Towards the total synthesis of thiopeptide antibiotics

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By

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This thesis is dedicated to Arron.

ABSTRACT

The Bohlmann–Rahtz intermediates (aminodienones) have provided a viable route to 2,3,6–trisubstituted and 2,3,5,6–tetrasubstituted pyridines (a substitution pattern hitherto unreported for Bohlmann–Rahtz intermediates), using new mild facile reaction conditions. These new methods provide the potential for both complimentary and independent pathways to access many of the thiopeptide antibiotics as well as other pyridine containing natural products. Degradation studies of thiopeptide antibiotics have led to the isolation of fragments that help in the structural elucidation of the parent molecules. Our efforts to synthesize degradation fragments from thiopeptide antibiotics have led to the total synthesis of the methyl sulfomycinate **39** in 9 steps from H-Thr-OMe and 15% overall yield, sulfomycinic amide **38**, sulfomycinine **37** and the synthesis of saramycetic acid I **47**, the latter in 9 steps and 11% overall yield. Furthermore progress towards the total synthesis of micrococcin P_1 has demonstrated that enamine and alkynone precursors to the Bohlmann–Rahtz pyridine synthesis are viable intermediates en route to the core of micrococcin P_1.

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ABBREVIATIONS

Ac	Acetyl
APcI	atmospheric pressure chemical ionization
aq	Aqueous
Ar	Unspecified aryl substituent
Boc	*tert*–butoxycarbonyl
Boc_2O	Di–*tert*–butyldicarboxylate
Bu	Butyl
BuLi	Butyllithium
c	Concentration
cat.	Catalytic/catalyst
Cbz	Benzyloxycarbonyl
CF	Continuous Flow
CI	Chemical Ionisation
COSY	Correlation Spectroscopy
Cys	Cysteine
DAST	Diethylaminosulphur–trifluoride
DBU	1,8–Diaza[5.4.0]undec–7–ene
DCC	Dicyclohexylcarbodiimide
Deoxofluor	[Bis(2–methoxyethyl)amino]sulphur trifluoride
Dept/dept	Distorntionless enhancement by polarisation transfer
DIBAL	Diisobutylaluminium hydride
DIEA	Diisopropylethylamine
DMAP	*N,N*–Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	*N,N*–Dimethylformamide
DMP	Dimethoxypropane
DMSO	Dimethyl sulphoxide
ε	Molar absorbtivity
EDCI	1– [3– (Dimethylamino)propyl]–3–ethylcarbodiimide hydrochloride
ee	Enantiomeric excess
EI	Electron Impact
equiv. or eq.	Equivalent
Et	Ethyl
exch.	Exchange

FAB	Fast Atom Bombardment
FTIR	Fourrier Transform Infra Red
g	Grams
GC–MS	Gas Chromatography Mass Spectrometry
h	hour/s
HPLC	High Pressure Liquid Chromatography
HR–FAB–MS	High Resolution Fast Atom Bombardment Mass Spectrometry
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
IBX	*o*-Iodoxybenzoic acid
IC_{50}	Concentration of an inhibitor that is required for 50% inhibition of an enzyme *in vitro*
IR	Infra Red
J	Coupling constant (in Hz)
L	Laevorotatory
L11	Ribosomal protein
LDA	Lithium di *iso*–propylamine
lit.	Literature
LRMS	Low Resolution Mass Spectrometry
M	Molar
MAOS	Microwave–Assisted Organic Synthesis
m–CPBA	*meta*–Chloroperbenzoic acid
MCR	Multiple Component Reaction
Me	Methyl
MHz	Megahertz
min	Minutes
μM	Micromolar
mol	Mole
Mp	Melting point
MS	Mass Spectrometry
NCS	*N*–Chlorosuccinimide
NBS	*N*–Bromosuccinimide
NIS	*N*–Iodosuccinimide
NMM	*N*–methyl morpholine
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser effect

NOSEY	Nuclear Overhauser effect Spectroscopy
P	*Para*
P	Protecting group
Ph	Phenyl
PhMe	Toluene
Ppm	parts per million
PPTS	pyridinium *p*–toluenesulphonate
p–TsOH	*p*–Toluenesulphonic acid
pyBOP	Benzotriazole–1–yloxytripyrrolidinophosphonium hexafluorophosphate
quant.	Quantitative
R	Specified substituent
R_f	Retention factor
rRNA	ribosomal–Ribose Nucleic Acid
RT	Room Temperature
Ser	Serine
Silica/SiO_2	Merck Kieselgel 60 H silica or Matrex silica 60
sp.	Species
Subsp.	Sub species
TBAF	Tetra–*n*–butylammonium fluoride
TBDMSCl	*tert*–Butyldimethylsilyl chloride
TBS	*tert*–Butyldimethylsilyl
Tert	Tertiary
Tf_2O	Triflic anhydride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
Trt	Triphenyl methyl
Ts	Tosyl (*para*–toluene sulphonyl)
UV	Ultraviolet
Vs.	Versus

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Chapter One – Introduction

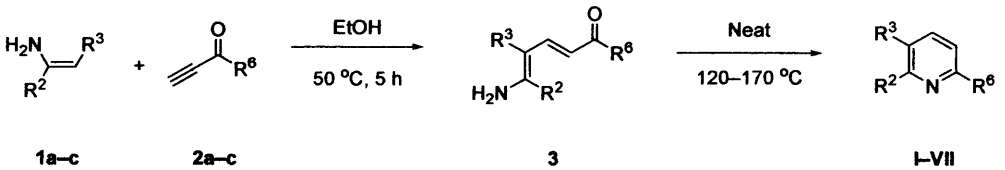
1 The Bohlmann–Rahtz pyridine synthesis

1.1 Discovery

Many different methods for the synthesis of pyridines are available, encompassing a number of prominent procedures that have earned their inventors great recognition in tribute to the discovery of useful heterocyclic methodology. Invariably these procedures are judged on a number of familiar criteria: efficiency, selectivity (including regio–, chemo– and even stereoselectivity), substrate tolerance, opportunities for diversity, atom economy, ecological value, etc. However, inevitably, with so many varied routes to these targets being unearthed throughout the years, some transformations following their discovery, in spite of having the potential to satisfy most if not all of the criteria, become largely forgotten. The Bohlmann–Rahtz pyridine synthesis, first reported in 1957,[1] until very recently was one such procedure.

Trisubstituted pyridines are synthesized in a two–step procedure by the reaction of enamines **1** and alkynyl ketones or aldehydes, **2**, (Scheme 1). Note that throughout this document the R group number system corresponds to the final position that a given R group is located around the pyridine ring. Bohlmann and Rahtz observed that the condensation of ethyl β–aminocrotonate **1a**, 2–aminopent–2–ene–4–one **1b**, or β–aminocrotonitrile **1c** and 1–phenylprop–2–yn–1–one **2a**, but–3–yn–2–one **2b**, or propargylic aldehyde **2c** is completely regioselective and proceeds by Michael addition and enamine *C*–alkylation to give an aminodiene intermediate **3** that can be isolated in high yield. In a subsequent procedure, heating intermediates **3** to a temperature of 120–170 °C, induces spontaneous cyclodehydration (presumably via isomerisation of double bond geometry) to give 2,3,6–trisubstituted pyridines **I–VI** in excellent overall yield and with total regiocontrol. Although it is related to the corresponding reaction of enamines with enones and hence to the well–known Hantzsch dihydropyridine synthesis,[2] the use of ynones leads to an heteroaromatic product directly thus obviating the need for a final aromatizing oxidative step.[3]

Scheme 1. The traditional Bohlmann–Rahtz pyridine synthesis



1a–c **2a–c** **3** **I–VII**

I, R^2=Me, R^3=COMe, R^6=H; **II**, R^2=Me, R^3=COMe, R^6=Me; **III**, R^2=Me, R^3=CN, R^6=Me; **IV**, R^2=Me, R^3=CN, R^6=H; **V**, R^2=Ph, R^3=CN, R^6=Me; **VI**, R^2=Me, R^3=CO_2Et, R^6=H;

1.2 Recent improvements in methodology

Within the last decade the potential of the Bohlmann–Rahtz pyridine synthesis has began to be unravelled. Bagley and co–workers have published numerous works regarding methodology improvement, including a range of cataylsis and solvent effects, tandem processes and microwave technology. The immediate result of these works is the identification of modified Bohlmann–Rahtz procedures that offer a growing range of tri– and tetrasubstituted pyridines that are regio–, chemo– and even stereospecific. Moreover the modified procedures offer the potential for greater application in synthetic chemistry due to milder, improved reaction conditions compared to the original Bohlmann–Rahtz pyridine synthesis. This chapter will review the contributions made from the literature since Bohlmann–Rahtz first discovered the reaction in 1957 (including much of the authors findings for completeness).

1.2.1 Use of catalysts and solvents

Avoiding the use of high cyclodehydration temperatures, extending this protocol for use in the synthesis of tetrasubstituted pyridines and developing a single one–step experimental procedure would increase the scope and utility of the Bohlmann–Rahtz reaction.[4] In order to investigate these improvements, intermediate aminodienone **3a** was prepared by standard Bohlmann–Rahtz conditions,[1,4] from ethyl β–aminocrotonate **1a** and but–3–yn–2–one **2a** in ethanol (EtOH) at 50 °C, to give the pure cyclodehydration precursor in 98% yield following purification. However, due to shortages in the availability of butynone **2a**, concerns about its volatility and in order to expand the scope of viable substrates, an alternative route to intermediate **3a** was sought. To that end a mixture of ethyl β–aminocrotonate **1a** and 4–(trimethylsilyl)but–3–yn–2–one **2d**, which was cheaper than **2a**, more readily available and less volatile, was heated to 50 °C in a range of different solvents (Table 1).[4]

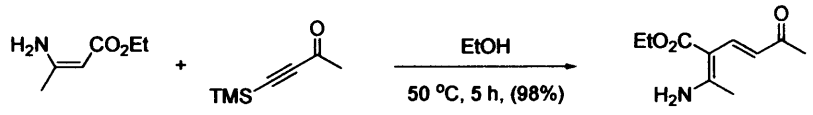
Table 1. Michael addition of **1a** and **2d** in various solvents at 50 °C

Solvent	Result	Solvent	Result
Acetone	No reaction	Diethyl ether	No reaction
Toluene	No reaction	Neat	No reaction
Dichloromethane	No reaction	Dimethylsulphoxide	Product **3a** (59%)
Chloroform	No reaction	Ethanol	Product **3a** (98%)

In most cases only unreacted starting materials were isolated from the reaction mixture. However, when a solution of crotonate **1a** and butynone **2d** was stirred in ethanol (the original solvent of choice from Bohlmann–Rahtz conditions) or dimethylsulphoxide (DMSO) at 50 °C for

5 hours, aminodienone **3a** was produced in 98 or 59% yield, respectively, after purification. Clearly the reaction favours the use of polar solvents, although ethanol, a protic solvent gave better yields over DMSO, an aprotic solvent. In both of these solvents protodesilylation occurred spontaneously under the reaction conditions, and, in view of the improvements that this transformation offered over the original procedure, this became the method of choice for the synthesis of aminodienone **3a** (Scheme 2).

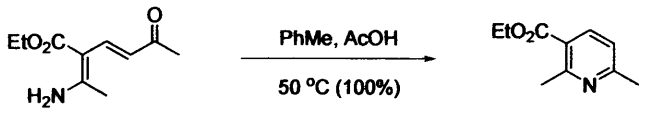
Scheme 2. Synthesis of aminodiene **3a** using a modified alkynone **2d**



1a **2d** **3a**

In view of the fact that the Bohlmann–Rahtz synthesis requires a Michael donor (enamine) and a Michael acceptor (alkynone), it was postulated that the use of a Brønsted[5] (namely acetic acid, AcOH) or Lewis acid[6] may promote the conjugate addition as well as double bond isomerisation of the intermediate aminodienone **3a**, thus facilitating spontaneous cyclodehydration to pyridine **4a**. Furthermore the presence of an acid could enable the reaction to proceed at lower temperature than traditional Bohlmann–Rahtz conditions, by acid catalysed protonation of double bonds present in the intermediate **3a** thus obviating the need to isolate the conjugate addition product. Validation of this hypothesis was achieved by stirring aminodienone **3a** in toluene (PhMe) and acetic acid (5:1) to generate pyridine **4a** in excellent yield and without need for further purification (Scheme 3). After verifying the potential for Brønsted acid catalysis from the

Scheme 3. Pyridine formation from aminodienone by Brønsted acid catalysis

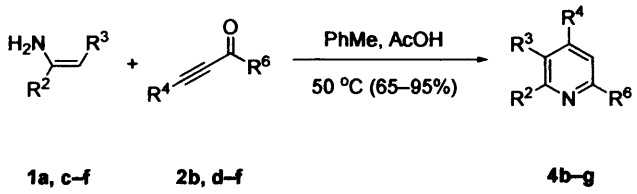


3a **4a**

aminodienone to the corresponding pyridine, a range of enaminoesters **1a, c–f** was prepared according to a modified literature procedure.[7] These were reacted with alkynones **2b, d–f** at 50 °C in toluene–acetic acid (5:1) to provide highly functionalised pyridines **4b–g** in a single step in

good to excellent yield (Bagley, Dale and Bower. Scheme 4, Table 2).[5] Only pyridines **4h–j** failed to form, which in examples **4h** and **4j** was attributed to acid catalyzed decomposition of the starting enamine. In the case of **4i** the reaction is likely to have failed due to the lack of an ethyl ester/electron withdrawing group in the R^6 position of the alkynone, which clearly enhances the formation of **4d** (95% yield).

Scheme 4. Synthesis of functionalised pyridines **4b–g**



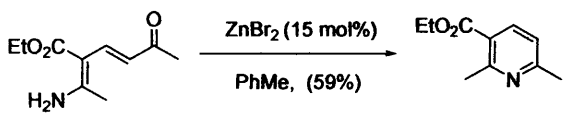
1a, c–f **2b, d–f** **4b–g**

Table 2. Synthesis of functionalised pyridines **4b–j**

R^2	R^3	R^4	R^6	Product	Yield%
Me	EtO_2C	Me_3Si	Me	**4b**	79
Me	EtO_2C	Et	Me	**4c**	85
Me	EtO_2C	Ph	EtO_2C	**4d**	95
Ph	EtO_2C	Et	Me	**4e**	65
Ph	EtO_2C	H	Me	**4f**	73
2–furyl	EtO_2C	Me_3Si	Me	**4g**	80
Me	NC	Et	Me	**4h**	0
Me	EtO_2C	Ph	Me	**4i**	0
Me	$^tBuO_2C$	Me_3Si	Me	**4j**	0

Investigations by the Bagley research group during 2001 and 2002 using Lewis acid catalysis as an alternative to Brønsted acids have been shown to be largely successful. Aminodiene **3a** was treated with various Lewis acids at low temperature, but the use of $BF_3{\cdot}OEt_2$ failed to generate even a trace of pyridine **4a**, whereas zinc(II) bromide (15 mol%) or ferric chloride (10 mol%) in dichloromethane (CH_2Cl_2) did promote the cyclodehydration. Full conversion was achieved when a solution of aminodienone **3a** and zinc(II) bromide (15 mol%) in toluene was heated at reflux for 5 hours, to give pyridine **4a** as the only product in 59% yield (Scheme 5,[6] Table 3[4]).

Scheme 5. Pyridine formation from aminodienone by Lewis acid catalysis



3a **4a**

Table 3. Lewis acid catalysed cyclodehydration of aminodiene **3a**

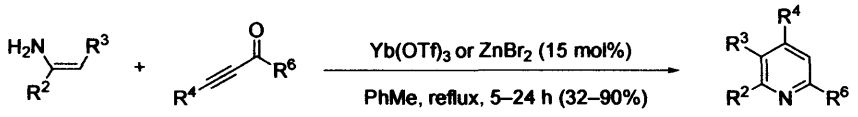
Lewis acid	Conditions	Compound
$BF_3.OEt_2$	CH_2Cl_2, reflux, 4 h	**3a**
$FeCl_2$ (10 mol%)	CH_2Cl_2, reflux, 3.5 h	**3a**, **4a** (3:2)
$Sc(OTf)_3$ (100 mol%)	CH_2Cl_2, reflux, 24 h	**3a**, **4a** (3:2)
$ZnBr_2$ (100 mol%)	CH_2Cl_2, reflux, 24 h	**4a** (46%)
$ZnBr_2$ (15 mol%)	PhMe, reflux, 5 h	**4a** (59%)

Since it was clear that Lewis acid catalysis had the potential to generate the pyridine at relatively low temperatures from the corresponding aminodienone, a range of different Lewis acids was screened in a known Bohlmann–Rahtz heteroannelation reaction in an effort to provide an

alternative one step procedure. A solution of ethyl β–aminocrotonate **1a** and 4–(trimethylsilyl)but–3–yn–2–one **2d** in either dichloromethane or toluene was heated at reflux overnight in the presence of 10–100 mol% of a Lewis acid. No reaction occurred in the absence of a Lewis acid catalyst, but in all other experiments pyridine **4a** was formed, showing that Lewis acid catalysis promotes both the Michael addition and subsequent spontaneous cyclodehydration. The best conditions found used either ytterbium(III) triflate (20 mol%), refluxing in toluene for 18 hours (90% yield) or zinc(II) bromide (15 mol%), refluxing in toluene for 5 hours (90% yield). Neither the aminodienone intermediate **3a** or its trimethylsilyl derivative were isolated from any of these reactions, leading to the conclusion that, in the presence of a Lewis acid, cyclodehydration occurred spontaneously under the reaction conditions. It is worthy noting however, mechanistic studies for the use of Lewis acids were not undertaken and it is assumed that Lewis acid catalysis is facilitated by co ordination to any of the heteroatoms present in the reactant material resulting in isomerisation and cyclodehydration.

In order to extend the scope of this methodology a series of enamines **1a,c,f,g** and alkynones **2b, d–g** was submitted to the Lewis acid catalyzed heteroannelation conditions (Scheme 6), in toluene in the presence of either zinc(II) bromide or ytterbium(III) triflate (Table 4).

Scheme 6. Lewis acid catalysed procedure for the one step synthesis of tri– or tetra–substituted pyridines



1a,b,f,g **2b, d–g** **4b–f, m–p**

Table 4. Heteroannelation catalysed by $Yb(OTF)_3$ or $ZnBr_2$

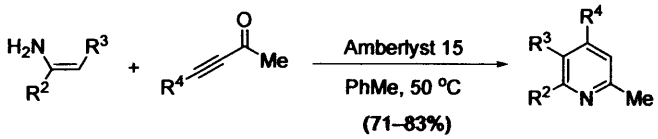
R^2	R^3	R^4	R^6	Product	$Yb(OTf)_3$ Yield%	$ZnBr_2$ Yield%
Me	EtO_2C	Me_3Si	Me	**4b**	90[a]	90
Me	EtO_2C	Et	Me	**4c**	83	67
Me	EtO_2C	Ph	EtO_2C	**4d**	55	85[b]
Me	EtO_2C	Me_3Si	EtO_2C	**4m**	33	44[c]
Ph	EtO_2C	Et	Me	**4e**	32	72
Ph	EtO_2C	Ph	Me	**4n**	68	62
Ph	EtO_2C	Ph	EtO_2C	**4o**	44	65
Ph	EtO_2C	H	Me	**4f**	58	70
2–Pyridyl	EtO_2C	Et	Me	**4p**	68	62

[a] 20 mol% catalyst was used. [b] 10 mol% catalyst was used. [c] protodesilylated pyridine (R^4 = H) was also isolated.

In general, reactions catalyzed by zinc(II) bromide were faster (complete after 5 hours as opposed to 24 hours) and more efficient than those conducted in the presence of ytterbium(III) triflate, with a few notable exceptions (such as the synthesis of pyridine **4c, 4n** and **4p**). It is noteworthy to consider that in the case of zinc(II) bromide it transpires that protodesilylation occurs giving rise to the trisubstituted pyridine (entry **4m**) in one step from the enamine and alkynone rather than two steps in accordance with the traditional Bohlmann–Rahtz procedure. Furthermore control over the retention of the silyl group in the R^4 position could provide a useful synthetic strategy to alternative functionality at the R^4 position, although methodology to employ this feature has not been explored to date. In all cases the Lewis acid catalyzed Bohlmann–Rahtz heteroannelation reaction was successful to give tri– and tetrasubstituted pyridines **4b–f, m–p**,[6] following spontaneous cyclodehydration.

Bagley, Dale and Bower explored milder conditions for conjugate addition/cyclodehydration that would be compatible with acid sensitive substrates, enamines **1a, c–e** and alkynones **2d,e,g** were stirred in toluene at 50 °C in the presence of amberlyst 15 ion exchange resin for 26 hours (Scheme 7). Although reactions involving β–aminocrotonitrile **1c** were unsuccessful, in all other experiments pyridines **4g, i–l** were formed in good yield (Table 5).[5] This procedure is a compelling alternative to those already discussed due to the simplicity of the purification, requiring filtration only to yield the pure product.[4] It was interesting to note that in the case of pyridine **4g**, protodesilylation did not occur in the presence of an acidic resin, yet for pyridine **4j**, protodesilylation was spontaneous under the reaction conditions.

Scheme 7. Synthesis of pyridines using amberlyst 15 ion exchange resin as the catalyst



1a, c–e **2d,e,g** **4g, i–l**

Table 5. Synthesis of functionalised pyridines using amberlyst 15 ion exchange resin

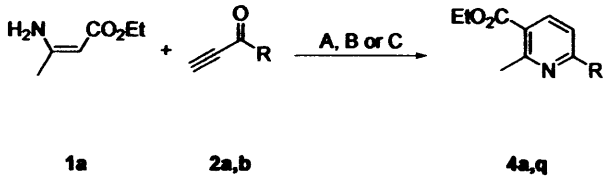
R^2	R^3	R^4	Product	Yield%
2–Furyl	EtO_2C	Me_3Si	**4g**	73
Me	EtO_2C	Ph	**4i**	71
Me	$^tBuO_2C$	Me_3Si	**4j**	83[a]
Me	$^tBuO_2C$	Et	**4k**	80
Me	$^tBuO_2C$	Ph	**4l**	76

[a] Product is the protodesilylated pyridine (R^4=H).

With the success of these facile one–step cyclodehydration reactions firmly established, it remained to investigate the scope and versatility of the Brønsted and Lewis acid catalyzed methods and compare these with the original Bohlmann–Rahtz reaction. Ethyl β–aminocrotonate **1a** was reacted with either but–3–yn–2–one **2a** or 1–phenylprop–2–yn–1–one **2b** using the traditional two–step procedure (Method A) and both of the new one–step acid–catalyzed methods

(Methods B and C) (Scheme 8). All three reactions gave the desired products **4a** and **4q** in excellent yield, the least efficient route to pyridine **4q** being the traditional two–step procedure (Table 6). In contrast the direct study shows that the one–step reactions using but–3–yn–2–one **2a**, although more convenient, were still not as efficient as the traditional Bohlmann–Rahtz method for this transformation.[4] Clearly the yield of products in the original procedure is equally as good as the two catalyzed procedures however, the new methods represented a breakthrough in the search for improved Bohlmann–Rahtz methodology. The catalyzed procedures are suitable for application in synthesis of tri– and tetrasubstituted pyridines that avoided the very harsh conditions of the traditional Bohlmann–Rahtz procedure. More importantly, these new methods can be conducted in a single preparative step and combine favourable facile reaction conditions.

Scheme 8. Bohlmann–Rahtz methodology comparisons



1a **2a,b** **4a,q**

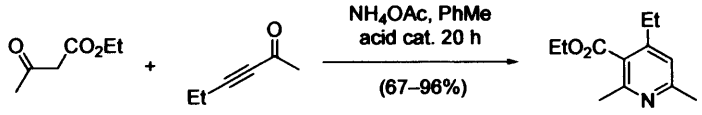
Reagents and conditions: (A) EtOH, 50 °C, 5 h; then 140–160 °C; (B) PhMe–AcOH (5:1), 50 °C, 5.5 h; (C) $ZnBr_2$ (15 mol%), PhMe, reflux, 5.5 h.

Table 6. Comparing the three Bohlmann–Rahtz heteroannelation methods

Alkynone	R	Method	Catalyst	Product	Yield%
2a	Me	A	None	**4a**	85
2a	Me	B	AcOH	**4a**	77
2a	Me	C	$ZnBr_2$	**4a**	65
2b	Ph	A	None	**4q**	80
2b	Ph	B	AcOH	**4q**	85
2b	Ph	C	$ZnBr_2$	**4q**	86

In order to overcome problems of poor substrate availability (enamines **1**), to improve the facility of the whole process and in the discovery of new multiple–component condensation reactions in heterocyclic chemistry, Bagley, Dale and Bower proposed that a three–component condensation of a β–keto ester **5**, alkynone **2** and ammonia would provide a much more efficient approach towards poly–substituted pyridines **4**.[8] To that end ethyl acetoacetate **5a** was reacted with either one or two equivalents of hex–3–yn–2–one **2e** and ammonium acetate in toluene, heated at reflux in the presence of either acetic acid or zinc(II) bromide (Scheme 9). In all cases, pyridine **4c** was formed directly as the only reaction product and isolated in good to excellent yield in a single preparative step with total regiocontrol. With two equivalents of alkynone, the zinc(II) bromide catalyzed reaction gave the best result (96%), compared to acetic acid (78%), although when using one equivalent of alkynone the acetic acid method gave slightly higher yields (68% vs. 66%).

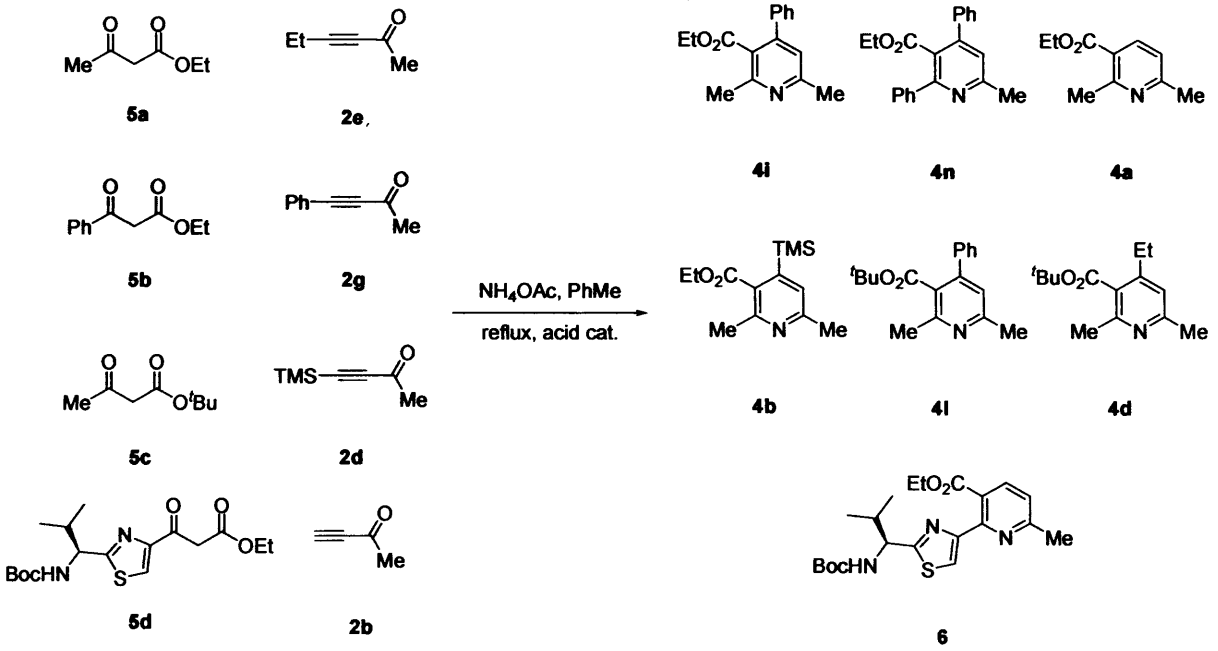
Scheme 9. Three component condensation reaction



5a **2e** **4c**

The scope of this reaction was established with a range of different β–keto esters **5a–d** and alkynones **2b,d,e,g**, which were heated at reflux in toluene under different acidic conditions in the presence of an excess of ammonium acetate (Scheme 10).

Scheme 10. Three component condensation reaction of β-keto ester subset **5**, alkynone subset **2** and ammonia under acid conditions



In all of the cases that were investigated (Table 7), pyridine **4** or **6** was isolated in good to excellent yield (55–96%). Again it was observed that the reaction of 4–(trimethylsilyl)but–3–yn–2–one **2d** in the presence of zinc(II) bromide resulted in partial protodesilylation to give a mixture of pyridine **4a** and **4b** (entry 5). However, by employing acetic acid as the catalyst, only a single pyridine **4a** was produced in 75% yield (entry 6). The use of *tert*–butyl acetoacetate **5c**, caused a reduction in the efficiency of the process but pyridine **4l** was still isolated in moderate yield under zinc(II) bromide or Amberlyst 15 ion exchange resin catalyzed conditions (entries 7 and 9). The synthesis of the *N–tert*–butoxycarbonyl–protected valine derived thiazolyl–pyridine **6**, resulted in a high degree of racemisation in toluene (entry 11), and is likely due to protonation of the nitrogen

Table 7. Examining the scope of the three–component heteroannelation reaction

Entry	β-Keto ester **5**	Alkynone **2**	Equivalents of **5**	Acid catalyst	Product	Yield%[a]
1	**5a**	**2e**	2	$ZnBr_2$	**4c**	96
2	**5a**	**2g**	2	AcOH	**4i**	80
3	**5b**	**2g**	2	AcOH	**4n**	70
4	**5b**	**2g**	2	$ZnBr_2$	**4n**	88
5	**5a**	**2d**	2	$ZnBr_2$	**4a,4b** (44:56)	55
6	**5a**	**2d**	2	AcOH	**4a**	75
7	**5c**	**2e**	3	$ZnBr_2$	**4l**	49
8	**5c**	**2g**	2	Amberlyst 15	**4l**	53
9	**5c**	**2g**	3	Amberlyst 15	**4l**	60
10	**5c**	**2e**	3	Amberlyst 15	**4k**	55
11	**5d**	**2b**	3	AcOH	**6**	68[b]
12	**5d**	**2b**	3	AcOH	**6**	71[b,c]

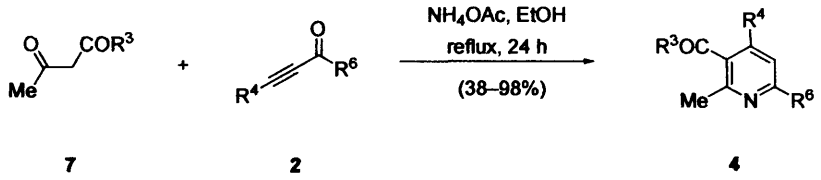
[a] Isolated yield of product. [b] Formed in 78% *ee* (entry 11) or 98% *ee* (entry 12) by HPLC analysis [Chiralpak AD column, hexane-IPA (92:8)]. [c] Reflux in benzene.

atom in the thiazole ring allowing C1 raceimsation however, by switching to benzene these problems were reduced (entry 12).[8]

Clearly the results from Bagley, Dale and Bower are showing that there is scope to react small, cheap and commercially available 1,3 dicarbonyls with alkynones to generate a range of differentially substituted pyridines in good yield, using methods developed within the group. Furthermore it is remarkable that simple 1,3 dicarbonyl compounds that have potentially numerous reactive pathways can undergo Michael addition with an alkynone generating pyridine **6** in good to excellent yield.

Later in 2004 Bagley, Chapernari and Xiong reacted a range of acid sensitive precursors in order to complement the multi–step three–component condensation process described above, for the synthesis of pyridine heterocycles. To that end, 1,3–dicarbonyl compounds **7a–c** and alkynones **2a,d,e,g–i** were heated in ethanol at reflux for 24 hours in the presence of one or ten equivalents of ammonium acetate (Scheme 11).

Scheme 11. Mild acid–free three–component condensation reaction



7 **2** **4**

In most experiments (Table 8), pyridine **4b,c,i–k,q–s,u,x** was generated in moderate to excellent yield (entries 1–12, 38–98%) as the only regioisomeric product. When 4–(trimethylsilyl)but–3–yn–2–one **2d** was used, only protodesilylated pyridines **4b** and **4j** were obtained. It is not clear why 10 equivalents of ammonium acetate result in a lower yield of product (entry 10) then when using 1 equivalent of the same reagent (entry 9). Clearly pyridine **4u** is generated in good yield without significant degradation when 10 equivalents of ammonium acetate are used (entry 8). It would be worth repeating these reactions comparing in each case the yields obtained when using 1 or 10 equivalents of ammonium acetate. However reaction was successful for a wide variety of substrates and constitutes a mild acid–free method incorporating a protic solvent, for the synthesis of poly–substituted pyridines from simple acid sensitive reagents.[9]

Table 8. Examining the scope of the three–component reaction

Entry	Compound 7	Alkynone 2	R^2	R^3	R^4	R^5	NH_4OAc Equivalents[a]	Product	Yield%[b]
1	**7a**	**2a**	Me	OEt	H	Ph	10	**4q**	95[c]
2	**7a**	**2h**	Me	OEt	H	4'–C_6H_4Cl	10	**4r**	84[c]
3	**7a**	**2i**	Me	OEt	H	4'–C_6H_4OMe	10	**4s**	90[c]
4	**7a**	**2e**	Me	OEt	Et	Me	10	**4c**	38
5	**7a**	**2d**	Me	OEt	TMS	Me	1	**4b**	90[d]
6	**7a**	**2d**	Me	OEt	TMS	Me	10	**4b**	90[d]
7	**7a**	**2g**	Me	OEt	Ph	Me	1	**4i**	51
8	**7b**	**2a**	Me	O^{t}Bu	H	Ph	10	**4u**	89
9	**7b**	**2e**	Me	O^{t}Bu	Et	Me	1	**4k**	71
10	**7b**	**2e**	Me	O^{t}Bu	Et	Me	10	**4k**	63
11	**7b**	**2d**	Me	O^{t}Bu	TMS	Me	1	**4j**	98[d]
12	**7c**	**2a**	Me	NH_2	H	Ph	1	**4x**	98

[a] with respect to 1,3–dicarbonyl compounds. [b] Isolated yield after purification. [c] An excess (1.7 equiv.) of 1,3–dicarbonyl compound was employed. [d] Only protodesilylated pyridine (R^4=H) was produced.

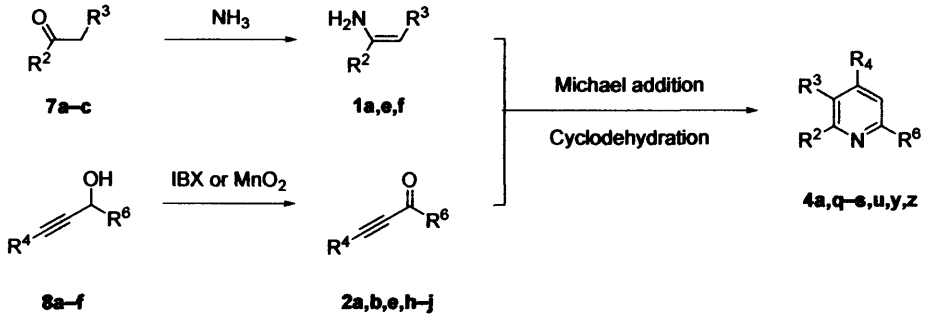
1.2.2 Tandem oxidation–heteroannelation Bohlmann–Rahtz reaction

The *in situ* tandem oxidation–heteroannelation of propargylic alcohols **8** with either *o*–iodoxybenzoic acid (IBX) or manganese dioxide provides 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 heteroannelation reactions proceed in good yield and with total regiocontrol of the pyridine **4** from either enamine **1** or β–keto ester **5** precursors.

Optimum conditions involved heating enamine **1a** and a one–fold excess of both the propargylic alcohol **8a** and *o*–iodoxybenzoic acid in dimethylsulphoxide–acetic acid (5:1) at 65 °C overnight to give pyridine **4a** in 70% isolated yield after purification on silica. With successful conditions

established for the tandem process, a range of propargylic alcohols **8a–e** was submitted to *in situ* oxidation–heteroannelation with ethyl β–aminocrotonate **1a** and *o*–iodoxybenzoic acid (Scheme 12).

Scheme 12. Tandem oxidation–heteroannelation of proparglic alcohols



It was found that the efficiency of the reaction was highly dependent upon the nature of the propargylic alcohol, generating pyridines **4a,q–s,y** in 20–73% yield (Table 9a). It was postulated

Table 9a. *In situ* tandem oxidation–heteroannelation of propogylic alcohols

Using IBX								
β–Keto ester **5**	Enamine **1**	Alcohol **8**	R^2	R^3	R^4	R^6	Pyridine **4**	Yield%
–	**1a**	**8a**	Me	EtO_2C	H	Ph	**4q**	70
–	**1a**	**8b**	Me	EtO_2C	H	4'–C_6H_4Cl	**4r**	73
–	**1a**	**8c**	Me	EtO_2C	H	4'–C_6H_4OMe	**4s**	53
–	**1a**	**8d**	Me	EtO_2C	H	Me	**4a**	45
–	**1a**	**8e**	Me	EtO_2C	H	Et	**4y**	20

that the moderate yield of the one–pot process was a consequence of the oxidative degradation of ethyl β–aminocrotonate **1a** under the reaction conditions. When a range of propargylic alcohols **8a–d,f,** β–keto esters **5a–c** and ammonium acetate were heated at reflux in toluene–acetic acid (5:1) in the presence of manganese dioxide (Scheme 12), pyridines **4a,a',r,s,u,z** were formed directly in up to 96% yield and with total regiocontrol (Table 9b). Thus, generating the enamine *in situ* by the condensation of the corresponding β–keto ester **5** and ammonia helped to reduced enamine degradation and furthermore establish a new and highly facile 3–component cyclocondensation route to these targets that affects four transformations in a single preparative step.[10]

Table 9b. *In situ* tandem oxidation–heteroannelation of propogylic alcohols

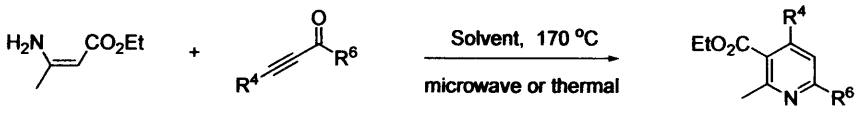
Using MnO_2								
5a	–	**8d**	Me	EtO_2C	H	Me	**4a**	66
5a	–	**8b**	Me	EtO_2C	H	4'–C_6H_4Cl	**4r**	96
5a	–	**8c**	Me	EtO_2C	H	4'–C_6H_4OMe	**4s**	85
5c	–	**8f**	Me	$^tBuO_2C$	Et	Me	**4z**	60
5c	–	**8a**	Me	$^tBuO_2C$	H	Ph	**4u**	73
5b	–	**8b**	Ph	EtO_2C	H	4'–C_6H_4Cl	**4a'**	63

1.2.3 Microwave synthesis

In recent years, the use of microwave dielectric heating in synthetic chemistry has emerged as a valuable alternative to conventional conductive heating methods.[11] This field of chemistry is known as 'Microwave–assisted organic synthesis' (MAOS) and with no direct contact between the chemical reactants and the energy source, microwave–assisted chemistry can be more efficient, in terms of the energy used, capable of providing faster heating rates and able to improve reaction rates and efficiencies. Recent advances in instrumentation, with the introduction of dedicated ovens for organic synthesis that focus microwaves in a monomodal cavity, have increased the popularity and reproducibility of microwave chemistry, increasing the methodology available for the development of new synthetic reactions and optimisation of existing procedures.[12]

In order to expand further the versatility of the Bohlmann–Rahtz reaction and in an effort to explore a microwave–assisted process, Bagley, Lunn and Xiong carried out investigations in a self–tuning single–mode CEM Discover™ Focused Synthesizer.[13] A solution of ethyl β–aminocrotonate **1a** and an excess of phenylpropynone **2a** (Scheme 13) was stirred in toluene or

Scheme 13. One–pot heteroannelation of enamine **1a** with alkynones **2** under microwave or thermal conditions to yield pyridines **4**



1a **2a,d,e,g–i** **4a,c,i,q–s**

dimethylsulphoxide (solvents that have been shown to promote Michael addition in traditional Bohlmann–Rahtz reactions in previous studies) at 170 °C by irradiating initially at 150 or 160 W (Table 10). The reaction conducted in toluene was found to be sluggish at best, providing

pyridine **4q** in 76% yield after 90 min (entry 1). The use of a more polar solvent, dimethylsulphoxide that can couple more efficiently with microwave radiation, resulted in a more

Table 10. Reactions in a sealed tube using either microwave–assisited conditions or conductive heating

Entry	microwave yield%[a]	Thermal yield%[a]	Solvent	Time (min)
1	76	54	PhMe	90
2	87	80	DMSO	20
3	80	33	PhMe–$ZnBr_2$ (15 mol%)	10
4	98	95	PhMe–AcOH (5:1)	10
5	84	93	Neat	20

[a] Isolated yield after purification on silica

rapid reaction. Michael addition and spontaneous cyclodehydration was complete after 20 min, to give pyridine **4q** in 87% yield (entry 2). As expected reactions conducted in toluene were accelerated dramatically by the presence of zinc(II) bromide (15 mol%) providing the product in 80% yield after 10 min at 170 °C (entry 3). However, the optimum conditions for this transformation employed acetic acid. After stirring for 10 min in a solution of toluene–acetic acid (5:1) at 170 °C (initial power 160 W), pyridine **4q** was isolated in 98% yield (entry 4). In a bid to explore solventless reaction conditions, a mixture of enamine **1a** and alkynone **2a** was irradiated at 170 °C (initial power 150 W) for 20 min to give pyridine **4q** in 84% yield (entry 5). Although this final experiment was not as efficient, the use of solventless reaction conditions does have some intrinsic ecological and chemical value by virtue of no waste solvent and thus ultimately no direct impact to the environment.

All of the microwave–assisted experiments facilitated both Michael addition and cyclodehydration in a single synthetic step and generated the target pyridine **4q** as a single regioisomer. A further investigation was carried out in order to establish if traditional conductive heating methods could also facilitate a similar one–pot transformation. The same range of reactions was repeated in a sealed tube using an oil bath as an external heat source and the results were compared with the microwave–assisted reactions (Table 10). In almost all of the experiments, the microwave–assisted conditions gave the product in a higher yield, in particular for the reaction conducted in toluene in the presence of 15 mol% of zinc(II) bromide (entry 3), although in many instances comparable yields were obtained (entries 2, 4 and 5). Only the solventless reaction (entry 5) gave superior results in a Carius tube, this is likely due to the lack of energy transfer when neat reagents were radiated under microwave conditions.

In order to test the scope of the microwave–assisted reaction, ethyl β–aminocrotonate **1a** was reacted with alkynones **2a,d,e,g–i** by irradiating a solution of the reagents in dimethylsulphoxide at 170 °C for 20 min (Scheme 13). In all of the experiments, a single regioisomeric pyridine was formed (Table 11). Although the efficiency of the reaction of enamine **1a** and 4–phenylbutynone **2g** was low (entry 1),[13] this alkynone has been noted to be problematic in similar heteroannelation reactions.[5]

Table 11. Microwave–assisited synthesis of pyridines **4**

Entry	Alkynone 2	R^4	R^6	Product 4	Yield%[a]
1	**2g**	Ph	Me	**4i**	24
2	**2d**	H	Me	**4a**	62
3	**2e**	Et	Me	**4c**	94
4	**2a**	H	Ph	**4q**	87
5	**2h**	H	4'–C_6H_4Cl	**4r**	75
6	**2i**	H	4'–C_6H_4OMe	**4s**	66

[a] Isolated yield after purification on silica

The remaining microwave–assisted reactions gave pyridine products **4a,c,q–s** in good yields after purification by column chromatography (entries 2–6), illustrating that the one–pot microwave–assisted Bohlmann–Rahtz reaction represents a simple and highly–expedient route to tri– and tetrasubstituted pyridines. This is a new and extremely simple method to facilitate Michael addition and cyclodehydration in only 20 min using microwave irradiation, which proceeds with total control of regiochemistry.[13] Nonetheless the microwave procedures described are only viable to substrates that can tolerate the high temperature conditions and therefore should not be considered a general method.

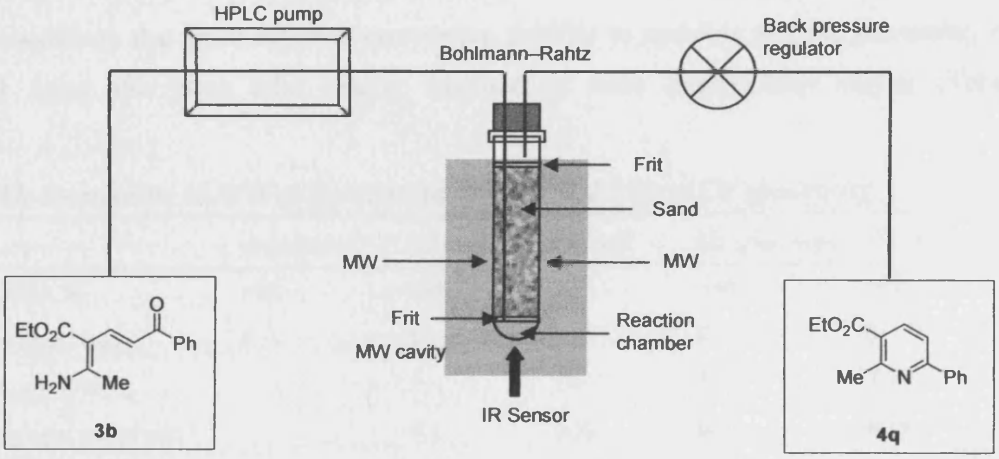
1.2.4 Continuous flow reactors

From the early experiments in domestic ovens to the use of multimodal or monomodal instruments designed for organic synthesis, this technology has been implemented worldwide and continues to be developed.[12] However, although modern monomodal instruments dedicated for MAOS are very successful in small scale operations, efforts to process this technology in continuous flow (CF) reactors are frustrated by the physical limitations of microwave heating, with a penetration depth of only a few centimetres and the limited dimensions of the standing wave cavity. Current technology has attempted to overcome these obstacles with conventional instruments by the use of CF reactors that pump the reagents through a small heated coil that

winds in and out of the cavity, with external temperature monitoring using a fibre optic sensor, although alternative methods, such as using a multimode batch or CF reactor, have also been described.

More recently a new method for carrying out MAOS under CF processing using a commercially available monomodal microwave synthesizer has been described by Bagley, Jenkins, Lubinu, Mason and Wood.[14] The flow cell was inserted into the cavity of a self–tunable monomodal microwave synthesizer, irradiated, and stabilized at the required reaction temperature through moderation of microwave power before the introduction of reagents into the reactor (Figure 1). The reactant, aminodienone **3b** is dissolved in solvent and clearly introduced into the microwave through a small tube (~5 mm internal diameter) via the HPLC pump, the solvent/reactant mixture passes through a layer of sand (~10 g) (necessary to minimize dispersion and create micro–channels) via the small tube and is affected by microwave irradiation in the reaction chamber at the bottom of the reaction tube (10 ml volume). As a result of the continuous pressure exerted by the HPLC pump and that of the back pressure regulator (sand is stopped from escaping the reaction tube by the presence of two frits, shown in white) the reacting mixture is forced up the reaction tube. The mixture passes through the micro–channels in the sand and is collected in a flask and purified initially by column chromatography until the procedure is optimised such that the product, pyridine **4q** can be isolated as a single product by simply evaporating off the solvent.

Figure 1. Schematic diagram of the CF microwave reactor



The development of a new microwave–assisted CF process shown above for the synthesis of pyridines based upon the Bohlmann–Rahtz reaction was one of the first systems to be tested. Aminodienone **3b** was prepared and cyclodehydrated with CF processing under homogeneous

processing rate, mmo

total energy,[g] kJ

---

[a] Batch experiment in

reactor charged with

microwave cavity.

[f] Residency in the hea

Additionally, CF reactions run at the same flow rate used less magnetron energy in a glass tube (method c) than in the Teflon heating coil (method b) (Table 12), demonstrating that a glass tube CF reactor offers (i) improved heating efficiency, (ii) the potential for operation on a large scale, (iii) successful transfer from batch (method a) to CF processing (method c), and (iv) improved performance over commercial Teflon heating coils. As a result of these findings based partially on the Bohlmann–Rahtz synthesis, the scene has been set to transfer the process technology to large–scale operations and ultimately to an industrial based process.

1.3 Applications

As a direct result of the increased awareness of the Bohlmann–Rahtz pyridine synthesis and due to improved Bohlmann–Rahtz experimental procedures, the application of this reaction to the synthesis of a variety of heterocyclic targets is now in evidence. These heterocycles not only include tri– and tetrasubstituted pyridines but also pyrido[2,3–*d*]pyrimidines in combinatorial libraries and as building blocks in natural product synthesis. Although the predominant contributor to the versatility of the Bohlmann–Rahtz pyridine synthesis remains Bagley and co–workers, contributions from both Moody and Baldwin must be acknowledged.

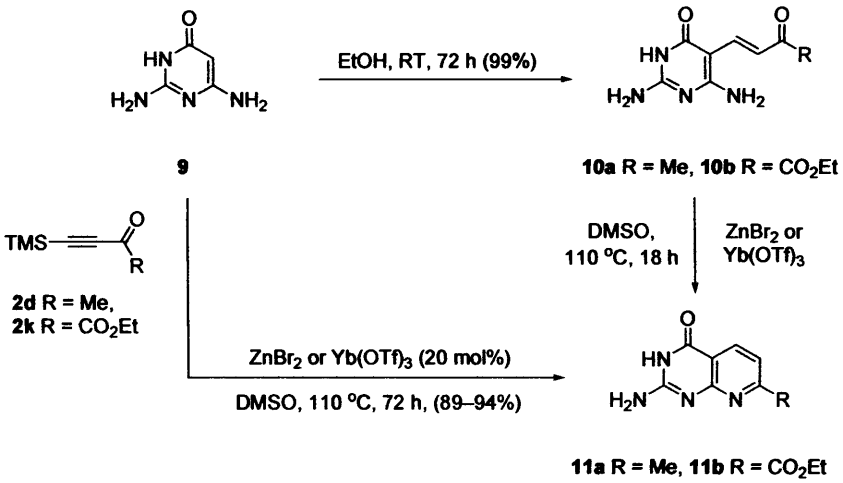
1.3.1 Synthesis of pyrido[2,3–*d*]pyrimidines and uracil derivatives

Pyrido[2,3–*d*]pyrimidine heterocycles (also known as 5–deazapteridines) have received much less attention in the literature in spite of their structural relationship to both pyridines and pterins, the latter isolated from the wing pigments of European butterflies as long ago as the nineteenth century.[15] Interest in pyrido[2,3–*d*]pyrimidine derivatives has increased dramatically in recent years, based upon a diverse range of biological properties and the potential for folate antagonists[16] to elicit highly species–specific responses as antitumour,[17] antibacterial,[18] anti–inflammatory[19] and insecticidal agents.[20]

To continue interests in the synthesis of nitrogen–containing heterocycles, the development of new methods for the synthesis of highly–functionalised 5–deazapterins, by modifying the Bohlmann–Rahtz pyridine synthesis has been reported, for the rapid assembly of a targeted library of folate antagonists. Central to the approach was the need to develop a novel method, using readily available starting materials and simple experimental procedures, for the rapid synthesis of structurally diverse heterocycles with complete control of regiochemistry. Following the success of a number of modified Bohlmann–Rahtz conditions for the synthesis of pyridines,[4–6,8] it was proposed by Bagley, Hughes, Lloyd and Powers that similar Michael addition–cyclodehydration strategies should be successful for the synthesis of highly functionalised 5–deazapteridine heterocycles.[21]

Earlier work by Bagley, Dale, Hughes, Ohnesorge, Phillips and Bower showed that Lewis acid methodology could be applied to the synthesis of pyrido[2,3–*d*]pyrimidines **11** from the corresponding aminodienone pyrimidine derivative **10** or from pyrimidinone **9** and alkynone **2d,k**. The study showed that when a solution of aminodienone pyrimidine **10a** or **10b** and zinc(II) bromide (20 mol%) in dimethylsulphoxide was stirred overnight at 110 °C the heteroannelated product, **11a** or **11b** respectively, was isolated in quantitative yield (Scheme 15). Furthermore when a solution of pyrimidinone **9** and either alkynone **2d** or **2k** in dimethylsulphoxide was stirred at 110 °C for 72 hours in the presence of either zinc(II) bromide (20 mol%) or ytterbium(III) triflate (20 mol%), the zinc(II) catalyzed reactions gave pure pyrido[2,3–*d*]pyrimidine **11a,b** in 92 and 89% yield, respectively, whereas the ytterbium(III) catalyzed process gave a slightly impure product **11a,b** in 94 and 91% respective yield (Scheme 15).[6]

Scheme 15. Synthesis of pyrido[2,3–*d*]pyrimidines **11a,b** using Lewis acid catalysis

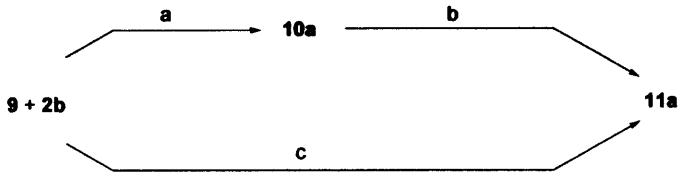


The application of Bohlmann–Rathz methodology to the synthesis of pyridopyrimidones was expanded further by assessing the treatment of pyrimidinone **9** with one equivalent of alkynone **(2b)** in a range of solvents at room temperature or 50 °C according to standard conditions for Bohlmann–Rahtz pyridine synthesis (Table 13). The optimum conditions to facilitate the reaction involved stirring at room temperature, where the product yield was equal to (entries 1–3) or better than (entries 4–8) the corresponding process run at 50 °C. It is noteworthy that the choice of solvent had a large influence upon the course of the reaction. In experiments using acetone, acetonitrile or 1,2–dimethoxyethane (DME) at 50 °C, only a trace of Michael addition product

depending upon

addition product

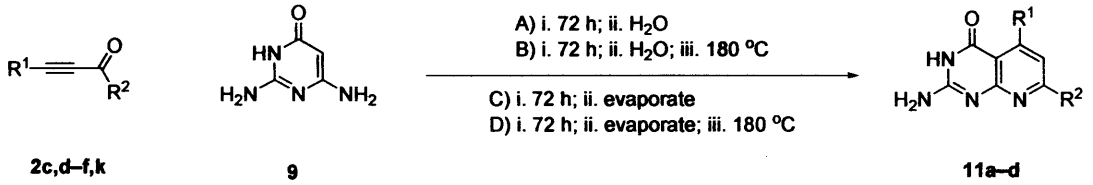
Scheme 16. Summary of conditions to synthesize **11a**



Reagents and conditions: (a) EtOH, MeOH or DMSO, 50 °C, 72 h (72–95%); (b) 180 °C (100%); (c) AcOH or DMF, 50 °C, 72 h (90–92%).

Bagley and Hughes set out to ascertain whether the methodology could be extended to provide a new general route to pyrido[2,3–*d*]pyrimidine derivatives, pyrimidinone **9** was treated with a range of 4–substituted alkynones **2b,d–g,k** at room temperature in a range of different solvents; acetic acid, ethanol, *N,N*–dimethylformamide or dimethylsulphoxide according to a number of procedures (A–D) (Scheme 17). Details of the optimum results are shown in Table 14.[21]

Scheme 17. Reaction of pyrimidinone **9** and alkynone **2c,d–g,k** in different solvents by method A, B, C or D



2c,d–f,k **9** **11a–d**

It was found that the course of the reaction varied with the choice of alkynone and solvent. Alkynone substitution appeared to slow down the reaction, as evidenced by the presence of starting material after stirring at room temperature for 72 hours in dimethylsulphoxide or *N,N*–dimethylformamide (data not shown). The only reliable method, that tolerated a wide range of substituents, was by stirring pyrimidinone **9** with one equivalent of alkynone **2b,d–f,k** in dimethylsulphoxide for 72 hours at either room temperature or 60 °C, followed by addition of water and filtration of the precipitated solid. As before, in cases where cyclodehydration did not occur spontaneously under the reaction conditions, pyridine annelation could then be affected by heating the resultant solid to 180 °C to give pyrido[2,3–*d*]pyrimidine **11a** or **11b** in excellent yield (entries 3, 5–7). The purity of pyrido[2,3–*d*]pyrimidines **11a–d** prepared by method A or B in dimethylsulphoxide (>95% by ^{1}H NMR) was much higher than similar reactions conducted in either ethanol or *N,N*–dimethylformamide and thus the use of this solvent became the method of choice for the synthesis of these heterocycles.

Table 14. Reaction of pyrimidinone **9** with alkynones **2b,d–g,k** in various solvents and under different reaction conditions

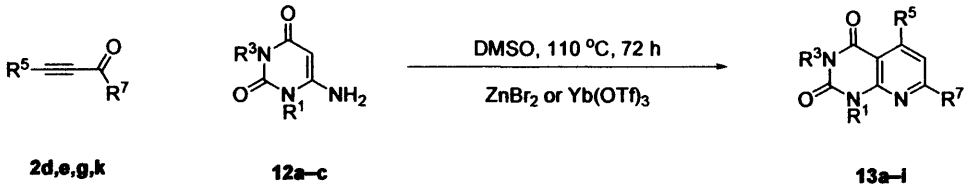
Entry	2	R^1	R^2	Solvent	Method	Temperature	Product	R^1	R^2	Yield%
1	**2e**	Et	Me	DMSO	A	60 °C	**11c**	Et	Me	**84**
2	**2f**	Ph	EtO_2C	DMSO	A	RT	**11d**	Ph	EtO_2C	79
3	**2b**	H	Me	DMSO	B	RT	**11a**	H	Me	86[a]
4	**2d**	TMS	Me	DMSO	B	RT	**11a**	H	Me	96[a,b]
5	**2k**	TMS	EtO_2C	DMSO	B	60 °C	**11b**	H	EtO_2C	95[a,b]
6	**2d**	TMS	Me	DMF	C	60 °C	**11a**	H	Me	71[a,b]
7	**2d**	TMS	Me	EtOH	D	60 °C	**11a**	H	Me	96[a,b]

[a] Cyclodehydration was facilitated by heating to 180 °C in a two–step process. [b] Spontaneous protodesilylation accompanied Michael addition.

The Michael addition and subsequent cyclodehydration of pyrimidinone **9** and a range of alkynones can generate pyrido[2,3–*d*]pyrimidines in excellent yield (optimum yield for preparation of **11a–d** varies between 71 and 98%). This method is effective in a number of different solvents but is most reliable and versatile in dimethylsulphoxide. The experimental procedure is facile and lends itself well to combinatorial methods, requiring no further purification and providing the target heterocycles in one or two steps with total regiocontrol.[21]

Bagley and Hughes also established successful Bohlmann–Rahtz conditions for the synthesis of pyridopyrimidine **13a** from 6–aminouracil **12a** and 4–(trimethylsilyl)–butynone **2d** in dimethylsulphoxide at 110 °C for 72 hours in the presence of either zinc(II) bromide or ytterbium(III) triflate (20 mol%), to give the products in 60 and 52% yield, respectively (Scheme 18). Further application of this reaction showed that 5–deazapterin **13b–i** was generated in good yield from alkynones **2d,e,g,k** and 6–aminouracils **12a–c** (Scheme 18, Table 15). When trimethylsilyl–substituted alkynones **2d,k** were reacted with uracils **12a,b** (entries 1–4 and 7–10, Table 15) spontaneous protodesilylation accompanied Michael addition–cyclodehydration to generate pyridopyrimidines **13a,b,d,e** (R^5 = H). When these reactions were conducted either in the absence of a Lewis acid (entry 2 and 8) or at room temperature (entry 9) the efficiency of reaction was reduced. The optimum experimental conditions reacted uracils **12a–c** with alkynones **2,e,g,k** at 110 °C in the presence of zinc(II) bromide for 72 h to give products **13d–i** in good yield (60–75%). Replacing zinc(II) bromide with ytterbium(III) triflate (entries 12 and 16) caused a small reduction in the efficiency of the cyclocondensation process. It was apparent that the Lewis acid catalyzed cyclocondensation was appropriate for a number of different alkynones and a range of uracil derivatives **12a–c**.[22]

Scheme 18. Reaction of 6–aminouracil **12a–c** and alkynone **2d,e,g,k** in DMSO with a Lewis acid catalyst



2d,e,g,k **12a–c** **13a–l**

Table 15. Reaction of uracil **12a–c** with alkynones **2d,e,g,k**

Entry	12	R^1	R^3	2	R^5	R^7	Lewis acid	Product	Yield%[a]
1	**12a**	H	H	**2d**	TMS	Me	$ZnBr_2$	**13a**	60[b]
2	**12b**	Me	H	**2d**	TMS	Me	None	**13b**	74[b]
3	**12b**	Me	H	**2d**	TMS	Me	$ZnBr_2$	**13b**	94[b]
4	**12b**	Me	H	**2d**	TMS	Me	$Yb(OTf)_3$	**13b**	90[b]
5[c]	**12c**	Me	Me	**2d**	TMS	Me	$ZnBr_2$	**13c**	53[b]
6[c]	**12c**	Me	Me	**2d**	TMS	Me	$Yb(OTf)_3$	**13c**	54[b]
7	**12a**	H	H	**2k**	TMS	EtO_2C	$ZnBr_2$	**13d**	60[b]
8	**12b**	Me	H	**2k**	TMS	EtO_2C	None	**13e**	43[b]
9	**12b**	Me	H	**2k**	TMS	EtO_2C	$ZnBr_2$	**13e**	39[b,d]
10	**12b**	Me	H	**2k**	TMS	EtO_2C	$ZnBr_2$	**13e**	65[b]
11	**12b**	Me	H	**2e**	Et	Me	$ZnBr_2$	**13f**	71
12	**12b**	Me	H	**2e**	Et	Me	$Yb(OTf)_3$	**13f**	68
13	**12b**	Me	H	**2g**	Ph	Me	$ZnBr_2$	**13g**	62
14	**12c**	Me	Me	**2e**	Et	Me	None	**13h**	42
15	**12c**	Me	Me	**2e**	Et	Me	$ZnBr_2$	**13h**	75
16	**12c**	Me	Me	**2e**	Et	Me	$Yb(OTf)_3$	**13h**	72
17	**12c**	Me	Me	**2g**	Ph	Me	$ZnBr_2$	**13i**	72

[a] Isolated yield. [b] Spontaneous protodesilylation accompanied the reaction (R^5 = H). [c] Reactions were run over 96 hours, isolating the Bohlmann–Rahtz intermediate only. [d] Reaction was carried out at room temperature.

The application of a number of modified Bohlmann–Rahtz procedures has led to new general methods for the synthesis of a number of potentially biologically active heterocycles based upon modified uracil derivatives that could be used for the preparation of diverse 5–deazapterin libraries as inhibitors of folate–dependent enzymes. Given the known difficulties surrounding these complex heterocycles (synthesis and handling) it is remarkable to see modified Bohlmann–Rahtz procedures being applied to the synthesis of these compounds in excellent yields using relatively simple methodology.

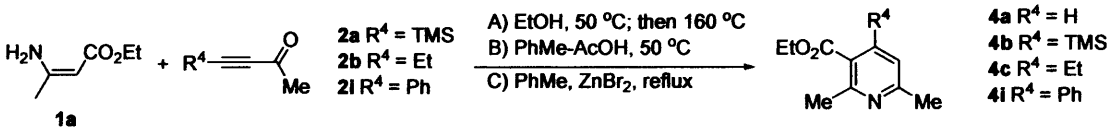
1.3.2 Combinatorial library synthesis

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

application of the Bohlmann–Rahtz heteroannelation reaction has been successful for the combinatorial synthesis of a small pyridine library in solution, establishing the potential of this methodology in combinatorial chemistry.

In all of the reactions investigated, a complete pyridine library was generated with varying product ratios (determined by integration of the ^{1}H NMR for the pyridine 4–H or 5–H resonances compared with the ^{1}H NMR of pure pyridines isolated in previous studies) and high library purity (determined by reference to a known quantity of tetramethylsilane as an internal standard according to established methodology) using a simple acid–base extraction workup procedure. For example, an excess of ethyl β–aminocrotonate **1a** was reacted with an equimolar mixture of three alkynones **2d,e,g** which varied in the nature of the 4–substituent and that have been reported to react differently in heteroannelation processes. A small library of pyridines **4a–c,i** (Scheme 19) was generated using either traditional Bohlmann–Rahtz conditions (method A), stirring in

Scheme 19. Combinatorial synthesis of pyridines **4a–c,i**



acetic acid–toluene (method B), or the Lewis acid catalyzed heteroannelation process (method C) (Table 16). Using the traditional two step procedure (method A), heating the mixture to 50 °C in ethanol, isolating the intermediate, and then heating to 160 °C for 3 h to effect cyclodehydration, resulted in the isolation of four pyridines **4a–c, i** with protodesilylation occurring throughout the course of reaction. With this in mind, the product ratio (*R*) was large and varied between $1 < R < 25$, although the overall yield and product purity were good. Some protodesilylation also accompanied the reaction conducted in toluene–acetic acid (method B) and although the product ratio (*R*) improved, varying between $1 < R < 8.3$, the overall yield was low (30%). However, the Lewis acid–catalyzed conditions (method C) resulted in no protodesilylation and thus gave rise to three pyridines **4b,c,i** in high purity, good overall yield, and in a product ratio (*R*) that only varied between $1 < R < 2$. It was curious to note that the product ratios varied between the different heteroannelation procedures, as did the identity of the major product, indicating that the alkynones display different reactivity profiles according to the method used. The Lewis acid–catalyzed methods appeared to provide the best overall yield, product ratios, and library purity for the solution phase combinatorial synthesis of pyridines from ethyl β–aminocrotonate (**1a**) in the Bohlmann–Rahtz synthesis.

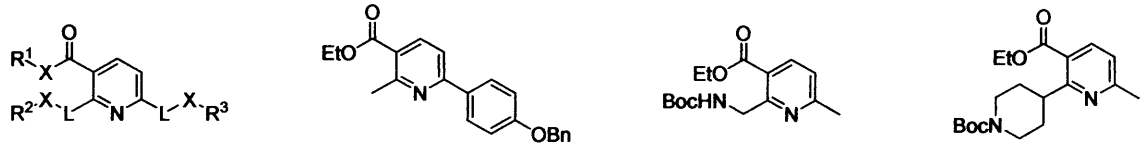
Table 16. Comparison of Bohlmann–Rahtz heteroannelation procedures for the combinatorial synthesis of pyridines **4a–c,i**

Method	Products	Yield%	Ratio	Library purity%
A	**4a–c,i**	62	54:100:65:4	75
B	**4a–c,i**	30	12:52:100:67	80
C	**4b,c,i**	64	100:73:50	86

This approach has the potential to access highly substituted and heavily functionalized pyridines directly from readily available starting materials without any need for subsequent purification and so should complement other published procedures for the solution phase combinatorial synthesis of pyridine libraries.[23]

Simple low molecular weight heterocyclic molecules make ideal scaffolds on which to base the high throughput synthesis of libraries of drug–like compounds. Examples include the use of 1,2,5–thiadiazolidin–3–one–1,1–dioxide and pyrimidine scaffolds in the synthesis of serine proteinase and kinase inhibitors respectively,[24] and the use of simple pyridine scaffolds to generate libraries of inhibitors of HIV–1 protease and Factor Xa.[25] In 2003, Moody and co–workers were involved in a programme designed to develop new heterocyclic scaffolds **14** for library synthesis, trisubstituted pyridines **15–17** containing two points of potential diversity were synthesized using Bohlmann–Rahtz methodology (Figure 2).

Figure 2. Pyridine scaffold **14** and corresponding trisubtituted pyridines **15–17**



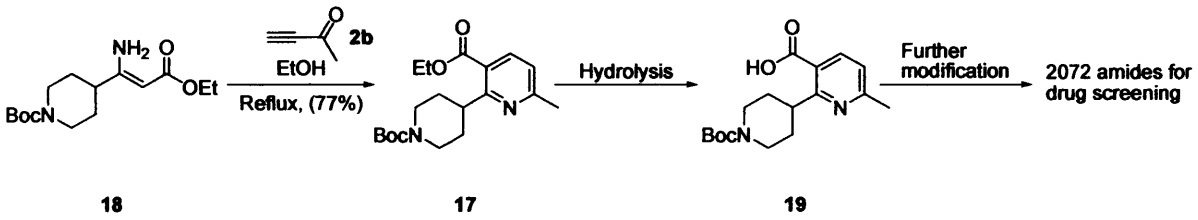
14 **15** **16** **17**

R^1, R^2, R^3 points of diversity; X = O, NH; L = Linker

The conditions for the synthesis of pyridine **17** were optimised by the reaction of enamine **18** with butynone **2b** in boiling ethanol to give product **17** in 77% yield. Hydrolysis of ester **17** gave acid **19** that was then taken on to scale up. Following a design process directed towards drug–like products, acid **19** was coupled to a range of amines to provide 14 protected intermediates in 61–

87% yield on a 100 mmol scale, that were then subjected to reaction with 148 carboxylic acids generating 2072 amides for drug screening (Scheme 20). In summary the use of modified Bohlmann–Rahtz methodology has been demonstrated in an efficient large scale synthesis of the pyridine scaffold **19** containing two points of diversity and applied in library synthesis.[26]

Scheme 20. Drug design library synthesis applying the Bohlmann–Rahtz pyridine synthesis

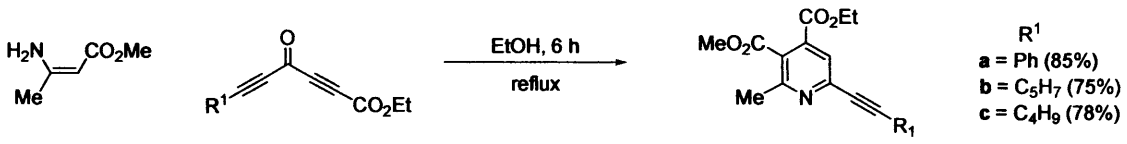


18 **17** **19**

1.3.3 Natural product chemistry

Baldwin and co–workers previously reported that by stirring bis–acetylenes **20a–c** with enamine **21** in ethanol at reflux afforded the corresponding pyridines **22a–c** in good isolated yield (Scheme 21).

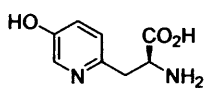
Scheme 21. Bohlmann–Rahtz reaction of bis–acetylenes **20a–c** and enamine **21**



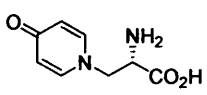
21 **20a–c** **22a–c**

The initial studies by Baldwin (shown above) clearly inspired the synthesis of the pyridine analogue derived from the natural product, azatyrosine **23**. Heterocyclic α–amino acids azatyrosine **23**, mimosine **24**, and lathyrine **25** have been observed to display antibiotic, antitumor, wool growth and pollen growth inhibitory activities (Figure 3).[27]

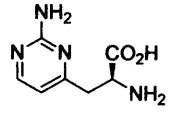
Figure 3. Heterocyclic α–amino acids, azatyrosine **23**, mimosine **24** and lathyrine **25**



L–Azatyrosine **23**



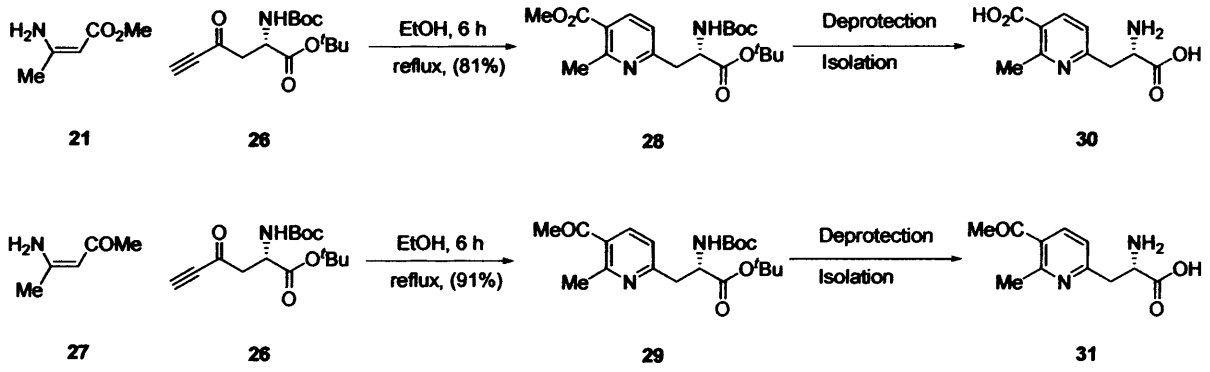
L–Mimosine **24**



L–Lathyrine **25**

The condensation of alkynone **26** and 3–aminobut–2–enoic acid methyl ester **21** or 4–aminopent–3–en–2–one **27**, in ethanolic solution at reflux, gave the corresponding trisubstituted pyridine, β–alanines **28** and **29** in 81 and 91% yield respectively. Subsequent deprotection and purification by ion exchange chromatography afforded the free amino acids **30** and **31** as analogues of the α–amino acid L–azatyrosine (Scheme 22).[28]

Scheme 22. Bohlmann–Rahtz synthesis of analogues **30** and **31** of L–azatyrosine **23**



The most significant application of the Bohlmann–Rahtz reaction is in the synthesis of thiopeptide antibiotics, a class of naturally occurring sulphur–containing, highly modified, macrocyclic peptides, nearly all of which inhibit protein synthesis in bacteria. Structurally, these antibiotics consist of a tri– or tetrasubstituted 6–membered nitrogen heterocycle clustered in a central polyazole domain in a macrocyclic framework of modified heterocyclic residues, including thiazoles, oxazoles, indoles, and dehydroamino acids. Notable examples include; thiostrepton **32**, Sch 40832 **33**, micrococcin P_1 **34** and nosiheptide **35** (Figure 4).[29]

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 containing well over 76 structurally distinct entities. Classification of the thiopeptide antibiotic families is achieved according to their structure and in particular, in the nature of the central heterocyclic domain. Essentially there are five distinct classes of these natural products, assigned based upon the oxidation state of the central pyridine/piperidine (Table 17). All of the series *a* and *b* thiopeptides can be identified by their piperidine or dehydropiperidine central heterocyclic domain, series *c* represents the smallest class of these natural products and to date is made up of only one thiopeptide antibiotic (Sch 40832), possessing a unique central domain consisting of a fully saturated piperidine heterocycle fused to a imidazoline ring. The series *d* family is by far the largest of the series, making up 19 of

Figure 4. Notable examples of the thiopeptide antibiotics representing different families

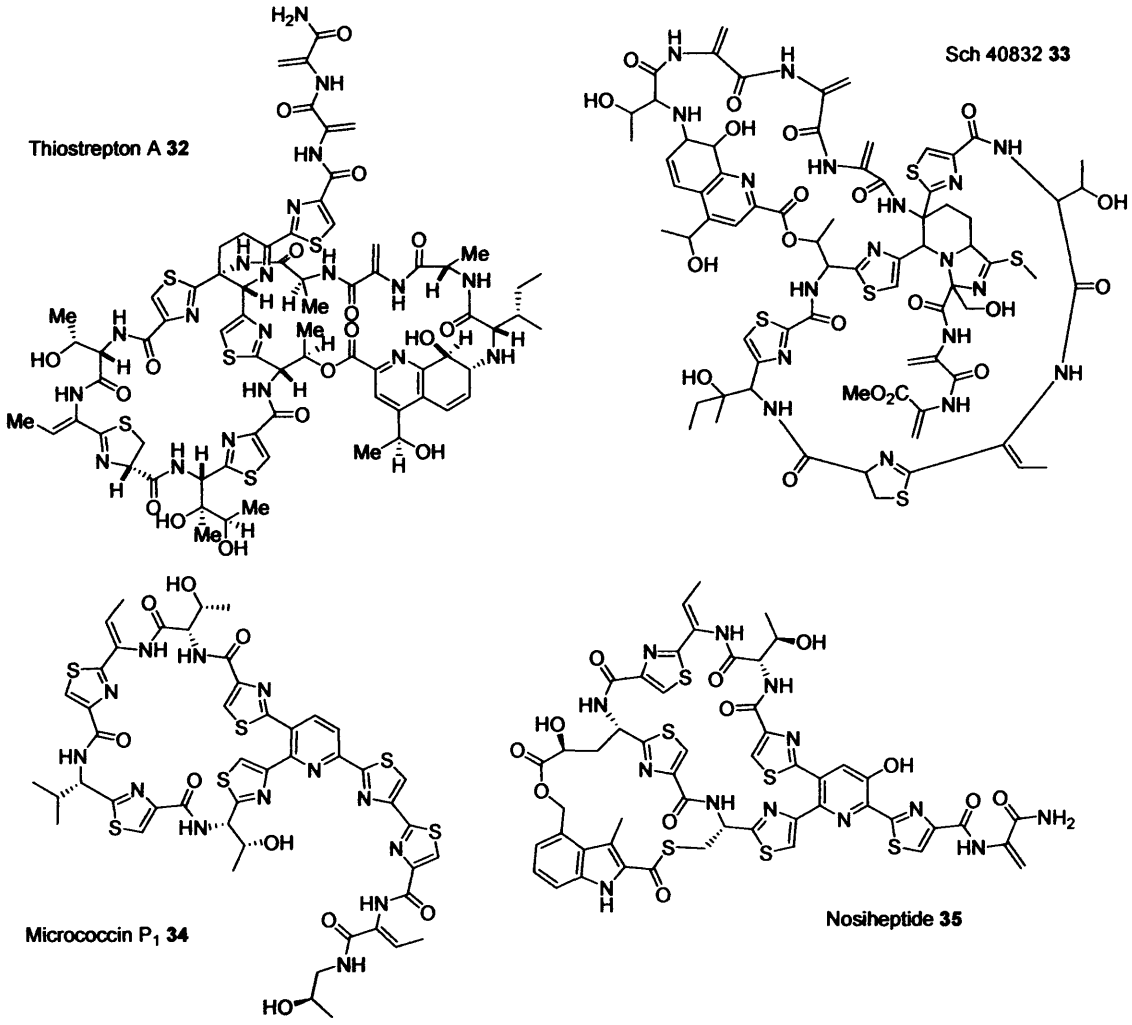


Table 17. Thiopeptide antibiotic families classified according to their central heterocyclic domain

series *a* and *b*	series *c*	series *d*		series *e*
bryamycin (A–8506)[a]	Sch 40832	A10255	promothiocin	glycothiohexide α
Sch 18640 (68–1147)		amythiamicin	AN3323	MJ347–81F4
siomycin		berninamycin	radamycin	multhiomycin[b]
thiactin[a]		cyclothiazomycin	sulfomycin	nocathiacin
thiopeptin		GE2270	thioactin	nosiheptide
thiostrepton		GE37468	thiocillin	S–54832
		geninthiocin	thiotipin	
		methylsulfomycin	thioxamycin	
		micrococcin	YM-266183–4	
		promoinducin		

[a] Shown to be identical to thiostrepton. [b] Shown to be identical to nosiheptide.

the 29 different antibiotic families and containing a trisubstituted pyridine central domain. The series *e* thiopeptide antibiotics contain a 2,3,5,6–tetrasubstituted pyridine with a 5–hydroxy

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Sulfomycin II
Sulfomycin II

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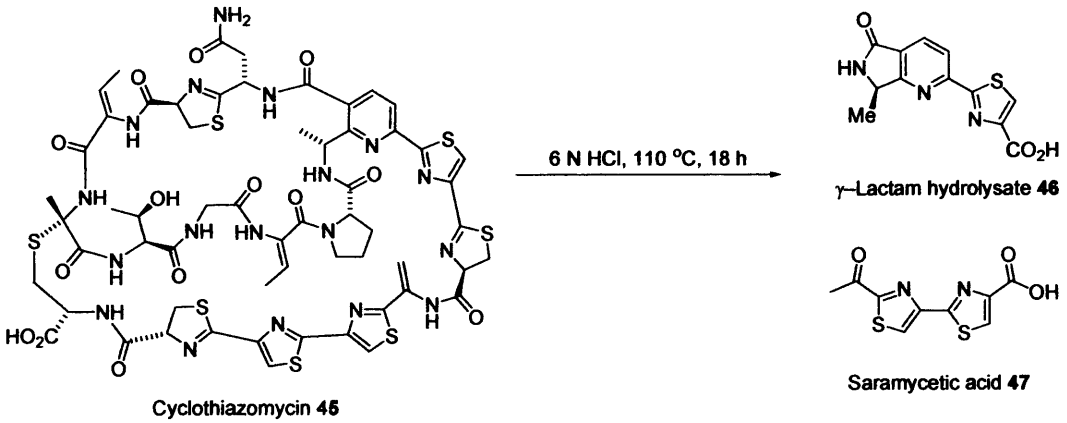
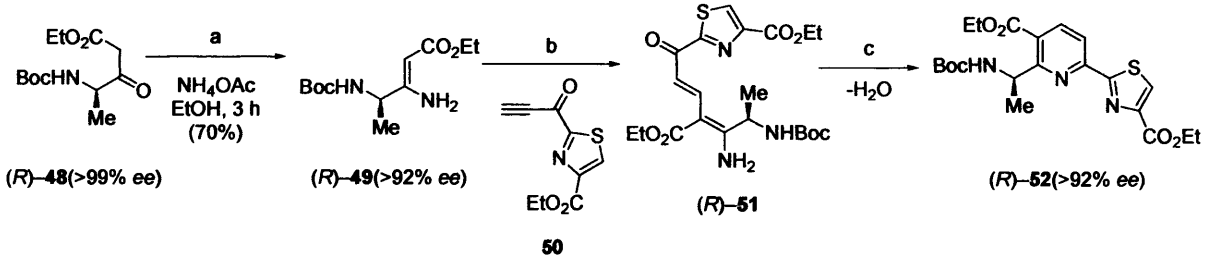
Scheme 25. Sturcture and degradation products of cyclothiazomycin **45**



The preparation of some of the unusual structural motifs present in cyclothiazomycin has attracted synthetic interest,[35] but until recently the stereospecific synthesis of its γ–amino acid central heterocyclic domain had not been addressed. In 2004, Bagley and Xiong approached the synthesis of γ–lactam **46** by constructing the cyclothiazomycin central domain **52** utilizing a Bohlmann–Rahtz reaction to assemble the pyridine from acyclic precursors, starting from the corresponding amino acid **48** (Scheme 26).[36] However, the effectiveness of a Bohlmann–Rahtz strategy, in this case relied upon the ready availability of chiral enamine **49**, which needed to proceed through the heteroannelation without racemization. Reacting the known (*R*)–β–keto ester **48**[37] with ammonium acetate gave enamine **49**; however, during its formation or purification, this chiral intermediate partially racemized on exposure to heat (ethanol at reflux), Brønsted acid (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 (a). The

Scheme 26. Synthesis of cyclothiazomycin central domain **52**



Bohlmann–Rahtz reaction of (*R*)–β–keto ester **48**, enamine **49** and readily available propynone **50** was further investigated by Bagley and Xiong in order to optimise conditions (Scheme 26, Table

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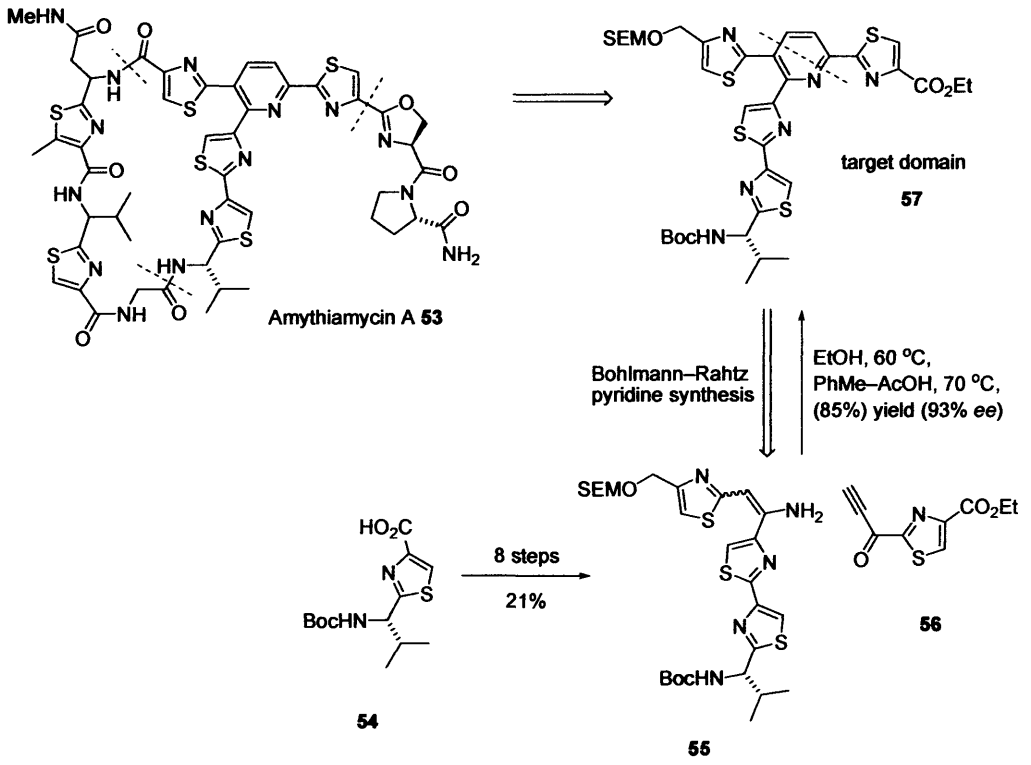
procedure, the total synthesis of γ–lactam **46** was achieved in a remarkable 4 steps from readily available precursors in overall 30% yield and 88% *ee*.[36]

Retrosynthetic studies of the thiopeptide amythiamicin A **53** provided a further application of the Bohlmann–Rahtz reaction by Michael addition–cyclodehydration of enamine **55** and alkynone **56** (Scheme 27). Utilizing conditions successful for the synthesis of other pyridines, Michael addition of enamine **55** and alkynone **56** in ethanol followed by acetic acid–catalyzed cyclodehydration at 70 °C, gave the amythiamicin heterocyclic domain **57** with total regiocontrol in 85% yield and 93% *ee*. This work by Bagley, Dale, Jenkins and Bower, represents the first synthesis of the heterocyclic cluster of amythiamicin, in protected form, generated in only 9 steps and 18% overall yield from **54**, and constitutes a rapid route to the amythiamicin antibiotic family.[38]

Scheme 27. Retrosynthetic analysis of amythiamycin A **53** and the forward synthesis of the target domain **57**



Promothiocin A **58** is a member of the series *d* family of thiopeptide antibiotics and is isolated from *Streptomyces* sp. SF2741. Its structure was elucidated in 1994 by Yun, Hidaka, Furihata and Seto using NMR spectroscopy[39] and although the stereochemistry of the natural product was not reported, it was assumed that the three stereogenic centres originated from natural L–amino acids. The synthesis of this thiopeptide antibiotic is not only one of the first examples to use a traditional

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[19] Deyanov, A. B.;
Konshin, M. E. *Kh*
[20] Heckler, R. E.; J

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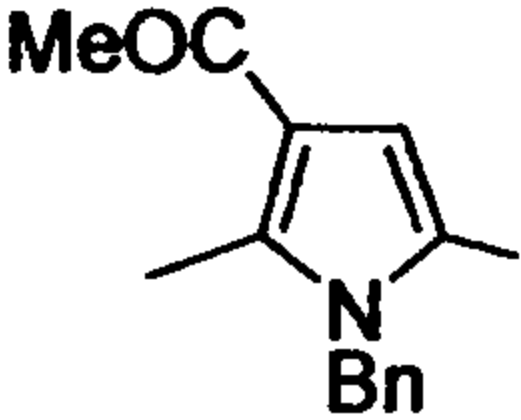

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oxidation of the aminodienone in order to access the series *e* core domain directly are not feasible. There is some scope to revisit this methodology at a later date particularly in view of the poor solvent choice for methods a–c, which is likely to be reacting with the oxidising reagents.

2.4.3 Further investigation of halo pyridines

The lack of promising results to access the series *e* core domain led us to reinvestigate the synthesis of halopyridines. Rather than using bromine as the halogen; we considered synthesizing pyridines bearing chlorine or iodine. In order to investigate halogenation, the corresponding *N*–halosuccinimide and aminodienone **3e** was stirred in ethanol at 0 °C for 1 hour according to our previously reported method for bromocyclization[9] (Scheme 43).

Scheme 43. Reaction of *N*–halosuccimides with aminodienone **3e**

3e **89** X = halogen **3e** **4f**

Reagents and conditions: (a) EtOH, 0 °C, *N*–halosuccinimide, 1 h; (b) EtOH, 0 °C, *N*–iodosuccinimide, 1 h (100%).

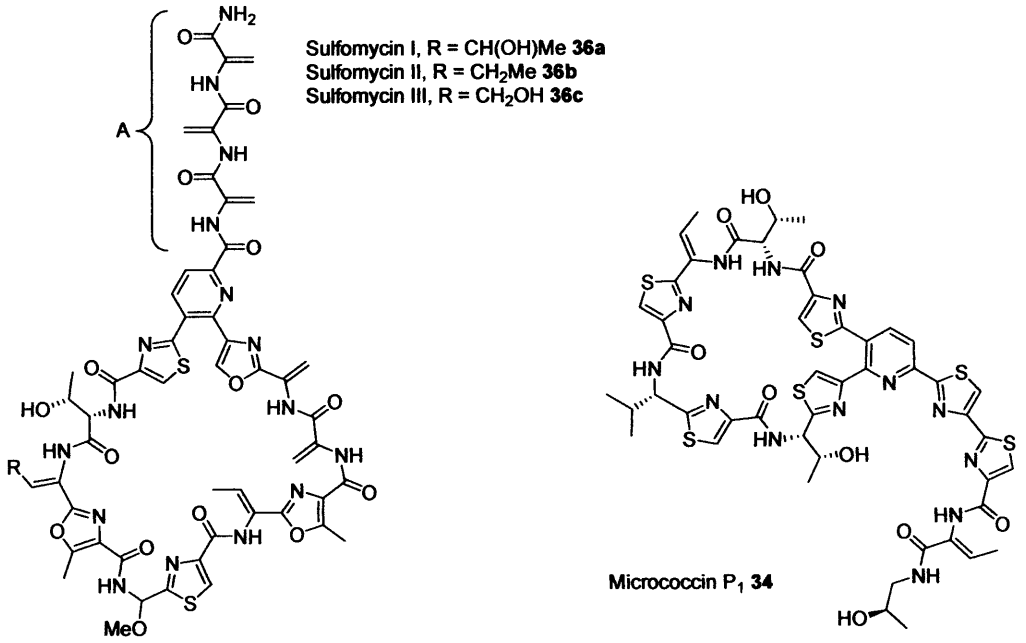
Reaction of aminodienone **3e** with *N*–chlorosuccinimide (NCS) (method a) resulted in a complex mixture of crude products by ^{1}H NMR spectroscopy, no chloropyridine **89** was isolated and after purification only trace quantities of trisubstituted pyridine **4f** could be identified. Surprisingly the same reaction using *N*–iodosuccinimide (NIS) (method b) returned only trisubstituted pyridine **4f** and after purification a quantitative yield was recorded. No halopyridines were isolated using either method, however the method using *N*–iodosuccinimide revealed an astonishing and unexpected result that could be used in the synthesis of the core pyridine domain of the series *d* thiopeptide antibiotics (**2.1**, Figure 5) and therefore demanded further investigation.

2.5 Series *d* thiopeptide antibiotics

Series *d* thiopeptide antibiotics predominantly contain only one macrocyclic peptide loop centred around a 2,3,6–trisubstituted pyridine core domain clustered with thiazole and/or oxazole heterocycles with a peptide side chain consisting of heterocyclic or dehydrated amino acid residues attached at the 6–position of the pyridine ring. As well as being the dominant class of thiopeptide antibiotics they contain 49 distinct entities, which show diversity in structure and biological activity. Notable examples include the sulfomycins I–III **36a–c** and the first reported

thiopeptide antibiotic to be discovered in 1948, found in the sewage from the city of Oxford, micrococcin **34** (Figure 7).[1]

Figure 7. Examples of series *d* thiopeptide antibiotics



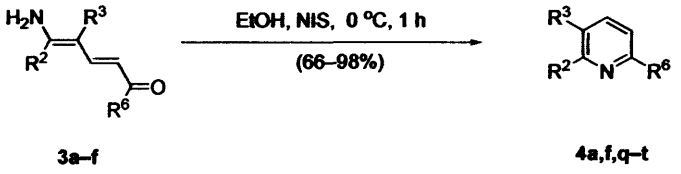
It is noteworthy to discuss the function of the string of dehydroalanine residues (A) attached to the 6–position of core pyridine domain found in the sulfoymicins **36a–c**. So called dehydroalanine due to the fact that investigations have shown this string of residues is constructed biosynthetically from modified alanine amino acid residues.[1] As revealed (Chapter One, **1.3.3**) thiopeptide antibiotics are secondary metabolites from Actinomycetes, these organisms were once thought to be a part of the Fungi kingdom, indicating their need to produce antibiotics. Later it became clear that these organisms displayed features that were both eukaryotic (multi cellular organism) and prokaryotic (single cellular organism) and as a result the antibiotics produced in defence of bacteria could endanger the Actinomycetes existence. In order to combat this self generated threat and regulate thiopeptide production many Actinomycetes exhibit this string of dehydroalanine residues. The mechanisms that bring about this resistance and regulation are beyond the scope of this work, however, suffice to know that the dehydroalanine residues (A) is recognised and docks into specific sites on cell proteins that in turn bring about regulation and resistance. Dehydroalanine residues are not exclusive to the series *d* thiopeptide antibiotics and can be seen in many other thiopeptides. In fact the cell proteins involved in the recognition of the dehydroalanine residues are known collectively as TIP (Thiostrepton Induced Proteins). Clearly

then thiostrepton a series *b*, thiopeptide antibiotic contains the dehydroalanine resiudes recognition site and work that elucidated the function of this feature was originally carried out on thiostrepton.[14] The fact that not all the thiopeptide antibiotics contain the dehydroalanine residues is a clear indication that mechanisms for antibiotic resistance and regulation in Actinomycetes are numerous and complex.[1]

2.5.1 NIS mediated cyclodehydration

In order to investigate the *N*–iodosuccinimide mediated cyclodehydration, aminodienones **3a–f** were stirred with 1.2 equivalents of *N*–iodosuccinimide in ethanol at 0 °C for one hour. The reaction was evaporated *in vacuo* and purified by column chromatography to reveal cyclodehydrated products **4a,f,q–t** in good to excellent yield (Scheme 44).

Scheme 44. NIS mediated cyclodehydration



3a–f **4a,f,q–t**

Remarkably the lowest yield recorded for these facile reactions was when aminodienone **3a** was used to generate trisubstituted pyridine **4a** in 66% yield (entry 1, Table 23), although when the

Table 23. Cyclodehydration of aminodienones **3** mediated by NIS

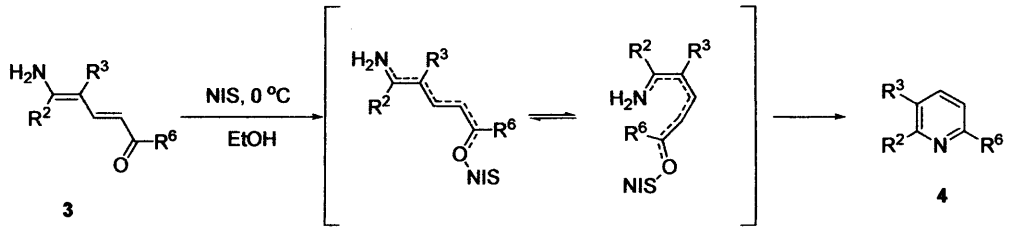
Entry	Dienone **3**	R^2	R^3	R^6	Product **4**	Yield%[a]
1	**3a**	Me	EtO_2C	Me	**4a**	66
2	**3a**	Me	EtO_2C	Me	**4a**	71[b]
3	**3a**	Me	EtO_2C	Me	**4a**	84[d]
4	**3e**	Ph	EtO_2C	Me	**4f**	>98
5	**3b**	Me	EtO_2C	Ph	**4q**	>98
6	**3b**	Me	EtO_2C	Ph	**4q**	>98[c]
7	**3c**	Me	EtO_2C	4'–C_6H_4Cl	**4r**	97
8	**3d**	Me	EtO_2C	4'–C_6H_4OMe	**4s**	>98
9	**3f**	Me	$^tBuO_2C$	Me	**4t**	>98
10	**3f**	Me	$^tBuO_2C$	Me	**4t**	>98[b]

[a] Isolated yield after purification on silica. [b] Reactions were run in the presence of $NaHCO_3$. [c] A catalytic (20 mol%) quantity of NIS was used. [d] Reaction was run over the course of 4 h rather than 1 h.

reaction was run for 4 hours rather than 1 hour the yield increased to 84% (entry 3). Furthermore the use of only a catalytic quantity of *N*–iodosuccinimide (20 mol%) did not adversely affect the course or efficiency of reaction, generating pyridine **4q** in quantitative yield (entry 6).

It is proposed that the Lewis acidity of *N*–iodosuccinimide was responsible for this astonishingly facile cyclodehydration, and this was supported by further experimentation. Repeating the process in the absence of *N*–iodosuccinimide returned unreacted starting material **3** only. When *N*–iodosuccinimide was purified by recrystallization prior to use, or employed in the presence of sodium hydrogen carbonate (to remove HI present or generated throughout the course of the reaction), this did not adversely affect the yield of pyridine **4** and in some instances improved the reaction efficiency (entries 2 & 10). Although no mechanistic studies were performed it is hypothesized that NIS is behaving as a Lewis acid and is likely coordinating with oxygen or at times with nitrogen at various stages of the transformation simply facilitating geometric bond isomerization at low temperature (Scheme 45). The use of *N*–iodosuccinimide in ethanol represents a new mild method for the low temperature cyclodehydration of Bohlmann–Rahtz intermediates for the synthesis of 2,3,6–trisubstituted pyridines **4** in excellent yield.[9]

Scheme 45. Proposed mechanism for NIS mediated cyclodehydration

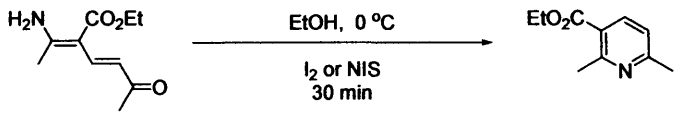


3 4

2.5.2 Iodine mediated cyclodehydration

Although it was evident that traces of HI had not catalyzed the NIS process, it could not be ruled out that traces of iodine (generated by photochemical decomposition) were mediating the reaction. To test this hypothesis aminodienone **3a**, the least efficient substrate in the *N*–iodosuccinimide cyclodehydration reactions, was reacted with a stoichiometric amount of either iodine or *N*–iodosuccinimide in ethanol at 0 °C for 30 minutes (Scheme 46). Under these conditions both reactions gave efficient conversion to pyridine **4a**, but the iodine cyclodehydration was superior, generating the product in quantitative yield after only a simple work up. These transformations

Scheme 46. Cyclodehydration of aminodienone **3a**



3a **4a**

were then repeated in the presence of two equivalents of sodium thiosulphate, added prior to the cyclodehydrating agent, to establish if iodine generated from *N*–iodosuccinimide was responsible for the reaction's facility (Table 24). As expected, the iodine mediated reaction now failed,

Table 24. Comparing stoichiometic I_2 and NIS

Reagent	Yield%	Yield%[a]
I_2	>98	9[b]
NIS	88[c]	80[c]

[a] Reaction was run in the presence on $Na_2S_2O_3$. [b] Starting material **3a** was recovered (91%). [c] Purification on silica was required.

giving only a 9% yield of product and predominantly returning unreacted starting material **3a** (91% recovery). In contrast, the cyclodehydration mediated by *N*–iodosuccinimide was chiefly unaffected by the presence of sodium thiosulphate and gave pyridine **4a** in 80% yield, supporting the hypothesis that it was the Lewis acidity of *N*–iodosuccinimide and not the generation of iodine *insitu*, that was responsible for the reactivity.

Following the success of this study, an alternative aminodienone **3f** was reacted with iodine (0.1–100 mol%) in ethanol at room temperature for 30 minutes in an effort to establish if a process could be developed that was catalytic in reagent. Essentially quantitative conversions to pyridine **4t** were observed under catalytic conditions even at very low iodine concentrations (0.5 mol%, Table 25).

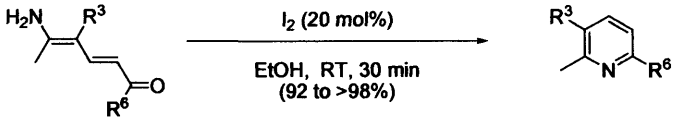
Table 25. Room temperature cyclodehydration of **3g** varying concentration of I_2

I_2 (mol%)[c]	Yield%
100	>98
50	>98
20	>98
10	>98
1.0	>98
0.5	>98
0.1	18[a,b]

[a] From 1H NMR analysis of the crude reaction mixture. [b] A mixture of **3f**:**4t** (5.3:1) was obtained. [c] Reaction was run for 30 minutes

With conditions established for catalytic cyclodehydration, a range of aminodienones **3a–d,f–i** were reacted with catalytic iodine (20 mol%) in ethanol at room temperature for 30 minutes (Scheme 47). After a simple aqueous work up with sodium thiosulphate solution, the 2,3,6–trisubstituted pyridines **4a,q–w** were obtained in excellent yield (Table 26). Only in case of the iodine catalyzed cyclodehydration of **3d** (entry 4) was any further purification required and this was attributed to the poor solubility of the substrate in ethanol.[15]

Scheme 47. Catalytic cyclodehydration of aminodienones **3**



3a–d,f–i **4a,q–w**

Table 26. Cyclodehydration of aminodienones **3** using catalytic quantities of iodine (20 mol%)

Entry	Dienone **3**	R^3	R^6	Product **4**	Yield %[a]
1	**3a**	EtO_2C	Me	**4a**	>98
2	**3b**	EtO_2C	Ph	**4q**	>98
3	**3c**	EtO_2C	4'–C_6H_4Cl	**4r**	>98
4	**3d**	EtO_2C	4'–C_6H_4OMe	**4s**	92[b]
5	**3f**	$^tBuO_2C$	Me	**4t**	>98
6	**3g**	$^tBuO_2C$	Ph	**4u**	97
7	**3h**	$^tBuO_2C$	4'–C_6H_4Cl	**4v**	92
8	**3i**	$^tBuO_2C$	4'–C_6H_4OMe	**4w**	>98

[a] Isolated yield of pure **4** after an aqueous work up. [b] Yield of pyridine **4s** from analysis of ^1H NMR spectrum which showed dienone **3d** (8%) was also present.

2.6 Conclusion

We have been able to access 5–bromopyridines in excellent yield from the corresponding Bohlmann–Rahtz intermediates, a substitution pattern hitherto not accessible from these intermediates. Efforts to elaborate the bromopyridines in order to access the core domain found in the series *e* thiopeptide antibiotics were unsuccessful. However methodology originally anticipated to be valuable in synthesising the core domain of series *e* thiopeptide antibiotics has been applied to the synthesis of the core domain of the series *d* thiopeptide antibiotics. Treatment with *N*–iodosuccinimide represents a new mild method for the synthesis of 2,3,6–trisubstituted

pyridines at low temperature, whilst iodine can be used to mediate the same transformation at ambient temperature with a number of advantages over *N*–iodosuccinimide or other Lewis acids. The procedure is simple to perform, giving the product in excellent yield, without the need for column chromatography and with total regiocontrol.

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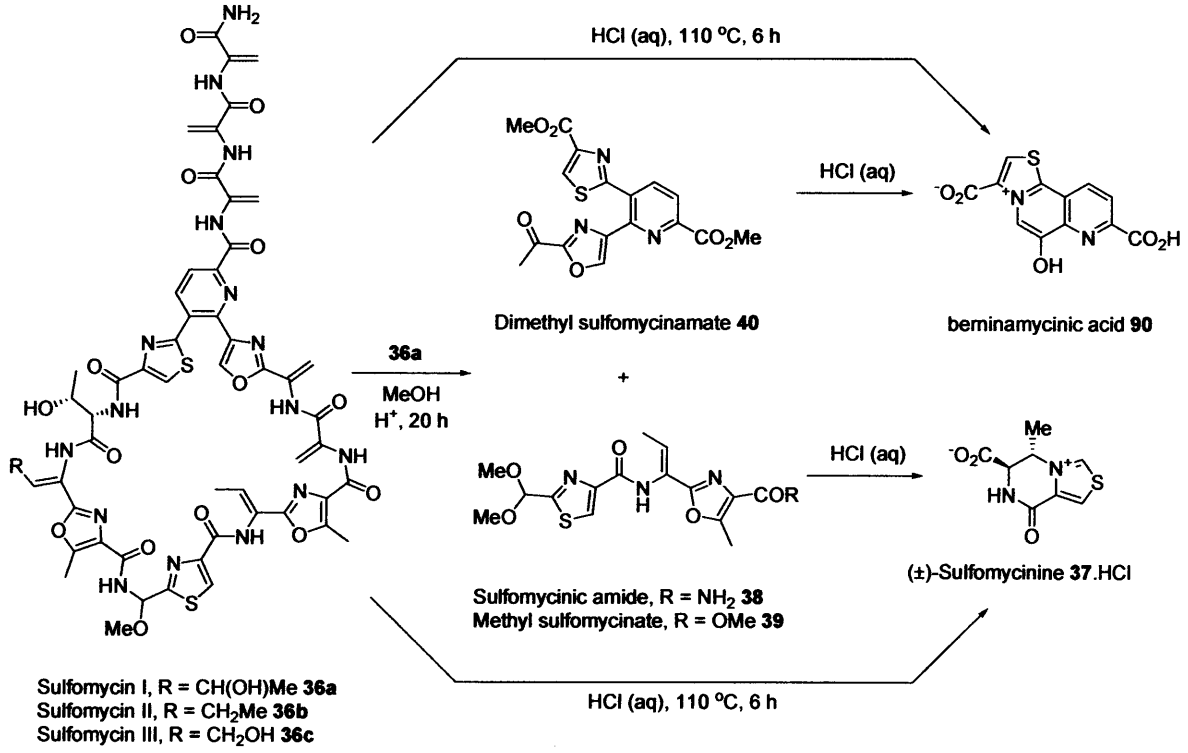
Chapter Three – Results and Discussion

3 Synthesis of degradation products from thiopeptide antibiotics

3.1 Sulfomycins

The sulfomycins **36a–c** are one class of thiopeptide antibiotic isolated from *Streptomyces viridochromogenes* subsp. *sulfomycini* ATCC 29776 and MCRL–0368 with strong inhibitory activity against Gram–positive bacteria.[1] Chemical degradation studies,[2,3] in combination with ^{1}H and ^{13}C NMR spectroscopic and FAB mass spectrometric data,[4,5] elucidated their structure which contains an oxazole–thiazole–pyridine series *d* domain but differs between factors in the identity of one alkenyl substituent (R) located on the cyclic peptide backbone. In these degradation experiments, the acid hydrolysis of sulfomycin I in concentrated hydrochloric acid at 110 °C gave berninamycinic acid **87** and sulfomycinine hydrochloride **37**, both of which have been identified by X–ray crystallographic methods and chemical synthesis.[6,7] Acidic methanolysis of sulfomycin using Amberlyst 15 ion–exchange resin gave dimethyl sulfomycinamate **40**, sulfomycinic amide **38**, and methyl sulfomycinate **39**, the latter of which was transformed to **37** in order to provide corroborating data on the structure (Scheme 48).

Scheme 48. Structure and chemical degradation of the sulfomycins **36a–c**



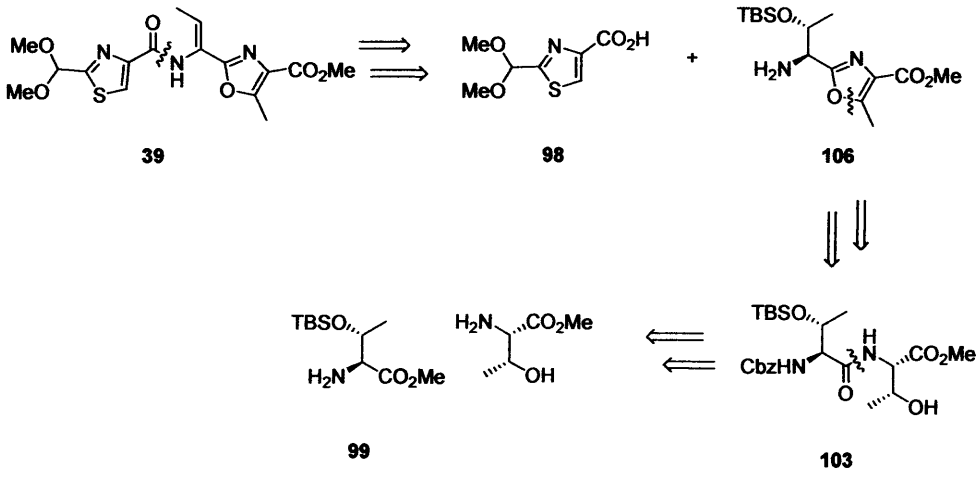
The synthesis of dimethyl sulfomycinamate **40**,[8] by Bohlmann–Rahtz reaction of an oxazolylenamine, further verified the identity of this acidic methanolysis product to complement the findings of Kelly,[9] but the structures of degradation products **38** and **39**, which were proposed on the basis of IR, UV, ^{1}H NMR spectroscopic and MS data for **39**,[2] are unconfirmed. In order to validate a route towards the sulfomycins and as part of our interest in the total synthesis[10] and stereochemistry of thiopeptide antibiotics,[11] we set out to address this shortfall and establish a convergent synthesis of methyl sulfomycinate **39**, which was then converted to sulfomycinic amide **38** by aminolysis. In order to further confirm Abe's assignment of the degradation fragments **38** and **39** we also treated methyl sulfomycinate with 6N hydrochloric acid, isolating sulfomycinine hydrochloride **37** with identical spectroscopic data previously reported.

3.2 Synthesis of degradation products 37, 38 and 39 from the sulfomycins

3.2.1 Retrosynthesis of methyl sulfomycinate

Retrosynthesis of methyl sulfomycinate reveals that its structure is a modified tripeptide that consists of two fragments: an oxazole–containing dipeptide **106** related to the known (–)–muscoride A building block,[12] and a 2–formylthiazole–4–carboxylate residue **98** that is also a component of the antibiotic althiomycin,[13] present as its dimethyl acetal (Scheme 49).

Scheme 49. Retrosynthesis of methyl sulfomycinate

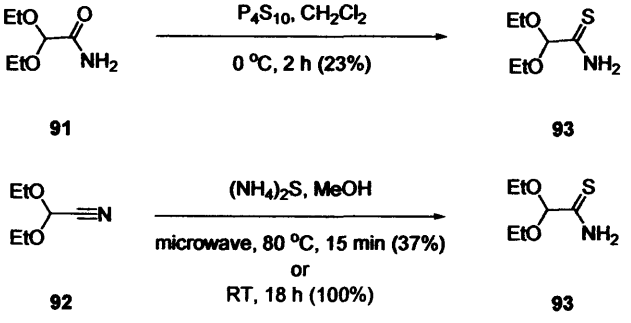


Our convergent strategy assembled each heterocyclic component in turn and then coupled them together with subsequent elaboration to give methyl sulfomycinate **39**.

3.2.2 Synthesis of 2–formylthiazole–4–carboxylate residue 98

Starting with 2,2–diethoxyacetamide **91**, treatment with phosphorous pentasulphide in dichloromethane for 2 hours at 0 °C, according to a modified literature procedure,[14] gave thioamide **93** in 23% yield. However recent studies within the research group identified the effective application of ammonium sulphide in the thionation of nitriles to give thioamides in good to excellent yield.[15] To test whether this method could be used in the synthesis of residue **98**, 2,2–diethoxyacetonitrile **92** was stirred in methanol and ammonium sulphide either under microwave–assisted or traditional thermal conditions. This approach gave thioamide **93** in higher yield than the corresponding reaction with phosphorus pentasulphide. Although quicker than traditional thermal conditions, the microwave–assisted reaction gave the desired product in 37% yield (loss of yield attributed to the solubility of **93** in aqueous phase during aqueous work up), as compared to a quantitative yield (no aqueous work up necessary) when run under ambient conditions for 18 hours. Both methods are viable, however the method using microwave irradiation requires an aqueous work up to purify the reaction mixture, this results in an appreciable loss of yield. As a result of a cleaner reaction under ambient conditions, a superior yield was observed making this the method of choice for the synthesis of residue **98** (Scheme 50).

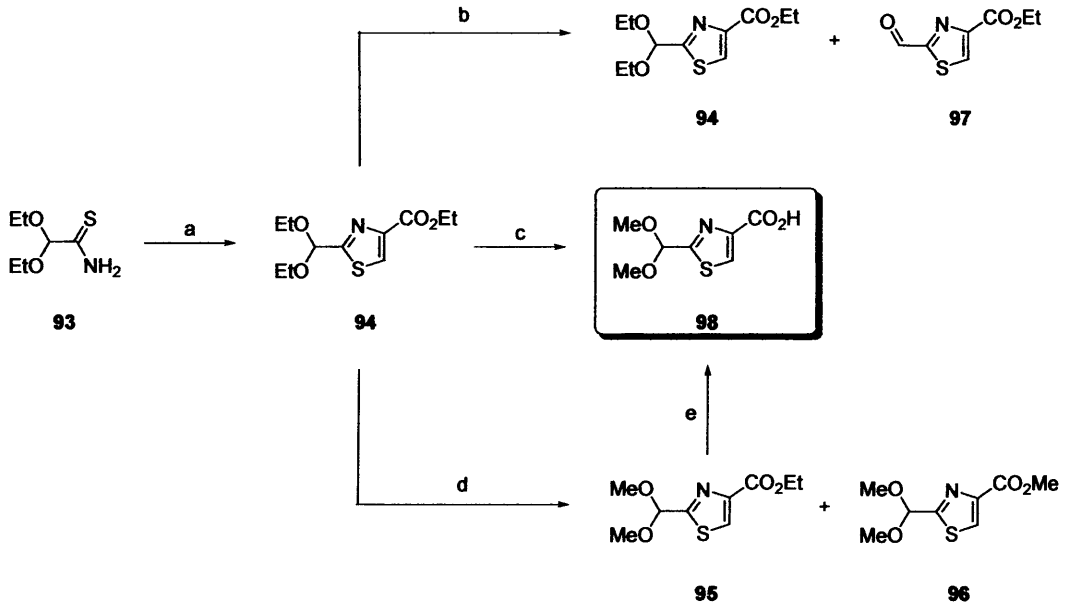
Scheme 50. Synthesis of thioamide **93** from the corresponding amide or nitrile



Ethyl 2–(diethoxymethyl)thiazole–4–carboxylate **94** was prepared by reaction of thioamide **93** with ethyl bromopyruvate in ethanol in the presence of 4Å molecular sieves (to prevent acetal deprotection) under Hantzsch conditions. Thiazole **94** was isolated in quantitative yield after purification twice by column chromatography. Transacetalization was necessary to facilitate the dimethoxy–acetal present in the target compound **39**, a feature commercial unavailable in a corresponding nitrile. The transformation of **94** occurred under acidic conditions with *p*–toluenesulphonic acid (*p*–TsOH) in methanol at reflux for 6 hours gave ethyl 2–(dimethoxymethyl)thiazole–4–carboxylate **95** in good yield (66%). Saponification of compound

95 using lithium hydroxide–water gave the desired 2–(dimethoxymethyl)thiazole–4–carboxylic acid residue **95** in 64% yield. It is noteworthy to discuss that the transacetalization reaction can suffer from the transesterification side reaction, giving a mixture of ethyl and methyl esters, compounds **95** and **96** respectively. This makes purification problematic, due to the compounds' similar R_f values, and can cause a reduction in yield. In order to try and mitigate transesterification the milder acid catalyst, pyridinium *p*–toluenesulphonate (PPTS) was employed. However when thiazole **94** was stirred in methanol at reflux, with PPTS for 6 or 18 hours, unreacted starting material was isolated with some acetal deprotection product **97** (identified by ^{1}H NMR spectroscopy), although no transacetalization appeared to have occurred. In an effort to circumvent this problem, reduce the total number of steps to compound **98** and improve the yield over the two steps, we decided to perform an intuitive one–pot transacetalization and saponification of thiazole **94**. The advantage of this procedure would be any transesterification that may occur in the first part of the reaction would still yield the desired residue **98** upon hydrolysis. Thiazole **94** was stirred in methanol with *p*–toluenesulphonic acid at reflux for 6 hours. After this time an excess of six equivalents of lithium hydroxide monohydrate was added and the reaction mixture was stirred for 48 hours at room temperature. After acidic work up, the desired compound **98** was isolated in 54% overall yield, an increase of 12% over the previous two step method (42%) (Scheme 51).

Scheme 51. Synthesis of residue **98**

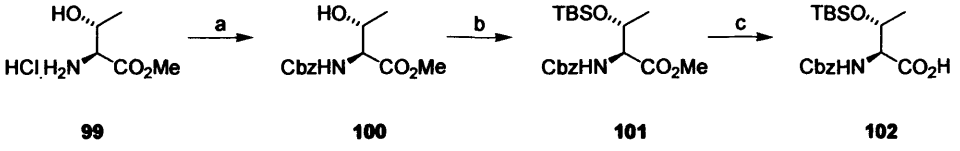


Reagents and conditions: (a) ethyl bromopyruvate, EtOH, reflux, 1 h (100%); (b) PPTS, MeOH, reflux, 6 or 18 h; (c) *p*–TsOH, MeOH, reflux, 6 h; LiOH MeOH–H_2O, 48 h (54%); (d) *p*–TsOH, MeOH, reflux, 6 h (**95**, 66%); (e) LiOH, MeOH–H_2O, rt, 18 h (64%).

3.2.3 Synthesis of oxazole 106

Oxazole **106** was prepared according to a modified method of Pattenden12 from an *O*–silyl ether derivative of L–threonine (Thr) **102**. Synthesis of *N*–benzyloxycarbonylthreonine (Cbz) ester **100** was achieved in good yield (93%) from the corresponding amine hydrochloride, HCl.H–L–Thr–OMe **99**. *O*–Silyl protection of **100** in dry *N,N*–dimethylformamide using *tert*–butyldimethylsilyl–(TBS)chloride (TBDMSCl), imidazole and *N,N*–dimethylaminopyridine (DMAP), by stirring at room temperature for 36 hours, gave Cbz–L–Thr(TBS)–OMe **101** in quantitative yield. Saponification of **101** in methanol–water using lithium hydroxide monohydrate gave the desired Cbz–L–Thr(TBS)–carboxylic acid **102**,[16] in 96% yield (Scheme 52).

Scheme 52. Synthesis of Cbz–L–Thr(TBS)–OMe **102**



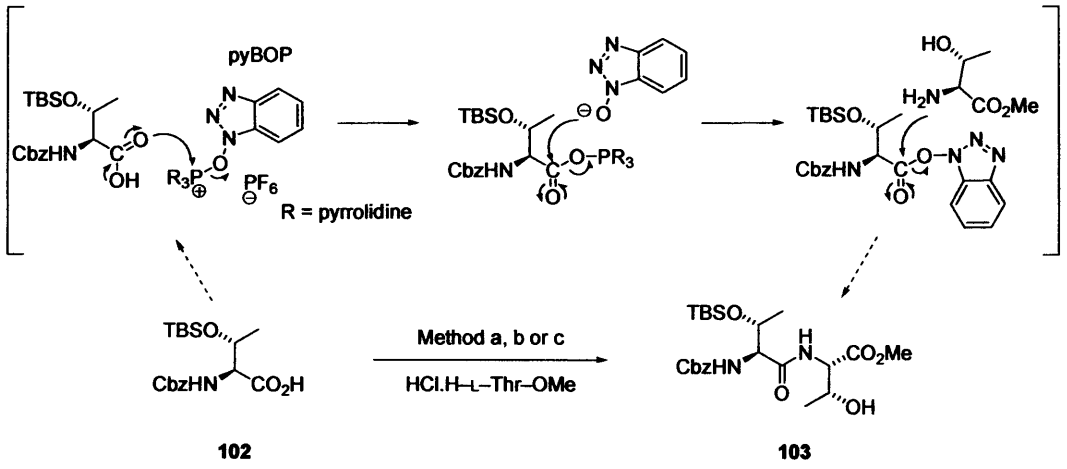
99 **100** **101** **102**

Reagents and conditions: (a) Benzyl chloroformate, $NaHCO_3$, H_2O, RT, 72 h (93%); (b) TBDMSCl, imidazole, DMAP, DMF, RT, 16 h (98%); (c) LiOH, MeOH–H_2O, RT, 18 h (96%).

Peptide coupling was investigated using three different coupling reagents, 1–[3–(dimethylamino)propyl]–3–ethylcarbodiimide (EDCI) (method a), *N*–methyl morpholine (NMM) and isobutylchloroformate (method b)[17] or benzotriazole–1–yloxytripyrrolidinophosphonium hexafluorophosphate (pyBOP) (method c)[12] (Scheme 53). Cbz–L–Thr(TBS)–OH **102**, was reacted according to methods a–c and, after purification by column chromatography, the yields were compared. Method a provided dipeptide **103** in 23% yield, whilst method b provided a much improved yield of 66% in a shorter period of time. However the use of pyBOP furnished a superior yield to either methods a or b, giving dipeptide **103** in 83% yield (method c). pyBOP is from a class of peptide coupling reagents which burst onto the scene in the very early 1990s, championed by Castro, Coste and Le–Nguyen.[18] The coupling reagent incorporates the more traditional hydroxybenzotriazole functionality as well as a phosphonium cation within its structure. Benefits from using pyBOP over hydroxybenzotriazole are measured by the increased activation induced by the phosphonium cation, which helps to ensure immediate activation of a carboxylic acid functionality to aminolysis of a reacting amino acid. The life time of an activated carboxy group is reduced and therefore the common risk associated with coupling amino acid (racemization) is reduced. Furthermore the procedure is very simple, allowing all of the reagents

and substrates to be added to the reaction mixture at the on set of the reaction, purification is relatively easy as the side products of this reaction are usually insoluble and/or do not move off the base line in thin layer chromatography analysis, in elution solvents used to isolate the product. The mechanism for the reaction proceeds via numerous routes and is facilitated by the presence of an organic base (triethylamine), however examples of the fundamental processes involved are illustrated in Scheme 53.

Scheme 53. Synthesis of dipeptide **103**

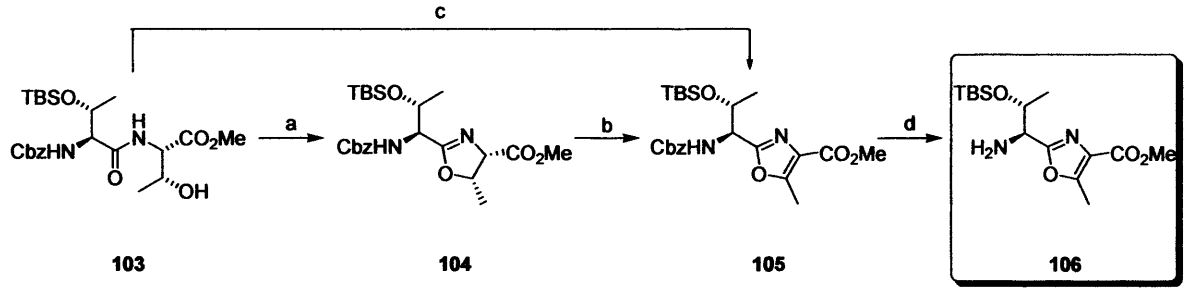


Reagents and conditions: (a) Et_3N, EDCI, CH_2Cl_2, 0 °C, 1 h; then amino acid, RT, 16 h (23%); (b) NMM, isobutylchloroformate, THF, 0 °C, 30 min, then amino acid, 0 °C, 4 h (66%); (c) amino acid, pyBOP, Et_3N, CH_2Cl_2, 0 °C–RT, 18 h (83%).

Cyclization of dipeptide **103** to oxazoline **104** was attempted according to a number of reported procedures. The first method utilized *p*–toluenesulphonic acid in toluene at reflux over a Dean and Stark apparatus,[19] and after purification by column chromatography returned predominantly starting material (50% yield) and two unidentifiable fractions by ^{1}H NMR. Method two used thionylchloride in *N,N*–dimethylformamide at 0 °C followed by stirring at room temperature overnight[20] and after purification by column chromatography returned material that made up 30% of the original mass, however the mixture was unidentifiable (^{1}H NMR) and did not contain either compound **103** or **104**. The third method utilized [bis(2–methoxyethyl)amino]sulphur trifluoride (DeoxoFluor) with stirring in dichloromethane at –20 °C for 18 hours[21] after a basic work up and purification by column chromatography gratifyingly the desired oxazoline **104** was isolated in 65% yield (Scheme 54). The mechanisms surrounding the use of Deoxofluor are discussed later in the context of an analogous reagent, diethyl amino sulphur trifluoride (DAST) (Chapter Four–**4.5**).

Oxidation of oxazoline **104** in dichloromethane using bromotrichloromethane and 1,8–diaza[5.4.0]undec–7–ene (DBU),[22] gave oxazole **105** in 52% yield. It was observed that the ^1H NMR spectrum of the crude material from the cyclization step, after work up, contained no starting material whereas, in some instances, after further purification by column chromatography contamination from dipeptide **103** was observed. This indicated that some hydrolysis of oxazoline **104** occurs in the presence of silica. The hypothesis was further supported by the difficulty observed when low and high resolution mass spectrometry analysis repeatedly found the mass ion (MH^+, 483) for dipeptide **103**, and only after repeated efforts did we observe the mass ion (MH^+, 465) for oxazoline **104**. In an effort to circumvent the instability of compound **104**, and in accordance with a reported procedure,[21] dipeptide **103** was subjected to cyclization and oxidation in one pot giving oxazole **105** in 52% overall yield. A one–pot two–step method not only reduces the overall number of synthetic steps but also improves the yield of oxazole **105**. Hydrogenolysis of the benzyloxycarbonyl protecting group over palladium on carbon in methanol gave the target oxazole **106** in 93% yield (Scheme 54).

Scheme 54. Cyclization and modification of dipeptide **103**

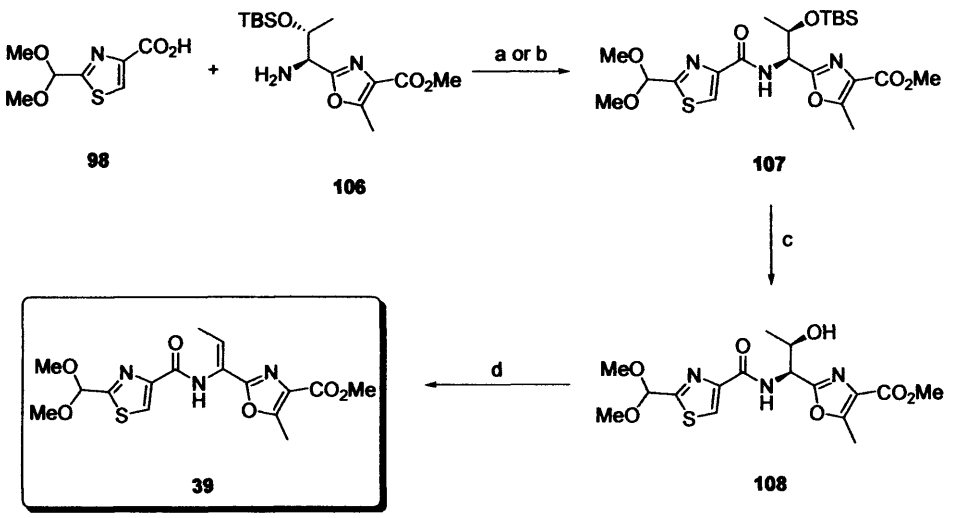


Reagents and conditions: (a) DeoxoFluor, CH_2Cl_2, -20 °C, 18 h (65%); (b) $CBrCl_3$, DBU, CH_2Cl_2, -20 °C, 18 h (52%); (c) DeoxoFluor, CH_2Cl_2, -20 °C, 18 h; then $CBrCl_3$, DBU, CH_2Cl_2, -20 °C, 18 h (52%); (d) H_2, Pd–C, MeOH, RT, 3 h (98%).

3.2.4 Synthesis of methyl sulfomycinate 39

Residues **98** and **106** were coupled to give tripeptide **107** using either *iso*–butyl chloroformate or pyBOP. As previously observed, when using pyBOP there was a notable increase in yield over isobutylchloroformate (88% and 43% respectively). Protodesilylation of **107** by treatment with tetra–*n*–butylammmoniumfluoride (TBAF) in tetrahydrofuran (THF) gave the free alcohol **108** in 77% yield, which was dehydrated via the corresponding methanesulphonate derivative to give the desired methyl sulfomycinate **39** in 60% yield (Scheme 55).

Scheme 55. Methyl sulfomycinate **39**



Reagents and conditions: (a) NMM, isobutylchloroformate, THF, 0 °C, 30 min; then **106**, 0 °C, 4 h (43%); (b) pyBOP, Et_3N, CH_2Cl_2, 0 °C–RT, 18 h (88%); (c) TBAF, THF, 0 °C–RT, 5 h (77%); (d) $MeSO_2Cl$, CH_2Cl_2, RT, 1 h; Et_3N, CH_2Cl_2, RT, 18 h (60%).

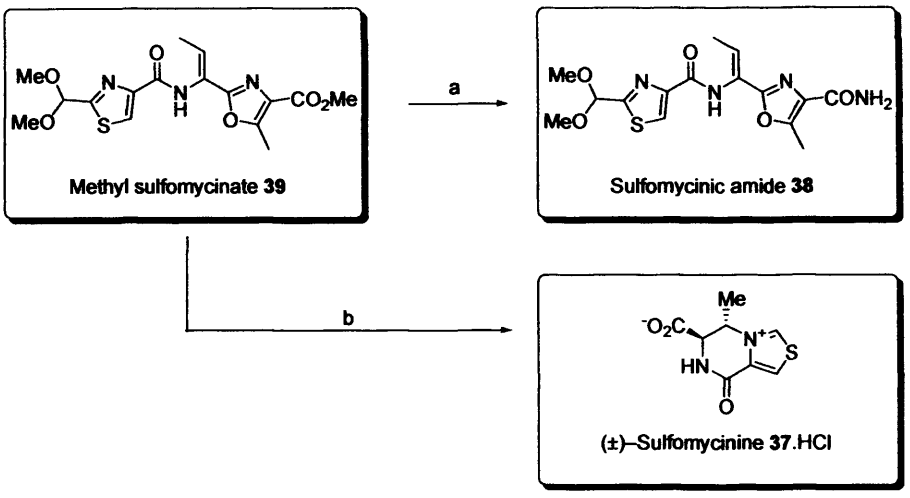
The physical and spectroscopic data of the synthetic material [mp 122–123 °C; IR (KBr)/cm^{-1} 1719, 1684; ^1H NMR ($CDCl_3$) 8.65 (1H, bs, NH), 8.14 (1H, s, CH) 6.68 (1H, q, *J* 7.2, CH), 5.55 (1H, s, CH), 3.82 (3H, s, OMe), 3.41 (6H, s, OMe), 2.57 (3H, s, Me), 1.84 (3H, d, *J* 7.2, CH*Me*); UV (MeOH)/nm λmax 246 (log ε 4.40)] were in excellent agreement with the literature data on the degradation product [mp 124–125 °C; IR (KBr)/cm^{-1} 1715, 1685; ^1H NMR ($CDCl_3$) 8.70 (1H, bs, NH), 8.20 (1H, s, CH), 6.68 (1H, q, *J* 7.2, CH), 5.58 (1H, s, CH), 3.86 (3H, s, OMe), 3.46 (6H, s, OMe), 2.61 (3H, s, Me), 1.87 (3H, d, *J* 7.2, CH*Me*); UV (MeOH)/nm λmax 247 (log ε 4.38)],[2] confirming the outcome of Abe's methanolysis studies[2] and providing a viable route to this region of the sulfomycin cyclic peptides. Furthermore the geometric stereochemistry of methyl sulfomycinate **39** has been assigned as *Z* based upon 2D NOE analysis;[4] by design, anti–elimination of the precursor **108** would give the desired *Z*–configuration. In order to verify the predicted configuration, 2D NOE was used on our synthetic material and gratifyingly we identified a significant correlation between the methyl group of the propene moiety and the NH of the amide.

3.2.5 Synthesis of sulfomycinic amide and sulfomycinine

To complement the degradation studies described and to further confirm the validity of our synthetic material we carried out both the known ammonolysis and hydrolysis of **39**, in methanolic ammonia at room temperature for the former or in hydrochloric acid at 110 °C for the

latter, to obtain sulfomycinic amide **38** and sulfomycinine **37** in moderate yield (24% and 27% respectively) (Scheme 56).

Scheme 56. Degradation products from the sulfomycins



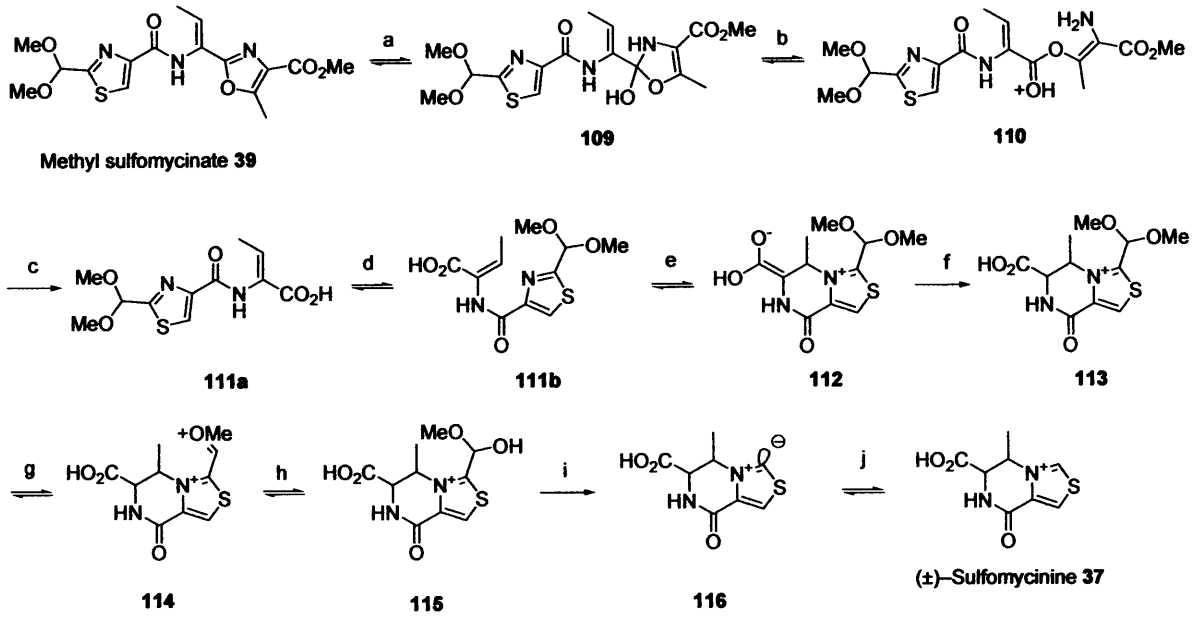
Reagents and conditions: (a) NH_3, MeOH, RT, 2 d (24%); (b) HCl (aq) 110 °C, 2 h (27%).

The physical properties of compounds **37** and **38** corroborate known data.[2,3,7] This convergent synthesis thus confirms both the structures of methyl sulfomycinate **39** and the outcome of its chemical degradation, as well as providing a method for the preparation of this fragment applicable to the total synthesis of the sulfomycin thiopeptide antibiotics in 9 steps from the commercially available H–L–Thr–OMe **96** .HCl and 15% overall yield.[23]

The chemical degradation of compounds **38** or **39** in 6N hydrochloric acid will generate sulfomycinine **37**, the mechanism by which this is achieved is complex as a consequence of the many possible degradation pathways that may occur. The following discussion focuses direct mechanistic pathways necessary to form the degradation product, the reader should be aware that this is by no means the only mechanistic pathway occurring under the conditions described (Scheme 57).

It is evident that the final degradation product **37** must lose the oxazole fragment at some point during the reaction, this is a good a place as any to start. The solution is proton rich therefore the nitrogen of the oxazole ring may become protonated and thus activated for nucleophilic attack by water (a), further hydrolysis will generate compound **109**. Protonation of the same nitrogen on the ring, in conjunction with the donation of a pair of electrons from one of the two possible oxygen atoms (b) will result in formation of intermediate **110**. The final step in the removal of

Scheme 57. Degradation of methyl sulfomycinate **39** to (±)–sulfomycinine **37**

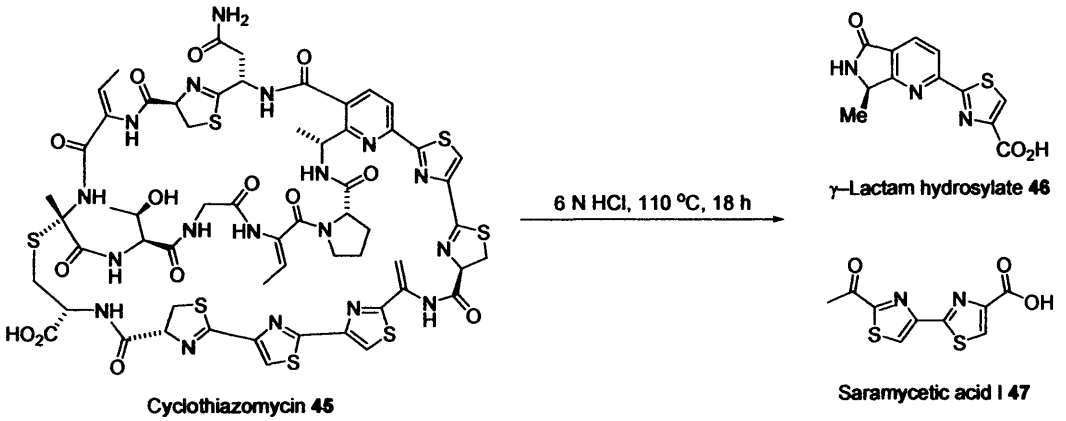


the oxazole (c) requires another molecule of water to attack the activated ester functionality, driving off the unwanted fragment generating **111a**. It should be noted that step c is not shown in equilibrium and would not favour reforming compound **110**, effectively making the transformation unidirectional. In order to precede compound **111a** must undergo geometric isomerization about the dehydrothreonine functionality (d) generating a new conformation, compound **111b**. The α,β unsaturated carbonyl functionality of the compound will now undergo attack from the nitrogen in the thiazole ring (e) to form the thiazolium compound **112**, this can easily reform to the carboxylic acid under the reaction conditions (f) forming compound **113**. The acetal moiety is not required on the thiazolium ring and through protonation of one the methoxy groups and intramolecular attack (g), oxonium ion **114** is generated. Water may now attack (h) to form hemiacetal **115**, this may now undergo elimination in a process common to thiazolium compounds (i), generating a stablized anion adjacent to the cationic nitrogen **116**. Finally sulfomycinine **37** can be formed by protonation of the anion (j).

3.3 Cyclothiazomycin

Cyclothiazomycin is an unusual series *d* thiazolylpeptide that possesses a number of unique structural features. It lacks the 2– and 3– azole substituents on the central core domain, a feature common to series *d* core domains. Instead it contains an alanine derived heterocyclic residue of (*R*)–configuration, quaternary sulphide, and two macrocyclic loops (Scheme 58).[24] Although no

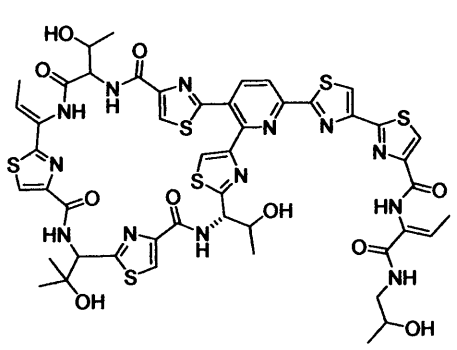
Scheme 58. Cyclothiazomycin **45** and its hydrolysates



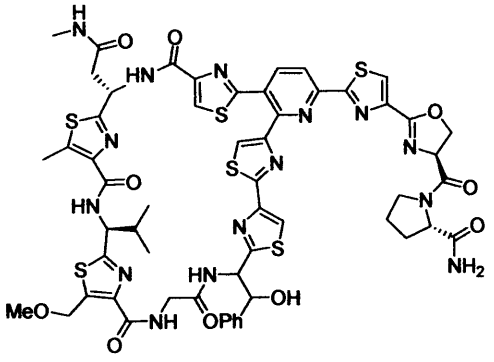
antibacterial data have been associated with cyclothiazomycin, which also lacks the characteristic polydehydroalanine side chain, this thiopeptide is still worthy of note as a novel and selective inhibitor of human plasma renin with an IC_{50} of 1.7 μM. First isolated from the fermentation broth of *Streptomyces* sp. NR0516 from a soil sample collected at Kanagawa, Japan, and purified first by column chromatography and then by preparative HPLC,[25] initial structure determination, using high–resolution HR–FAB–MS mass spectrometry, elemental analysis, and ^{1}H and ^{13}C NMR spectroscopic data, was supported by chemical degradation studies, acidic hydrolysis generating an unusual pyridine containing γ–amino acid as lactam **46**,[26] the identity of which has been verified by synthesis, and saramycetic acid I **47** (Scheme 59).[27]

Saramycetic acid I **47**, also described as 2–(2–acetylthiazol–4–yl)–4–thiazolecarboxylic acid, was isolated as long ago as 1967 from saramycetin, an unidentified antifungal antibiotic. It consists essentially of a bisthiazole moiety with an acetyl substituent at the 2–position and a carboxylic acid in the 4'–position.[28] Bisthiazoles are of interest because they are present in many natural products with useful biological properties. For example it can be found within the side chain of a large number of other thiopeptide antibiotics, including micrococcin, all of the thiocillins (I, **117**, Figure 8), YM–266183, YM–266184, QN3323A, QN3323B and QN3323Y,[1] and in the macrocyclic loops of all of the amythiamicins, the GE2270 factors (A, **118**, Figure 8) and GE37468A.[29]

Figure 8. Examples of thiopeptide antibiotics containing a bisthiazole moiety



Thiocillin I **117**

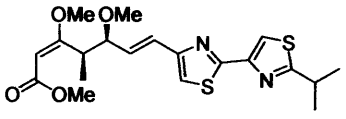


GE2270A **118**

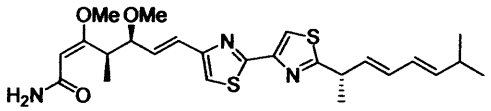
3.4 Natural source and uses of bisthiazoles

Over the last two decades, unique natural products containing directly linked thiazoles have been isolated from natural sources. Owing to their important biological activities, many of these compounds are leads for drug development and have therefore drawn the attention of various research groups. Although there are two natural sources of compounds that contain two or more 2,4–linked azoles, microorganism cultures have predominantly furnished the linked thiazole–containing natural products. Isolation of a family of antifungal cystothiazoles including the potent cystothiazole A **119** (Figure 9) from the culture broth of myxobacterium *Cystobacter fuscus* has been reported.[30] Structurally related to the cystothiazoles and also representative of bisthiazole

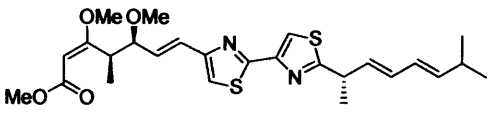
Figure 9. Additional natural products containing a bisthiazole moiety



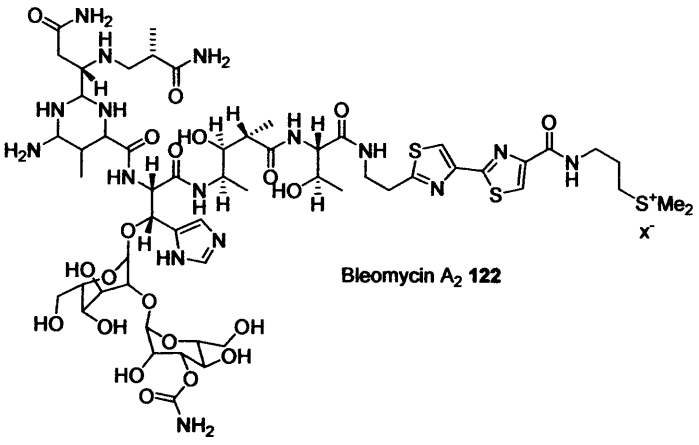
Cystothiazole A **119**



Myxothiazole A **120**



Myxothiazole Z **121**



Bleomycin A_2 **122**

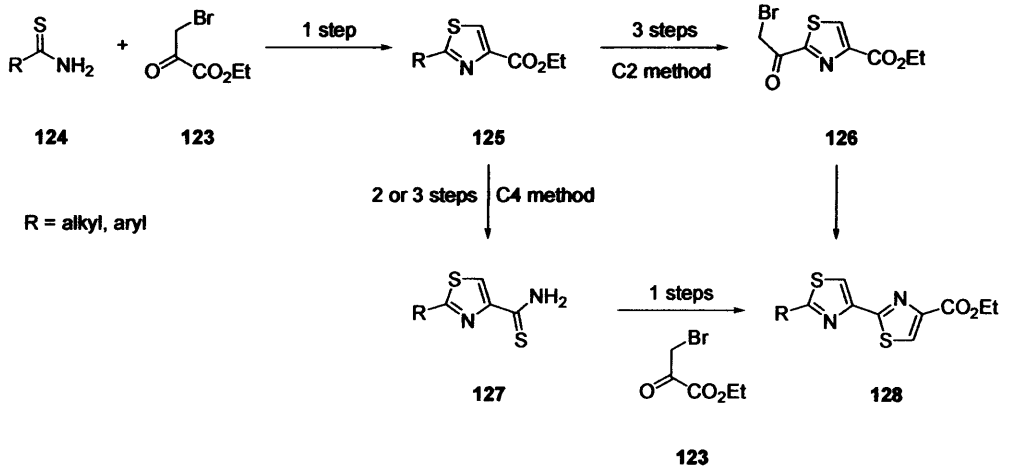
families are myxothiazols[31] **120** and **121** (Figure 9) and melithiazoles.[32] Evidently included within this family are many of the thiopeptide antibiotics discussed above, along with a group of glycopeptide antibiotics isolated from the microorganism *Streptomyces verticillus*.[33] In particular, bleomycin A_2 **122** (Figure 9) is the major component of the anticancer drug bleonoxane, which has found clinical use in combination chemotherapy for the treatment of a range of cancer related illnesses.[34]

3.5 Synthesis of bisthiazole fragments

3.5.1 Hantzsch cyclization

The Hantzsch synthesis is one of the most utilized approaches to prepare polythiazoles. This one pot reaction of a α–haloketone, often ethyl bromopyruvate **123**, with a thioamide **124** mediates the cyclization and subsequent dehydration to give the corresponding thiazole **125**. Once the first ring is prepared, derivatization at the C2 or C4 positions can facilitate formation of the bisthiazole via an iterative process. The C2 route requires transformation to the corresponding α–haloketone **126**, thus generating one of the substrates required for the Hantzsch. However if the more popular C4 route is chosen then transformation to a thioamide **127**, often by ester hydrolysis, amide formation and thionation by Lawesson's reagent or phosphorus pentasulphide generates the Hantzsch substrate. To demonstrate this principal, if α–haloketone **126** and thioamide **124** or thioamide **127** and ethyl bromopyruvate **123** were reacted together then bisthiazole **128** would be generated (Scheme 59).

Scheme 59. Hantzsch synthesis of bisthiazoles



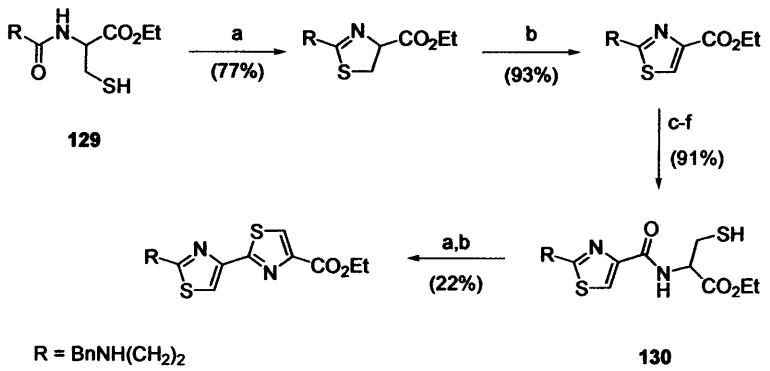
Although no conditions are shown, yields vary from 35–95% on a range of reactions; it is noteworthy that the C2 method has been used to prepare the bisthiazole fragment found in

bleomycin A_2,[35] whereas C4 methodology has been applied to the synthesis of derivatives of the antibiotic GE2270A.[36]

3.5.2 Formation and oxidation of thiazolines

Inspired by the biosynthesis of thiazoles, several synthetic routes to bisthiazoles employ cysteine derivatives. This strategy has also been applied to the synthesis of a bisthiazole fragment of bleomycin B_2.[37] The procedure is based on sequential formation of thiazoline rings from cysteine derived peptides **129** and **130** with hydrochloric acid, followed by oxidation with nickel peroxide (Scheme 60).

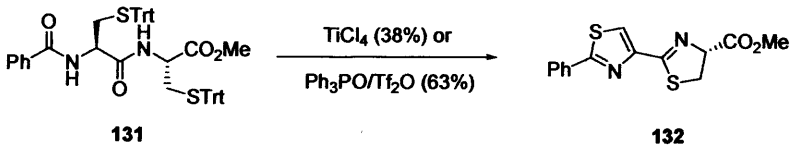
Scheme 60. Biomimetically inspired synthesis of bisthiazoles



Reagents and conditions: (a) HCl, $CHCl_3$, 0 °C; (b) NiO_2, $CHCl_3$; (c) KOH, aq dioxane; (d) H–Cys(Trt)–OEt, DCC, THF; (e) $AgNO_3$, pyridine, MeOH; (f) H_2S, MeOH.

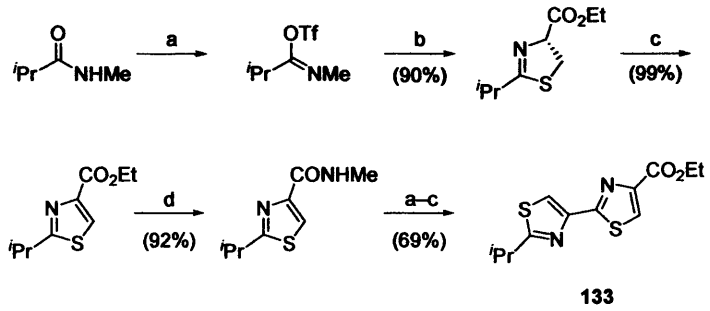
In addition to sequential strategies employed in bisthiazole formation, a one–pot preparation of a thiazole–thiazoline fragment **132** has been reported.[38] This relies on the preparation of a protected cysteine–cysteine dipeptide **131**, and when activated the thiazole–thiazoline is formed by rapid oxidation of one ring. Although further oxidation is not described, one could envisage bisthiazole formation in one step from compound **132** (Scheme 61).

Scheme 61. Synthesis of thiazole–thiazoline **132**



Furthermore thiazoline formation can be achieved by electrophilic activation of an amide, employing triflic anhydride followed by condensation with cysteine. This strategy has been employed to generate (+)–cystothiazole A from **133** in excellent overall yield (Scheme 62).[39]

Scheme 62. Electrophilic activation to give a bisthiazole

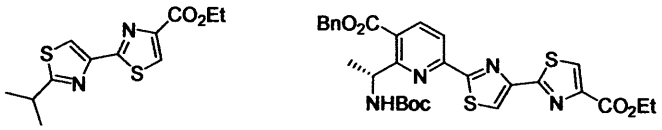


133

Reagents and conditions: (a) Tf_2O, Pyridine, CH_2Cl_2; (b) H–Cys–OEt.HCl, pyridine; (c) $CBrCl_3$, DBU; (d) $MeNH_2$, MeOH.

Combinations of methods discussed above have been utilized to synthesize bisthiazoles. These include compound **134** for the use in the synthesis of cystothiazole A and C, the A–C fragment of micrococcin P_1 and a fragment present in cyclothiazomycin **135** (Figure 10).[34]

Figure 10. Examples of bisthiazoles prepared by Hantzsch or thiazoline chemistry



134 **135**

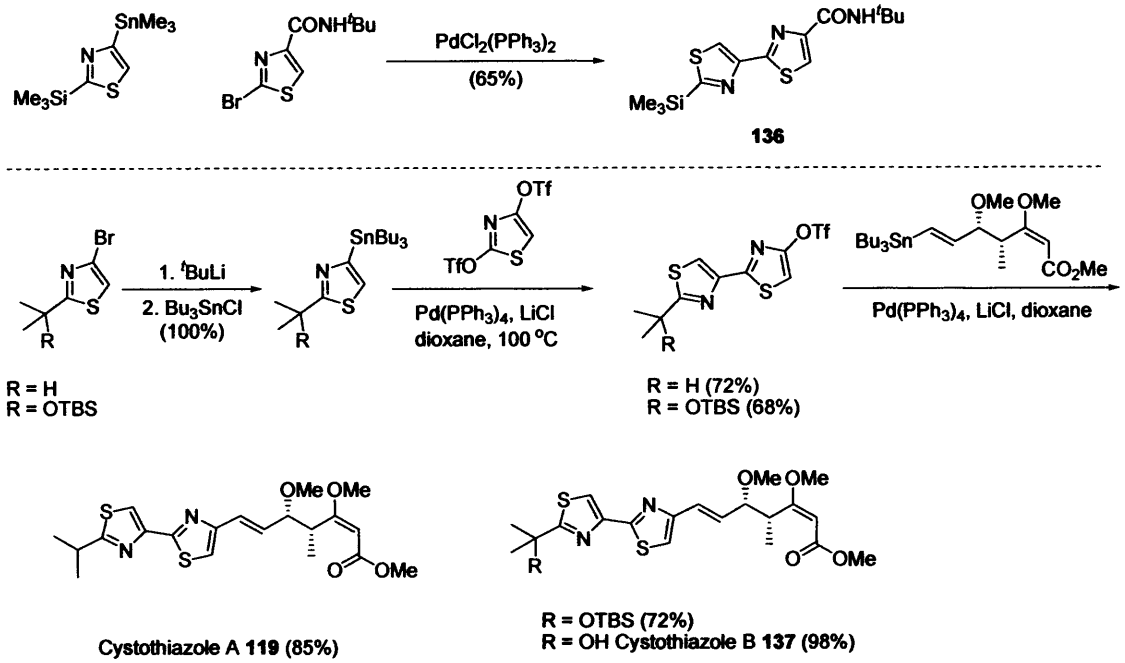
3.5.3 Cross–coupling reactions

Palladium catalyzed cross coupling reactions although useful for aryl–aryl or aryl–heteroaryl bond formation have seen little use in forming 2,4'–bisthiazole bonds probably as a consequence of the difficulty in obtaining the required starting materials. Nonetheless with the appropriate starting material 2,4' bond formations have been described in the synthesis of a number of bisthiazoles including micrococcinic acid,[40] cystothiazole A, B[41] and E[42] and GE2270D2.[43]

The bisthiazole in micrococcinic acid **136** (Scheme 63) was achieved by a Stille cross–coupling reaction, whilst a range of 2,4'–thiazole–thiazole bond formations have been achieved in

moderate to excellent yield, depending on the substitution pattern, by implementing Stille or Negishi methods.[44] Furthermore this strategy has been applied successfully in the total synthesis of cystothiazole A **119**, cystothiazole B **137** (Scheme 64), cystothiazole E and also for the synthesis of the bisthiazole fragment of the thiopeptide antibiotic GE2270D2.34

Scheme 63. Cross–coupling reactions to form bisthiazoles



3.5.4 Summary

Of the different polyazole containing natural products with important biological features, the bisthiazoles are common structures to be observed. In general Hantzsch cyclization has been the preferred method to prepare mono– and bisthiazoles; however commercially available cysteine has also been shown worthy as an alternative strategy to these interesting structures. Limitations on the use of palladium catalyzed cross–coupling strategies have been attributed to difficulties involved in accessing suitable precursors. However there can be no doubt of the effectiveness of cross–coupling reactions in the formation of bisthiazoles.

3.6 Synthesis of saramycetic acid I 47

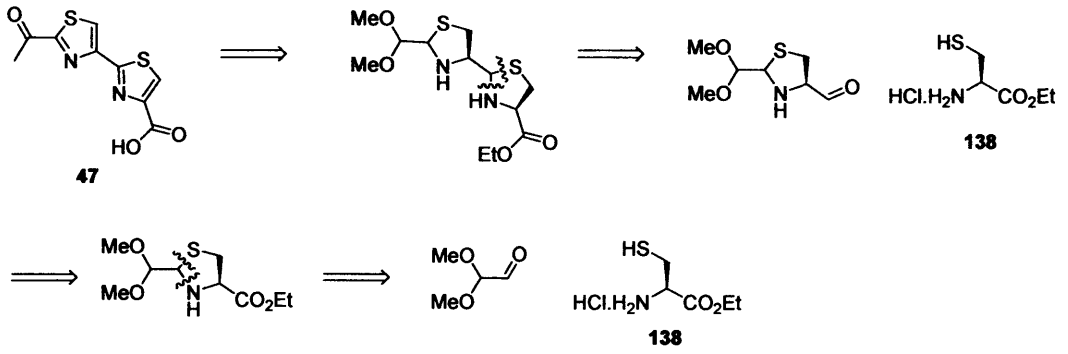
In order to consolidate the methods described above, and as part of our interest in the total synthesis10 of thiopeptide antibiotics, we set out to design and implement a route towards saramycetic acid I **47**, the acid hydrolysate of cyclothiazomycin **45**. Due to the plethora of methods that could lead to the synthesis of the target molecule we decided to neglect

organometallic methods favouring instead a range of other methods investigated in parallel in order to optimise efficiency to the target molecule.

3.6.1 Cysteine–derived synthesis of saramycetic acid I

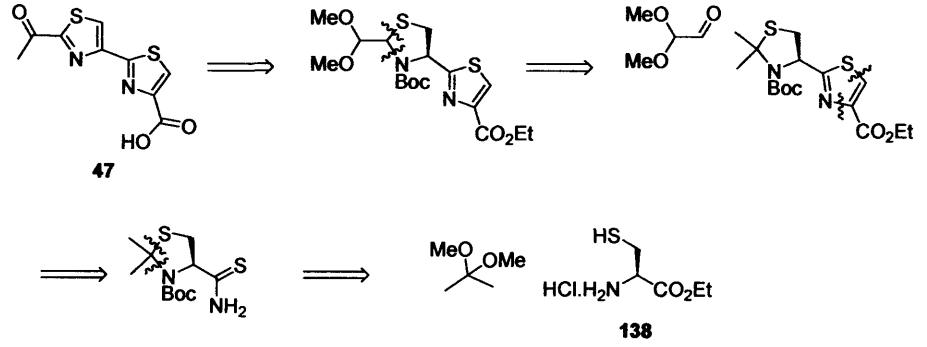
The overall aim of this synthesis was to prepare a bisthiazolidine fragment and investigate its potential for both rings to undergo oxidation in one step, a method not previously reported (Scheme 64).

Scheme 64. Retrosynthesis of a bisthiazolidine fragment



In addition we also considered the potential for thiazolidine formation and oxidation followed by derivatization and elaboration to give a second thiazoline (Scheme 65).

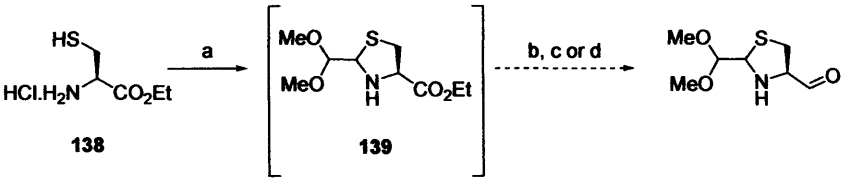
Scheme 65. Retrosynthesis of an iterative bisthiazole fragment



In order to test these hypotheses our initial goal was the synthesis of a thiazolidine ring containing a latent aldehyde functionality **139** that could be manipulated towards the target molecule once the second thiazolidine ring was successfully incorporated. In addition investigations of the susceptibility of thiazolidine **139** by oxidation using manganese dioxide as reported for similar

structures,[45] and its propensity for ester reduction using diisobutylaluminium hydride (DIBAL) were undertaken. Thiazolidine **139** was prepared by the condensation of the free base derivative of the hydrochloride salt of H–L–cysteine–ethyl ester **138** and dimethoxyacetaldehyde in toluene. The compound isolated after evaporation and drying over sodium sulphate in chloroform was characterized by ^{1}H NMR spectroscopic analysis and taken on to the next step without further purification. The material was reacted according to three procedures using DIBAL as the reducing agent (Scheme 66).[46]

Scheme 66. Preparation and reduction of thiazolidine **139**

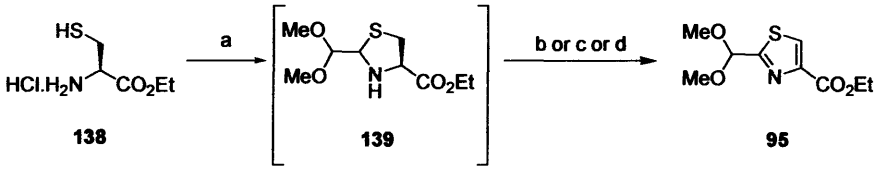


138 **139**

Reagents and conditions: (a) Et_3N, RT, 3 h, filter; then dimethoxyacetaldehyde, RT, 18 h; (b) 1.5 eq. DIBAL, CH_2Cl_2, -84 °C, 2.5 h; (c) 1.5 eq. DIBAL, PhMe, -84 °C, 2.5 h; (d) 1.5 eq. DIBAL, THF, -84 °C, 2.5 h.

The ^{1}H NMR spectrum showed in each case (methods b–d) that after filtration the presence of mostly starting material was observed. There was no significant signal in the spectrum to indicate the presence of an aldehyde CH, suggesting that reduction of thiazolidine **139** had been unsuccessful. In addition oxidation of **139** was performed in a range of solvents, thermally and under microwave irradiation (Scheme 67, Table 27). The results show that when the reaction was

Scheme 67. Oxidation of thiazolidine **139**



138 **139** **95**

Reagents and conditions: (a) Et_3N, RT, 3 h, filter, then dimethoxyacetaldehyde, RT, 18 h; (b) 10 eq. MnO_2, PhMe, microwave, 140 °C, 1 h (9%); (c) 10 eq. MnO_2, PhMe, microwave, 140 °C, 2 h (9%); (c) 4 eq. MnO_2, PhMe, reflux, 18 h (11%).

carried out in toluene, oxidation of thiazolidine **139** to the desired compound **95** occurred in low yield (entries 3–5). If the solvent was changed for either acetonitrile (MeCN) or dichloromethane under microwave irradiation, no oxidation occurred and return of starting material was observed

with a small loss of yield (entries 1 & 2). Ten equivalents of manganese dioxide and an elevated temperature were required for reactions in the microwave to proceed (entries 3 & 4). Under thermal conditions the reaction was run for a longer period of time to achieve comparable yields to those run in the microwave; however only four equivalents of reagent were required at a lower reaction temperature (entry 5).

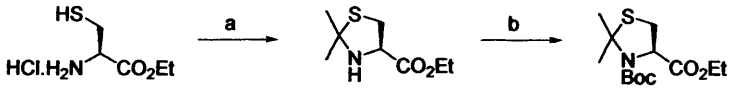
Table 27. Oxidation of compound **139** using MnO_2

Entry	Solvent	Conditions	Equivalents MnO_2	Compound	Yield%[a]
1	Acetonitrile	microwave, 100 °C, 1 h	4	**139**	95[b]
2	Dichloromethane	microwave, 100 °C, 1 h	4	**139**	92[b]
3	Toluene	microwave, 140 °C, 1 h	10	**95**	9
4	Toluene	microwave, 140 °C, 2 h	10	**95**	9
5	Toluene	thermal, reflux, 18 h	4	**95**	11

[a] Isolated yield after purification by column chromatography. [b] Crude recovery of starting material after filtration.

We have demonstrated that the reduction of thiazolidine **139** was not possible using DIBAL in a range of solvents at –84 °C, although this may be due to the low temperature in which the reactions were carried out. More importantly however, is the result showing that oxidation of thiazolidine **139** using manganese dioxide is not an effective method to form thiazole **95**, which therefore undermines this strategy. In contrast, in parallel to the above strategy, preparation of thiazolidine **140** according to a modified procedure,[47] gave the desired product in excellent yield (97%). The second step of the route although apparently relatively straight forward did not give good yields of the desired *tert*–butoxycarbonyl (Boc) protected amine under a range of conditions (Scheme 68, Table 28).

Scheme 68. Formation of thiazolidine **140** and Boc protection



138 **140** **141**

Reagents and conditions: (a) Et_3N, acetone, RT, 1 h, filter, then 1,3-dimethoxypropane, acetone, 60 °C, 2 h (97%); (b) 1.1 eq. DIEA, 1.5 eq. Boc_2O, MeCN, 50 °C, 2 d (9%).

Table 28. Methods attempted to protect thiazolidine **140**

Entry	Solvent	Base	Equivalents of base	Equivalents of Boc	Temperature /°C	Time /h	Catalyst	Compound	Yield %[a]
1	CH_2Cl_2	Et_3N	2.3	2	25	18	—	**140**	**88**
2	MeCN	$^iPr_2NEt$	1.1	1.2	25	48	—	**140**	77
3	MeCN	$^iPr_2NEt$	1.1	1.5	50	48	—	**140/141**	63/9
4	MeCN	$^iPr_2NEt$	1.1	1.5	90	60	DMAP	**140**	53

[a] Isolated yield after purification by column chromatogrpahy

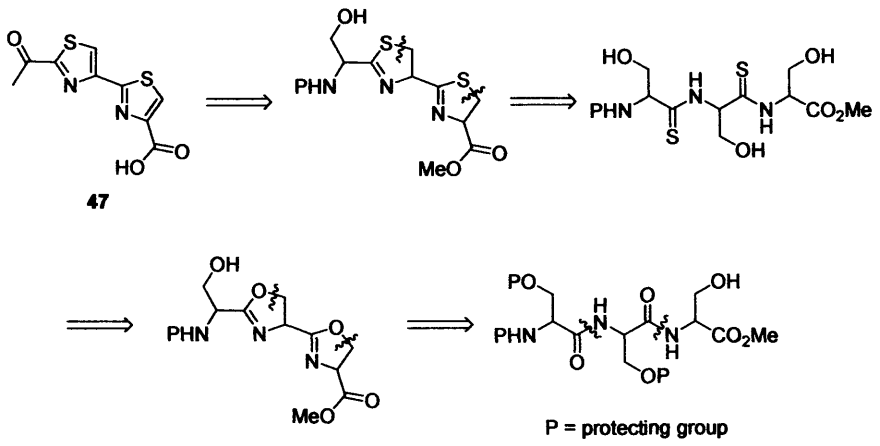
All of the attempts to generate compound **141** returned starting material (entries 1–4). By increasing the temperature of the reaction to 50 °C we isolated the desired product **141** in low yield (9%) along with appreciable starting material (63%) (entry 3). In an effort to improve the yield we increased the reaction temperature to reflux, increased the reaction time and included a catalyst in the form of *N,N*–dimethylaminopyridine (entry 4), however to our surprise no desired product was observed and a lower yield of starting material was returned (53%).

Even though the synthesis of thiazolidine **140** was superior to that of thiazolidine **139**, the failure of **140** to undergo Boc protection raised some serious doubts about the validity of this strategy. This in combination with the lack of susceptibility of thiazolidine **139** to undergo reduction, and the poor yields obtained for the oxidation provided early evidence that these strategies were too problematic to validate an elegant synthetic approach to saramycetic acid I **47**.

3.6.2 Tripeptide–based synthesis of saramycetic acid I

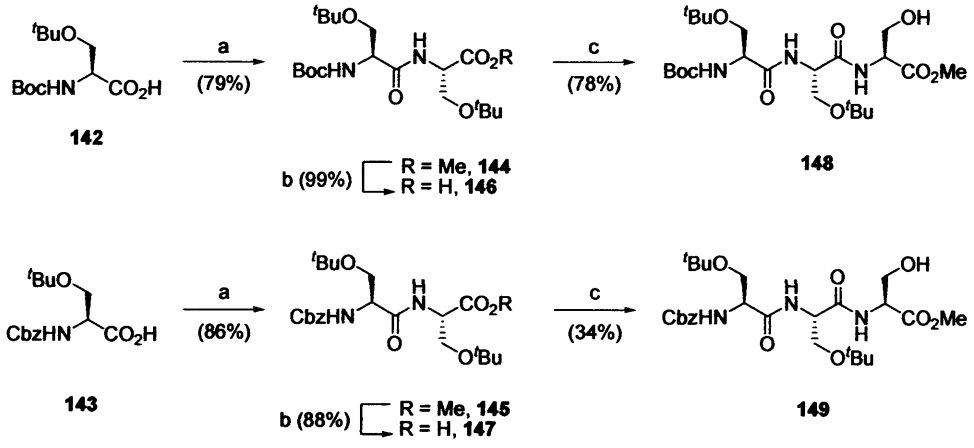
Further retrosynthetic analysis of saramycetic acid I led to the hypothesis that it could be prepared from a tripeptide made up of serine amino acid residues. Cyclization of the tripeptide using diethylaminosulphur–trifluoride (DAST) would give the corresponding oxazoline. Subsequent thionation using hydrogen sulphide to give the thioamide tripeptide could then be cyclization to the bisthiazoline. Oxidation using DBU and bromotrichloromethane rather then manganese dioxide would give the corresponding bisthiazole. After amine deprotection the compound could be activated by protonation, this in turn would result in elimination and hydrolysis to give the methyl ketone. The desired compound **47** could then be isolated after soaponification of the ester functionality (Scheme 69).

Scheme 69. Tripeptide inspired retrosynthesis of saramycetic acid **47**



Orthogonal protection of the amine terminus of the fragment would be necessary. Therefore the parallel synthesis of two tripeptides was undertaken, one with a Boc protected amine **142** and the other with a Cbz protected amine **143**. The *N*–protected L–serine carboxylic acids, side chain protected as the *tert*–butyl ethers were stirred with *N*–methyl morpholine, isobutyl chloroformate in tetrahydrofuran at 0 °C for 20 minutes before adding H–L–Ser(ᵗBu)–OMe, to give dipeptides **144** and **145** in good yield (79 and 86% respectively). Hydrolysis of the esters with lithium hydroxide monohydrate in methanol–water gave the corresponding carboxylic acids (**146**, 99%; **147**, 88%) in good yield. Tripeptides **148** and **149** were prepared by using the same coupling method with H–L–serine–OMe (Scheme 70).

Scheme 70. Synthesis of tripeptides **148** and **149**



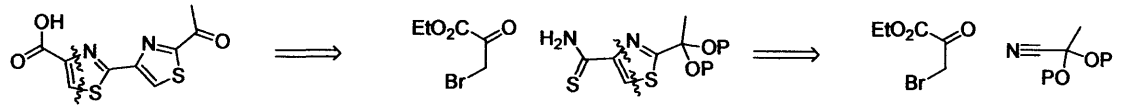
Reagents and condtions: (a) NMM, isobutylchloroformate, THF, 0 °C, 20 min; then H–L–Ser(ᵗBu)–OMe, 0 °C, 5 h; (b) LiOH, MeOH–H_2O, RT, 18 h; (c) NMM, isobutylchloroformate, THF, 0 °C, 20 min, then H–L–Ser–OMe, 0 °C, 5 h.

Tripeptide **148** was isolated in good overall yield (61%) from amino acid **142**, whereas tripeptide **149** was isolated in moderate overall yield (26%) from amino acid **143**. In order for cyclization to be performed the tripeptide side chains were deprotected according to literature procedures,[48] in dichloromethane–trifluoroacetic acid (10:1) at 0 °C for 4 hours. The ^{1}H NMR spectrum of the crude material from both reactions indicated that multiple side reactions had occurred and it was difficult to identify a solvent system by thin layer chromatography that would separate pure material. Due to the difficulties experienced in preparing the deprotected tripeptides in combination with the problematic thiazolidine chemistry, we decided to concentrate our efforts on an Hantzsch inspired synthesis of saramycetic acid I **47**.

3.6.3 Hantzsch–inspired synthesis of saramycetic acid I

Encouraged by our reported method for the synthesis of acetal protected thiazole **94 (3.2.2)** in the synthesis of degradation products of the sulfomycins,[23] and in combination with our work on the simple thionation of nitriles to give thioamides,[15] we investigated a similar method for the preparation of saramycetic acid I **47** (Scheme 71).

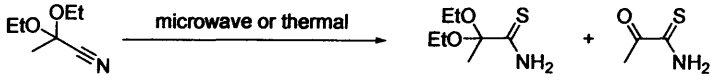
Scheme 71. Hantzsch–inspired retrosynthesis of saramycetic acid I **47**



47 **123** **123**

Commercially available 2,2–dimethoxypropionitrile **150** was stirred in methanol and ammonium sulphide (50 wt% in water) under a range of reaction conditions (Scheme 72, Table 29).

Scheme 72. Hantzsch thiazole synthesis from 2,2–diethoxypropionitrile **150**



150 **151** **152**

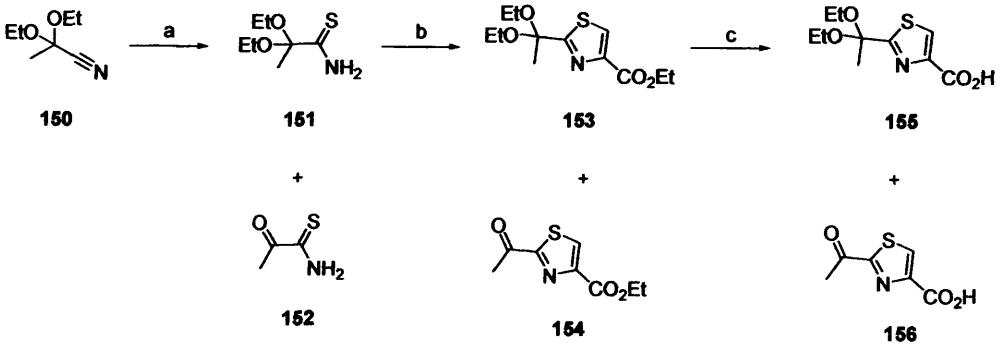
Table 29. Thionation of 2,2–diethoxypropionitrile **150**

Entry	Condtions	Temperature /°C	Time /h	Product (Yield%)	
1	microwave	80	0.25	**151** (7)	**152** (4)
2	microwave	130	0.5	**151** (5)	**152** (7)
3	thermal	RT	18	**151** (7)	**152** (1)
4	thermal	50	4	**151** (21)	
5	thermal	RT	2.5	**151** (47)[a]	

[a] No aqueous workup was employed

When compound **150** was reacted according to reported procedures,15 a low yield of isolated thioamide **151** was observed and deprotected ketal **152** (determined by ^{1}H NMR spectroscopy) was also isolated as a separate fraction by column chromatography under microwave or thermal conditions (entries 1–3). When the reaction was repeated under thermal conditions at 50 °C an increased yield was observed (entry 4), indicating that the acetal protected compounds may not be stable to high temperatures or microwave irradiation. Furthermore when compound **150** was reacted in methanol at room temperature for two and a half hours without an aqueous work up (as previously used), the desired ketal protected thioamide **151** was isolated in moderate yield (47%) (entry 5), indicating that compound **151** may be soluble in aqueous medium. To our disappointment when thioamide **151** was reacted under our reported Hantzsch conditions,[23] deprotection of the acetal occurred to give a mixture of protected and unprotected ketones **153** and **154** (determined by ^{1}H NMR spectroscopy). This trend was observed when attempts to hydrolyze the ketal–protected thiazole ester **153** to the corresponding carboxylic acid gave the mixed ketal/ketone thiazole carboxylic acids **154** and **156** (determined by ^{1}H NMR spectroscopy) (Scheme 73).

Scheme 73. Hantzsch thiazole synthesis from 2,2–diethoxypropionitrile **150**

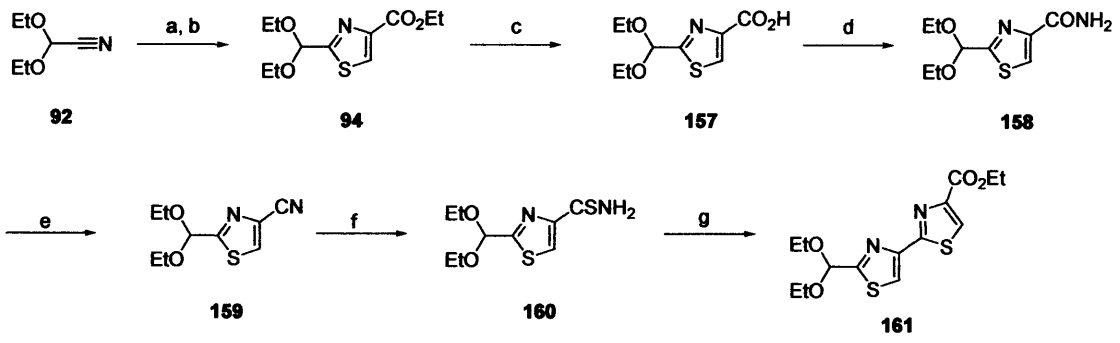


Reagents and condtions: (a) $(NH_4)_2S$, MeOH, RT, 2.5 h (47%); (b) ethyl bromopyruvate, EtOH, reflux, 1 h (41%); (c) LiOH, MeOH–H_2O, 18 h (40%).

Even though synthesis of thiazole **153** was unsuccessful our strategy was still viable by utilizing our reported synthesis of thiazole **94**. Notably thiazole **94** has an acetal functionality whereas thiazole **153** differs only in that it has a ketal functionality. Furthermore we have already reported good yields for the reaction of thioamide **92** under Hantzsch with subsequent hydrolysis (**3.2.2**).[23] Therefore if the bisthiazole fragment was constructed by a second Hantzsch thiazole synthesis via elaboration of the 4–substituent of thiazole **94**, deprotection of the acetal would follow and Grignard addition–oxidation–hydrolysis steps would give the desired acetyl moiety.

In order to access the necessary thioamide, saponification of thiazole ester **94** with lithium hydroxide in methanol–water gave carboxylic acid **157** in good yield (64%) followed by ammonolysis in tetrahydrofuran with ethyl chloroformate, triethylamine and saturated ammonia solution to give amide **158** in 85% yield. Amide **158** was then treated with Lawesson's reagent in dichloromethane at either reflux or room temperature for 18 hours. The desired thioamide **160** was not formed and even after column chromatography and isolation of numerous fractions no starting material was identified either, indicating that Lawesson's reagent caused amide **158** to undergo mixed reactions. In order to circumvent thionation of amide **158** and to explore the versatility of our reported procedure for thionation of nitriles, amide **158** was reacted with phosphorous oxychloride whilst stirring in pyridine at room temperature for two hours to give nitrile **159** in good yield (83%). Gratifyingly when nitrile **159** was stirred in methanol with ammonium sulphide at room temperature for 18 hours thioamide **160** was isolated in quantitative yield, with no need for purification by column chromatography. Furthermore when thioamide **160** was reacted according to our Hantzsch thiazole procedure bisthiazole **161** was isolated after simple extraction in quantitative yield (Scheme 74).

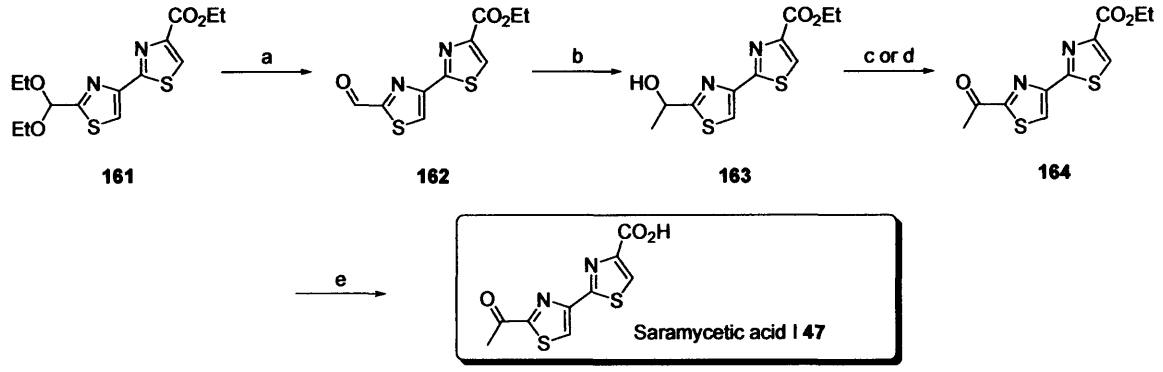
Scheme 74. Synthesis of bisthiazole **161**



Reagents and conditions: (a) $(NH_4)_2S$, MeOH, RT, 18 h (100%); (b) ethyl bromopyruvate, EtOH, reflux, 1 h (100%); (c) LiOH, MeOH–H_2O, RT, 18 h (64%); (d) ethyl chloroformate, Et_3N, THF, 0 °C, 1 h; then NH_4OH, 0 °C, 1 h (85%); (e) $POCl_3$, pyridine, 0 °C–RT, 2 h (83%); (f) $(NH_4)_2S$, MeOH, RT, 18 h (100%); (g) ethyl bromopyruvate, EtOH, reflux, 1 h (100%).

With the synthesis of the bisthiazole fragment completed, deprotection of acetal **161** was achieved in 87% yield to give aldehyde **162**. Grignard addition with methylmagnesium bromide in dichloromethane at 0 °C for 18 hours gave the methyl alcohol **163** in 41% yield and returned starting material in 38% yield. Compound **163** was oxidized to ketone **164** using either manganese dioxide (52%) in dichloromethane or *o*–iodoxybenzoic acid (IBX) (50%) in dimethylsulphoxide. Hydrolysis of **164** by stirring in a slight excess of lithium hydroxide in methanol–water for 18 hours, gave saramycetic acid I **47** in 11 steps and 8% overall yield (Scheme 75).

Scheme 75. Synthesis of saramycetic acid I **47**



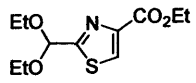
Reagents and conditions: (a) 2M HCl (aq), acetone, reflux, 1 h (87%); (b) MeMgBr, CH_2Cl_2, 0 °C–RT, 18 h (41%); (c) MnO_2, CH_2Cl_2, RT, 40 min (52%); (d) IBX, DMSO, RT, 1 h (50%); (e) LiOH, MeOH–H_2O, 18 h (100%).

The spectroscopic data of the synthetic material [^{1}H NMR $SO(CD_3)_2$) δ 8.85 (0.18H, s, 5'–H), 8.82 (0.82H, s, 5'–H), 8.71 (0.18H, s, 5–H), 8.63 (0.82H, s, 5–H), 2.77 (3H, s, Me); ^{13}C NMR (125 MHz; $SO(CD_3)_2$) δ 191.4 (C), 167.9 (C), 162.4 (C), 161.7 (C), 149.8 (C), 148.8 (C), 130.1 (CH), 125.8 (CH), 26.3 (Me); *m/z* (APcI) 255 (MH^+, 40%); UV (MeOH)/nm λ_{max} 220 (log ε 4.29), 291 (log ε 4.14)] were in good agreement with the literature data on the degradation product [^{1}H NMR ($SO(CD_3)_2$) δ 8.97 (1H, s, 5–H), 7.92 (1H, s, 5–H), 2.70 (3H, s, Me); ^{13}C NMR ($SO(CD_3)_2$) δ 189.6, 165.4, 162.4, 158.4, 157.5, 122.7, 120.7, 24.4; UV (MeOH)/nm λ_{max} 218 (log ε 4.32), 291 (log ε 4.16)][27] confirming the outcome of the degradation studies[27,28] and providing a new viable route to bisthiazoles that could be applied to the synthesis of a range of thiopeptide antibiotics.

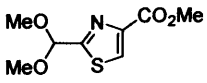
Efforts to improve the synthesis of saramycetic acid I was undertaken at various stages of the procedure. Initially an investigation of direct ammonolysis under thermal[49] and under microwave

conditions,[50] of either thiazole **96** or **94** in methanolic ammonia under various conditions was attempted (Table 30).

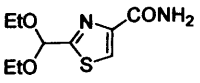
Table 30. Ammonolysis of thiazole esters **94** and **96**



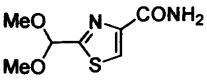
94



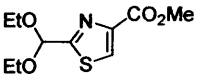
96



158



165



166

Entry	Starting material	Conditions[a]	Temperature /°C	Time	Compound (Yield%) Ester	Amide	Overall yield%
1	**96**	microwave	60	40 min	**96** (81)	**165** (19)	100
2	**96**	microwave	60	4 h	**96** (19)	**165** (77)	95
3	**96**	microwave	60	40 min	**96** (79)	**165** (21)	85
4	**96**	microwave	100	80 min	**96** (45)	**165** (40)	85
5	**94**	microwave	80	4 h	**166** (15)	**158** (29)	44
6	**94**	microwave	120	2 h	**166** (9)	**158** (9)	18
7	**94**	microwave	100	2 h	**166** (34)	**158** (66)	100
8	**94**	microwave	100	4 h	**166** (9)	**158** (33)	42
9	**94**	microwave[b]	100	1 h	**166** (28)	**158** (21)	49
10	**94**	microwave[c]	80	1 h	**94**[e]	—	—
11	**94**	microwave[d]	80	1 h	**94**[e]	—	—
12	**94**	thermal	50	1 h	**166** (18)	**158** (51)	69
13	**94**	thermal	25	3 d	**166** (2)	**158** (41)	43
14	**94**	thermal	50	4 d	—	**158** (50)	50
15	**94**	thermal	25	36 h	—	**158** (53)	53
16	**94**	thermal	45	7 d	**166** (7)	**158** (65)	72

[a] Reactions carried out in methanol. [b] Reaction run without cooling air on. [c] Reaction run in THF as solvent. [d] Reaction run with acetonitrile as solvent. [e] Compound identified by ^{1}H NMR spectrum of crude residue.

When thiazoles **94** and **96** were reacted under microwave conditions (entries 1–11) the overall yields were found to range between 42–100%, the greatest recorded yield of amide **158** and **165** was 77% and 67%, respectively (entry 2 and 7). Temperatures between 60–100 °C did not adversely effect the reaction however at 120 °C an overall yield of only 18% was observed (entry 6) compared to an overall yield of 100% when the same reaction was run at 100 °C (entry 7). When thiazole **96** was reacted for 40 minutes a ratio of 4:1 in favour of the ester was recorded (entry 1), however when the same reaction was run for 4 hours a ratio of 4:1 in favour of amide formation was observed (entry 2), and when the reaction was run for 80 minutes (although at higher temperature) a ratio of 1:1 was observed (entry 4).

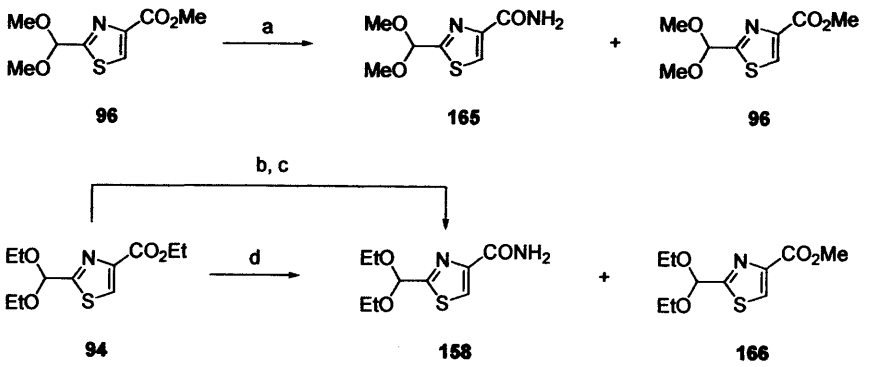
It was noteworthy to identify that methanol was also acting as a competing nucleophile in the reaction, demonstrated by the isolation of methyl ester **166** (identified by ^{1}H NMR spectroscopy)

when using ethyl ester thiazole **94**. In order to prevent competition of ammonium with methanol, two other solvents were investigated, acetonitrile due to its comparable dielectric constant with methanol and tetrahydrofuran for its capacity to dissolve ammonia. When thiazole **94** was reacted in the microwave using either acetonitrile or tetrahydrofuran under conditions known to promote amide formation, no reaction occurred (entries 10 and 11). Although changing the solvent system prevented competing reactions from taking place, the chosen solvents also prevented ammonia attacking the ester, returning thiazole **94**. In the case of acetonitrile this could be explained by the poor solubility of ammonia. In the case of tetrahydrofuran we envisage poor energy transfer is probably to blame due to the low dielectric, concluding methanol is the best solvent to use out of those investigated.

When reactions were attempted under thermal conditions, there was a notable reduction in the amount of ester isolated at the end of the reaction period (entries 12–16). At 25 °C, when the reaction was run for 36 hours, amide **158** was isolated in 53% yield with no return of starting material (entry 15), whereas when the same reaction was run for 3 days amide **158** was isolated in 41% yield and starting material **166** in 2% yield (entry 13). When the reaction was run at 50 °C for 1 hour or 4 days no adverse effect on the yield of amide **158** (51% and 50% respectively) was observed (entries 12 and 14). Curiously the best yield of amide **158** was recorded under thermal conditions (65%) when stirred for 7 days at 45 °C (entry 16).

Even though microwave conditions gave a good yield of amide in a short period of time, (entries 2 and 7), the thermal conditions are more favourable. At 45 °C in methanolic ammonia over a period of 7 days amide **158** was produced in 65% yield (entry 16). More importantly, in comparison to our original procedure which gave amide **158** in 54% yield over two steps, we have successfully improved the overall yield and reduced the number of steps (Scheme 76).

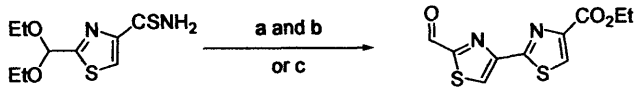
Scheme 76. Optimising the ammonolysis of thiazole **94** and **96**



Reagents and conditions: (a) MeOH–NH_3, microwave, 60 °C, 4 h (66%); (b) LiOH, MeOH–H_2O, RT, 18 h (64%); (c) ethyl chloroformate, Et_3N, THF, 0 °C, 1 h; then NH_4OH, 0 °C, 1 h (85%); (d) MeOH–NH_3, 45 °C, 7 d (65%).

Given the ease with which thioamide **160** undergoes a Hantzsch thiazole synthesis and the simplicity of the deprotection method to give aldehyde **162**, we tested the hypothesis that these two transformations could be achieved in one step without any loss of yield and without the need for purification by column chromatography. Therefore thioamide **160** was reacted according to our Hantzsch thiazole procedure. After evaporation *in vacuo* the residue was dissolved in a solution of acetone–aqueous hydrochloric acid (2M) and stirred at reflux for a further 1.5 hours. Gratifyingly after a simple aqueous work up with dichloromethane, aldehyde **162** was isolated in excellent yield (100%), providing a more direct route to saramycetic acid I **47** without compromising yield. In fact the overall yield over the two steps was increased by 17% (Scheme 77).

Scheme 77. Direct route to aldehyde **162**



160 **162**

Reagents and conditions: (a) ethyl bromopyruvate, EtOH, reflux, 1 h (100%); (b) 2M HCl (aq), acetone, reflux, 1 h (87%); (c) ethyl bromopyruvate, EtOH, reflux, 1 h, then 2M HCl (aq), acetone, reflux, 1.5 h (100%).

It is noteworthy at this time to consider the reactivity of aldehyde **162.** The reaction of **162** with methylmagnesium bromide to form ethyl alcohol **163** (Table 31), identified that the electrophilic potential of the aldehyde carbonyl appeared to be significantly reduced making nucleophilic attack at this position difficult. When the reaction was carried out over 1.5 hours with 1.2 equivalents of methylmagnesium bromide a mixture of starting material and product was formed, the combined yields of which made up only 21% of the original quantity of starting material

Table 31. Reaction of aldehyde **162** under a range of conditions

Entry	Grignard reagent	Equivalents of reagent	Solvent	Temperature /°C	Time /h	Compound (Yield%)		Overall yield%
1	MeMgBr	1.2	THF	0–RT	1.5	**162** (8)	**163** (13)	21
2	MeMgBr	1.1 + 1.2	THF	0–RT	0.33 + 0.66	**162** (10)	**163** (15)	25
3	MeMgBr	1 + 0.5	THF	0–RT	1 + 0.5	**162** (3)	**163** (39)	42
4	MeMgBr	1.03	CH_2Cl_2	0–RT	18	**162** (38)	**163** (41)	79
5	MeLi	1.03	THF	0	1	**162**	–	–
6	MeLi	1.03	THF	0	1.5	**162** (10)	–	10
7	MeLi[a]	1.03	THF	0	1	**162** (6)	–	6

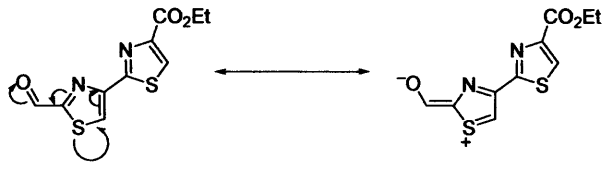
[a] 12–Crown–4–ether used

(entry 1). By reducing the reaction time and adding an additional aliquot of methylmagnesium bromide there was no significant difference in the quantity of isolated material and overall yield (entry 2). However when 1.5 equivalents of methylmagnesium bromide were added to a reaction and stirred for 1.5 hours a significant decrease in isolated starting material **162** and an increase in the isolated yield of the desired product **163** was observed (entry 3). When the reaction was run for 18 hours in dichloromethane with only 1.03 equivalents of Grignard reagent the best isolated yield of desired product (41%) was obtained with a substantial amount of unreacted starting material (38%) (entry 4).

In an attempt to the improve yield, reactions were run using 1.03 equivalents of methyl lithium, an alternative nucleophile. When the reaction was run for 1 hour only starting material was observed by ^{1}H NMR spectroscopy (entry 5). Increasing the reaction time to 1.5 hours resulted in isolation of starting material (10%) and a mixture of unidentifiable products. 12–crown–4–ether was added in order to manipulate the reactivity of the nucleophile, however only 6% of unreacted starting material was isolated and again a mixture of unidentifiable products, (entry 6).

In summary efforts to improve the nucleophilic addition to bisthiazole–aldehyde **162** gave little success, identifying the unreactivity of this moiety is probably a consequence of the resonance of the thiazole ring (Figure 11).

Figure 11. Deactivation of the carbonyl group of aldehyde **162**



Lewis acid activation was not attempted and it is likely that it too, would deactivate compound **162** as shown in figure 11, however, it would be worth investigating in the future. Nonetheless as a result of improvements in the formation of amide **158** and bisthiazole **162**, saramycetic acid I **47** was synthesized in 9 steps and 11% overall yield.

3.7 Conclusion

Degradation studies of thiopeptide antibiotics have led to the isolation of fragments that can help in the structural elucidation of the parent molecules. Many of the fragments have been assigned based on spectroscopic data, although very few of these hypotheses have been verified by chemical synthesis. Our efforts to synthesize degradation fragments from thiopeptide antibiotics

have helped to address this short fall, validating known data and consolidating methods that could lead to the total synthesis of the parent antibiotics.

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Chapter Four – Results and Discussion

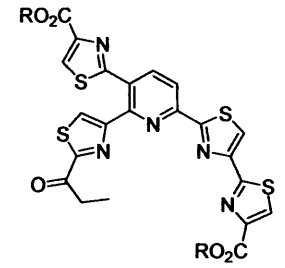
4 Towards the total synthesis of the core of micrococcin

4.1 Micrococcin

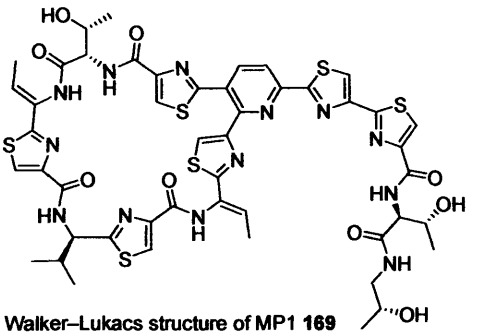
Micrococcin was the first example of a thiopeptide antibiotic to be recorded, appearing in "The British Journal of Experimental Pathology" in 1948 and was discovered from a strain of *Micrococcus* found in the sewage from the city of oxford,[1] although no structural data on this thiopeptide was ever reported. Structural investigation of material obtained from soil collected in East Africa containing a spore–bearing bacillus called *Bacillus pumilus*[2] demonstrated a considerable degree of homology with the antibiotic isolated from *Micrococcus*, suggesting two members of a closely related family and as such this new material was named micrococcin P. Interestingly this antibiotic consisted of two distinct components, present in a ratio of 7:1 and designated micrococcin P_1 and P_2, respectively.[3] More recently micrococcin P_1 has been isolated from *Staphylococcus equorum* WS2733 found in French Raclette cheese,[4] a gratifying discovery due to the difficulties experienced in obtaining the compounds from their original sources. The isolate has been shown to inhibit growth of the malaria parasite *Plasmodium falciparum* and ribosomal protein biosynthesis in Gram–positive bacteria, the latter through binding to the complex of protein L11 and 23S rRNA in the bacterial 50S subunit.[5] Work spanning the 50 year period following initial isolation to elucidate the correct structure and stereochemistry of micrococcin P_1, tells an extraordinary story. Despite considerable spectroscopic and synthetic advances in combination with a number of total syntheses the correct structure remains unresolved.3

Initially due to the lack of structural data, micrococcin P_1 (also described as micrococcin P1 and MP1), was characterized by examination of its acid hydrolysates.[6] Analysis of the acid soluble component gave a laevorotatory hydrochloride that was assigned as L–threonine.HCl by IR spectroscopy. Further analysis identified the central heterocyclic domain as micrococcinic acid **167** and methyl micrococcinate **168** and an aminoalcohol that was later identified as (*R*)–isoalaninol from ^{13}C NMR studies of the natural product. The net result of these studies was the proposal of the Walker–Lukacs structure **169** which assumes structural homology with other thiopeptide antibiotics, such as thiostrepton and nosiheptide (Figure 12).[7]

Figure 12. Hydrolysates of micrococcin P_1 and the Walker–Lukacs structure



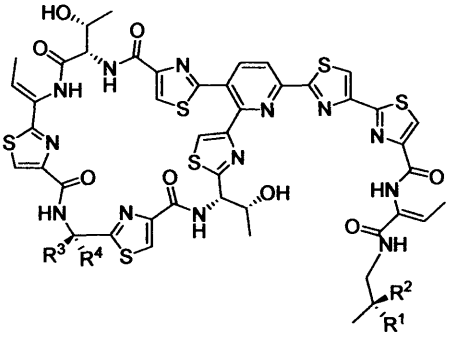
R = H, micrococcinic acid **167**
R = Me, methyl micrococcinate **168**



Walker–Lukacs structure of MP1 **169**

Bycroft and Gowland separated micrococcin P_1 and micrococcin P_2 in order to carry out their own studies and analyses of the acid hydrolysates of micrococcin P_1. Only one mole of threonine was produced in these experiments, which was in contrast to the findings of Walker and Lukacs. As a result the Bycroft–Gowland structures of micrococcin P_1 **170** and P_2 **171** were proposed (Figure 13).[8]

Figure 13. Proposed structures of the micrococcins



Bycroft–Gowland structure of:
MP1 **170** R^1 = OH, R^2 = H, R^3 = $CHMe_2$, R^4 = H;
MP2 **171** R^1,R^2 = O (π–bond), R^3 = $CHMe_2$, R^4 = H;
Shin's synthetic epimer of:
MP1 **172** R^1 = H, R^2 =OH, R^3 = $CHMe_2$, R^4 = H;
Probable structure of:
MP1 **173** R^1 = OH, R^2 = H, R^3 = H, R^4 = $CHMe_2$;
MP2 **174** R^1,R^2 = O (π–bond), R^3 = H, R^4 = $CHMe_2$.

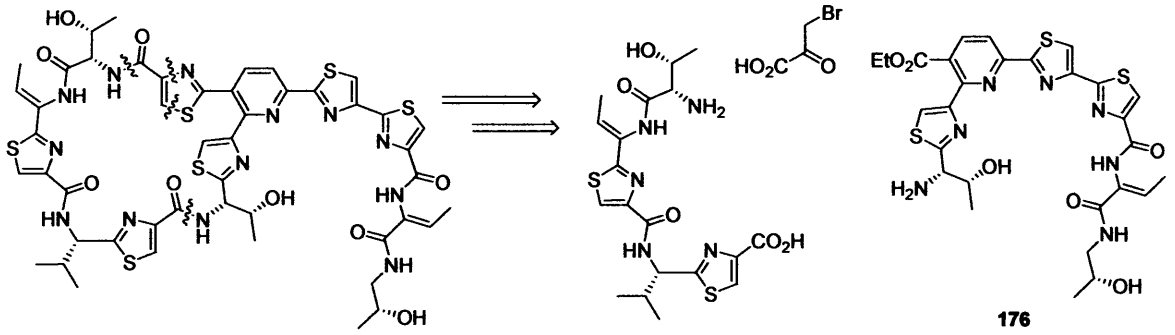
The proposed structure was assumed to be correct for twenty years until in 1999 Ciufolini completed an elegant total synthesis of the Bycroft–Gowland micrococcin P_1 structure and found that the data of the synthetic material did not correspond to that of the natural product.[9] The situation was further confounded by Shin's synthesis of two epimeric substances of Walker–Lukacs and Bycroft–Gowland structures, described as micrococcin P and micrococcin P_1 **172**[10] (Figure 13). Later Ciufolini drew together all of these findings and validated the order of the residues in micrococcin P_1 according to the Bycroft–Gowland hypothesis, discovering that the correct order had been postulated. This result indicated that the real structure of micrococcin P_1 was different from Bycroft–Gowland's structure only in its stereochemistry. Therefore the origin

of this discrepancy must be in either the configuration of the L–threonine–derived thiazole at the 3–position of the pyridine core domain and/or in the (*R*)–valine–derived thiazole in the peptide macrocycle, both of which were proposed in the absence of reliable experimental evidence.[6,11] Synthetic studies performed recently within our own group have provided evidence in support of Walker's isolation of laevorotatory valine, indicating that the correct stereochemistry of the valine–derived residue is *S* **173** (Figure 13).[5]

4.2 Retrosynthesis of micrococcin P_1

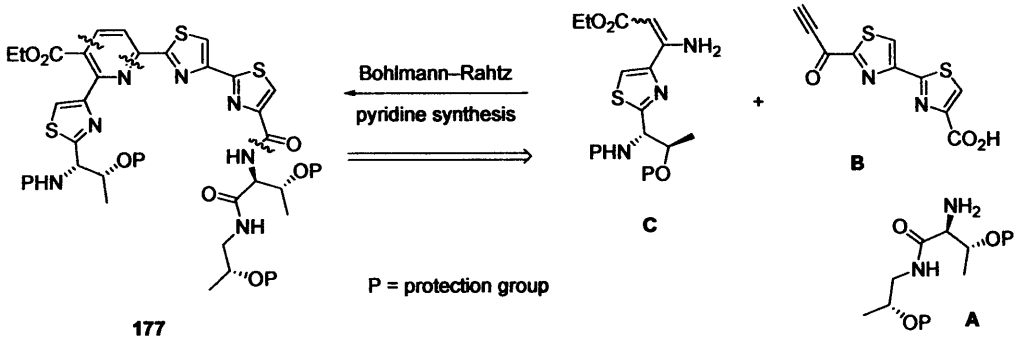
In view of our recent studies into the stereochemistry of micrococcin P_1, the challenge has been presented to synthesize this ageing molecule with the correct stereochemistry in order to solve the long standing mystery surrounding this relatively simple thiopeptide antibiotic. Retrosynthetic analysis has provided a number of target molecules, the backbone of micrococcin P_1 **175** and the core of micrococcin P_1 **176**, (Figure 14).

Figure 14. Retrosynthesis of the proposed structure of micrococcin P_1



It follows that the forward synthesis of molecule **175** is proposed to be achieved by methodology originating from the Bagley laboratory.[5] The core of micrococcin P_1 **176** consists of an ester in the 3–position and a side chain attached to the 6–position of the pyridine ring via a 2–bisthiazole which is further substituted at the 4'–position by a demethyldehydrovaline and (*R*)–isoalaninol moiety. Furthermore an L–threonine–derived thiazole moiety is attached at the 2–position of the pyridine core via the 4–position of the thiazole. Retrosynthetic analyses of the protected core **177** reveals fragments **A**, **B** and **C** which identify the potential for the forward synthesis to incorporate a Bohlmann–Rahtz pyridine synthesis (Scheme 78).

Scheme 78. Bohlmann–Rahtz inspired retrosynthesis of the protect core of micrococcin P_1 **177**



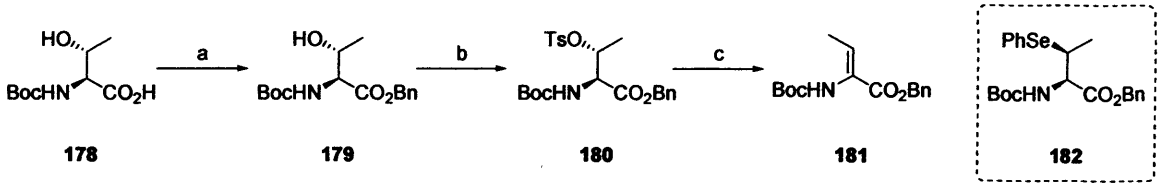
The remainder of this chapter explores the synthesis of fragments **A**, **B** and **C** in order to identify a viable and convergent route to the synthesis of **177**, that could be utilized in the total synthesis of micrococcin P_1 **173**.

4.3 Synthesis of dipeptide A

The synthesis of peptide **A** was undertaken with the view to incorporate a protected alcohol on the threonine that was orthogonal to the protection group of the alcohol found in the (*R*)–isoalaninol moiety. Furthermore we decided to attempt the synthesis of a selenide protection group for a threonine amino acid according to literature procedures,[12] this would provide chemical differentiation between other threonine protection groups and therefore orthogonality..

Boc–L–Thr–OH **178** was treated with benzyl bromide and triethylamine in *N,N*–dimethylformamide at 0 °C for 3 hours. After warming to room temperature the solution was stirred for 2 days and purified to give the benzyl protected threonine **179** in good yield (65%). Tosylation of the free alcohol was achieved by treatment with tosyl chloride (TsCl) in pyridine at 0 °C over 2 days. After purification by column chromatography, tosyl–protected threonine **180** was isolated in moderate yield (45%). Tosylate displacement of **180** was carried out according to the literature procedure but gave the elimination product **181** in 60% yield, instead of the desired residue, phenyl selenide **182** (Scheme 79).

Scheme 79. Phenyl selenide: a masked enamine

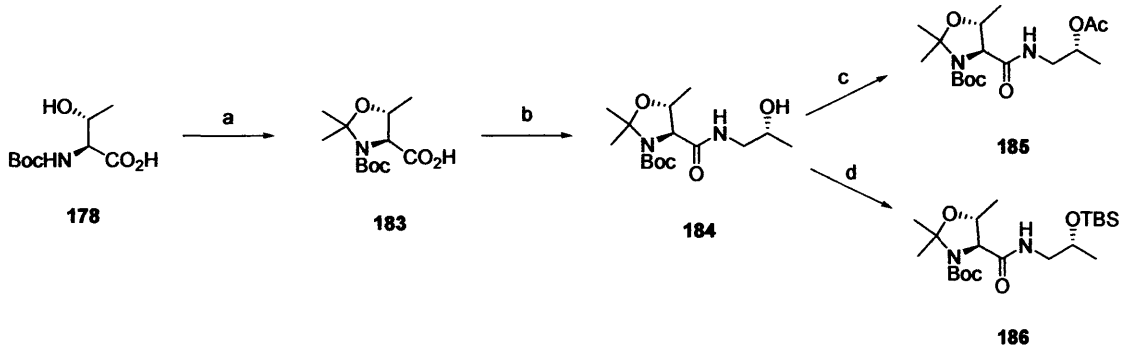


Reagents and conditions: (a) BnBr, Et_3N, DMF, 0 °C, 3 h then RT, 2 d (65%); (b) TsCl, pyridine, 0 °C, 2 d (45%); (c) PhSeK, DMF, 48 h (60%).

In an effort to avoid elimination, the less basic selenophenol (PhSeH) was stirred with **180** for either 4 days at room temperature or for 2 days at reflux, but in both cases only starting material was returned with no trace of elimination product **181** or the desired selenide product **182.** Due to the limited information available in the literature on how to perform the procedure this method was abandoned in preference for alternative protecting group strategies.

Boc–L–Thr–OH **178** was treated with *p*–toluenesulphonic acid and 2,2–dimethoxypropane in tetrahydrofuran at reflux for 18 hours to give oxazolidine **183** in excellent yield (99%). Commercially available (*R*)–isoalaninol was coupled with oxazolidine **183,** by treatment with pyBOP and triethylamine in dichloromethane for 18 hours to give dipeptide **184** in excellent yield (100%). In order to provide orthogonal protection of the free alcohol of (*R*)–isoalaninol, reaction with either acetic anhydride in pyridine for 36 hours or *tert*–butyldimethylsilylchloride, imidazole and *N,N*–dimethylaminopyridine in *N,N*–dimethylformamide for 36 hours gave fully protected dipeptide **185** or **186** in 73 and 98% yield, respectively (Scheme 80).

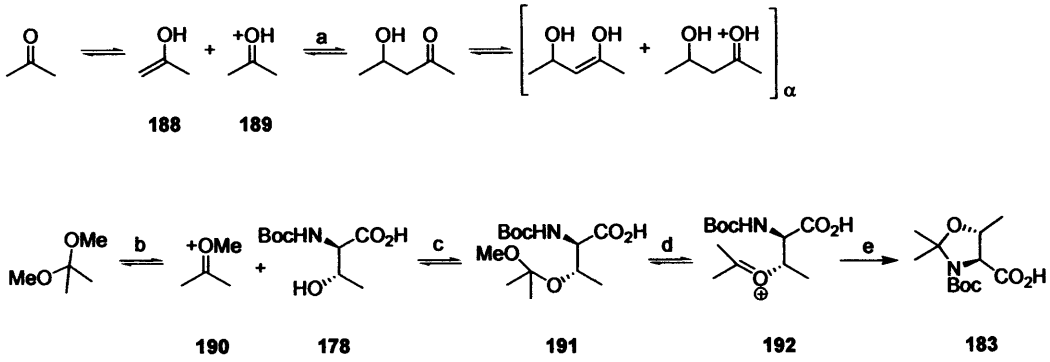
Scheme 80. Synthesis of **185** and **186,** the side chain of micrococcin P_1



Reagents and conditions: (a) *p*–TsOH, 2,2–dimethoxypropane, THF, reflux, 18 h (99%); (b) (*R*)–isoalaninol, Et_3N, pyBOP, CH_2Cl_2, 0 °C–RT, 18 h (100%); (c) acetic anhydride, pyridine, RT, 36 h (73%); (d) imidazole, DMAP, TBDMSCl, DMF, 36 h (98%).

Before proceeding it is worth considering the first reaction in Scheme 80, where Boc–L–Thr–OH **178,** 2,2–dimethoxypropane and *p*–toluenesulphonic acid in THF generates oxazolidine **183.** 2,2–dimethoxypropane is the acetal protected acetone and interestingly this reaction does not proceed if the acetal was substituted for the unprotected ketone. The reason for this is quite obvious, the free ketone under the conditions above will undergo mixed reactions, the acetone is easily converted into an enol **188** as well as the activated oxonium ion **189.** The two species would freely react in an aldol reaction (a) (Scheme 81) and continue to do so forming mixed aldol products. 2,2–dimethoxypropane is therefore used to mitigate against competing side reactions.

Scheme 81. Mechanism for oxazolidine formation **183**

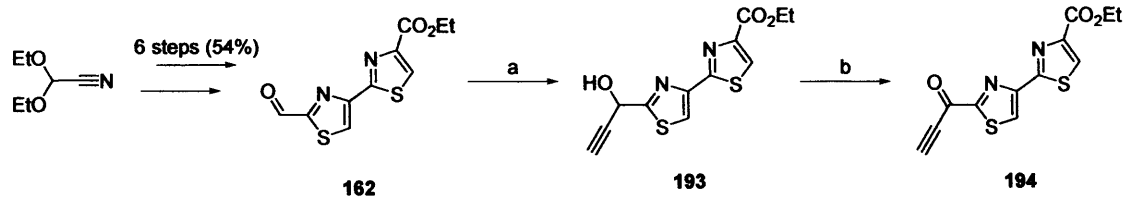


The mechanism of the reaction to form oxazolidine **183** begins with the protonation of 2,2–dimethoxypropane by *p*–TsOH present in catalytic amounts followed by intramolecular displacement of methanol (b), forming the activated oxonium ion **190**. Boc–L–Thr–OH **178** will attack oxonium ion **190** through the hydroxyl group (c) forming acetal **191**. Through protonation of the remaining methoxy group and intramolecular displacement (d) oxonium ion **192** is generated. The lone pair of electrons on the nitrogen is more available then if it was an amide due to the carbarmate functionality present as a consequence of the Boc group. Therefore nitrogen attacks oxonium ion **192** (e) forming oxazolidine **183**.

4.4 Synthesis of alkynone B

Synthesis of bisthiazole aldehyde **162** was achieved in 54% yield over 6 steps using methodology developed in the synthesis of saramycetic acid I **47** (Chapter 3–**3.6.3**). Grignard addition of the corresponding ethynylmagnesium bromide with aldehyde **162** gave the propargylic alcohol **193** in moderate yield (54%). Attempts to hydrolyze the terminal ester of **193** to a carboxylic acid for incorporation of the threonine–(*R*)–isoalaninol side chain were unsuccessful, resulting in the isolation of unidentified material. Gratifyingly oxidation of the propargylic alcohol **193** to give the desired alkynone **194** was achieved by treatment with *o*–iodoxybenzoic acid in dimethylsulphoxide for 2 hours in excellent yield (95%). Typically alkynone **194** was unstable in air and characterization of the compound could only be achieved from ^{1}H and ^{13}C NMR spectroscopic data. Nonetheless synthesis of compound **194** demonstrates that a Bohlmann–Rahtz precursor based on the side chain of micrococcin P_1 could be synthesized in preparation for pyridine synthesis (Scheme 82).

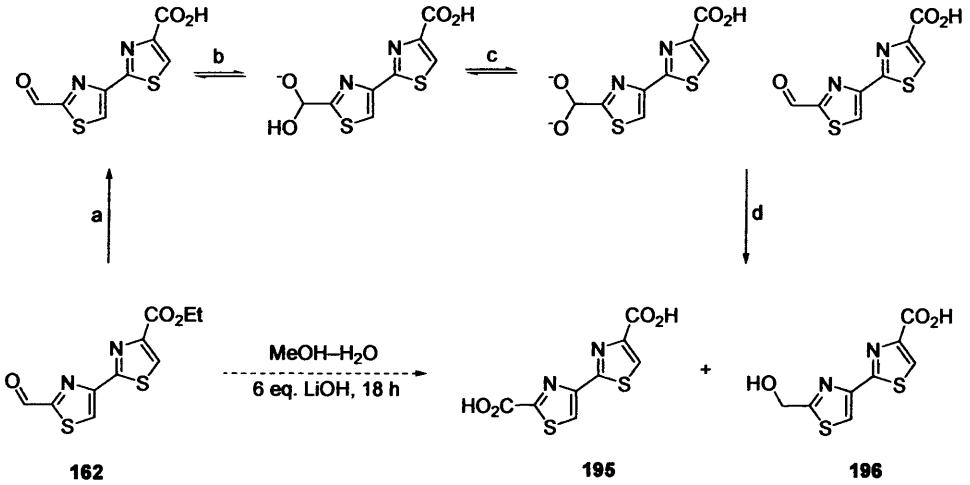
Scheme 82. Synthesis of alkynone **194**



Reagents and conditions: (a) HCCMgBr, THF, 0 °C–RT, 1 h (47%); (b) IBX, DMSO, RT, 2 h (95%).

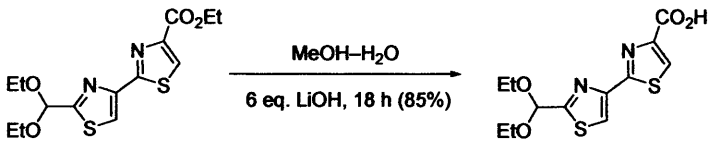
In order to incorporate the whole of the side chain of micrococcin P_1 in an alkynone suitable for a Bohlmann–Rahtz synthesis we explored the hydrolysis of the 4'–ester of bisthiazole **162**. Initially aldehyde **162** was treated with six equivalents of lithium hydroxide in methanol–water and stirred for 18 hours. After acidic work up, ^{1}H NMR spectroscopic analysis indicated multiple reaction products which could be indicative of the Cannizzaro reaction[13] (side reaction of aldehydes in concentrated base), giving dicarboxylic acid **195** and alcohol **196**. This was further supported by low resolution mass spectrometry, identifying both parent ions. The mechanism by which compounds **195** and **196** were generated follows a relatively simple pathway (Scheme 83). Ester hydrolysis would occur (a), in concentrated base the resulting aldehyde may undergo nucleophilic attack from excess hydroxide ions (b) forming an anion which in turn is deprotonated by another hydroxide ion (c) forming a dianion. The dianion is so electron rich that it can donate a hydride to a corresponding aldehyde, generating, after acidic work up the diacid **195** and alcohol **196** (d).

Scheme 83. Hydrolysis of aldehyde **162**



When one equivalent of lithium hydroxide was used, there was no evidence of the Canizarro products by ^1H NMR spectroscopy, but in addition to starting material other unidentifiable products were present that could not be removed using available methods. Due to the difficulties experienced in hydrolysing aldehyde **162**, it was decided to attempt the hydrolysis of the ester from the diethoxyacetal–protected bisthiazole **161** which was synthesized in 6 steps and 54% overall yield in the synthesis of saramycetic acid I **47**. Gratifyingly when compound **161** was treated with 6 equivalents of lithium hydroxide in methanol–water for 18 hours the desired acetal protected carboxylic acid **197** was isolated cleanly and in good yield (85%) after simple acidic extraction (Scheme 84).

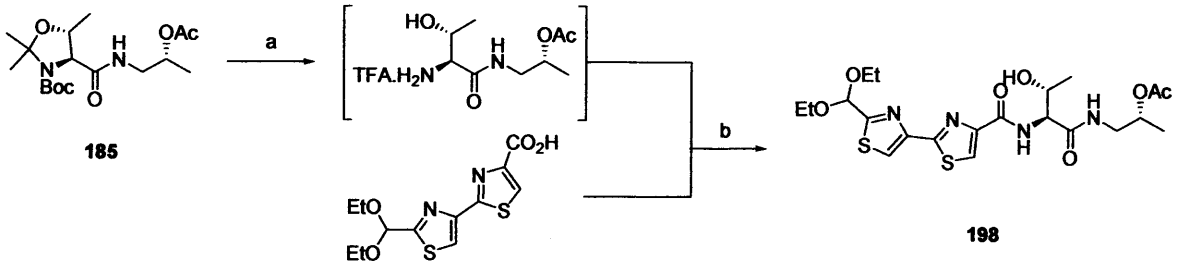
Scheme 84. Hydrolysis of bisthiazole **161**



161 **197**

Compound **185** was *N*–deprotected according to literature procedures[9] to give the amine as its TFA–acetate salt which, after evaporation *in vacuo*, was submitted for peptide coupling with triethylamine, pyBOP and acid **197** in dichloromethane for 4 hours. Purification by column chromatography gave the desired acetal protected side chain of micrococcin P_1 **198** in limited yield (7%), perhaps due to steric hinderance from the threonine hydroxyl group. This demonstrated the potential for the preparation of a Bohlmann–Rahtz precursor containing the micrococcin P_1 side chain, but limited material prevented further progress in this study (Scheme 85).

Scheme 85. Synthesis of the acetal protected side chain of micrococcin P_1



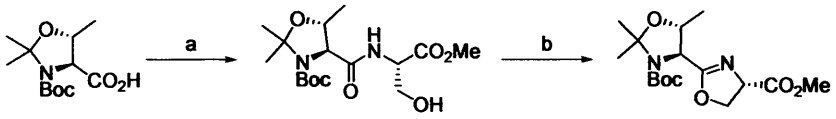
185 **197** **198**

Reagents and conditions: (a) TFA–CH_2Cl_2, RT, 4 h; (b) Et_3N, pyBOP, CH_2Cl_2, 0 °C–RT, 4 h (7%).

4.5 Synthesis of fragment C

Starting with oxazolidine **183**, peptide coupling was achieved by activation with ethyl chloroformate in the presence of triethylamine and H–L–serine–OMe in tetrahydrofuran at 0 °C for 3.5 hours to give the desired oxazolidine dipeptide **199** after column chromatography in excellent yield (93%). Subsequent treatment with DAST in dichloromethane at –78 °C for 3 hours according to literature procedures[14] gave cyclized oxazoline **200** in excellent yield (89%) (Scheme 86).

Scheme 86. Synthesis of oxazoline **200**



183 **199** **200**

Reagents and conditions: (a) ethyl chloroformate, Et_3N, THF, 0 °C, 1 h; then HCl.H–Ser–OMe, 0 °C, 2.5 h (94%); (c) DAST, CH_2Cl_2, -78 °C, 3 h (89%).

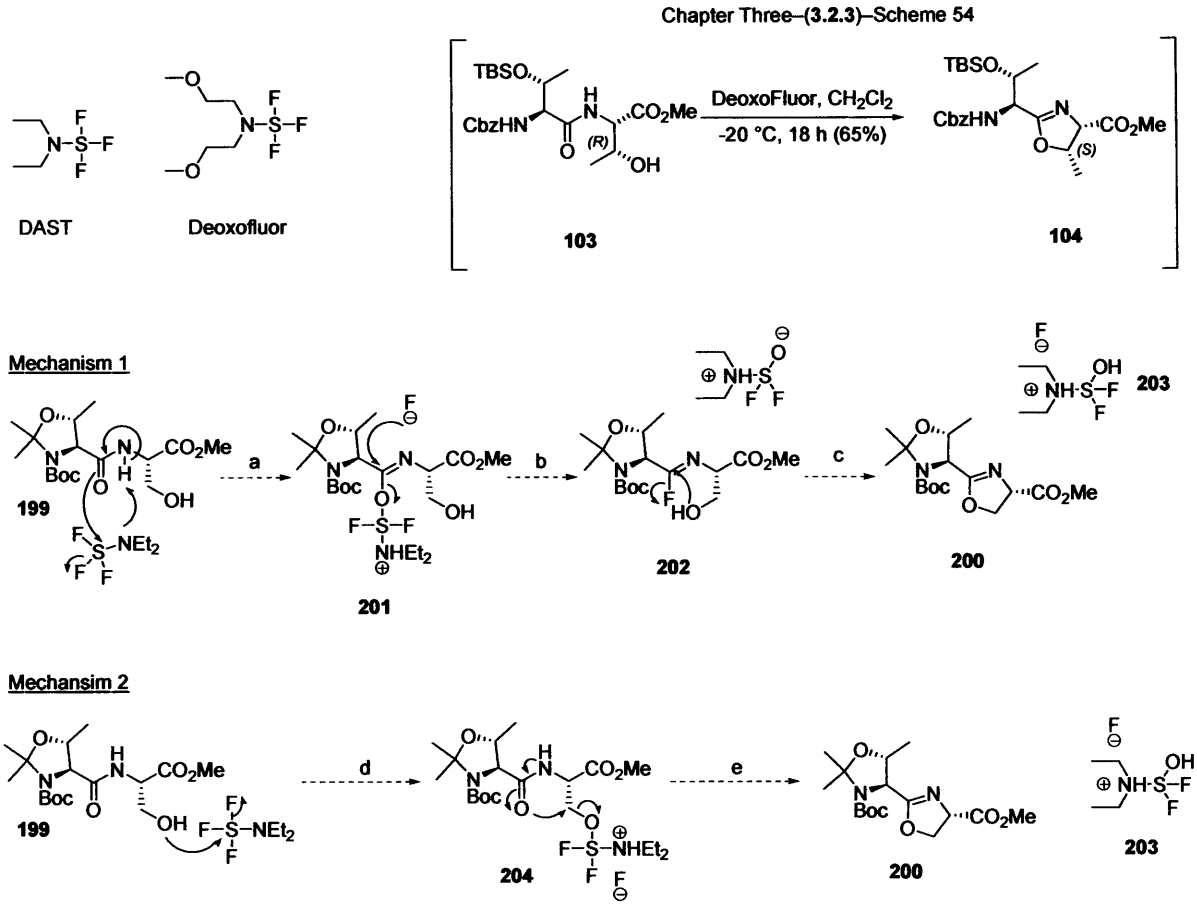
According to the literature, there are two possible mechanisms that facilitate cyclization using DAST,[15] these mechanisms can be consider to be identical for Deoxofluor (an analogous reagent to DAST) that exhibits superior thermal stability then DAST and therefore can be used at a higher temperature (Chapter Three–**3.2.3**). Neither mechanism has been shown by mechanistic studies however they are still worthy of consideration, in the example mechanisms (Scheme 87) to follow the cyclization agent used will be DAST.

Mechanism 1 is thought to initiate through the carbonyl of the amide in compound **199** by nucleophilic attack on the sulphur atom of DAST. This process is enhanced by proton abstraction by the amine functionality of the cyclizing agent and furthermore results in loss of fluoride (a). Curiously the imidate **201** is thought to undergo nucleophilic attack from fluoride (b) causing loss of cyclizing agent possibly as the zwitterion intermediate and generation of fluoroimine **202**. Clearly fluoroimine **202** is activated for intramolecular nucleophilic attack from the hydroxyl group (c), giving the desired product **200** and hydrogen fluoride as a leaving group. It is likely that hydrogen fluoride and the zwitterion intermediate forming a tight ion complex, compound **203**, as any free hydrogen fluoride generated in the reaction would have deleterious effects on silyl protection groups, a feature not observed when using either DAST or Deoxofluor.[16]

The explanation for mechanism 2 begins with nucleophilic attack from the oxygen of the hydroxyl group in compound **199** to the sulphur of the cyclizing agent (d) generating an ionic complex. Activation of the hydroxyl group now undergoes nucleophilic substitution from the

amide functionality in compound **204** (e), resulting in generation of the desired product **200** and the tight ion complex **203**.

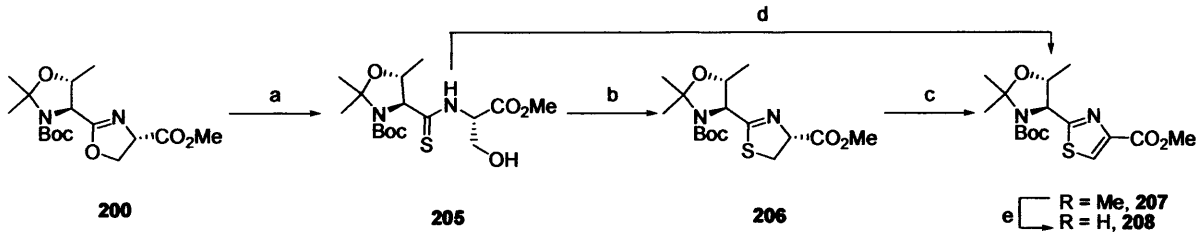
Scheme 87. Mechanism for oxazoline formation from Deoxofluor or DAST



It is unlikely that mechanism 1 is a viable route to cyclization for two reasons, firstly fluoride is not a good nucleophile and therefore intermolecular attack process (b) would not be expected to occur over intramolecular attack from the hydroxyl group. Secondly and equally importantly, is the example used in the synthesis of the oxazole fragment for methyl sulfomycinate (Chapter 3–**3.2.3**). The dipeptide **103** underwent cyclization and inversion at the 5' position on the oxazoline ring **104** (Scheme 87). It is accepted that inversion at the β–position will occur when using these cyclizing agents,[17] this could not be achieved through mechanism 1. Although the actually mechanism is not clear, mechanism 2 is more favourable since it is simpler and does not over complicate the process. It is remarkable to observe that silyl protection groups tolerate the reaction conditions and is evidence in support of tight ion complex formation which would be a side product of both mechanisms.

Thionation of oxazoline **200** was achieved by treatment with hydrogen sulphide gas and triethylamine in methanol at room temperature for 4 hours to give thioamide **205** in moderate yield (45%). Cyclization using DAST in dichloromethane at –78 °C for 3 hours gave thiazoline **206**, followed by treatment with DBU and bromotrichloromethane at –20 °C for 18 hours gave thiazole **207** in 62% isolated yield. Moreover thiazole **207** was isolated in 60% yield from a one–pot two–step reaction of thioamide **205**, a transformation not previously reported, by treatment with DAST in dichloromethane at –78 °C for 3 hours, immediately followed by a temperature raise to –20 °C, the addition of DBU and bromotrichloromethane and stirring the reaction for 18 hours. Saponification of thiazole **207** with lithium hydroxide in methanol–water gave carboxylic acid **208** in quantitative yield (Scheme 88).

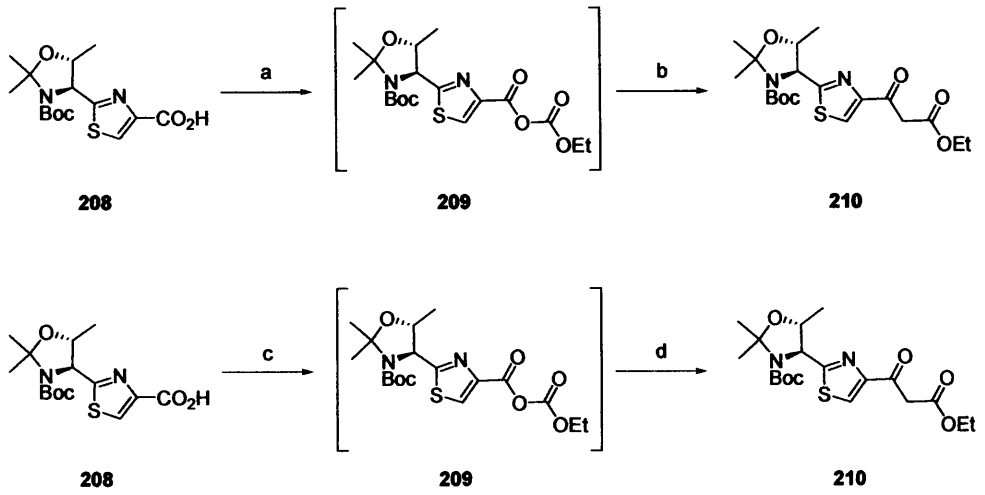
Scheme 88. Synthesis of carboxylic acid **208**



Reagents and conditions: (a) H_2S, MeOH–Et_3N, RT, 4 h (45%); (b) DAST, CH_2Cl_2, -78 °C, 3 h (100%); (c) $CBrCl_3$, DBU, CH_2Cl_2, -20 °C, 18 h (62%); (d) DAST, CH_2Cl_2, -78 °C, 3 h; then $CBrCl_3$, DBU, -20 °C, 18 h (60%); (e) LiOH, MeOH–H_2O, RT, 18 h (100%).

Activation of **208** by treatment with ethyl chloroformate and triethylamine in tetrahydrofuran at 0 °C gave the mixed anhydride intermediate **209**. Reaction of **209** with either the lithium enolate of ethyl acetate or the magnesium enolate of ethyl potassium malonate gave β–keto ester **210** as a mixture of tautomers in 16 and 58% isolated yield, respectively (Scheme 89).

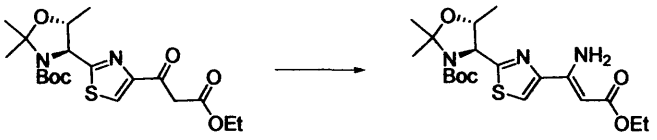
Scheme 89. Synthesis of β–keto ester **210**



Reagents and conditions: (a) Ethyl chloroformate, Et_3N, THF, 0 °C, 1.5 h; (b) ^nBuLi, $^iPr_2NH$, THF, 0 °C; then -78 °C, EtOAc, 30 min; **187**, 1.5 h (16%); (c) ethyl chloroformate, Et_3N, THF, 0 °C, 20 min; (d) MeMgBr, potassium ethyl malonate, THF, 0 °C, 30 min; RT, 1.5 h; **187**, 3.5 h (58%).

Thus far β–keto ester **210** has been generated in 7 steps and 13% overall yield by preparing a thiazole directly from the secondary thioamide in a hitherto unreported procedure. Generation of enamine **211** from β–keto ester **210** was attempted under various conditions (Scheme 90, Table 32), but was not easily achieved. All of the conditions used to synthesize **211** failed to generate the enamine (entries 1–10). Reacting the material for longer periods of time did not promote enamine formation (entries 2,3,9 & 10). When microwave irradiation was used at high temperature and elevated pressure, the reaction was irreproducible (entries 5 & 6). Even the use of toluene–acetic acid (5:1), a tried and tested method for enamine formation, gave only starting material by ^1H NMR spectroscopy or after column chromatography. A 14 fold excess of ammonium acetate under Dean–Stark conditions also proved unsuccessful (entry 9), and the use of aqueous ammonium hydroxide in methanol in large excess (1:3) only returned starting material (entry 10). It is not clear why enamine formation proved so difficult to achieve, the presence of the oxazolidine ring may be contributing to steric effects. There appears to be only one example in the literature for the transformation of a β–keto–ester thiazole to an enamine thiazole.[18] This method was attempted (entry 5 and 6) but returned only starting material.

Scheme 90. Generation of enamine **211**



210 **211**

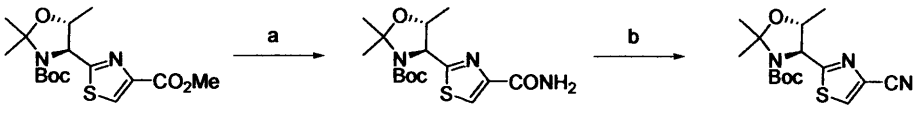
Table 32. Efforts to synthesis enamine **211** from **210**

Entry	NH_3 source	Equivalents of NH_3	Solvent	Temperature /°C	Conditions	Time	Compound[a]
1	NH_4OAc	5	EtOH	50	thermal	4 h	**210**
2	NH_4OAc	5	PhMe	120	thermal	18 h	**210**
3	NH_4OAc	10	PhMe–AcOH (5:1)	120	thermal	18 h	**210**
4	NH_4OAc	10	PhMe–AcOH (5:1)	120	thermal	4 h	**210**
5	NH_4OAc	10	PhMe–AcOH (5:1)	120	microwave[b]	30 min	–
6	NH_4OAc	10	PhMe–AcOH (5:1)	120	microwave[b]	30 min	**210**[c]
7	NH_4OAc	12	PhMe	120	microwave[b]	30 min	**210**
8	NH_4OAc	12	PhMe–AcOH (5:1)	120	microwave[b]	30 min	**210**
9	NH_4OAc	14	PhMe–AcOH (5:1)	120	thermal, Dean–Stark	18 h	**210**[d]
10	NH_4OH	–	MeOH–NH_4OH (aq) (3:1)	25	thermal	18 h	**210**

[a] Outcome determined by ^1H NMR spectroscopic analysis only. [b] simultaneous cooling. [c] 50% isolated recovery of starting material by column chromatography. [d] 4% isolated recovery of starting material by column chromatography.

In an effort to circumvent the need to synthesize β–keto ester **210** in the synthesis of enamine **211**, the ready conversion of nitriles to enamines was investigated.[19] Thiazole ester **207** was stirred in methanolic ammonia for 18 hours at room temperature and after purification gave the corresponding amide **212** in quantitative yield. Treatment of the amide with phosphorous oxychloride in pyridine at 0 °C for 2 hours gave the corresponding nitrile **213** in good yield (77%) (Scheme 91).

Scheme 91. Synthesis of nitirile **213**



207 **212** **213**

Reagents and conditions: (a) NH_3, MeOH, RT, 18 h (100%); (b) $POCl_3$, pyridine, 0 °C–RT, 2 h (77%).

Nitrile **213** was reacted according to a modified literature procedure[19] with the magnesium enolate of ethyl acetate generated from lithium diisopropylamine and methylmagnesium bromide. After stirring at 0 °C for 1 hour the reaction was quenched with saturated ammonium chloride and extracted with dichloromethane, starting material was recovered in quantitative yield. When the reaction was attempted using potassium ethyl malonate as the nucleophile, only starting material was isolated. In an effort to ensure protonation of the reaction intermediate the reaction was carried out in a 25% w/v sodium methoxide–methanol solution and stirred in the microwave under cooling conditions at 110 °C for 1 hour. A congealed brown gel was isolated directly from the microwave which was partitioned between water and dichloromethane, with the addition of ethyl acetate to aid solvation of the suspension. In contrast no starting material was observed in the ^{1}H NMR spectrum of the extracted material, but multiple products were formed which did not support enamine formation but suggested some degradation of the oxazolidine ring.

4.7 Conclusion

Steps towards the total synthesis of micrococcin P_1 have proven to be challenging, requiring efforts that have been both disappointing and rewarding. Although greater diversity of protection group chemistry utilizing selenium chemistry was unsuccessful, the synthesis of two orthogonally protected side chain dipeptides was achieved. Our study demonstrates that the synthesis of a precursor to the Bohlmann–Rahtz pyridine is viable en route to the core of micrococcin P_1. Synthesis of a bisthiazole–alkynone was achieved in 8 steps and 24% overall yield. In addition, we have investigated the hydrolysis of various bisthiazole fragments, identifying that the acetal protected bisthiazole can be successfully hydrolyzed and coupled to a side chain precursor for elaboration to an alkynone in the total synthesis of micrococcin P_1.

The corresponding Bohlmann–Rahtz precursor, namely β–keto ester **210** has been synthesized in 7 steps and 13% overall yield. It is noteworthy to recognize that this strategy appears to be the only example in the literature that cyclizes and oxidises a secondary thioamide to the corresponding thiazole in one pot. Enamine formation was unsuccessful from the β–keto ester under the conditions described and is likely due in part to steric hindrance by the butoxycarbonyloxazolidine and secondly to the increased propensity of the thiazole adjacent to the β–keto ester to promote formation of the enol tautomer. Recently however the enamine was isolated within the group using copious quantities of ammonium acetate under harsh microwave–assisted conditions giving consistently 37% yield from the β–keto ester. Indeed a one–pot two– or three–component reaction as described in the introduction may be the way forward. In addition further investigation of nucleophilic addition to the corresponding nitrile may yield the desired enamine when more encouraging conditions have been found.

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Chapter Five – Summary

5 Summary

The re–emergence of the Bohlmann–Rahtz pyridine synthesis over the last decade has provided a viable route to 2,3,6–trisubstituted, 2,3,4,6–tetrasubstituted and 2,3,5,6–tetrasubstituted pyridines, the latter providing a substitution pattern hitherto unreported from Bohlmann–Rahtz intermediates. These methods have been broadened further by the introduction of mild reaction conditions using either NIS or iodine to facilitate facile cyclodehydration of the Bohlmann–Rahtz intermediate with total regiocontrol. In particular NIS methods have been shown to provide a valuable route to the core domain of the series *d* thiopeptide antibiotic, cyclothiazomycin. Furthermore these new methods have the potential to provide complementary and independent pathways to access many of the thiopeptide antibiotics.

Degradation studies of thiopeptide antibiotics have led to the isolation of fragments that can help in the structural elucidation of the parent molecules. Although many of the fragments have been assigned based on spectroscopic data, very few of these hypotheses have been verified by chemical synthesis. Our efforts to synthesize degradation fragments from thiopeptide antibiotics have helped to address this short fall, validating known data and consolidating methods that could lead to the total synthesis of the parent antibiotics.

Successes include the synthesis of methyl sulfomycinate **39** in 9 steps from H-Thr-OMe and 15% overall yield, and elaboration of this target molecule to give sulfomycinic amide **38** and sulfomycinine **37**. This has enabled us to confirm the outcome of Abe's methanolysis studies and has provided a viable route to this region of the sulfomycin cyclic peptides. The total synthesis of saramycetic acid I **47** in 9 steps and 11% overall yield using methodology developed within the research group provided bisthiazoles for application in the total synthesis of the thiopeptide antibiotics.

Steps towards the total synthesis of micrococcin P_1 have demonstrated that the synthesis of enamine and alkynone precursors of Bohlmann–Rahtz pyridines is viable en route to the core of micrococcin P_1. The bisthiazole–alkynone **194** was synthesized in 8 steps and 24% overall yield, whilst the corresponding β–keto ester **210** was synthesized in 7 steps and 13% overall yield. These methods could be used in the future to construct micrococcin P_1 in order to specify once and for all the correct stereochemistry of this biologically important thiopeptide antibiotic.

Chapter Six – Experimental

6 Experimental

6.1 Experimental techniques

Commercially available reagents were used without further purification; solvents were dried by standard procedures. Light petroleum refers to the fraction with bp 40–60 °C, ether (Et_2O) refers to diethyl ether and EtOAc refers to ethyl acetate. Column 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). Melting points (mp) were determined on a Kofler hot stage apparatus and are uncorrected. Infra–red (IR) spectra were recorded in the range 4000–600 cm^{-1} on a Perkin–Elmer 1600 series FTIR spectrometer using KBr disks for solid samples and thin films between NaCl plates for liquid samples or as a nujol mull and are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ at 25 °C unless stated otherwise using a Bruker DPX 400 instrument operating at 400 MHz for ^1H spectra and 100 MHz for ^{13}C spectra and were reported in ppm; *J* values were recorded in Hz and multiplicities were expressed by the usual conventions (s=singlet, d=doublet, t=triplet, app=apparent, m=multiplet). Low–resolution mass spectra (MS) were determined using a Fisons VG Platform II Quadrupole instrument using atmospheric pressure chemical ionization (APcI) unless otherwise stated. ES refers to electrospray ionization, CI refers to chemical ionization (ammonia) and EI refers to electron impact. High-resolution mass spectra were obtained courtesy of the EPSRC Mass Spectrometry Service at Swansea, UK using the ionisation methods specified. Microanalyses were recorded using a Perkin–Elmer 240C Elemental Analyzer. Specific rotations were measured at the indicated temperature using an AA–1000 (Optical Activity Ltd) polarimeter at the sodium D line and are given in deg $cm^3g^{-1}dm^{-1}$ with concentration *c* in 10^{-2} gcm^{-3}. *In vacuo* refers to evaporation at reduced pressure using a rotary evaporator and diaphragm pump, followed by the removal of trace volatiles using a vacuum (oil) pump. Microwave experiments were carried out in a CEM Discovery microwave synthesiser at the temperature and initial power stated.

6.2 General experimental procedures

6.2.1 General procedure for Michael addition of enamines and alkynones

A solution of enamine **1** (0.36 mmol, 1 equiv.) and alkynone **2** (0.56 mmol, 1.5 equiv.) in EtOH (5 ml) was stirred at 50 °C for 1–7 h, cooled and evaporated *in vacuo* to give the crude aminodienone **3**.

6.2.2 General procedure for the cyclodehydration of aminodienones using NIS

A solution of aminodienone **3** (0.2 mmol, 1 equiv.) and *N*–iodosuccinimide (0.25 mmol, 1.2 equiv.) in EtOH (4 ml) was stirred at 0 °C for 1 h and then evaporated *in vacuo*, to give the crude pyridine **4**.

6.2.3 General procedure for the catalytic cyclodehydration of aminodienones using iodine

A solution of aminodienone **3** (0.22 mmol, 1 equiv.) and iodine (0.04 mmol, 20 mol%) in EtOH (4 ml) was stirred at RT for 30 min and an aqueous solution of sodium thiosulphate (10% w/v, 10 ml) was added. The mixture was extracted with CH_2Cl_2 (3 x 20 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to give pyridine **4**.

6.2.4 General procedure for the bromocyclization of aminodienones using NBS

A solution of aminodienone **3** (0.28 mmol, 1 equiv.) and *N*–bromosuccinimide (0.34 mmol, 1.2 equiv.) in EtOH (5 ml) was stirred at 0 °C for 1 h and evaporated *in vacuo*, to give the crude bromopyridine **70**.

6.2.5 General procedure for the synthesis of propargylic alcohols from aldehydes

A solution of aldehyde **67** (5.0 mmol) in dry THF (10 ml) was added to a stirred solution of ethynylmagnesium bromide in THF (0.5 M; 15 ml, 7.5 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred overnight. Saturated aqueous NH_4Cl solution (2 ml) was added, the mixture was evaporated *in vacuo* and partitioned between Et_2O (30 ml) and saturated aqueous ammonium chloride solution (30 ml). The ethereal layer was washed with brine (30 ml), dried (Na_2SO_4) and evaporated *in vacuo*.

6.3 Experimental procedures

tert–Butyl β–aminocrotonate (1e)

Ammonium hydroxide solution (35%, 40 ml) was added to a mixture of *tert*–butyl acetoacetate **7b** (4 ml, 24.2 mmol) in MeOH (40 ml) and stirred at 50 °C for 18 h. After cooling, the solution was evaporated *in vacuo* and partitioned between H_2O (40 ml) and Et_2O (40 ml). The aqueous layer was further extracted with EtOAc (2 x 35 ml) and the combined organic extracts were washed with brine (25 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound*[1] as a clear oil (3.72 g, 98%) (Found: MH^+, 158.1178. $C_8H_{16}NO_2$, requires *MH*$^+$, 158.1176); IR (film)/cm^{-1} ν_{max} 3554, 3341, 2980, 2919, 1666, 1622, 1567, 1454, 1390, 1366, 1296, 1150, 983,

790; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.20 (1H, bs, NH), 4.20 (1H, bs, NH), 4.35 (1H, s, CH), 1.80 (3H, s, Me), 1.38 (9H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (C), 158.8 (C), 85.9 (CH), 78.2 (C), 28.6 (Me), 22.4 (Me); *m/z* (APcI) 158 (MH^+, 77%).

Ethyl 3–amino–3–phenylpropenoate (1f)

Ammonium acetate (13.4 g, 0.17 mol) was added to a solution of ethyl benzoylacetate **7d** (5 ml, 29.0 mmol) and the mixture was heated at reflux in toluene–glacial acetic acid (5:1; 40 ml) for 20 h. After partitioning between H_2O (100 ml) and Et_2O (60 ml), the aqueous layer was further extracted with Et_2O (2 x 25 ml) and the combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$ solution (50 ml) and brine (25 ml), dried ($MgSO_4$) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (3:1) (R_f 0.11), gave the *title compound*[2] as a pale yellow oil (3.32 g, 60%) (Found: MNH_4^+, 209.1289. $C_{11}H_{17}N_2O_2$, requires MNH_4^+, 209.1285); IR (film)/cm^{-1} ν_{max} 3441, 3326, 2979, 2936, 1663, 1617, 1555, 1492, 1364, 1176, 1095, 1025, 796, 772, 699; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.35 (1H, bs, NH), 7.33–7.12 (5H, PhH), 7.00 (1H, bs, NH), 4.75 (1H, s, CH), 3.95 (2H, q, *J* 7.1, OCH_2Me), 1.05 (3H, t, *J* 7.1, CH_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C), 160.5 (C), 137.7 (C), 130.3 (CH), 128.9 (CH), 126.2 (CH), 84.7 (CH), 59.0 (CH_2), 14.6 (Me); *m/z* (APcI) 192 (MH^+, 100%) and 146 (13).

1–Phenylprop–2–yn–1–one (2a)

A solution of *o*–iodoxybenzoic acid (IBX) (3.65 g, 13.0 mmol) in DMSO (110 ml) was stirred for 15 min at room temperature until homogeneous. A solution of 1–phenyl–2–propyn–1–ol **68a** (1.32 g, 10.0 mmol) in DMSO (10 ml) was added and the mixture was stirred for 5 h. H_2O (30 ml) was added and the mixture was stirred at room temperature for 10 min, cooled in ice and partitioned between H_2O (120 ml) and Et_2O (90 ml). The mixture was filtered through Celite® and the aqueous layer was further extracted with Et_2O (50 ml). The organic extracts were combined, washed sequentially with H_2O (3 x 50 ml), saturated aqueous ($NaHCO_3$) solution (70 ml) and

brine (70 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound* as a pale yellow solid (1.0 g, 77%), mp 49–50 °C (MeOH) (lit.[3] mp 47–48 °C) (Found: M^+, 130.0414. C_9H_6O, requires M^+, 130.0413); IR (KBr)/cm^{-1} ν_{max} 3231, 2094, 1645, 1593, 1578, 1452, 1317, 1261, 1173, 1005, 695; ^1H NMR (400 MHz; $CDCl_3$) δ 8.12 (2H, m, *o*–Ph*H*), 7.55 (1H, m, *p*–Ph*H*), 7.45 (2H, m, *m*–Ph*H*), 3.36 (1H, s, CH); ^{13}C NMR (100 MHz; $CDCl_3$) δ 177.5 (C), 136.1 (C), 134.6 (CH), 129.7 (CH), 128.7 (CH), 80.9 (C), 80.3 (CH); *m/z* (EI) 130 ($M^{\cdot+}$, 16%), 77 (32), 53 (100).

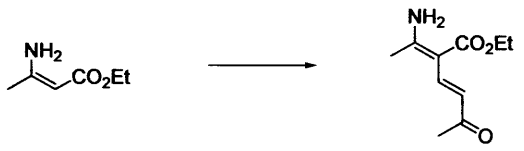
1–(4–Chlorophenyl)prop–2–yn–1–one (2h)

A solution of *o*–iodoxybenzoic acid (IBX) (5.84 g, 20.8 mmol) in DMSO (120 ml) was stirred for 15 min at room temperature until homogeneous. A solution of 1–(4–chlorophenyl)prop–2–yn–1–ol **68b** (2.72 g, 16.3 mmol) in DMSO (20 ml) was added and the mixture was stirred for 5 h. H_2O (40 ml) was added and the mixture was stirred at room temperature for 10 min, cooled in ice and partitioned between H_2O (120 ml) and Et_2O (90 ml). The mixture was filtered through Celite® and the aqueous layer was further extracted with Et_2O (50 ml). The organic extracts were combined, washed sequentially with H_2O (3 × 50 ml), saturated aqueous $NaHCO_3$ solution (70 ml) and brine (70 ml), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with $CHCl_3$ (R_f 0.45), gave the *title compound*[4] as a pale yellow solid (2.50 g, 93%), mp 68–69 °C (MeOH) (lit.[4] mp 68–69 °C) (Found: M^+, 165.9991. $C_9H_5Cl^{37}O$, requires M^+, 165.9994); IR (KBr)/cm^{-1} ν_{max} 2921, 2853, 2360, 1662, 1462, 1377, 1248, 1094, 1003, 722; ^1H NMR (400 MHz; $CDCl_3$) δ 7.98 (2H, appd, *J* 8.6, 3',4'–PhH), 7.41 (2H, appd, *J* 8.6, 2',5'–PhH), 3.38 (1H, s, CH); ^{13}C NMR (100 MHz; $CDCl_3$) δ 176.1 (C), 141.3 (C), 134.5 (C), 131.0 (CH), 129.1 (CH), 81.4 (C), 79.9 (CH); *m/z* (EI) 166 ($M^{\cdot+}$, 7%), 164 (M^+, 21), 138 (11), 136 (33), 113 (3), 111 (12), 53 (100).

1–(4–Methoxyphenyl)prop–2–yn–1–one (2i)

A solution of *o*–iodoxybenzoic acid (IBX) (5.14 g, 18.3 mmol) in DMSO (120 ml) was stirred for 15 min at room temperature until homogeneous. A solution of 1–(4–methoxyphenyl)prop–2–yn–1–ol **68c** (2.27 g, 14.0 mmol) in DMSO (20 ml) was added and the mixture was stirred for 5 h. Water (40 ml) was added and the mixture was stirred at room temperature for 10 min, cooled in ice and partitioned between H_2O (120 ml) and Et_2O (90 ml). The mixture was filtered through Celite® and the aqueous layer was further extracted with Et_2O (80 ml). The organic extracts were combined, washed sequentially with H_2O (3 × 50 ml), saturated aqueous $NaHCO_3$ solution (70 ml) and brine (70 ml), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with $CHCl_3$ (R_f 0.29), gave the *title compound*[5] as a pale yellow solid (2.24 g, 89%), mp 86–87 °C (MeOH) (lit.[4] mp 85–87 °C) (Found: MH^+, 161.0597. $C_{10}H_9O_2$, requires MH^+, 161.0597); IR (KBr)/cm^{-1} ν_{max} 3297, 2092, 1641, 1597, 1572, 1511, 1423, 1252, 1170, 1116, 1023, 841, 758, 710, 685; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.05 (2H, appd, *J* 8.7, 2',6–PhH), 6.88 (2H, appd, *J* 8.7, 3',5'–PhH), 3.88 (3H, s, OMe), 3.29 (1H, s, CH); ^{13}C NMR (100 MHz; $CDCl_3$) δ 176.0 (C), 164.8 (C), 132.2 (CH), 129.2 (CH), 113.9 (CH), 80.4 (C), 80.1 (C), 55.6 (Me); *m/z* (APcI) 161 (MH^+, 100%).

(4*E*)–2–Amino–3–ethoxycarbonylhexa–2,4–diene–6–one (3a)

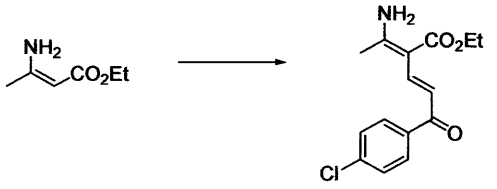


Aminodienone **3a** was prepared according to the general procedure **6.2.1** using ethyl β–aminocrotonate **1a** and 4–(trimethylsilyl)but–3–yn–2–one **2d**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.23), gave the *title compound* as a yellow solid (58 mg, 82 %), mp 125–126 °C (light petroleum–EtOAc) (lit.[3] mp 125.5–126.4 °C) (Found: C, 60.6; H, 7.7; N, 7.0. Calc. for $C_{10}H_{15}NO_3$: C, 60.1; H, 7.7; N, 7.1%) (Found: MH^+, 198.1125. $C_{10}H_{16}NO_3$ requires MH^+, 198.1125); IR (KBr)/cm^{-1} ν_{max} 3334, 3193, 2977,1647, 1546, 1488, 1459, 1362, 1319, 1286, 1205, 1180, 1112, 1024, 970, 950, 844; ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.65 (1H, bs, NH), 7.55 (1H, d, *J* 15.6, 4–H), 6.50 (1H, d, *J* 15.6, 5–H), 5.5 (1H, bs, NH), 4.22 (2H, q, *J* 7.1, OCH_2Me), 2.22 (3H, s, Me), 2.15 (3H, s, Me), 1.28 (3H, t, *J* 7.1, CH_2Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ199.0 (C), 169.7 (C), 165.7 (C), 139.5 (CH), 121.1 (CH), 94.4 (C), 60.0 (CH_2), 28.4 (Me), 22.6 (Me), 14.4 (Me); *m/z* (APcI) 198 (MH^+, 100%) and 181 (48).

(4*E*)–2–Amino–3–ethoxycarbonyl–6–phenylhexa–2,4–diene–6–one (3b)

Aminodienone **3b** was prepared according to the general procedure **6.2.1** using ethyl β–aminocrotonate **1a** and 1–phenylprop–2–yn–1–one **2a**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.24), gave the *title compound*[6] as a yellow solid (79 mg, 85 %), mp 156–157 °C (light petroleum–EtOAc) (Found: C, 69.4; H, 6.6; N, 5.2. Calc. for $C_{15}H_{18}NO_3$: C, 69.5; H, 6.6; N, 5.4%) (Found: MH^+, 260.1282. $C_{15}H_{17}NO_3$ requires MH^+, 260.1281); IR (KBr)/cm^{-1} ν_{max} 3342, 3203, 2976, 1623, 1580, 1539, 1497, 1378, 1354, 1320, 1286, 1223, 1205, 1178, 1110, 1055, 1036, 1023, 976, 847, 705, 626; ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.67 (1H, bs, NH), 7.94 (2H, m, *o*–Ph*H*), 7.86 (1H, d, *J* 15.0, 4–H), 7.43 (3H, *m,p*–Ph*H*), 7.39 (1H, d, *J* 15.0, 5–H), 5.70 (1H, bs, NH), 4.24 (2H, q, *J* 7.1, OC*H*$_2$Me), 2.33 (3H, s, Me), 1.35 (3H, t, *J* 7.1, CH$_2$*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 190.9 (C), 169.8 (C), 166.7 (C), 141.1 (CH), 139.7 (C), 131.8 (CH), 128.4 (CH), 128.1 (CH), 115.8 (CH), 95.6 (C), 60.0 (CH_2), 22.6 (Me), 14.5 (Me); *m/z* (APcI) 260 (MH^+, 100%).

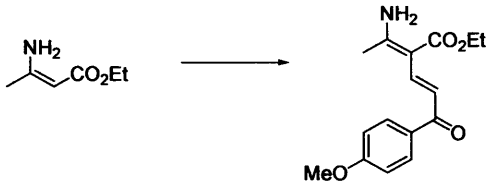
(4*E*)–2–Amino–3–ethoxycarbonyl–6–(4–chlorophenyl)hexa–2,4–diene–6–one (3c)



Aminodienone **3c** was prepared according to the general procedure **6.2.1** using ethyl β–aminocrotonate **1a** and 1–(4–chlorophenyl)prop–2–yn–1–one **2h**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.18), gave the *title compound*[6] as a yellow solid (91 mg, 86 %), mp 163–164 °C (light petroleum–EtOAc) (Found: MH^+, 294.0893. $C_{15}H_{17}ClNO_3$ requires MH^+, 294.0891); IR (KBr)/cm^{-1} ν_{max} 3315, 3168, 2964, 1653, 1630, 1591, 1571, 1539, 1485, 1350, 1323, 1288, 1224, 1177, 1120, 1089, 1057, 1037, 1012, 975, 856, 824, 743, 713, 642, 589, 538, 502; ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.71 (1H, bs, NH), 7.88 (1H, d, *J* 15.0, 4–H), 7.84 (2H, appd, *J* 8.5, 2',6'–PhH), 7.36 (2H, appd, *J* 8.5, 3',5'–

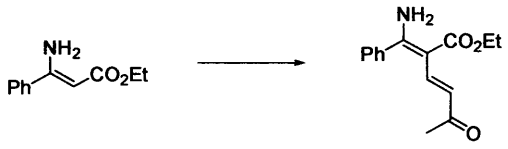
PhH), 7.33 (1H, d, *J* 15.0, 5–H), 5.78 (1H, bs, NH), 4.20 (2H, q, *J* 7.1, OC*H*$_2$Me), 2.27 (3H, s Me), 1.35 (3H, t, *J* 7.1, CH$_2$*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 189.5 (C), 169.7 (C), 167.0 (C), 141.6 (CH), 138.0 (C), 138.0 (C), 129.5 (CH), 128.6 (CH), 115.1 (CH), 95.7 (C), 60.1 (CH_2), 22.6 (Me), 14.5 (Me); *m/z* (APcI) 296 (MH^+, 32%), 294 (MH^+, 100).

(4*E*)–2–Amino–3–ethoxycarbonyl–6–(4–methoxyphenyl)hexa–2,4–diene–6–one (3d)



Aminodienone **3d** was prepared according to the general procedure **6.2.1** using ethyl β–aminocrotonate **1a** and 1–(4–methoxyphenyl)prop–2–yn–1–one **2i**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.50), gave the *title* compound[6] as a yellow solid (99 mg, 95 %), mp 155–156 °C (light petroleum–EtOAc) (lit.[8] 159 °C) (Found: MH^+, 290.1386. $C_{16}H_{20}NO_4$ requires *MH*$^+$, 290.1387); IR (KBr)/cm^{-1} ν_{max} 3297, 3145, 2974, 1717, 1627, 1598, 1576, 1539, 1511, 1475, 1362, 1321, 1300, 1263, 1231, 1168, 1126 1021, 834, 586; ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.63 (1H, bs, NH), 7.91 (2H, appd, *J* 8.8, 2',6'–PhH), 7.82 (1H, d, *J* 15.0, 4–H), 7.38 (1H, d, *J* 15.0, 5–H), 6.85 (1H, appd, *J* 8.8, 3',5'–PhH), 5.63 (1H, bs, NH), 4.23 (2H, q, *J* 7.2, OC*H*$_2$Me), 3.83 (3H, s, OMe), 2.25 (3H, s Me), 1.35 (3H, t, *J* 7.2, CH$_2$*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 189.3 (C), 169.9 (C), 166.3 (C), 162.7 (C), 140.2 (CH), 132.4 (C), 130.2 (CH), 115.7 (CH), 113.6 (CH), 95.5 (C), 60.0 (CH_2), 55.4 (Me), 22.6 (Me), 14.5 (Me); *m/z* (APcI) 290 (MH^+, 100%).

(4*E*)–2–Amino–3–ethoxycarbonyl–1–phenylhexa–2,4–diene–6–one (3e)



Aminodienone **3e** was prepared according to the general procedure **6.2.1** using ethyl 3–amino–3–phenylpropenoate **1f** and 4–(trimethylsilyl)but–3–yn–2–one **2d**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.24), gave the *title compound*[6] as a yellow solid (22 mg, 23%), mp 104–105 °C (light petroleum–EtOAc) (Found: C,

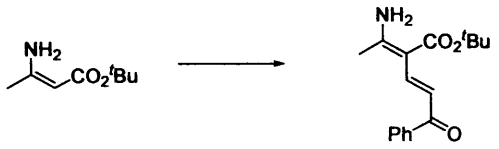
69.2; H, 6.6; N, 5.2. Calc. for $C_{15}H_{17}NO_3$: C, 69.5; H, 6.6; N, 5.4%); IR (KBr)/cm^{-1} ν_{max} 3317, 3120, 2980, 1654, 1586, 1507, 1461, 1345, 1285, 1257, 1210, 1119, 1023, 1002, 979, 927, 852, 768, 702, 632, 358; ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.40 (1H, bs, NH), 7.50–7.30 (5H, PhH), 7.08 (1H, d, *J* 15.9, 4–H), 6.45 (1H, d, *J* 15.9, 5–H), 5.45 (1H, bs, NH), 4.25 (2H, q, *J* 7.1, OCH_2Me), 1.88 (3H, s, Me), 1.35 (3H, t, *J* 7.1, CH_2Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 190.2 (C), 169.8 (C), 167.4 (C), 142.1 (CH), 136.8 (CH), 130.5 (CH), 128.9 (CH), 128.4 (CH), 122.6 (CH), 95.5 (C), 60.3 (CH_2), 26.8 (Me), 14.5 (CH_2); *m/z* (APcI) 260 (MH^+, 100%) and 243 (12).

Alternative procedure: A solution of ethyl 3–amino–3–phenylpropenoate **1f** (1.25 g, 6.54 mmol) and 4–(trimethylsilyl)but–3–yn–2–one **2d** (1.29 g, 9.2 mmol) in EtOH (20 ml) was stirred at 50 °C for 18 h, cooled and then evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.24), gave the *title compound*[6] as a yellow solid (0.79 g, 47%) with identical physical and spectroscopic properties.

(4*E*)–2–Amino–3–*tert*–butoxycarbonylhexa–2,4–diene–6–one (3f)

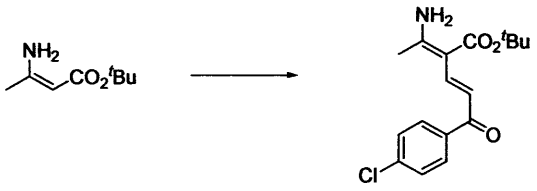
Aminodienone **3f** was prepared according to the general procedure **6.2.1** using *tert*–Butyl β–aminocrotonate **1e** and 4–(trimethylsilyl)but–3–yn–2–one **2d**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.26), gave the *title compound*[6] as a yellow solid (55 mg, 68 %), mp 142–143 °C (light petroleum–EtOAc) (Found: C, 63.8; H, 8.5; N, 6.1. Calc. for $C_{12}H_{19}NO_3$: C, 64.0; H, 8.5; N, 6.2%) (Found: MH^+, 226.1437. $C_{12}H_{20}NO_3$, requires MH^+, 226.1438); IR (KBr)/cm^{-1} ν_{max} 3341, 3198, 2975, 1654, 1539, 1485, 1454, 1362, 1323, 1295, 1218, 1160, 1111, 1034, 970, 840, 692, 572; ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.60 (1H, bs, NH), 7.48 (1H, d, *J* 15.4, 4–H), 6.45 (1H, d, *J* 15.4, 5–H), 5.39 (1H, bs, NH), 2.20 (3H, s, Me), 2.18 (3H, s, Me), 1.48 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 198.8 (C), 169.1 (C), 165.2 (C), 139.9 (CH), 120.8 (CH), 95.7 (C), 80.8 (C), 28.5 (Me), 28.4 (Me), 22.7 (Me); *m/z* (APcI) 226 (MH^+, 98%), 208 (54), and 152 (100).

(4*E*)–2–Amino–3–*tert*–butoxycarbonyl–6–phenylhexa–2,4–diene–6–one (3g)



Aminodienone **3g** was prepared according to the general procedure **6.2.1** using *tert*–butyl β–aminocrotonate **1e** and 1–phenylprop–2–yn–1–one **2a**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.21), gave the *title compound*[7] as a yellow solid (87 mg, 84 %), mp 150–151 °C (light petroleum–EtOAc) (Found: MH^+, 288.1599. $C_{17}H_{22}NO_3$ requires MH^+, 288.1594); IR (KBr)/cm^{-1} ν_{max} 3307, 3126, 2970, 1654, 1614, 1521, 1393, 1359, 1298, 1214, 1158, 1122, 1053, 1022, 974, 840, 818, 786, 698; ^1H NMR (400 MHz; $CDCl_3$) δ 9.72 (1H, bs, NH), 7.90 (2H, m, *o*–Ph*H*), 7.87 (1H, d, *J* 15.0, 4–H), 7.40 (3H, *m*, *p*–Ph*H*), 7.36 (1H, d, *J* 15.0, 5–H), 5.55 (1H, bs, NH), 2.25 (3H, s, Me), 1.55 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 190.9 (C), 169.4 (C), 166.9 (C), 141.8 (CH), 139.8 (C), 131.7 (CH), 128.4 (CH), 128.0 (CH), 115.8 (CH), 96.8 (C), 80.7 (C), 29.7 (Me), 22.6 (Me); *m/z* (APcI) 288 (MH^+, 100%).

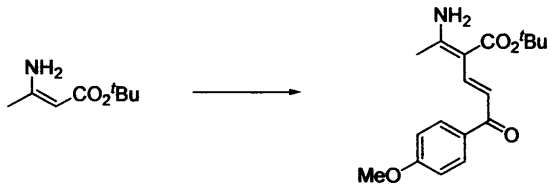
(4*E*)–2–Amino–3–*tert*–butoxycarbonyl–6–(4–chlorophenyl)hexa–2,4–diene–6–one (3h)



Aminodienone **3h** was prepared according to the general procedure **6.2.1** using *tert*–butyl β–aminocrotonate **1e** and 1–(4–chlorophenyl)prop–2–yn–1–one **2h**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.21), gave the *title compound*[7] as a yellow solid (85 mg, 73 %), mp 158–159 °C (light petroleum–EtOAc) (Found: MH^+, 322.1203. $C_{17}H_{21}ClNO_3$ requires MH^+, 322.1204); IR (KBr)/cm^{-1} ν_{max} 3324, 3178, 2976, 2915, 2361, 1660, 1629, 1588, 1587, 1570, 1482, 1345, 1319, 1299, 1233, 1209, 1157, 1109, 1089, 1052, 1024, 1010, 978; ^1H NMR (500 MHz; $CDCl_3$) δ 9.75 (1H, bs, NH), 7.88 (1H, d, *J* 14.8, 4–H), 7.85 (2H, appd, *J* 8.6, 2',6'–PhH), 7.37 (2H, appd, *J* 8.6, 3',5'–PhH), 7.34 (1H, d, *J* 14.8, 5–H), 5.56 (1H, bs, NH), 2.27 (3H, s, Me), 1.58 (9H, s, CMe_3); ^{13}C NMR (100 MHz;

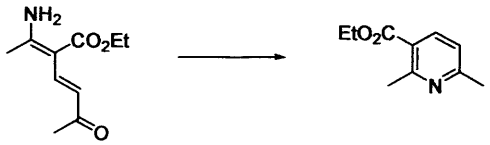
$CDCl_3$) δ 189.4 (C), 169.3 (C), 166.9 (C), 142.0 (CH), 138.2 (C), 137.9 (C), 129.4 (CH), 128.6 (CH), 114.7 (CH), 96.9 (C), 80.9 (C), 28.6 (Me), 22.8 (Me); *m/z* (APcI) 322 (MH^+, 100%).

(4*E*)–2–Amino–3–*tert*–butoxycarbonyl–6–(4–methoxyphenyl)hexa–2,4–diene–6–one (3i)



Aminodienone **3i** was prepared according to the general procedure **6.2.1** using *tert*–butyl β–aminocrotonate **1e** and 1–(4–methoxyphenyl)prop–2–yn–1–one **2i**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.21), gave the *title compound*[7] as a yellow solid (98 mg, 86 %), mp 149–150 °C (light petroleum–EtOAc) (Found: MH^+, 318.1702. $C_{18}H_{24}NO_4$ requires *MH*$^+$, 318.1700); IR (KBr)/cm^{-1} ν_{max} 3311, 3176, 1708, 1634, 1602, 1577, 1547, 1417, 1323, 1291, 1258, 1233, 1171, 1122, 1024, 953, 829, 745; ^1H NMR (400 MHz; $CDCl_3$) δ 9.71 (1H, bs, NH), 7.90 (2H, appd, *J* 8.5, 2',6'–PhH), 7.84 (1H, d, *J* 14.6, 4–H), 7.36 (1H, appd, *J* 14.6, 5–H), 6.86 (2H, d, *J* 8.5, 3',5'–PhH), 6.24 (1H, bs, NH), 3.78 (3H, s, OMe), 2.26 (3H, s, Me), 1.55 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 189.6 (C), 169.4 (C), 167.0 (C), 162.6 (C), 141.2 (CH), 132.6 (C), 130.2 (CH), 114.6 (CH), 113.6 (CH), 96.6 (C), 80.6 (C), 55.4 (C), 28.7 (Me), 22.4 (Me); *m/z* (APcI) 318 (MH^+, 100%).

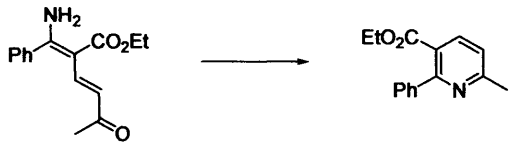
Ethyl 2,6–dimethylpyridine–3–carboxylate (4a)



Pyridine **4a** was prepared using aminodienone **3a** according to the general procedure **6.2.2**, purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.44), gave the *title compound*[8] as a pale yellow solid (24 mg, 66%). Pyridine **4a** was also prepared using aminodienone **3a** according to the general procedure **6.2.3** to give the *title compound*[8] as a pale yellow solid (39 mg, 98%), mp 59–60 °C (ethanol) (lit.[8] 60 °C) (Found: MH^+, 180.1017. $C_{10}H_{14}NO_2$ requires *MH*$^+$, 180.1019); IR (film)/cm^{-1} ν_{max} 2924, 2825, 1730,

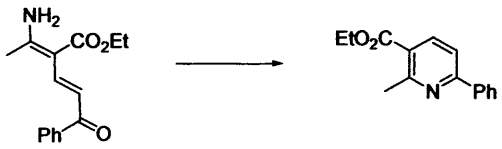
1593, 1462, 1377, 1272, 1236, 1148, 1079, 770, 722; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.03 (1H, d, *J* 8.0, 4–H), 7.00 (1H, d, *J* 8.0, 5–H), 4.27 (2H, q, *J* 7.2, OC*H*$_2$Me), 2.75 (3H, s, Me), 2.50 (3H, s, Me), 1.33 (3H, t, *J* 7.2, CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 166.7 (C), 161.1 (C), 159.4 (C), 138.9 (CH), 122.8 (C), 120.5 (CH), 61.1 (CH_2), 24.8 (Me), 24.6 (Me), 13.7 (Me); *m/z* (APcI) 180 (MH^+, 100%).

Ethyl 6–methyl–2–phenylpyridine–3–carboxylate (4f)



Pyridine **4f** was prepared using aminodienone **3e** according to the general procedure **6.2.2.** Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (*R*$_f$ 0.56), gave the *title compound*[8] as a pale yellow solid (47 mg, 98%), mp 45–46 °C (ethanol) (lit.[8] 46 °C) (Found: MH^+, 242.1175. $C_{15}H_{16}NO_2$ requires *MH*$^+$, 242.1176); IR (film)/cm^{-1} ν_{max} 3056, 2963, 2915, 2855, 2363, 1715, 1589, 1440, 1356, 1278 1211, 1137, 1106, 1053, 797, 767, 740, 699, 650; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.95 (1H, d, *J* 8.0, 4–H), 7.33–7.25 (5H, PhH), 7.12 (1H, d, *J* 8.0, 5–H), 4.05 (2H, q, *J* 7.1, OC*H*$_2$Me), 2.55 (3H, s, Me), 0.94 (3H, t, *J* 7.1, CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.2 (C), 160.8 (C), 158.7 (C), 140.5 (C), 138.4 (CH), 128.5 (CH), 128.4 (CH), 128.1 (CH), 124.4 (C), 121.3 (CH), 61.3 (CH_2), 24.8 (Me), 13.6 (Me); *m/z* (APcI) 242 (MH^+, 100%).

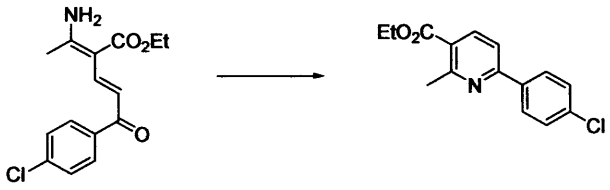
Ethyl 2–methyl–6–phenylpyridine–3–carboxylate (4q)



Pyridine **4q** was prepared using aminodienone **3b** according to the general procedure **6.2.2,** purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (*R*$_f$ 0.62), gave the *title compound*[8] as a pale yellow solid (47 mg, 98%), mp 44–45 °C (ethanol) (lit. 44–45 °C) Pyridine **4q** was also prepared using aminodienone **3b** according to the general procedure **6.2.3** to give the *title compound*[8] as a pale yellow solid (52 mg, 98%) mp 44–45 °C

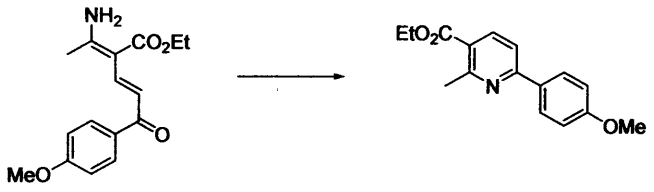
(methanol) (lit.[8] mp 44 °C) (Found: MH^+, 242.1176. $C_{15}H_{16}NO_2$ requires MH^+, 242.1179); IR (KBr)/cm^{-1} ν_{max} 2981, 1715, 1582, 1496, 1455, 1382, 1264, 1185, 1154, 1091, 1026, 922, 848, 798, 757, 692; ^1H NMR (400 MHz; $CDCl_3$) δ 8.21 (1H, d, *J* 8.2, 4–H), 8.02 (2H, m, *o*–Ph*H*), 7.60 (1H, d, *J* 8.2, 5–H), 7.42 (3H, *m,p*–Ph*H*) 4.32 (2H, q, *J* 7.2, OCH_2Me), 2.85 (3H, s, Me), 2.45 (3H, t, *J* 7.2, CH_2Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 166.9 (C), 160.0 (C), 159.1 (C), 139.3 (CH), 138.5 (C), 129.7 (CH), 128.8 (CH), 127.3 (CH), 123.7 (C), 117.4 (CH), 61.2 (CH_2), 25.3 (Me), 14.3 (Me); *m/z* (APcI) 242 (MH^+, 100%).

Ethyl 2–methyl–6–(4–chlorophenyl)pyridine–3–carboxylate (4r)



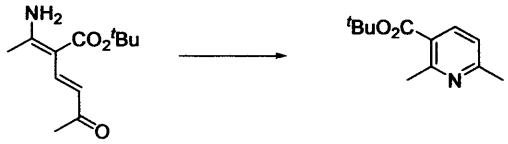
Pyridine **4r** was prepared using aminodienone **3c** according to the general procedure **6.2.2,** purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.68), gave the *title compound*[4] as a pale yellow solid (54 mg, 98%). Pyridine **4r** was also prepared using aminodienone **3c** according to the general procedure **6.2.3** to give the *title compound* as a pale yellow solid (59 mg, 97%) mp 48–49 °C (aqueous EtOH) (lit.[4] mp 47–48 °C) (Found: MH^+, 276.0784. $C_{15}H_{15}ClNO_2$ requires MH^+, 276.0786); IR (KBr)/cm^{-1} ν_{max} 2985, 1720, 1585, 1492, 1455, 1372, 1265, 1180, 1155, 1096, 1013, 896, 833, 786, 742, 705; ^1H NMR (400 MHz; $CDCl_3$) δ 8.23 (1H, d, *J* 8.2, 4–H), 7.95 (2H, appd, *J* 8.5, 2',6'–PhH), 7.54 (1H, d, *J* 8.2, 5–H), 7.40 (2H, appd, *J* 8.5, 3',5'–PhH), 4.38 (2H, q, *J* 7.1, OCH_2Me), 1.60 (3H, s, Me), 1.35 (3H, t *J* 7.1, CH_2Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 166.5 (C), 160.1 (C), 157.7 (C), 139.4 (CH), 136.9 (C), 135.9 (C), 129.0 (CH), 128.6 (CH), 123.9 (C), 117.4 (CH), 61.2 (CH_2), 25.3 (Me), 14.3 (Me); *m/z* (APcI) 276 (MH^+, 100%), 278 (MH^+, 33).

Ethyl 2–methyl–6–(4–methoxyphenyl)–3–carboxylate (4s)



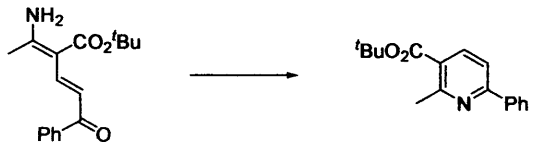
Pyridine **4s** was prepared using aminodienone **3d** according to the general procedure **6.2.2**, purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.63), gave the *title compound*[4] as a pale yellow solid (53 mg, 98%). Pyridine **4s** was also prepared using aminodienone **3d** according to general procedure **6.2.3** to give the *title compound*[4] as a pale yellow oil (55 mg, 92%) mp 68–69 °C (aq. EtOH) (lit.[4] mp 68–69 °C) (Found: MH^+, 272.1284. $C_{16}H_{18}NO_3$ requires *MH*$^+$, 272.1281); IR (KBr)/cm^{-1} ν_{max} 2989, 2895, 1717, 1605, 1579, 1509, 1452, 1439, 1389, 1362, 1311, 1264, 1172, 1112, 1088, 1071, 1031, 832, 785; ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.20 (1H, d, *J* 8.2, 4–H), 7.95 (2H, appd, *J* 8.7, 2',6'–PhH), 7.53 (1H, d, *J* 8.2, 5–H), 7.37 (2H, appd, *J* 8.7, 3',5'–PhH), 4.34 (2H, q, *J* 7.1, OC*H*$_2$Me), 3.82 (3H, s, OMe), 2.85 (3H, s, Me), 1.36 (3H, t *J* 7.1, CH$_2$*Me*); ^{13}C NMR (125 MHz; $CDCl_3$) δ 166.8 (C), 161.0 (C), 160.0 (C), 158.7 (C), 139.3 (CH), 131.7 (C), 128.7 (CH), 122.8 (C), 116.5 (CH), 114.2 (CH), 61.1 (CH_2), 55.4 (Me), 25.4 (Me), 14.4 (Me); *m/z* (APcI) 272 (MH^+, 100%).

***tert*–Butyl 2,6–dimethylpyridine–3–carboxylate (4t)**



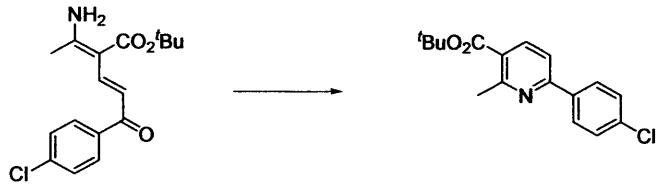
Pyridine **4t** was prepared using aminodienone **3f** according to the general procedure **6.2.2**, purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.59), gave the *title compound*[3] as a pale yellow oil (41 mg, 98%) Pyridine **4t** was also prepared using aminodienone **3f** according to general procedure **6.2.3** to give the *title compound*[3] as a pale yellow oil (45 mg, 98%) (Found: MH^+, 208.1329. $C_{12}H_{18}NO_2$ requires *MH*$^+$, 208.1332); IR (film)/cm^{-1} ν_{max} 2921, 2852, 1724, 1592, 1462, 1377, 1284, 1175, 1144, 1080, 771, 722; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.95 (1H, d, *J* 8.0, 4–H), 6.95 (1H, d, *J* 8.0, 5–H), 2.68 (3H, s, Me), 2.45 (3H, s, Me), 1.50 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 166.1 (C), 160.6 (C), 158.7 (C), 138.8 (CH), 124.5 (C), 120.5 (CH), 81.7 (C), 28.2 (Me), 24.8 (Me), 24.6 (Me); *m/z* (APcI) 208 (MH^+, 100%).

tert–Butyl 2–methyl–6–phenylpyridine–3–carboxylate (4u)



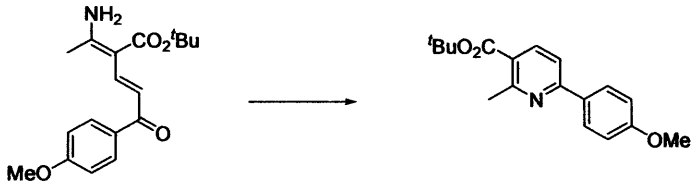
Pyridine **4u** was prepared using aminodienone **3g** according to the general procedure **6.2.3** to give the *title* compound[7] as a pale yellow oil (58 mg, 98%) (Found: MH^+, 270.1490. $C_{17}H_{20}NO_2$ requires *MH*$^+$, 270.1489); IR (film)/cm^{-1} ν_{max} 2976, 2925, 1714, 1581, 14554, 1367, 1282, 1172, 1147, 1091, 1075, 838, 758, 690; ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.11 (1H, d, *J* 8.3, 4–H), 7.96 (2H, m, *o*–Ph*H*), 7.52 (1H, d, *J* 8.3, 5–H), 7.38 (3H, *m,p*–Ph*H*), 2.81 (3H, s, Me), 1.54 (9H, s, CMe_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1 (C), 159.4 (C), 158.7 (C), 139.2 (CH), 138.7 (C), 129.5 (CH), 128.8 (CH), 127.3 (CH), 125.4 (C), 117.4 (CH), 81.8 (C), 28.3 (Me), 25.4 (Me); *m/z* (APcI) 270 (MH^+, 100%).

tert–Butyl 2–methyl–6–(4–chlorophenyl)pyridine–3–carboxylate (4v)



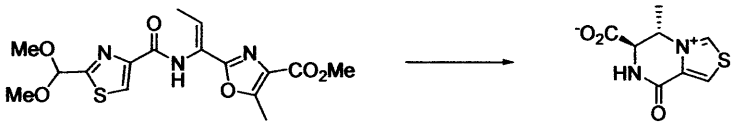
Pyridine **4v** was prepared using aminodienone **3h** according to the general procedure **6.2.3** to give the *title compound*[7] as a pale yellow oil (61 mg, 92%) (Found: MH^+, 304.1100. $C_{17}H_{19}ClNO_2$ requires *MH*$^+$, 304.1099); IR (film)/cm^{-1} ν_{max} 2974, 2925, 1703, 1577, 1556, 1491, 1453, 1410, 1365, 1293, 1254, 1172, 1146, 1096, 1010, 902, 828, 782; ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.09 (1H, d, *J* 8.2, 4–H), 7.91 (2H, appd, *J* 8.6, 2',6'–PhH), 7.48 (1H, d, *J* 8.2, 5–H), 7.36 (2H, appd, *J* 8.6, 3',5'–PhH), 2.79 (3H, s, Me), 1.54 (9H, s, CMe_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 165.0 (C), 158.4 (C), 156.2 (C), 138.3 (CH), 136.0 (C), 134.7 (C), 127.9 (CH), 127.5 (CH), 124.6 (C), 116.5 (CH), 80.9 (C), 27.2 (Me), 24.3 (Me); *m/z* (APcI) 304 (MH^+, 100%).

tert-Butyl 2-methyl-6-(4-methoxyphenyl)-3-carboxylate (4w)



Pyridine **4w** was prepared using aminodienone **3i** according to the general procedure **6.2.3** to give the *title compound*[7] as a pale yellow oil (65 mg, 98%) (Found: MH^+, 300.1591. $C_{18}H_{22}NO_3$ requires *MH*$^+$, 300.1594); IR (film)/cm^{-1} ν_{max} 2978, 2936, 1712, 1603, 1580, 1511, 1454, 1369, 1283, 1252, 1170, 1152, 1111, 1092, 1072, 1029, 832, 787; ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.06 (1H, d, *J* 8.3, 4–H), 7.93 (2H, appd, *J* 8.7, 2',6'–PhH), 7.45 (1H, d, *J* 8.3, 5–H), 6.91 (2H, appd, *J* 8.7, 3',5'–PhH), 3.77 (3H, s, OMe), 2.79 (3H, s, Me), 1.53 (9H, s, CMe_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 166.2 (C), 160.0 (C), 159.3 (C), 158.3 (C), 139.2 (CH), 131.2 (C), 128.7 (CH), 124.6 (C), 116.5 (CH), 114.2 (CH), 81.6 (C), 55.4 (Me), 28.3 (Me), 25.4 (Me); *m/z* (APcI) 300 (MH^+, 100%).

(±)-Sulfomycinine.HCl (37). [6-Carboxy-5-methyl-8-oxo-5,6,7,8-tetrahydrothiazolo(3,4-*a*)pyrazinium chloride]



Methyl sulfomycinate **39** (67 mg, 0.76 mmol) was stirred in hydrochloric acid (6 M; 9 ml) in a Carius tube at 110 °C for 2 h. After cooling, the mixture was evaporated *in vacuo*, by forming an azeotrope with MeOH. The residue was triturated with MeOH to give the *title compound* as a colourless solid (12 mg, 27%), mp 203–204 °C (dec.) (EtOAc) (lit.[9] mp 205–207 °C) (Found: $[M-Cl]^+$ 213.0325. $C_8H_8N_2O_3S$ requires *[M–Cl]*$^+$, 213.0328); IR (KBr)/cm^{-1} ν_{max} 3455, 3197, 3096, 3055, 2370, 2288, 1740, 1686, 1575, 1436, 1192, 887, 761; UV (MeOH)/nm λ_{max} 230 (log ε 3.76) [lit.[9] 230 (log ε 3.85)]; ^{1}H NMR (500 MHz; D_2O) δ 10.15 (1H, d, *J* 2.4, exch. D_2O, 3–H), 8.77 (1H, d, *J* 2.4, 1–H), 5.51 (1H, dq, *J* 1.5, 6.9, 5–H), 4.46 (1H, d, *J* 1.5, 6–H), 1.61 (3H, d, *J* 6.9, Me); ^{13}C NMR (125 MHz; D_2O) δ 172.3 (C), 159.7 (CH), 156.7 (C), 136.1 (C), 131.5 (CH), 59.8 (CH), 57.5 (CH), 19.6 (Me); *m/z* (ES) 215 ($[M-Cl]^+$, 100%).

Sulfomycinic amide (38). 2–[1–{[2–(Dimethoxymethyl)thiazol–4–yl]carbonylamino}–1–propenyl]–5–methyloxazole–4–carboamide

A saturated solution of methanolic NH_3 (20 ml) was added to methyl sulfomycinate **39** (70 mg, 0.18 mmol) at room temperature. The mixture was stirred at this temperature for 2 d and then evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with EtOAc, gave the *title compound* as a colourless solid (16 mg, 24%), mp 188–189 °C (EtOAc) (lit.[10] mp 194.5–195 °C); (Found: MH^+ 367.1070. $C_{15}H_{19}N_4O_5S$ requires *MH*$^+$, 367.1071); IR (KBr)/cm^{-1} ν_{max} 3474, 3360, 3284, 3153, 3090, 2940, 2842, 1687, 1636, 1604, 1555, 1531, 1497, 1482, 1449, 1419, 1369, 1333, 1305, 1229, 1211, 1195, 1168, 1104, 1082, 1042, 1016, 990, 968, 956, 836, 789, 762, 721, 707, 684; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.71 (1H, bs, NH), 8.13 (1H, s, 5''–H) 6.84 (1H, bs, N*H*H), 6.61 (1H, q, *J* 7.2, 2'–H), 5.58 (1H, bs, NH*H*), 5.53 (1H, s, CH), 3.37 (6H, s, OMe), 2.55 (3H, s, 5–Me), 1.84 (3H, d, *J* 7.2, 2'–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.2 (C), 164.0 (C), 159.0 (C), 156.7 (C), 153.9 (C), 149.7 (C), 129.4 (C), 128.7 (CH), 125.7 (CH), 122.2 (C), 99.8 (CH), 53.6 (Me), 14.5 (Me), 11.9 (Me); *m/z* (APcI) 367 (MH^+, 18%).

Methyl sulfomycinate (39). Methyl 2–(1–{[2–(Dimethoxymethyl)thiazol–4–yl]carbonylamino}–1–propenyl)–5–methyloxazole–4–carboxylate

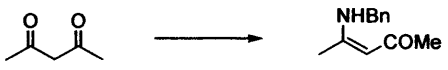
A solution of alcohol **108** (344 mg, 0.86 mmol), Et_3N (1.2 ml, 8.6 mmol) and MsCl (0.53 ml, 6.9 mmol) in dry CH_2Cl_2 (20 ml) was stirred for 1 h at room temperature. The solution was partitioned between H_2O (45 ml) and CH_2Cl_2 (30 ml) and the aqueous layer was further extracted with $CHCl_3$ (2 x 40 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was dissolved in dry CH_2Cl_2 (20 ml), Et_3N (2.0 ml, 14.4 mmol) was added and the mixture was stirred for 18 h. After evaporating *in vacuo*, purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.44), gave the *title compound* as a colourless solid (198 mg, 60%), mp 122–123 °C (EtOAc) (lit.[10] mp 124–124.5 °C) (Found: MH^+ 382.1066.

$C_{16}H_{20}N_3O_6S$ requires *MH*$^+$, 382.1067); IR (KBr)/cm^{-1} ν_{max} 3372, 3116, 2924, 2845, 2363, 2344, 1719, 1684, 1618, 1527, 1469, 1439, 1351, 1233, 1189, 1101, 1062; UV (MeOH)/nm λ_{max} 246 (log ε 4.40) [lit.[10] 247 (log ε 4.38)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.65 (1H, bs, NH), 8.14 (1H, s, 5''–H), 6.68 (1H, q, *J* 7.2, 2'–H), 5.55 (1H, s, CH), 3.82 (3H, s, OMe), 3.41 (6H, s, OMe), 2.57 (3H, s, 5–Me), 1.84 (3H, d, *J* 7.2, 2'–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.1 (C), 162.7 (C), 159.0 (C), 157.5 (C), 156.5 (C), 149.7 (C), 128.9 (CH), 128.1 (C), 125.7 (CH), 122.1 (C), 99.9 (CH), 53.6 (Me), 52.0 (Me), 14.5 (Me), 12.2 (Me); *m/z* (CI$^+$) 382 (MH$^+$, 100%).

Saramycetic acid I (47). 2–[2–(1–Acetyl)thiazol–4–yl]thiazole–4–carboxylic acid

LiOH monohydrate (17 mg, 0.41 mmol) was added to a stirred solution of ethyl ester **164** (110 mg, 0.40 mmol) in MeOH–H_2O (5:1) (12 ml) and the solution was stirred for 20 h. After evaporating *in vacuo*, the mixture was partitioned between aqueous $KHSO_4$ solution (10% w/v; 30 ml) and CH_2Cl_2 (50 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 45 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound*[11] as a colourless solid (102 mg, 100%), mp 244–245 °C (dec.) (MeOH) (Found: MH$^+$, 254.9893. $C_9H_7N_2O_3S_2$ requires *MH*$^+$, 254.9893); IR (KBr)/cm^{-1} ν_{max} 3406, 3103, 2936, 2584, 2514, 2362, 1734, 1689, 1492, 1412, 1356, 1296, 1220, 1192, 1097, 1061, 1008, 942, 868, 809, 753, 716; UV (MeOH)/nm λ_{max} 220 (log ε 4.29), 291 (log ε 4.14) [lit.[12] 218 (log ε 4.32), 291 (log ε 4.16)]; ^{1}H NMR (500 MHz; $SO(CD_3)_2$) δ 8.85 (0.18H, s, 5'–H), 8.82 (0.82H, s, 5'–H), 8.71 (0.18H, s, 5–H), 8.63 (0.82H, s, 5–H), 2.77 (3H, s, Me); ^{13}C NMR (125 MHz; $SO(CD_3)_2$) δ 191.4 (C), 167.9 (C), 162.4 (C), 161.7 (C), 149.8 (C), 148.8 (C), 130.1 (CH), 125.8 (CH), 26.3 (Me); *m/z* (APcI) 255 (MH$^+$, 40%).

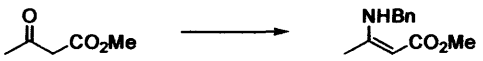
***N*–Benzyl–4–aminopent–3–en–2–one (66a)**



2,4 Pentadione **7e** (1.20 ml, 12 mmol) was added to a solution of benzylamine (1.10 ml, 10 mmol) in MeOH (50 ml). The mixture was stirred at reflux for 21 h and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f

0.42), gave the *title compound*[13] as a yellow oil (1.88 g, 99%) (Found: MH^+, 190.1227. $C_{12}H_{16}NO$ requires *MH*$^+$, 190.1226); IR (film)/cm^{-1} ν_{max} 3428, 3002, 1610, 1573, 1514, 1437, 1355, 1294, 1237, 1102, 1072, 1027, 986, 966, 933, 747, 697; ^{1}H NMR (400 MHz; $CDCl_3$) δ 11.1 (1H, bs, NH), 7.27 (2H, m, *o*–Ph*H*), 7.19 (3H, *m,p*–Ph*H*), 4.97 (1H, s, 3–H), 4.39 (2H, d, *J* 6.5, CH_2), 1.96 (3H, s, Me), 1.84 (3H, s, Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 195.4 (C), 163.2 (C), 138.0 (C), 128.8 (CH), 127.4 (CH), 126.7 (CH), 95.9 (CH), 46.7 (CH_2), 29.0, (Me), 18.9 (Me); *m/z* (APcI) 190 (MH^+, 100%).

Methyl 3–(benzylamino)but–2–enoate (66b)



Methyl acetoacetate **7f** (1.30 ml, 12 mmol) was added to a solution of benzylamine (1.10 ml, 10 mmol) in MeOH (50 ml). The mixture was stirred at reflux for 21 h and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.63), gave the *title compound*[14] as an orange oil (1.44 g, 70%) (Found: MH^+, 206.1176. $C_{12}H_{16}NO_2$ requires *MH*$^+$, 206.1175); IR (film)/cm^{-1} ν_{max} 3292, 2947, 1652, 1606, 1496, 1440, 1276, 1236, 1171, 1117, 1013, 931, 785, 731, 697; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.87 (1H, bs, NH), 7.27 (2H, m, *o*–Ph*H*), 7.19 (3H, *m,p*–Ph*H*), 4.47 (1H, s, 2–H), 4.36 (2H, d, *J* 6.5, CH_2), 3.56 (3H, s, OMe), 1.85 (3H, s, 4–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.9 (C), 162.0 (C), 138.7 (C), 128.9 (CH), 127.8 (CH), 126.7 (CH), 82.7 (CH), 50.1 (Me), 46.8 (CH_2), 19.4 (Me); *m/z* (APcI) 206 (MH^+, 100%).

1–(4–Chlorophenyl)prop–2–yn–1–ol (68b)

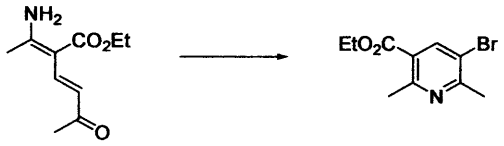
Propargylic alcohol **68b** was prepared using aldehyde **67b** according to the general procedure **6.2.5.** Purification by column chromatography on SiO_2, eluting with CH_2Cl_2 (R_f 0.27), gave the *title compound*[6] as a pale yellow oil (635 mg, 76%) (Found: M^+, 166.0181. C_9H_7ClO, requires *M*$^+$, 166.0180); IR (film)/cm^{-1} ν_{max} 3418, 3296, 2884, 2119, 1904, 1645, 1597, 1490, 1406, 1257, 1192, 1092, 1015, 950, 909, 835, 791, 734, 650; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.48 (2H, appd, *J* 8.4, 2',6'–PhH), 7.33 (2H, appd, *J* 8.4, 3',5'–PhH), 5.45 (1H, s, 1–H), 2.68 (1H, s, 3–H), 2.28

(1H, s, OH); ^{13}C NMR (100 MHz; $CDCl_3$) δ 138.4 (C), 134.4 (C), 128.8 (CH), 128.0 (CH), 83.1 (CH), 75.3 (C), 63.7 (CH); *m/z* (EI) 166 ($M^{\cdot+}$, 9%), 164 (27), 113 (5), 111 (14), 53 (100).

1–(4–Methoxyphenyl)prop–2–yn–1–ol (68c)

Propargylic alcohol **68c** was prepared using aldehyde **67c** according to the general procedure **6.2.5**. Purification by column chromatography on SiO_2, eluting with CH_2Cl_2 (R_f 0.10), gave the *title compound*[15] as a pale yellow oil (513 mg, 63%) (Found: $[MH]^+$, 161.0596. $C_{10}H_{10}O_2$, requires $[MH]^+$, 161.0597); IR (film)/cm^{-1} ν_{max} 3438, 3284, 3003, 2935, 2837 1892 1611, 1512, 1464, 1442, 1304, 1249, 1174, 1112, 1032, 948, 833, 768; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.50 (2H, appd, *J* 8.6, 2',6'–PhH), 6.90 (2H, appd, *J* 8.6, 3',5'–PhH), 5.42 (1H, s, 1–H), 3.85 (3H, s, OMe), 2.65 (1H, s, 3–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 159.8 (C), 132.4 (C), 128.1 (CH), 114.0 (CH), 83.7 (CH), 74.7 (C), 64.0 (CH), 55.4 (Me); *m/z* (EI) 162 ($M^{\cdot+}$, 100%), 161 (54), 145 (35), 131 (38), 89 (57), 53 (43).

Ethyl 5–bromo–2,6–dimethylpyridine–3–carboxylate (70a)

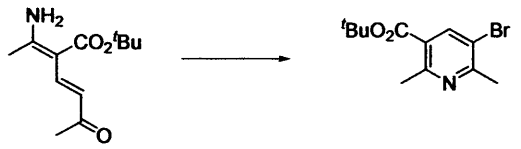


Bromopyridine **70a** was prepared using aminodienone **3a** according to the general procedure **6.2.4**. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.68), gave the *title compound*[6] as an off–white solid (69 mg, 96 %), mp 31–32 °C (light petroleum–EtOAc) (lit.[6] mp 32.1–32.2 °C) (Found: C, 46.5; H, 5.0; N, 5.4. Calc. for $C_{10}H_{12}BrNO_2$: C, 46.5; H, 4.7; N, 5.4%) (Found: MH^+, 258.0128. $C_{10}H_{13}BrNO_2$, requires MH^+, 258.0124); IR (KBr)/cm^{-1} ν_{max} 2924, 2852, 1732, 1572, 1462, 1377, 1247, 1093, 1016, 781, 722; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.22 (1H, s, 4–H), 4.30 (2H, q, *J* 7.2, OC*H*$_2$Me), 2.70 (3H, s, Me), 2.58 (3H, s, Me), 1.35 (3H, t, *J* 7.2, CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 165.2 (C), 160.0 (C), 158.0 (C), 141.9 (CH), 124.4 (C), 118.0 (C), 61.5 (CH_2), 24.0 (Me), 24.2 (Me), 14.3 (Me); *m/z* (APcI) 260 (MH^+, 88%), 258 (MH^+, 100%).

Ethyl 5–bromo–2–phenyl–6–methylpyridine–3–carboxylate (70b)

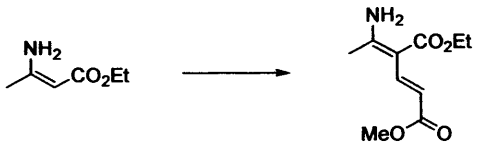
Bromopyridine **70b** was prepared using aminodienone **3e** according to the general procedure **6.2.4**. Purification by column chromatography on SiO_2, eluting with light petroleum–Et_2O (1:1) (R_f 0.50), gave the *title compound*[6] as a brown oil (88 mg, 98 %), (Found: MH^+, 320.0281. $C_{15}H_{15}BrNO_2$, requires MH^+, 320.0281); IR (film)/cm^{-1} ν_{max} 2918, 2853, 1725, 1572, 1462, 1377, 1295, 1242, 1112, 1061, 1027, 771, 722, 696; ^1H NMR (400 MHz; $CDCl_3$) δ 8.18 (1H, s, 4–H), 7.50–7.34 (5H, PhH), 4.05 (2H, q, *J* 7.1, OCH_2Me), 2.68 (3H, s, Me), 1.00 (3H, t, *J* 7.1, CH_2Me); ^{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); *m/z* (APcI) 322 (MH^+, 100%), 320 (98).

***tert*–Butyl 5–bromo–2,6–dimethylpyridine–3–carboxylate (70f)**



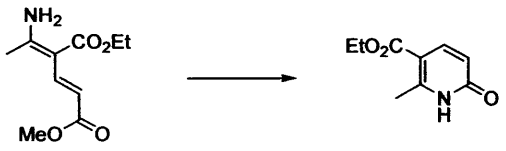
Bromopyridine **70f** was prepared using aminodienone **3f** according to the general procedure **6.2.4**. Purification by column chromatography on SiO_2, eluting with light petroleum–Et_2O (1:1) (R_f 0.53), gave the *title compound*[6] as a brown oil (78 mg, 97 %), (Found: M^+, 285.0359. $C_{12}H_{16}BrNO_2$, requires MH^+, 285.0359); IR (film)/cm^{-1} ν_{max} 2922, 2852, 1726, 1579, 1462, 1377, 1277, 1167, 1095, 970, 848, 782, 722; ^1H NMR (400 MHz; $CDCl_3$) δ 8.11 (1H, s, 4–H), 2.81 (3H, s, Me), 2.62 (3H, s, Me), 1.55 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 177.3 (C), 165.1 (C), 159.9 (C), 157.9 (C), 142.0 (CH), 124.5 (C), 118.1 (C), 24.9 (Me), 24.1 (Me), 14.3 (Me); *m/z* (APcI) 288 (MH^+, 100%), 286 (94).

(2*E*,4*Z*)–Methyl 5–amino–4–ethoxycarbonyl–hexa–2,4–dienoate (76)



Methyl propiolate **78** (0.65 g, 7.75 mmol) was added to a solution of ethyl β–aminocrotonate **1a** (0.5 g, 3.85 mmol) in MeOH (13 ml). The mixture was stirred at reflux for 18 h and then evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (4:1) (R_f 0.48), gave the *title compound* as a yellow solid (0.51g 67%), mp 107–108 °C (light petroleum–EtOAc) (lit.[16] mp 108 °C) (Found: MH^+, 214.1073. $C_{10}H_{16}NO_4$ requires MH^+, 214.1074); IR (KBr)/cm^{-1} ν_{max} 3331, 3187, 1669, 1590, 1433, 1331, 1273, 1217, 1163, 1122, 1030, 978, 950, 845; ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.53 (1H, bs, NH), 7.59 (1H, d, *J* 15.6, 3–H), 6.11 (1H, d, *J* 15.6, 2–H), 5.34 (1H, bs, NH), 4.19 (2H, q, *J* 7.3, OC*H*$_2$Me), 3.67 (3H, s, OMe), 2.21 (3H, s, 6–H), 1.29 (3H, t, *J* 7.3, CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.9 (C), 169.8 (C), 165.0 (C), 140.4 (CH), 110.7 (CH), 94.4 (C), 59.9 (CH_2), 51.2 (Me), 22.5 (Me), 14.5 (Me); *m/z* (APcI) 214 (MH^+, 100%), 168 (50).

6–Methyl–5–ethoxycarbonylpyrid–2–one (77)



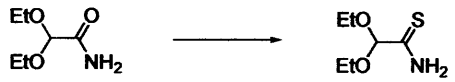
Methyl propiolate **78** (0.65 g, 7.75 mmol) was added to a solution of ethyl β–aminocrotonate **1a** (0.5 g, 3.85 mmol) in MeOH (13 ml). The mixture was stirred at reflux for 18 h and then evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (4:1) (R_f 0.12), gave the *title compound* as a yellow solid (0.18 g 26%), mp 206–207 °C (light petroleum–EtOAc) (lit.[17] mp 207 °C) (Found: MH^+, 182.0810. $C_9H_{12}NO_3$ requires MH^+, 182.0812); IR (KBr)/cm^{-1} ν_{max} 2940, 2094, 2952, 2358, 1712, 1608, 1465, 1378, 1259, 1198, 1147, 1097, 1028, 917, 850, 780, 727; ^{1}H NMR (400 MHz; $CDCl_3$) δ 12.94 (1H, bs, NH), 7.98 (1H, d, *J* 9.5, 4–H), 6.35 (1H, d, *J* 9.5, 3–H), 4.25 (2H, q, *J* 7.0, OC*H*$_2$Me), 2.68 (3H, s, 6–Me), 1.30 (3H, t, *J* 7.0, CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 165.5 (C), 164.9 (C), 153.0

(C), 142.9 (CH), 116.2 (CH), 109.0 (C), 60.2 (CH_2), 19.8 (Me), 14.3 (Me); *m/z* (APcI) 182 (MH^+, 100%), 154 (57).

Diethoxythioacetamide (93)

A mixture of 2,2–diethoxyacetonitrile **92** (1.12 ml, 8.06 mmol) and ammonium sulphide (50 wt. % in H_2O; 1.5 ml, 11.0 mmol) in MeOH (100 ml) was stirred overnight. The mixture was evaporated *in vacuo* to give the *title compound* as a pale yellow solid (1.5 g, 100%), mp 92–93 °C (lit.[18] mp 81–82 °C) (Found: MH^+, 164.0743. $C_6H_{14}NO_2S$ requires MH^+, 164.0740) (Found: C, 44.1; H, 8.0; N, 8.4; S, 19.5. Calc. for $C_6H_{13}NO_2S$: C, 44.2; H, 8.0; N, 8.6; S, 19.6%); IR (KBr)/cm^{-1} ν_{max} 3371, 3256, 2971, 2916, 1604, 1424, 1368, 1246, 1121, 1057, 960, 918, 824, 724; ^1H NMR (400 MHz; $CDCl_3$) δ 7.87 (1H, bs, NH), 7.62 (1H, bs, NH), 5.05 (1H, s, CH), 3.76 (2H, dq, *J* 9.5, 7, 2OC*H*HMe), 3.67 (2H, dq, *J* 9.5, 7, 2OCH*H*Me), 1.18 (6H, t, *J* 7, CH_2*Me*); ^{13}C NMR (125 MHz; $CDCl_3$) δ 202.2 (C), 103.0 (CH), 62.9 (CH_2), 15.1 (Me); *m/z* (EI) 163 ($M^{\cdot+}$, 2%), 118 (15), 103 (87).

Diethoxythioacetamide (93)



Phosphorous pentasulphide (30.0 g, 68 mmol) was added to a solution of 2,2–diethoxyacetamide **91** (20.0 g, 0.136 M) in CH_2Cl_2 (250 ml). The mixture was stirred for 2 h at 0°C, filtered through Celite®, washing with CH_2Cl_2 (2 x 20 ml), and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (3:2), followed by recrystalization from light petroleum–Et_2O (7:3) gave the *title compound*[18] as a pale orange solid (5 g, 23%) with identical physical and spectroscopic properties.

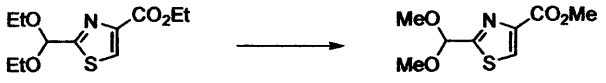
Ethyl 2–(diethoxymethyl)thiazole–4–carboxylate (94)

Ethyl bromopyruvate (1.71 ml, 13.6 mmol) was added to a stirred solution of 2,2–diethoxythioacetamide **93** (1.22 g, 7.47 mmol) in EtOH (16 ml) over 4Å molecular sieves (14 g) and the mixture was heated at reflux for 1 h. The solution was allowed to cool, filtered through Celite® and evaporated *in vacuo*. Purification twice by column chromatography on SiO_2, eluting with light petroleum–Et_2O (4:1) and light petroleum–Et_2O (1:1) (R_f 0.28), gave the *title compound*[19] as a pale yellow oil (1.94 g, 100%) (Found: MH^+, 260.0956. $C_{11}H_{18}NO_4S$ requires MH^+, 260.0951); IR (film)/cm^{-1} ν_{max} 3441, 3113, 2983, 2359, 1694, 1556, 1454, 1391, 1368, 1332, 1216, 1101, 1020, 953, 882, 860, 767, 704; ^1H NMR (400 MHz; $CDCl_3$) δ 8.10 (1H, s, 5–H), 5.63 (1H, s, CH), 4.32 (2H, q, *J* 7.1, OCH_2Me), 3.69 (2H, dq, *J* 9.5, 7, 2OC*H*H), 3.60 (2H, dq, *J* 9.5, 7, 2OCH*H*), 1.35 (3H, t, *J* 7.1, CH_2Me), 1.18 (6H, app t, *J* 7.1, $2CH_2Me$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.2 (C), 161.4 (C), 147.1 (C), 128.5 (CH), 98.7 (CH), 62.8 (CH_2), 61.5 (CH_2), 15.1 (Me), 14.4 (Me); *m/z* (APcI) 260 (MH^+, 37%).

Ethyl 2–(dimethoxymethyl)thiazole–4–carboxylate (95)

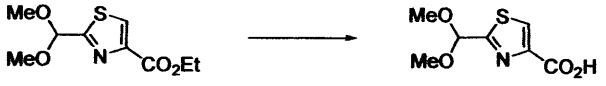
p–TsOH monohydrate (30 mg, 20 mol%) was added to a stirred solution of ethyl 2–(diethoxymethyl)thiazole–4–carboxylate **94** (200 mg, 0.77 mmol) in dry MeOH (5 ml) and the mixture heated at reflux for 6 h. After evaporating *in vacuo*, the mixture was partition between saturated aqueous $NaHCO_3$ solution (15 ml) and CH_2Cl_2 (30 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 30 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with EtOAc–light petroleum (1:1) (R_f 0.41), gave the *title compound*[19] as a brown oil (118 mg, 66%) (Found: MH^+, 232.0638. $C_9H_{14}NO_4S$ requires MH^+, 232.0637); IR (film)/cm^{-1} ν_{max} 3439, 3106, 2981, 1730, 1454, 1369, 1332, 1248, 1216, 1095, 1021, 950.1, 860, 766; ^1H NMR (400 MHz; $CDCl_3$) δ 8.14 (1H, s, 5–H), 5.52 (1H, s, CH), 4.35 (2H, q, *J* 7.1, OCH_2Me), 3.39 (6H, s, 2OMe), 1.34 (3H, t, *J* 7.1, CH_2Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.9 (C), 161.4 (C), 147.3 (C), 128.6 (CH), 100.3 (CH), 61.6 (CH_2), 54.0 (Me), 14.4 (Me); *m/z* (APcI) 232 (MH^+, 73 %).

Methyl 2–(dimethoxymethyl)thiazole–4–carboxylate (96)



p–TsOH monohydrate (293 mg, 40 mol%) was added to a stirred solution of thiazole **94** (1.0 g, 3.86 mmol) in MeOH (25 ml) and the mixture heated at reflux overnight. After evaporating *in vacuo*, the mixture was partition between saturated aqueous $NaHCO_3$ solution (40 ml) and EtOAc (50 ml). The aqueous layer was further extracted with EtOAc (2 x 40 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with EtOAc–light petroleum (1:1) (R_f 0.29), gave the *title compound* as a clear oil (506 mg, 60%) (Found: MH^+, 218.0482. $C_8H_{12}NO_4S$ requires *MH*$^+$, 218.0482); IR (film)/cm^{-1} ν_{max} 3111, 2953, 2835, 2360, 1737, 1489, 1435, 1328, 1248, 1217, 1092, 1068, 1000, 973, 919, 864, 763; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.14 (1H, s, 5–H), 5.55 (1H, s, CH), 3.92 (3H, s, OMe), 3.39 (6H, s, 2OMe); ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.0 (C), 161.8 (C), 146.9 (C), 128.8 (CH), 100.3 (CH), 54.0 (Me), 52.5 (Me); *m/z* (APcI) 218 (MH^+, 100 %).

2–(Dimethoxymethyl)thiazole–4–carboxylic acid (98)

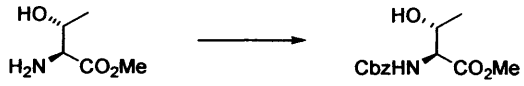


LiOH monohydrate (108 mg, 2.6 mmol) was added to a stirred solution of thaizole **95** (100 mg, 0.43 mmol) in MeOH–H_2O (5:1) (4 ml) and the solution was stirred overnight. After evaporating *in vacuo*, the mixture was partitioned between aqueous citric acid (1 M; 8 ml) and CH_2Cl_2 (35 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 20 ml) and the organic extracts were combined, washed with brine (30 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound*[19] as a brown solid (56 mg, 64%), mp 112–113 °C (EtOAc) (Found: MH^+ 204.0325. $C_7H_{10}NO_4S$ requires *MH*$^+$ 204.0325); IR (KBr)/cm^{-1} ν_{max} 3391, 2885, 2698, 2586, 2511, 1726, 1503, 1484, 1459, 1399, 1320, 1205, 1092, 1062, 984, 953, 905, 872, 820, 770, 732, 670; ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.40 (1H, bs, OH), 8.25 (1H, s, 5–H), 5.52 (1H, s, CH), 3.36 (6H, s, 2OMe); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.5 (C), 161.3 (C), 147.4 (C), 129.1 (CH), 100.2 (CH), 53.1 (Me); *m/z* (APcI) 204 (MH^+, 100 %).

2–(Dimethoxymethyl)thiazole–4–carboxylic acid (98) from 94

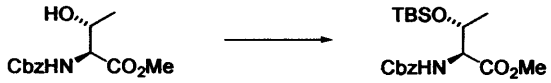
p–TsOH monohydrate (300 mg, 156 mmol) was added to a stirred solution of ethyl 2–(diethoxymethyl)thiazole–4–carboxylate **94** (2 g, 7.7 mmol) in MeOH (60 ml) and the mixture heated at reflux for 6.5 h. After cooling to room temperature, LiOH monohydrate (1.9 g, 46 mmol) and water (12 ml) were added and the solution was stirred for 48 h at room temperature. After evaporating *in vacuo*, the mixture was partitioned between aqueous citric acid (1 M; 40 ml) and CH_2Cl_2 (80 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 60 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound*[19] as an off–white solid (0.85 g, 54%), mp 115–116 °C, with identical spectroscopic properties.

(2*S*,3*R*)–Methyl 2–benzyloxycarbonylamino–3–hydroxybutanoate (100). (Z–L–Thr–OMe)



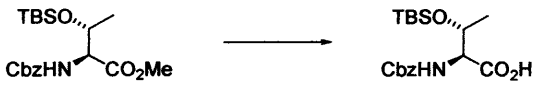
Benzyl chloroformate (4.90 ml, 32.5 mmol) was added to a solution of HCl.H–Thr–OMe **99** (5.0 g, 29.5 mmol) and sodium hydrogen carbonate (5.45 g, 64.9 mmol) in H_2O (250 ml). The solution was stirred at RT for 72 h and extracted with $CHCl_3$ (200 ml). The aqueous layer was further extracted with $CHCl_3$ (2 x 150 ml) and the combined organic extracts were dried (Na_2SO_4), and evaporated *in vacuo* to give the *title compound* as a colourless solid (7.36 g, 93%), mp 92–93 °C (EtOAc) (lit.[20] mp 93–96 °C) (Found: MH^+, 268.1178. $C_{13}H_{18}NO_5$ requires MH^+, 268.1179); $[\alpha]_D^{28}$ –12.33 (*c*1.20, $CHCl_3$) {lit.[20] $[\alpha]_D^{20}$ –13.7 (*c*1.20, $CHCl_3$)}; IR (KBr)/cm^{-1} ν_{max} 3471, 3314, 3058, 3032, 2979, 2955, 2930, 2899, 1720, 1689, 1546, 1498, 1468, 1455, 1441, 1477, 1335, 1247, 1176, 1130, 1092, 1072, 1020, 982, 967, 939, 914, 867, 841, 790, 756, 729, 698; ^1H NMR (400 MHz; $CDCl_3$) δ 7.28 (5H, m, PhH), 5.58 (1H, d, *J* 9.0, NH), 5.07 (1H, d, *J* 12.8, OC*H*H), 5.04 (1H, d, *J* 12.8, OCH*H*), 4.27 (1H, s, 2–H), 4.25 (1H, s, 3–H), 3.69 (3H, s, OMe), 1.17 (3H, d, *J* 6.5, 4–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.7 (C), 156.7 (C), 136.1 (C), 128.6 (CH), 128.3 (CH), 128.1 (CH), 68.0 (CH), 67.2 (CH_2), 59.0 (Me), 52.7 (CH), 19.9 (Me); *m/z* (APcI) 268 (MH^+, 100%).

(2*S*,3*R*)–Methyl 2–benzyloxycarbonylamino–3–(*tert*–butyldimethylsilyl)oxybutanoate (101). (Z–L–Thr(TBS)–OMe)



Imidazole (1.5 g, 22 mmol), DMAP (0.92 g, 7.5 mmol) and TBDMSCl (2.3 g, 15 mmol) were added successively to a solution of Z–L–Thr–OMe **100** (3.0 g, 11 mmol) in DMF (11 ml). The mixture was stirred at room temperature for 36 h, acidified with hydrochloric acid (1 M; 15 ml) and extracted with Et_2O (3 x 40 ml). The combined organic extracts were washed with H_2O (3 x 60 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound* as a pale yellow oil (4.3 g, 100%) (Found: MH^+, 382.2048. $C_{19}H_{32}NO_5Si$ requires *MH*$^+$, 382.2044); $[\alpha]_D^{30}$ –6.6 (*c*2.05, $CHCl_3$) {lit.[21] $[\alpha]_D^{24}$ –7.31 (*c*3.55, $CHCl_3$)}; IR (film)/cm^{-1} ν_{max} 3449, 2954, 2856, 1730, 1507, 1472, 1436, 1378, 1344, 1315, 1255, 1209, 1174, 1129, 1101, 1071, 1030, 1004, 963, 838, 810, 777; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.35 (5H, PhH), 5.42 (1H, bd, *J* 9.8, NH), 5.13 (2H, s, OCH_2), 4.42 (1H, dq, *J* 6.3, 1.7, 3–H), 4.25 (1H, dd, *J* 9.8, 1.7, 2–H), 3.67 (3H, s, OMe), 1.17 (3H, d, *J* 6.3, 4–H) 0.8 (9H, s, CMe_3), –0.01 (3H, s, *Me*SiMe), –0.05 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.4 (C), 156.8 (C), 136 .3 (C), 128.6 (CH), 128.5 (CH), 122.3 (CH), 69.8 (CH), 67.2 (CH_2), 59.9 (CH), 52.3 (Me), 25.6 (Me), 20.8 (Me), 17.8 (C), –4.39 (Me), –5.34 (Me); *m/z* (APcI) 382 (MH^+, 100%).

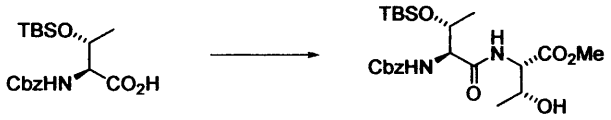
(2*S*)–Benzyloxycarbonylamino–(3*R*)–(*tert*–butyldimethylsilyl)oxybutanoic acid (102). (Z–L–Thr(TBS)–OH)



LiOH monohydrate (1.05 g, 25.0 mmol) was added to a stirred solution of Z–L–Thr(TBS)–OMe **101** (1.5 g, 3.93 mmol) in MeOH–H_2O (5:1) (40 ml). After stirring for 18 h, the mixture was partitioned between hydrochloric acid (1 M; 40 ml) and CH_2Cl_2 (60 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 60 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound* as a colourless solid (1.38 g, 96%), mp 149–150 °C (triturated with light petroleum–EtOAc) (lit.[22] mp 154–157 °C) (Found: MH^+, 368.1883. $C_{18}H_{30}NO_5Si$ requires *MH*$^+$, 368.1888); $[\alpha]_D^{31}$ +10.3 (*c*2.82, $CHCl_3$) {lit.[22] $[\alpha]_D^{22}$ +10.5 (*c*1.69, $CHCl_3$)}; IR (KBr)/cm^{-1} ν_{max} 3440, 3035, 2956, 2856, 1702, 1600, 1514, 1455, 1413, 1344, 1308,

1257, 1102, 1042, 1005, 975, 836, 813, 778, 733, 696; ^{1}H NMR (500 MHz; $CDCl_3$) δ 9.6 (1H, bs, CO_2H), 7.3 (5H, PhH), 5.43 (1H, bd, *J* 8.6, NH), 5.04 (2H, s, OCH_2), 4.40 (1H, dq, *J* 2.4, 6.3, 3–H), 4.24 (1H, dd, *J* 8.6, 2.4, 2–H), 1.11 (3H, d, *J* 6.3, 4–H), 0.75 (9H, s, CMe_3), –0.02 (3H, s, *Me*SiMe), –0.03 (3H, s, MeSi*Me*); ^{13}C NMR (125 MHz; $CDCl_3$) δ 175.7 (C), 156.7 (C), 136.1 (C), 128.6 (CH), 128.3 (CH), 128.2 (CH), 68.5 (CH), 67.3 (CH_2), 59.4 (CH), 25.7 (Me), 20.3 (Me), 17.9 (C), –4.6 (Me), –5.1 (Me); *m/z* (CI) 368 (MH^+, 28%), 277 (100).

(2*S*,3*R*,2'*S*,3'*R*)–Methyl 2–({(2–benzyloxycarbonylamino)–3–[*tert*–butyldimethylsilyloxy]–butanoyl}amino)–3–hydroxybutanoate (103). Z–L–Thr(TBS)–L–Thr–OMe



Et_3N (0.95 ml, 6.8 mmol) was added dropwise over 30 min to a stirred solution of acid **102** (1.0 g, 2.7 mmol), HCl.H–L–Thr–OMe (0.509 g, 3.0 mmol) and pyBOP (1.56 g, 3.0 mmol) in dry CH_2Cl_2 (13 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 18 h. After evaporating *in vacuo*, purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.49), gave the *title compound*[19] as a colourless solid (1.1 g, 83%), mp 133–134 °C (light petroleum–EtOAc) (Found: MH^+, 483.2521. $C_{23}H_{39}N_2O_7Si$ requires *MH*$^+$, 483.2521); $[\alpha]_D^{32}$ +13.1 (*c*1.05, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3446, 3357, 2955, 1752, 1723, 1670, 1507, 1253, 1207, 1132, 1102, 1078, 967, 839, 780; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.43 (1H, bd, *J* 8.8, NH), 7.20 (5H, PhH), 5.74 (1H, bd, *J* 5.7, NH), 5.01 (1H, d, *J* 12, OC*H*H), 4.92 (1H, d, *J* 12, OCH*H*), 4.42 (1H, dd, *J* 8.8, 1.7, 2–H), 4.21 (2H, 2 and 2'–H), 4.14 (1H, m, 2'–H), 3.60 (3H, s, OMe), 2.05 (1H, bs, OH), 1.05 (3H, d, *J* 6.4, Me), 1.01 (3H, d, *J* 6.2, Me), 0.78 (9H, s, CMe_3), 0.04 (3H, s, *Me*SiMe), 0.00 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.1 (C), 170.1 (C), 156.2 (C), 136.1 (C), 128.6 (CH), 128.2 (CH), 128.1 (CH), 68.4 (CH), 67.6 (CH), 67.0 (CH_2), 59.1 (CH), 57.3 (CH), 52.5 (Me), 25.7 (C), 19.9 (Me), 17.9 (Me), 17.4 (Me), –4.8 (Me), –5.0 (Me); *m/z* (APcI) 483 (MH^+, 100%).

(4*S*,5*S*,1'*S*,2'*R*)–Methyl 2–[1–(benzyloxycarbonylamino)–2–(*tert*–butyldimethylsilyloxy)–prop–1–yl]–4,5–dihydro–5–methyloxazole–4–carboxylate (104)

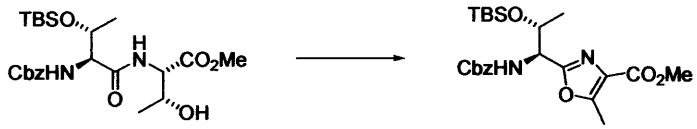
[Bis(2–methoxyethyl)amino]sulfur trifluoride (Deoxo–Fluor) (0.37 ml, 2.0 mmol) was added dropwise to a stirred solution of Z–L–Thr(TBS)–L–Thr–OMe **103** (0.95 g, 1.97 mmol) in dry CH_2Cl_2 (30 ml) at –20 °C. The solution was stirred for 18 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (30 ml). After warming to room temperature, the mixture was extracted with CH_2Cl_2 (3 x 40 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (R_f 0.39), gave the *title compound*[19] as a colourless oil (595 mg, 65%); (Found: MH^+ 465.2418. $C_{23}H_{37}N_2O_6Si$ requires *MH*$^+$, 465.2415); $[\alpha]_D^{32}$ +7.4 (*c*1.05, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3330, 3034, 2954, 2856, 2358, 1731, 1674, 1504, 1383, 1258, 1212, 1101, 836, 778, 698; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.33 (5H, PhH), 5.45 (1H, bd, *J* 9.5, NH), 5.11 (1H, d, *J* 13.7, OC*H*H), 5.08 (1H, d, *J* 13.7, OCH*H*), 4.90 (1H, dq, *J* 10.4, 6.3, 5–H), 4.78 (1H, d, *J* 10.4, 4–H), 4.42 (1H, d, *J* 9.5, 1'–H), 4.35 (1H, q, *J* 6.3, 2'–H), 3.75 (3H, s, OMe), 1.25 (3H, d, *J* 6.3, 5–Me), 1.17 (3H, d, *J* 6.3, 2'–Me), 0.80 (9H, s, CMe_3), 0.00 (3H, s, *Me*SiMe), –0.06 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.0 (C), 168.8 (C), 156.4 (C), 136.4 (C), 128.6 (CH), 128.5 (CH), 128.1 (CH), 78.1 (CH), 71.3 (CH), 69.3 (CH), 67.0 (CH_2), 55.5 (CH), 52.1 (Me), 25.7 (Me), 20.6 (Me), 17.9 (C), 16.3 (Me), –4.4 (Me), –5.0 (Me); *m/z* (APcI) 465 (MH^+, 50%).

(1'*S*,2'*R*)–Methyl 2–[1–(benzyloxycarbonylamino)–2–(*tert*–butyldimethylsilyloxy)prop–1–yl]–5–methyloxazole–4–carboxylate (105)

$BrCCl_3$ (0.88 ml, 8.9 mmol) and DBU (0.88 ml, 5.9 mmol) were added successively to a stirred solution of the oxazoline **104** (0.60 g, 1.28 mmol) in dry CH_2Cl_2 (24 ml) at –20 °C. After stirring for 18 h, the mixture was poured into saturated aqueous $NaHCO_3$ solution (40 ml) and extracted with ethyl acetate (3 x 60 ml). The organic extracts were combined, dried (Na_2SO_4) and

evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (R_f 0.63), gave the *title compound*[19] as a colourless oil (0.31 g, 52%) (Found: MH^+ 463.2258. $C_{23}H_{35}N_2O_6Si$ requires MH^+, 463.2259); $[\alpha]_D^{33}$ –5.7 (*c*2.75, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3436, 3352, 2954, 2925, 2857, 2361, 1732, 1622, 1587, 1504, 1442, 1352, 1253, 1211, 1100, 965, 915, 838, 810, 778, 739, 698; ^1H NMR (400 MHz; $CDCl_3$) δ 7.35 (5H, PhH), 5.72 (1H, bd, *J* 9.4, NH), 5.15 (2H, s, OCH_2), 4.93 (1H, dd, *J* 9.4, 2.1, 1'–H), 4.42 (1H, qd, *J* 6.2, 2.1, 2'–H), 3.90 (3H, s, OMe), 2.60 (3H, s, 5–Me), 1.26 (3H, d, *J* 6.2, 2'–Me) 0.78 (9H, s, CMe_3), 0.03 (3H, s, *Me*SiMe), –0.17 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 162.6 (C), 160.9 (C), 156.4 (C), 156.4 (C), 136.2 (C), 128.6 (CH), 128.2, (CH), 127.8 (CH), 127.5 (C), 70.0 (CH), 67.3 (CH_2), 55.5 (CH), 52.0 (Me), 25.5 (Me), 20.4 (Me), 17.8 (C), 11.9 (Me), –4.7 (Me), –5.5 (Me); *m/z* (APcI) 463 (MH^+, 100%).

Oxazole 105 from Z–L–Thr(TBS)–L–Thr–OMe (103)



Deoxo–Fluor (0.72 ml, 3.9 mmol) was added dropwise to a stirred solution of Z–L–Thr(TBS)–L–Thr–OMe **103** (1.87 g, 3.87 mmol) in dry CH_2Cl_2 (60 ml) at –20 °C. The solution was stirred for 18 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (30 ml). After warming to room temperature, the mixture was extracted with CH_2Cl_2 (3 x 100 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to give the crude oxazoline **104** as a brown oil (2.1 g). The residue was dissolved in dry CH_2Cl_2 (70 ml) and cooled to –20 °C. $BrCCl_3$ (1.7 ml, 17.4 mmol) and DBU (2.6 ml, 17.4 mmol) were added and the solution was stirred at –20 °C for 18 h. The mixture was poured into saturated aqueous $NaHCO_3$ solution (70 ml) and extracted with ethyl acetate (3 x 60 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–Et_2O (R_f 0.15), gave the *title compound*[19] as a colourless oil (0.94 g, 52%) with identical physical and spectroscopic properties.

(1'*S*,2'*R*)–Methyl 2–[1–amino–2–(*tert*–butyldimethylsilyloxy)prop–1–yl]–5–methyloxazole–4–carboxylate (106)

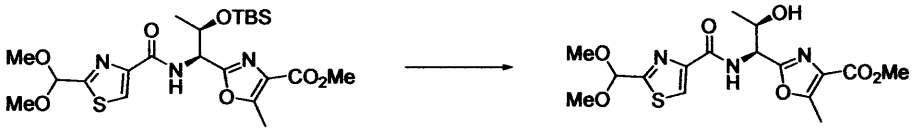
A solution of oxazole **105** (0.94 g, 2.0 mmol) in MeOH (34 ml) was stirred over Pd–C (10 wt. %; 817 mg) under an atmosphere of hydrogen for 3 h. The mixture was filtered through Celite® and evaporated *in vacuo* to give the *title compound*[19] as a colourless solid, mp 75–76 °C (623 mg, 93%) (Found: MH^+ 329.1890, $C_{15}H_{29}N_2O_4Si$ requires MH^+ 329.1891); $[\alpha]_D^{31}$ –29.3 (*c*2.78, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3387, 2955, 2857, 1721, 1621, 1441, 1351, 1256, 1205, 1098, 970, 837, 776, 666; ^1H NMR (500 MHz; $CDCl_3$) δ 8.80–6.10 (2H, bs, NH), 4.45 (2H, 1',2'–H), 3.85 (3H, s, OMe), 2.53 (3H, s, 5–Me) 1.35 (3H, bs, 2'–Me) 0.75 (9H, s, CMe_3), 0.05 (3H, s, *Me*SiMe), –0.1 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 164.0 (C), 162.8 (C), 156.3 (C), 127.2 (C), 70.7 (CH), 56.5 (CH), 52.0 (Me), 25.7 (Me), 20.5 (Me), 17.8 (C), 11.9 (Me), –4.5 (Me), –5.3 (Me); *m/z* (APcI) 329 (MH^+, 100%).

(1'*S*,2'*R*)–Methyl 2–(1–{[2–(Dimethoxymethyl)thiazol–4–ylcarbonylamino]–2–*tert*–butyldimethylsilyloxypropyl})–5–methyloxazole–4–carboxylate (107)

Et_3N (0.50 ml, 3.6 mmol) was added dropwise over 30 min to a stirred solution of acid **98** (325 mg, 1.6 mmol), amine **106** (420 mg, 1.3 mmol) and pyBOP (830 mg, 1.6 mmol) in dry CH_2Cl_2 (13 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 18 h. After concentrating *in vacuo*, purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.31), gave the *title compound*[19] as a colourless oil (580 mg, 88%) (Found: MH^+ 514.2044, $C_{22}H_{36}N_3O_7SSi$ requires MH^+, 514.2038); $[\alpha]_D^{25}$ +16 (*c*2.3, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3409, 3116, 2956, 2857, 2400, 1731, 1682, 1621, 1538, 1499, 1471, 1352, 1258, 1186, 1098, 973, 910, 838, 756, 666; ^1H NMR (400 MHz; $CDCl_3$) δ 8.30 (1H, s, 5''–H), 8.20 (1H, d, *J* 8.9, NH), 5.72 (1H, s, CH), 5.41 (1H, dd, *J* 8.9, 2.8, 1'–H), 4.65 (1H, qd, *J* 6.1, 2.8, 2'–H), 4.07 (3H, s, OMe), 3.68 (3H, s, OMe), 3.40 (3H, s, OMe), 2.75 (3H, s, Me), 1.41 (3H,

d, *J* 6.1, 2'–Me), 1.05 (9H, s, CMe_3), 0.22 (3H, s, *Me*SiMe), 0.03 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 167.9 (C), 162.7 (C), 161.1 (C), 160.7 (C), 156.4 (C), 149.5 (C), 127.5 (C), 125.0 (CH), 100.3 (CH), 70.0 (CH), 54.1 (Me), 53.8 (Me), 53.4 (CH), 52.0 (Me), 25.5 (Me), 20.7 (Me), 17.8 (C), 12.0 (Me), –4.6 (Me), –5.4 (Me); *m/z* (CI^+) 514 (MH^+, 95%).

(1'*S*,2'*R*)–Methyl 2–(1–{1–[2–(Dimethoxymethyl)thiazol–4–ylcarbonylamino]–2–hydroxy}-propyl)–5–methyloxazole–4–carboxylate (108)



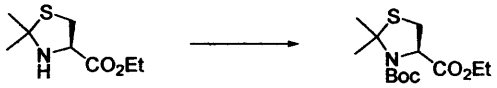
A solution of TBAF (1.0 M; 1.8 mmol) in THF (1.8 ml) was added to a stirred solution of silyl ether **107** (580 mg, 1.1 mmol) in dry THF (21 ml) at 0 °C. After warming to room temperature, the mixture was stirred for 4.5 h and then partitioned between H_2O (15 ml) and EtOAc (20 ml). The aqueous layer was further extracted with EtOAc (2 x 20 ml) and the combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.31), gave the *title compound*[19] as a colourless solid (344 mg, 77%), mp 159–160 °C (light petroleum–EtOAc) (1:3) (Found: MH^+ 400.1171. $C_{16}H_{22}N_3O_7S$ requires MH^+, 400.1173); $[\alpha]_D^{27}$ –14.7 (*c*2.3, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3458, 3277, 3123, 2956, 2825, 2362, 1715, 1668, 1540, 1497, 1453, 1391, 1376, 1334, 1247, 1217, 1192, 1148, 1098, 1063, 982, 828, 797; ^1H NMR (400 MHz; $CDCl_3$) δ 8.11 (1H, s, 5''–H), 8.03 (1H, d, *J* 9.3, NH) 5.45 (1H, s, CH), 5.24 (1H, dd, *J* 9.3, 2.9, 1'–H), 4.51 (1H, qd, *J* 6.3, 2.9, 2'–H), 3.82 (3H, s, OMe), 3.61 (1H, s, OH), 3.37 (6H, s, OMe), 2.53 (3H, s, 5–Me), 1.22 (3H, d, *J* 6.3, 2'–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.2 (C), 162.4 (C), 161.3 (C), 160.6 (C), 156.9 (C), 149.3 (C), 127.3 (C), 125.5 (CH), 99.9 (CH), 67.7 (CH), 53.7 (Me), 52.0 (Me), 51.9 (CH), 19.2 (Me), 12.1 (Me); *m/z* (CI^+) 400 (MH^+, 100%).

(4*R*)–2,2–Dimethyl–4–(ethoxycarbonyl)thiazolidine (140)

Et_3N (4.0 ml, 28.7 mmol) was added to a solution of HCl.H–L–Cys–OEt **138** (5.40 g, 28.8 mmol) in acetone (analytical grade) (90 ml). The mixture was stirred for 1 h, filtered over Celite® and

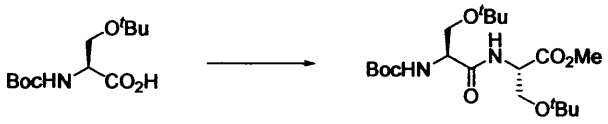
washed with acetone (30 ml). The solution was stirred at 60 °C for 2 h, cooled and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.57), gave the *title compound*[23] as a brown oil (5.31 g, 97%) (Found: MH^+ 190.0898, $C_8H_{16}NO_2S$ requires MH^+ 190.0896); $[\alpha]_D^{23}$ –68.3 (*c*1.30, MeOH); IR (film)/cm^{-1} ν_{max} 3306, 2978, 2931, 1738, 1447, 1371, 1334, 1227, 1187, 1027, 856, 801, 650; ^1H NMR (400 MHz; $CDCl_3$) δ 4.17 (2H, q, *J* 7.0, OCH_2Me), 4.00 (1H, dd, *J* 9.0, 7.0, 4–H), 3.37 (1H, dd, *J* 10.5, 7.0, 5–*H*H), 2.95 (1H, dd, *J* 10.5, 9.0, 5–H*H*), 2.49 (1H, bs, NH), 1.64 (3H, s, 2–Me), 1.49 (3H, s, 2–Me), 1.23 (3H, t, *J* 7.0, CH_2Me); ^{13}C (100 MHz; $CDCl_3$) δ 171.6 (C), 75.8 (C), 65.5 (CH), 61.6 (CH_2), 40.4 (CH_2), 32.7 (Me), 30.5 (Me), 14.2 (Me); *m/z* (APcI) 190 (MH^+, 98%), 150 (100), 118 (75).

(4*R*)–2,2–Dimethyl–3–(*tert*–butoxycarbonyl)–4–(ethoxycarbonyl)thiazolidine (141)



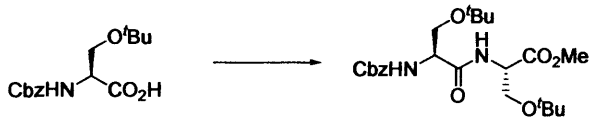
DIEA (1.00 ml, 5.81 mmol) was added to a solution of thiazolidine **140** (1.0 g, 5.28 mmol) and di–*tert*–butylbicarbonate (1.73 g, 7.93 mmol) in dry MeCN (10 ml) under nitrogen. The solution was stirred at 50 °C for 2 d, then allowed to cool and evaporated *in vacuo*. Purification twice by column chromatography, eluting with light petroleum–Et_2O (1:1) and light petroleum–Et_2O (4:1) (R_f 0.14), gave the *title compound* as a clear oil (80 mg, 6%) (Found: MH^+ 290.1422, $C_{13}H_{24}NO_4S$ requires MH^+ 290.1421); $[\alpha]_D^{23}$ –66.6 (*c*2.95, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 2976, 2933, 1754, 1704, 1454, 1362, 1291, 1257, 1192, 1172, 1125, 1069, 1031, 959, 934, 858, 818, 772; ^1H NMR (500 MHz; $CDCl_3$) δ 4.88 (0.45H, s, 4–H), 4.74 (0.55H, s, 4–H), 4.14 (2H, m, OCH_2Me), 3.20 (1H, m, 5–*H*H), 3.03 (1H, m, 5–H*H*), 1.79 (1.65H, s, 2–Me), 1.74 (1.35H, s, 2–Me), 1.72 (1.65H, s, 2–Me), 1.68 (1.35H, s, 2–Me), 1.43 (4H, s, CMe_3), 1.34 (5H, s, CMe_3), 1.21 (3H, m, CH_2Me); ^{13}C NMR (125 MHz; $CDCl_3$) δ 171.1 (C), 170.5 (C), 152.9 (C), 151.7 (C), 80.8 (C), 80.3 (C), 71.6 (C), 69.9 (C), 66.2 (CH), 65.5 (CH), 61.3 (CH_2), 31.0 (Me), 30.5 (CH_2), 30.1 (CH_2), 29.7 (Me), 28.3 (Me), 28.2 (Me), 27.7 (Me), 27.3 (Me), 14.2 (Me), 14.1 (Me); *m/z* (APcI) 290 (MH^+, 48%), 251 (30), 190 (75).

(2*S*,2'*S*)–Methyl 2–{[(2–*tert*–butoxycarbonylamino)–3–(*tert*–butoxy)propanoyl]amino}–3–(*tert*–butoxy)butanoate (144). Boc–Ser(^{t}Bu)–Ser(^{t}Bu)–OMe



Boc–L–Ser(^{t}Bu)–OH.DCHA **142** (250 mg, 0.56 mmol) was dissolved in dry THF (1.1 ml) at 0 °C. NMM (0.12 ml, 1.12 mmol) and isobutyl chloroformate (0.10 ml, 0.76 mmol) were added dropwise and the mixture was stirred for 20 min. H–Ser(^{t}Bu)–OMe (142 mg, 0.67 mmol) was added and the solution was stirred at 0 °C for 5 h, evaporated *in vacuo* and partitioned between aqueous $KHSO_4$ solution (10% w/v, 15 ml) and CH_2Cl_2 (20 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 15 ml) and the combined organic layers were washed with brine (50 ml), dried (Na_2SO_4), filtered and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.53) gave the *title compound* as a clear oil (229 mg, 98%) (Found: MH^+ 419.2753. $C_{20}H_{39}N_2O_7$ requires MH^+, 419.2752); $[\alpha]_D^{23}$ +9.12 (*c*2.73, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3342, 2975, 2925, 2875, 2360, 1755, 1717, 1681, 1484, 1392, 1365, 1293, 1236, 1192, 1170, 1097, 1048, 1023, 881; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.56 (1H, d, *J* 8.0, NH), 5.44 (1H, d, *J* 5.5, NH), 4.64 (1H, dt, *J* 8.0, 3.0, 2–H), 4.16 (1H, m, 2'–H), 3.77 (1H, dd, *J* 9.0, 3.0, 3–*H*H), 3.70 (1H, dd, *J* 8.0, 3.0, 3'–*H*H), 3.66 (3H, s, OMe), 3.47 (1H, dd, *J* 9.0, 3.0, 3–H*H*), 3.35 (1H, m, 3'–H*H*), 1.39 (9H, s, CMe_3), 1.16 (9H, s, CMe_3), 1.07 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.8 (C), 170.6 (C), 155.5 (C), 79.8 (C), 74.1 (C), 73.4 (C), 62.0 (CH_2), 61.9 (CH_2), 53.8 (Me), 53.1 (CH), 52.3 (CH), 29.7 (Me), 27.4 (Me), 27.3 (Me); *m/z* (APcI) 419 (MH^+, 100%), 383 (80).

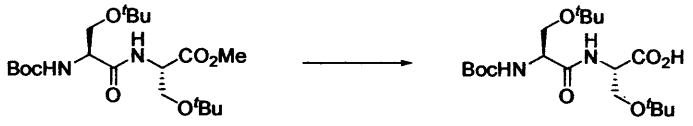
(2*S*,2'*S*)–Methyl 2–{[(2–benzyloxycarbonylamino)–3–(*tert*–butoxy)propanoyl]amino}–3–(*tert*–butoxy)butanoate (145). Z– Ser(^{t}Bu)–Ser(^{t}Bu)–OMe



NMM (0.19 ml, 1.7 mmol) and isobutyl chloroformate (0.13 ml, 0.94 mmol) were added dropwise to a solution of Z–Ser(^{t}Bu)–OH **143** (250 mg, 0.85 mmol) in dry THF (1.7 ml) at 0 °C and the mixture was stirred for 20 min. H–Ser(^{t}Bu)–OMe (196 mg, 0.94 mmol) was added and

the solution was stirred at 0 °C for 5 h, evaporated *in vacuo* and partition between aqueous $KHSO_4$ solution (10% w/v, 15 ml) and CH_2Cl_2 (15 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 15 ml) and the combined organic layers were washed with brine (50 ml), dried (Na_2SO_4), filtered and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.54), gave the *title compound* as a colourless oil (340 mg, 88%) (Found: MH^+ 453.2590. $C_{23}H_{37}N_2O_7$ requires MH^+, 453.2595); $[\alpha]_D^{21}$ +42.3 (*c*2.50, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3340, 2975, 2877, 1730, 1681, 1504, 1392, 1365, 1194, 1142, 1098, 1049, 985, 931, 875, 848, 755, 698; ^1H NMR (400 MHz; $CDCl_3$) δ 7.57 (1H, d, *J* 8.5, NH), 7.28 (5H, m, PhH) 5.71 (1H, d, *J* 6.0, NH), 5.02 (2H, s, OCH_2), 4.63 (1H, dt, *J* 8.5, 3.0, 2–H), 4.22 (1H, m, 2'–H), 3.78 (1H, dd, *J* 9.0, 3.0, 3–*H*H), 3.76 (1H, m, 3'–*H*H), 3.66 (3H, s, OMe), 3.47 (1H, dd, *J* 9.0, 3.0, 3–H*H*), 3.37 (1H, app t, *J* 9, 3'–H*H*), 1.17 (9H, s, CMe_3), 1.10 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.5 (C), 170.4 (C), 156.0 (C), 136.3 (C), 128.7 (CH), 128.2 (CH), 128.1 (CH), 74.3 (C), 66.9 (CH_2), 62.0 (CH_2), 61.8 (CH_2), 54.1 (Me), 53.1 (CH), 52.3 (CH), 27.4 (Me), 27.3 (Me); *m/z* (APcI) 453 (MH^+, 100%).

(2*S*,2'*S*)–2–{[(2–*tert*–butoxycarbonylamino)–3–(*tert*–butoxy)propanoyl]amino}–3–(*tert*–butoxy)propanoic acid (146). Boc–Ser(^{t}Bu)–Ser(^{t}Bu)–OH



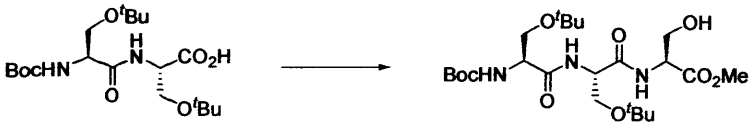
LiOH monohydrate (107 mg, 2.5 mmol) was added to a solution of Boc–Ser(^{t}Bu)–Ser(^{t}Bu)–OMe **144** (175 mg, 0.42 mmol) in MeOH–H_20 (5:1) (4.0 ml). The mixture was stirred for 18 h at room temperature. The solution was evaporated *in vacuo* and partitioned between hydrochloric acid (1M , 30 ml) and CH_2Cl_2 (50 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 30 ml) and the organic extracts were combined, dried (Na_2SO_4), filtered and evaporated *in vacuo* to give the *title compound*[24] as a colourless oil (240 mg, 99%) (Found: MH^+ 405.2594. $C_{19}H_{37}N_2O_7$ requires MH^+, 405.2595); $[\alpha]_D^{23}$ +19.4 (*c*3.20, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3425, 3327, 2975, 2251, 1713, 1669, 1486, 1392, 1365, 1194, 1100, 1023, 910, 881, 733; ^1H NMR (400 MHz; $CDCl_3$) δ 7.50 (1H, d, *J* 7.5, NH), 6.30 (1H, bs, OH), 5.68 (1H, bs, NH), 4.47 (1H, m, 2–H), 4.20 (1H, m, 2'–H), 3.78 (1H, m, 3–*H*H), 3.67 (1H, m, 3'–*H*H), 3.52 (1H, m, 3–H*H*), 3.40 (1H, m, 3'–H*H*), 1.37 (9H, s, CMe_3), 1.13 (9H, s, CMe_3), 1.09 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 173.8 (C), 170.8 (C), 156.2 (C), 136.2 (C), 128.6 (CH), 128.4 (CH), 128.2 (CH), 74.4 (C), 73.9

(C), 67.1 (CH_2), 61.7 (CH_2), 61.6 (CH_2), 54.2 (CH), 52.9 (CH), 27.3 (Me), 27.1 (Me); *m/z* (ES) 403 ([M–H$^-$], 100%), 329 (60).

(2*S*,2'*S*)–2–{[(2–benzyloxycarbonylamino)–3–(*tert*–butoxy)propanoyl]amino}–3–(*tert*–butoxy)propanoic acid (147). Z–Ser(^{t}Bu)–Ser(^{t}Bu)–OH

LiOH monohydrate (170 mg, 4.0 mmol) was added to a solution of Z–Ser(^{t}Bu)–Ser(^{t}Bu)–OMe **145** (300 mg, 0.66 mmol) in MeOH–H_2O (5:1) (7.0 ml). The mixture was stirred for 18 h at room temperature. The solution was evaporated *in vacuo* and partitioned between hydrochloric acid (1M, 25 ml) and CH_2Cl_2 (35 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 35 ml) and the organic extracts were combined, dried (Na_2SO_4), filtered and evaporated *in vacuo* to give the *title compound*[24] as a colourless oil (255 mg, 88%) (Found: MH$^+$ 439.2438. $C_{22}H_{35}N_2O_7$ requires *MH*$^+$, 439.2436); $[\alpha]_D^{23}$ +19.7 (*c*2.67, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3328, 2972, 2614, 1731, 1679, 1654, 1504, 1392, 1365, 1195, 1100, 875, 755, 698, 650; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.48 and 7.40 (1H, *J* 8.0, NH), 7.26 (5H, m, PhH), 6.33 (1H, bs, OH), 5.79 (1H, d, *J* 6.0, NH), 5.05 (2H, s, OCH_2), 4.63 (1H, m, 2–H), 4.25 (1H, m, 2'–H), 3.83 (1H, m, 3–*H*H), 3.71 (1H, m, 3'–*H*H), 3.47 (1H, m, 3–H*H*), 3.37 (1H, m, 3'–H*H*), 1.13 (9H, s, CMe_3), 1.09 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 173.8 (C), 170.8 (C), 156.2 (C), 136.2 (C), 128.6 (CH), 128.4 (CH), 128.2 (CH), 74.4 (C), 73.9 (C), 67.1 (CH_2), 61.7 (CH_2), 61.6 (CH_2), 54.2 (CH), 52.9 (CH), 27.3 (Me), 27.1 (Me); *m/z* (APcI) 439 (MH$^+$, 100%), 363 (95).

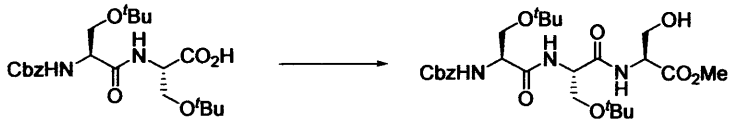
(2*S*,2'*S*,2''*S*)–Methyl 2–[({[(2–*tert*–butoxycarbonylamino)–3–(*tert*–butoxy)propanoyl]-amino}–3–(*tert*–butoxy)propanoyl)amino]–3–hydroxypropanoate (148). Boc–Ser(^{t}Bu)–Ser(^{t}Bu)–Ser–OMe



NMM (0.13 ml, 1.2 mmol) and isobutyl chloroformate (0.10 ml, 0.76 mmol) were added dropwise to a solution of Boc–Ser(^{t}Bu)–Ser(^{t}Bu)–OH **146** (240 mg, 0.59 mmol) in dry THF (1.0

ml) at 0 °C and the mixture was stirred for 20 min. H–Ser–OMe (140 mg, 0.90 mmol) was added and the solution was stirred at 0 °C for 5 h, evaporated *in vacuo* and partitioned between aqueous $KHSO_4$ solution (10% w/v, 25 ml) and CH_2Cl_2 (35 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 35 ml) and the combined organic layers were washed with brine (50 ml), dried (Na_2SO_4), filtered and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.44), gave the *title compound* as a colourless oil (234 mg, 78%) (Found: MH^+ 506.3080. $C_{23}H_{44}N_3O_9$ requires MH^+, 506.3072); $[\alpha]_D^{23}$ +16.2 (*c*2.60, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3404, 2975, 1710, 1650, 1504, 1366, 1169, 1091, 1017, 878, 730, 650; ^1H NMR (400 MHz; $SO(CD_3)_2$) δ 8.23 (1H, m, 2–NH), 7.87 (0.5H, d, *J* 8.0, 2'–NH) 7.77 (0.5H, d, *J* 8.0, 2'–NH), 6.86 (0.5H, d, *J* 8.0, 2''–NH), 6.75 (0.5H, d, *J* 8.0, 2''–NH), 5.14 (1H, m, 3–*H*H), 4.51 (1H, m, 2'–H), 4.48 (1H, m, 2–H), 4.23 (0.5H, m, 2''–H), 4.11 (0.5, m, 2''–H), 3.82–3.41 (5H, $2CH_2$ and CH*H*), 3.74 (3H, s, OMe), 3.41 (1H, s, OH), 1.45 (9H, s, CMe_3), 1.17 (18H, s, CMe_3); ^{13}C NMR (100 MHz; $SO(CD_3)_2$) δ 171.8 (C), 171.4 (C), 171.3 (C), 171.2 (C), 170.3 (C), 170.1 (C), 155.7 (C), 155.6 (C), 78.7 (C), 78.7 (C), 73.4 (C), 73.3 (C), 73.2 (C), 70.5 (C), 62.3 (CH_2), 61.8 (CH_2), 61.7 (CH_2), 55.4 (CH), 55.2 (CH), 55.0 (CH), 53.3 (CH), 53.2 (CH), 53.0 (CH), 52.3 (Me), 28.6 (Me), 27.6 (Me), 27.5 (Me); *m/z* (APcI) 506 (MH^+, 100%).

(2*S*,2'*S*,2''*S*)–Methyl 2–[({[((2–benzyloxycarbonylamino)–3–(*tert*–butoxy)propanoyl]-amino}–3–(*tert*–butoxy)propanoyl)amino]–3–hydroxypropanoate (149). Z–Ser(^{t}Bu)–Ser(^{t}Bu)–Ser–OMe



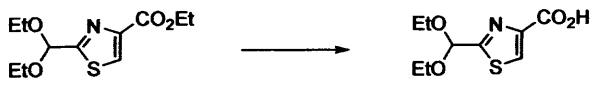
NMM (0.13 ml, 1.2 mmol) and isobutyl chloroformate (0.10 ml, 0.76 mmol) were added dropwise to a solution of Z–Ser(^{t}Bu)–Ser(^{t}Bu)–OH **147** (255 mg, 0.58 mmol) in dry THF (1.0 ml) at 0 °C and the mixture was stirred for 20 min. H–Ser–OMe (140 mg, 0.90 mmol) was added and the solution was stirred at 0 °C for 5 h, evaporated *in vacuo* and partitioned between aqueous $KHSO_4$ solution (10% w/v, 25 ml) and CH_2Cl_2 (35 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 35 ml) and the combined organic layers were washed with brine (50 ml), dried (Na_2SO_4), filtered and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.52), gave the *title compound* as a colourless oil (182 mg, 34%) (Found: MH^+ 540.2910. $C_{26}H_{42}N_3O_9$ requires MH^+, 540.2916); $[\alpha]_D^{23}$ +16.4 (*c*1.27, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3332, 2974, 1730, 1660, 1518, 1366, 1195, 1086; ^1H NMR (400 MHz; $SO(CD_3)_2$) δ 8.89

(1H, bs, NH), 8.25 (0.5H, d, *J* 8.0, NH), 8.21 (0.5H, d, *J* 8.0, NH), 7.42–7.35 (5H, PhH), 5.11 (2H, s, OCH_2), 4.81 (1H, bs, NH), 4.42 (2H, 2CH), 4.24–3.42 (8H, $3CH_2$, CH and OH), 3.68 (3H, s, OMe), 1.13 (18H, s, $2CMe_3$); ^{13}C NMR (100 MHz; $SO(CD_3)_2$) δ 171.9 (C), 171.3 (C), 170.1 (C), 156.4 (C), 137.4 (C), 128.8 (CH), 128.1 (CH), 127.1 (CH), 73.4 (C), 73.3 (C), 65.9 (CH_2), 63.3 (CH_2), 62.3 (CH_2), 61.7 (CH_2), 55.8 (CH), 55.7 (CH), 55.4 (CH), 55.0 (CH), 53.2 (CH), 53.1 (CH), 52.3 (Me), 27.6 (Me), 27.6 (Me); *m/z* (APcI) 540 (MH^+, 12%), 464 (100).

2,2–Diethoxy–3–thioamide (151)

A mixture of 2,2 diethoxypropionitrile **150** (0.5 ml, 3.2 mmol) and ammonium sulphide (50 wt.% in H_2O; 1.0 ml, 7.4 mmol) in MeOH (25 ml) was stirred for 2.5 h. The mixture was evaporated *in vacuo*, purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.64), to give the *title compound* as an off white solid (267 mg, 47%), mp 110–111 °C (light petroleum:EtOAc) (Found: MH^+, 178.0892. $C_7H_{15}NO_2S$ requires MH^+, 178.0896); IR (KBr)/cm^{-1} ν_{max} 3427, 3305, 2975, 2933, 2891, 2362, 1597, 1428, 1265, 1192, 1144, 1129, 1051, 962, 864, 823, 728, 677; ^1H NMR (400 MHz; $CDCl_3$) δ 8.16 (2H, bs, NH_2), 3.49 (2H, dq, *J* 9.0, 7.0, 2OC*H*HMe), 3.44 (2H, dq, *J* 9.0, 7.0, 2OCH*H*Me), 1.60 (3H, s, Me), 1.16 (6H, appt, *J* 7.0, $2CH_2$*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 206.6 (C), 103.0 (C), 58.0 (CH_2), 24.7 (Me), 15.2 (Me); *m/z* (APcI) 178 (MH^+, 30%), 132 (100).

2–(Diethoxymethyl)thiazole–4–carboxylic acid (157)



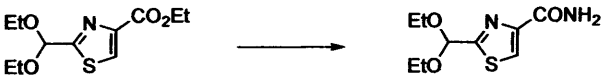
LiOH monohydrate (2.73 g, 65.1 mmol) was added to a stirred solution of thiazole **94** (2.55 g, 11.0 mmol) in MeOH–H_2O (5:1) (100 ml) and the solution was stirred overnight. After evaporating *in vacuo*, the mixture was partitioned between citric acid (1 M; 40 ml) and CH_2Cl_2 (150 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 100 ml) and the organic extracts were combined, washed with brine (60 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound* as a white solid (1.63 g, 64%), mp 97–98 °C (EtOAc) (Found: MH^+

232.0639. $C_9H_{14}NO_4S$ requires *MH*$^+$ 232.0638); IR (KBr)/cm^{-1} ν_{max} 3105, 2978, 2865, 2725, 2634, 2534, 1684, 1482, 1410, 1360, 1274, 1227, 1191, 1070, 1010, 941, 848, 779, 746; ^{1}H NMR (400 MHz; $CDCl_3$) δ 10.88 (1H, bs, OH), 8.21 (1H, s, 5–H), 5.68 (1H, s, CH), 3.64 (2H, dq, *J* 9.0, 7.0, 2OC*H*HMe), 3.58 (2H, dq, *J* 9.0, 7.0, 2OCH*H*Me), 1.18 (6H, app t, *J* 7.0, 2CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.3 (C), 165.2 (C), 147.6 (C), 129.0 (CH), 98.4 (CH), 62.6 (CH_2), 15.0 (Me); *m/z* (APcI) 232 (MH^+, 100 %).

2–(Diethoxymethyl)thiazole–4–carboxamide (158)

Ethyl chloroformate (0.68 ml, 7.15 mmol) was added to a solution of thiazole **157** (1.63 g, 7.05 mmol) and Et_3N (1.0 ml, 7.15 mmol) in dry THF (30 ml) and stirred at 0 °C for 1 h. Aqueous NH_4OH (35% w/v) (5.2 ml) was added and the solution stirred at 0 °C for 1h, allowed to warm to RT and evaporated *in vacuo*. The residue was partitioned between H_2O (70 ml) and EtOAc (150 ml), aqueous layer was further extracted with EtOAc (2 x 100 ml), the combined layers were washed with brine (60 ml), dried (Na_2SO_4), in evaporated *in vacuo*, purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.30), gave the *title compound*[17] as a colourless solid (1.38 g, 85%) mp 115–116 °C (EtOAc) (Found: MH^+, 231.0801. $C_9H_{15}N_2O_3S$ requires *MH*$^+$, 231.0798); IR (KBr)/cm^{-1} ν_{max} 3385, 3207, 3096, 2975, 2925, 2885, 1679, 1648, 1609, 1574, 1522, 1488, 1391, 1322, 1195, 1087, 1059, 937, 902, 841, 784, 708; ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.12 (1H, s, 5–H), 7.20 (1H, bs, NH), 6.46 (1H, bs, NH), 5.61 (1H, s, CH), 3.63 (4H, m, 2OCH_2), 1.25 (6H, m, 2Me); ^{13}C NMR (125 MHz; $CDCl_3$) δ 169.2 (C), 163.3 (C), 149.7 (C), 125.3 (CH), 98.1 (CH), 62.1 (CH_2), 15.1 (Me); *m/z* (APcI) 231 (MH^+, 100%).

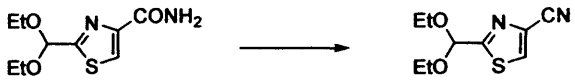
2–(Diethoxymethyl)thiazole–4–carboxamide (158)



Thiazole **94** (12.45 g, 54 mmol) was dissolved in a saturated solution of ammonia in MeOH (220 ml) and stirred at 45 °C for 7 d. The solution was evaporated *in vacuo*, purification by column

chromatography on SiO_2, eluting with EtOAc (R_f 0.30), gave the *title compound*[17] as a colourless solid (8.12 g, 65%) with the same spectroscopic properties.

2–(Diethoxymethyl)thiazole–4–carbonitrile (159)

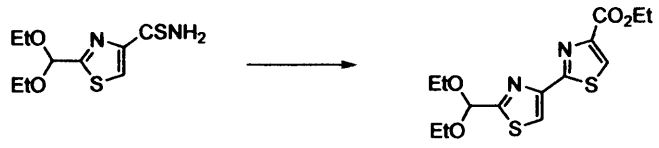


Phosphorous oxylchlroide (7.56 ml, 81.0 mmol) was added dropwise to a cold (0 °C) solution of thiazole **158** (8.12 g, 35.3 mmol) in pyridine (120 ml). The solution was warmed to RT and stirred for 2 h and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.56), gave the title compound as a brown oil (6.20 g, 83%) (Found: MH^+, 213.0690. $C_9H_{13}N_2O_2S$ requires MH^+, 213.0692); IR (film)/cm^{-1} ν_{max} 3106, 2979, 2931, 2889, 2240, 1496, 1444, 1392, 1371, 1319, 1062, 951, 906, 868, 787; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.93 (1H, s, 5–H), 5.66 (1H, s, CH), 3.71 (2H, dq, *J* 9.5, 7.0, 2OC*H*HMe), 3.62 (2H, dq, *J* 9.5, 7.0, 2OCH*H*Me), 1.21 (6H, app t, *J* 7.0, $2CH_2Me$); ^{13}C NMR (125 MHz; $CDCl_3$) δ 171.4 (C), 131.5 (CH), 126.9 (C), 113.9 (C), 98.2 (CH), 62.7 (CH_2), 15.0 (Me); *m/z* (APcI) 213 (MH^+, 97%), 139 (100).

2–(Diethoxymethyl)thiazole–4–thioamide (160)

A mixture of thiazole **159** (2.51 g, 11.83 mmol) and ammonium sulphide (50 wt. % in H_2O; 3.2 ml, 23.4 mmol) in MeOH (50 ml) was stirred overnight. The mixture was evaporated *in vacuo* to give the *title compound* as a pale yellow solid (2.9 g, 100%), mp 80–81 °C (EtOAc) (Found: MH^+, 247.0568. $C_9H_{15}N_2O_2S_2$ requires MH^+, 247.0569); IR (KBr)/cm^{-1} ν_{max} 3330, 3247, 3150, 2973, 2929, 1635, 1611, 1505, 1444, 1400, 1370, 1338, 1316, 1212, 1159, 1130, 1057, 1007, 970, 909, 878, 836; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.59 (1H, bs, NH), 8.40 (1H, s, 5–H), 7.50 (1H, bs, NH), 5.60 (1H, s, CH), 3.64 (2H, dq, *J* 9.0, 7.0, 2OC*H*HMe), 3.60 (2H, dq, *J* 9.0, 7.0, 2OCH*H*Me), 1.21 (6H, appt, *J* 7.0, $2CH_2Me$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 190.6 (C), 168.9 (C), 153.3 (C), 128.9 (CH), 98.0 (CH), 62.1 (CH_2), 15.1 (Me); *m/z* (APcI) 247 230 (100%), 247 (MH^+, 95).

Ethyl 2–[2–(diethoxymethyl)thiazol–4–yl]thiazole–4–carboxylate 161



Ethyl bromopyruvate (0.56 ml, 4.48 mmol) was added to a stirred solution of thiazole **160** (0.92 g, 3.73 mmol) in EtOH (10 ml) over 4Å molecular sieves (2.5 g) and the mixture was heated at reflux for 1 h. The solution was allowed to cool, filtered through Celite® and evaporated *in vacuo*. The residue was partitioned between saturated $NaHCO_3$ (80 ml) and $CHCl_3$ (80 ml), the aqueous layer was further extracted with $CHCl_3$ (2 x 60 ml), washed with brine (85 ml), dried Na_2SO_4 and evaporated *in vacuo* to give the *title compound* as a pale green solid (1.28 g, 100%) mp 85–86 °C (light petroleum–Et_2O) (Found: MH^+, 343.0786. $C_{14}H_{19}N_2O_4S_2$ requires MH^+, 343.0781); IR (KBr)/cm^{-1} ν_{max} 3120, 2980, 2925, 2885, 1724, 1540, 1476, 1440, 1392, 1368, 1327, 1290, 1210, 1058, 954, 894, 876, 845, 822, 801; ^1H NMR (400 MHz; $CDCl_3$) δ 8.12 (2H, s, 5 and 5'–H), 5.65 (1H, s, CH), 4.40 (2H, q, *J* 7.0, CH_2Me), 3.70 (2H, dq, *J* 9.5, 7.0, 2OC*H*HMe), 3.64 (2H, dq, *J* 9.5, 7.0, 2OCH*H*Me), 1.35 (3H, t, *J* 7.0, CH_2Me), 1.18 (6H, app t, *J* 7.0, $2CH_2Me$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.9 (C), 163.4 (C), 161.5 (C), 148.5 (C), 147.9 (C), 127.8 (CH), 118.5 (CH), 98.5 (CH), 62.4 (CH_2), 61.6 (CH_2), 15.1 (Me), 14.4 (Me); *m/z* (APcI) 343 (MH^+, 100%), 297 (50).

Ethyl 2–(2–formylthiazol–4–yl)thiazole–4–carboxylate 162

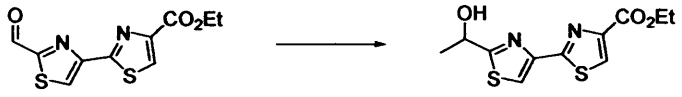
Bisthiazole **161** (278 mg, 0.81 mmol) was dissolved in acetone–HCl_{aq} (2M) (8:1) (9 ml) and stirred at reflux for 1 h, evaporated *in vacuo*, and separated in H_2O (10 ml) and CH_2Cl_2 (3 x 20 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound* as a yellow solid (190 mg, 87%) mp 155–156 °C (EtOAc) (lit.[25] mp 134–135.5 °C) (Found: MH^+, 269.0053. $C_{10}H_9N_2O_3S_2$ requires MH^+, 269.0049); IR (KBr)/cm^{-1} ν_{max} 3423, 3127, 3094, 2977, 2839, 2361, 1724, 1701, 1470, 1419, 1367, 1333, 1297, 1212, 1182, 1094, 1044, 1021, 955, 901, 881, 827, 812, 787, 773, 687, 660; ^1H NMR (400 MHz; $CDCl_3$) δ 9.98 (1H, s, CH), 8.44 (1H, s, 5'–H), 8.15 (1H, s, 5–H), 4.38 (2H, q, *J* 7.1, OCH_2Me), 1.37 (3H, t, *J* 7.1,

CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 183.3 (C), 165.9 (C), 162.0 (C), 161.2 (C), 151.1 (C), 148.3 (C), 128.5 (CH), 124.0 (CH), 61.8 (CH_2), 14.4 (Me); *m/z* (APcI) 269 (MH^+, 100%).

Ethyl 2–(2–formylthiazol–4–yl)thiazole–4–carboxylate (162)

Ethyl bromopyruvate (1.63 ml, 13.0 mmol) was added to a stirred solution of thiazole **160** (2.90 g, 11.77 mmol) in EtOH (40 ml) and the mixture was heated at reflux for 2 h and evaporated *in vacuo*. The residue was dissolved in acetone–HCl_{aq} (2M) (5:2) (70 ml) and stirred at reflux for 1.5 h, evaporated *in vacuo*, and separated in H_2O (60 ml) and CH_2Cl_2 (3 x 60 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound* as a yellow solid (3.15 g, 100%) with the same spectroscopic properties.

Ethyl 2–{2–[1–(1–hydroxyethyl)]thiazol–4–yl}thiazole–4–carboxylate (163)



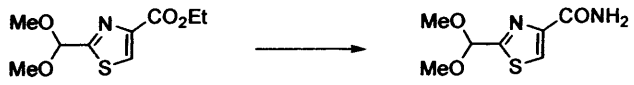
Methyl magnesium bromide (3.0 M in Et_2O) (0.77 ml, 2.31 mmol) was added dropwise to a solution of bisthiazole **162** (600 mg, 2.24 mmol) in dry CH_2Cl_2 (25 ml) at 0 °C. The solution was warmed to RT and stirred for 18 h, saturated aqueous NH_4Cl (20 ml) was added and evaporated *in vacuo*. The residue was partitioned between H_2O (35 ml) and CH_2Cl_2 (50 ml), the aqueous layer was further extracted with CH_2Cl_2 (2 x 40 ml), the combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.26) gave the title compound as colourless solid (260 mg, 41%) mp 109–110 °C (EtOAc) (Found: MH^+, 285.0359. $C_{11}H_{13}N_2O_3S_2$ requires MH^+, 285.0362); IR (KBr)/cm^{-1} ν_{max} 3422, 3106, 2975, 2925, 1734, 1654, 1560, 1541, 1298, 1216, 1098, 1017, 780; ^1H NMR (400 MHz; $CDCl_3$) δ 8.11 (1H, s, 5–H), 8.07 (1H, s, 5'–H), 5.14 (1H, q, *J* 6.5, CH), 4.38 (2H, q, *J* 7.0, OCH_2Me), 2.98 (1H, bs, OH), 1.63 (3H, d, *J* 6.5, Me), 1.36 (3H, t, *J* 7.0, CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 176.7 (C), 163.4 (C), 161.5 (C), 148.0 (C), 147.9 (C), 127.8 (CH), 117.5 (CH), 68.2 (CH), 61.6 (CH_2), 24.1 (Me), 14.4 (Me); *m/z* (APcI) 285 (MH^+, 100%).

Ethyl 2-(2-acetylthiazol-4-yl)thiazole-4-carboxylate (164)

Method A: IBX (618 mg, 2.20 mmol) was added to DMSO (15 ml) and stirred until homogenous (~15 min), bisthiazole **163** (250 mg, 0.88 mmol) was added and the solution was stirred for 1 h, partitioned between H_2O (15 ml) and CH_2Cl_2 (40 ml). The aqueous layer was further extracted CH_2Cl_2 (2 x 40 ml) and the combined organic layers washed saturated aqueous $NaHCO_3$ (40 ml), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.69), gave the *title compound* as a yellow solid (124 mg, 50%) mp 159–160 °C (EtOAc) (Found: MH^+, 283.0208. $C_{11}H_{11}N_2O_3S_2$ requires *MH*$^+$, 283.0206); IR (KBr)/cm^{-1} ν_{max} 3422, 3127, 3033, 1724, 1686, 1654, 1466, 1412, 1359, 1336, 1298, 1211, 1096, 1054, 937, 772; ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.38 (1H, s, 5'–H), 8.15 (1H, s, 5–H), 4.39 (2H, q, *J* 7.1, OC*H*$_2$Me), 2.72 (3H, s, Me), 1.38 (3H, t, *J* 7.1, CH$_2$*Me*); ^{13}C NMR (125 MHz; $CDCl_3$) δ 191.2 (C), 167.2 (C), 162.4 (C), 161.3 (C), 150.2 (C), 148.2 (C), 128.2 (CH), 124.1 (CH), 61.7 (CH_2), 26.0 (Me), 14.4 (Me); *m/z* (APcI) 283 (MH^+, 100%).

Method B: MnO_2 (activate on carbon) (64 mg, 0.74 mmol) was added to a solution of bis–thiazole (**97**) (21 mg, 74 μmol) in CH_2Cl_2 (4 ml) and stirred for 40 min. The solution was filtered over Celite®, washed with MeOH (15 ml) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–Et_2O (1:1) (R_f 0.48), gave the title compound as a yellow solid (11 mg, 52%) with the same physical and spectroscopic properties.

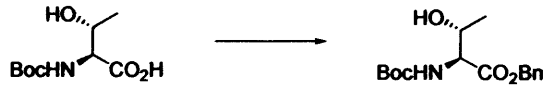
2-(Dimethoxymethyl)thiazole-4-carboxamide (165)



Thiazole **96** (109 mg, 0.50 mmol) was dissolved in a saturated solution of ammonia in MeOH (8 ml), the reaction was stirred in a sealed continuous microwave, irradiated at 300 watts, 60 °C, with cooling for 4 h. The solution was evaporated *in vacuo*, purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.28) gave the title compound as a colourless solid (77 mg, 76%) mp 110–111 °C (EtOAc) (Found: MH^+, 203.0488. $C_7H_{11}N_2O_3S$ requires *MH*$^+$, 203.0485); IR (KBr)/cm^{-1} ν_{max} 3405, 3290, 3161, 3124, 2941, 2903, 2834, 1689, 1612, 1517, 1495, 1370, 1358, 1327, 1285, 1187, 1095, 1054, 990, 940, 902, 812, 792, 705; ^{1}H NMR (400

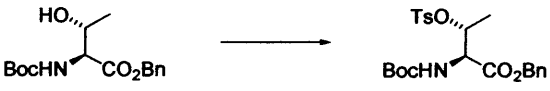
MHz; $CDCl_3$) δ 8.12 (1H, s, 5–H), 7.19 (1H, bs, NH), 5.78 (1H, bs, NH), 5.53 (1H, s, CH), 3.35 (6H, s, OMe); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.0 (C), 162.8 (C), 149.8 (C), 125.5 (CH), 99.7 (CH), 54.4 (Me); *m/z* (APcI) 203 (MH^+, 100 %).

(2*S*,3*R*)–Benzyl 2–(*tert*–butoxycarbonylamino)–3–hydroxybutanoate (179). (Boc–Thr–Bn)



Benzyl bromide (6.5 ml, 54.8 mmol) was added to a solution of Boc–Thr–OH **178** (3.0 g, 13.7 mmol) and Et_3N (2.0 ml, 14.4 mmol) in dry DMF (75 ml) at 0 °C and stirred for 3 h, allowed to warm to RT and stirred for 2 d. The solution was partitioned between H_2O (450 ml) and Et_2O (300 ml), the aqueous layer was further extracted with Et_2O (2 x 150 ml), the combined organic layers were washed with H_2O (2 x 180 ml), dried (Na_2SO_4), filtered and evaporated *in vacuo*. Purification twice by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) and light petroleum–Et_2O (1:1) (R_f 0.13), gave the *title compound* as a pale yellow oil (2.77 g, 65%) (Found: MH^+, 310.1649. $C_{16}H_{24}NO_5$ requires MH^+, 310.1649); $[\alpha]_D^{26}$ –12.2 (*c*2.15, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3453, 2978, 2925, 1740, 1716, 1503, 1455, 1368, 1256, 1163, 1068, 1001, 880, 850, 755, 698; ^1H NMR (400 MHz; $CDCl_3$) δ 7.29 (5H, m, PhH), 5.31 (1H, d, *J* 9.0, NH), 5.16 (1H, d, *J* 12.5, OC*H*H), 5.10 (1H, d, *J* 12.5, OCH*H*), 4.24 (2H, 2–H and 3–H), 2.09 (1H, d, *J* 4.5, OH), 1.37 (9H, s, CMe_3) 1.16 (3H, d, *J* 6.5, 3–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.4 (C), 156.2 (C), 135.3 (C), 128.6 (CH), 128.5 (CH), 128.2 (CH), 80.1 (C), 68.2 (CH), 67.3 (CH_2), 58.8 (CH), 28.3 (Me), 20.0 (Me); *m/z* (APcI) 310 (MH^+, 55%), 271 (100).

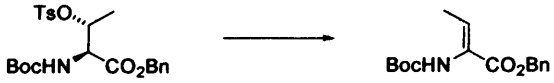
(2*S*,3*R*)–Benzyl 2–(*tert*–butoxycarbonylamino)–3–(*p*–toluenesulphonyloxy)butanoate (180). Boc–Thr(Ts)–Bn



TsCl (2.34 g, 12.3 mmol) was added portion wise to a solution of (Boc)–Thr–Bn **179** (1.52 g, 4.91 mmol) in pyridine (15 ml) at 0 °C and stirred for 2 d. The solution was warmed to RT and EtOAc (100 ml) was added, the solution was washed with aqueous $KHSO_4$ solution (5% w/v) (5 x 30 ml) and the organic layer dried (Na_2SO_4), evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (3:1) (R_f 0.35), gave the *title*

compound[26] as a colourless solid (1.03 g, 45%) mp 121–122 °C (EtOAc) (Found: MH^+, 464.1737. $C_{23}H_{30}NO_7S$ requires *MH*$^+$, 464.1737) $[\alpha]_D^{26}$ +50.30 (*c*1.15, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3412, 2977, 1751, 1720, 1599, 1512, 1455, 1388, 1362, 1314, 1247, 1207, 1163, 1089, 1055, 1022, 990, 957, 940, 909, 844, 818, 777, 767, 751, 701, 666; ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.66 (2H, d, *J* 8.2, *o*–Ph*H*), 7.23–7.33 (7H, m, PhH), 5.18 (1H, d, *J* 9.7, NH), 5.11 (1H, dq, *J* 2.0, 6.4, 3–H), 5.04 (1H, d, *J* 12.1, OC*H*H), 4.81 (1H, d, *J* 12.1, OCH*H*), 4.37 (1H, dd, *J* 2.0, 9.7, 2–H), 2.36 (3H, s, Me), 1.36 (9H, s, CMe_3), 1.26 (3H, d, *J* 6.4, 3–Me); ^{13}C NMR (125 MHz; $CDCl_3$) δ 169.1 (C), 155.6 (C), 145.0 (C), 134.9 (C), 133.6 (C), 129.9 (CH), 128.6 (CH), 128.6 (CH), 128.5 (CH), 127.9 (CH), 80.5 (C), 78.7 (CH), 67.9 (CH_2), 57.7 (CH), 28.2 (Me), 21.7 (Me), 18.2 (Me); *m/z* (ES) 464 (MH^+, 7%), 408 (85), 364 (100).

(*Z*)–Benzyl 2–(*tert*–butoxycarbonylamino)but–2–enoate (181)



Phenyl selenol (621 mg, 3.95 mmol) was added to a solution of KOH (188 mg, 3.35 mmol) in MeOH (6 ml) and stirred for 5 min, evaporated *in vacuo*, and dried in a vacuum dessicator for 36 h. Boc–Thr(Ts)–OBn **180** (400 mg, 0.86 mmol) was added to the residue in dry DMF under a nitrogen atmosphere at 0 °C and stirred for 48 h. The solution was partitioned between H_2O (40 ml) and Et_2O (50 ml), the aqueous layer was further extracted with Et_2O (2 x 50 ml) and the combined organic layers where washed with H_2O (40 ml), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.48), gave a colourless solid (150 mg, 60%) mp 85–86 °C (light petroleum–EtOAc) (lit.[27] mp 84–85 °C) (Found: MH^+, 292.1540. $C_{16}H_{22}NO_4$ requires *MH*$^+$, 292.1543); IR (KBr)/cm^{-1} ν_{max} 3336, 2985, 2360, 1723, 1695, 1659, 1499, 1457, 1393, 1367, 1348, 1291, 1245, 1172, 1107, 1057, 991, 907, 867, 780, 752, 700; ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.29 (5H, m, PhH), 6.66 (1H, q, *J* 7.0, 3–H), 5.96 (1H, bs, NH), 5.13 (2H, s, OCH_2), 1.73 (3H, d, *J* 7.0, 3–Me), 1.38 (CMe_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 164.8 (C), 135.7 (CH), 132.5 (C), 129.9 (C), 128.6 (CH), 128.5 (CH), 128.3 (CH), 80.5 (C), 78.7 (C), 67.9 (CH_2), 28.2 (Me), 14.4 (Me); *m/z* (APcI) 292 (MH^+, 25%), 192 (100), 175 (68).

(4*S*,5*R*)–2,2,5–Trimethyl–3–(*tert*–butoxycarbonyl)oxazolidine–4–carboxylic acid (183)

p–TsOH (5.70 g, 22.8 mmol) was added to a solution of Boc–Thr–OH **178** (15.0 g, 68.4 mmol) and 2,2–dimethoxypropane (84 ml, 684 mmol) in THF (500 ml) and stirred at reflux for 18 h. The solution was allowed to cool and evaporated *in vacuo*, partitioned between H_2O (200 ml) and EtOAc (300 ml), the aqueous layer was further extracted with EtOAc (2 x 200 ml) and the combined organic layers were dried (Na_2SO_4), filtered and evaporated *in vacuo* to give the title compound as a orange oil (17.35 g, 99%) (Found: MH^+ 260.1494, $C_{12}H_{22}NO_5$ requires MH^+ 260.1492); $[\alpha]_D^{31}$ –55.4 (*c*2.95, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3468, 3016, 2983, 2936, 1725, 1700, 1478, 1455, 1394, 1369, 1320, 1260, 1216, 1172, 1132, 1094, 988, 945, 856; ^1H NMR (400 MHz; $CDCl_3$) δ 10.47 (1H, bs, OH), 4.16 (1H, dq, *J* 8.0, 6.0, 5–H), 3.93 (0.38H, d, *J* 8.0, 4–H), 3.86 (0.62H, d, *J* 8.0, 4–H), 1.59 (1.86H, s, 2–Me), 1.53 (1.14H, s, 2–Me), 1.51 (1.86H, s, 2–Me), 1.48 (1.14H, s, 2–Me), 1.42 (3.43H, s, CMe_3), 1.37 (3H, d, *J* 6.0, 5–Me), 1.34 (5.57H, s, CMe_3); ^{13}C (100 MHz; $CDCl_3$) δ 171.2 (C), 175.8 (C), 152.3 (C), 150.9 (C), 95.3 (C), 94.7 (C), 81.4 (C), 80.8 (C), 73.9 (CH), 73.5 (CH), 66.0 (CH), 66.0 (CH), 28.3 (Me), 28.2 (Me), 27.7 (Me), 26.5 (Me), 24.8 (Me), 23.9 (Me), 18.9 (Me); *m/z* (APcI) 160 (MH^+, 100%).

(4*S*,5*R*,2'*R*)–4–[(2–Hydroxypropyl)aminocarbonyl]–2,2,5–trimethyl–3–(*tert*–butoxcarbonyl)oxazolidine (184)

Et_3N (2.78 ml, 20 mmol) was added dropwise over 30 min to a stirred solution of oxazolidine **183** (2.49 g, 9.68 mmol), (*R*)–1–amino–2–propanol (0.71 ml, 9.0 mmol) and pyBOP (5.2 g, 10 mmol) in dry CH_2Cl_2 (19 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 18 h. After concentrating *in vacuo*, purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.29), gave the *title compound* as a colourless solid (2.84 g, 100%), mp 118–119 °C (light petroleum–Et_2O) (Found: MH^+, 317.2072. $C_{15}H_{29}N_2O_5$ requires MH^+, 317.2071); $[\alpha]_D^{31}$ –62.0 (*c*1.50, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3488, 3286, 3116, 2978, 2925, 2865, 1672, 1570, 1366, 1259, 1135, 989, 967, 945, 909, 855, 781, 765, 690; ^1H NMR (400 MHz;

$CDCl_3$) δ 6.44 (1H, bs, NH), 4.22 (1H, bs, 5–H), 3.91 (1H, m, 2'–H), 3.76 (1H, d, *J* 7.5, 4–H), 3.40 (1H, bs, 1'–*H*H), 3.14 (1H, bs, 1'–H*H*), 2.97 (1H, bs, OH), 1.60 (3H, s, 2–Me), 1.58 (3H, s, 2–Me), 1.44 (9H, s, CMe_3), 1.37 (3H, d, *J* 6.0, 5–Me), 1.17 (3H, d, *J* 6.5, 2'–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.2 (C), 152.8 (C), 94.7 (C), 81.5 (C), 73.9, (CH), 67.9 (CH), 66.8 (CH), 47.1 (CH_2), 28.3 (Me), 27.6 (Me), 25.2 (Me), 20.2 (Me), 18.6 Me); *m/z* (APcI) 317 (MH^+, 100%), 261 (40).

(4*S*,5*R*,2'*R*)–4–[(2–Acetoxypropyl)aminocarbonyl]–2,2,5–trimethyl–3–(*tert*–butoxycarbonyl)oxazolidine (185)

Acetic anhydride (0.64 ml, 6.78 mmol) was added to a solution of oxazolidine **184** (714 mg, 2.26 mmol) in dry pyridine (20 ml) and stirred for 36 h. The solution was evaporated *in vacuo*, purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.24), gave the *title compound* as a colourless solid (810 mg, 73%), mp 124–125 °C (light petroleum–Et_2O) (Found: MH^+, 359.2179. $C_{17}H_{31}N_2O_6$ requires MH^+, 359.2177); $[\alpha]_D^{26}$ –34.1 (*c*1.35, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3382, 2985, 2945, 1735, 1711, 1663, 1532, 1478, 1459, 1376, 1241, 1221, 1175, 1126, 1096, 1076, 1057, 1026, 986, 972, 954, 894, 857, 783; ^1H NMR (500 MHz; $CDCl_3$) δ 6.36 (1H, bs, NH), 4.99 (1H, ddq, *J* 6.0,4.0,6.0, 2'–H), 4.22 (1H, bs, 5–H), 3.70 (1H, d, *J* 6.5, 4–H), 3.55 (1H, ddd, *J* 14.2, 6.0, 4.0, 1'–*H*H), 3.37 (1H, ddd, *J* 14.2, 6.0, 7.0, 1'–H*H*), 2.05 (3H, s, Me), 1.60 (3H, s, 2–Me) 1.55 (3H, s, 2–Me), 1.49 (9H, s, CMe_3), 1.42 (3H, d, *J* 6.0, 2'–Me), 1.30 (3H, d, *J* 6.5, 5–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.6 (C), 170.0 (C), 166.4 (C), 94.6 (C), 80.7 (C), 73.9 (CH), 69.6 (CH), 67.4 (CH), 43.8 (CH_2), 28.2 (Me), 27.7 (Me), 25.0 (Me), 21.1 (Me), 18.8 (Me), 17.5 (Me); *m/z* (APcI) 359 (MH^+, 100%), 303 (25), 259 (85).

(4*S*,5*R*,2'*R*)–4–[(2–*tert*–Butyldimethylsilyloxy)aminocarbonyl]–2,2,5–trimethyl–3–(*tert*–butoxycarbonyl)oxazolidine (186)

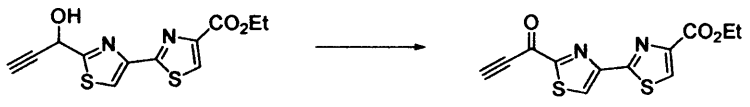
Imidazole (400 mg, 5.90 mmol), DMAP (122 mg, 1.0 mmol) and TBDMSCl (690 mg, 4.6 mmol) were added successively to a solution of oxazolidine **184** (1.04 g, 3.27 mmol) in DMF (25 ml). The mixture was stirred at room temperature for 36 h, acidified with hydrochloric acid (1 M; 80 ml) and extracted with Et_2O (3 x 90 ml). The combined organic extracts were washed with H_2O (3 x 300 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound* as a pale green oil (1.39 g, 98%) (Found: MH^+, 431.2934. $C_{21}H_{43}N_2O_5Si$ requires *MH*$^+$, 431.2936); $[\alpha]_D^{23}$ –26.4 (*c*0.75, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3329, 2976, 2931, 2858, 2359, 1710, 1669, 1530, 1462, 1367, 1258, 1175, 1133, 1006, 940, 860, 837, 777; ^{1}H NMR (500 MHz; $CDCl_3$) δ 6.10 (1H, bs, NH), 4.11 (1H, bs, 5–H), 3.89 (1H, m, 2'–H), 3.69 (1H, bs, 4–H), 3.23 (2H, 1'–CH_2), 1.55 (3H, s, 2–Me), 1.52 (3H, s, 2–Me), 1.36 (9H, s, CMe_3), 1.32 (3H, d, *J* 6.0, 5–Me), 1.07 (3H, d, *J* 6.0, 2'–Me), 0.82 (9H, s, CMe_3), 0.00 (3H, s, *Me*SiMe), 0.01 (3H, s, MeSi*Me*) ; ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.9 (C), 151.5 (C), 94.9 (C), 80.7 (C), 77.3 (C), 74.7 (CH), 68.1 (CH), 67.2 (CH), 46.3 (CH_2), 28.3 (Me), 25.8 (Me), 21.1 (Me), 19.2 (Me), 18.0 (Me), –3.6 (Me), –4.4 (Me); *m/z* (APcI) 431 (MH^+, 80%), 375 (100), 331 (75), 285 (45).

Ethyl 2–{2–[1–(1–hydroxyprop–2–yn)]thiazol–4–yl}thiazole–4–carboxylate (193)

Ethynyl magnesium bromide (0.5 M; THF) (10.40 ml, 5.2 mmol) was added dropwise to a solution of bisthiazole **162** (1.0 g, 3.73 mmol) in THF (25 ml) at 0 °C, was warmed to RT whilst stirring 1 h. The reaction was quenched with saturated aqueous NH_4Cl (20 ml), evaporated *in vacuo* and partitioned between H_2O (70 ml) and CH_2Cl_2 (75 ml), the aqueous layer was further extracted with CH_2Cl_2 (2 x 75 ml), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.40), gave the title compound as a colourless solid (513 mg, 47%) mp 153–154 °C (EtOAc) (Found: MH^+, 295.0202. $C_{12}H_{11}N_2O_3S_2$ requires *MH*$^+$, 295.0206); IR (KBr)/cm^{-1} ν_{max} 3297, 3249, 3112, 2983,

2118, 1715, 1541, 1498, 1442, 1370, 1326, 1296, 1214, 1155, 1063, 1019, 926, 776, 758; ^{1}H NMR (500 MHz; CD_3OD) δ 8.29 (1H, s, 5'–H), 8.22 (1H, s, 5–H), 5.16 (1H, s, 1''–H), 4.32 (2H, q, *J* 7.2, OCH_2Me), 3.06 (1H, s, 3''–H), 1.31 (3H, t, *J* 7.2, CH_2Me); ^{13}C NMR (125 MHz; CD_3OD) δ 171.2 (C), 163.5 (C), 161.2 (C), 147.9 (C), 147.2 (C), 128.2 (CH), 118.3 (CH), 81.4 (C), 74.7 (CH), 61.2 (CH), 60.9 (CH_2), 13.2 (Me); *m/z* (APcI) 295 (MH^+, 63%).

Ethyl 2–{2–[1–(prop–2–yn–1–oyl)]thiazol–4–yl}thiazole–4–carboxylate (194)



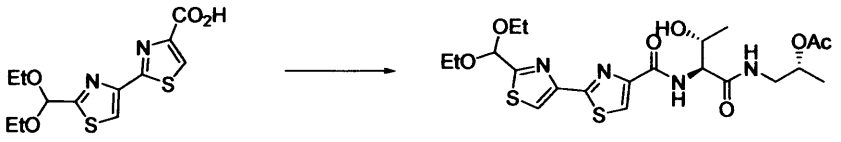
IBX (302 mg, 1.08 mmol) was added to DMSO (10 ml) and stirred until homogenous (~15 min), bisthiazole **193** (127 mg, 0.43 mmol) was added and the solution was stirred for 2 h, partitioned between H_2O (15 ml) and CH_2Cl_2 (30 ml). The aqueous layer was further extracted CH_2Cl_2 (2 x 25 ml) and the combined organic layers washed saturated aqueous $NaHCO_3$ (40 ml), dried (Na_2SO_4) and evaporated *in vacuo*, to give the *title compound* as a yellow solid (120 mg, 95%) ^{1}H NMR (100 MHz; $CDCl_3$) δ 8.47 (1H, s, 5'–H), 8.13 (1H, s, 5–H), 4.39 (2H, q, *J* 7.1, OCH_2Me), 3.65 (1H, s, 3''–H), 1.36 (3H, t, *J* 7.1, CH_2Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.6 (C), 165.7 (C), 162.0, (C), 161.2 (C), 151.1 (C), 148.22 (C), 128.8 (CH), 124.8 (CH), 84.0 (C), 77.3 (CH), 61.7 (CH_2), 14.4 (Me).

2–[2–(Diethoxymethyl)thiazol–4–yl]thiazole–4–carboxylic acid (197)

LiOH monohydrate (939 mg, 22.8 mmol) was added to a stirred solution of bisthiazole **161** (1.28 g, 3.73 mmol) in MeOH–H_2O (5:1) (60 ml) and the solution was stirred for 18 h. After evaporating *in vacuo*, the mixture was partitioned between $KHSO_4$ (2M; 150 ml) and EtOAc (100 ml). The aqueous layer was further extracted with EtOAc (2 x 80 ml) and the organic extracts were combined, dried (Na_2SO_4), azeotroped MeOH (3 x 40 ml) and evaporated *in vacuo* to give the *title compound* as a colourless solid (966 mg, 85%), mp > 315 °C (MeOH) (Found: MH^+ 315.0466. $C_{12}H_{15}N_2O_4S_2$ requires MH^+ 315.0468); IR (KBr)/cm^{-1} ν_{max} 3388, 3111, 2975, 2931,

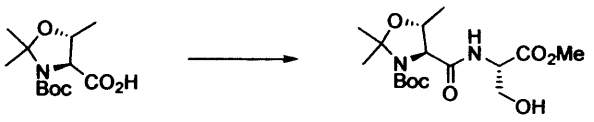
2888, 1585, 1514, 1442, 1382, 1322, 1303, 1272, 1178, 1102, 1062, 958, 892, 864, 846, 807, 783, 714; ^{1}H NMR (400 MHz; $SO(CD_3)_2$) δ 8.54 (1H, s, 5–H), 7.98 (1H, s, 5'–H), 5.85 (1H, s, CH), 3.75 (2H, dq, *J* 9.5, 7.0, 2OC*H*HMe), 3.73 (2H, dq, *J* 9.5, 7.0, 2OCH*H*Me), 1.25 (6H, app t, *J* 7.0, $2CH_2$*Me*); ^{13}C NMR (100 MHz; $SO(CD_3)_2$) δ 170.4 (C), 164.4 (C), 160.8 (C), 157.7 (C), 148.8 (C), 122.9 (CH), 118.8 (CH), 98.3 (CH), 62.3 (CH_2), 15.5 (Me); *m/z* (ES) 315 (MH^+, 100 %).

(2*R*,2'*S*,3'*R*)–1–(2–{2–[2–(Diethoxymethyl)thiazol–4–yl]thiazol–4–yl}carbonylamino)–3–[(hydroxybutanoyl)amino]prop–2–yl acetate (198)



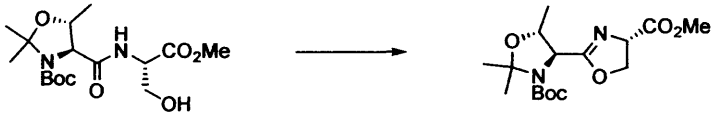
Et_3N (1.48 ml, 10.65 mmol) was added dropwise over 30 min to a stirred solution of bisthiazole **197** (800 mg, 2.54 mmol), TFA.H–L–Thr–aminopropanacetate (3.0 mmol) and pyBOP (1.73 g, 3.32 mmol) in dry CH_2Cl_2 (25 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 4 h. Evaporated *in vacuo*, purification by column chromatography on SiO_2, eluting with MeOH–CH_2Cl_2 (5% v/v) (R_f 0.21) and tritration (Et_2O), gave the *title compound* as a colourless solid (88 mg, 7%), mp 150–151 °C (Et_2O) (Found: MH^+ 515.1627. $C_{21}H_{31}N_4O_7S_2$ requires *MH*$^+$ 515.1629); IR (KBr)/cm^{-1} ν_{max} 3308, 2977, 2925, 1734, 1653, 1544, 1474, 1377, 1326, 1243, 1172, 1132, 1102, 1070, 1017, 842; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.35 (1H, d, *J* 8.0, 2'–NH), 8.28 (1H, s, 5'''–H), 8.25 (1H, s, 5''–H), 7.18 (1H, app t, *J* 6.0, 3–NH), 5.88 (1H, s, CH), 5.17 (1H, m, 2–H), 4.68 (1H, dq, *J* 6.5, 1.5, 3'–H), 4.62 (1H, dd, *J* 8.0, 1.5, 2'–H), 3.91 (2H, dq, *J* 9.5, 7.0, 2OC*H*HMe), 3.89 (2H, dq, *J* 9.5, 7.0, 2OCH*H*Me), 3.65 (1H, ddd, *J* 14.1, 6.0, 9.0, 3–*H*H), 3.46 (1H, ddd, *J* 14.1, 6.5, 7.0, 3–H*H*), 2.18 (3H, s, Me), 1.44 (6H, app t, *J* 7.0, $2CH_2$*Me*), 1.39 (3H, d, *J* 6.5, 3'''–Me), 1.36 (3H, d, *J* 6.0, 2–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.5 (C), 170.9 (C), 170.2 (C), 163.0, (C), 162.2 (C), 149.6 (C), 148.4 (C), 124.4 (CH), 118.4 (CH), 98.5 (CH), 69.5 (CH), 66.3 (CH), 62.4 (CH_2), 56.7 (CH), 43.7 (CH_2), 21.2 (Me), 18.3 (Me), 17.6 (Me), 15.1 (Me); *m/z* (ES) 553 ($M+K^+$, 45%), 537 ($M+Na^+$, 100), 515 (MH^+, 35).

(2*S*,4'*S*,5'*R*)–Methyl 2–{[2,2,5–Trimethyl–3–(*tert*–butoxycarbonyl)oxazolidin–4–yl]carbonylamino}–3–hydroxypropanoate (199)



Ethyl chloroformate (7.9 ml, 80.4 mmol) was added to a solution of oxazolidine **183** (17.35 g, 67.0 mmol) and Et_3N (28.0 ml, 0.2 mol) in THF (150 ml) and stirred at 0 °C for 1 h. HCl.H–Ser–OMe (12.47 g, 80.4 mmol) was added to the reaction and stirred at 0 °C for a further 2.5h. The solution was evaporated *in vacuo* and partitioned between aqueous $KHSO_4$ solution (10% w/v) (200 ml) and EtOAc (200 ml), the aqueous layer was further extracted with EtOAc (2 x 150 ml). The combined organic extracts were washed sequentially with $NaHCO_3$ (150 ml) and H_2O (150 ml), dried (Na_2SO_4), filtered and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.17), gave the *title compound* as a clear oil (22.95 g, 94 %) (Found: MH^+, 361.1969. $C_{16}H_{29}N_2O_7$ requires MH^+, 361.1968); $[\alpha]_D^{26}$ –12.2 (*c*2.15, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3432, 3337, 298, 2360, 1748, 1673, 1538, 1403, 1369, 1258, 1212, 1175, 1135, 1081, 942, 859, 757; ^1H NMR (400 MHz; $CDCl_3$) δ 6.85 (1H, d, *J* 6.0, NH) 4.55 (1H, m, 2–H), 4.23 (1H, m, 5'–H), 4.11 (1H, m, 3–*H*H), 3.80 (1H, m, 3–H*H*), 3.73 (3H, s, OMe), 3.71 (1H, m, 4'–H), 3.44 (1H, bs, OH), 1.53 (6H, s, 2'–2Me), 1.38 (9H, s, CMe_3), 1.37 (3H, bs, 5'–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171 (C), 169 (C), 152 (C), 94.7 (C), 81.6 (C), 73.6 (CH), 67.7 (CH), 61.7 (CH_2), 55.5 (CH), 52.7 (Me), 28.3 (Me), 24.7 (Me), 19.0 (Me), 18.0 (Me); *m/z* (APcI) 361 (MH^+, 100%), 261 (60), 58 (65).

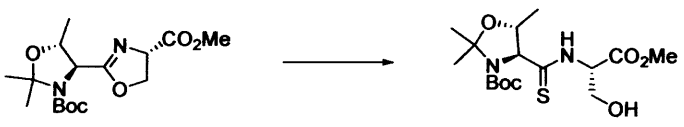
(4*S*,4'*S*,5'*R*)–2–[2,2,5–Trimethyl–3–(*tert*–butoxycarbonyl)oxazolidin–4–yl]–4,5–dihydro–4–methoxycarbonyloxazoline (200)



DAST (0.38 ml, 3.06 mmol) was added dropwise to a stirred solution of oxazoldine **199** (0.92 g, 2.55 mmol) in dry CH_2Cl_2 (45 ml) at –78 °C. The solution was stirred for 3 h and then quenched by the addition of $KHCO_3$ 423 mg, 3.06 mmol). After warming to room temperature, the mixture was extracted with saturated aqueous $NaHCO_3$ (30 ml) and CH_2Cl_2 (3 x 35 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo*, purification by column

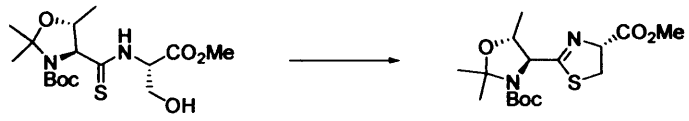
chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.19), gave the *title compound* as a clear oil (0.76 g, 89%) (Found: MH^+, 343.1863. $C_{16}H_{27}N_2O_6$ requires MH^+, 343.1864); $[\alpha]_D^{27}$ +36.52 (*c*1.15, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3387, 2980, 2925, 1745, 1711, 1664, 1519, 1474, 1438, 1389, 1263,1209, 1133, 1073, 980, 939, 921, 897, 776, 756; ^1H NMR (400 MHz; $CDCl_3$) δ 4.76 (1H, dd, *J* 10.4, 9.0, 4–H), 4.55 (1H, dd, *J* 9.0, 9.0, 5–C*H*H), (1H, dd, *J* 10.4, 9.0, 5–CH*H*), 4.06 (1H, m, 4'–H), 4.04 (1H, m, 5'–H), 3.73 (3H, s, OMe), 1.61 (3H, s, 2'–Me), 1.52 (3H, s, 2'–Me), 1.33 (12H, bs, CMe_3 and 5'–Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.0 (C), 168.0 (C), 151.1 (C), 95.2 (C), 80.4 (C), 74.0 (CH), 69.5 (CH_2), 68.3 (CH), 61.4 (CH), 52.7 (Me), 28.2 (Me), 26.5 (Me), 24.0 (Me), 18.5 (Me); *m/z* (APcI) 343 (MH^+, 100%), 287 (75), 261 (55).

(2*S*,4'*S*,5'*R*)–Methyl 2–{[2,2,5–trimethyl–3–(*tert*–butoxycarbonyl)oxazolidin–4–yl]thiocarbonylamino}–3–hydroxypropanoate (205)



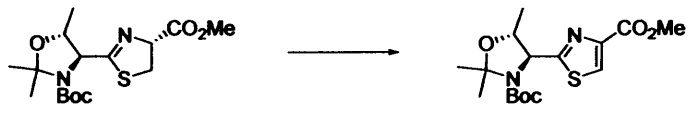
Oxazoline **200** (22.94 g, 67.0 mmol) was dissolved in MeOH–Et_3N (2:1) (150 ml), hydrogen sulphide gas was bubbled through the solution whilst stirring at RT for 5 h. The solution was evaporated *in vacuo*, purification by column chromatography on SiO_2, eluting with light petroleum–Et_2O (4:1) (R_f 0.14), gave the *title compound* as a brown oil (11.43 g, 45%) (Found: MH^+, 377.1741. $C_{16}H_{29}N_2O_6S$ requires MH^+, 377.1741); $[\alpha]_D^{28}$ +24.60 (*c*1.00, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3468, 3354, 2980, 2925, 2361, 1746, 1711, 1518, 1438, 1378, 1263, 1207, 1173, 1133, 1092, 982, 940, 923, 897, 858, 758; ^1H NMR (400 MHz; $CDCl_3$) δ 8.45 (1H, d, *J* 6.5, NH), 4.72 (1H, dd, *J* 10.5, 8.5, 2–H), 4.55 (1H, dd, *J* 9.0, 8.5, 3–*H*H), 4.44 (1H, dd, *J* 10.5, 9.0, 3–H*H*), 4.07 (2H, 4'–H and 5'–H), 3.76 (1.3H, s, OMe), 3.73 (1.7H, s, OMe), 1.60 (1.3H, s, 2'–Me), 1.58 (1.7H, s, 2'–Me), 1.55 (1.3, s, 2'–Me), 1.50 (1.7H, s, 2'–Me), 1.40 (0.36H, s, 5'–Me), 1.36 (3.9H, s, CMe_3), 1.33 (0.64H, s, 5'–Me),1.31 (5.1H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.9 (C), 168.0, (C), 151.1 (C), 95.2 (C), 80.4 (C), 74.0 (CH), 69.5 (CH_2), 68.3 (CH), 61.5 (CH), 53.0 (Me), 52.7 (Me), 28.3 (Me), 28.2 (Me), 26.5 (Me), 24.0 (Me), 18.5 (Me); *m/z* (APcI) 377 (MH^+, 48%), 343 (100).

(4*S*,4'*S*,5'*R*)-2-[2,2,5-Trimethyl-3-(*tert*-butoxycarbonyl)oxazolidin-4-yl]-4,5-dihydro-4-methoxycarbonylthiazole (206)



DAST (0.28 ml, 2.29 mmol) was added dropwise to a stirred solution of oxazolidine **205** (661 mg, 1.76 mmol) in dry CH_2Cl_2 (20 ml) at –78 °C. The solution was stirred for 3 h and then quenched by the addition of $KHCO_3$ 243 mg, 1.76 mmol). After warming to room temperature, the mixture was extracted with aqueous saturated $NaHCO_3$ (20 ml) and CH_2Cl_2 (3 x 30 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo*, purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.53), gave the *title compound* as a brown oil (628 mg, 100%) (Found: MH^+, 359.1636. $C_{16}H_{27}N_2O_5S$ requires MH^+, 359.1635); $[\alpha]_D^{26}$ +10.00 (*c*1.60, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3351, 2980, 2936, 2359, 1699, 1623, 1520, 1478, 1438, 1376, 1310, 1263, 1212, 1172, 1133, 1089, 984, 938, 898, 756; ^1H NMR (400 MHz; $CDCl_3$) δ 4.72 (1H, dd, *J* 10.0, 9.5, 4–H), 4.55 (1H, dd, *J* 9.5, 9.0, 5–*H*H), 4.43 (1H, dd, *J* 10.0, 9.0, 5–H*H*), 4.07 (2H, 4'–H and 5'–H), 3.73 (3H, s, OMe), 1.58 (3H, s, 2'–Me), 1.50 (3H, s, 2'–Me), 1.33 (3H, s, 5'–Me), 1.31, (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.3 (C), 168.0 (C), 151.4 (C), 95.1 (C), 80.4 (C), 74.0 (CH), 69.9 (CH_2), 68.1 (CH), 61.4 (CH), 52.7 (Me), 28.2 (Me), 24.0 (Me), 18.2 (Me), 15.1 (Me); *m/z* (APcI) 359 (MH^+, 100%), 303 (90), 259 (57), 215 (96).

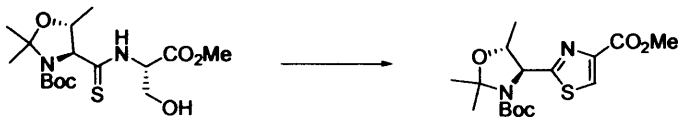
(4*S*,5*R*)-4-(4-Methoxycarbonylthiazol-2-yl)-2,2,5-trimethyl-3-(*tert*-butoxycarbonyl) oxazolidine (207)



$BrCCl_3$ (0.40 ml, 4.06 mmol) and DBU (0.6 ml, 4.01 mmol) were added dropwise to a solution of oxazolidine **206** (300 mg, 0.84 mmol) in dry CH_2Cl_2 (20 ml) at –20 °C and was stirred for 18 h. The mixture was poured into saturated aqueous $NaHCO_3$ solution (25 ml) and extracted with $CHCl_3$ (3 x 40 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.60), gave the *title compound* as a colourless solid (184 mg, 62%) mp 123–124 °C (EtOAc) (Found: MH^+, 357.1475. $C_{16}H_{25}N_2O_5S$ requires MH^+, 357.1479) $[\alpha]_D^{31}$ –67.60 (*c*0.55, $CHCl_3$); IR

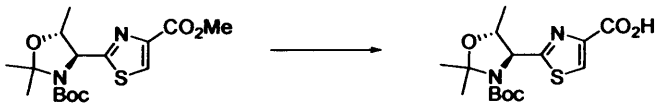
(KBr)/cm^{-1} ν_{max} 3098, 2890, 2943, 2911, 1708, 1479, 1457, 1436, 1366, 1301, 1258, 1219, 1153, 1134, 1100, 989, 931, 856, 840, 782, 754, 737; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.08 (1H, s, 5'–H), 4.72 (1H, d, *J* 7.5, 4–H), 4.12 (1H, dq, *J* 7.5, 6.0, 5–H), 3.86 (3H, s, OMe), 1.62 (6H, s, 2–2Me), 1.35 (3H, d, *J* 6.0, 5–Me), 1.19 (9H, bs, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 173.7 (C), 161.7 (C), 151.3 (C), 146.3 (C), 127.6 (CH), 95.3 (C), 80.8 (C), 77.9 (CH), 66.0 (CH), 52.5 (Me), 28.1 (Me), 26.5 (Me), 25.9 (Me), 17.8 (Me); *m/z* (APcI) 357 (MH^+, 55%), 102 (100).

(4*S*,5*R*)–4–(4–Methoxycarbonylthiazol–2–yl)–2,2,5–trimethyl–3–(*tert*–butoxycarbonyl) oxazolidine (207)



DAST (0.28 ml, 2.28 mmol) was added dropwise to a stirred solution of oxazolidine **205** (661 mg, 1.76 mmol) in dry CH_2Cl_2 (20 ml) at –78 °C. The solution was stirred for 3 h. $BrCCl_3$ (0.80 ml, 8.13 mmol) and DBU (1.2 ml, 8.02 mmol) were added and the solution was stirred at –20 °C for 18 h. The mixture was poured into saturated aqueous $NaHCO_3$ solution (40 ml) and extracted with $CHCl_3$ (3 x 60 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.60), gave the *title compound* as a colourless solid (626 mg, 60%) with identical physical and spectroscopic properties.

(4'*S*,5'*R*)–2–[2,2,5–Trimethyl–3–(*tert*–butoxycarbonyl)oxazolidin–4–yl]thiazole–4–carboxylic acid (208)



LiOH monohydrate (1.53 g, 36.5 mmol) was added to a stirred solution of oxazolidine **207** (2.17 g, 6.08 mmol) in MeOH–H_2O (5:1) (200 ml) and the solution was stirred overnight. After evaporating *in vacuo*, the mixture was partitioned between aqueous HCl (3M; 70 ml) and EtOAc (100 ml). The aqueous layer was further extracted with EtOAc (2 x 100 ml) and the organic extracts were combined, washed with brine (60 ml), dried (Na_2SO_4) and evaporated *in vacuo*, gave the *title compound* as a colourless solid (2.08 g, 100%) mp 159–160 °C (EtOAc) (Found:

MH^+, 343.1321. $C_{15}H_{23}N_2O_5S$ requires MH^+, 343.1322) $[\alpha]_D^{22}$ –58.17 (*c*1.75, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3106, 2975, 2925, 2865, 2725, 2634, 2544, 1715, 1687, 1509, 1489, 1458, 1375, 1300, 1265, 1226, 1163, 1135, 1111, 1092, 1066, 1050, 985, 946, 859, 844, 775, 752, 698; ^1H NMR (400 MHz; $CDCl_3$) δ 8.68 (1H, bs, OH), 8.18 (1H, s, 5–H), 4.75 (1H, d, *J* 7.0, 4'–H), 4.12 (1H, m, 5'–H), 1.58 (6H, s, 2'–2Me), 1.34 (3H, d, *J* 6.0, 5'–Me), 1.20 (9H, bs, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 174.0 (C), 164.7 (C), 151.3 (C), 145.7 (C), 129.0 (CH), 95.4 (C), 81.0 (C), 77.8 (CH), 65.9 (CH), 28.7 (Me), 26.5 (Me), 25.9 (Me), 17.8 (Me); *m/z* (ES) 341 ([M–H$^-$], 100%), 297 (50).

(4''*S*,5''*R*)–Ethyl 3–{2–[2,2,5–trimethyl–3–(*tert*–butyloxycarbonyl)oxazolidin–4–yl]thiazol–4–yl}–3–oxopropanoate (210)

Method A:

i.) Methyl magnesium bromide in THF (3M; 0.33 ml, 1.0 mmol) was added to a stirred suspension of potassium ethyl malonate (170 mg, 1.0 mmol), in dry THF (3 ml) at 0 °C. The mixture was stirred at 0 °C for 30 min then rapidly warmed to RT and stirred for a further 1.5 h.

ii.) Ethyl chloroformate (0.05 ml, 0.55 mmol) was added dropwise to a stirred solution of oxazolidine **208** (176 mg, 0.51 mmol), Et_3N (0.1 ml, 0.72 mmol) in dry THF (3 ml) at 0 °C and stirred for 20 min. The solution from i.) was added and stirred for 3.5 h, the reaction was quenched with saturated aqueous NH_4Cl (15 ml) and evaporated *in vacuo*. The residue was partitioned between H_2O (20 ml) and $CHCl_3$ (30 ml), the aqueous layer was further extracted with $CHCl_3$ (2 x 30 ml) and the combined organic extracts were washed sequentially with acetic acid (5% w/v; 20 ml), saturated aqueous $NaHCO_3$ (25 ml) and brine (20 ml), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.70), gave the title compound as a brown oil (128 mg, 58%) (Found: MH^+, 413.1737. $C_{19}H_{29}N_2O_6S$ requires MH^+, 413.1741) $[\alpha]_D^{24}$ –31.0 (*c*2.5, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3115, 2979, 2935, 1740, 1704, 1631, 1479, 1366, 1258, 1219, 1136, 1091, 1035, 984, 936, 857, 756, 666; ^1H NMR (400 MHz; $CDCl_3$) δ 12.0 (0.4H, s, OH), 8.10 (0.6H, s, 5'–H), 7.70 (0.4H, s, 5'–H), 5.95 (0.4H, s, 2–H), 4.7 (0.4H, bs, 4''–H), 4.6 (1H, m 5''–H), 4.2 (0.8H, q, *J* 7, OCH_2Me), 4.1 (1.2H, q, *J* 7, OCH_2Me), 4.1 (0.6H, bs, 4''–H), 4.05 (0.6H, d, *J* 16, 2–*H*H), 3.95 (0.6H, d, *J* 16, 2–H*H*), 1.7–1.0 (18H, 2'',2'',5''–Me and CMe_3), 1.3 (1.2H, t, *J* 7, CH_2Me), 1.2

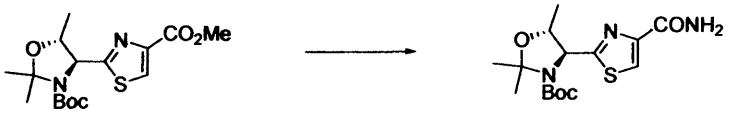
(1.8H, t, *J* 7, CH_2*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 187.5 (C), 173.4 (C), 172.4 (C), 171.2 (C), 167.5 (C), 166.6 (C), 164.8 (C), 153.4 (C), 152.3 (C), 151.3 (C), 149.6 (C), 125.9 (CH), 120.1 (CH), 95.3 (C), 95.2 (C), 94.8 (C), 89.5 (CH), 81.2 (C), 80.7 (C), 80.5 (C), 65.9 (CH), 65.7 (CH), 62.1 (CH_2), 61.6 (CH_2), 61.5 (CH_2), 61.2 (CH_2), 60.4 (CH_2), 49.0 (C), 46.9 (CH_2), 41.7 (CH_2), 29.1 (C), 28.1 (C), 26.5 (Me), 26.2 (Me), 26.1 (Me), 25.4 (Me), 17.6 (Me), 14.4 (Me), 14.3 (Me), 14.1 (Me), 14.0 (Me), 14.0 (Me); *m/z* (ES) 435 ($M+Na^+$, 37%), 313 (100).

Method B:

i.) n–BuLi in hexanes (2.5 M; 4.0 ml, 10.0 mmol) was added dropwise to a stirred solution of diisopropylamine (1.40 ml, 10.0 mmol) in dry THF (10 ml) at 0 °C and stirred for 10 min. The solution was cooled to –78 °C and dry EtOAc (0.49 ml, 5.01 mmol) was added dropwise and stirred for 30 min.

ii.) Ethyl chloroformate (0.52 ml, 5.5 mmol) was added dropwise to a stirred solution of oxazolidine **208** (1.70 g, 4.96 mmol), Et_3N (0.76 ml, 5.46 mmol) in dry THF (10 ml) at 0 °C and stirred for 1.5 h. The mixture was filtered and added dropwise to i.), stirred at –78 °C for 1h, warmed to RT whilst stirring for a further 2 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (60 ml) and evaporated *in vacuo*. The residue was partition between H_2O (60 ml) and EtOAc (120 ml), the aqueous layer was further extracted with EtOAc (2 x 40 ml) and the combined organic layers were washed with brine (60 ml), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–Et_2O (1:1) (R_f 0.56), gave the title compound as a brown oil (333 mg, 16%), with the same spectroscopic properties.

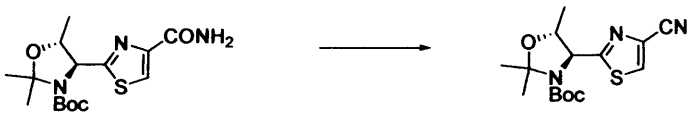
(4'*S*,5'*R*)–2–(2,2,5–trimethyl–3–*tert*–butoxycarbonyloxazolidin–4–yl)thiazole–4–carboxamide (212)



A saturated solution of methanolic ammonia (20 ml) was added to ester **207** (160 mg, 0.45 mmol) and stirred at room temperature for 18 h. The mixture was evaporated *in vacuo*, purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.21), gave the *title compound* as a colourless solid (153 mg, 100%), mp 55–56 °C (EtOAc) (Found: MH^+ 342.1485. $C_{15}H_{24}N_3O_4S$ requires MH^+, 342.1482); $[\alpha]_D^{24}$ –49.0 (*c*1.02, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3465, 2979, 2925, 1701, 1591, 1458, 1367, 1308, 1261, 1173, 1135, 1089, 984, 946, 857,

757; ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.06 (1H, s, 5–H), 7.08 (1H, bs, NH*H*) 6.39 (0.6H, bs, N*H*H), 6.26 (0.4H, bs, N*H*H), 4.65 (0.4H, bs, 4'–H), 4.57 (0.6H, d, *J* 7.3, 4'–H), 4.13 (1H, m, 5'–H), 1.62 (6H, s, 2'–2Me), 1.36 (3H, d, *J* 5.0, 5'–Me), 1.12 (9H, s, CMe_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 172.1 (C), 171.2 (C), 163.1 (C), 149.1 (C), 124.0 (CH), 95.3 (C), 81.3 (C), 77.6 (CH), 65.8 (CH), 28.3 (Me), 26.5 (Me), 25.5 (Me), 21.1 (Me) ; *m/z* (ES) 364 ($M+Na^+$, 36%), 242 (100).

(4'*S*,5'*R*)–2–(2,2,5–trimethyl–3–*tert*–butoxycarbonyloxazolidin–4–yl)thiazole–4–carbonitrile (213)



Phosphorous oxychloride (0.1 ml, 1.07 mmol) was added dropwise to a cold (0 °C) solution of thiazole **212** (153 mg, 0.45 mmol) in pyridine (8 ml). The solution was warmed to RT and stirred for 2 h and evaporated *in vacuo*. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (1:1) (R_f 0.66), gave the title compound as a brown oil (112 mg, 77%) (Found: MH^+, 324.1376. $C_{15}H_{22}N_3O_3S$ requires MH^+, 324.1376); $[\alpha]_D^{23}$ –66.38 (*c*0.93, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3020, 1703, 1369, 1215, 1135, 932, 760, 668; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.95 (1H, bs, 5–H), 4.72 (0.5H, m, 4'–H), 4.65 (0.5H, m, 4'–H), 4.13 (0.5H, m, 5'–H), 4.10 (0.5H, m, 5'–H), 1.65 (6H, s, 2'–2Me), 1.40 (3H, d, *J* 6.0, 5'–Me), 1.96 (9H, s, CMe_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 174.5 (C),173.1 (C), 152.3 (C), 151.1 (C), 130.3 (CH), 126.3 (C), 113.8 (C), 95.5 (C), 95.0 (C), 81.5 (C), 81.0 (C), 77.4 (CH), 76.7 (CH), 65.8 (CH), 65.4 CH), 28.1 (Me), 26.5 (Me), 26.2 (Me), 25.8 (Me), 17.9 (Me), 17.7 (Me); *m/z* (APcI) 324 ([M–H^+], 3%).

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