 7.94 (2 H, d, J = 8.6, *o*-Ph*H*), 7.52 (1 H, d, J = 8.2, PyH), 7.37 (2 H, d, J = 8.6, *m*-Ph*H*), 4.32 (2 H, q, J = 7.1, OCH_2Me), 2.83 (3 H, s, Me), 1.34 (3 H, t, J = 7.1, OCH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9 (C), 160.4 (C), 158.1 (C), 139.8 (CH), 137.2 (C), 136.3 (C), 129.4 (CH), 129.0 (CH), 124.3 (C), 117.5 (CH), 61.6 (CH_2), 25.7 (Me), 14.7 (Me); MS (APcI) *m/z* (relative intensity) 278 (MH^+, 25%), 276 (MH^+, 100%), 150 (10); HRMS calcd for $C_{15}H_{14}{}^{35}ClNO_2$ (M) 275.0713, found 275.0714.



Ethyl 2-methyl-6-(4-methoxyphenyl)pyridine-3-carboxylate (129)

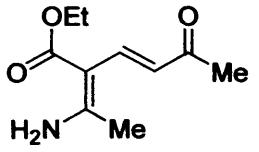
I. Preparation from 1-(4-methoxyphenyl)prop-2-yn-1-one (123) and ethyl β-aminocrotonate (126). Ethyl β-aminocrotonate (**126**) (65 mg, 0.5 mmol) and 1-(4-methoxyphenyl)prop-2-yn-1-one (**123**) were reacted according to general procedure **GP06** or **GP08**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:2), gave the *title compound* (68 mg, 50%, for method **GP06**; 45 mg, 66%, for method **GP08**) as a pale yellow solid.

II. Preparation from 1-(4-methoxyphenyl)prop-2-yn-1-one (123) and ethyl acetoacetate (74). 1-(4-Methoxyphenyl)prop-2-yn-1-one (**123**) (96 mg, 0.6 mmol) and ethyl acetoacetate (**74**) (130 mg, 1.0 mmol) were reacted according to general procedure **GP09**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:2), gave the *title compound* (146 mg, 90%) as a pale yellow solid.

III. Preparation from 1-(4-methoxyphenyl)prop-2-yn-1-ol (120) and ethyl β-aminocrotonate (126). 1-(4-Methoxyphenyl)prop-2-yn-1-ol (**120**) (162 mg, 1.0 mmol) and ethyl β-aminocrotonate (**126**) (65 mg, 0.5 mmol) were reacted according to general

procedure **GP10**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:2), gave the *title compound* (72 mg, 53%) as a pale yellow solid.

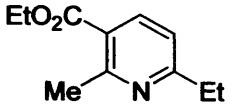
IV. Preparation from 1-(4-methoxyphenyl)prop-2-yn-1-ol (120) and ethyl acetoacetate (74). 1-(4-Methoxyphenyl)prop-2-yn-1-ol (**120**) (97 mg, 0.6 mmol) and ethyl acetoacetate (**74**) (39 mg, 0.3 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:2), gave the *title compound* (69 mg, 85%) as a pale yellow solid, mp 68–69 °C (from methanol) (lit.[192] mp 68–69 °C): IR (KBr) 2979, 2933, 2835, 1717, 1584, 1514, 1460, 1269, 1187, 1094, 1036, 828, 786 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (1 H, d, J = 8.2, PyH), 7.96 (2 H, d, J = 8.8, *o*-Ph*H*), 7.48 (1 H, d, J = 8.2, PyH), 6.92 (2 H, d, J = 8.8, *m*-Ph*H*), 4.31 (2 H, q, J = 7.1, OCH_2Me), 3.79 (3 H, s, OMe), 2.82 (3 H, s, Me), 1.34 (3 H, t, J = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2 (C), 161.4 (C), 160.3 (C), 159.1 (C), 139.7 (CH), 131.5 (C), 129.1 (CH), 123.2 (C), 116.9 (CH), 114.6 (CH), 61.5 (CH_2), 55.8 (Me), 25.8 (Me), 14.7 (Me); MS (APcI) *m/z* (relative intensity) 272 (MH^+, 100%), 150 (10); HRMS calcd for $C_{16}H_{17}NO_3$ (M) 271.1208, found 271.1206.



(4*E*)-2-Amino-3-ethoxycarbonylhepta-2,4-dien-6-one (130)

Ethyl *β*-aminocrotonate (**126**) (0.13 g, 1.0 mmol) and 4-(trimethylsilyl)but-3-yn-2-one (**80**) (0.25 mL, 1.5 mmol) were reacted for 6 h according to general procedure **GP03**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (0.16 g, 82%) as a yellow solid, mp 125.5–126.4 °C (from light petroleum) (lit.[156] mp 135 °C): IR (Nujol) 3335, 3194, 2924, 1643, 1548, 1462 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.59 (1 H, br s, N*H*H), 7.53 (1 H, d, J = 15.5, 4-H), 6.50 (1 H, d, J = 15.5, 5-H), 5.57 (1 H, br s, NH*H*), 4.20 (2 H, q, J = 7.1, OCH_2Me), 2.23 (3 H, s, Me), 2.17 (3 H, s, Me), 1.31 (3 H, t, J = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.7 (C), 170.5 (C), 166.5 (C), 140.4 (CH), 122.0 (CH), 95.3 (C), 59.9 (CH_2), 29.2 (Me), 23.5 (Me), 15.3 (Me); MS (APcI) *m/z* (relative intensity) 198 (MH^+, 9%), 181 (100), 152 (29), 107 (9); HRMS

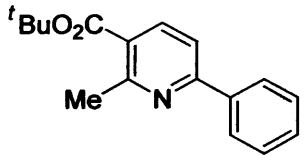
calcd for $C_{10}H_{16}NO_3$ (MH) 198.1129, found 198.1130; Anal. Calcd for $C_{10}H_{15}NO_3$: C, 60.5; H, 7.6; N, 7.0. Found: C, 60.9; H, 7.6; N, 7.1.



Ethyl 2-methyl-4-ethylpyridine-3-carboxylate (133)

I. Preparation from pent-1-yn-3-ol (132) and ethyl *β*-aminocrotonate (126). Pent-1-yn-3-ol (**132**) (0.17 g, 2.0 mmol) and ethyl *β*-aminocrotonate (**126**) (0.13 g, 1.0 mmol) were reacted according to general procedure **GP10**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (0.04 g, 20%) as a pale yellow oil.

II. Preparation from pent-1-yn-3-ol (132) and ethyl acetoacetate (74). Ethyl acetoacetate (**74**) (39 mg, 0.3 mmol) and pent-1-yn-3-ol (**132**) (51 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (28 mg, 49%) as a pale yellow oil: IR (KBr) 2961, 2934, 1726, 1591, 1464, 1443, 1398, 1261, 1096, 1022, 866, 804, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (1 H, d, J = 8.1, PyH), 7.06 (1 H, d, J = 8.1, PyH), 4.35 (2 H, q, J = 7.1, OCH_2Me), 2.82 (2 H, q, J = 7.6, CH_2Me), 2.81 (3 H, s, Me), 1.38 (3 H, t, J = 7.1, OCH_2Me), 1.29 (3 H, t, J = 7.6, CH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8 (C), 166.3 (C), 159.4 (C), 138.9 (CH), 122.8 (C), 119.0 (CH), 61.0 (CH_2), 31.6 (CH_2), 24.9 (Me), 14.3 (Me), 13.8 (Me); MS (APcI) *m/z* (relative intensity) 194 (MH^+, 100%); HRMS calcd for $C_{11}H_{16}NO_2$ (MH) 194.1176, found 194.1177.



***tert*-Butyl 2-methyl-6-phenylpyridine-3-carboxylate (134)**

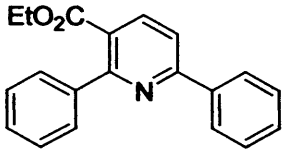
tert-Butyl acetoacetate (**76**) (47 mg, 0.3 mmol) and 1-phenylprop-2-yn-1-ol (**121**) (79 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (59 mg, 73%) as a colourless oil: IR (KBr) 3006, 2980, 2933, 1699, 1580, 1560,

1446, 1384, 1371, 1305, 1259, 1172, 1146, 1102, 840, 760, 688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (1 H, d, J = 8.2, PyH), 7.91 (2 H, m, *o*-Ph*H*), 7.44 (1 H, d, J = 8.2, PyH), 7.29 (3 H, *m,p*-Ph*H*), 2.77 (3 H, s, Me), 1.49 (9 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1 (C), 159.4 (C), 158.6 (C), 139.2 (CH), 138.6 (C), 129.5 (CH), 128.8 (CH), 127.3 (CH), 125.3 (C), 117.4 (CH), 81.7 (C), 28.3 (Me), 25.4 (Me); MS (APcI) *m/z* (relative intensity) 270 (MH^+, 90%), 214 (100); HRMS calcd for $C_{17}H_{20}NO_2$ (MH) 270.1489, found 270.1487.

S-Ethyl 3-oxo-3-phenylthiopropionate (136) and S-ethyl 3-hydroxy-3-phenylthiopropenoate

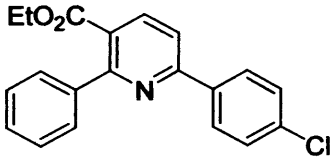
(i) Formation of lithium enolate of *S*-ethyl thioacetate. To a stirred solution of diisopropylamine (1.40 mL, 10.0 mmol) in dry tetrahydrofuran (10 mL) was added *n*-butyllithium in hexanes (2.5 M; 4.00 mL, 10.0 mmol) at 0 °C. The mixture was stirred for 10 min and cooled to –65 to –70 °C. Freshly distilled *S*-ethyl thioacetate (0.53 mL, 5.0 mmol) was added and the solution was stirred for 30 min. **(ii) Formation of *S*-ethyl 3-oxo-3-phenylthiopropionate (136) and *S*-ethylthio 3-hydroxy-3-phenylpropenoate.** To a stirred solution of benzoic acid (**223**) (0.61 g, 5.0 mmol) in dry THF (10 mL) was added triethylamine (1.39 mL, 10.0 mmol) followed by ethyl chloroformate (0.96 mL, 10.0 mmol) at 0 °C. After the mixture was stirred for 30 min at 0 °C, triethylamine hydrochloride was filtered off. The filtrate was added dropwise to the above solution of the lithium enolate of *S*-ethyl thioacetate at –68 °C. The mixture was stirred at –68 °C for 20 min and saturated aqueous ammonium chloride solution (50 mL) was added. The resulting mixture was warmed to room temperature. After extracting with ethyl acetate (50 mL), the organic extract was washed with brine (30 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–chloroform (2:1), gave the *title compound* (0.73 g, 70%) as a pale yellow oil: IR (film) 3062, 2970, 2931, 2874, 1698, 1672, 1611, 1574, 1451, 1381, 1266, 1096, 1054, 911, 757, 688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.16 (0.47 H, s, enolOH), 7.88 (1.06 H, m, *o*-Ph*H*), 7.70 (0.94 H, m, *o*-Ph*H*), 7.51 (0.53 H, m, *p*-Ph*H*), 7.39 (1.41 H, *m,p*-Ph*H*), 7.33 (1.06 H, m, *m*-Ph*H*), 6.00 (0.47 H, s, CH), 4.13 (1.06 H, s, CH_2), 2.91 (0.94 H, q, J = 7.4, SCH_2Me), 2.85 (1.06 H, q, J

= 7.4, SC*H*$_2$Me), 1.25 (1.41 H, t, *J* = 7.4, SCH$_2$*Me*), 1.18 (1.59 H, t, *J* = 7.4, SCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.5 (C), 193.1 (C), 192.5 (C), 169.1 (C), 136.4 (C), 134.2 (CH), 133.3 (C), 132.1 (CH), 129.3 (CH), 129.2 (CH), 129.0 (CH), 126.8 (CH), 97.7 (CH), 54.4 (CH_2), 24.5 (CH_2), 23.4 (CH_2), 15.4 (Me), 14.9 (Me); (MS (APcI) *m/z* (relative intensity) 209 (MH^+, 2%), 147 (9), 105 (100); HRMS calcd for $C_{11}H_{12}O_2S$ (M) 208.0552, found 208.0557.



Ethyl 2,6-diphenylpyridine-3-carboxylate (137)

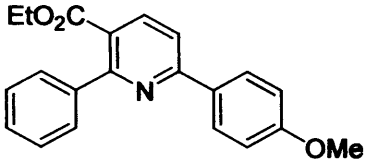
Ethyl benzoylacetate (**75**) (58 mg, 0.3 mmol) and 1-phenylprop-2-yn-1-ol (**121**) (79 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with chloroform, gave the *title compound* (37 mg, 41%) as a pale yellow oil: IR (KBr) 2970, 1716, 1586, 1574, 1437, 1380, 1261, 1177, 1142, 1111, 1018, 802, 760, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (1 H, d, *J* = 8.2, PyH), 8.06 [2 H, d, *J* = 7.2, 6-(*o*-Ph*H*)], 7.70 (1 H, d, *J* = 8.2, PyH), 7.57 [2 H, m, 2-(*o*-Ph*H*)], 7.43–7.37 (6 H, m, PhH), 4.10 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 1.00 (3 H, t, *J* = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3 (C), 157.7 (C), 157.4 (C), 139.5 (C), 137.9 (CH), 137.2 (C), 128.7 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.0 (CH), 126.3 (CH), 124.3 (C), 116.8 (CH), 60.4 (CH_2), 12.6 (Me); MS (APcI) *m/z* (relative intensity) 304 (MH^+, 100%); HRMS calcd for $C_{20}H_{18}NO_2$ (MH) 304.1332, found 304.1332.



Ethyl 2-phenyl-6-(4-chlorophenyl)pyridine-3-carboxylate (138)

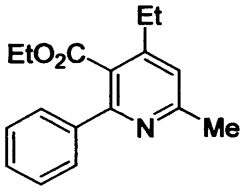
Ethyl benzoylacetate (**75**) (58 mg, 0.3 mmol) and 1-(4-chlorophenyl)prop-2-yn-1-ol (**119**) (100 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–chloroform (1:2), gave the *title compound* (64 mg, 63%) as a pale yellow oil: IR (KBr) 3056, 2986, 1720, 1585, 1572, 1491, 1436, 1375, 1290, 1181, 1144, 1092, 1052, 1012, 830, 802, 769, 699 cm^{-1}; ^{1}H NMR (400

MHz, $CDCl_3$) δ 8.10 (1 H, d, *J* = 8.2, PyH), 8.00 [2 H, d, *J* = 8.5, 6-(*o*-Ph*H*)], 7.66 (1 H, d, *J* = 8.2, PyH), 7.55 [2 H, m, 2-(*o*-Ph*H*)], 7.37 [2 H, d, *J* = 8.5, 6-(*m*-Ph*H*)], 7.37 [3 H, 2-(*m,p*-Ph*H*)], 4.10 (2 H, q, *J* = 7.1, OCH_2Me), 0.99 (3 H, t, *J* = 7.1, OCH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2 (C), 158.8 (C), 157.1 (C), 140.4 (C), 139.1 (CH), 136.7 (C), 136.0 (C), 129.0 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.1 (CH), 125.6 (C), 117.6 (CH), 61.5 (CH_2), 13.7 (Me); MS (APcI) *m/z* (relative intensity) 340 (MH^+, 30%), 338 (MH^+, 100); HRMS calcd for $C_{20}H_{17}{}^{35}ClNO_2$ (MH) 338.0942, found 338.0946.



Ethyl 2-phenyl-6-(4-methoxyphenyl)pyridine-3-carboxylate (139)

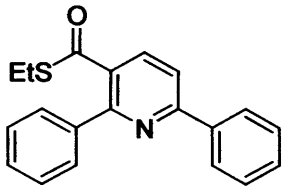
Ethyl benzoylacetate (**75**) (58 mg, 0.3 mmol) and 1-(4-methoxyphenyl)prop-2-yn-1-ol (**120**) (97 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with chloroform, gave the *title compound* (60 mg, 60%) as a pale yellow solid, mp 93–95 °C (from methanol): IR (KBr) 2984, 2936, 1715, 1605, 1586, 1572, 1513, 1454, 1436, 1422, 1379, 1293, 1176, 1148, 1018, 830, 772, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (1 H, d, *J* = 8.6, PyH), 8.03 [2 H, d, *J* = 9.1, 6-(*o*-Ph*H*)], 7.63 (1 H, d, *J* = 8.6, PyH), 7.56 [2 H, m, 2-(*o*-Ph*H*)], 7.37 [3 H, 2-(*m,p*-Ph*H*)], 6.93 [2 H, d, *J* = 9.1, 6-(*m*-Ph*H*)], 4.09 (2 H, q, *J* = 7.0, OCH_2Me), 3.80 (3 H, s, OMe), 0.99 (3 H, t, *J* = 7.0, OCH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4 (C), 161.1 (C), 158.8 (C), 158.1 (C), 140.7 (C), 138.9 (CH), 130.9 (C), 128.9 (CH), 128.8 (CH), 128.5 (CH), 128.0 (CH), 124.5 (C), 117.0 (CH), 114.2 (CH), 61.3 (CH_2), 55.4 (Me), 13.7 (Me); MS (APcI) *m/z* (relative intensity) 334 (MH^+, 100%); HRMS calcd for $C_{21}H_{20}NO_3$ (MH) 334.1438, found 334.1440.



Ethyl 4-ethyl-6-methyl-2-phenylpyridine-3-carboxylate (140)

Ethyl benzoylacetate (**75**) (58 mg, 0.3 mmol) and hex-3-yn-2-ol (**135**) (59 mg, 0.6 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (3:1), gave the *title compound* (45 mg, 56%)

as a pale yellow oil: IR (film) 2976, 2937, 1723, 1555, 1145, 1086, 1015, 918, 870, 768 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 (2 H, m, *o*-Ph*H*), 7.30 (3 H, *m,p*-Ph*H*), 6.96 (1 H, s, PyH), 4.00 (2 H, q, *J* = 7.6, OCH_2Me), 2.63 (2 H, q, *J* = 7.1, CH_2Me), 2.52 (3 H, s, Me), 1.18 (3 H, t, *J* = 7.6, OCH_2Me), 0.88 (3 H, t, *J* = 7.1, CH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5 (C), 159.4 (C), 156.8 (C), 151.9 (C), 140.8 (C), 128.8 (CH), 128.7 (CH), 128.7 (CH), 126.5 (C), 121.8 (CH), 61.7 (CH_2), 26.6 (CH_2), 25.0 (Me), 15.1 (Me), 14.0 (Me); MS (APcI) *m/z* (relative intensity) 270 (MH^+, 100%), 107 (4), 77 (4), 59 (49); HRMS calcd for $C_{17}H_{20}NO_2$ (MH) 270.1495, found 270.1494.

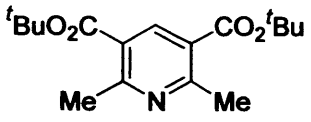


***S*-Ethyl 2,6-diphenylpyridine-3-thiocarboxylate (141)**

I. Preparation from *S*-ethyl 3-amino-3-phenylthiopropenoate (233). *S*-Ethyl 3-amino-3-phenylthiopropenoate (**233**) (518 mg, 2.50 mmol) and 1-phenylprop-2-yn-1-one (**124**) (390 mg, 3.00 mmol) were reacted according to general procedure **GP05** or **GP06**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (3:2), gave the *title compound* (375 mg, 47%, for method **GP05**; 144 mg, 18%, for method **GP06**) as pale yellow crystals.

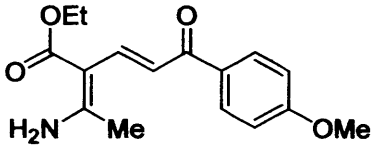
II. Preparation from *S*-ethyl benzoylthioacetate (136) and *S*-ethyl 3-hydroxy-3-phenylthiopropenoate. *S*-Ethyl benzoylthioacetate (**136**) and *S*-ethyl 3-hydroxy-3-phenylthiopropenoate (63 mg, 0.30 mmol) and 1-phenylprop-2-yn-1-ol (79 mg, 0.60 mmol) were reacted according to general procedure **GP11**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (3:2), gave the *title compound* (56 mg, 58%) as pale yellow crystals, mp 75.5–76 °C (from light petroleum): IR (KBr) 3036, 2962, 1588, 1566, 1452, 1438, 1262, 1182, 1098, 1018, 802, 754, 691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 [2 H, m, 6-(*o*-Ph*H*)], 7.90 (1 H, d, *J* = 8.1, PyH), 7.64 (1 H, d, *J* = 8.1, PyH), 7.63 [2 H, m, 2-(*o*-Ph*H*)], 7.41–7.34 (6 H, PhH), 2.90 (2 H, q, *J* = 7.4, SCH_2), 1.19 (3 H, t, *J* = 7.4, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.6 (C), 158.4 (C). 156.5 (C), 139.4 (C), 138.2 (C), 137.0 (CH), 132.8 (C), 129.8 (CH), 129.4 (CH), 129.0 (CH), 128.9 (CH), 128.2 (CH),

127.3 (CH), 117.8 (CH), 24.5 (CH_2), 14.5 (Me); MS (APcI) *m/z* (relative intensity) 320 (MH^+, 100%); HRMS calcd for $C_{20}H_{18}NOS$ (MH) 320.1104, Found 320.1102.



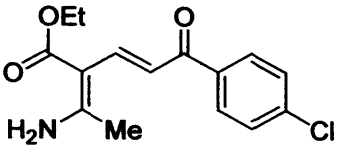
Di-*tert*-butyl 2,6-dimethylpyridine-3,5-dicarboxylate (142)

Pale yellow solid, mp 111–113 °C (from ethanol) (lit.[193] mp 108–110 °C): IR (KBr) 2978, 1715, 1596, 1546, 1365, 1297, 1261, 1154, 1112, 1022, 802 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.46 (1 H, s, PyH), 2.73 (6 H, s, Me), 1.54 (18 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5 (C), 161.2 (C), 140.8 (CH), 124.6 (C), 82.2 (C), 28.2 (Me), 25.0 (Me); MS (APcI) *m/z* (relative intensity) 308 (MH^+, 100%), 252 (32), 196 (8); HRMS calcd for $C_{17}H_{26}NO_4$ (MH) 308.1856, found 308.1852.



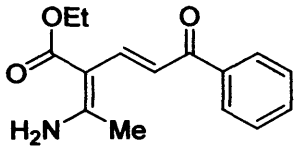
(4*E*)-2-Amino-3-ethoxycarbonyl-6-(4-methoxyphenyl)hexa-2,4-dien-6-one (146)

Ethyl *β*-aminocrotonate (**126**) (0.13 g, 1.0 mmol) and 1-(4-methoxyphenyl)prop-2-yn-1-one (**123**) (0.24 g, 1.5 mmol) were reacted for 6 h according to general procedure **GP03**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (0.27 g, 95%) as an orange solid, mp 151 °C (from light petroleum): IR (KBr) 3298, 3143, 1627, 1598, 1574, 1533, 1361, 1320, 1301, 1231, 1168, 1126, 1021, 833, 687, 667 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.63 (1 H, br s, N*H*H), 7.91 (2 H, d, J = 8.7, *o*-Ph*H*), 7.83 (1 H, d, J = 15.0, 4-H), 7.39 (1 H, d, J = 15.0, 5-H), 6.88 (2 H, d, J = 8.7, *m*-Ph*H*), 5.81 (1 H, br s, NH*H*), 4.25 (2 H, q, J = 7.1, OCH_2Me), 3.80 (3 H, s, OMe), 2.28 (3 H, s, Me), 1.38 (3 H, t, J = 7.1, OCH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.8 (C), 170.2 (C), 166.8 (C), 163.0 (C), 140.6 (CH), 132.8 (CH), 130.6 (CH), 116.0 (CH), 114.0 (CH), 95.9 (C), 60.3 (CH_2), 55.8 (Me), 23.0 (Me), 14.9 (Me); MS (APcI) *m/z* (relative intensity) 290 (MH^+, 100%), 273 (96), 272 (64), 271 (43), 244 (27); HRMS calcd for $C_{16}H_{20}NO_4$ (MH) 290.1392, found 290.1393; Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.4; H, 6.6; N, 4.8. Found: C, 66.4; H, 6.9; N, 4.8.



(4*E*)-2-Amino-3-ethoxycarbonyl-6-(4-chlorophenyl)hexa-2,4-dien-6-one (147)

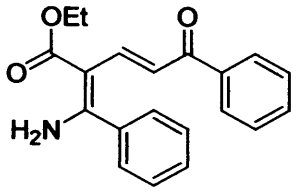
Ethyl *β*-aminocrotonate (**126**) (0.13 g, 1.0 mmol) and 1-(4-chlorophenyl)prop-2-yn-1-one (**122**) (0.25 g, 1.5 mmol) were reacted for 6 h according to general procedure **GP03**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (0.25 g, 86%) as a yellow solid, mp 178–179 °C (from light petroleum): IR (KBr) 3320, 3185, 2978, 1626, 1589, 1571, 1530, 1348, 1322, 1285, 1223, 1120, 1091, 1012, 976, 830, 744, 677 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.71 (1 H, br s, N*H*H), 7.86 (1 H, d, *J* = 14.9, 4-H), 7.83 (2 H, d, *J* = 8.5, *o*-Ph*H*), 7.36 (2 H, d, *J* = 8.5, *m*-Ph*H*), 7.33 (1 H, d, *J* = 14.9, 5-H), 5.82 (1 H, br s, NH*H*), 4.25 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 2.29 (3 H, s, Me), 1.37 (3 H, t, *J* = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.9 (C), 170.1 (C), 167.5 (C), 142.0 (CH), 138.41 (C), 138.39 (C), 129.9 (CH), 129.0 (CH), 115.4 (CH), 96.0 (C), 60.5 (CH_2), 23.0 (Me), 14.9 (Me); MS (APcI) *m/z* (relative intensity) 296 (MH^+, 8%), 294 (MH^+, 28), 277 (100), 248 (11); HRMS calcd for $C_{15}H_{17}{}^{35}ClNO_3$ (MH) 294.0897, found 294.0896.



(4*E*)-2-Amino-3-ethoxycarbonyl-6-phenylhexa-2,4-dien-6-one (148)

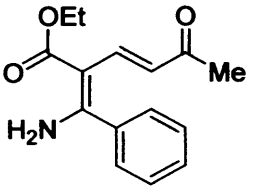
Ethyl *β*-aminocrotonate (**126**) (0.13 g, 1.0 mmol) and 1-phenylprop-2-yn-1-one (**124**) (0.20 g, 1.5 mmol) were reacted for 1 h according to general procedure **GP03**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (0.22 g, 85%) as yellow crystals, mp 156–157 °C (from light petroleum) (lit.[190] mp 164 °C): IR (KBr) 3344, 3204, 2976, 1622, 1579, 1539, 1354, 1321, 1286, 1224, 1023, 977, 705 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.68 (1 H, br s, N*H*H), 7.90 (2 H, m, *o*-Ph*H*), 7.86 (1 H, d, *J* = 15.0, 4-H), 7.41 (3 H, *m,p*-Ph*H*), 7.38 (1 H, d, *J* = 15.0, 5-H), 5.56 (1 H, br s, NH*H*), 4.25 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 2.29 (3 H, s, Me), 1.38 (3 H, t, *J* = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.6 (C), 170.2 (C), 168.0 (C), 142.2 (CH), 140.1 (C), 132.3 (CH), 128.8 (CH), 128.5 (CH), 115.4 (CH), 95.8 (C), 60.4 (CH_2), 22.6

(Me), 14.9 (Me); MS (EI) *m/z* (relative intensity) 259 (M^+, 10%), 241 (12), 214 (10), 196 (13), 186 (100), 154 (28), 126 (25), 115 (35), 105 (75), 77 (100); HRMS calcd for $C_{15}H_{18}NO_3$ (MH) 260.1286, found 260.1285.



(3*E*)-1-Amino-2-ethoxycarbonyl-1,5-diphenylpenta-1,3-dien-5-one (149)

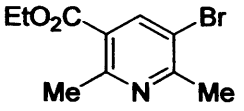
Ethyl 3-amino-3-phenylpropenoate (**125**) (0.19 g, 1.0 mmol) and 1-phenylprop-2-yn-1-one (**124**) (0.20 g, 1.5 mmol) were reacted for 7 h according to general procedure **GP03**. Purification by flash chromatography on silica, eluting with dichloromethane, gave the *title compound* (0.22 g, 68%) as a yellow solid, mp 121–122 °C (from light petroleum): IR (KBr) 3350, 3129, 2976, 2905, 1670, 1634, 1582, 1540, 1477, 1330, 1289, 1222, 1177, 1116, 1044, 1024, 776, 693 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.54 (1 H, br s, N*H*H), 7.75 (2 H, m, *o*-Ph*H*), 7.46 (1 H, d, *J* = 15.1, 3-H), 7.42–7.31 (8 H, m, Ph*H*), 7.27 (1 H, d, *J* = 15.1, 4-H), 5.50 (1 H, br s, NH*H*), 4.32 (2 H, q, *J* = 7.0, OC*H*$_2$Me), 1.42 (3 H, t, *J* = 7.0, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.2 (C), 170.4 (C), 168.7 (C), 143.6 (CH), 139.8 (C), 137.2 (C), 132.0 (CH), 130.9 (CH), 129.4 (CH), 128.7 (CH), 128.6 (CH), 128.4 (CH), 117.0 (CH), 96.9 (C), 60.7 (CH_2), 14.9 (Me); MS (APcI) *m/z* (relative intensity) 322 (MH^+, 100%), 305 (99), 276 (84); HRMS calcd for $C_{20}H_{20}NO_3$ (MH) 322.1443, found 322.1443.



(3*E*)-1-Amino-1-phenyl-2-ethoxycarbonylhexa-1,3-dien-5-one (150)

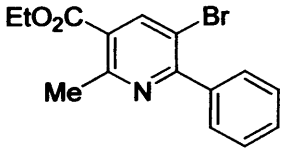
Ethyl 3-amino-3-phenylpropenoate (**125**) (0.19 g, 1.0 mmol) and 4-(trimethylsilyl)but-3-yn-2-one (**80**) (0.25 mL, 1.5 mmol) were reacted for 3 h according to general procedure **GP03**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (0.06 g, 23%) as yellow crystals, mp 115.8–117.5 °C (from light petroleum): IR (KBr) 3321, 3122, 2980, 1653, 1586, 1460, 1284, 1257, 1210, 1119, 1022, 1002, 978, 768, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.46 (1 H, br s, N*H*H), 7.41

(3 H, *o,p*-Ph*H*), 7.31 (2 H, m, *m*-Ph*H*), 7.06 (1 H, d, J = 15.9, 3-H), 6.42 (1 H, d, J = 15.9, 4-H), 5.47 (1 H, br s, NH*H*), 4.26 (2 H, q, J = 7.1, OC*H*$_2$Me), 1.89 (3 H, s, Me), 1.34 (3 H, t, J = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.2 (C), 169.8 (C), 167.4 (C), 142.1 (CH), 136.8 (C), 130.5 (CH), 128.9 (CH), 128.2 (CH), 122.6 (CH), 95.5 (C), 60.3 (CH_2), 26.7 (Me), 14.4 (Me); MS (APcI) *m/z* (relative intensity) 260 (MH^+, 100%), 243 (7); HRMS calcd for $C_{15}H_{18}NO_3$ (MH) 260.1286, found 260.1286.



Ethyl 5-bromo-2,6-dimethylpyridine-3-carboxylate (151)

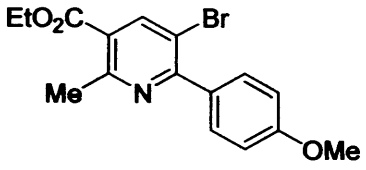
(4*E*)-2-Amino-3-ethoxycarbonylhepta-2,4-dien-6-one (**130**) (55 mg, 0.28 mmol) was reacted according to general procedure **GP12**. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (3:7), gave the *title compound* (69 mg, 96%) as a pale yellow solid, mp 34.5–36.5 °C (from aqueous methanol): IR (KBr) 2984, 1725, 1576, 1542, 1436, 1392, 1366, 1267, 1232, 1100, 1025, 970, 780, 680 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (1 H, s, PyH), 4.30 (2 H, q, J = 7.1, OC*H*$_2$Me), 2.69 (3 H, s, Me), 2.60 (3 H, s, Me), 1.33 (3 H, t, J = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6 (C), 160.3 (C), 158.4 (C), 142.0 (CH), 124.7 (C), 118.3 (C), 61.8 (CH_2), 25.4 (Me), 24.7 (Me), 14.6 (Me); MS (APcI) *m/z* (relative intensity) 260 (MH^+, 100%), 258 (MH^+, 95); HRMS calcd for $C_{10}H_{13}{}^{79}BrNO_2$ (MH) 258.0129, found 258.0124; Anal. Calcd for $C_{10}H_{12}BrNO_2$: C, 46.5; H, 4.7; N, 5.4. Found: C, 46.5; H, 5.0; N, 5.4.



Ethyl 5-bromo-2-methyl-6-phenylpyridine-3-carboxylate (152)

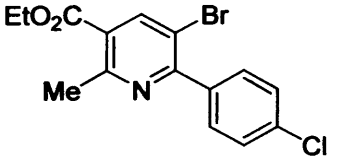
(4*E*)-2-Amino-3-ethoxycarbonyl-6-phenylhexa-2,4-dien-6-one (**148**) (73 mg, 0.28 mmol) was reacted according to general procedure **GP12**. Purification by flash chromatography on silica, eluting with light petroleum–chloroform (1:1), gave the *title compound* (83 mg, 92%) as a pale yellow solid, mp 88–89 °C (from aqueous methanol): IR (KBr) 2974, 2925, 1730, 1571, 1432, 1286, 1259, 1090, 1016, 928, 835, 780, 694 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (1 H, s, PyH), 7.64 (2 H, m, *o*-Ph*H*), 7.41 (3 H, *m,p*-Ph*H*), 4.34 (2 H, q, J = 7.1,

OC*H*$_2$Me), 2.77 (3 H, s, Me), 1.36 (3 H, t, *J* = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6 (C), 160.2 (C), 158.8 (C), 143.8 (CH), 139.3 (C), 129.8 (CH), 129.7 (CH), 128.5 (CH), 125.4 (C), 116.6 (C), 62.0 (CH_2), 25.0 (Me), 14.7 (Me); MS (APcI) *m/z* (relative intensity) 322 (MH^+, 36%), 320 (MH^+, 37), 242 (9), 93 (23), 79 (100); HRMS calcd for $C_{15}H_{15}{}^{79}BrNO_2$ (MH) 320.0286, found 320.0286.



Ethyl 5-bromo-2-methyl-6-(4-methoxyphenyl)pyridine-3-carboxylate (153)

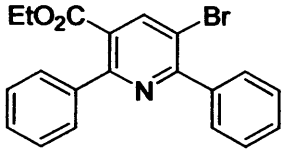
(4*E*)-2-Amino-3-ethoxycarbonyl-6-(4-methoxyphenyl)hexa-2,4-dien-6-one (**146**) (81 mg, 0.28 mmol) was reacted according to general procedure **GP12**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (2:1), gave the *title compound* (86 mg, 88%) as a colourless solid, mp 90–91 °C (from methanol): IR (KBr) 2963, 1724, 1609, 1570, 1512, 1433, 1257, 1178, 1087, 1027, 803, 701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (1 H, s, PyH), 7.66 (2 H, d, *J* = 8.8, *o*-Ph*H*), 6.91 (2 H, d, *J* = 8.8, *m*-PhH), 4.34 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 3.79 (3 H, s, OMe), 2.75 (3 H, s, Me), 1.34 (3 H, t, *J* = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7 (C), 160.8 (C), 159.6 (C), 158.7 (C), 143.8 (CH), 131.6 (C), 131.4 (CH), 124.8 (C), 116.2 (C), 113.8 (CH), 61.9 (CH_2), 55.8 (Me), 25.0 (Me), 14.7 (Me); MS (APcI) *m/z* (relative intensity) 352 (MH^+, 32%), 350 (MH^+, 50); HRMS calcd for $C_{16}H_{17}{}^{79}BrNO_3$ (MH) 350.0392, found 350.0393.



Ethyl 5-bromo-2-methyl-6-(4-chlorophenyl)pyridine-3-carboxylate (154)

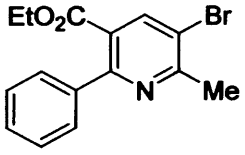
(4*E*)-2-Amino-3-ethoxycarbonyl-6-(4-chlorophenyl)hexa-2,4-dien-6-one (**147**) (82 mg, 0.28 mmol) was reacted according to general procedure **GP12**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (5:1), gave the *title compound* (88 mg, 89%) as a pale yellow solid, mp 106–108 °C (from aqueous ethanol): IR (KBr) 2973, 1730, 1595, 1570, 1534, 1492, 1432, 1259, 1089, 1016, 928, 835, 780 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (1 H, s, PyH), 7.61 (2 H, d, *J* = 8.6, *o*-Ph*H*), 7.37 (2 H, d, *J*

= 8.6, *m*-Ph*H*), 4.34 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 2.75 (3 H, s, Me), 1.35 (3 H, t, *J* = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1 (C), 158.5 (C), 158.4 (C), 143.5 (CH), 137.2 (C), 135.4 (C), 130.8 (CH), 128.3 (CH), 125.3 (C), 116.0 (C), 61.7 (CH_2), 24.5 (Me), 14.3 (Me); MS (APcI) *m/z* (relative intensity) 358 (MH^+, 16%), 356 (MH^+, 100), 354 (MH^+, 71), 117 (38), 71 (30); HRMS calcd for $C_{15}H_{14}{}^{79}Br^{35}ClNO_2$ (MH) 353.9896, found 353.9896.



Ethyl 5-bromo-2,6-diphenylpyridine-3-carboxylate (155)

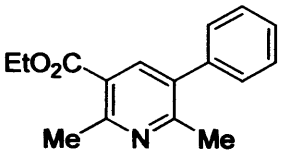
(3*E*)-1-Amino-2-ethoxycarbonyl-1,5-diphenylpenta-1,3-dien-5-one (**149**) (90 mg, 0.28 mmol) was reacted at –10 °C according to general procedure **GP12**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (5:1), gave the *title compound* (89 mg, 83%) as a pale yellow solid, mp 80–83 °C (from methanol): IR (KBr) 2976, 1732, 1558, 1426, 1372, 1244, 1114, 1088, 1017, 922, 772, 687 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (1 H, s, PyH), 7.71 (2 H, m, *o*-Ph*H*), 7.50 (2 H, m, *o*-Ph*H*), 7.43–7.33 (6 H, *m,p*-Ph*H*), 4.12 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 1.02 (3 H, t, *J* = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7 (C), 159.2 (C), 157.2 (C), 143.0 (CH), 139.2 (C), 138.8 (C), 129.6 (CH), 129.3 (CH), 128.9 (CH), 128.7 (CH), 128.1 (CH), 128.0 (CH), 126.5 (C), 117.3 (C), 61.8 (CH_2), 13.7 (Me); MS (APcI) *m/z* (relative intensity) 384 (MH^+, 100%), 382 (MH^+, 80), 304 (15); HRMS calcd for $C_{20}H_{16}{}^{79}BrNO_2$ (M) 381.0364, found 381.0360.



Ethyl 5-bromo-2,6-diphenylpyridine-3-carboxylate (156)

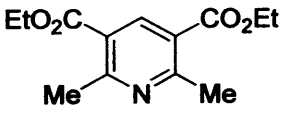
(3*E*)-1-Amino-1-phenyl-2-ethoxycarbonylhexa-1,3-dien-5-one (**150**) (73 mg, 0.28 mmol) was reacted according to general procedure **GP12**. Purification by flash chromatography on silica, eluting with light petroleum–chloroform (1:1), gave the *title compound* (88 mg, 98%) as a pale yellow oil: IR (nujol) 2918, 2853, 1725, 1572, 1462, 1377, 1295, 1242, 1112, 1061, 1027, 771, 722, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (1 H, s, PyH), 7.50–7.34 (5 H, Ph*H*), 4.05 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 2.68 (3 H, s, Me), 1.00 (3 H, t, *J* = 7.1, OCH$_2$*Me*);

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6 (C), 159.6 (C), 157.2 (C), 141.3 (CH), 139.5 (C), 128.7 (CH), 128.5 (CH), 128.2 (CH), 125.8 (C), 119.3 (C), 61.7 (CH_2), 25.3 (Me), 13.7 (Me); MS (APcI) *m/z* (relative intensity) 322 (MH^+, 100%), 320 (MH^+, 98); HRMS calcd for $C_{15}H_{15}{}^{79}BrNO_2$ (MH) 320.0281, found 320.0281.



Ethyl 2,6-dimethyl-5-phenylpyridine-3-carboxylate (157)

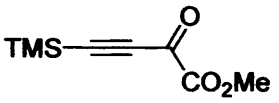
To a stirred solution of ethyl 5-bromo-2,6-dimethylpyridine-3-carboxylate (**151**) (26 mg, 0.1 mmol) and tetrakis(triphenylphosphine)palladium(0) (3.6 mg, 3.1 μmol) in toluene (2 mL), under a nitrogen atmosphere, was added aqueous sodium carbonate solution (2.0 M; 0.5 mL, 1.0 mmol, 10 equiv.) and a solution of benzeneboronic acid (15 mg, 0.12 mmol) in methanol (0.5 mL). The reaction mixture was then stirred vigorously at 80 °C for 3.5 h. The resulting mixture was extracted with ethyl acetate (2 x 10 mL), the combined organic extracts were washed sequentially with water (15 mL) and brine (15 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with dichloromethane, gave the *title compound* (17 mg, 67%) as pale yellow crystals, mp 46–49 °C (from methanol): IR (KBr) 2984, 1721, 1547, 1450, 1290, 1254, 1224, 1190, 1086, 1020, 772, 736, 705 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (1 H, s, PyH), 7.37 (2 H, m, *m*-Ph*H*), 7.32 (1 H, m, *p*-Ph*H*), 7.25 (2 H, m, *o*-Ph*H*), 4.29 (2 H, q, J = 7.1, OCH_2Me), 2.78 (3 H, s, Me), 2.45 (3 H, s, Me), 1.30 (3 H, t, J = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6 (C), 158.5 (C), 158.0 (C), 139.5 (CH), 139.0 (C), 134.3 (C), 129.0 (CH), 128.5 (CH), 127.6 (CH), 122.9 (C), 61.1 (CH_2), 24.5 (Me), 23.5 (Me), 14.3 (Me); MS (APcI) *m/z* (relative intensity) 256 (MH^+, 100%); HRMS calcd for $C_{16}H_{18}NO_2$ (MH) 256.1337, found 256.1336.



Diethyl 2,6-dimethylpyridine-3,5-dicarboxylate (158)

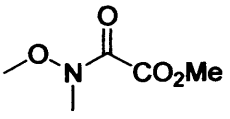
Pale yellow solid, mp 71–71.5 °C (from ethanol) (lit.[194] mp 70–72 °C): IR (KBr) 2977, 2930, 1720, 1591, 1556, 1444, 1368, 1296, 1223, 1124, 1045, 866, 772, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (1 H, s, PyH), 4.33 (4 H, q, J = 7.1, OCH_2Me), 2.78 (6 H, s, Me), 1.35

(6 H, t, J = 7.1, OCH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4 (C), 162.7 (C), 141.4 (CH), 123.5 (C), 61.8 (CH_2), 25.4 (Me), 14.7 (Me); MS (APcI) *m/z* (relative intensity) 252 (MH^+, 100%), 224 (8), 208 (20), 167 (25); HRMS calcd for $C_{13}H_{18}NO_4$ (MH) 252.1236, found 252.1232.



Methyl 2-oxo-4-(trimethylsilyl)but-3-ynoate (216)

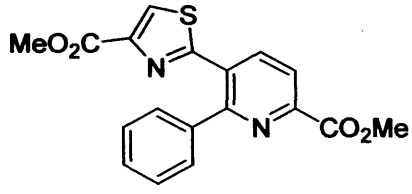
A solution of *n*-butyllithium in hexanes (2.5 M; 3.1 mL, 7.82 mmol) was added dropwise over a 10 min period to a stirred solution of trimethylsilylacetylene (**221**) (0.7 mL, 6.8 mmol) in dry tetrahydrofuran (30 mL) at –78 °C. The solution was stirred for 30 min and added dropwise to a solution of monomethyloxalic acid *N*-methoxy-*N*-methylamide (**220**) (1.0 g, 6.8 mmol) in dry tetrahydrofuran (60 mL) at –78 °C. The mixture was stirred for 30 min, warmed to room temperature and poured over ice (20 g), then aqueous orthophosphoric acid solution (20%; 30 mL) was added. The mixture was concentrated *in vacuo* and partitioned between water (20 mL) and diethyl ether (50 mL) and the aqueous layer further extracted with diethyl ether (2 x 30 mL). The combined organic extracts were washed sequentially with aqueous orthophosphoric acid solution (10%; 20 mL), saturated aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum-diethyl ether (5:1) gave the *title compound* (0.70 g, 56%) as a yellow oil: IR (film) 2960, 2150, 1746, 1685, 1438, 1254, 1103, 850, 763 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.71 (3 H, s, OMe), 0.08 (9 H, s, $SiMe_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8 (C), 159.3 (C), 107.0 (C), 100.0 (C), 53.7 (Me), –1.0 (Me); MS (EI) *m/z* (relative intensity) 184 (M^+, 3%), 169 (20), 158 (100); HRMS calcd for $C_8H_{12}O_3Si$ (M) 184.0550, found 184.0545.



Monomethyloxalic acid-*N*-methoxy-*N*-methylamide (220)

Triethylamine (11.4 mL, 81.6 mmol) was added dropwise over a 10 min period to a solution of *N,O*-dimethylhydroxylamine hydrochloride (**219**) (3.98 g, 40.8 mmol) and methyl oxalyl chloride (**218**) (3.75 mL, 40.8 mmol) in dry dichloromethane (270 mL) at 0 °C and the

mixture was stirred for 3.5 h. Methanol (5 mL, 0.12 mol) was added and the mixture was evaporated *in vacuo*. Tetrahydrofuran (100 mL) was added and the precipitate was filtered under suction, washing with tetrahydrofuran (2 x 100 mL). The mother liquor was evaporated *in vacuo* and distilled to give the *title compound* (6.47 g, 94%) as a colourless oil, bp 114–120 °C (2–3 Torr): IR (film) 2945, 1748, 1680, 1394, 1257, 1175, 1092, 999, 962, 784 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.83 (3 H, s, Me), 3.70 (3 H, s, Me), 3.18 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.8 (C), 161.8 (C), 62.3 (Me), 52.6 (Me), 31.4 (Me); MS (CI) *m/z* (relative intensity) 165 ($M + NH_4^+$, 100%), 148 (MH^+, 24), 135 (55), 118 (10); HRMS calcd for $C_5H_{13}N_2O_4$ (MNH_4) 165.0870, found 165.0870.

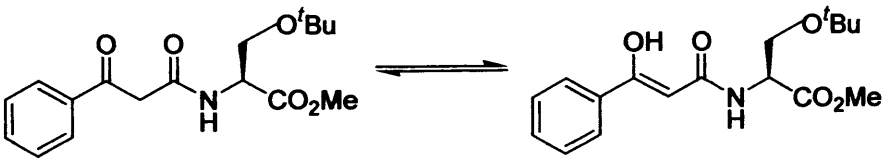


Methyl 2-phenyl-3-[4-(methoxycarbonyl)thiazol-2-yl]pyridine-6-carboxylate (222)

A mixture of (*R*)-methyl 2-phenyl-3-(4-methoxycarbonyl-2-thiazolin-2-yl)pyridine-6-carboxylate (**244**) (36 mg, 0.10 mmol) and activated manganese(IV) oxide (174 mg, 2.0 mmol) in dichloromethane (3 mL) was irradiated at 100 °C (initial power 300 W) for 10 min in a sealed pressure-rated reaction tube (10 mL) using a CEM Discover™ Microwave Synthesizer. The mixture was cooled rapidly to room temperature in a flow of compressed air for 5 min, filtered through Celite® washing with dichloromethane (2 x 10 mL) and evaporated *in vacuo* to give the *title compound* (35 mg, 100%) as a pale yellow solid, mp 173–175 °C (from methanol): IR (KBr) 2962, 1719, 1262, 1096, 1023, 801 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.53 (1 H, d, J = 8.1, PyH), 8.15 (1 H, d, J = 8.1, PyH), 8.06 (1 H, s, SCH), 7.41–7.32 (5 H, PhH), 3.95 (3 H, s, OMe), 3.91 (3 H, s, OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3 (C), 165.1 (C), 161.7 (C), 158.2 (C), 148.2 (C), 146.8 (C), 139.3 (CH), 138.1 (C), 130.9 (C), 130.2 (CH), 130.0 (CH), 129.6 (CH), 128.9 (CH), 123.7 (CH), 53.2 (Me), 52.6 (Me); MS (APcI) *m/z* (relative intensity) 355 (MH^+, 100%); HRMS calcd for $C_{18}H_{15}N_2O_4S$ (MH) 355.0747, Found 355.0747.

(*S*)-*N*-(3-Oxo-3-phenylpropanoyl)serine methyl ester (226) and (*S*)-*N*-(3-hydroxy-3-phenylpropenoyl)serine methyl ester

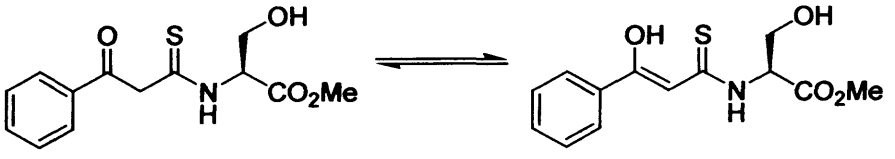
S-Ethyl benzoylthioacetate(**136**) and *S*-ethyl 3-hydroxy-3-phenylthiopropenoate (0.42 g, 2.0 mmol) were reacted with L-serine methyl ester hydrochloride (**224**) (0.31 g, 2.0 mmol) according to general procedure **GP13**. Purification by flash chromatography on silica, eluting with diethyl ether, followed by recrystallization from light petroleum–ethyl acetate gave the *title compound* (0.51 g, 96%) as a colourless solid, mp 102.5–103.5 °C (from light petroleum–ethyl acetate): $[\alpha]_D^{24}$ +24.8 (*c* 1.00, $CHCl_3$); IR (KBr) 3507, 3289, 3089, 2965, 1741, 1691, 1642, 1561, 1449, 1434, 1354, 1210, 1175, 1137, 1081, 1033, 1015, 761, 690 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.74 (0.13 H, s, enolOH), 7.91 (1.74 H, m, *o*-Ph*H*), 7.74 (0.87 H, d, *J* = 6.8, NH), 7.68 (0.26 H, m, *o*-Ph*H*), 7.55 (0.87 H, m, *p*-Ph*H*), 7.42 (1.74 H, m, *m*-Ph*H*), 7.35 (0.39 H, *m,p*-Ph*H*), 6.35 (0.13 H, d, *J* = 6.6, NH), 5.57 (0.13 H, s, CH), 4.72 (0.13 H, m, α-CH), 4.65 (0.87 H, m, α-CH), 4.00 (0.87 H, d, *J* = 17.0, C*H*H), 3.95 (2 H, app m, β-CH_2), 3.94 (0.87 H, d, *J* = 17.0, CH*H*), 3.75 (0.39 H, s, OMe), 3.72 (2.61 H, s, OMe), 2.78 (0.87 H, t, *J* = 5.8, OH), 2.60 (0.13 H, t, *J* = 6.4, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.5 (C), 184.1 (C), 172.0 (C), 170.8 (C), 170.1 (C), 166.7 (C), 136.0 (C), 134.2 (CH), 134.0 (C), 130.8 (CH), 128.9 (CH), 128.6 (CH), 128.5 (CH), 125.8 (CH), 88.4 (CH), 63.3 (CH_2), 62.8 (CH_2), 55.0 (CH), 54.2 (CH), 52.9 (Me), 52.8 (Me), 45.5 (CH_2); MS (APcI) *m/z* (relative intensity) 266 (MH^+, 100%), 248 (47), 120 (25); HRMS calcd for $C_{13}H_{16}NO_5$ (MH) 266.1023, Found 266.1024.



(*S*)-*N*-(3-Oxo-3-phenylpropanoyl)-*O*-*tert*-butylserine methyl ester (227) and (*S*)-*N*-(3-hydroxy-3-phenylpropenoyl)-*O*-*tert*-butylserine methyl ester

S-Ethyl benzoylthioacetate (**136**) and *S*-ethyl 3-hydroxy-3-phenylthiopropenoate (0.42 g, 2.0 mmol) were reacted with *O*-*tert*-butyl-L-serine methyl ester hydrochloride (**225**) (0.42 g, 2.0

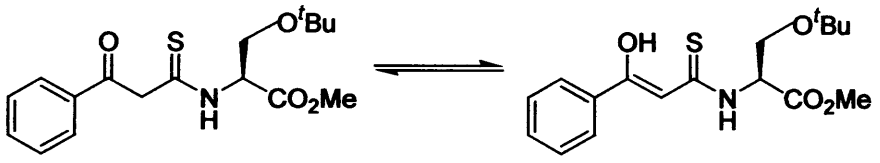
mmol) according to general procedure **GP13**. Purification by flash chromatography on silica, eluting with dichloromethane–diethyl ether (3:1), gave the *title compound* (0.58 g, 91%) as a colourless solid, mp 48–50 °C (from methanol): $[\alpha]_D^{22}$ +28.3 (*c* 2.00, $CHCl_3$); IR (KBr) 2968, 1752, 1691, 1669, 1639, 1534, 1450, 1364, 1209, 1100, 1051, 1021, 774, 760, 691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.89 (0.18 H, s, enolOH), 7.94 (1.64 H, m, *o*-Ph*H*), 7.69 (0.36 H, m, *o*-Ph*H*), 7.59 (0.82 H, d, *J* = 7.8, NH), 7.54 (0.82 H, m, *p*-Ph*H*), 7.42 (1.64 H, m, *m*-Ph*H*), 7.35 (0.54 H, *m,p*-Ph*H*), 6.19 (0.18 H, d, *J* = 8.3, NH), 5.56 (0.18 H, s, CH), 4.75 (0.18 H, m, α-CH), 4.67 (0.82 H, m, α-CH), 3.97 (0.82 H, d, *J* = 16.4, C*H*H), 3.91 (0.82 H, d, *J* = 16.4, CH*H*), 3.80 (0.18 H, dd, *J* = 9.1, 2.8, β-C*H*H), 3.76 (0.82 H, dd, *J* = 9.1, 3.1, β-C*H*H), 3.70 (0.54 H, s, OMe), 3.66 (2.46 H, s, OMe), 3.56 (0.18 H, dd, *J* = 9.1, 3.1, β-CH*H*), 3.51 (0.82 H, dd, *J* = 9.1, 3.3, β-CH*H*), 1.08 (1.62 H, s, CMe_3), 1.07 (7.38 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.0 (C), 171.6 (C), 171.0 (C), 170.7 (C), 170.0 (C), 165.8 (C), 136.2 (C), 134.1 (C), 133.9 (CH), 130.8 (CH), 128.8 (CH), 128.6 (CH), 128.4 (CH), 125.8 (CH), 88.5 (CH), 73.6 (C), 73.5 (C), 62.1 (CH_2), 61.8 (CH_2), 53.1 (CH), 52.5 (Me), 52.4 (CH), 52.4 (Me), 45.6 (CH_2) 27.3 (Me), 27.3 (Me); MS (APcI) *m/z* (relative intensity) 322 (MH^+, 23%), 266 (100); HRMS calcd for $C_{17}H_{24}NO_5$ (MH) 322.1649, Found 322.1652.



(*S*)-*N*-(3-Oxo-3-phenylthiopropanoyl)serine methyl ester (228) and (*S*)-*N*-(3-hydroxy-3-phenylthiopropenoyl)serine methyl ester

A solution of (*S*)-*N*-(3-oxo-3-phenylthiopropanoyl)-*O*-*tert*-butylserine methyl ester (**229**) and (*S*)-*N*-(3-hydroxy-3-phenylthiopropenoyl)-*O*-*tert*-butylserine methyl ester (0.28 g, 0.83 mmol) in trifluoroacetic acid–dichloromethane (1:1) (20 mL) was stirred at room temperature for 30 min and evaporated *in vacuo*. Purification by flash column chromatography on silica, eluting with ethyl acetate, gave the *title compound* (0.17 g, 73%) as a pale yellow oil: $[\alpha]_D^{24}$ +124.0 (*c* 0.30, $CHCl_3$); IR (KBr) 3458, 3324, 3046, 2958, 1740, 1684, 1616, 1527, 1438, 1325, 1261, 1213, 1074, 1022, 802, 769, 689 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 14.23 (0.33 H, s, OH), 9.45 (0.67 H, br s, NH), 7.93 (1.34 H, m, *o*-Ph*H*), 7.70 (0.33 H, br s, NH), 7.70 (0.66 H, m, *o*-Ph*H*), 7.55 (0.67 H, m, *p*-Ph*H*), 7.44–7.32 (2.33

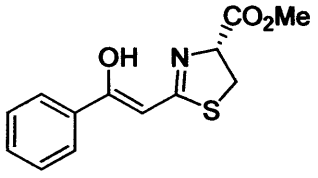
H, *m,p*-Ph*H*), 6.10 (0.33 H, s, CH), 5.23 (1 H, m, α-CH), 4.57 (0.67 H, d, *J* = 17.1, C*H*H), 4.42 (0.67 H, d, *J* = 17.1, CH*H*), 4.19 (0.67 H, dd, *J* = 11.5, 3.3, β-C*H*H), 4.08 (0.66 H, app d, *J* = 2.4, β-CH_2), 4.03 (0.67 H, dd, *J* = 11.5, 2.6, β-CH*H*), 3.75 (0.99 H, s, OMe), 3.74 (2.01 H, s, OMe), 2.53 (1 H, br s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.8 (C), 195.8 (C), 191.9 (C), 170.7 (C), 170.2 (C), 170.0 (C), 135.7 (C), 135.1 (C), 134.4 (CH), 131.1 (CH), 128.9 (CH), 128.8 (CH), 128.6 (CH), 126.1 (CH), 98.0 (CH), 62.4 (CH_2), 61.6 (CH_2), 60.2 (CH), 57.6 (CH), 53.6 (CH_2), 53.0 (Me), 53.0 (Me); MS (EI) *m/z* (relative intensity) 281 (M^+, 16%), 267 (6), 221 (13), 207 (82), 105 (100), 77 (85); HRMS calcd for $C_{13}H_{16}NO_4S$ (MH) 282.0795, Found 282.0796.



(*S*)-*N*-(3-Oxo-3-phenylthiopropanoyl)-*O*-*tert*-butylserine methyl ester (229) and (*S*)-*N*-(3-hydroxy-3-phenylthiopropenoyl)-*O*-*tert*-butylserine methyl ester

A solution of (*S*)-*N*-(3-oxo-3-phenylpropanoyl)-*O*-*tert*-butylserine methyl ester (**227**) and (*S*)-*N*-(3-hydroxy-3-phenylpropenoyl)-*O*-*tert*-butylserine methyl ester (0.49 g, 1.52 mmol) and Lawesson's reagent (0.61 g, 1.52 mmol) in benzene (25 mL) was heated at 40 °C for 60 h. The mixture was allowed to cool, evaporated *in vacuo*, and purified by flash chromatography on silica eluting with dichloromethane to give the *title compound* (0.46 g, 89%) as a pale yellow oil: $[\alpha]_D^{23}$ +70.4 (*c* 1.00, $CHCl_3$); IR (KBr) 3322, 2975, 1742, 1677, 1614, 1576, 1522, 1392, 1334, 1290, 1235, 1200, 1095, 768, 690 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.28 (0.46 H, s, OH), 9.58 (0.54 H, d, *J* = 7.2, NH), 7.92 (1.08 H, m, *o*-Ph*H*), 7.70 (0.46 H, d, *J* = 7.8, NH), 7.68 (0.92 H, m, *o*-Ph*H*), 7.49 (0.54 H, m, *p*-Ph*H*), 7.39–7.28 (2.46 H, *m,p*-Ph*H*), 6.07 (0.46 H, s, CH), 5.25 (1 H, m, α-CH), 4.47 (0.54 H, d, *J* = 16.9, C*H*H), 4.37 (0.54 H, d, *J* = 16.9, CH*H*), 3.81 (0.46 H, dd, *J* = 9.5, 2.8, β-C*H*H), 3.79 (0.54 H, dd, *J* = 9.4, 2.9, β-C*H*H), 3.69 (1 H, app m, β-CH*H*), 3.67 (1.62 H, s, OMe), 3.65 (1.38 H, s, OMe), 1.06 (4.86 H, s, CMe_3), 1.05 (4.14 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.4 (C), 195.4 (C), 191.4 (C), 170.4 (C), 169.7 (C), 169.5 (C), 136.0 (C), 135.2 (C), 134.0 (CH), 131.0 (CH), 128.8 (CH), 128.7 (CH), 128.5 (CH), 126.1 (CH), 98.0 (CH), 73.8 (C), 73.7 (C), 61.5 (CH_2), 60.9 (CH_2), 58.9 (CH), 56.5 (CH), 53.1 (CH_2), 52.6 (Me), 52.6 (Me), 27.3 (Me),

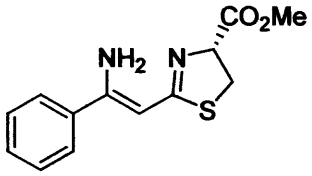
27.2 (Me); MS (APcI) *m/z* (relative intensity) 338 (MH^+, 100%); HRMS calcd for $C_{17}H_{24}NO_4S$ (MH) 338.1421, Found 338.1422.



(*R*)-1-Hydroxy-1-phenyl-2-(4-methoxycarbonyl-2-thiazolin-2-yl)ethene (230)

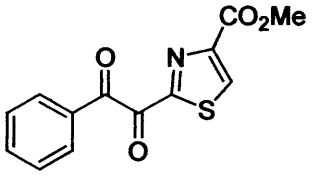
I. Preparation from (*S*)-*N*-(3-oxo-3-phenylthiopropanoyl)serine methyl ester (228) and (*S*)-*N*-(3-hydroxy-3-phenylthiopropenoyl)serine methyl ester. A solution of (*S*)-*N*-(3-oxo-3-phenylthiopropanoyl)serine methyl ester (**228**) and (*S*)-*N*-(3-hydroxy-3-phenylthiopropenoyl)serine methyl ester (84 mg, 0.30 mmol) and Burgess reagent (79 mg, 0.33 mmol) in dry tetrahydrofuran (15 mL) was stirred at reflux for 5 h and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (46 mg, 58%) as a dark foam.

II. Preparation from (*S*)-*N*-(3-oxo-3-phenylthiopropanoyl)-*O*-*tert*-butylserine methyl ester (229) and (*S*)-*N*-(3-hydroxy-3-phenylthiopropenoyl)-*O*-*tert*-butylserine methyl ester. A solution of (*S*)-*N*-(3-oxo-3-phenylthiopropanoyl)-*O*-*tert*-butylserine methyl ester (**229**) and (*S*)-*N*-(3-hydroxy-3-phenylthiopropenoyl)-*O*-*tert*-butylserine methyl ester (154 mg, 0.45 mmol) in trifluoroacetic acid–dichloromethane (1:1) (10 mL) was stirred at room temperature for 48 h and evaporated *in vacuo*. Purification by flash column chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (86 mg, 71%) as a dark foam: $[\alpha]_D^{24}$ –7.3 (*c* 0.63, $CHCl_3$); IR (KBr) 3438, 3237, 2961, 1742, 1596, 1574, 1508, 1460, 1437, 1262, 1204, 1102, 1058, 1024, 801, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (2 H, m, *o*-Ph*H*), 7.37 (3 H, *m,p*-Ph*H*), 5.97 (1 H, s, CH), 4.74 (1 H, dd, *J* = 7.5, 5.8, NCH), 3.75 (3 H, s, OMe), 3.47 (2 H, app m, SCH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.0 (C), 169.9 (C), 168.3 (C), 139.1 (C), 131.2 (CH), 128.3 (CH), 127.3 (CH), 88.2 (CH), 62.9 (CH), 53.2 (Me), 31.3 (CH_2); MS (CI) *m/z* (relative intensity) 264 (MH^+, 100%), 248 (8), 234 (12), 220 (12), 204 (16), 177 (31), 148 (46), 139 (79), 122 (40); HRMS calcd for $C_{13}H_{14}NO_3S$ (MH) 264.0689, Found 264.0689.



(*R*)-1-Amino-1-phenyl-2-(4-methoxycarbonyl-2-thiazolin-2-yl)ethene (231)

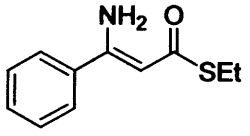
A solution of (*R*)-1-hydroxy-1-phenyl-2-(4-methoxycarbonyl-2-thiazolin-2-yl)ethene (**230**) (66 mg, 0.25 mmol) and ammonium acetate (385 mg, 5.0 mmol) was heated at reflux in benzene–glacial acetic acid (5:1) (12 mL) for 24 h using a Dean and Stark trap. The mixture was allowed to cool and partitioned between saturated aqueous sodium hydrogen carbonate solution (30 mL) and ethyl acetate (30 mL), the aqueous layer was further extracted with ethyl acetate (2 x 15 mL) and the combined organic layers were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (15 mL) and brine (15 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (3:2), gave the *title compound* (32 mg, 49%) as a pale yellow oil: $[\alpha]_D^{22}$ +7.5 (*c* 0.27, $CHCl_3$); IR (KBr) 3430, 2956, 2924, 1734, 1617, 1541, 1493, 1222, 1049, 745, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.20–6.00 (2 H, br s, NH_2), 7.48 (2 H, m, *o*-Ph*H*), 7.34 (3 H, *m,p*-Ph*H*), 5.11 (1 H, t, *J* = 8.7, NCH), 5.06 (1 H, s, CH), 3.74 (3 H, s, OMe), 3.41 (1 H, app d, *J* = 3.9, SC*H*H), 3.39 (1 H, app d, *J* = 4.1, SCH*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3 (C), 169.3 (C), 151.2 (C), 137.7 (C), 129.9 (CH), 128.8 (CH), 126.2 (CH), 126.1 (CH), 88.1 (CH), 52.6 (Me), 34.5 (CH_2); MS (APcI) *m/z* (relative intensity) 263 (MH^+, 100%), 107 (25); HRMS calcd for $C_{13}H_{15}N_2O_2S$ (MH) 263.0849, Found 263.0850.



Methyl 2-(2-oxo-2-phenylacetyl)thiazole-4-carboxylate (232)

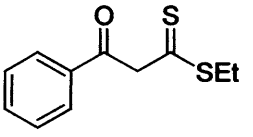
A mixture of (*R*)-1-hydroxy-1-phenyl-2-(4-methoxycarbonyl-2-thiazolin-2-yl)ethene (**230**) (150 mg, 0.57 mmol) and activated manganese(IV) oxide (496 mg, 5.70 mmol) in chloroform (30 mL) was stirred at reflux for 24 h and allowed to cool to room temperature, filtered through Celite® washing with chloroform (2 x 10 mL) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with dichloromethane, gave the *title*

compound (108 mg, 69%) as a pale yellow solid, mp 113–115 °C (from methanol): IR (KBr) 3122, 2963, 1721, 1682, 1594, 1488, 1450, 1424, 1316, 1261, 1214, 1094, 1019, 872, 797, 767, 732, 686 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.52 (1 H, s, SCH), 7.94 (2 H, m, *o*-Ph*H*), 7.62 (1 H, m, *p*-Ph*H*), 7.47 (2 H, m, *m*-Ph*H*), 3.86 (3 H, s, OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.7 (C), 185.0 (C), 163.4 (C), 161.0 (C), 149.7 (C), 135.3 (CH), 134.3 (CH), 132.1 (C), 130.2 (CH), 129.1 (CH), 52.8 (Me); MS (APcI) *m/z* (relative intensity) 276 (MH^+, 100%); HRMS calcd for $C_{13}H_{10}NO_4S$ (MH) 276.0325, Found 276.0325.



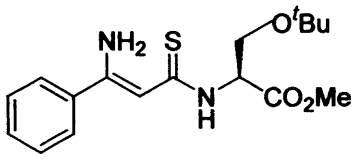
S-Ethyl 3-amino-3-phenylthiopropenoate (233)

A solution of *S*-ethyl benzoylthioacetate (**136**) and *S*-ethyl 3-hydroxy-3-phenylthiopropenoate (0.50 g, 2.4 mmol) and ammonium acetate (1.16 g, 15.0 mmol) in toluene–glacial acetic acid (5:1) (25 mL) was heated at reflux for 20 h. The mixture was partitioned between saturated aqueous sodium hydrogen carbonate solution (50 mL) and ethyl acetate (50 mL). The aqueous layer was further extracted with ethyl acetate (2 x 25 mL) and the combined organic layers were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (25 mL) and brine (25 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (1:1), gave the *title compound* (0.38 g, 77%) as a pale yellow oil: IR (film) 3428, 3297, 2967, 2924, 1605, 1573, 1534, 1489, 1305, 1114, 1020, 883, 756, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.30-8.00 (1 H, br s, NH), 7.44 (2 H, m, *o*-Ph*H*), 7.33 (3 H, *m,p*-Ph*H*), 5.70-4.30 (1 H, br s, NH), 5.31 (1 H, s, CH), 2.84 (2 H, q, J = 7.4, CH_2), 1.22 (3H, t, J = 7.4, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9 (C), 157.7 (C), 136.8 (C), 130.6 (CH), 129.0 (CH), 126.6 (CH), 94.6 (CH), 22.7 (CH_2), 15.5 (Me); MS (APcI) *m/z* (relative intensity) 208 (MH^+, 100%), 146 (82), 120 (17), 103 (4); HRMS calcd for $C_{11}H_{14}NOS$ (MH) 208.0790, Found 208.0792.



S-Ethyl benzoyldithioacetate (236)

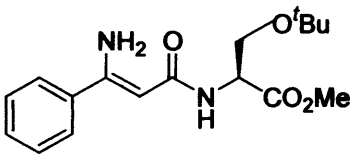
(i) Formation of lithium enolate of *S*-ethyl dithioacetate. To a stirred solution of diisopropylamine (0.70 mL, 5.0 mmol) in dry tetrahydrofuran (5 mL) was added *n*-butyllithium in hexanes (2.5 M; 2.00 mL, 5.0 mmol) at 0 °C. The mixture was stirred for 10 min and cooled to –65 to –70 °C. Freshly distilled *S*-ethyl dithioacetate (**235**) (0.29 mL, 2.5 mmol) was added and the solution was stirred for 30 min. **(ii) Formation of *S*-ethyl benzoyldithioacetate (236).** To a stirred solution of benzoic acid (**223**) (0.30 g, 2.5 mmol) in dry THF (5 mL) was added triethylamine (0.70 mL, 5.0 mmol) followed by ethyl chloroformate (0.48 mL, 5.0 mmol) at 0 °C. After the mixture was stirred for 30 min at 0 °C, triethylamine hydrochloride was filtered off. The filtrate was added dropwise to the above solution of the lithium enolate of *S*-ethyl dithioacetate (**235**) at –68 °C. The mixture was stirred at –68 °C for 20 min and saturated aqueous ammonium chloride solution (30 mL) was added. The resulting mixture was warmed to room temperature. After extracting with ethyl acetate (30 mL), the organic extract was washed with brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–dichloromethane (3:1), gave the *title compound* (0.49 g, 88%) as a pale yellow oil: IR (film) 3063, 2968, 2927, 2865, 1729, 1686, 1599, 1581, 1448, 1264, 1204, 1176, 1094, 895, 772, 712, 687 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.88 (2 H, m, *o*-Ph*H*), 7.49 (1 H, m, *p*-Ph*H*), 7.36 (2 H, m, *m*-Ph*H*), 5.79 (1 H, d, J = 1.0, C*H*H), 5.71 (1 H, d, J = 1.0, CH*H*), 2.76 (2 H, q, J = 7.4, SCH_2Me), 1.23 (3 H, t, J = 7.4, SCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.8 (C), 136.2 (C), 133.9 (CH), 132.4 (C), 128.8 (CH), 127.5 (CH), 125.3 (CH_2), 28.1 (CH_2), 13.4 (Me); MS (CI) *m/z* (relative intensity) 242 (M + NH_4^+, 5%), 225 (MH^+, 100), 139 (40), 122 (27), 121 (39), 105 (64); HRMS calcd for $C_{11}H_{13}OS_2$ (MH) 225.0402, found 225.0402.



(*S*)-*N*-(3-Amino-3-phenylthiopropenoyl)-*O*-*tert*-butylserine methyl ester (237)

A mixture of (*S*)-*N*-(3-oxo-3-phenylthiopropanoyl)-*O*-*tert*-butylserine methyl ester (**229**) and (*S*)-*N*-(3-hydroxy-3-phenylthiopropenoyl)-*O*-*tert*-butylserine methyl ester (273 mg, 0.81 mmol) and ammonium acetate (375 mg, 4.86 mmol) in toluene–glacial acetic acid (5:1) (15 mL) was heated under reflux for 20 h using a Dean and Stark trap. The mixture was allowed

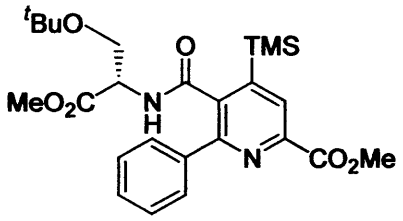
to cool and partitioned between saturated aqueous sodium hydrogen carbonate solution (30 mL) and ethyl acetate (30 mL). The aqueous layer was further extracted with ethyl acetate (2 x 15 mL) and the combined organic layers were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (15 mL) and brine (15 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with dichloromethane, gave the *title compound* (155 mg, 57%) as a pale yellow oil: $[\alpha]_D^{23}$ +33.0 (*c* 1.00, $CHCl_3$); IR (KBr) 3378, 3109, 2970, 1740, 1611, 1492, 1364, 1232, 1198, 1092, 768, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.38–6.20 (2 H, br s, NH_2), 7.45 (2 H, m, *o*-Ph*H*), 7.34 (3 H, *m,p*-Ph*H*), 7.15 (1 H, d, *J* = 8.0, NH), 5.40 (1 H, m, α-CH), 5.36 (1 H, s, CH), 3.81 (1 H, dd, *J* = 9.2, 2.8, β-C*H*H), 3.68 (3 H, s, OMe), 3.65 (1 H, dd, *J* = 9.2, 3.1, β-CH*H*), 1.06 (9 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.6 (C), 171.2 (C), 159.8 (C), 139.2 (C), 130.1 (CH), 128.9 (CH), 126.4 (CH), 96.6 (CH), 73.6 (C), 62.0 (CH_2), 56.4 (CH), 52.4 (CH_3), 27.3(Me); MS (APcI) *m/z* (relative intensity) 337 (MH^+, 100%), 281 (8), 247 (10), 162 (17); HRMS calcd for $C_{17}H_{25}N_2O_3S$ (MH) 337.1580, Found 337.1581.



(*S*)-*N*-(3-Amino-3-phenylpropenoyl)-*O*-*tert*-butylserine methyl ester (239)

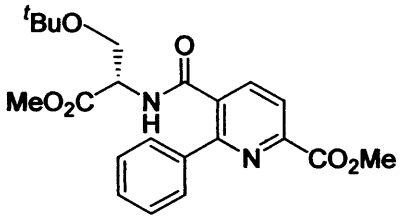
A mixture of (*S*)-*N*-(3-oxo-3-phenylpropanoyl)-*O*-*tert*-butylserine methyl ester (**227**) and (*S*)-*N*-(3-hydroxy-3-phenylpropenoyl)-*O*-*tert*-butylserine methyl ester (1.05 g, 3.27 mmol) and ammonium acetate (2.52 g, 32.70 mmol) in toluene–glacial acetic acid (5:1) (30 mL) was heated under reflux for 20 h using a Dean and Stark trap. The mixture was allowed to cool and partitioned between saturated aqueous sodium hydrogen carbonate solution (50 mL) and ethyl acetate (50 mL), the aqueous layer was further extracted with ethyl acetate (2 x 25 mL) and the combined organic layers were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (25 mL) and brine (25 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (2:1), gave the *title compound* (0.61 g, 58%) as a pale yellow oil: $[\alpha]_D^{23}$ +27.2 (*c* 1.39, $CHCl_3$); IR (KBr) 3433, 3307, 2972, 1747, 1627, 1554, 1499, 1364, 1343, 1196, 1090, 771, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.47 (2 H, m, *o*-Ph*H*), 7.34

(3 H, *m,p*-PhH), 6.52 (2 H, br s, NH_2), 5.90 (1 H, d, J = 8.1, NH), 4.83 (1 H, s, CH), 4.74 (1 H, m, α-CH), 3.78 (1 H, dd, J = 8.9, 2.9, β-CHH), 3.68 (3 H, s, OMe), 3.52 (1 H, dd, J = 8.9, 3.4, β-CHH), 1.07 (9 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8 (C), 169.8 (C), 158.3 (C), 138.3 (C), 129.9 (CH), 128.8 (CH), 126.1 (CH), 87.1 (CH), 73.4 (C), 62.4 (CH_2), 52.4 (CH), 52.3 (CH_3), 27.3(Me); MS (APcI) *m/z* (relative intensity) 321 (MH^+, 100%), 265 (14); HRMS calcd for $C_{17}H_{25}N_2O_4$ (MH) 321.1809, Found 321.1809.



(*S*)-*O*-*tert*-Butyl-*N*-[(2-phenyl-4-trimethylsilyl-6-methoxycarbonylpyridin-3-yl)carbonyl]serine methyl ester (240)

(*S*)-*N*-(3-Amino-3-phenylpropenoyl)-*O*-*tert*-butylserine methyl ester (**239**) (65 mg, 0.20 mmol) and methyl 2-oxo-4-(trimethylsilyl)but-3-ynoate (**216**) (74 mg, 0.40 mmol) were reacted according to general procedure **GP05** or **GP06**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (2:1), gave the *title compound* [24 mg, 25%, for method **GP05** (reaction time 80 min); 62 mg, 64%, for method **GP06** (reaction time 70 min)] as a pale yellow oil: $[\alpha]_D^{22}$ +51.0 (*c* 2.91, $CHCl_3$); IR ($CHCl_3$) 3431, 2960, 1742, 1664, 1508, 1439, 1365, 1261, 1213, 1096, 1020, 846, 807 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.20 (1 H, s, PyH), 7.62 (2 H, m, *o*-PhH), 7.32 (3 H, *m,p*-PhH), 6.12 (1 H, d, J = 8.5, NH), 4.62 (1 H, m, α-CH), 3.94 (3 H, s, $PyCO_2Me$), 3.55 (3 H, s, OMe), 3.50 (1 H, dd, J = 8.9, 2.8, β-CHH), 2.90 (1 H, dd, J = 8.9, 3.2, β-CHH), 0.87 (9 H, s, CMe_3), 0.33 (9 H, s, $SiMe_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (C), 168.7 (C), 165.9 (C), 155.6 (C), 151.9 (C), 146.8 (C), 139.2 (C), 139.1 (C), 129.1 (CH), 129.0 (CH), 128.6 (CH), 128.5 (CH), 73.2 (C), 61.6 (CH_2), 53.1 (CH), 53.0 (Me), 52.3 (Me), 27.1 (Me), –0.6 (Me); MS (APcI) *m/z* (relative intensity) 487 (MH^+, 100%), 431 (11); HRMS calcd for $C_{25}H_{35}N_2O_6Si$ (MH) 487.2259, found 487.2257.

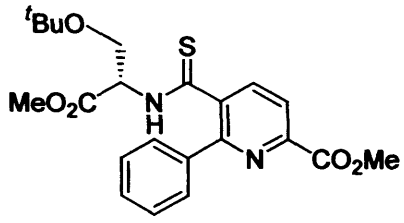


(*S*)-*O*-*tert*-Butyl-*N*-[(2-phenyl-6-methoxycarbonylpyridin-3-yl)carbonyl]serine methyl ester (241)

I. Preparation from (*S*)-*O*-*tert*-butyl-*N*-[(2-phenyl-4-trimethylsilyl-6-methoxycarbonylpyridin-3-yl)carbonyl]serine methyl ester (240). To a solution of (*S*)-*O*-*tert*-butyl-*N*-[(2-phenyl-4-trimethylsilyl-6-methoxycarbonylpyridin-3-yl)carbonyl]serine methyl ester (**240**) (26 mg, 0.05 mmol) in tetrahydrofuran (10 mL) was added tetra-*n*-butylammonium fluoride in tetrahydrofuran (1 M; 0.06 mL, 0.06 mmol) at 0 °C. The mixture was stirred at this temperature for 2 h, concentrated *in vacuo* and partitioned between water (10 mL) and chloroform (10 mL). The aqueous layer was further extracted with chloroform (2 x 10 mL) and the combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (17 mg, 81%) as a pale yellow oil.

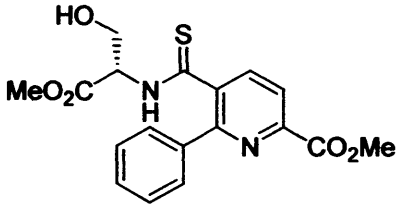
II. Preparation from (*S*)-*N*-(3-amino-3-phenylpropenoyl)-*O*-*tert*-butylserine methyl ester (239). A solution of (*S*)-*N*-(3-amino-3-phenylpropenoyl)-*O*-*tert*-butylserine methyl ester (**239**) (26 mg, 0.08 mmol) and methyl 2-oxo-4-(trimethylsilyl)but-3-ynoate (**216**) (19 mg, 0.10 mmol) in methanol (15 mL) was stirred at room temperature for 60 h and evaporated *in vacuo*. The crude product was purified by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), to give the *title compound* (32 mg, 95%) as a pale yellow oil: $[\alpha]_D^{23}$ +48.3 (*c* 2.83, $CHCl_3$); IR (KBr) 3428, 3341, 2976, 1750, 1654, 1522, 1433, 1392, 1364, 1321, 1228, 1192, 1168, 1096, 972, 859, 740, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (2 H, app s, 4,5-PyH), 7.66 (2 H, m, *o*-Ph*H*), 7.37 (3 H, *m,p*-Ph*H*), 6.28 (1 H, d, *J* = 8.3, NH), 4.66 (1 H, m, α-CH), 3.95 (3 H, s, $PyCO_2Me$), 3.64 (1 H, dd, *J* = 9.0, 2.9, β-C*H*H), 3.63 (3 H, s, OMe), 3.18 (1 H, dd, *J* = 9.0, 3.2, β-CH*H*), 0.91 (9 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (C), 167.4 (C), 165.2 (C), 156.7 (C), 148.7 (C), 138.4 (CH), 138.2 (C), 133.6 (C), 129.5 (CH), 129.2 (CH), 128.7 (CH), 123.2 (CH), 73.4

(C), 61.3 (CH_2), 53.2 (CH), 53.1 (Me), 52.5 (Me), 27.1 (Me); MS (APcI) *m/z* (relative intensity) 415 (MH^+, 100%); HRMS calcd for $C_{22}H_{27}N_2O_6$ (MH) 415.1864, found 415.1863.



(*S*)-*O*-*tert*-Butyl-*N*-[(2-phenyl-6-methoxycarbonylpyridin-3-yl)thiocarbonyl]serine methyl ester (242)

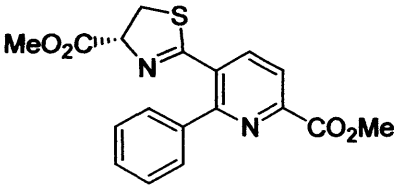
A solution of (*S*)-*O*-*tert*-butyl-*N*-[(2-phenyl-6-methoxycarbonylpyridin-3-yl)carbonyl]-serine methyl ester (**241**) (67 mg, 0.16 mmol) and Lawesson's reagent (52 mg, 0.13 mmol) in benzene (25 mL) was heated under reflux for 66 h. The mixture was allowed to cool, evaporated *in vacuo*, and purified by flash chromatography on silica, eluting with light petroleum–ethyl acetate (4:3), to give the *title compound* (66 mg, 96%) as a pale yellow oil: $[\alpha]_D^{28}$ +99.7 (*c* 1.52, $CHCl_3$); IR (KBr) 3294, 2973, 1744, 1512, 1432, 1392, 1364, 1321, 1223, 1153, 1095, 915, 848, 802, 743, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (1 H, d, *J* = 8.0, PyH), 8.04 (1 H, d, *J* = 8.0, PyH), 7.72 (1 H, d, *J* = 7.9, NH), 7.71 (2 H, m, *o*-Ph*H*), 7.35 (3 H, *m,p*-Ph*H*), 5.12 (1 H, m, α-CH), 3.95 (3 H, s, $PyCO_2Me$), 3.64 (1 H, dd, *J* = 9.2, 2.5, β-C*H*H), 3.62 (3 H, s, OMe), 3.14 (1 H, dd, *J* = 9.2, 3.1, β-CH*H*), 0.89 (9 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.3 (C), 169.1 (C), 165.2 (C), 154.0 (C), 148.3 (C), 140.1 (C), 139.5 (CH), 138.1 (C), 129.3 (CH), 129.0 (CH), 128.6 (CH), 123.1 (CH), 73.7 (C), 60.5 (CH_2), 58.8 (CH), 53.1 (Me), 52.7 (Me), 27.1 (Me); MS (EI) *m/z* (relative intensity) 430 (M^+, 100%); HRMS calcd for $C_{22}H_{27}N_2O_5S$ (MH) 431.1635, found 431.1637.



(*S*)-*N*-[(2-Phenyl-6-methoxycarbonylpyridin-3-yl)thiocarbonyl]serine methyl ester (243)

Trifluoroacetic acid (5 mL) was added dropwise to a solution of (*S*)-*O*-*tert*-butyl-*N*-[(2-phenyl-6-methoxycarbonylpyridin-3-yl)thiocarbonyl]serine methyl ester (**242**) (26 mg, 0.06 mmol) in dichloromethane (5 mL) at room temperature. The reaction mixture was stirred for

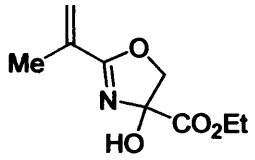
72 h and concentrated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:3) to give the *title compound* (20 mg, 87%) as a pale yellow oil: $[\alpha]_D^{27}$ +67.4 (*c* 1.00, $CHCl_3$); IR (KBr) 3438, 3285, 3026, 2958, 1734, 1559, 1522, 1432, 1399, 1321, 1295, 1261, 1225, 1148, 1078, 1026, 802, 745, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (1 H, d, *J* = 8.0, PyH), 8.07 (1 H, d, *J* = 8.0, PyH), 7.74 (2 H, m, *o*-Ph*H*), 7.73 (1 H, d, *J* = 7.4, NH), 7.41 (3 H, *m,p*-Ph*H*), 5.10 (1 H, m, α-CH), 3.95 (3 H, s, $PyCO_2Me$), 3.77 (1 H, dd, *J* = 11.8, 2.3, β-C*H*H), 3.73 (1 H, dd, *J* = 11.8, 2.8, β-CH*H*), 3.66 (3 H, s, OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.0 (C), 169.5 (C), 165.0 (C), 153.8 (C), 148.4 (C), 140.5 (C), 139.1 (CH), 138.6 (C), 129.5 (CH), 129.1 (CH), 128.8 (CH), 123.4 (CH), 61.0 (CH_2), 60.1 (CH), 53.2 (Me), 53.1 (Me); MS (CI) *m/z* (relative intensity) 375 (MH^+, 63%), 239 (100), 228 (38), 214 (36), 181 (16), 122 (20), 102 (33), 90 (22); HRMS calcd for $C_{18}H_{19}N_2O_5S$ (MH) 375.1009, found 375.1013.



(*R*)-Methyl 2-phenyl-3-(4-methoxycarbonyl-2-thiazolin-2-yl)pyridine-6-carboxylate (244)

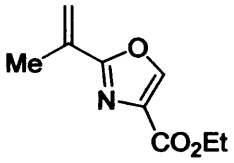
A solution of (*S*)-*O*-*tert*-butyl-*N*-[(2-phenyl-6-methoxycarbonylpyridin-3-yl)thiocarbonyl]-serine methyl ester (**242**) (61 mg, 0.14 mmol) in trifluoroacetic acid (10 mL) was heated under reflux for 48 h. The solution was allowed to cool and evaporated *in vacuo*. The residue was purified by flash chromatography on silica, eluting with light petroleum–ethyl acetate (2:3), to give the *title compound* (50 mg, 99%) as a colourless solid, mp 121–122.5 °C (from methanol): $[\alpha]_D^{28}$ +3.8 (*c* 1.05, $CHCl_3$); IR (KBr) 2955, 1740, 1723, 1584, 1440, 1426, 1320, 1227, 1177, 1130, 1104, 1027, 804, 749, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (2 H, app s, 4,5-PyH), 7.57 (2 H, m, *o*-Ph*H*), 7.35 (3 H, *m,p*-Ph*H*), 5.15 (1 H, app t, *J* = 9.1, NCH), 3.94 (3 H, s, $PyCO_2Me$), 3.73 (3 H, s, OMe), 3.62 (1 H, dd, *J* = 11.2, 8.5, SC*H*H), 3.52 (1 H, dd, *J* = 11.2, 9.6, SCH*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (C), 170.4 (C), 165.2 (C), 158.1 (C), 148.8 (C), 138.8 (CH), 138.1 (C), 131.4 (C), 129.6 (CH), 129.4 (CH), 128.3 (CH), 123.0 (CH), 77.8 (CH), 53.2 (Me), 53.0 (Me), 36.9 (CH_2); MS

(APcI) *m/z* (relative intensity) 357 (MH^+, 100%); HRMS calcd for $C_{18}H_{17}N_2O_4S$ (MH) 357.0904, Found 357.0904.



Ethyl 4-hydroxy-2-(2-propenyl)-2-oxazoline-4-carboxylate (247)

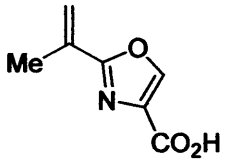
Ethyl bromopyruvate (**246**) (0.9 mL, 7.17 mmol) was added to a solution of 2-methacrylamide (**245**) (0.5 g, 6.02 mmol) and $NaHCO_3$ (2.50 g, 29.8 mmol) in dry tetrahydrofuran (60 mL). The mixture was heated at reflux for 18 h, filtered through Celite® and concentrated *in vacuo*. The crude solid was recrystallized from light petroleum–diethyl ether to give the *title compound* (0.96 g, 80%) as a colourless solid, mp 80–81 °C: IR (Nujol) 1749, 1654, 1602, 1459, 1376, 1224, 1154, 1083, 1016, 954 cm^{-1}; ^1H NMR (400 MHz, d_4-methanol) δ 5.89 (1 H, m, CC*H*H), 5.52 (1 H, m, CCH*H*), 4.59 (1 H, d, J = 10.0, OC*H*H), 4.15 (2 H, q, J = 7.1, CH_2Me), 4.12 (1 H, d, J = 10.0, OCH*H*), 1.86 (3 H, m, Me), 1.21 (3 H, t, J = 7.1, CH_2*Me*); ^{13}C NMR (100 MHz, d_4-methanol) δ 170.6 (C), 168.6 (C), 132.2 (C), 123,8 (CH_2), 97.1 (C), 76.1 (CH_2), 62.0 (CH_2), 17.9 (Me), 13.0 (Me); MS (APcI) *m/z* (relative intensity) 200 (MH^+, 100%), 182 (32); HRMS calcd for $C_9H_{14}NO_4$ (MH) 200.0917, found 200.0916; Anal. Calcd for $C_9H_{13}NO_4$: C, 54.3; H, 6.6; N, 7.0. Found: C, 54.0; H, 6.4; N, 6.7.



Ethyl 2-(propenyl)oxazole-4-carboxylate (248)

A solution of 2,6-lutidine (8.12 mL, 77.1 mmol) and trifluoroacetic anhydride (4.73 mL, 33.5 mmol) in dry tetrahydrofuran (10 mL) was added to a solution of ethyl 4-hydroxy-2-(2-propenyl)-2-oxazoline-4-carboxylate (**247**) (5.55 g, 27.9 mmol) at 0 °C. After stirring for 30 min, water (50 mL) was added and the mixture was concentrated *in vacuo*. Purification by flash chromatography on silica, gradient eluting with light petroleum to light petroleum–ethyl acetate (3:1), gave the *title compound* (5.06 g, 94%) as a colourless oil: IR (film) 3155, 2984, 1744, 1575, 1543, 1448, 1391, 1315, 1254, 1176, 115, 982, 916, 763 cm^{-1}; ^1H NMR

(400 MHz, $CDCl_3$) δ 8.12 (1 H, s, 5-H), 6.15 (1 H, d, J = 1.4, C*H*H), 5.39 (1 H, d, J = 1.4, CH*H*), 4.32 (2 H, q, J = 7.1, CH_2Me), 2.12 (3 H, app s, Me), 1.30 (3 H, t, J = 7.1, CH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1 (C), 161.3 (C), 143.5 (CH), 134.2 (C), 131.1 (C), 119.9 (CH_2), 61.2 (CH_2), 19.0 (Me), 14.3 (Me); MS (EI) *m/z* (relative intensity) 181 (M^+, 100%); HRMS calcd for $C_9H_{12}NO_3$ (MH) 182.0817, found 182.0810.



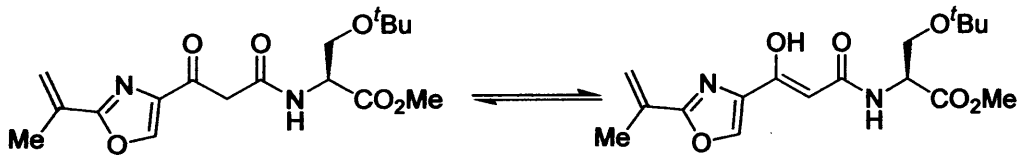
2-(2-Propenyl)oxazole-4-carboxylic acid (249)

Lithium hydroxide monohydrate (8.77 g, 209 mmol) was added to a solution of ethyl 2-(2-propenyl)oxazole-4-carboxylate (**248**) (4.76 g, 26.4 mmol) in methanol–water (1:1) (120 mL). The mixture was stirred at room temperature for 1 h, concentrated *in vacuo* and partitioned between water (100 mL) and chloroform (50 mL). The aqueous layer was further extracted with chloroform (2 x 50 mL), acidified to pH 5–6 with dilute hydrochloric acid (3 N) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with brine (25 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by recyrstalisation from light petroleum–ethyl acetate (7:3) gave the *title compound* (5.00 g, 94%) as a colourless solid, mp 121.5–122 °C: IR (Nujol) 3136, 1691, 1562, 1462, 1377, 1260, 1186, 1124, 984, 910, 853, 766, 665 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.26 (1 H, br s, CO_2H), 8.23 (1 H, s, 5-H), 6.00 (1 H, s, C*H*H), 5.44 (1 H, s, CH*H*), 2.14 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1 (C), 163.5 (C), 145.0 (CH), 133.4 (C), 130.9 (C), 120.6 (CH_2), 19.0 (Me); MS (APcI) *m/z* (relative intensity) 154 (MH^+, 100%), 136 (80); HRMS calcd for $C_7H_8NO_3$ (MH) 154.0499, found 154.0498; Anal. Calcd for $C_7H_7NO_3$: C, 54.9; H, 4.6; N, 9.2. Found: C, 54.6; H, 4.6; N, 8.9.

***S*-Ethyl {[2-(2-propenyl)oxazol-4-yl]carbonyl}thioacetate and *S*-ethyl 3-hydroxy-3-[2-(2-propenyl)oxazol-4-yl]thiopropenoate (250)**

(i) Formation of lithium enolate of *S*-ethyl thioacetate. A solution of n-butyllithium in hexanes (2.5 M; 6.24 mL, 15.6 mmol) was added to a stirred solution of diisopropylamine

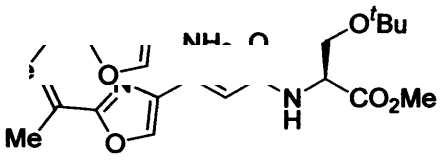
(2.20 mL, 15.6 mmol) in tetrahydrofuran (15.7 mL) at 0 °C. The mixture was stirred for 10 min and cooled to -78 °C. Freshly distilled S-ethyl thioacetate (0.83 mL, 7.8 mmol) was added and the solution was stirred for 30 min. **(ii) Formation of *S*-ethyl [(oxazol-4-yl)carbonyl]thioacetate.** Ethyl chloroformate (0.54 mL, 5.7 mmol) was added dropwise to a stirred solution of 2-(2-propenyl)oxazole-4-carboxylic acid (**249**) (800 mg, 5.22 mmol) and triethylamine (0.80 mL, 5.7 mmol) in dry tetrahydrofuran (11 mL) at 0 °C. After stirring for 30 min, the mixture was filtered, cooled to –78 °C and a solution of the lithium enolate of S-ethyl thioacetate was added dropwise. The mixture was stirred at –78 °C for 30 min and partitioned between saturated aqueous ammonium chloride solution (60 mL) and ethyl acetate (60 mL). The organic extract was washed with brine (40 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with dichloromethane, gave the *title compounds* (0.94 g, 75%) as a pale yellow oil: IR (KBr) 2966, 2925, 1704, 1648, 1589, 1538, 1452, 1408, 1330, 1246, 1120, 1080, 775, 722 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.54 (0.65 H, s, OH), 8.15 (0.35 H, s, OxaH), 7.93 (0.65 H, s, OxaH), 6.20 (0.65 H, s, CH), 5.94 (0.35 H, s, C*H*H), 5.91 (0.65 H, s, C*H*H), 5.40 (0.35 H, s, CH*H*), 5.37 (0.65 H, s, CH*H*), 4.10 (0.70 H, s, CH_2), 2.90 (1.30 H, q, *J* = 7.4, CH_2Me), 2.86 (0.70 H, q, *J* = 7.4, CH_2Me), 2.09 (3 H, s, Me), 1.24 (1.95 H, t, *J* = 7.4, CH_2*Me*), 1.19 (1.05 H, t, *J* = 7.4, CH_2*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.5 (C), 192.0 (C), 186.9 (C), 163.0 (C), 162.8 (C), 161.0 (C), 142.8 (CH), 140.7 (C), 139.7 (CH), 136.6 (C), 131.2 (C), 131.0 (C), 120.1 (CH_2), 119.6 (CH_2), 98.3 (CH), 54.5 (CH_2), 24.0 (CH_2), 22.9 (CH_2), 18.91 (Me), 18.88 (Me), 14.8 (Me), 14.5 (Me); MS (APcI) *m/z* (relative intensity) 240 (MH^+, 100%), 210 (44); HRMS calcd for $C_{11}H_{14}NO_3S$ (MH) 240.0689, found 240.0688.



(*S*)-*N*-{3-Oxo-3-[2-(2-propenyl)oxazol-4-yl]propanoyl}-*O*-*tert*-butylserine methyl ester (251) and (*S*)-*N*-{3-hydroxy-3-[2-(2-propenyl)oxazol-4-yl]propenoyl}-*O*-*tert*-butylserine methyl ester

S-Ethyl {[2-(2-propenyl)oxazol-4-yl]carbonyl}thioacetate and *S*-ethyl 3-hydroxy-3-[2-(2-propenyl)oxazol-4-yl]thiopropenoate (**250**) (274 mg, 1.14 mmol) were reacted with *O*-*tert*-butyl-L-serine methyl ester hydrochloride (**225**) (242 mg, 1.14 mmol) according to general

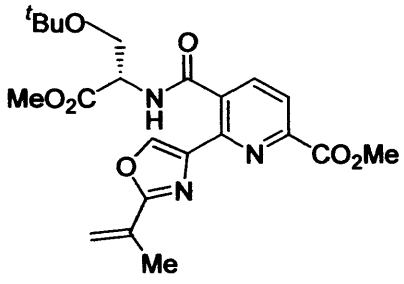
procedure **GP13**. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (4:1), gave the *title compounds* (0.33 g, 83%) as a pale yellow oil: $[\alpha]_D^{24}$ +41.7 (*c* 1.13, $CHCl_3$); IR (KBr) 3366, 2971, 1750, 1694, 1661, 1609, 1549, 1438, 1364, 1248, 1204, 1093, 1054, 1020, 915, 812, 780, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.36 (0.36 H, s, OH), 8.24 (0.64 H, s, OxaH), 7.87 (0.36 H, s, OxaH), 7.72 (0.64 H, d, *J* = 8.2, NH), 6.42 (0.36 H, d, *J* = 8.5, NH), 5.95 (0.64 H, s, 2"-H), 5.90 (0.36 H, s, 2"-H), 5.79 (0.36 H, s, CH), 5.42 (0.64 H, s, 2"-H), 5.35 (0.36 H, s, 2"-H), 4.71 (0.36 H, m, α-CH), 4.66 (0.64 H, m, α-CH), 3.93 (0.64 H, d, *J* = 15.6, C*H*H), 3.89 (0.64 H, d, *J* = 15.6, CH*H*), 3.78 (0.36 H, dd, *J* = 8.9, 2.9, β-C*H*H), 3.74 (0.64 H, dd, *J* = 9.1, 3.0, β-C*H*H), 3.68 (1.08 H, s, OMe), 3.65 (1.92 H, s, OMe), 3.54 (0.36 H, dd, *J* = 8.9, 3.2, β-CH*H*), 3.49 (0.64 H, dd, *J* = 9.1, 3.2, β-CH*H*), 2.11 (1.92 H, s, Me), 2.08 (1.08 H, s, Me), 1.06 (1.08 H, s, CMe_3), 1.04 (1.92 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.8 (C), 171.3 (C), 170.8 (C), 170.6 (C), 165.2 (C), 162.8 (C), 162.7 (C), 162.2 (C), 143.2 (CH), 140.7 (C), 138.1 (CH), 137.4 (C), 131.2 (C), 130.9 (C), 120.2 (CH_2), 119.2 (CH_2), 89.9 (CH), 73.4 (C), 73.3 (C), 61.9 (CH_2), 61.8 (CH_2), 53.1 (CH), 52.4 (Me), 52.3 (Me), 52.3 (CH), 47.0 (CH_2), 27.2 (Me), 27.2 (Me), 18.9 (Me), 18.8 (Me); MS (APcI) *m/z* (relative intensity) 353 (MH^+, 44%), 297 (100), 279 (12), 120 (37); HRMS calcd for $C_{17}H_{25}N_2O_6$ (MH) 353.1707, found 353.1701.



(*S*)-*N*-{3-Amino-3-[2-(2-propenyl)oxazol-4-yl]propenoyl}-*O*-*tert*-butylserine methyl ester (252)

Ammonium acetate (120 mg, 1.56 mmol) was added to a solution of (*S*)-*N*-{3-hydroxy-3-[2-(2-propenyl)oxazol-4-yl]propenoyl}-*O*-*tert*-butylserine methyl ester and (*S*)-*N*-{3-oxo-3-[2-(2-propenyl)oxazol-4-yl]propanoyl}-*O*-*tert*-butylserine methyl ester (**251**) (92 mg, 0.26 mmol) in toluene (3 mL) under nitrogen and the mixture irradiated at 120 °C (initial power 100 W) in a CEM Discover™ microwave synthesizer for 30 min. After cooling to room temperature, the mixture was partitioned between saturated aqueous sodium hydrogen carbonate solution (10 mL) and ethyl acetate (10 mL) and the aqueous layer further extracted with ethyl acetate (10 mL). The combined organic extracts were washed

sequentially with saturated aqueous sodium hydrogen carbonate solution (8 mL) and brine (8 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (60 mg, 66%) as a pale yellow oil: $[\alpha]_D^{24}$ +51.2 (*c* 1.00, $CHCl_3$); IR (KBr) 3452, 3324, 2974, 1748, 1644, 1598, 1540, 1363, 1198, 1098, 1050, 1021, 976, 913, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (1 H, s, OxaH), 6.82 (2 H, br s, NH_2), 5.90 (1 H, s, C*H*H), 5.89 (1 H, d, *J* 8.5, NH), 5.36 (1 H, s, CH*H*), 5.00 (1 H, s, CH), 4.72 (1 H, m, α-CH), 3.78 (1 H, dd, *J* = 8.9, 2.9, β-C*H*H), 3.68 (3 H, s, OMe), 3.51 (1 H, dd, *J* = 8.9, 3.2, β-CH*H*), 2.10 (3 H, s, Me), 1.08 (9 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8 (C), 169.8 (C), 162.6 (C), 147.8 (C), 138.6 (C), 135.5 (CH), 131.3 (C), 119.1 (CH_2), 84.2 (CH), 73.4 (C), 62.4 (CH_2), 52.34 (CH), 52.30 (Me), 27.3 (Me), 19.0 (Me); MS (APcI) *m/z* (relative intensity) 353 (100%), 352 (MH^+, 67); HRMS calcd for $C_{17}H_{26}N_3O_5$ (MH) 352.1867, found 352.1871.

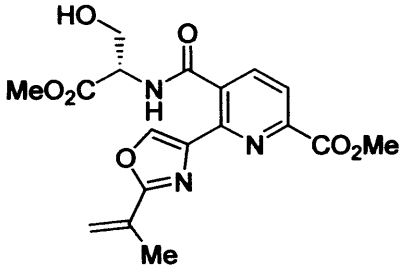


(*S*)-*O-tert*-Butyl-*N*-({2-[2-(2-propenyl)oxazol-4-yl]-6-methoxycarbonylpyridin-3-yl}carbonyl)serine methyl ester (253)

I. Preparation from (*S*)-*N*-{3-amino-3-[2-(2-propenyl)oxazol-4-yl]propenoyl}-*O-tert*-butylserine methyl ester (252). A solution of (*S*)-*N*-{3-amino-3-[2-(2-propenyl)oxazol-4-yl]propenoyl}-*O-tert*-butylserine methyl ester (**252**) (88 mg, 0.25 mmol) and methyl 2-oxo-4-(trimethylsilyl)but-3-ynoate (**216**) (61 mg, 0.33 mmol) in methanol (10 mL) was stirred at room temperature for 24 h and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:2), gave the *title compound* (104 mg, 93%) as a pale yellow oil.

II. Preparation from (*S*)-*N*-{3-oxo-3-[2-(2-propenyl)oxazol-4-yl]propanoyl}-*O-tert*-butylserine methyl ester (251) and (*S*)-*N*-{3-hydroxy-3-[2-(2-propenyl)oxazol-4-yl]propenoyl}-*O-tert*-butylserine methyl ester. To a solution of (*S*)-*N*-{3-oxo-3-[2-(2-propenyl)oxazol-4-yl]propanoyl}-*O-tert*-butylserine methyl ester (**251**) and (*S*)-*N*-{3-

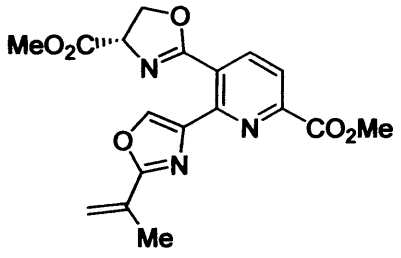
hydroxy-3-[2-(2-propenyl)oxazol-4-yl]propenoyl}-*O*-*tert*-butylserine methyl ester (35 mg, 0.10 mmol) and methyl 2-oxo-4-(trimethylsilyl)but-3-ynoate (**216**) (49 mg, 0.25 mmol) in methanol (10 mL) was added ammonium acetate (77 mg, 1.00 mmol). The mixture was stirred at reflux for 5 h, allowed to cool and evaporated *in vacuo*. The residue was partitioned between saturated aqueous sodium hydrogen carbonate solution (5 mL) and ethyl acetate (8 mL) and the aqueous layer was further extracted with ethyl acetate (5 mL). The combined organic extracts were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (5 mL) and brine (5 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:2), gave the *title compound* (36 mg, 81%) as a pale yellow oil: $[\alpha]_D^{29}$ +12.0 (*c* 1.78, $CHCl_3$); IR (KBr) 2966, 1751, 1670, 1540, 1436, 1364, 1323, 1262, 1099, 801, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (1 H, s, OxaH), 8.05 (1 H, d, *J* = 8.0, PyH), 8.01 (1 H, d, *J* = 8.0, PyH), 7.00 (1 H, d, *J* = 8.0, NH), 5.93 (1 H, s, C*H*H), 5.35 (1 H, s, CH*H*), 4.85 (1 H, m, α-CH), 3.95 (3 H, s, $PyCO_2$*Me*), 3.80 (1 H, dd, *J* = 9.1, 3.0, β-C*H*H), 3.69 (3 H, s, OMe), 3.57 (1 H, dd, *J* = 9.1, 3.2, β-CH*H*), 2.10 (3 H, s, Me), 1.01 (9 H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C), 167.0 (C), 165.0 (C), 162.6 (C), 148.5 (C), 147.5 (C), 139.5 (C), 139.0 (CH), 138.1 (CH), 133.3 (C), 131.4 (C), 123.6 (CH), 119.0 (CH_2), 73.6 (C), 61.8 (CH_2), 53.5 (CH), 53.1 (Me), 52.5 (Me), 27.2 (Me), 19.0 (Me); MS (APcI) *m/z* (relative intensity) 446 (MH^+, 100%); HRMS calcd for $C_{22}H_{28}N_3O_7$ (MH) 446.1927, found 446.1925.



(*S*)-*N*-({2-[2-(2-Propenyl)oxazol-4-yl]-6-methoxycarbonylpyridin-3-yl}carbonyl)serine methyl ester (254)

A solution of (*S*)-*O*-*tert*-butyl-*N*-({2-[2-(2-propenyl)oxazol-4-yl]-6-methoxycarbonylpyridin-3-yl}carbonyl)serine methyl ester (**253**) (60 mg, 0.14 mmol) in trifluoroacetic acid–dichloromethane (1:1) (20 mL) was stirred at room temperature for 20 min and evaporated *in vacuo*. Purification by flash column chromatography on silica, eluting

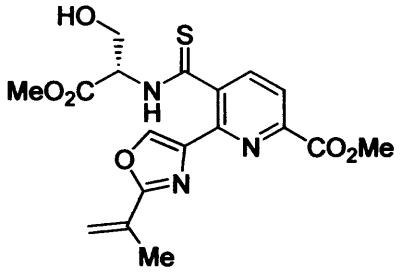
with ethyl acetate, gave the *title compound* (50 mg, 96%) as colourless crystals, mp 74–76 °C (from aqueous ethanol): $[\alpha]_D^{25}$ –8.8 (*c* 0.50, $CHCl_3$); IR (KBr) 3439, 2954, 1734, 1654, 1542, 1438, 1323, 1293, 1234, 1174, 1140, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.22 (1 H, s, OxaH), 7.89 (1 H, d, *J* = 7.9, PyH), 7.84 (1 H, d, *J* = 7.9, PyH), 7.50 (1 H, d, *J* = 7.1, NH), 5.89 (1 H, s, C*H*H), 5.35 (1 H, s, CH*H*), 4.70 (1 H, m, α-CH), 4.32 (1 H, br s, OH), 4.01 (1 H, dd, *J* = 8.2, 3.1, β-C*H*H), 3.94 (1 H, dd, *J* = 8.2, 3.7, β-CH*H*), 3.91 (3 H, s, $PyCO_2$*Me*), 3.68 (3 H, s, OMe), 2.05 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C), 167.6 (C), 164.9 (C), 162.8 (C), 148.0 (C), 147.2 (C), 142.2 (C), 139.7 (CH), 137.8 (CH), 132.8 (C), 131.0 (C), 123.5 (CH), 119.9 (CH_2), 62.2 (CH_2), 55.6 (CH), 53.2 (Me), 52.8 (Me), 18.9 (Me); MS (APcI) *m/z* (relative intensity) 390 (MH^+, 100%); HRMS calcd for $C_{18}H_{20}N_3O_7$ (MH) 390.1296, found 390.1301.



(*S*)-Methyl 2-[2-(2-propenyl)oxazol-4-yl]-3-(4-methoxycarbonyl-2-oxazolin-2-yl)pyridine-6-carboxylate (255)

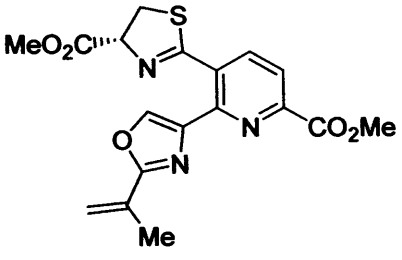
A solution of (*S*)-*N*-({2-[2-(2-propenyl)oxazol-4-yl]-6-methoxycarbonylpyridin-3-yl}carbonyl)serine methyl ester (**254**) (41 mg, 0.10 mmol) and Burgess reagent (28 mg, 0.11 mmol) in dry tetrahydrofuran (5 mL) was stirred at 70 °C for 1 h and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:2), gave the *title compound* (24 mg, 63%) as a pale yellow oil: $[\alpha]_D^{29}$ +29.1 (*c* 0.87, $CHCl_3$); IR (KBr) 2956, 1741, 1654, 1437, 1325, 1286, 1228, 1139, 1052, 958, 762 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (1 H, s, OxaH), 8.06 (1 H, d, *J* = 8.0, PyH), 8.01 (1 H, d, *J* = 8.0, PyH), 5.93 (1 H, s, C*H*H), 5.36 (1 H, s, CH*H*), 4.94 (1 H, dd, *J* = 10.7, 8.6, OC*H*H), 4.64 (1 H, app t, *J* = 8.6, NCH), 4.57 (1 H, dd, *J* = 10.7, 8.6, OCH*H*), 3.95 (3 H, s, $PyCO_2$*Me*), 3.77 (3 H, s, OMe), 2.11 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (C), 165.9 (C), 164.9 (C), 162.3 (C), 149.6 (C), 149.0 (C), 140.2 (C), 139.8 (CH), 139.1 (CH), 131.3 (C), 124.9 (C), 123.1 (CH), 118.8 (CH_2), 70.3 (CH_2), 68.8 (CH), 53.1 (Me), 52.8 (Me),

19.0 (Me); MS (APcI) *m/z* (relative intensity) 372 (MH^+, 100%); HRMS calcd for $C_{18}H_{18}N_3O_6$ (MH) 372.1191, found 372.1191.



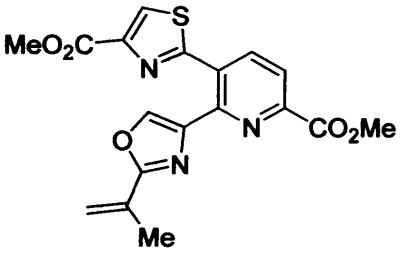
(*S*)-*N*-({2-[2-(2-Propenyl)oxazol-4-yl]-6-methoxycarbonylpyridin-3-yl}thiocarbonyl)serine methyl ester (256)

A solution of (*S*)-methyl 2-[2-(2-propenyl)oxazol-4-yl]-3-(4-methoxycarbonyl-2-oxazolin-2-yl)pyridine-6-carboxylate (**255**) (23 mg, 0.06 mmol) in methanol–triethylamine (2:1) (3 mL) was saturated with hydrogen sulfide, stirred at room temperature for 3.5 h and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with diethyl ether–acetone (5:1), gave the *title compound* (17 mg, 71%) as a pale yellow oil: $[\alpha]_D^{20}$ –29.6 (*c* 0.90, $CHCl_3$); IR (KBr) 3400, 2956, 1736, 1542, 1437, 1388, 1293, 1262, 1232, 1140, 1087, 1027, 802, 761 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.18 (1 H, d, *J* = 6.1, NH), 8.31 (1 H, s, OxaH), 7.91 (1 H, d, *J* = 8.0, PyH), 7.81 (1 H, d, *J* = 8.0, PyH), 5.89 (1 H, s, C*H*H), 5.37 (1 H, s, CH*H*), 5.18 (1 H, m, α-CH), 4.34 (1 H, dd, *J* = 11.9, 3.2, β-C*H*H), 4.05 (1 H, dd, *J* = 11.9, 2.9, β-CH*H*), 3.93 (3 H, s, $PyCO_2$*Me*), 3.83 (1 H, br s, OH), 3.77 (3 H, s, OMe), 2.04 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9 (C), 169.6 (C), 164.9 (C), 162.7 (C), 146.7 (C), 144.8 (C), 140.2 (CH), 139.7 (C), 138.5 (C), 137.7 (CH), 130.8 (C), 123.3 (CH), 120.1 (CH_2), 61.2 (CH_2), 61.0 (CH), 53.2 (Me), 52.9 (Me), 18.9 (Me); MS (APcI) *m/z* (relative intensity) 406 (MH^+, 87%), 181 (68), 130 (100); HRMS calcd for $C_{18}H_{20}N_3O_6S$ (MH) 406.1067, found 406.1062.



(*R*)-Methyl 2-[2-(2-propenyl)oxazol-4-yl]-3-(4-methoxycarbonyl-2-thiazolin-2-yl)pyridine-6-carboxylate (257)

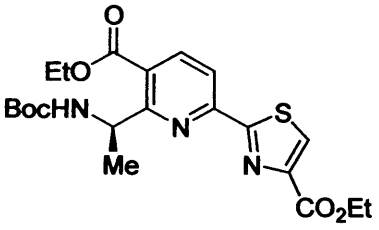
A solution of (*S*)-*N*-({2-[2-(2-propenyl)oxazol-4-yl]-6-methoxycarbonylpyridin-3-yl}thiocarbonyl)serine methyl ester (**256**) (33 mg, 0.08 mmol) and Burgess reagent (24 mg, 0.10 mmol) in dry tetrahydrofuran (5 mL) was stirred at 70 °C for 30 min and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with ethyl acetate, gave the *title compound* (27 mg, 87%) as a pale yellow oil: $[\alpha]_D^{23}$ +18.4 (*c* 1.06, $CHCl_3$); IR (KBr) 2952, 1742, 1618, 1436, 1323, 1281, 1227, 1137, 931, 851, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.16 (1 H, s, OxaH), 8.02 (1 H, d, *J* = 8.0, PyH), 7.91 (1 H, d, *J* = 8.0, PyH), 5.92 (1 H, s, C*H*H), 5.35 (1 H, s, CH*H*), 5.21 (1 H, app t, *J* = 9.7, NCH), 3.95 (3 H, s, $PyCO_2$*Me*), 3.77 (3 H, s, OMe), 3.77 (1 H, dd, *J* = 11.2, 9.7, SC*H*H), 3.67 (1 H, dd, *J* = 11.2, 9.7, SCH*H*), 2.12 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (C), 169.6 (C), 165.0 (C), 162.4 (C), 148.6 (C), 148.5 (C), 139.4 (C), 139.3 (CH), 138.6 (CH), 131.4 (C), 130.8 (C), 123.2 (CH), 78.6 (CH), 53.1 (Me), 53.0 (Me), 37.0 (CH_2), 19.1 (Me); MS (APcI) *m/z* (relative intensity) 388 (MH^+, 100%); HRMS calcd for $C_{18}H_{18}N_3O_5S$ (MH) 388.0962, found 388.0962.



Methyl 2-[2-(2-propenyl)oxazol-4-yl]-3-[4-(methoxycarbonyl)thiazol-2-yl]pyridine-6-carboxylate (258)

A mixture of (*R*)-methyl 2-[2-(2-propenyl)oxazol-4-yl]-3-(4-methoxycarbonyl-2-thiazolin-2-yl)pyridine-6-carboxylate (**257**) (27 mg, 0.07 mmol) and activated manganese(IV) oxide (121 mg, 1.39 mmol) in dichloromethane (3 mL) was irradiated at 100 °C (initial power 300 W) for 150 min in a sealed pressure-rated reaction tube (10 mL) using a CEM Discover™ Microwave Synthesizer. The mixture was cooled rapidly to room temperature in a flow of compressed air for 5 min, filtered through Celite® washing with dichloromethane (2 x 10 mL) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:2), gave the *title compound* (21 mg, 79%) as a pale yellow oil: IR

(KBr) 2956, 2919, 1724, 1560, 1542, 1438, 1322, 1229, 1139, 1087, 761 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (1 H, s, OxaH), 8.20 (1 H, d, J = 8.0, PyH), 8.10 (1 H, d, J = 8.0, PyH), 7.96 (1 H, s, SCH), 5.83 (1 H, s, C*H*H), 5.30 (1 H, s, CH*H*), 3.97 (3 H, s, $PyCO_2$*Me*), 3.91 (3 H, s, OMe), 1.95 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0 (C), 164.6 (C), 162.3 (C), 161.7 (C), 149.2 (C), 148.6 (C), 146.9 (C), 140.0 (CH), 139.3 (C), 139.1 (CH), 131.3 (C), 130.7 (C), 129.8 (CH), 123.6 (CH), 118.8 (CH_2), 53.2 (Me), 52.7 (Me), 18.9 (Me); MS (APcI) *m/z* (relative intensity) 386 (MH^+, 100%); HRMS calcd for $C_{18}H_{16}N_3O_5S$ (MH) 386.0805, found 386.0812.

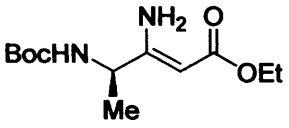


(*R*)-Ethyl 6-[4-(ethoxycarbonyl)thiazol-2-yl]-2-[1-(*N-tert*-butoxycarbonyl)aminoethyl]pyridine-3-carboxylate (311)

A solution of (*R*)-ethyl 4-(*N-tert*-butoxycarbonyl)amino-3-oxopentanoate (**320**) (107 mg, 0.41 mmol) and ammonium acetate (158 mg, 2.0 mmol) in ethanol (10 mL) was stirred at room temperature for 4 h then a solution of ethyl 2-(propynoyl)thiazole-4-carboxylate (**114**) (69 mg, 0.33 mmol) in ethanol (5 mL) was added. The reaction mixture was stirred at room temperature for 1 h and cooled to 0 °C. *N*-Iodosuccinimide (92 mg, 0.41 mmol) was added portionwise and the mixture was stirred at 0 °C for 15 min then concentrated *in vacuo*. The residue was partitioned between ethyl acetate (15 mL) and saturated aqueous sodium hydrogen carbonate solution (15 mL). The aqueous layer was further extracted with ethyl acetate (10 mL). The organic layers were combined, washed sequentially with saturated aqueous sodium hydrogen carbonate solution (10 mL), and brine (10 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (7:3), gave the *title compound*[i] (82 mg, 55%) as a pale yellow oil: $[\alpha]_D^{21}$ +39.0 (*c* 1.06, $CHCl_3$); IR (KBr) 3361, 2983, 2933, 1738, 1723, 1689, 1582, 1528, 1449, 1366, 1336, 1270, 1208, 1174, 1047, 1029, 855, 806, 756 cm^{-1}; ^1H NMR (400 MHz, d_6-acetone) δ 8.46 (1 H, s, SCH), 8.31 (1 H, d, J = 8.1, PyH), 8.08 (1 H, d, J = 8.1, PyH),

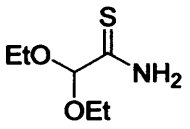
[i] HPLC: ee = 96%; ChiralPak AD, hexane—2-propanol (95:5), λ_{max} = 311 nm, 1.0 mL min^{-1}, R_T = 14.8 min (*S*-isomer has R_T = 12.4 min).

6.26 (1 H, d, J = 8.2, NH), 5.58 (1 H, m, C*H*Me), 4.31 (2 H, q, J = 7.2, OC*H*$_2$Me), 4.26 (2 H, q, J = 7.1, OC*H*$_2$Me), 1.37 (3 H, d, J = 6.8, CH*Me*), 1.30 (3 H, t, J = 7.1, OCH$_2$*Me*), 1.26 (3 H, t, J = 7.2, OCH$_2$*Me*), 1.24 (9 H, s, CMe$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7 (C), 165.2 (C), 162.9 (C), 161.3 (C), 155.2 (C), 151.7 (C), 148.7 (C), 140.3 (CH), 130.3 (CH), 125.2 (C), 118.1 (CH), 79.3 (C), 61.9 (CH_2), 61.7 (CH_2), 48.7 (CH), 28.4 (Me), 22.9 (Me), 14.4 (Me), 14.2 (Me); MS (APcI) *m/z* (relative intensity) 450 (MH^+, 100%), 394 (23), 350 (9); HRMS calcd for $C_{21}H_{28}N_3O_6S$ (MH) 450.1693, found 450.1691.



(*R*)-Ethyl 3-amino-4-(*N-tert*-butoxycarbonyl)aminopent-2-enoate (312)

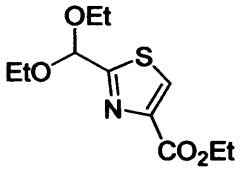
A mixture of (*R*)-ethyl 4-(*N-tert*-butoxycarbonyl)amino-3-oxopentanoate (**320**) (994 mg, 3.8 mmol) and ammonium acetate (1.47 g, 19.0 mmol) in ethanol (30 mL) was stirred at room temperature for 4 h and concentrated. The residue was partitioned between ethyl acetate (60 mL) and saturated aqueous sodium hydrogen carbonate solution (60 mL). The aqueous layer was extracted with ethyl acetate (60 mL). The organic layers were combined, washed sequentially with saturated aqueous sodium hydrogen carbonate solution (60 mL), and brine (60 mL), dried (Na_2SO_4), evaporated *in vacuo*, and purified by flash chromatography on silica, eluting with light petroleum–ethyl acetate (7:3), to give the *title compound*[i] (687 mg, 70%) as a colourless oil: $[\alpha]_D^{25}$ +17.0 (*c* 2.50, $CHCl_3$); IR (KBr) 3458, 3365, 3320, 2983, 2937, 1683, 1612, 1522, 1365, 1310, 1274, 1162, 1042, 861, 792 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (1 H, br s, N*H*Boc), 4.54 (1 H, s, =CH), 4.13 (1 H, q, J = 7.0, C*H*Me), 4.04 (2 H, q, J = 7.1, OC*H*$_2$Me), 1.37 (9 H, s, CMe$_3$), 1.28 (3 H, d, J = 7.0, CH*Me*), 1.19 (3 H, t, J = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C), 164.7 (C), 155.6 (C), 81.0 (CH), 80.3 (C), 58.7 (CH_2), 49.7 (CH), 28.3 (Me), 19.6 (Me), 14.5 (Me); MS (APcI) *m/z* (relative intensity) 259 (MH^+, 100%), 203 (55), 160 (23); HRMS calcd for $C_{12}H_{23}N_2O_4$ (MH) 259.1652, found 259.1648.



[i] HPLC: ee = 92%; ChiralPak AD, hexane–2-propanol (95:5), λ_{max} = 273 nm, 1.0 mL min^{-1}, R_T = 15.1 min (*S*-isomer has R_T = 18.0 min).

2,2-Diethoxythioacetamide (316)

According to a modified literature procedure,[i] phosphorus pentasulphide (15.0 g, 34.0 mmol) was added to a solution of 2,2-diethoxyacetamide (**315**) (10.0 g, 68.0 mmol) in dichloromethane (125 mL). The mixture was stirred for 2 h at 0 °C, the resulting dark purple mixture was filtered through Celite®, washed with dichloromethane (2 x 10 mL) and concentrated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum-ethyl acetate (1:1), followed by recrystallization from light petroleum–diethyl ether (7:3) gave the *title compound* (2.5 g, 23%) as a brown solid, mp 93–94 °C (from light petroleum–diethyl ether) (lit.[i] mp 81–82 °C): IR (Nujol) 3310, 3180, 1645, 1258, 1142, 1102, 1057, 981, 886, 836, 715 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (1 H, br s, NH), 7.90 (1 H, br s, NH), 4.98, (1 H, s, CH), 3.66 (2 H, q, J = 7.1, C*H*$_2$Me), 3.59 (2 H, q, J = 7.1, C*H*$_2$Me), 1.19 (6 H, app t, J = 7.1, CH_2*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.2 (C), 103.0 (CH), 62.9 (CH_2), 15.1 (Me); MS (APcI) *m/z* (relative intensity) 164 (MH^+, 7%), 118 (100), 90 (14); Anal. Calcd for $C_6H_{13}NO_2S$: C, 44.1; H, 8.0; N, 8.6. Found: C, 44.1; H, 8.0; N, 8.6.

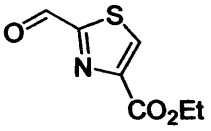


Ethyl 2,2-(diethoxymethyl)thiazole-4-carboxylate (317)

Ethyl bromopyruvate (**246**) (2.2 mL, 15.4 mmol) was added to a mixture of 2,2-diethoxythioacetamide (**316**) (2.3 g, 14.0 mmol) and 4Å molecular sieves (4 g) in ethanol (30 mL) and the mixture heated at reflux for 1 h. The solution was cooled, filtered through Celite® and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum-ethyl acetate (3:1) gave the *title compound* (2.5 g, 68%) as a pale yellow oil: IR (film) 3111, 2980, 2936, 2885, 1732, 1489, 1368, 1313, 1241, 1206, 1093, 1062, 1020, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (1 H, s, 5-H), 5.64 (1 H, s, CH), 4.36 (2 H, q, J = 7.1, C*H*$_2$Me), 3.69 (2 H, q, J = 7.1, OC*H*$_2$Me), 3.58 (2 H, q, J = 7.1, OC*H*$_2$Me), 1.33 (3 H, t, J = 7.1, CH_2*Me*), 1.19 (6 H, app t, J = 7.1, OCH_2*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1 (C), 161.4 (C), 147.1 (C), 128.5 (CH), 98.8 (CH), 62.8 (CH_2),

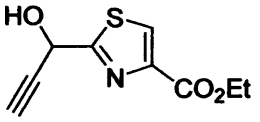
[i] Inami, K.; Shiba, T. *Bull Chem. Soc. Jpn.* **1985**, *58*, 352.

61.5 (CH_2), 15.1 (Me), 14.4 (Me); MS (APcI) *m/z* (relative intensity) 261 (M^+, 67%), 215 (100); HRMS calcd for $C_{11}H_{21}N_2O_4S$ (MNH_4) 277.1222, found 277.1218.



Ethyl 2-formylthiazole-4-carboxylate (318)

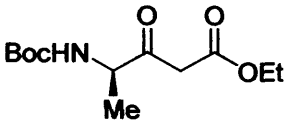
A solution of ethyl 2,2-(diethoxymethyl)thiazole-4-carboxylate (**317**) (2.11 g, 8.14 mmol) was heated at reflux in acetone (175 mL) with aqueous 2 N hydrochloric acid (17 mL) for 1 h, cooled, evaporated *in vacuo* and extracted with chloroform (100 mL). The aqueous layer was further extracted with chloroform (2 x 70 mL) and the combined organic extracts were dried (Na_2SO_4) and partially concentrated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum-ethyl acetate (3:2), gave the *title compound* (1.39 g, 92%) as a pale yellow solid, mp 64.3–65.6 °C (from light petroleum) (lit.[7] mp 67–68 °C): IR (Nujol) 3103, 1723, 1706, 1324, 1214, 1100, 1022, 946, 879, 767 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.01 (1 H, d, J = 1.2, CHO), 8.52 (1 H, d, J = 1.2, 5-H), 4.42 (2 H, q, J = 7.2, CH_2Me), 1.37 (3 H, t, J = 7.2, CH_2*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.7 (CH), 166.1 (C), 160.6 (C), 149.5 (C), 133.1 (CH), 62.1 (CH_2), 14.3 (Me); MS (APcI) *m/z* (relative intensity) 186 (MH^+, 100%), 171 (40), 158 (12), 140 (24); HRMS calcd for $C_7H_8NO_3S$ (MH) 186.0225, found 186.0219; Anal. Calcd for $C_7H_7NO_3S$: C, 45.5; H, 3.8; N, 7.6. Found: C, 45.5; H, 3.8; N, 7.5.



Ethyl 2-(1-hydroxyprop-2-ynyl)thiazole-4-carboxylate (319)

A solution of ethynyl magnesiumbromide in tetrahydrofuran (0.5 M; 14.1 mL, 7.07 mmol) was added dropwise over 5 min to a solution of ethyl 2-formylthiazole-4-carboxylate (**318**) (1.31 g, 7.07 mmol) in tetrahydrofuran (50 mL) at 0 °C and the mixture was stirred for 30 min at this temperature. A further aliquot of ethynyl magnesiumbromide in tetrahydrofuran (0.5 M; 2.8 mL, 1.41 mmol) was added and the solution stirred for a further 20 min. Saturated aqueous ammonium chloride solution (30 mL) was added and the mixture was concentrated *in vacuo* and partitioned between water (30 mL) and chloroform (60 mL). The aqueous layer was further extracted with chloroform (2 x 30 mL) and the combined organic

extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (2:3), gave the *title compound* (1.09 g, 73%) as a brown oil: IR (film) 3290, 3122, 2981, 2121, 1714, 1486, 1371, 1221, 1174, 1100, 1020, 958, 922, 878, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (1 H, s, 5-H), 5.70, (1 H, m, C*H*OH), 5.14, (1 H, bm, OH), 4.30 (2 H, q, J = 7.2, CH_2Me), 2.69 (1 H, d, J = 1.9, CCH), 1.29 (3 H, t, J = 7.2, CH_2*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3 (C), 161.3 (C), 146.6 (C), 128.6 (CH), 81.3 (C), 75.5 (CH), 61.7 (CH_2), 61.3 (CH), 14.3 (Me); MS (EI) *m/z* (relative intensity) 211 (M^+, 20%), 182 (74), 166 (58), 158 (22), 139 (51), 121 (26), 112 (82), 84 (34), 55 (100), 45 (58); HRMS calcd for $C_9H_{10}NO_3S$ (MH) 212.0381, found 212.0380.

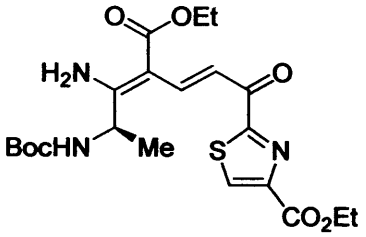


(*R*)-Ethyl 4-(*N-tert*-butoxycarbonyl)amino-3-oxopentanoate (320)[195]

(i) Formation of lithium enolate of ethyl acetate. A solution of *n*-butyllithium in hexanes (2.5 M; 4.00 mL, 10.0 mmol) was added to a stirred solution of diisopropylamine (1.40 mL, 10.0 mmol) in tetrahydrofuran (10 mL) at 0 °C. The mixture was stirred for 10 min and cooled to –78 °C. Ethyl acetate (0.49 mL, 5.0 mmol) was added and the solution was stirred for 30 min. **(ii) Formation of ethyl 4-amino-3-oxopentanoate.** Ethyl chloroformate (0.52 mL, 5.5 mmol) was added dropwise to a stirred solution of *N-tert*-butoxycarbonyl-D-alanine (946 mg, 5.0 mmol) and triethylamine (0.77 mL, 5.5 mmol) in dry tetrahydrofuran (10 mL) at –10 °C. After stirring for 30 minutes at this temperature, the mixture was filtered and added dropwise to the above solution of the lithium enolate of ethyl acetate at –78 °C. The mixture was stirred at –78 °C for 20 min and saturated aqueous ammonium chloride solution (60 mL) was added. The resulting mixture was warmed to room temperature. After extraction with ethyl acetate (60 mL), the organic extract was washed with brine (40 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (2:1), gave the *title compound*[i] (751 mg, 58%) as a colourless oil: $[\alpha]_D^{25}$ +7.6 (*c* 1.00, $CHCl_3$); IR (KBr) 3347, 2976, 1725, 1514, 1453, 1368,

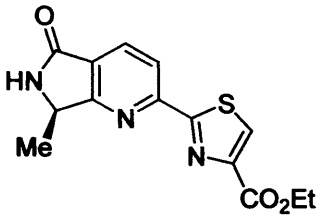
[i] HPLC: ee >99%; ChiralPak AD, hexane–2-propanol (99:1), λ_{max} = 244 nm, 1.0 mL min^{-1}, R_T = 30.3 min (*S*-isomer has R_T = 34.5 min).

1248, 1167, 1027, 946, 862, 786 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.14 (1 H, d, *J* = 6.4, NH), 4.30 (1 H, m, C*H*Me), 4.13 (2 H, q, *J* = 7.2, OC*H*$_2$Me), 3.53 (1 H, d, *J* = 16.0, C*H*H), 3.47 (1 H, d, *J* = 16.0, CH*H*), 1.38 (9 H, s, CMe_3), 1.29 (3 H, d, *J* = 7.2, CH*Me*), 1.21 (3 H, t, *J* = 7.2, OCH$_2$*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.5 (C), 166.9 (C), 155.1 (C), 80.1 (C), 61.5 (CH_2), 55.4 (CH), 45.9 (CH_2), 28.3 (Me), 17.1 (Me), 14.1 (Me); MS (APcI) *m/z* (relative intensity) 260 (MH^+, 4%), 204 (96), 160 (100); HRMS calcd for $C_{12}H_{22}NO_5$ (MH) 260.1492, found 260.1490.



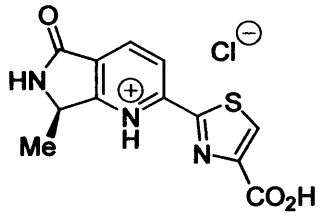
(*R*,*4E*)-Ethyl 2-[5-amino-6-(*N*-*tert*-butoxycarbonyl)amino-4-ethoxycarbonyl-hepta-2,4-dienoyl]-thiazole-4-carboxylate (321)

To a solution of ethyl 2-(propynoyl)thiazole-4-carboxylate (**114**) (29 mg, 0.14 mmol) in ethanol (5 mL) was added (*R*)-ethyl 3-amino-4-(*N*-*tert*-butoxycarbonyl)aminopent-2-enoate (**312**) (41 mg, 0.16 mmol). The reaction mixture was stirred at 50 °C for 10 min and concentrated *in vacuo*. Purification by flash chromatography on silica, eluting with light petroleum–ethyl acetate (1:1), gave the *title compound* (53 mg, 82%) as a yellow solid, mp 176–178 °C (from methanol): $[\alpha]_D^{25}$ –153.3 (*c* 1.25, $CHCl_3$); IR (KBr) 3424, 2976, 1720, 1654, 1559, 1509, 1458, 1368, 1328, 1278, 1222, 1022 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 9.77 (1 H, br s, N*H*H), 8.82 (1 H, s, SCH), 8.25 (1 H, d, *J* = 15.0, 3-H), 8.18 (1 H, br s, NH*H*), 7.59 (1 H, d, *J* = 15.0, 2-H), 7.38 (1 H, d, *J* = 5.6, N*H*Boc), 4.96 (1 H, m, C*H*Me), 4.34 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 4.25 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 1.40 (3 H, t, *J* = 7.1, OCH$_2$*Me*), 1.39 (9 H, s, CMe_3), 1.33 (3 H, t, *J* = 7.1, OCH$_2$*Me*), 1.33 (3 H, d, *J* = 6.7, CH*Me*); ^{13}C NMR (100 MHz, d_6-DMSO) δ 177.6 (C), 172.0 (C), 167.9 (C), 166.4 (C), 158.3 (C), 152.4 (C), 144.9 (C), 139.6 (CH), 131.8 (CH), 109.6 (CH), 90.3 (C), 76.6 (C), 58.7 (CH_2), 57.5 (CH_2), 44.9 (CH), 25.8 (Me), 17.5 (Me), 11.8 (Me), 11.7 (Me); MS (APcI) *m/z* (relative intensity) 468 (MH^+, 100%); HRMS calcd for $C_{21}H_{30}N_3O_7S$ (MH) 468.1799, found 468.1796.



(*R*)-2-[4-(Ethoxycarbonyl)thiazol-2-yl]-7-methyl-5-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridine (323)

A solution of (*R*)-ethyl 6-[4-(ethoxycarbonyl)thiazol-2-yl]-2-[1-(*N*-*tert*-butoxycarbonyl)aminoethyl]pyridine-3-carboxylate (**311**) (93 mg, 0.21 mmol) in 6 N aqueous hydrochloric acid (10 mL) was stirred at room temperature for 6 h. After neutralising with 5 N aqueous sodium hydroxide to pH 7, the mixture extracted with dichloromethane (3 x 20 mL). The organic layers were combined, dried (Na_2SO_4) and evaporated *in vacuo*. Purification by flash chromatography on silica, eluting with ethyl acetate, gave the *title compound*[i] (56 mg, 89%) as a colourless solid, mp 236–238 °C (from methanol): $[\alpha]_D^{24}$ +48.1 (*c* 0.54, $CHCl_3$); IR (KBr) 3200, 2926, 1700, 1600, 1412, 1247, 1098, 1022, 805 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 9.14 (1 H, s, NH), 8.79 (1 H, s, SCH), 8.33 (1 H, d, *J* = 8.0, PyH), 8.30 (1 H, d, *J* = 8.0, PyH), 4.83 (1 H, q, *J* = 6.8, C*H*Me), 4.42 (2 H, q, *J* = 7.1, OC*H*$_2$Me), 1.52 (3 H, d, *J* = 6.8, CH*Me*), 1.41 (3 H, t, *J* = 7.1, OCH$_2$*Me*); ^{13}C NMR (100 MHz, d_6-DMSO) δ 168.9 (C), 168.5 (C), 166.8 (C), 161.0 (C), 152.5 (C), 148.0 (C), 133.9 (CH), 132.6 (CH), 127.3 (C), 119.7 (CH), 61.5 (CH_2), 53.5 (CH), 19.0 (Me), 14.7 (Me); MS (APcI) *m/z* (relative intensity) 304 (MH^+, 100%); HRMS calcd for $C_{14}H_{14}N_3O_3S$ (MH) 304.0750, found 304.0750.



(*R*)-2-(4-Carboxythiazol-2-yl)-7-methyl-5-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridinium chloride (324)

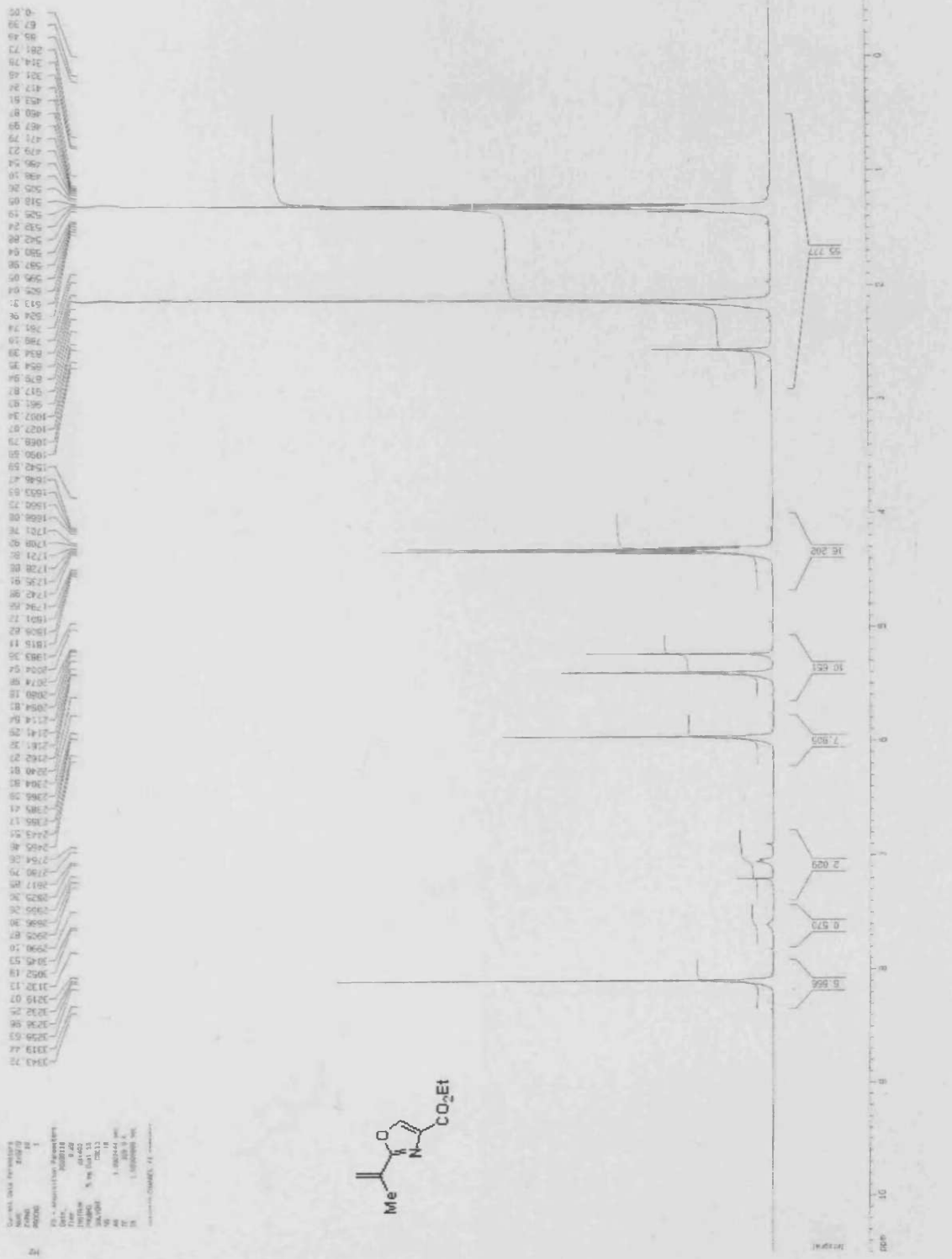
[i] HPLC: ee = 94%; ChiralPak OD, hexane—2-propanol (90:10), λ_{max} = 314 nm, 1.0 mL min^{-1}, R_T = 52.6 min (*S*-isomer has R_T = 34.8 min).

Lithium hydroxide monohydrate (25 mg, 0.60 mmol) was added to a solution of (*R*)-2-[4-(ethoxycarbonyl)thiazol-2-yl]-7-methyl-5-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyridine (**323**) (92 mg, 0.30 mmol) in tetrahydrofuran–water (1:1) (30 mL) at room temperature. The reaction solution was stirred for 3 h, evaporated *in vacuo*, and partitioned between water (30 mL) and dichloromethane (30 mL). The aqueous layer was separated, acidified to pH 6 with 3 N aqueous hydrochloric acid and concentrated *in vacuo*. The precipitate was filtered, washed sequentially with water (2 mL) and acetone (5 mL), and dried in a vacuum dessicator for 24 h to give the *title compound* (72 mg, 77%) as a colourless solid that was used without further purification, mp 287 °C dec (from aqueous methanol): $[\alpha]_D^{30}$ +30.8 (*c* 0.26, MeOH); UV λ_{max} (methanol) 234 nm (ε 12400), 314 nm (ε 17000); IR (KBr) 3234, 2925, 2517, 1890, 1716, 1648, 1601, 1412, 1364, 1221, 1099, 1013, 744, 725 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 9.14 (1 H, s, NH), 8.69 (1 H, s, SCH), 8.34 (1 H, d, *J* = 8.0, PyH), 8.31 (1 H, d, *J* = 8.0, PyH), 4.84 (1 H, q, *J* = 6.8, C*H*Me), 1.52 (3 H, d, *J* = 6.8, CH*Me*); ^{13}C NMR (100 MHz, d_6-DMSO) δ 168.4 (C), 167.6 (C), 166.3 (C), 161.9 (C), 152.2 (C), 148.7 (C), 133.3 (CH), 131.6 (CH), 126.7 (C), 119.2 (CH), 53.0 (CH), 18.5 (Me); MS (ES) *m/z* (relative intensity) 298 (M – HCl + Na^+, 100%), 276 (M – HCl + H^+, 53); HRMS calcd for $C_{12}H_{10}N_3O_3S$ (M – HCl + H) 276.0437, found 276.0441.

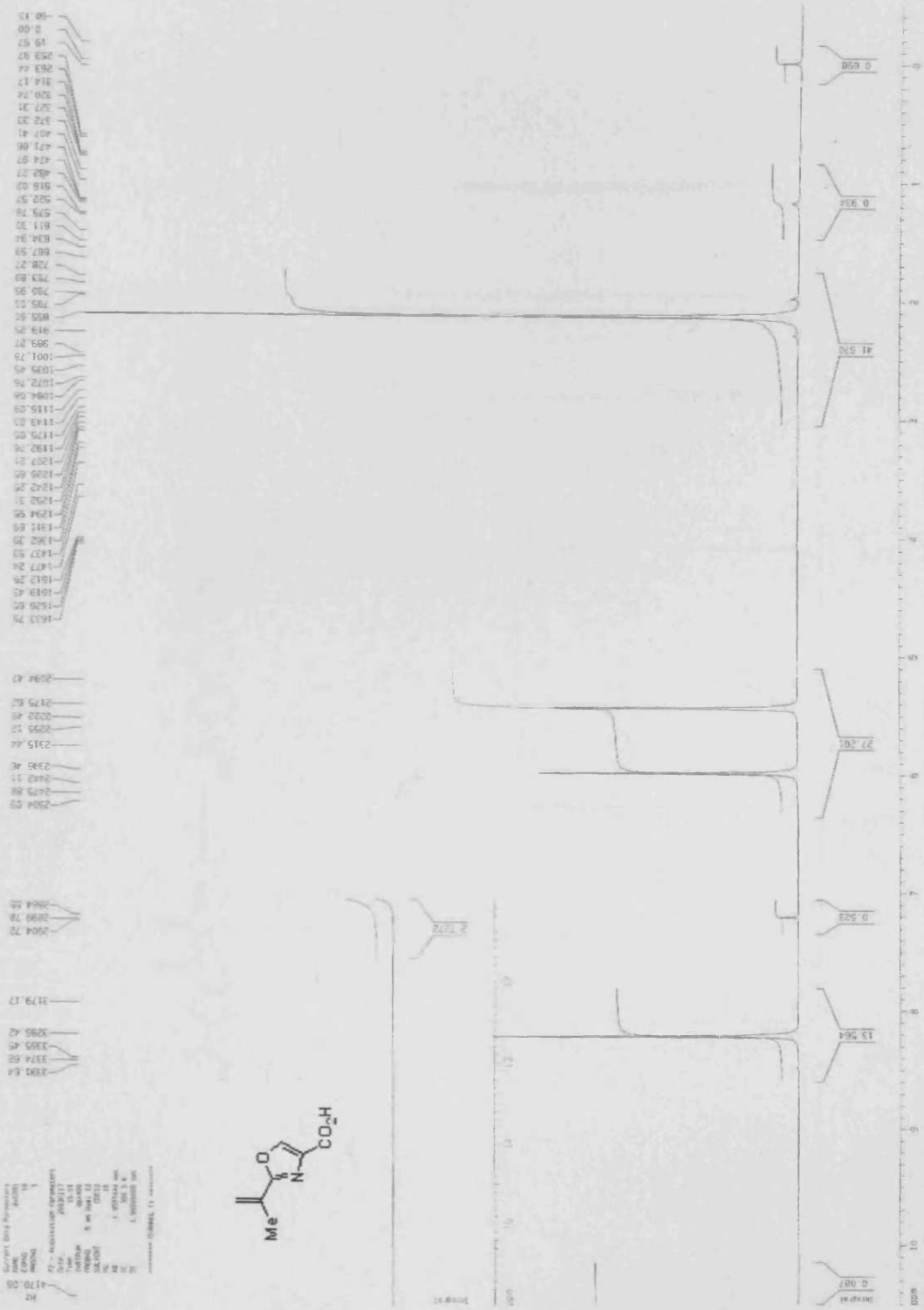
Chapter Five

Appendix

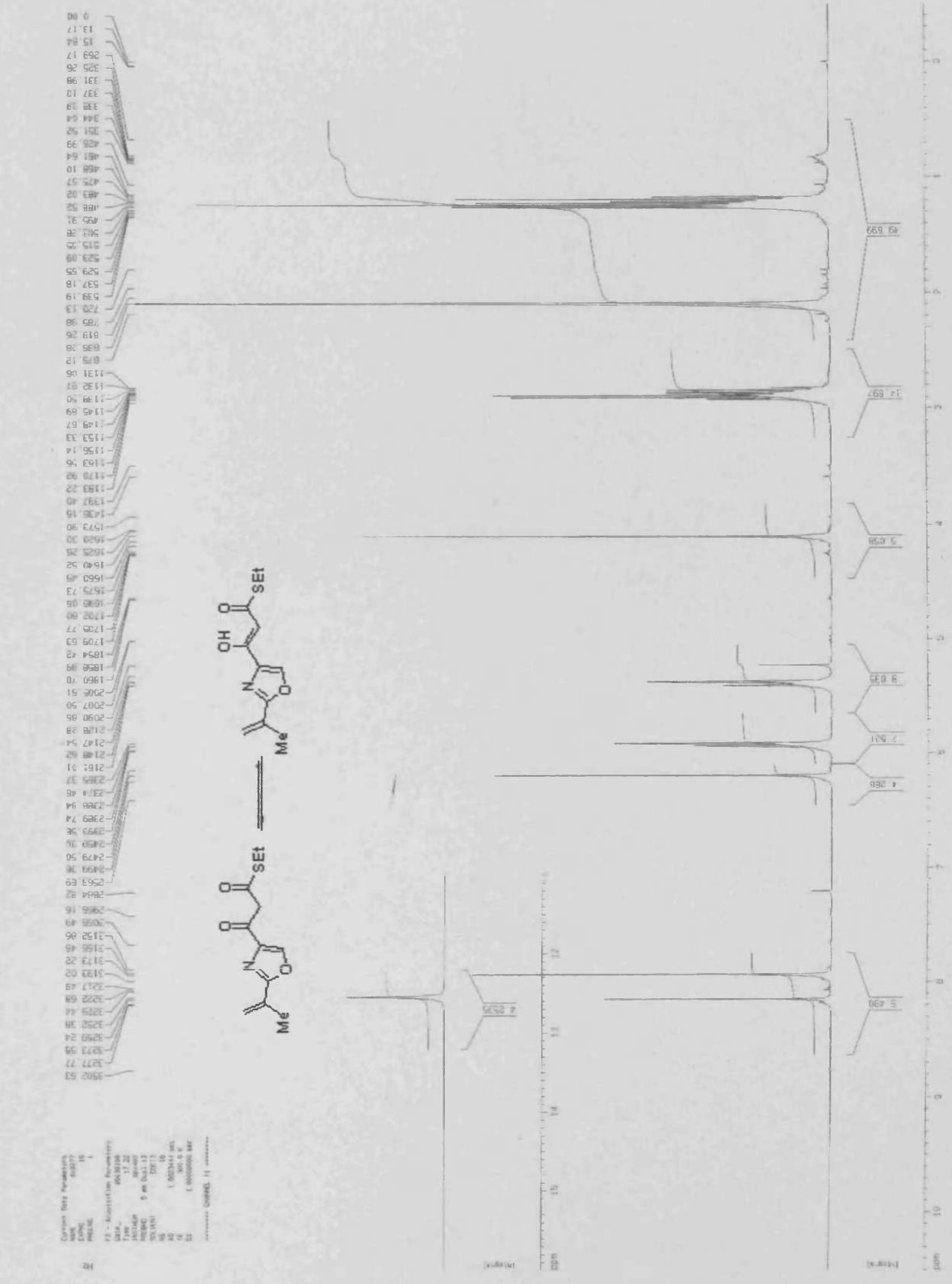
Appendix 1 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 248



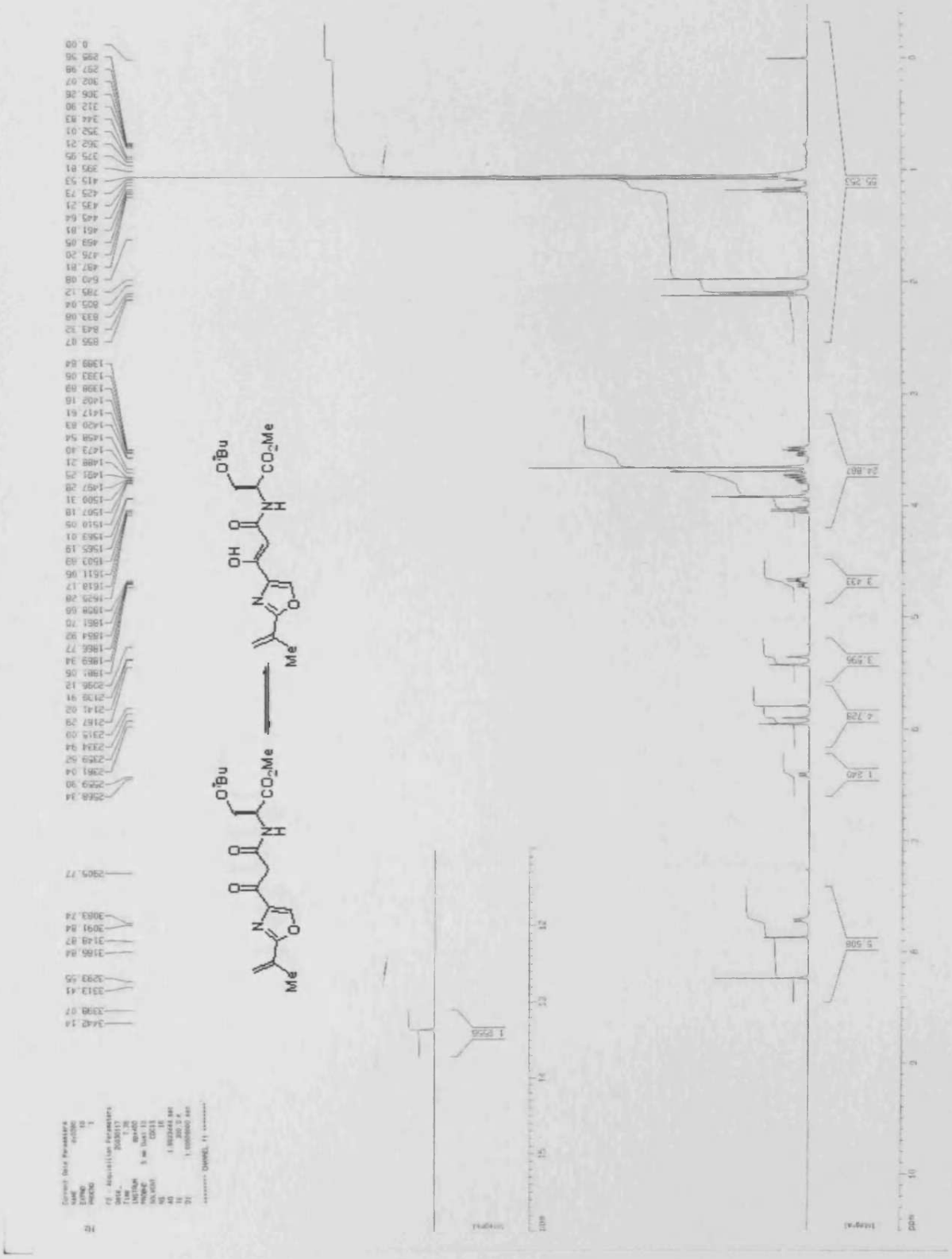
Appendix 2 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 249



Appendix 3 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 250

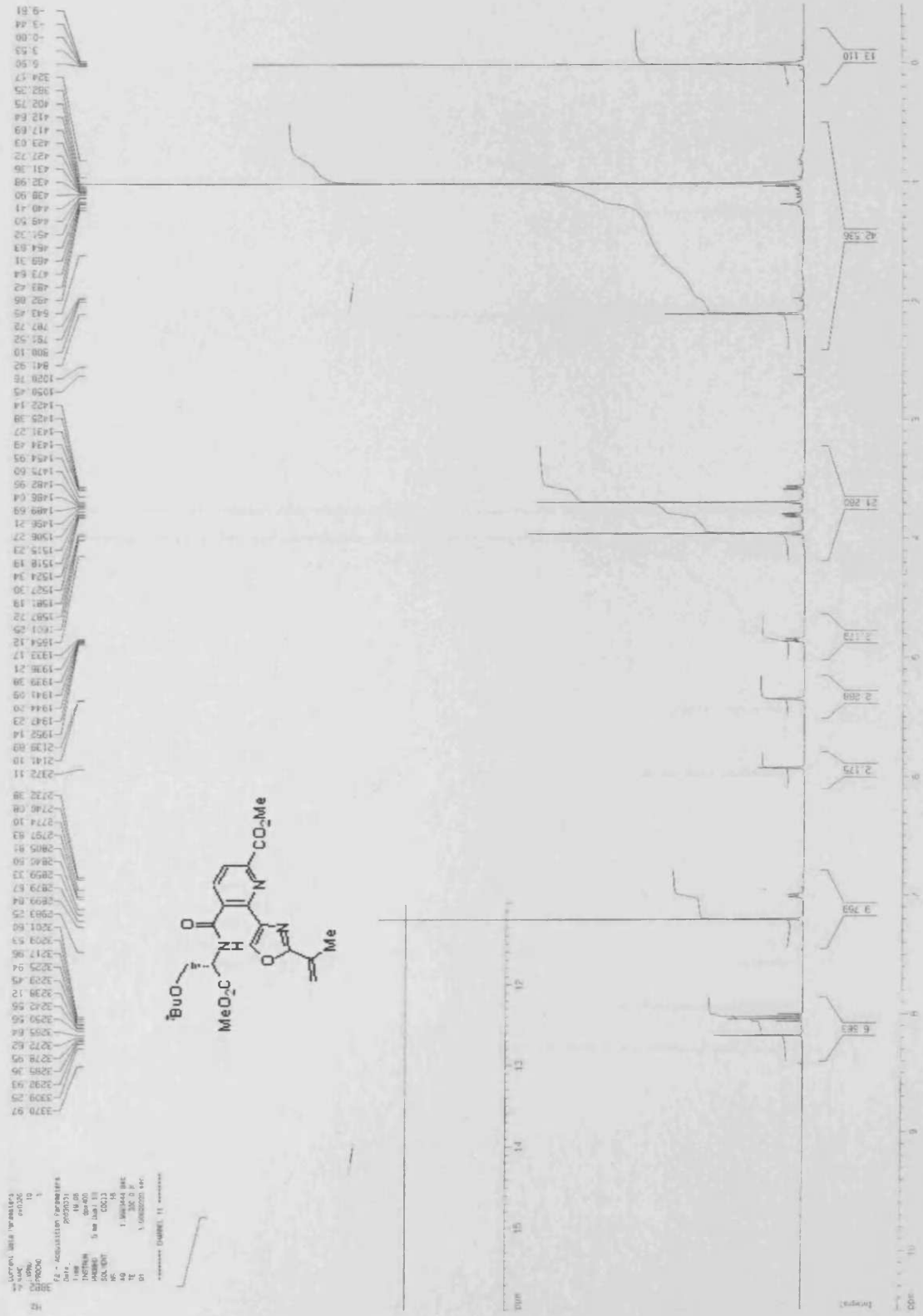


Appendix 4 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 251



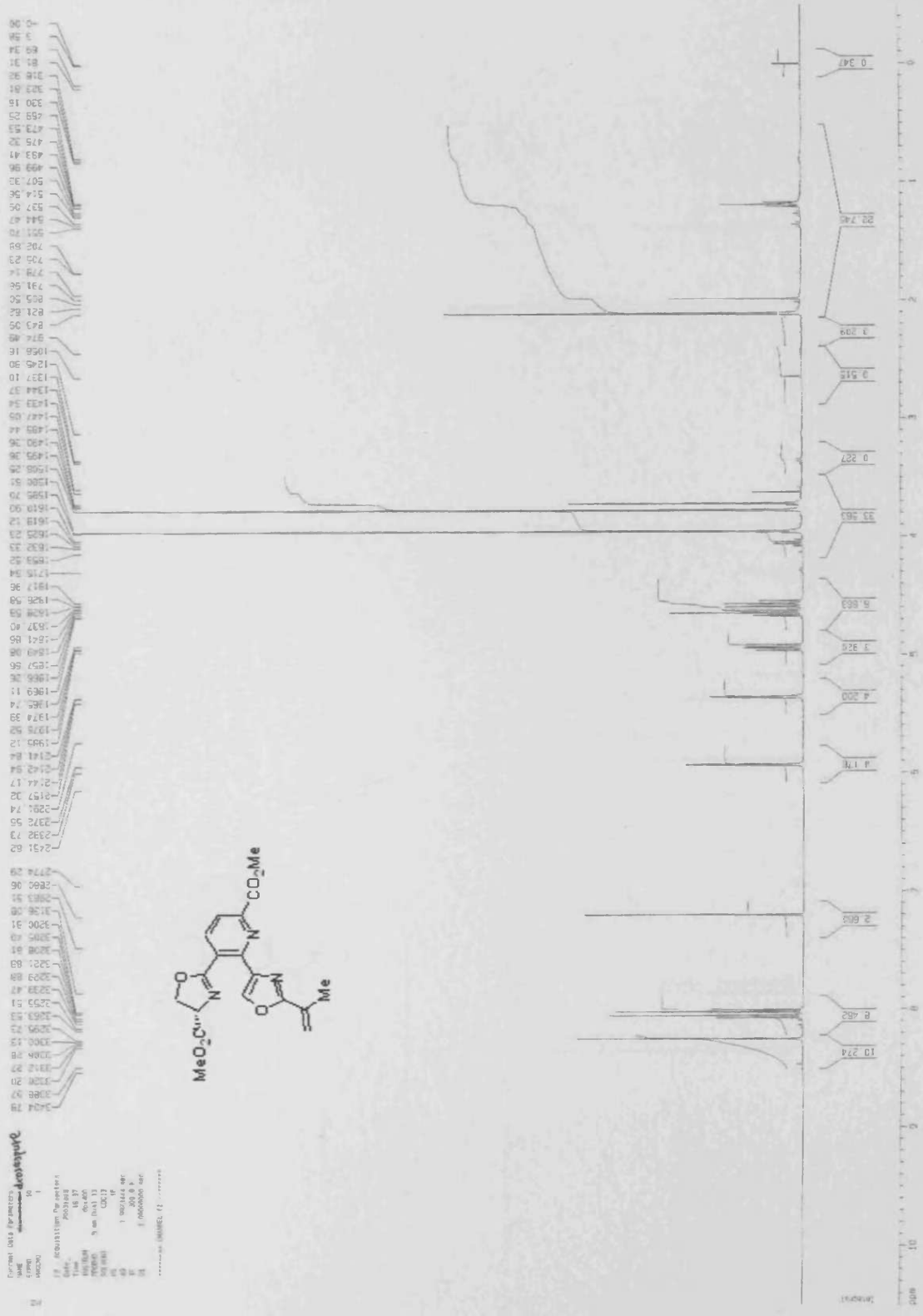
Appendix 5 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 252

Appendix 6 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 253

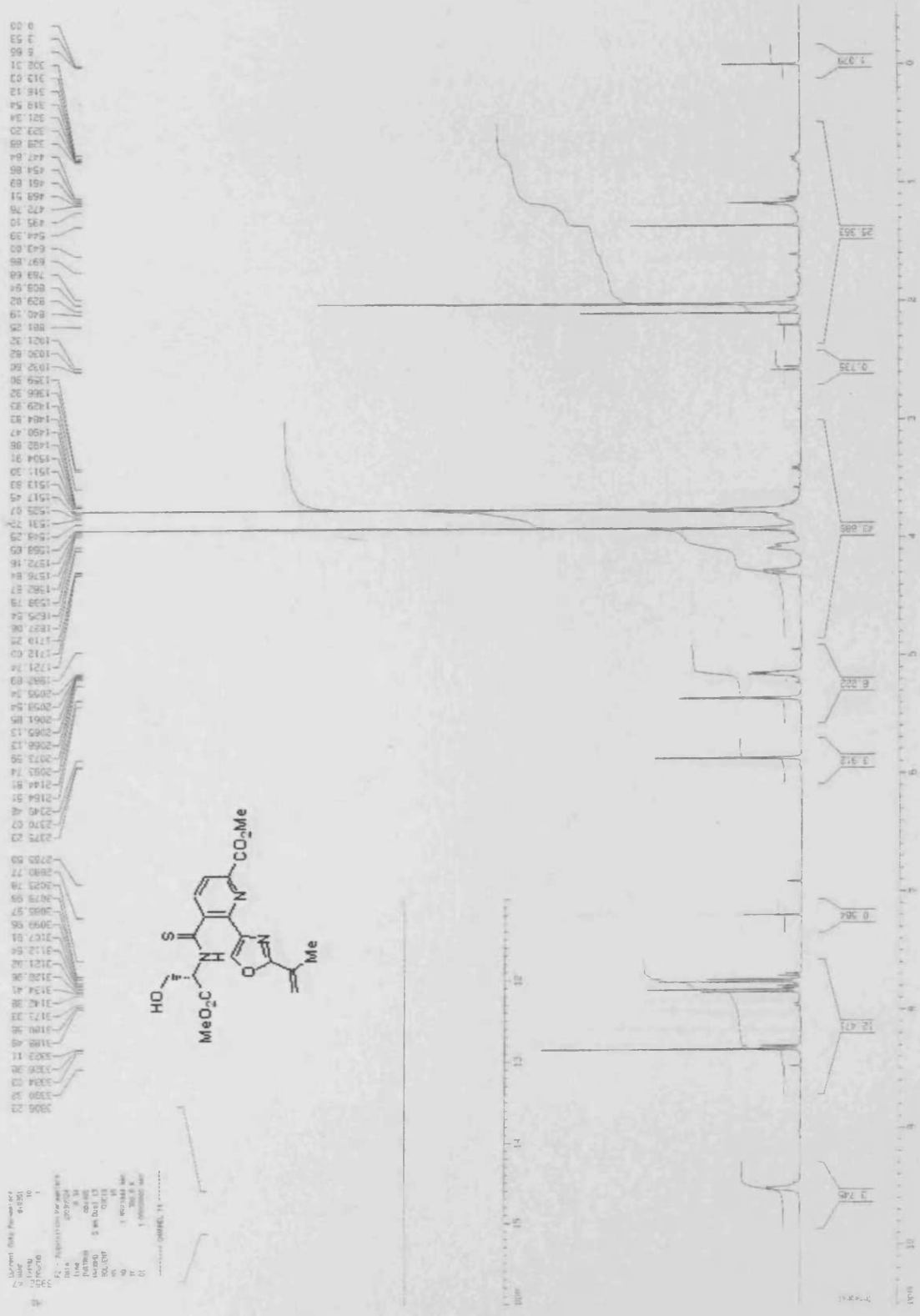


Appendix 7 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 254

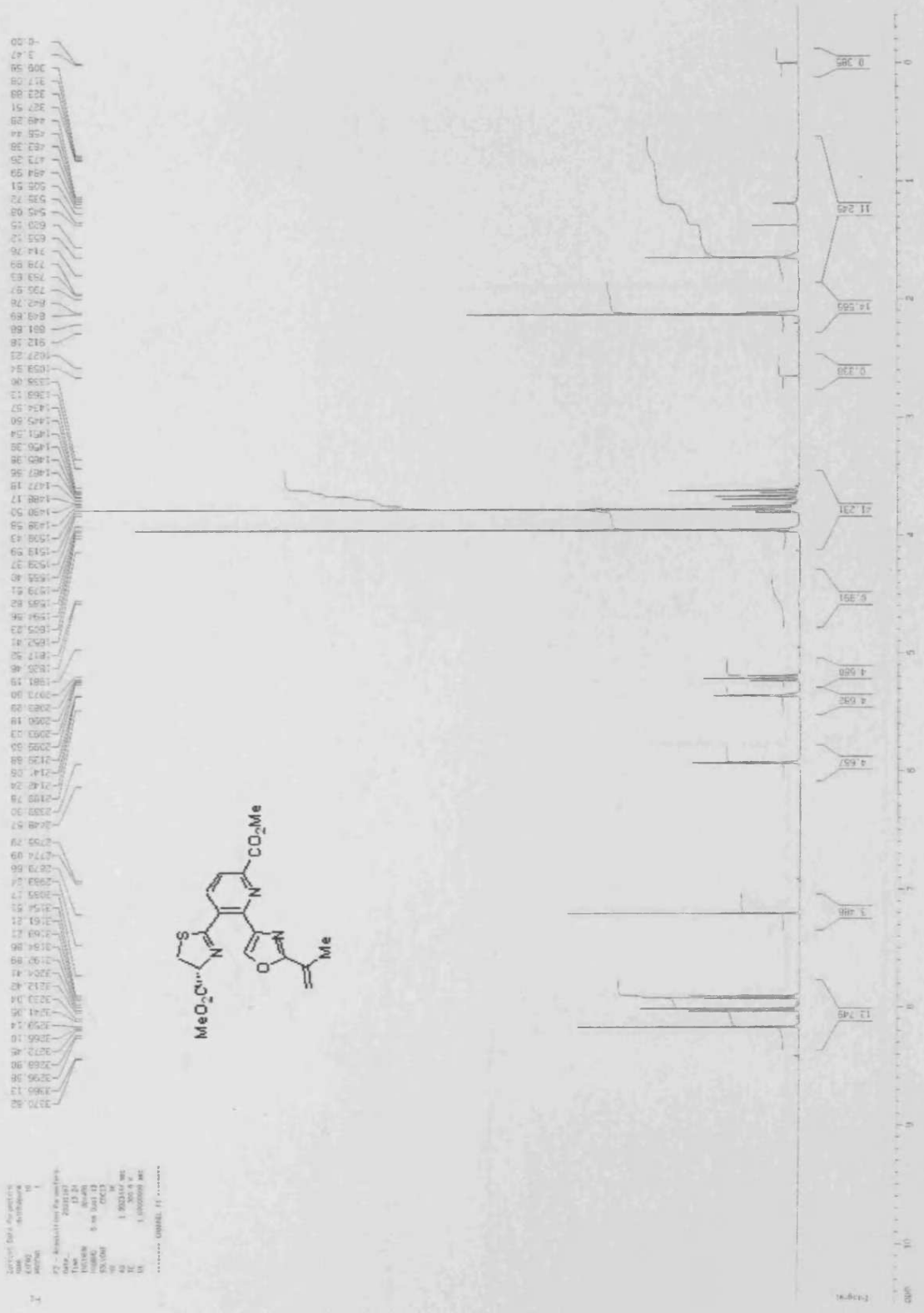
Appendix 8 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 255



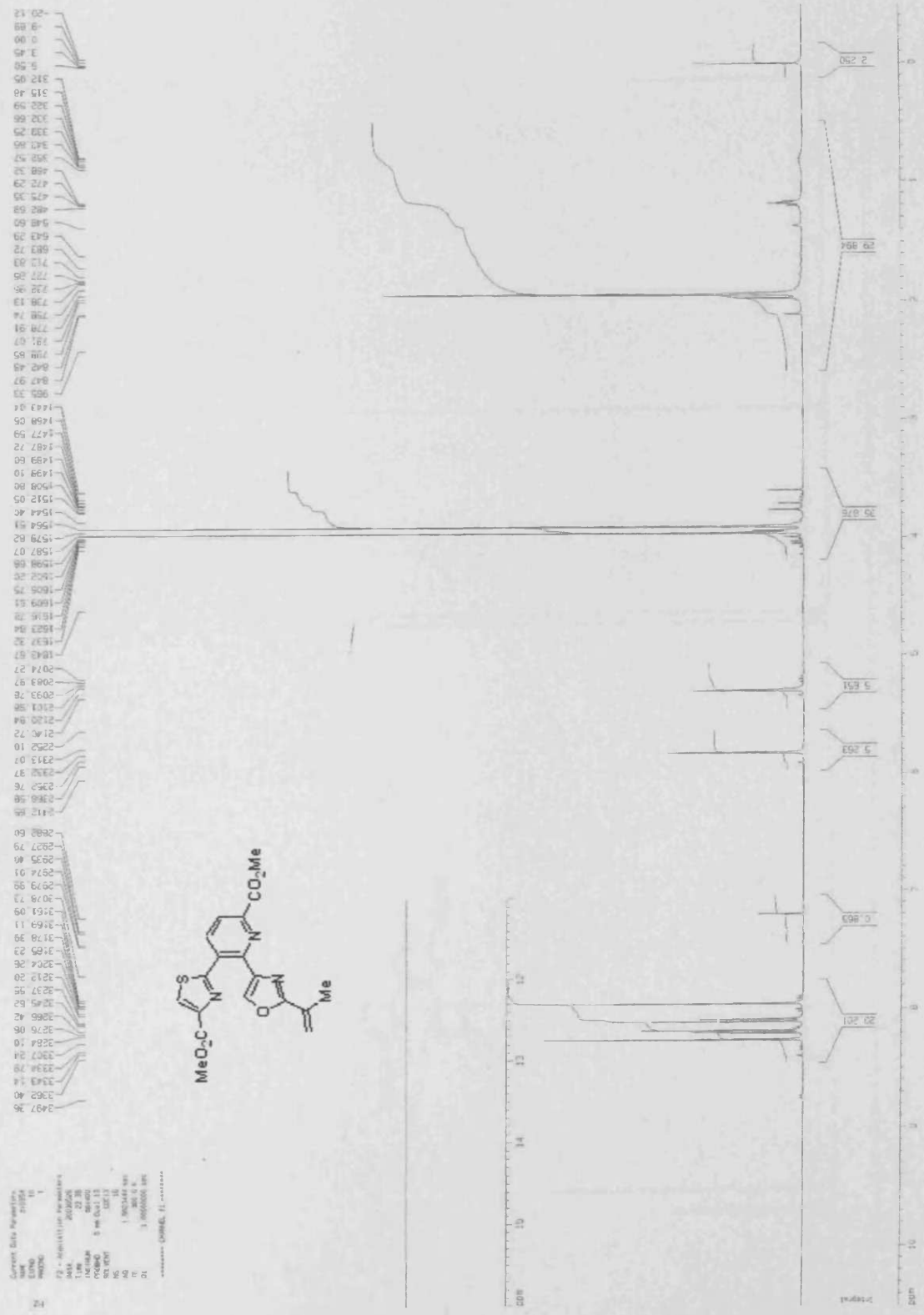
Appendix 9 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 256



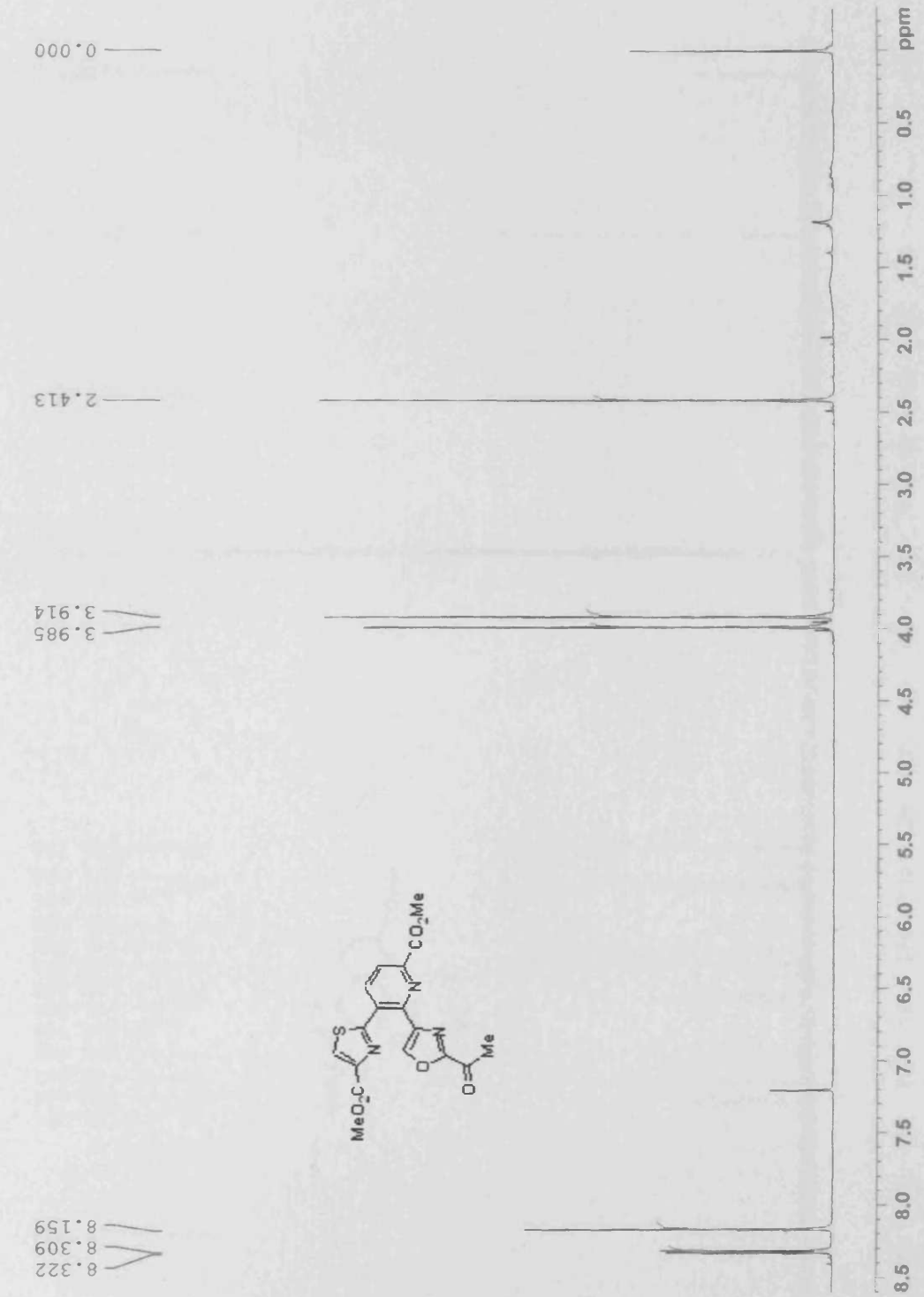
Appendix 10 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 257



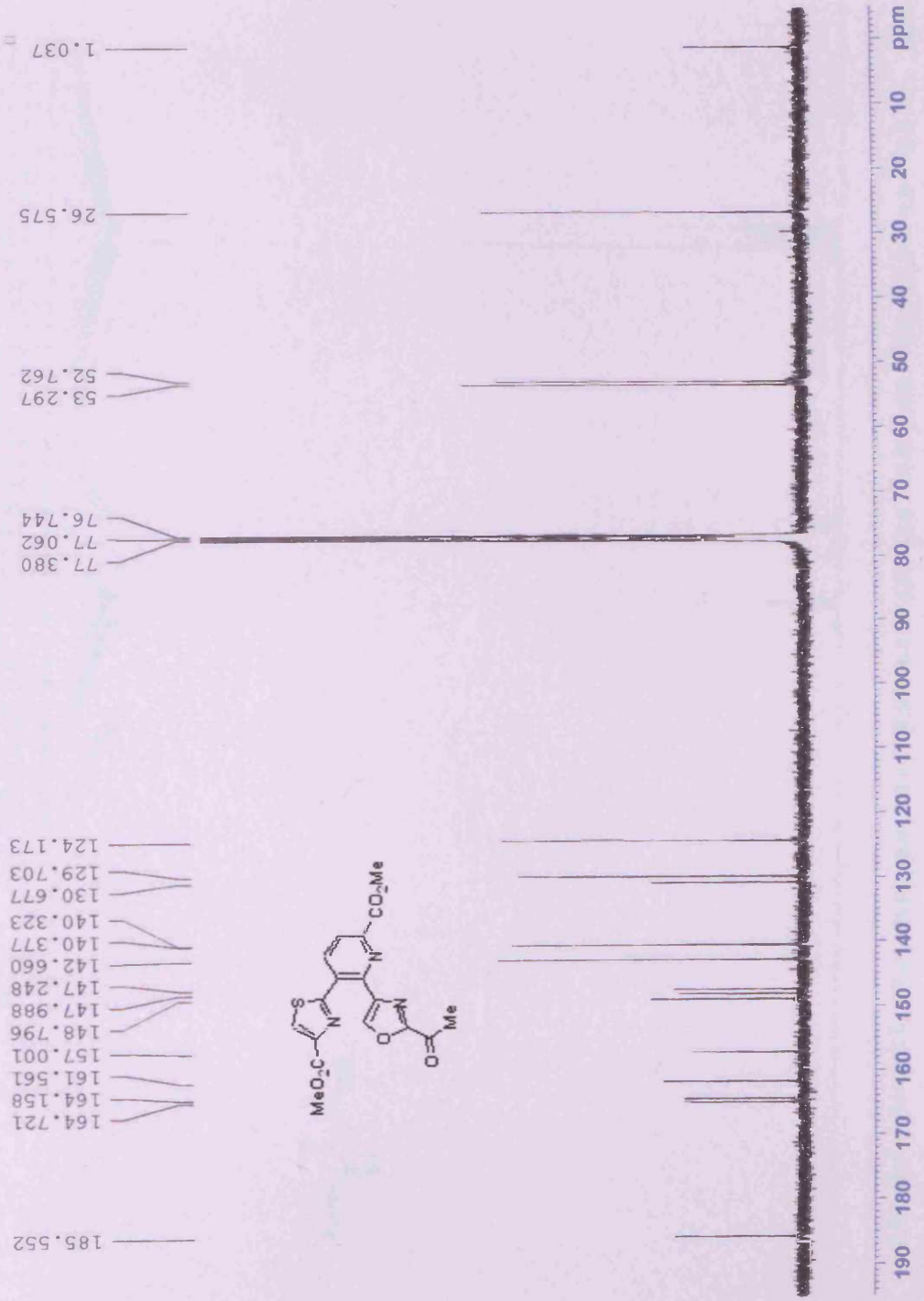
Appendix 11 ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 258



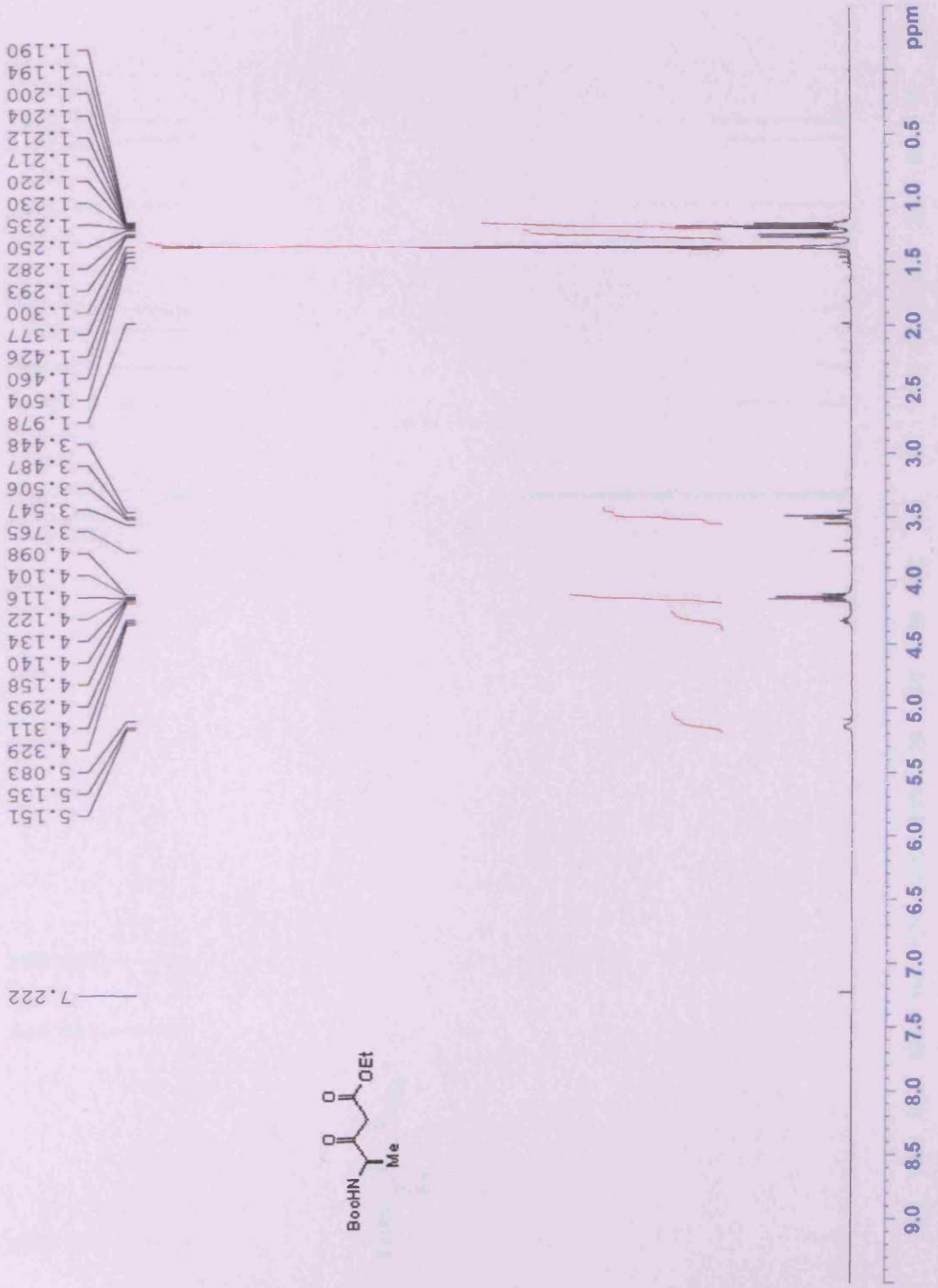
Appendix 12a ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of dimethyl sulfomycinamate (13)



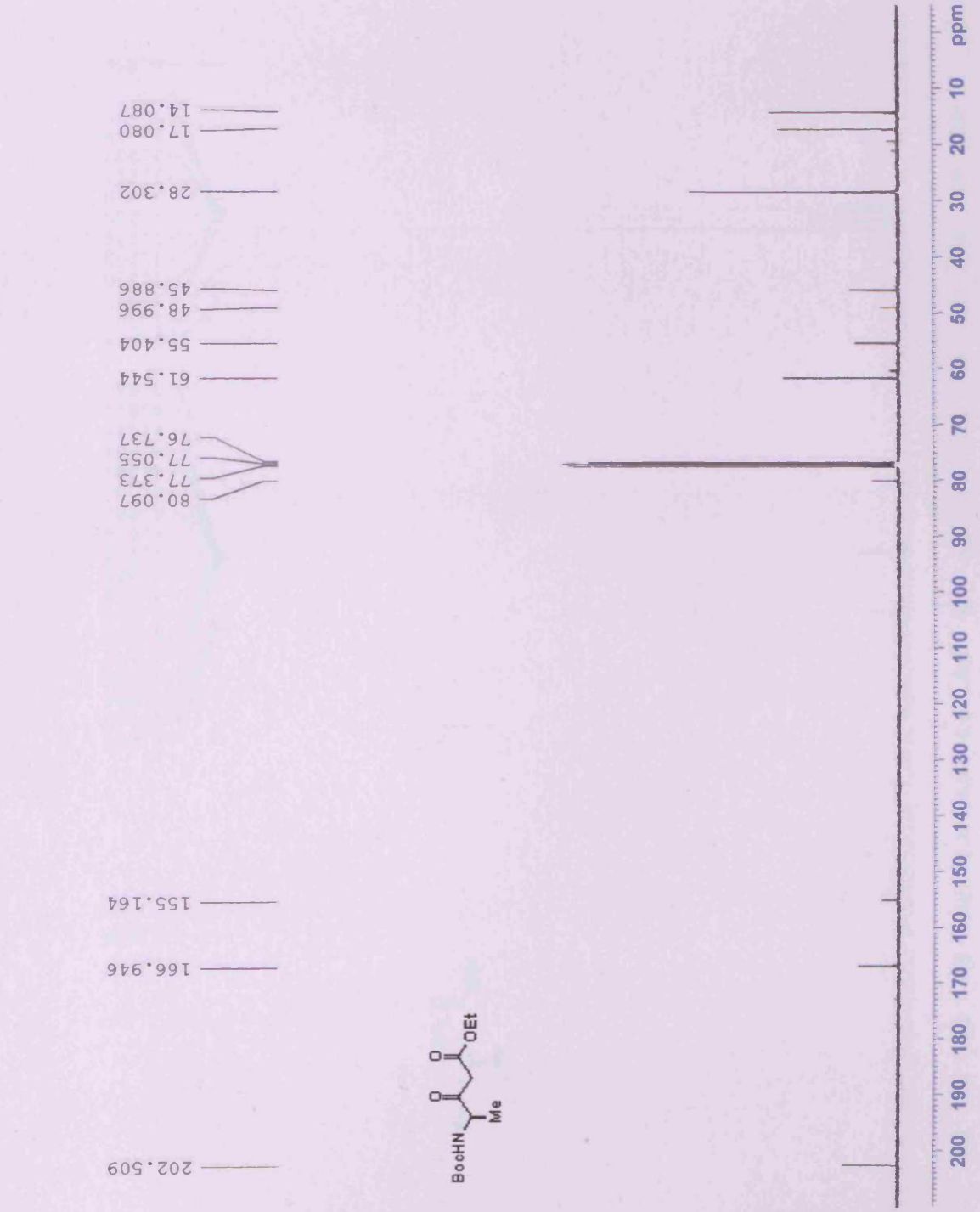
Appendix 12b ^{13}C NMR spectrum ($CDCl_3$, 100MHz) of dimethyl sulfomycinamate (13)



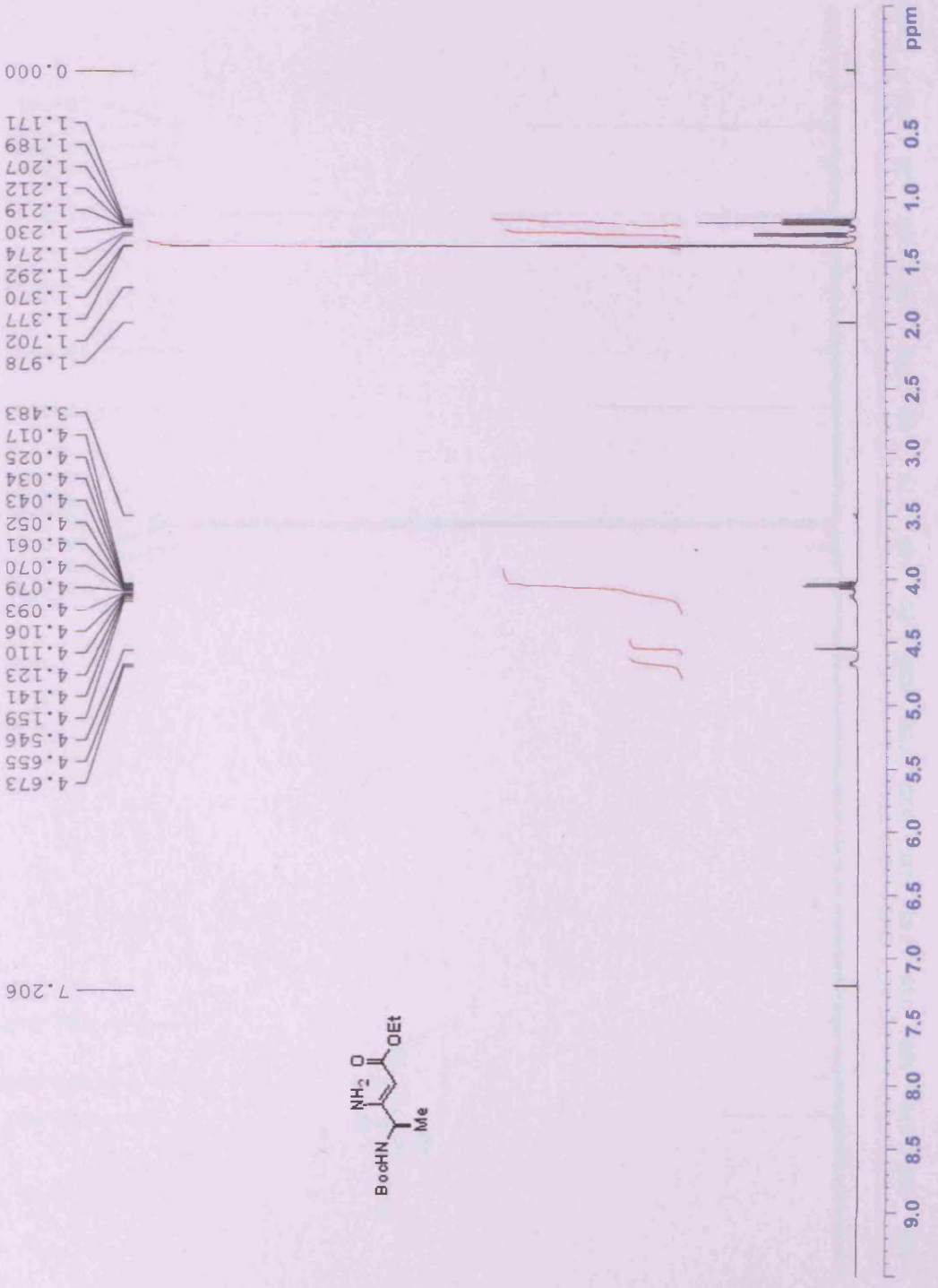
Appendix 13a ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 320



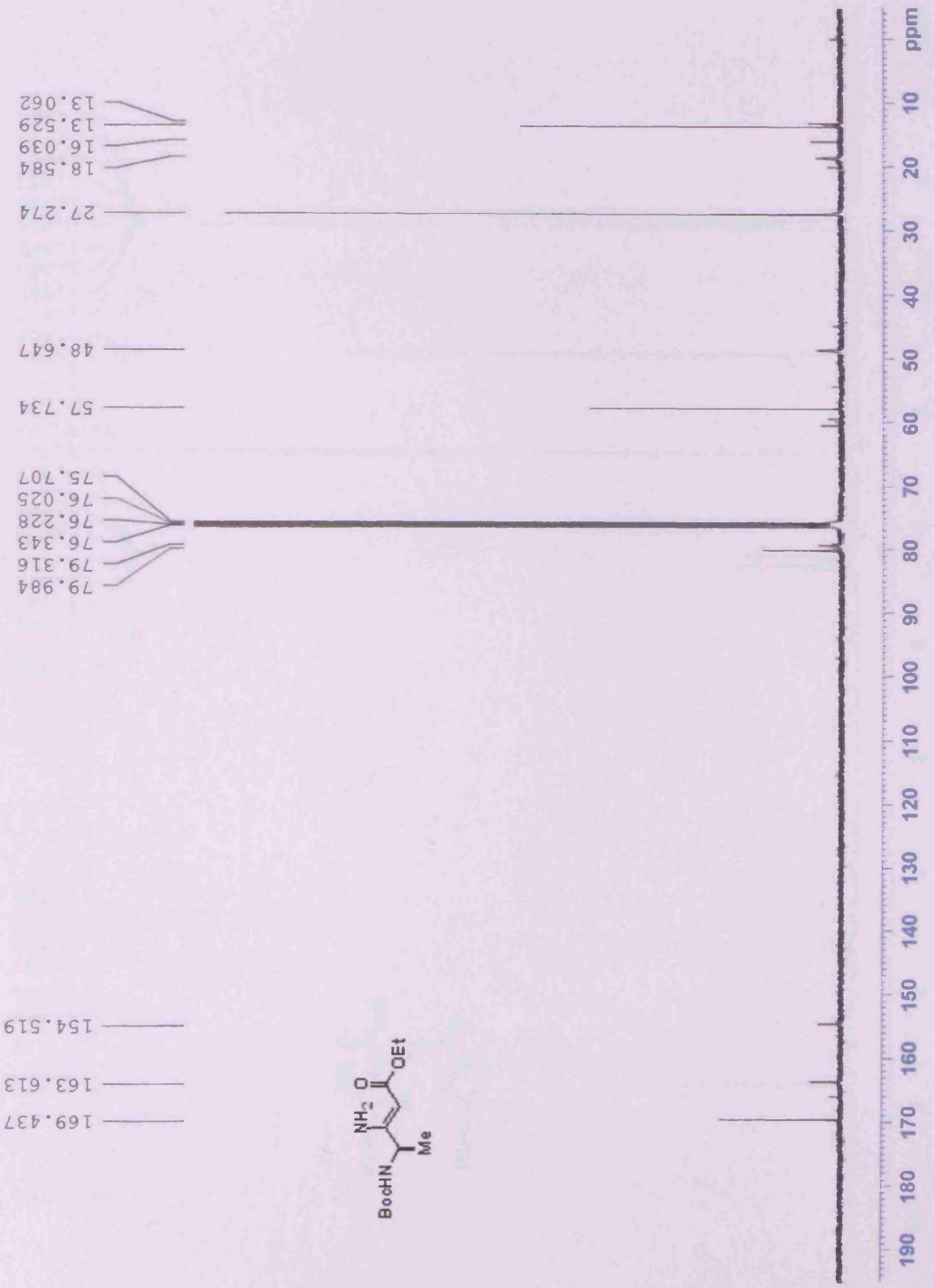
Appendix 13b ^{13}C NMR spectrum ($CDCl_3$, 100MHz) of compound 320



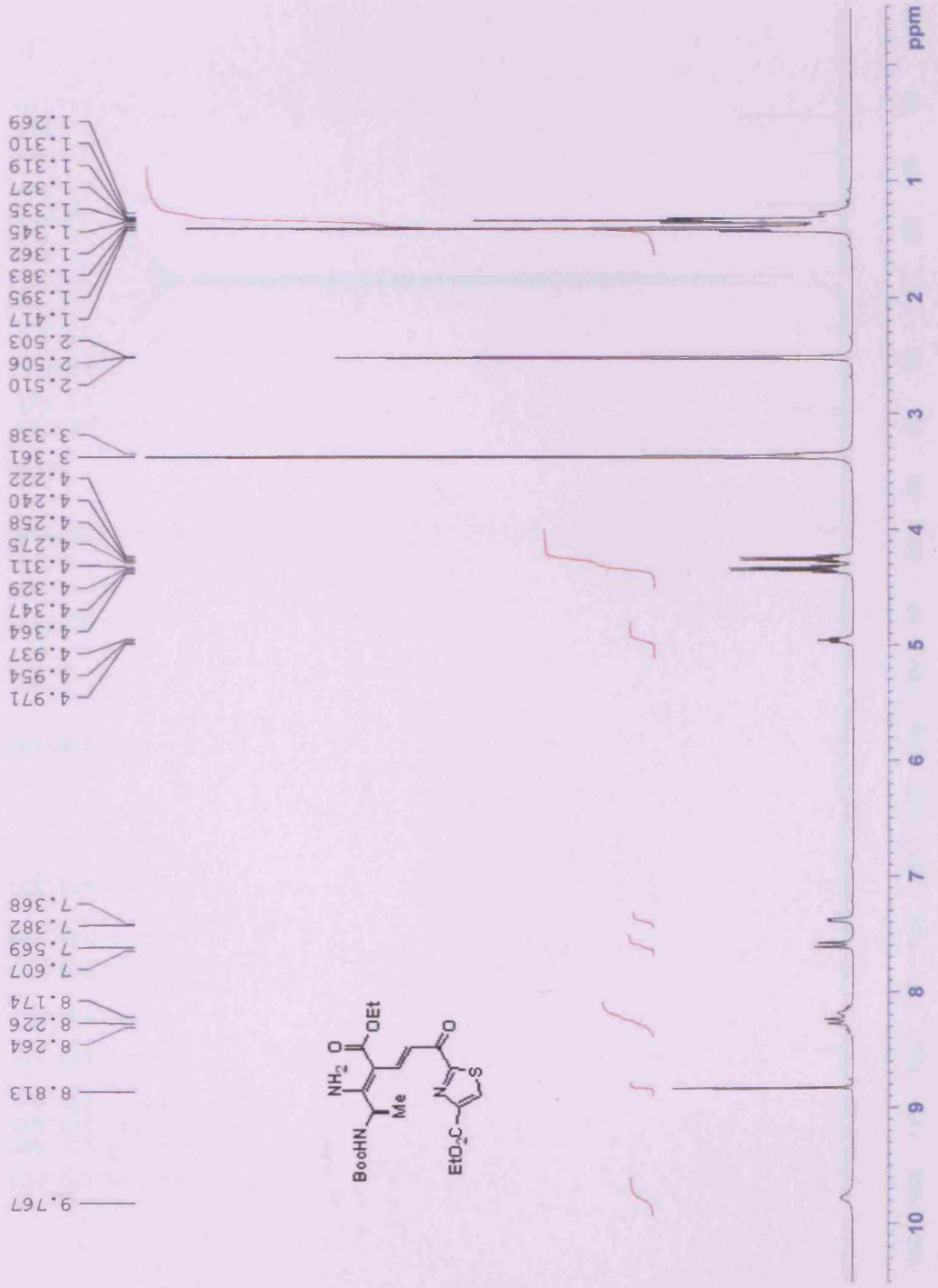
Appendix 14a ^{1}H NMR spectrum ($CDCl_3$, 400MHz) of compound 312



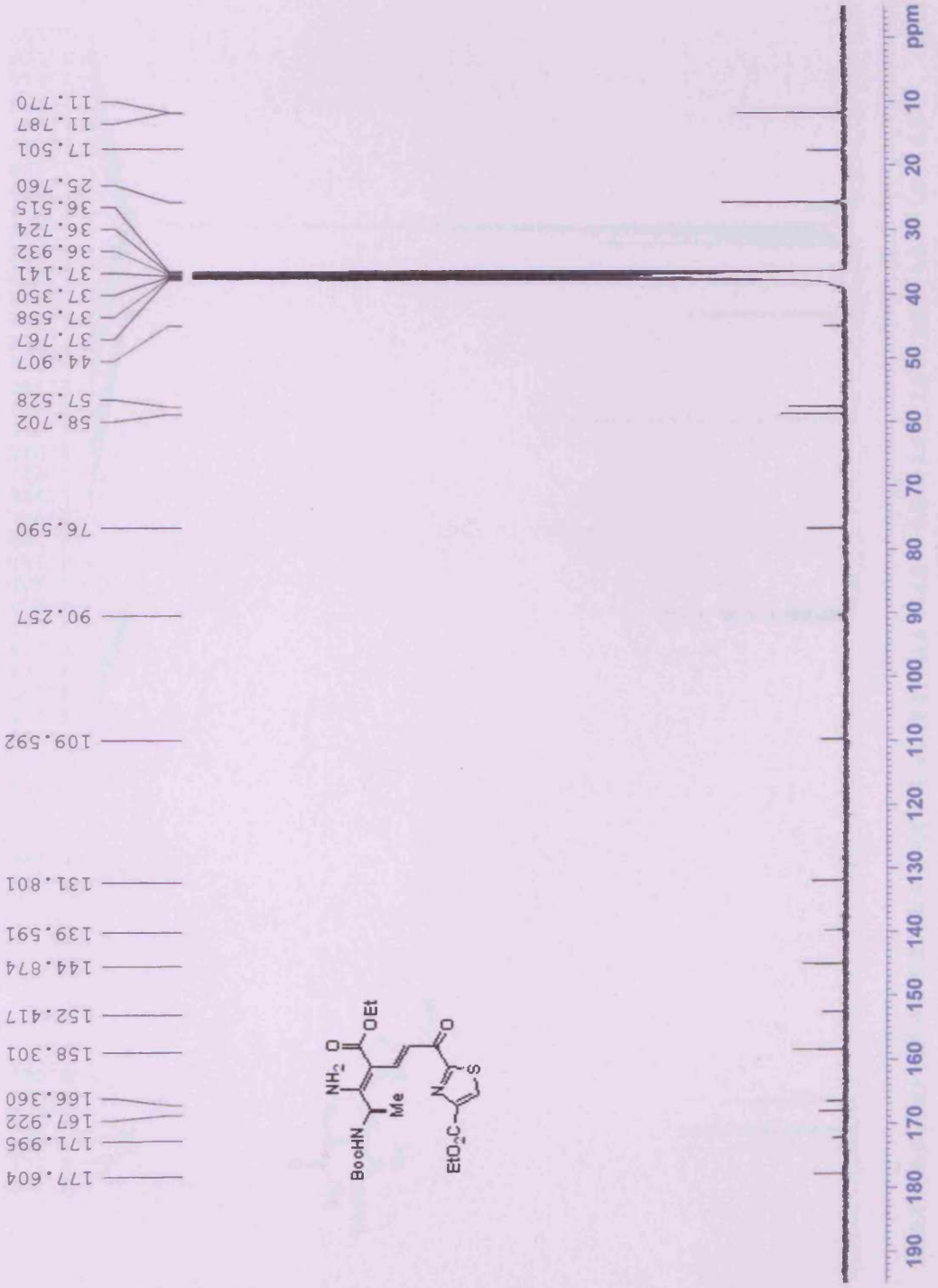
Appendix 14b ^{13}C NMR spectrum ($CDCl_3$, 100MHz) of compound 312



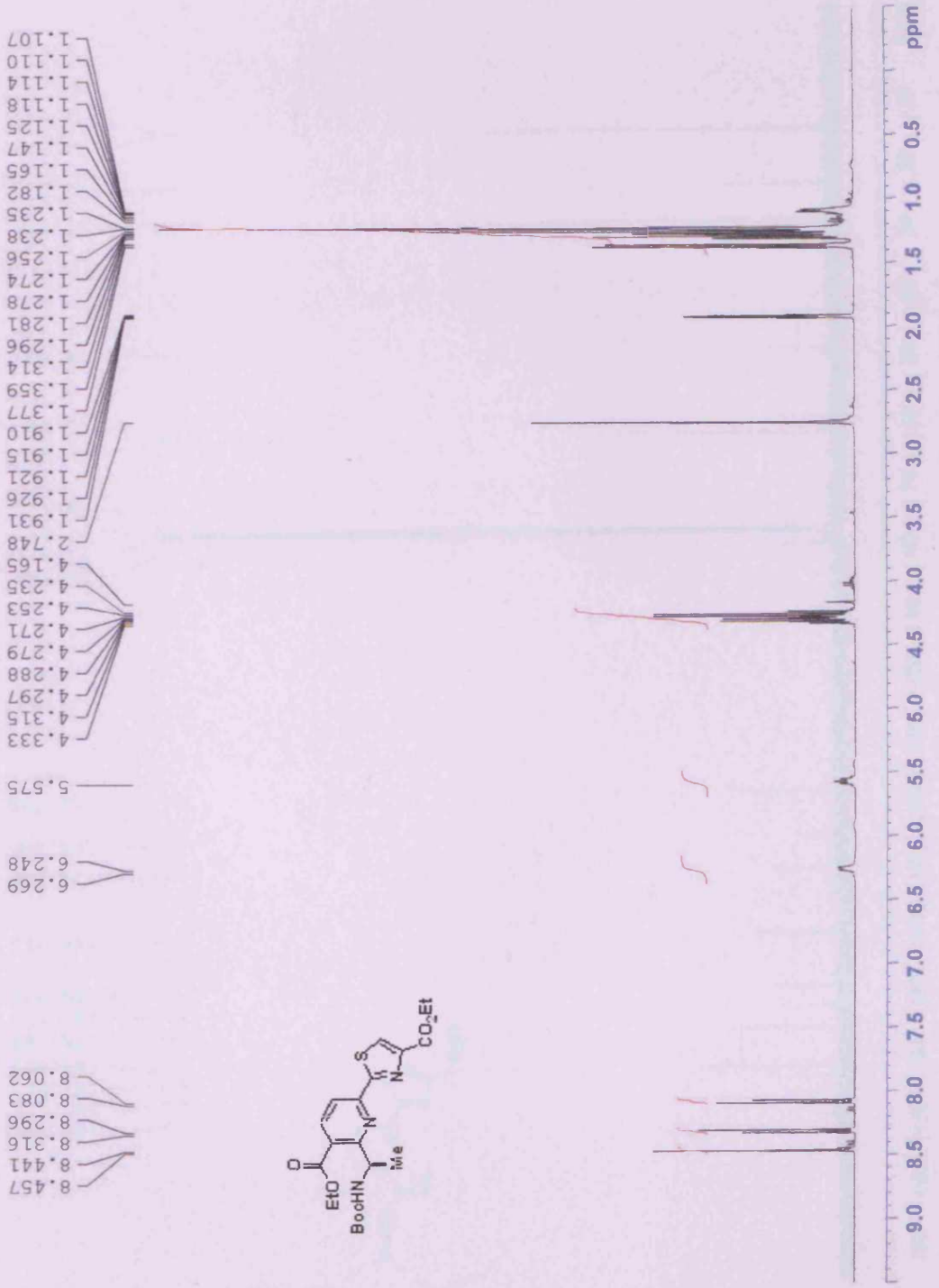
Appendix 15a ^{1}H NMR spectrum (d_6-DMSO, 400MHz) of compound 321



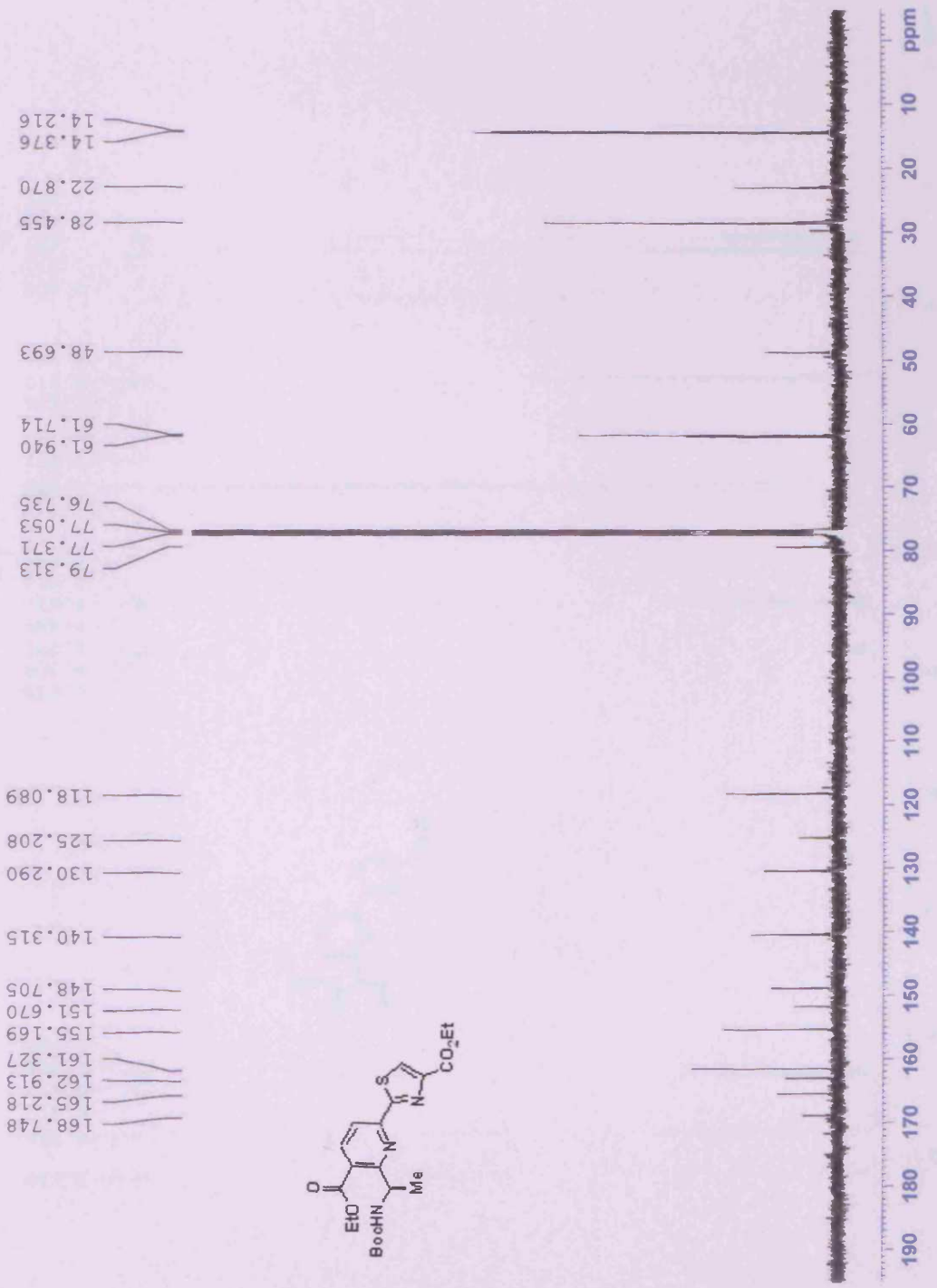
Appendix 15b ^{13}C NMR spectrum (d_6-DMSO, 100MHz) of compound 321



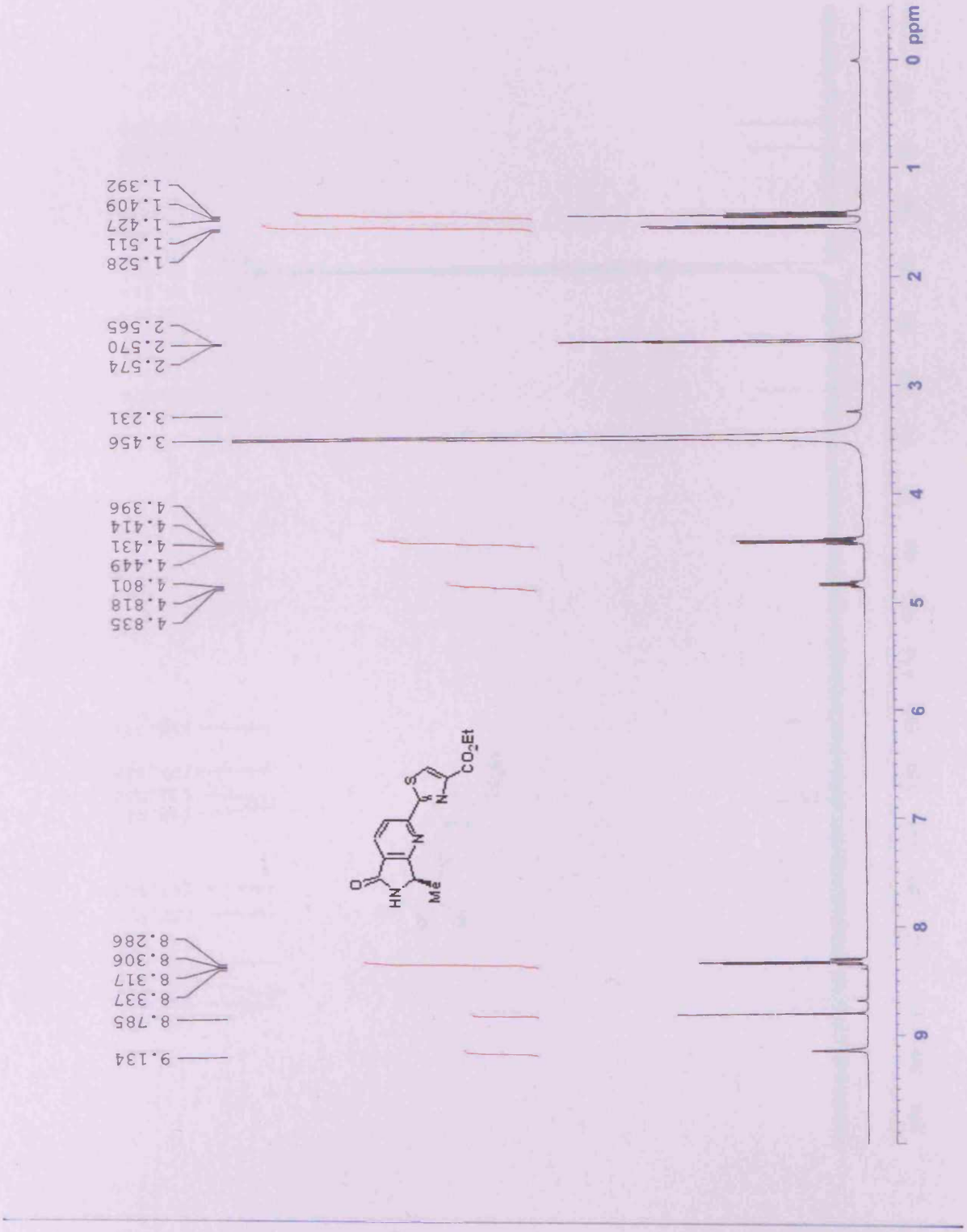
Appendix 16a ^{1}H NMR spectrum (d_6-acetone, 400MHz) of compound 311



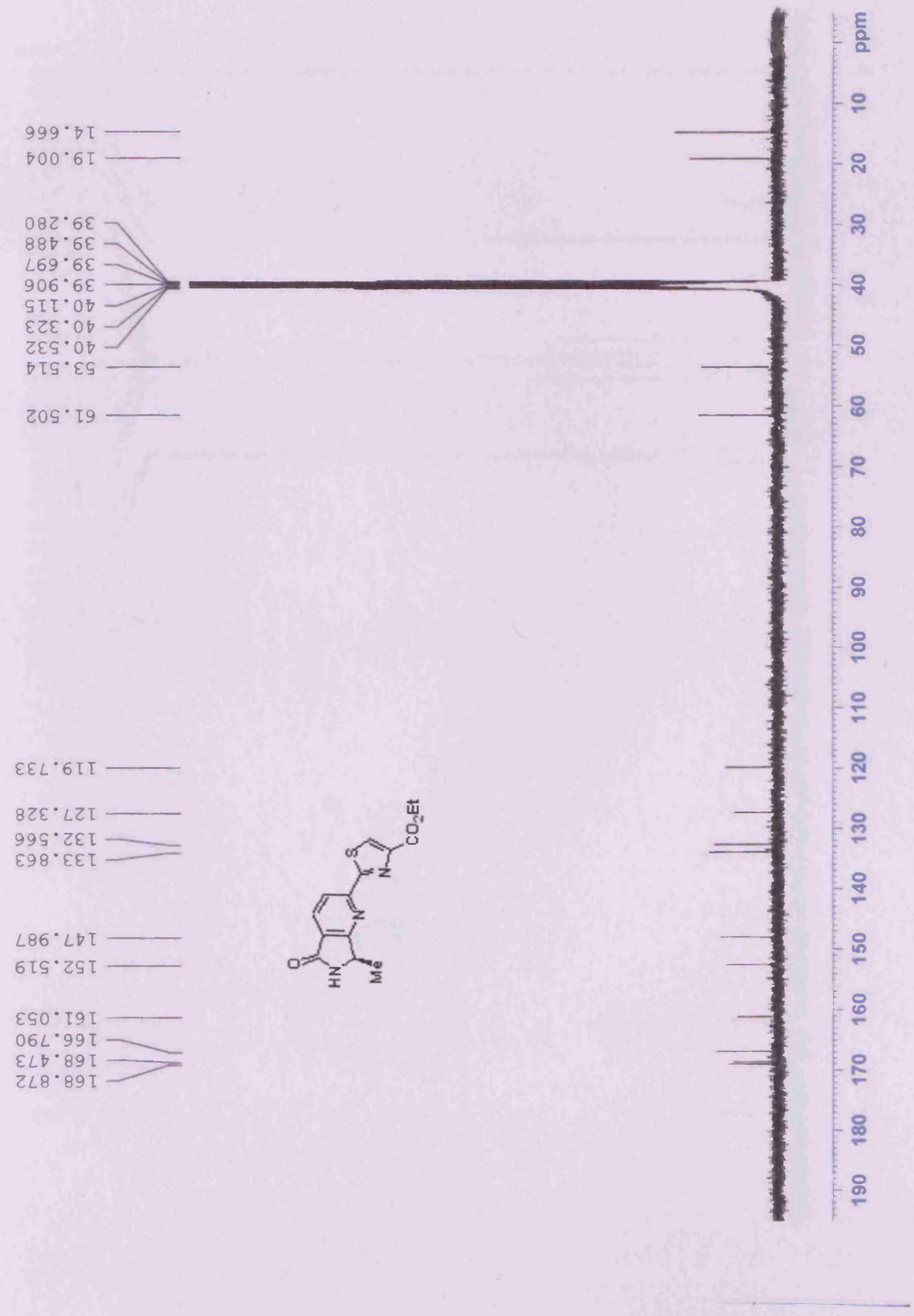
Appendix 16b ^{13}C NMR spectrum ($CDCl_3$, 100MHz) of compound 311



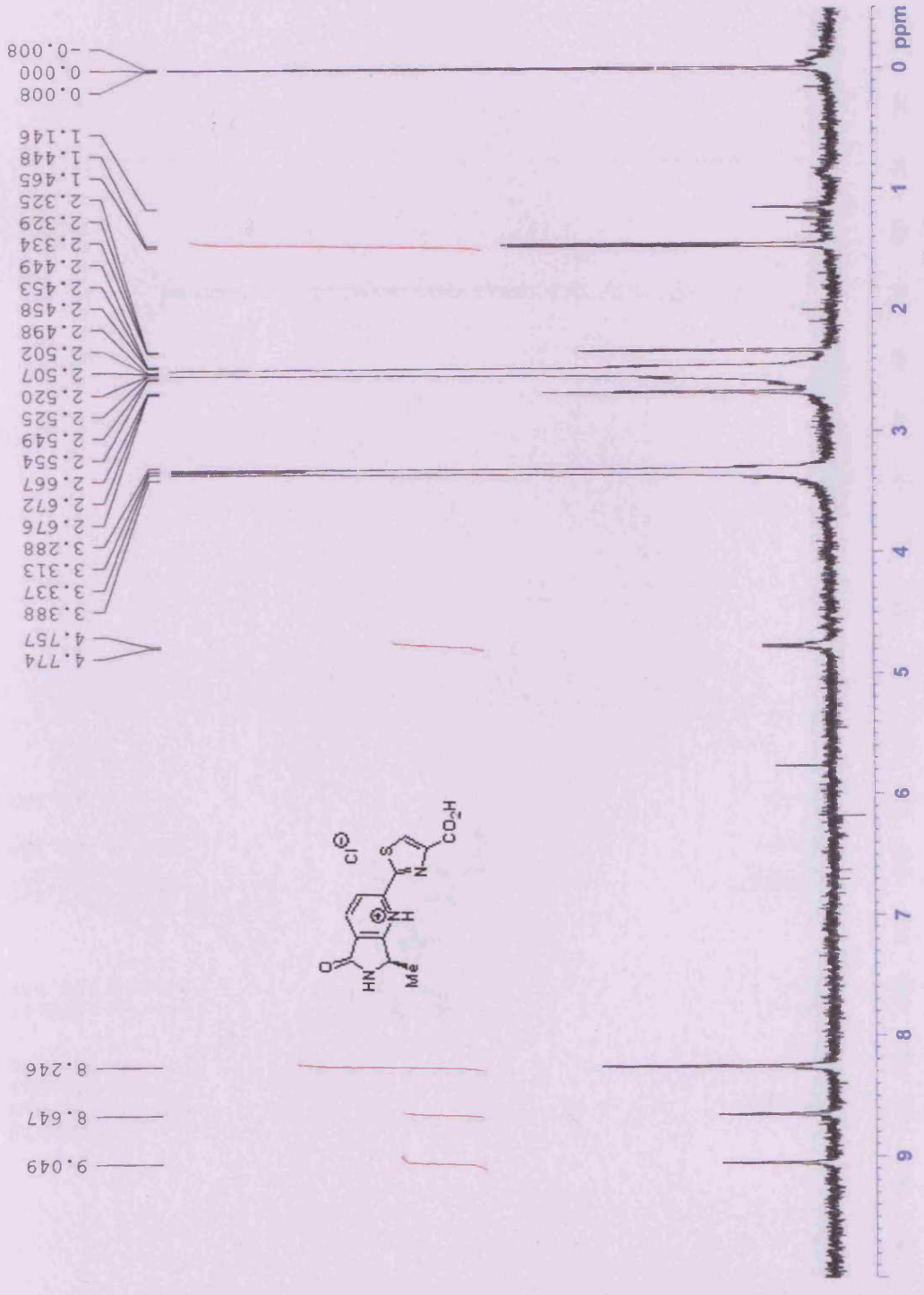
Appendix 17a ^{1}H NMR spectrum (d_6-DMSO, 400MHz) of compound 323



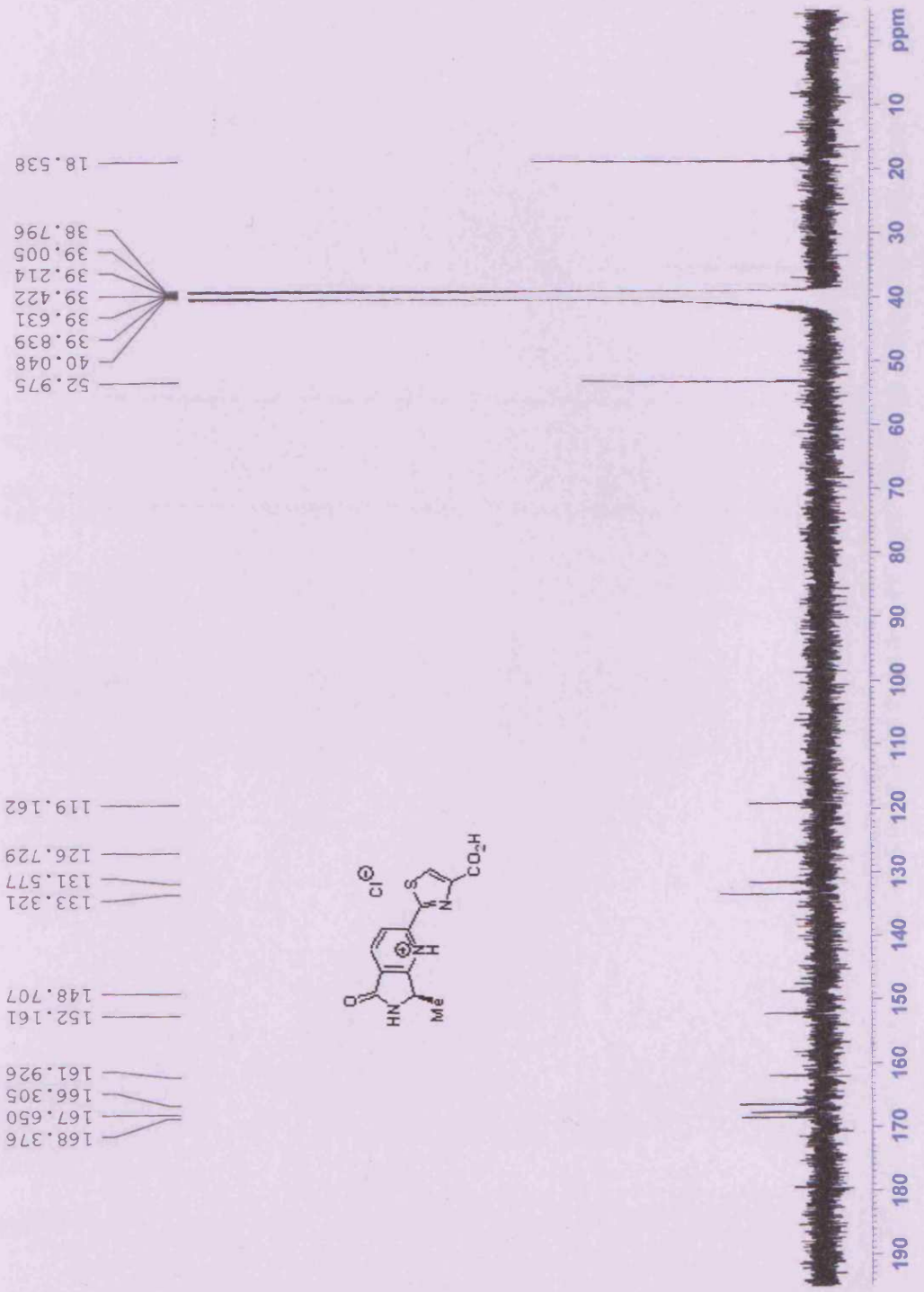
Appendix 17b ^{13}C NMR spectrum (d_6-DMSO, 100MHz) of compound 323



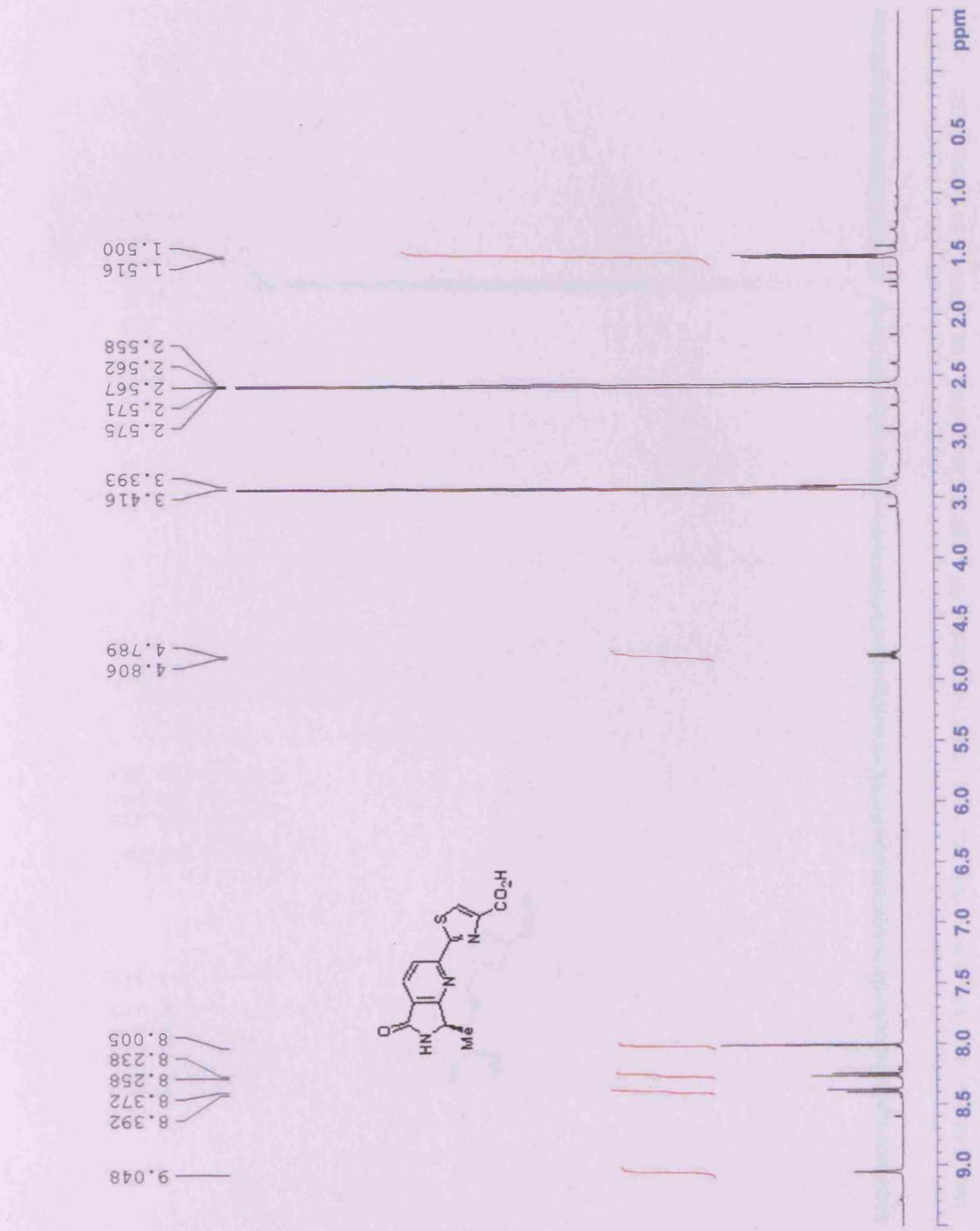
Appendix 18a ^{1}H NMR spectrum (d_6-DMSO, 400MHz) of compound 324



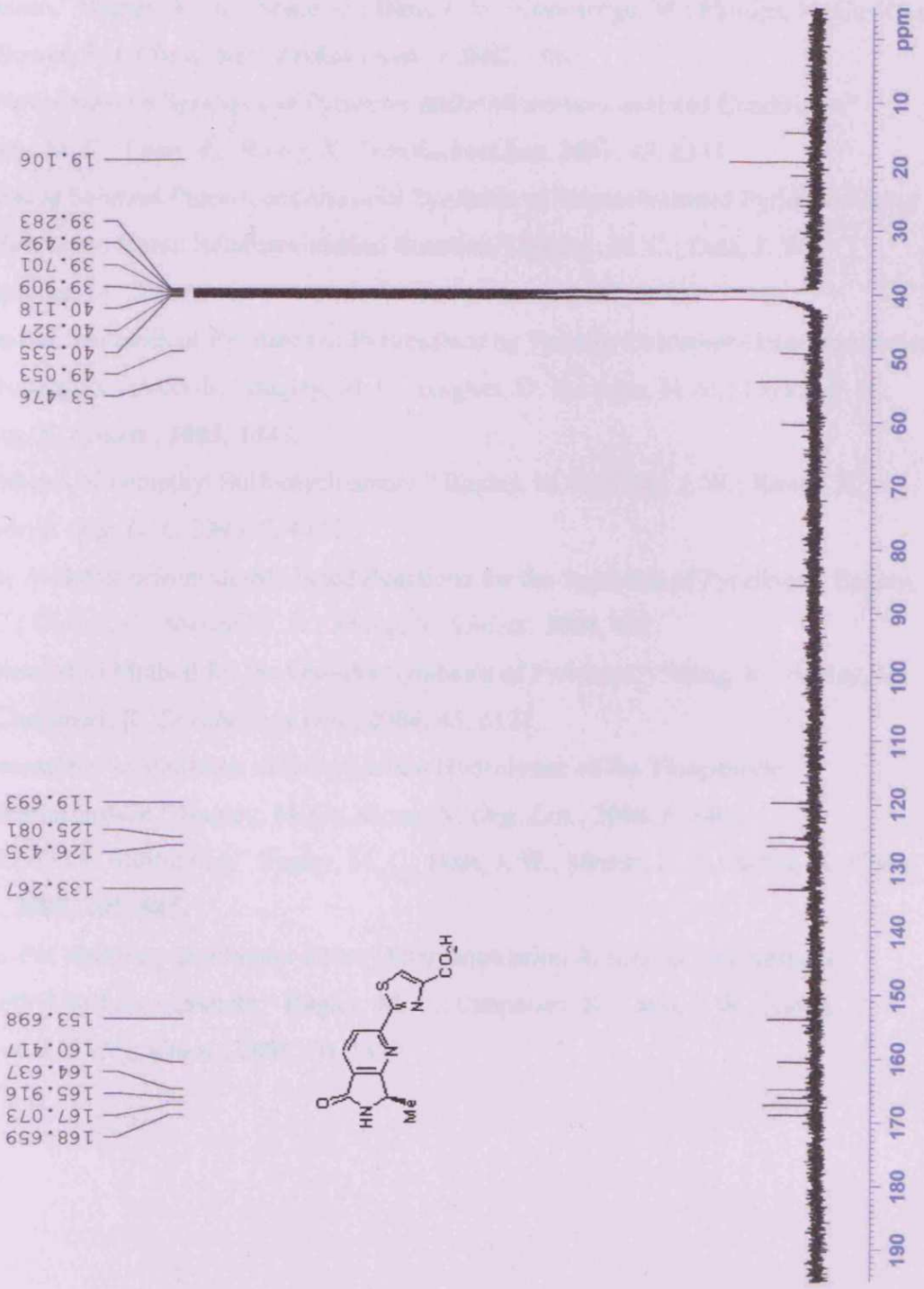
Appendix 18b ^{13}C NMR spectrum (d_6-DMSO, 100MHz) of compound 324



Appendix 19a ^{1}H NMR spectrum (d_6-DMSO, 400MHz) of compound 19



Appendix 19b ^{13}C NMR spectrum (d_6-DMSO, 100MHz) of compound 19



Appendix 20 List of graduate publications

1. "Synthesis of Tetrasubstituted Pyridines by The Acid-catalysed Bohlmann–Rahtz Reaction." Bagley, M. C.; Brace, C.; Dale, J. W.; Ohnesorge, M.; Phillips, N. G.; Xiong, X.; Bower, J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1663.
2. "A New One-step Synthesis of Pyridines under Microwave-assisted Conditions." Bagley, M. C.; Lunn, R.; Xiong, X. *Tetrahedron Lett.* **2002**, *43*, 8331.
3. "A Facile Solution Phase Combinatorial Synthesis of Tetrasubstituted Pyridines Using the Bohlmann-Rahtz Heteroannulation Reaction." Bagley, M. C.; Dale, J. W.; Ohnesorge, M.; Xiong, X.; Bower, J. *J. Comb. Chem.*, **2003**, 5, 41.
4. "One-Pot Synthesis of Pyridines or Pyrimidines by Tandem Oxidation-Heteroannulation of Propargylic Alcohols." Bagley, M. C.; Hughes, D. D.; Sabo, H. M.; Taylor, P. H.; Xiong, X. *Synlett.*, **2003**, 1443.
5. "Synthesis of Dimethyl Sulfomycinamate." Bagley, M. C.; Dale, J. W.; Xiong, X.; Bower, J. *Org. Lett.*, **2003**, 5, 4421.
6. "New *N*-Halosuccinimide-Mediated Reactions for the Synthesis of Pyridines." Bagley, M. C.; Glover, C.; Merritt, E. A.; Xiong, X. *Synlett.*, **2004**, 811.
7. "A New Mild Method for the One-Pot Synthesis of Pyridines." Xiong, X.; Bagley, M. C.; Chapaneri, K. *Tetrahedron Lett.*, **2004**, 45, 6121.
8. "Stereoselective Synthesis of the γ-Lactam Hydrolysate of the Thiopeptide Cyclothiazomycin." Bagley, M. C.; Xiong, X. *Org. Lett.*, **2004**, 6, 3401.
9. "Thiopeptide Antibiotics." Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.*, **2005**, 105, 685.
10. "One-Pot Multistep Bohlmann-Rahtz Heteroannulation Reactions: Synthesis of Dimethyl Sulfomycinamate." Bagley, M. C.; Chapaneri, K.; Dale, J. W.; Xiong, X.; Bower, J. *J. Org. Chem.*, **2005**, 70, 1389.

References

1. Niccolai, D.; Tarsi, L.; Thomas, R. J. *Chem. Commun.* **1997**, 2333.
2. Cundliffe, E. *Annu. Rev. Microbiol.* **1989**, *43*, 207.
3. Cundliffe, E. *Br. Med. Bull.* **1984**, *40*, 61.
4. Bérdy, J. *Adv. Appl. Microbiol.* **1974**, *18*, 309.
5. Hensens, O. D.; Albers-Schönberg, G. *Tetrahedron Lett.* **1978**, 3649.
6. Pagano, J. F.; Weinstein, M. J.; Stout, H. A.; Donovick, R. *Antibiotics Ann.* **1955/56**, 554.
7. Vandeputte, J.; Dutcher, J. D. *Antibiotics Ann.* **1955/56**, 560.
8. Steinberg, B. A.; Jambor, W. P.; Suydam, L. O.; Soriano, A. *Antibiotics Ann.* **1955/56**, 562.
9. (a) Jones, Jr, W. F.; Finland, M. *Antibiot. Chemother.* **1958**, *8*, 387. (b) Kutscher, A. H.; Seguin, L.; Rankow, R. M.; Piro, J. D. *Antibiot. Chemother.* **1958**, *8*, 576.
10. Cron, M. J.; Whitehead, D. F.; Hooper, I. R.; Heinemann, B.; Lein, J. *Antibiot. Chemother.* **1956**, *6*, 63.
11. Bodanszky, M.; Dutcher, J. D.; Williams, N. J. *J. Antibiotics* Ser. A **1963**, *16*, 76.
12. Bodanszky, M.; Fried, J.; Sheehan, J. T.; Williams, N. J.; Alicino, J.; Cohen, A. I.; Keeler, B. T.; Birkhimer, C. A. *J. Am. Chem. Soc.* **1964**, *86*, 2478.
13. Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature* **1970**, *225*, 233.
14. Tori, K.; Tokura, K.; Okabe, K.; Ebata, M.; Otsuka, H. *Tetrahedron Lett.* **1976**, 185.
15. Trejo, W. H.; Dean, L. D.; Pluscec, J.; Meyers, E.; Brown, W. E. *J. Antibiot.* **1977**, *30*, 639.
16. Hensens, O. D.; Albers-Schönberg, G. *J. Antibiot.* **1983**, *36*, 832.
17. Mocek, U.; Beale, J. M.; Floss, H. G. *J. Antibiot.* **1989**, *42*, 1649.
18. Bond, C. S.; Shaw, M. P.; Alphey, M. S.; Hunter, W. N. *Acta Cryst.* **2001**, *D57*, 755.
19. Tori, K.; Tokura, K.; Yoshimura, Y.; Terui, Y.; Okabe, K.; Otsuka, H.; Matsushita, K.; Inagaki, F.; Miyazawa, T. *J. Antibiot.* **1981**, *34*, 124.
20. Nishimura, H.; Okamoto, S.; Mayama, M.; Otsuka, H.; Nakajima, K.; Tawara, K.; Shimohira, M.; Shimaoka, N. *J. Antibiotics* Ser. A. **1961**, *14*, 255.
21. Ebata, M.; Miyazaki, K.; Otsuka, H. *J. Antibiot.* **1969**, *22*, 364.
22. Tori, K.; Tokura, K.; Yoshimura, Y.; Okabe, K.; Otsuka, H.; Inagaki, F.; Miyazawa, T. *J. Antibiot.* **1979**, *32*, 1072.
23. Miyairi, N.; Miyoshi, T.; Aoki, H.; Kohsaka, M.; Ikushima, H.; Kunugita, K.; Sakai, H.; Imanaka, H. *Antimicrob. Agents Chemother.* **1972**, *1*, 192.
24. Hensens, O. D.; Albers-Schönberg, G. *J. Antibiot.* **1983**, *36*, 814.
25. Muramatsu, I.; Motoki, Y.; Aoyama, M.; Suzuki, H. *J. Antibiot.* **1977**, *30*, 383.
26. Motoki, Y.; Muramatsu, I. *Pept. Chem.* 1979 (Pub. **1980**), 13; *Chem. Abstr.* **1981**, *94*, 103798m.
27. Puar, M. S.; Ganguly, A. K.; Afonso, A.; Brambilla, R.; Mangiaracina, P.; Sarre, O.; MacFarlane, R. D. *J. Am. Chem. Soc.* **1981**, *103*, 5231.
28. Puar, M. S.; Chan, T. M.; Hegde, V.; Patel, M.; Bartner, P.; Ng, K. J.; Pramanik, B. N.; MacFarlane, R. D. *J. Antibiot.* **1998**, *51*, 221.
29. Egawa, Y.; Umino, K.; Tamura, Y.; Shimizu, M.; Kaneko, K.; Sakurazawa, M.; Awataguchi, S.; Okuda, T. *J. Antibiot.* **1969**, *22*, 12.
30. Reusser, F. *Biochemistry* **1969**, *8*, 3303.
31. Abe, H.; Ikeda, M.; Takaishi, T.; Ito, Y.; Okuda, T. *Tetrahedron Lett.* **1977**, 735.
32. Liesch, J. M.; Millington, D. S.; Pandey, R. C.; Rinehart, Jr., K. L. *J. Am. Chem. Soc.* **1976**, *98*, 8237.
33. Abe, H.; Takaishi, T.; Ito, Y.; Okuda, T. *Heterocycles* **1977**, *8*, 461.
34. Abe, H.; Takaishi, T.; Okuda, T., Aoe, K.; Date, T. *Tetrahedron Lett.* **1978**, 2791.
35. (a) Kelly, T. R.; Lang, F. *Tetrahedron Lett.* **1995**, *36*, 5319. (b) Kelly, T. R.; Lang, F. *J. Org. Chem.* **1996**, *61*, 4623.

36. Bagley, M. C.; Dale, J. W.; Xiong, X.; Bower, J. *Org. Lett.* **2003**, *5*, 4421.
37. Kohno, J.; Kameda, N.; Nishio, M.; Kinumaki, A.; Komatsubara, S. *J. Antibiot.* **1996**, *49*, 1063.
38. Aoki, M.; Ohtsuka, T.; Yamada, M.; Ohba, Y.; Yoshizaki, H.; Yasuno, H.; Sano, T.; Watanabe, J.; Yokose, K. *J. Antibiot.* **1991**, *44*, 582.
39. Bagley, M. C.; Xiong, X. *Org. Lett.* **2004**, *6*, 3401.
40. Aoki, M.; Ohtsuka, T.; Itezono, Y.; Yokose, K.; Furihata, K.; Seto, H. *Tetrahedron Lett.* **1991**, *32*, 217.
41. Aoki, M.; Ohtsuka, T.; Itezono, Y.; Yokose, K.; Furihata, K.; Seto, H. *Tetrahedron Lett.* **1991**, *32*, 221.
42. Su, T. L. *Brit. J. Exp. Path.* **1948**, *29*, 473.
43. Kelly, B. K.; Miller, G. A.; Whitmarsh, J. M. Brit. Patent 711,593, 1954; *Chem. Abstr.* **1955**, *49*, 573.
44. Heatley, N. G.; Doery, H. M. *Biochem. J.* **1951**, *50*, 247.
45. Fuller, A. T. *Nature* **1955**, *175*, 722.
46. (a) Carnio, M. C.; Holtzel, A.; Rudolf, M.; Henle, T.; Jung, G.; Scherer, S. *Appl. Environ. Microbiol.* **2000**, *66*, 2378. (b) Carnio, M. C.; Stachelhaus, T.; Francis, K. P.; Scherer, S. *Eur. J. Biochem.* **2001**, *268*, 6390.
47. Brookes, P.; Fuller, A. T.; Walker, J. *J. Chem. Soc.* **1957**, 689.
48. Brookes, P.; Clark, R. J.; Fuller, A. T.; Mijović, M. P. V.; Walker, J. *J. Chem. Soc. C* **1960**, 916.
49. Mijović, M. P. V.; Walker, J. *J. Chem. Soc. C* **1960**, 909.
50. Walker, J.; Olesker, A.; Valente, L.; Rabanal, R.; Lukacs, G. *J. Chem. Soc., Chem. Commun.* **1977**, 706.
51. Bycroft, B. W.; Gowland, M. S. *J. Chem. Soc., Chem. Commun.* **1978**, 256.
52. Ciufolini, M. A.; Shen, Y.-C. *Org. Lett.* **1999**, *1*, 1843.
53. Shin, C.; Okumura, K.; Shigekuni, M.; Nakamura, Y. *Chem. Lett.* **1998**, 139.
54. Okumura, K.; Ito, A.; Yoshioka, D.; Shin, C. *Heterocycles* **1998**, *48*, 1319.
55. Fenet, B.; Pierre, F.; Cundliffe, E.; Ciufolini, M. A. *Tetrahedron Lett.* **2002**, *43*, 2367.
56. Dean, B. M.; Mijović, M. P. V.; Walker, J. *J. Chem. Soc.* **1961**, 3394.
57. Boeck, L. D.; Berry, D. M.; Mertz, F. P.; Wetzel, R. W. *J. Antibiot.* **1992**, *45*, 1222.
58. Shimanaka, K.; Kinoshita, N.; Iinuma, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 668.
59. Shimanaka, K.; Takahashi, Y.; Iinuma, H.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 1153.
60. Shimanaka, K.; Takahashi, Y.; Iinuma, H.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 1145.
61. Liesch, J. M.; Rinehart, Jr., K. L. *J. Am. Chem. Soc.* **1977**, *99*, 1645.
62. Liesch, J. M.; McMillan, J. A.; Pandey, R. C.; Paul, I. C.; Rinehart, Jr., K. L.; Reusser, F. *J. Am. Chem. Soc.* **1979**, *98*, 299.
63. Kelly, T. R.; Echavarren, A.; Chandrakumar, N. S.; Kŏksal, Y. *Tetrahedron Lett.* **1984**, *25*, 2127.
64. Stella, S.; Montanini, N.; Le Monnier, F.; Ferrari, P.; Colombo, L.; Landini, P.; Ciciliato, I.; Goldstein, B. P.; Selva, E.; Denaro, M. *J. Antibiot.* **1995**, *48*, 780.
65. Ferrari, P.; Colombo, L.; Stella, S.; Selva, E.; Zerilli, L. F. *J. Antibiot.* **1995**, *48*, 1304.
66. Selva, E.; Beretta, G.; Montanini, N.; Saddler, G. S.; Gastaldo, L.; Ferrari, P.; Lorenzetti, R.; Landini, P.; Ripamonti, F.; Goldstein, B. P.; Berti, M.; Montanaro, L.; Denaro, M. *J. Antibiot.* **1991**, *44*, 693.
67. Kettenring, J.; Colombo, L.; Ferrari, P.; Tavecchia, P.; Nebuloni, M.; Vékey, K.; Gallo, G. G.; Selva, E. *J. Antibiot.* **1991**, *44*, 702.
68. Colombo, L.; Tavecchia, P.; Selva, E.; Gallo, G. G.; Zerilli, L. F. *Org. Mass Spectrom.* **1992**, *27*, 219.
69. Tavecchia, P.; Gentili, P.; Kurz, M.; Sottani, C.; Bonfichi, R.; Lociuro, S.; Selva, E. *J. Antibiot.* **1994**, *47*, 1564.
70. Tavecchia, P.; Gentili, P.; Kurz, M.; Sottani, C.; Bonfichi, R.; Selva, E.; Lociuro, S.; Restelli, E.; Ciabatti, R. *Tetrahedron* **1995**, *51*, 4867.
71. Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 969.

72. Nadkarni, S. R.; Mukhopadhyay, T.; Jayvanti, K.; Vijaya Kumar, E. K. Eur. Patent 96111156,4, 1998; *Chem. Abstr.* **1998**, *128*, 153208.
73. Vijaya Kumar, E. K. S.; Kenia, J.; Mukhopadhyay, T.; Nadkarni, S. R. *J. Nat. Prod.* **1999**, *62*, 1562.
74. Holgado, G. G.; Rodríguez, J. C.; Hernández, L. M. C.; Díaz, M.; Fernández-Abalos, J. M.; Trujillano, I.; Santamaría, R. I. *J. Antibiot.* **2002**, *55*, 383.
75. Yun, B.-S.; Seto, H. *Biosci. Biotech. Biochem.* **1995**, *59*, 876.
76. Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 510.
77. Moody, C. J.; Bagley, M. C. *Chem. Commun.* **1998**, 2049.
78. Bagley, M. C.; Bashford, K. E.; Hesketh, C. L.; Moody, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 3301.
79. Kamigiri, K.; Watanabe, M.; Nagai, K.; Arao, N.; Suzumura, K.; Suzuki, K.; Kurane, R.; Yamaoka, M.; Kawano, Y. PCT Int. Appl. 072617, 2002; *Chem. Abstr.* **2002**, *137*, 246602.
80. Rodríguez, J. C.; Holgado, G. G.; Sánchez, R. I. S.; Cañedo, L. M. *J. Antibiot.* **2002**, *55*, 391.
81. Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 1541.
82. Shoji, J.; Hinoo, H.; Wakisaka, Y.; Koizumi, K.; Mayama, M.; Matsuura, S.; Matsumoto, K. *J. Antibiot.* **1976**, *29*, 366.
83. Shoji, J.; Kato, T.; Yoshimura, Y.; Tori, K. *J. Antibiot.* **1981**, *34*, 1126.
84. Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *Tetrahedron* **1994**, *50*, 11659.
85. Matsumoto, M.; Kawamura, Y.; Yasuda, Y.; Tanimoto, T.; Matsumoto, K.; Yoshida, T.; Shoji, J. *J. Antibiot.* **1989**, *42*, 1465.
86. Nagai, K.; Kamigiri, K.; Arao, N.; Suzumura, K.-I.; Kawano, Y.; Yamaoka, M.; Zhang, H.; Watanabe, M.; Suzuki, K. *J. Antibiot.* **2003**, *56*, 123.
87. Suzumura, K.-I.; Yokoi, T.; Funatsu, M.; Nagai, K.; Tanaka, K.; Zhang, H.; Suzuki, K. *J. Antibiot.* **2003**, *56*, 129.
88. Stella, S.; Montanini, N.; Le Monnier, F. J.; Colombo, L.; Selva, E.; Denaro, M. PCT Int. Appl. WO 94 14,838, 1994; *Chem. Abstr.* **1995**, *122*, 128491r.
89. Benazet, F.; Cartier, M.; Florent, J.; Godard, C.; Jung, G.; Lunel, J.; Mancy, D.; Pascal, C.; Renaut, J.; Tarridec, P.; Theilleux, J.; Tissier, R.; Dubost, M.; Ninet, L. *Experientia* **1980**, *36*, 414.
90. Depaire, H.; Thomas, J.-P.; Brun, A.; Olesker, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1403.
91. Depaire, H.; Thomas, J.-P.; Brun, A.; Olesker, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1397.
92. Depaire, H.; Thomas, J.-P.; Brun, A.; Hull, W. P.; Olesker, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1401.
93. Gasmi, G.; Massiot, G.; Nuzillard, J. M. *Magn. Reson. Chem.* **1996**, *34*, 185.
94. Depaire, H.; Thomas, J.-P.; Brun, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1395.
95. Prange, T.; Ducruix, A.; Pascard, C.; Lunel, J. *Nature* **1977**, *265*, 189.
96. Pascard, C.; Ducruix, A.; Lunel, J.; Prangé, T. *J. Am. Chem. Soc.* **1977**, *99*, 6418.
97. Tanaka, T.; Endō, T.; Shimazu, A.; Yoshida, R.; Suzuki, Y.; Ōtake, N.; Yonehara, H. *J. Antibiot.* **1970**, *23*, 231.
98. Endō, T.; Yonehara, H. *J. Antibiot.* **1978**, *31*, 623.
99. Casteels, M.; Bekaert, H.; Buysse, F. X. *Rev. Agric.* (*Brussels*) **1980**, *33*, 1069; *Chem. Abstr.* **1981**, *94*, 101869m.
100. Horii, S.; Oku, N. *J. AOAC Int.* **2000**, *83*, 17.
101. Tanaka, T.; Sakaguchi, K.; Yonehara, H. *J. Antibiot.* **1970**, *23*, 401.
102. Northcote, P. T.; Williams, D.; Manning, J. K.; Borders, D. B.; Maiese, W. M.; Lee, M. D. *J. Antibiot.* **1994**, *47*, 894.
103. Steinberg, D. A.; Bernan, V. S.; Montenegro, D. A.; Abbanat, D. R.; Pearce, C. J.; Korshalla, J. D.; Jacobus, N. V.; Petersen, P. J.; Mroczenski-Wildey, M. J.; Maiese, W. M.; Greenstein, M. *J. Antibiot.* **1994**, *47*, 887.
104. Northcote, P. T.; Siegel, M.; Borders, D. B.; Lee, M. D. *J. Antibiot.* **1994**, *47*, 901.

105. Keller-Juslen, C.; Kuhn, M.; DeLisle King, H. Ger. Offen. 2,921,148, 1979; *Chem. Abstr.* **1980**, *93*, 6124k.
106. Sasaki, T.; Otani, T.; Matsumoto, H.; Unemi, N.; Hamada, M.; Takeuchi, T.; Hori, M. *J. Antibiot.* **1998**, *51*, 715.
107. (a) Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. *J. Am. Chem. Soc.* **2002**, *124*, 7284. (b) Li, W.; Leet, J. E.; Ax, H. A.; Gustavson, D. R.; Brown, D. M.; Turner, L.; Brown, K.; Clark, J.; Yang, H.; Fung-Tomc, J.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 226. (c) Leet, J. E.; Li, W.; Ax, H. A.; Matson, J. A.; Huang, S.; Huang, R.; Cantone, J. L.; Drexler, D.; Dalterio, R. A.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 232.
108. (a) Mocek, U.; Zeng, Z.; O'Hagan, D.; Zhou, P.; Fan, L.-D. G.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7992. (b) Priestley, N. D.; Smith, T. M.; Shipley, P. R.; Floss, H. G. *Biorg. Med. Chem.* **1996**, *4*, 1135. (c) Smith, T. M.; Priestley, N. D.; Knaggs, A. R.; Nguyen, T.; Floss, H. G. *J. Chem. Soc., Chem. Commun.* **1993**, 1612.
109. Mocek, U.; Knaggs, A. R.; Tsuchiya, R.; Nguyen, T.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7557.
110. Fate, G. D.; Benner, C. P.; Grode, S. H.; Gilbertson, T. J. *J. Am. Chem. Soc.* **1996**, *118*, 11363.
111. Favret, M. E.; Paschal, J. W.; Elzey, T. K.; Boeck, L. D. *J. Antibiot.* **1992**, *45*, 1499.
112. Lau, R. C. M.; Rinehart, K. L. *J. Am. Chem. Soc.* **1995**, *117*, 7606.
113. De Pietro, M. T.; Marazzi, A.; Sosio, M.; Donadio, S.; Lancini, G. *J. Antibiot.* **2001**, *54*, 1066.
114. Nicolaou, K. C.; Nevalainen, M.; Safina, B. S.; Zak, M.; Bulat, S. *Angew. Chem. Int. Ed.* **2002**, *41*, 1941.
115. Moody, C. J.; Hughes, R. A.; Thompson, S. P.; Alcaraz, L. *Chem. Commun.* **2002**, 1760.
116. Roy, R. S.; Gehring, A. M.; Milne, J. C.; Belshaw, P. J.; Walsh, C. T. *Nat. Prod. Rep.* **1999**, *16*, 249.
117. Wilson, D. N.; Nierhaus, K. H. *Angew. Chem. Int. Ed.* **2003**, *42*, 3464.
118. Ban, N.; Nissen, P.; Hansen, J.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 905.
119. (a) Carter, A. P.; Clemons, W. M.; Broderson, D. E.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Nature* **2000**, *407*, 340. (b) Schluenzen, F.; Tocilj, A.; Zarivach, R.; Harms, J.; Gluehmann, M.; Janell, D.; Bashan, A.; Bartels, H.; Agmon, I.; Franceschi, F.; Yonath, A. *Cell* **2000**, *102*, 615. (c) Wimberly, B. T.; Broderson, D. E.; Clemons Jr, W. M.; Morgan-Warren, R. J.; Carter, A. P.; Vonrhein, C.; Hartsch, T.; Ramakrishnan, V. *Nature* **2000**, *407*, 327. (d) Protein Data Bank (PDB) ID: 1FFK, 1FKA and 1FLG. Credit also to David S. Goodsell, The Scripps Research Institute for diagrams.
120. Ramakrishnan, V. *Cell* **2002**, *108*, 557.
121. Bower, J.; Drysdale, M.; Hebdon, R.; Jordan, A.; Lentzen, G.; Matassova, N.; Murchie, A.; Powles, J.; Roughley, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2455.
122. Cundliffe, E. *Biochem. Biophys. Res. Commun.* **1971**, *44*, 912.
123. (a) Ryan, P. C.; Lu, M.; Draper, D. E. *J. Mol. Biol.* **1991**, *221*, 1257. (b) Conn, G. L.; Draper, D. E.; Lattman, E. E.; Gittis, A. G. *Science* **1999**, *284*, 1171.
124. Egebjerg, J.; Douthwaite, S. R.; Liljas, A.; Garrett, R. A. *J. Mol. Biol.* **1990**, *213*, 275.
125. Xing, Y.; Draper, D. E. *Biochemistry* **1996**, *35*, 1581.
126. (a) Blyn, L. B.; Risen, L. M.; Griffey, R. H.; Draper, D. E. *Nucleic Acids Res.* **2000**, *28*, 1778. (b) Wimberly, B. T.; Guymon, R.; McCutcheon, J. P.; White, S. W.; Ramakrishnan, V. *Cell* **1999**, *97*, 491.
127. Porse, B. T.; Leviev, I.; Mankin, A. S.; Garrett, R. A. *J. Mol. Biol.* **1998**, *276*, 391.
128. Rodnina, M. V.; Savelsbergh, A.; Katunin, V. I.; Wintermeyer, W. *Nature* **1997**, *385*, 37.
129. Landini, P.; Soffientini, A.; Monti, F.; Lociuro, S.; Marzorati, E.; Islam, K. *Biochemistry* **1996**, *35*, 15288.
130. Anborgh, P. H.; Parmeggiani, A. *EMBO J.* **1991**, *10*, 779.
131. Shimanaka, K.; Iinuma, H.; Hamada, M.; Ikeno, S.; S.-Tsuchiya, K.; Arita, M.; Hori, M. *J. Antibiot.* **1995**, *48*, 182.

132. Selva, E.; Montanini, N.; Stella, S.; Soffientini, A.; Gastaldo, L.; Denaro, M. *J. Antibiot.* **1997**, *50*, 22.
133. Kelly, T. R.; Jagoe, C. T.; Gu, Z. *Tetrahedron Lett.* **1991**, *32*, 4263.
134. (a) Higashibayashi, S.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2002**, *43*, 105. (b) Higashibayashi, S.; Kohno, M.; Goto, T.; Suzuki, K.; Mori, T.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2004**, *45*, 3707.
135. Koerber-Plé, K.; Massiot, G. *Synlett* **1994**, 759.
136. Umemura, K.; Tate, T.; Yamaura, M.; Yoshimura, J.; Yonezawa, Y.; Shin, C. *Synthesis* **1995**, 1423.
137. Umemura, K.; Noda, H.; Yoshimura, J.; Konn, A.; Yonezawa, Y.; Shin, C. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1391.
138. Bentley, D. J.; Fairhurst, J.; Gallagher, P. T.; Manteuffel, A. K.; Moody, C. J.; Pinder, J. L. *Org. Biomol. Chem.* **2004**, *2*, 701.
139. Moody, C. J.; Bagley, M. C. *Synlett* **1998**, 361.
140. Kayano, T.; Yonezawa, Y.; Shin, C. *Chem. Lett.* **2004**, *33*, 72.
141. Bagley, M. C.; Dale, J. W.; Jenkins, R. L.; Bower, J. *Chem. Commun.* **2004**, 102.
142. Yamada, T.; Okumura, K.; Yonezawa, Y.; Shin, C. *Chem. Lett.* **2001**, 102.
143. (a) Shin, C., Ito, A.; Okumura, K.; Nakamura, Y. *Chem. Lett.* **1995**, 45. (b) Okabe, A.; Ito, A.; Okumura, K.; Shin, C. *Chem. Lett.* **2001**, 380. (c) Shin, C.; Okabe, A.; Ito, A.; Ito, A.; Yonezawa, Y. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1583.
144. Endoh, N.; Yonezawa, Y.; Shin, C. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 643.
145. Umemura, K.; Ikeda, S.; Yoshimura, J.; Okumura, K.; Saito, H.; Shin, C. *Chem. Lett.* **1997**, 1203.
146. Okumura, K.; Saito, H.; Shin, C.; Umemura, K.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1863.
147. (a) Ciufolini, M. A.; Shen, Y. C. *J. Org. Chem.* **1997**, *62*, 3804. (b) Suzuki, S.; Yonezawa, Y.; Shin, C. *Chem. Lett.* **2004**, *33*, 814.
148. Hughes, R. A.; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *Chem. Commun.* **2004**, 946.
149. Nicolaou, K. C.; Safina, B. S.; Funke, C.; Zak, M.; Zécri, F. J. *Angew. Chem. Int. Ed.* **2002**, *41*, 1937.
150. Nicolaou, K. C.; Nevalainen, M.; Zak, M.; Bulat, S.; Bella, M.; Safina, B. S. *Angew. Chem. Int. Ed.* **2003**, *42*, 3418.
151. Nicolaou, K. C.; Safina, B. S.; Zak, M.; Estrada, A. A.; Lee, S. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 5087.
152. Nicolaou, K. C.; Zak, M.; Safina, B. S.; Lee, S. H.; Estrada, A. A. *Angew. Chem. Int. Ed.* **2004**, *43*, 5092.
153. Gill, N. S.; James, K. B.; Lions, F.; Potts, K. T. *J. Am. Chem. Soc.* **1952**, *74*, 4923.
154. Duhamel, P.; Hennequin, L.; Poirier, J. M.; Tavel, G.; Vottero, C. *Tetrahedron* **1986**, *42*, 4777
155. Hantzsch, A. *Liebigs Ann. Chem.* **1882**, *215*, 1.
156. Bohlmann, F.; Rahtz, D. *Chem. Ber.* **1957**, *90*, 2265.
157. (a) Bagley, M. C.; Dale J. W.; Bower J. *Synlett* **2001**, 1149. (b) Bagley, M. C.; Dale J. W.; Hughes D. D.; Ohnesorge M.; Phillips N. G.; Bower J. *Synlett* **2001**, 1523.
158. Bagley, M. C.; Brace, C.; Dale, J. W.; Ohnesorge, M.; Phillips, N. G.; Xiong, X.; Bower, J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1663.
159. Bagley, M. C.; Dale, J. W.; Bower, J. *Chem. Commun.* **2002**, 1682.
160. Bagley, M. C.; Glover, C.; Merritt, E. A.; Xiong, X. *Synlett* **2004**, 811.
161. Bagley, M. C.; Glover, C.; Chevis, D. *Synlett* **2005**, 649.
162. Moody, C. J.; Bagley, M. C. *Synlett* **1998**, 361.
163. Adlington, R. M.; Baldwin, J. E.; Catterick, D.; Pritchard, G. J.; Tang, L. T. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2311.
164. Bagley, M. C.; Hughes, D. D.; Lloyd, R.; Powers, V. E. C. *Tetrahedron Lett.* **2001**, *42*, 6585.

165. Bashford, K. E.; Burton, M. B.; Cameron, S.; Cooper, A. L.; Hogg, R. D.; Kane, P. D.; MacManus, D. A.; Matrunola, C. A.; Moody, C. J.; Robertson, A. A. B.; Warne, M. R. *Tetrahedron Lett.* **2003**, *44*, 1627.
166. Dale, J. W. Ph.D. Thesis, Cardiff University, 2003.
167. Gabriel, C.; Gabriel, S.; Grant, E. H.; Halstead, B. S. J.; Mingos, D. M. P. *Chem. Soc. Rev.* **1998**, *27*, 213.
168. Hayes, B. L. *Microwave Synthesis: Chemistry at the Speed of Light*; CEM Publishing: Matthews, NC, 2002.
169. Gedey, R.; Smith, F.; Westaway, K.; Ali, H.; Baldisera, L.; Laberge, L.; Roussel, J. *Tetrahedron Lett.* **1986**, *27*, 279.
170. For very recent reviews on microwave-assisted organic synthesis see: (a) Kappe, C. O. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250. (b) Hayes, B. L. *Aldrichimica Acta* **2004**, *37*, 66. (c) Kuhnert, N. *Angew. Chem. Int. Ed.* **2002**, *41*, 1863. (d) Lidström, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetradedron* **2001**, *57*, 9225.
171. (a) Bagley, M. C.; Hughes, D. D.; Taylor, P. H. *Synlett* **2003**, 259. (b) Bagley, M .C.; Singh, N. *Synlett* **2002**, 1718.
172. Bagley, M. C.; Lunn, R.; Xiong, X. *Tetrahedron Lett.* **2002**, *43*, 8331.
173. Loupy, A.; Petit, A.; Hamelin, J.; Texier-Boullet, F.; Jacquault, P.; Mathé, D. *Synthesis* **1998**, 1213.
174. (a) Foot, J. S.; Kanno, H.; Giblin, G. M. P.; Taylor, R. J. K. *Synthesis* **2003**, 1055. (b) Blackburn, L.; Kanno, H.; Taylor, R. J. K. *Tetrahedron Lett.* **2003**, *44*, 115. (c) Runcie, K. A.; Taylor, R. J. K. *Chem. Commun.* **2002**, 974. (d) Blackburn, L.; Pei, C.; Taylor, R. J. K. *Synlett* **2002**, 215. (e) Blackburn, L.; Taylor, R. J. K. *Org. Lett.* **2001**, *3*, 1637. (f) Wei, X.; Taylor, R. J. K. *J. Org. Chem.* **2000**, *65*, 617. (g) Blackburn, L.; Wei, X.; Taylor, R. J. K. *Chem. Commun.* **1999**, 1337. (h) Wei, X.; Taylor, R. J. K. *Tetrahedron Lett.* **1998**, *39*, 3815.
175. Crich, D.; Mo, X.-S. *Synlett* **1999**, 67.
176. Barrett, A. G. M.; Hamprecht, D.; Ohkubo, M. *J. Org. Chem.* **1997**, *62*, 9376.
177. Shuto, S.; Niizuma, S.; Matsuda, A. *J. Org. Chem.* **1998**, *63*, 4489.
178. Collins, I. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1921.
179. Baraldi, P. G.; Simoni, D.; Manfredini, S. *Synthesis* **1983**, 902.
180. (a) Agami, C.; Dechoux, L.; Hebbe, S. *Synlett* **2001**, 1440. (b) Agami, C.; Dechoux, L.; Hamon, L.; Hebbe, S. *Synthesis* **2003**, 859.
181. Agami, C.; Dechoux, L.; Hebbe, S.; Moulinas, J. *Synlett* **2002**, 79.
182. Roth, H. J.; Kleemann, A. In *Pharmaceutical Chemistry. Volume 1: Drug Synthesis*; John Wiley & Sons: New York, 1988.
183. (a) Nettekoven, M. *Synlett* **2001**, 1917. (b) Breitenbucher, J. G.; Figliozzi, G. *Tetrahedron Lett.* **2000**, *41*, 4311. (c) Brändli, C.; Ward, T. R. *J. Comb. Chem.* **2000**, *2*, 42. (d) Bhandari, A.; Li, B.; Gallop, M. A. *Synthesis* **1999**, 1951. (e) Grosche, P.; Höltzel, A.; Walk, T. B.; Trautwein, A. W.; Jung, G. *Synthesis* **1999**, 1961. (f) Chiu, C.; Tang, Z.; Ellingboe, J. W. *J. Comb. Chem.* **1999**, *1*, 73. (g) Lago, M. A.; Nguyen, T. T.; Bhatnagar, P. *Tetrahedron Lett.* **1998**, *39*, 3885. (h) Marzinzik, A. L.; Felder, E. R. *J. Org. Chem.* **1998**, *63*, 723. (i) Gordeev, M. F.; Patel, D. V.; Gordon, E. M. *J. Org. Chem.* **1996**, *61*, 924. (j) Gordeev, M. F.; Patel, D. V.; Wu, J.; Gordon, E. M. *Tetrahedron Lett.* **1996**, *37*, 4643. (k) Powers, D. G.; Casebier, D. S.; Fokas, D.; Ryan, W. J.; Troth, J. R.; Coffen, D. L. *Tetrahedron* **1998**, *54*, 4085.
184. Kim, H.-O.; Olsen, R. K.; Choi, O.-S. *J. Org. Chem.* **1987**, *52*, 4531.
185. Wipf, P.; Miller, C. P.; Venkatraman, S.; Fritch, P. C. *Tetrahedron Lett.* **1995**, *36*, 6395.
186. Yura, Y. *Chem. Pharm. Bull.* **1962**, *10*, 376.
187. Ameer, F.; Drewes, S. E.; Drewes, M. W.; Roos, G. H. P.; Watson, M. C. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1425.
188. Nishio, T.; Nakata, H.; Omote, Y. *J. Heterocyclic Chem.* **1986**, *23*, 1011.
189. Miller, R. D.; Reiser, O. *J. Heterocyclic Chem.* **1993**, *30*, 755.

190. Fetter, J.; Nagy, I.; Giang, L. T.; Kajtár-Peredy, M.; Rockenbauer, A.; Korecz, L.; Czira, G. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1131 and references cited therein.
191. Kochetkov, N. K.; Khomutova, E. D.; Likhosherstov, L. M. *Zh. Obshch. Khim.* **1959**, *29*, 1657.
192. Gräf, E.; Troschütz, R. *Synthesis* **1999**, 1216.
193. Roomi, M. W. *J. Med. Chem.* **1975**, *18*, 457.
194. Vanden Eynde, J.-J.; Mayence, A.; Maquestiau, A.; Anders, E. *Heterocycles* **1994**, *37*, 815.
195. Honoré, T.; Hjeds, H.; Krogsgaard-Larsen, P.; Christiansen, T. R. *Eur. J. Med. Chem.* **1978**, *13*, 429.