Stereoselective Pyrrolidine Synthesis and Approaches to Cyclic Tetrapeptides

A dissertation submitted for the degree of

Doctor of Philosophy

By

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For My Family

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Abstract

This thesis describes the synthesis of a family of amino acids containing a ketone functionalised side-chain (I, II, III), which are analogues of one of the amino acids found in the cyclic tetrapeptide apicidin (IV). Cross metathesis of the alkenes (V, VI, VII) with a range of unsaturated ketones, followed by hydrogenation, gave the target amino acids in excellent yields. The required alkenes were prepared in moderate to good yield by the copper-catalysed allylation of the homologous organozinc reagents (VIII, IX, X) with allyl chloride.

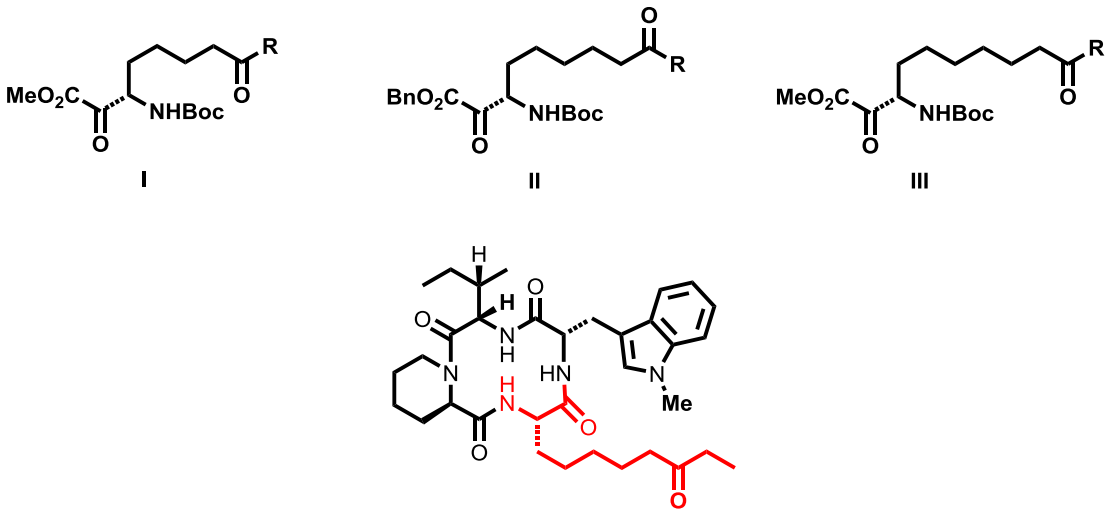
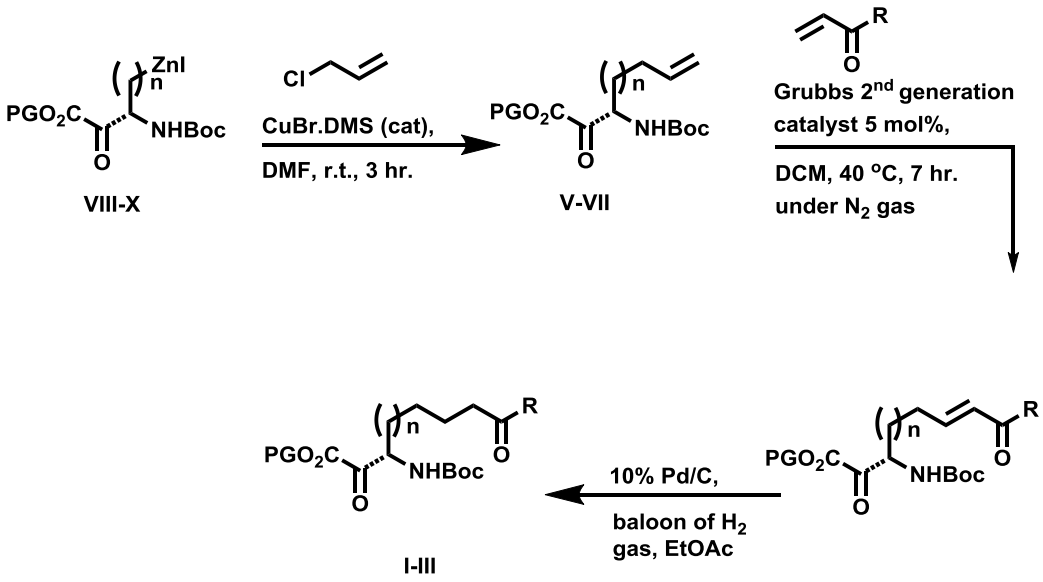
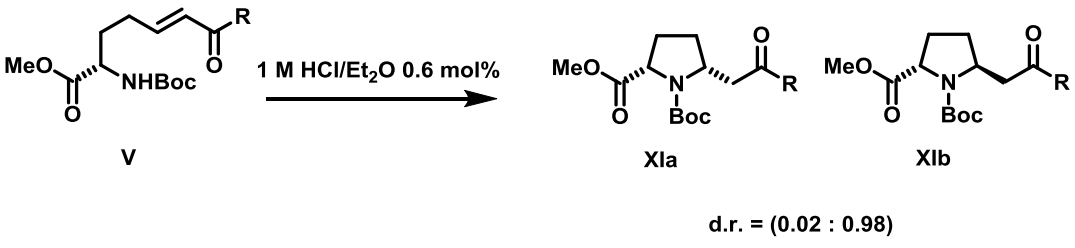


Figure for structures I–IV



Scheme for overall synthesis from zinc reagent to ketone

In initial cross-metathesis experiments using the alkene (V), a highly diastereoselective cyclisation to give the *trans*-pyrrolidines (XIb) was observed. After careful study, it was established that the cyclisation occurred on standing in $CDCl_3$, and that it could also be achieved in a more controlled way by using catalytic amounts of dry HCl in ether.



Scheme for a highly diastereoselective intramolecular aza-Michael cyclisation

The alkene (VI) was incorporated into the linear tetrapeptide (XII), which was then cyclised to give the cyclic tetrapeptide (XIII) along with the cyclic octapeptide (XIV). Cross metathesis of the cyclic tetrapeptide (XIII) with methyl vinyl ketone and propyl vinyl ketone, followed in each case by hydrogenation, gave the two modified cyclic peptides (XV) and (XVI) in good yield, demonstrating the feasibility of the cross-metathesis/hydrogenation strategy for the preparation of analogues of apicidin.

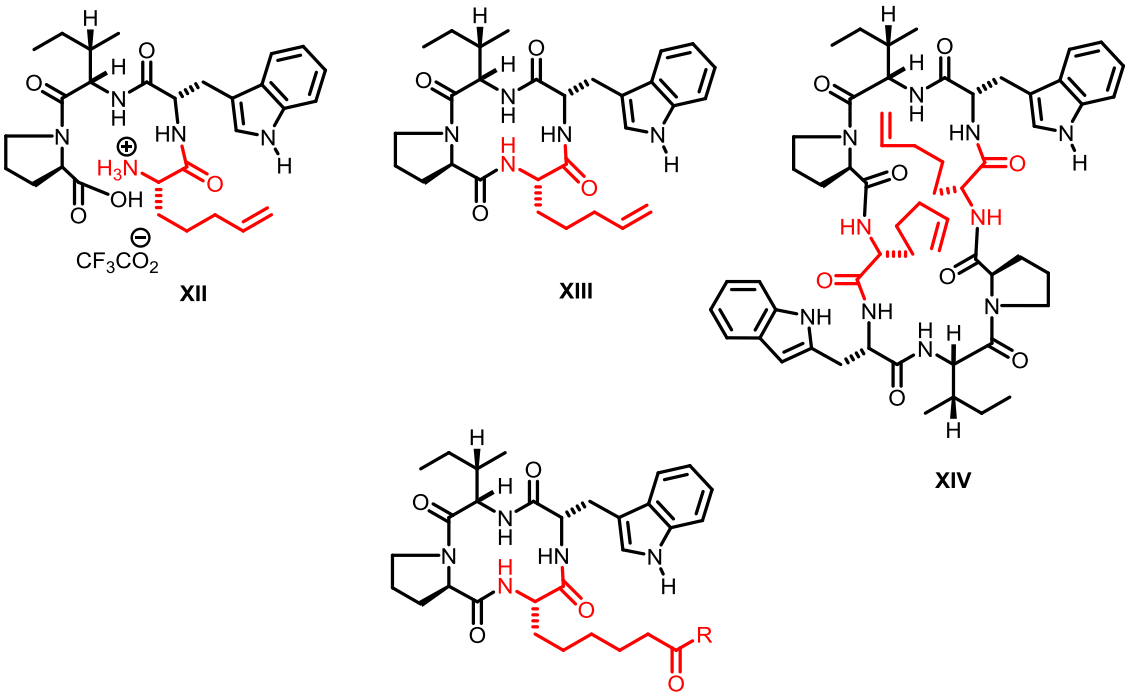


Figure for structures XII-XVI

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I would like to thank my supervisor Professor Richard Jackson for his support and guidance while conducting this project.

I also would like to say a big thank you to the members of the Jackson group, past and present. They are: Ghaith Ghaith, Mohamed Zreigh, Mansour Abdelsalam, Vicki Lovett, Phil Reeve, Akram Qaddo, Liam Marshall and Mehul Makwana; special thanks go to Matt Lilley and Martyn Chilton. I would also like to thank all the masters students, visiting academics and summer students, for making the group a good and enjoyable environment in which to work. Thanks to technical staff, without whom it would not have been possible to complete the thesis.

I would like to thank the Kurdistan regional government for the opportunity for the scholarship and generous support.

Abbreviations

$[\alpha]^{23}_{D}$	optical rotation
δ	chemical shift
)))	sonication
Ac	acetyl
AIBN	azobisisobutyronitrile
br.	broad
Bn	benzyl
$(Boc)_2O$	di-tert-butyl dicarbonate
Boc	*tert*-Butoxycarbonyl
BOP	(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluoro phosphate
Cat.	catalysis
2-ClTrt-Cl	2-chlorotrityl chloride
CM	cross-metathesis
CMP	cross-metathesis product
COP	cyclic octapeptide
CTP	cyclic tetrapeptide
Cy	cyclohexyl
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
d.r.	diastereomeric ratio
dt	doublet of triplets
DBU	1, 8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N'*-Dicyclohexylcarbodiimide

DCM	Dichloromethane
DDQ	2,3-Dichloro-5, 6-dicyano-1, 4-benzoquinone
DIBAL	Diisobutylaluminium hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N*, *N*-dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDAC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
eq.	equivalents
FGI	functional group interconversion
Fmoc	9-fluorenylmethyloxy carbonyl
GC	gas chromatography
hr.	hour
HAT	Histone acetyltransferases
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate
HBTU	*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluoro phosphate
HDAC	Histone deacetylases
HIV	Human immunodeficiency virus
HOBt	1-Hydroxybenzotriazole
HPLC	high performance liquid chromatography
hv	Photolysis
IR	infrared
J	coupling constant

Lit.	Literature
LiHMDS	Lithium bis(trimethylsilyl)amide
m	multiplet
m.p.	melting point
m/z	mass/charge ratio
NBS	*N*-Bromosuccinimide
NHC	*N*-heterocyclic carbenes
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
Nu	nucleophile
o/n	overnight
PG	protecting group
q	quartet
R_f	retention factor
r.t.	room temperature
s	singlet
SM	starting material
ST	substrate
TBAF	tetrabutylammonium fluoride
^{t}Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TFE	2,2,2-Trifluoroethanol
TIPS	Triisopropylsilane
TLC	thin layer chromatography
Ts	tosyl

UV	ultraviolet
Z	carboxybenzyl
Zn*	activated zinc

Chapter 1: Introduction

1.1 Background

Deoxyribonucleic acid (DNA) is a fundamental part of living organisms. Its function is the long term storage of genetic information. It is used in both transcription and translation to produce proteins and its structure is tightly bound in active chromosomes.[1-3] Transcription makes a copy of a single chain of double stranded helical DNA, or a section of it. In order to get transcription it is necessary to open the DNA double helix (Figure 1 fibre) so that the RNA polymerase can bind to a section of the DNA template (Figure 1 beads on a string structure). Remodeling of histone protein that package chromosomal DNA by acetylation is one method that can be used to change from fibre to beads on a string packing.[1,4-6]

The figure removed

Figure 1. Different stages of chromatin packing to give condensed chromosome[7]

Histone acetyltransferase (HAT) and histone deacetylase (HDAC) are enzymes that regulate the acetylation state of the histone core. HDAC has been found in bacteria, fungi, plants and animals and catalyses the deacetylation of N-acetylated lysine residues in histone proteins. The positively charged N-terminal tails of the lysine residues in the histones bind to negatively charged DNA. An increase in this interaction leads to cell division, and is controlled by lysine modification. Neutralization of the positive charge by HAT leads to decreased interactions between the histone and DNA, and it allows transcription factor to separate the DNA chain where the gene is

to be transcribed to make mRNA. Therefore, the acetylation of histones plays a vital role in the transcriptional response of the cell. A lack of balance between acetylation and deacetylation leads to abnormal behavior of the cells in morphology, cell cycle, differentiation, and carcinogenesis. For these reason HDAC has been considered as a target for cancer therapy.[8-15]

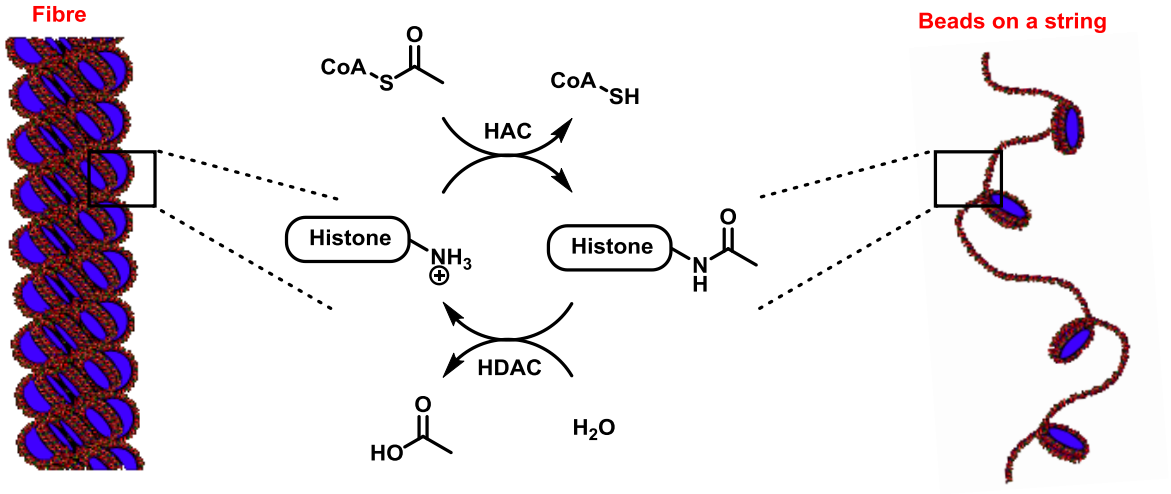
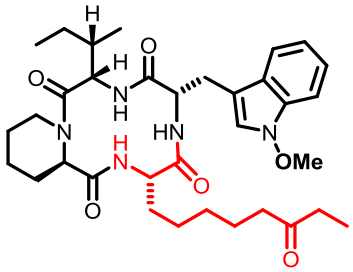


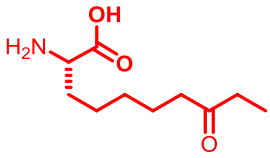
Figure 2. Acylation and deacetylation of lysine[7,8]

Inhibition of HDAC leads to a reduction in the concentration of the fibre structure (Figure 2) compared with the beads on a string structure (Figure 2). As a result RNA polymerase is free to bind and perform transcription because Zn^{2+} in the active site pocket of HDAC, binds to oxygen of N-acetylated histone lysine residues. Subsequent hydrolysis of the amide produces positively charged N-terminal tails.[6,7]

Cyclic tetrapeptides are natural products that have been shown to inhibit the activity of HDAC. This class of macrocyclic peptide consists of a large hydrophobic cap group with a long side-chain formed by incorporation of 2-amino-8-oxo-decanoic acid (Aoda), **2** or an analogue such as Aoe, **3**.[16-20] Apicidin **1**, a natural product isolated from endophytic fungi on twigs (Fusarium sp.) collected in Costa Rica[21,22] (Figure 3) is an example. Apicidin is structurally related to a family of α-epoxyketone cyclic tetrapeptides (**4-8**) which are known histone deacetylase inhibitors (HDACi) with pronounced anti neoplastic[23-30] and anti HIV activity.[31,32]

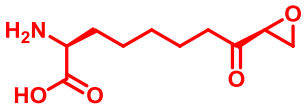


Apicidin, 1

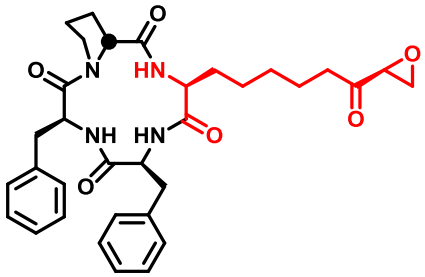


2-amino-8-oxo-decanoic acid (Aoda), 2

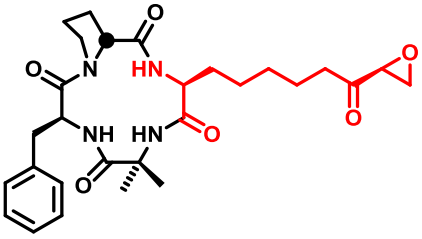
Figure 3. Cyclic tetrapeptides contain 2-amino-8-oxo-decanoic acid (Aoda)[10,21]



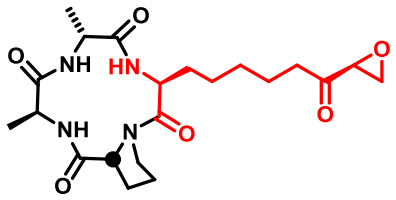
(2S, 9S)-2-amino-8-oxo-9, 10-epoxydecanoic acid (Aoe), 3



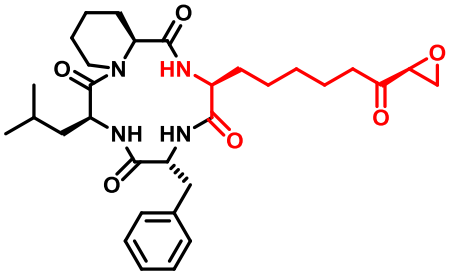
Trapoxin A, 4



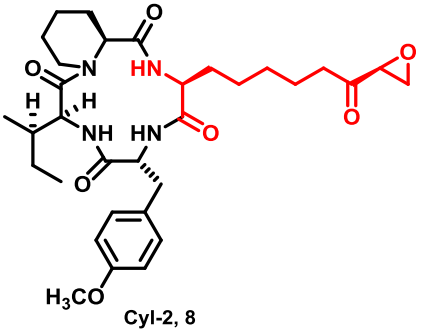
Chlamydocin, 5



HC toxin, 6



WF-3161, 7



Cyl-2, 8

Figure 4. Cyclic tetrapeptides containing (2S, 9S)-2-amino-8-oxo-9, 10-epoxydecanoic acid (Aoe)[8,33]

1.2 Biological activity

Bioactive cyclic peptides are synthetic targets for medicinal chemists. In general, due to enzymatic degradation linear peptides are not as stable as their cyclic equivalents in *vivo*. Stability is a crucial requirement for drug candidates in *vivo*, therefore the preparation small cyclic peptides is of great importance to medicinal chemists.[34,35] HDACi act to mimic access of HDAC to acetylated lysine residues. A suggested mechanism for this process is the binding of a zinc ion in the active site pocket of HDAC to the functional group in the side chain of the cyclic tetrapeptide. In the case of Aoda and Aoe, zinc binds to the ketone in the 8-position and in trichostatin TSA **9** to the hydroxamic acid (Figure 5).[6]

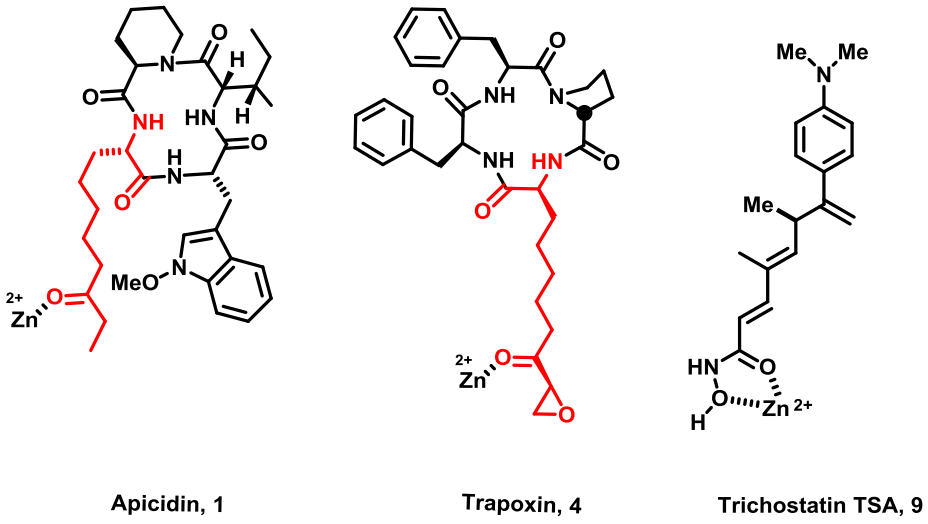


Figure 5. Proposed coordination to zinc in cyclic tetrapeptides and chelating in TSA, 9

Apicidin **1** induces both mammalian and parasite histone hyperacetylation. It has been shown to have broad biological action towards the apicomplexan family of protozoan parasites and prevents the growth of intracellular parasites that lead to diseases such as malaria and coccidisis.[21,36] Trapoxin A, **4** isolated from the fungus Helicoma ambiens, has been found to strongly affect mammalian cell growth and morphology[33] (Figure 4). Several groups have shown that removing the epoxide group by reduction or hydrolysis removes biological activity, suggesting trapoxin may bind irreversibly to its target.[29,33,37,38] Horinouchi and co-workers synthesized a novel, potent, analogue of trapoxin containing a hydroxamic acid instead of the

epoxyketone as the only modification. At low nanomolar concentration the hybrid compound was shown to act as a reversible HDAC inhibitor.[39] In contrast, when analogues of chlamydocin containing hydroxamic acid compounds **11**, **12** were tested they showed less potent HDAC inhibitory activity than reference compound **10** (Figure 6). The different inhibitory effects on HDAC of compounds **10-12** may be due to the presence of a cyclic amino acid residue in **10**, because it affects the orientation of the aromatic ring.[40]

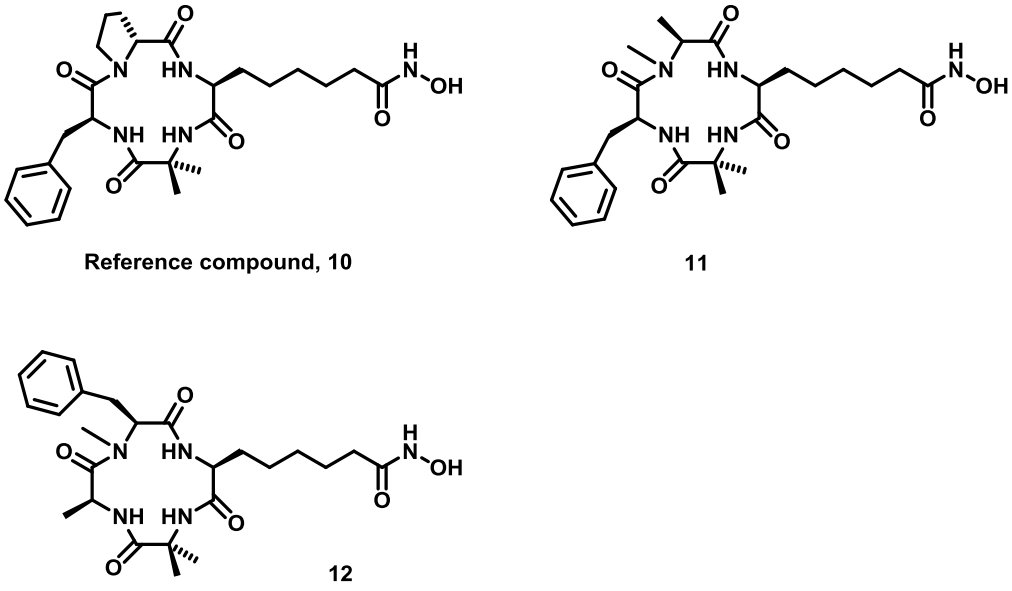


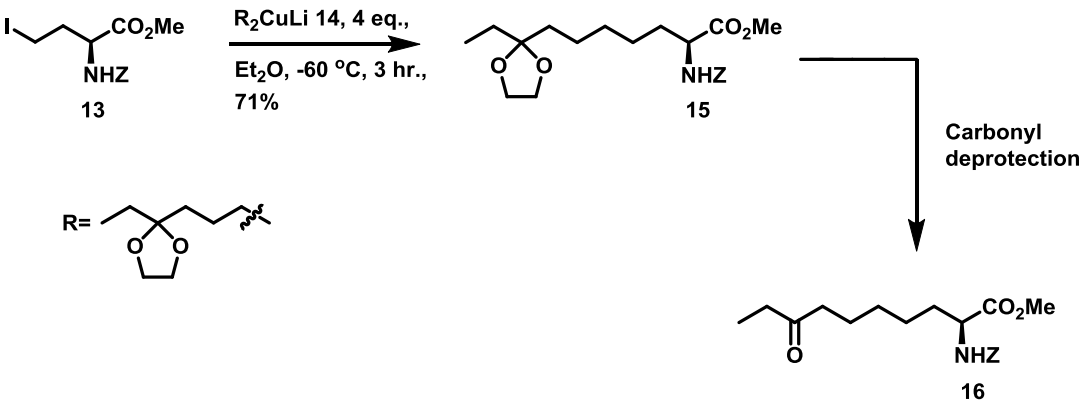
Figure 6. Chlamydocin containing hydroxamic acid

The varying cytotoxicity of cyclic tetrapeptide inhibitors can be attributed to a number of factors, including the conformation of the peptide backbone in solution, the overall polarity of the peptide, which in turn affects the ability of inhibitors to cross cellular and nuclear membranes, and the functionality of the amino acid residues which can coordinate to Zn^{2+}.[9]

Due to the limited supply of these biologically important compounds found in nature, they have attracted the interest of researchers. Therefore, the synthesis of several HDAC inhibitors with different chemical structures has been extensively studied.

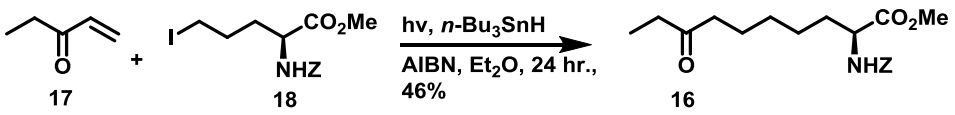
1.3 Different routes to synthesis 2-amino-8-oxo-decanoic acid 2

A number of routes towards Aoda have been reported. The first approach was reported in 1985 by Viallefont and co-workers[41] and involved reaction of the iodide **13**, derived from protected aspartic acid, with an organocuprate **14**. Subsequent deprotection of **15** gave the desired product **16** (Scheme 1).



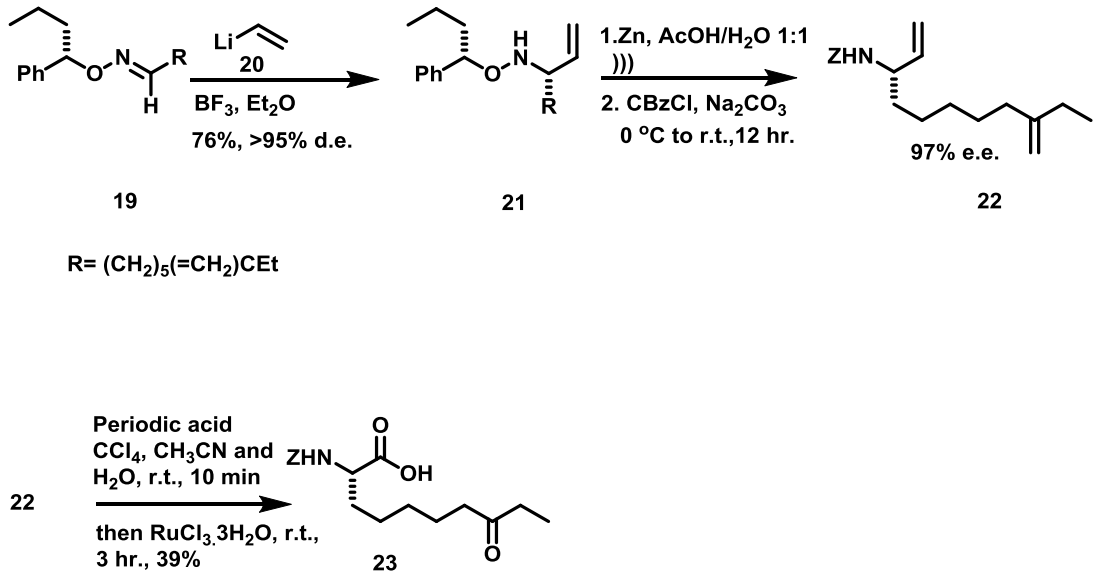
Scheme 1. Synthesis of Aoda

In 2001, Singh and Mou[42] reported the preparation of ketone **16** by the photochemically induced reaction of the iodide **18** with the unsaturated ketone **17** in the presence of tri-n-butyltin hydride and AIBN (Scheme 2).



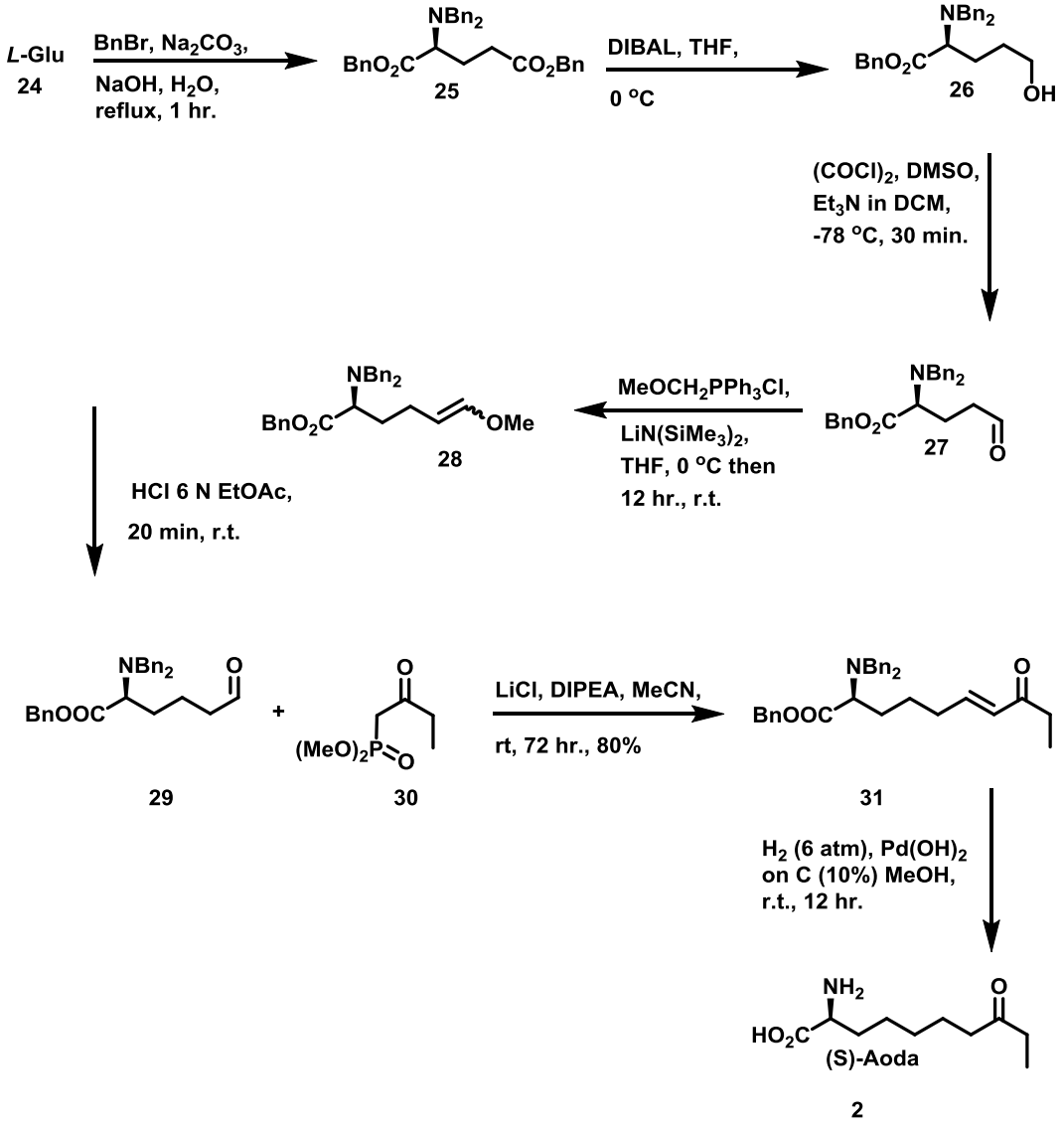
Scheme 2. Synthesis of Aoda by radical generation

In 2004 new methodology reported by Moody and co-workers[43] used a highly diastereoselective addition reaction between oxime **19** and vinyllithium **20** to give adduct **21**. The N-O bond was cleaved, followed by *N*-protection to give **22**. Oxidative cleavage of both alkenes gave the desired product **23** (Scheme 3).



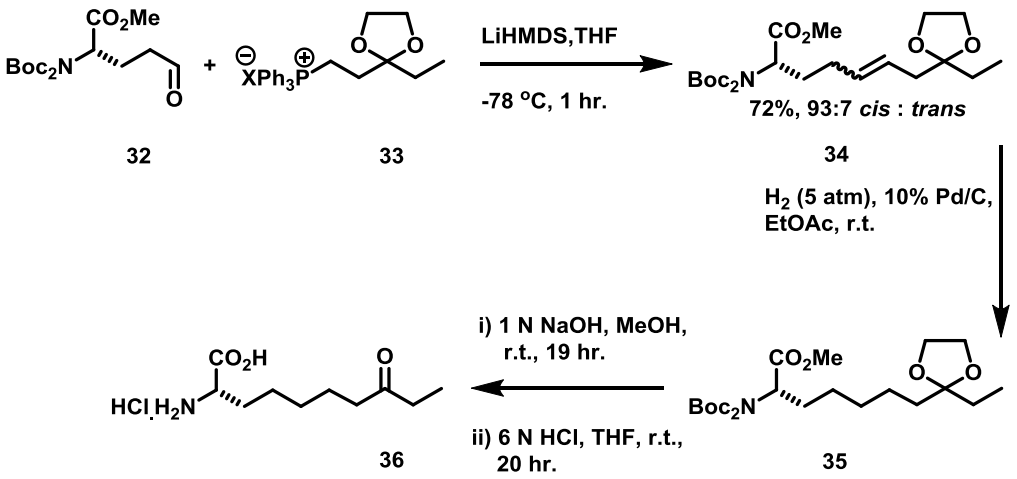
Scheme 3. New methodology: Asymmetric synthesis of Aoda

In 2006 Taddei and co-workers[16] used a different approach to elongate an enantiomerically pure amino acid, using a Wadsworth Emmons reaction between aldehyde **29** and phosphonate **30** (Scheme 4). Compound **29** was synthesized from *S*-glutamic acid **24** (Scheme 4), by double protection of the amine and -COOH using benzyl bromide, followed by selective reduction of the benzyl ester to give the corresponding alcohol **26** and oxidation under Swern conditions. Finally, homologation with methoxymethyl triphenylphosphonium chloride and LiHMDS, followed by acidic work up, gave the aldehyde, **29**. Aoda was then obtained through hydrogenation of enone **31**.



Scheme 4. Synthesis of Aoda by Wadsworth Emmons reaction from aldehyde 29

In the same year Alajarin and co-workers[44] prepared Aoda using the Wittig reaction, by treatment of phosphonium salt **33** with LiHMDS, followed by addition of aldehyde **32** derived from glutamic acid (Scheme 5). The salt **33** was prepared from 1-chloropentan-3-one. The alkene **34**, obtained as a mixture of isomers was converted into Aoda **36** as its hydrochloride by hydrogenation and acetal deprotection.

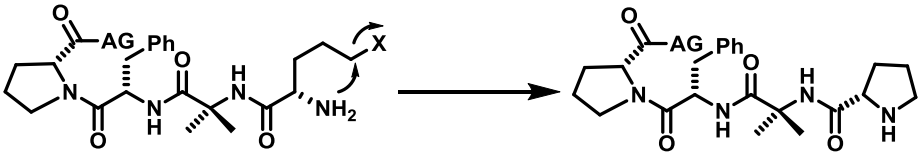


Scheme 5. Synthesis of Aoda by Wittig reaction

1.4 Previous total syntheses of natural macrocyclic tetrapeptides

1.4.1 Synthesis of chlamydocin

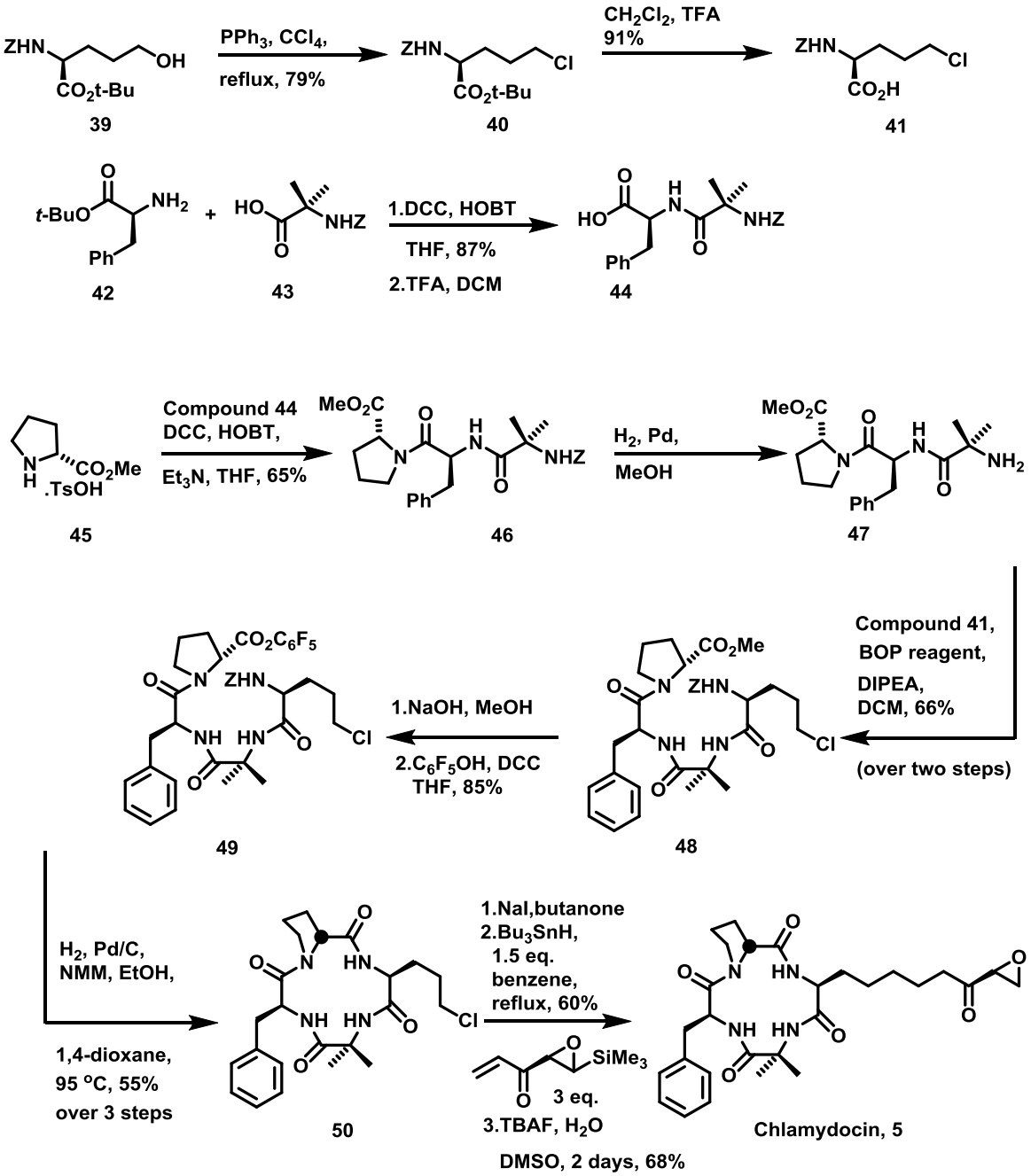
In 1993, Godfrey and co-workers[45] reported a synthesis of chlamydocin **5**. They chose to form the cyclic tetrapeptide by cyclization between the *N*-terminus of (Aoe) and the active ester of proline in the linear tetrapeptide precursor **37**. They preferred to use (*S*)-2-amino-5-chloropentanoic acid (ACP) rather than (*S*)-2-amino-5-iodopentanoic acid (AIP) as a residue in compound **37** because there is a greater chance of intramolecular nucleophilic displacement of the iodide group by reaction with the primary amino group to give **38** [46] (Scheme 6). In addition, the iodo group is not compatible with hydrogenolysis conditions. Chlorination of protected hydroxyamino acid **39** gave diprotected chloroamino acid **40**, which was deprotected using TFA to give *N*-Z-(*S*)-2-amino-5-chloropentanoic acid **41**. [47,48] Coupling of (*S*) phenylalanine *t*-butyl ester **42** with Z-α-aminoisobutyric acid **43** followed by ester deprotection gave dipeptide **44**, which was coupled with (*R*)-proline to give tripeptide **46**. Hydrogenolysis of **46** to give **47** followed by coupling with **41**, using BOP [49] produced the desired tetrapeptide **48**, which was saponified then reacted with pentafluorophenol. Chlorocyclopeptide **50** was converted to iodo cyclopeptide under a Finkelstein exchange and subsequent radical reaction and desilylation gave chlamydocin **5** (Scheme 7).[45,50]



37 Unwanted byproduct 38

AG = activating group
X = halogen

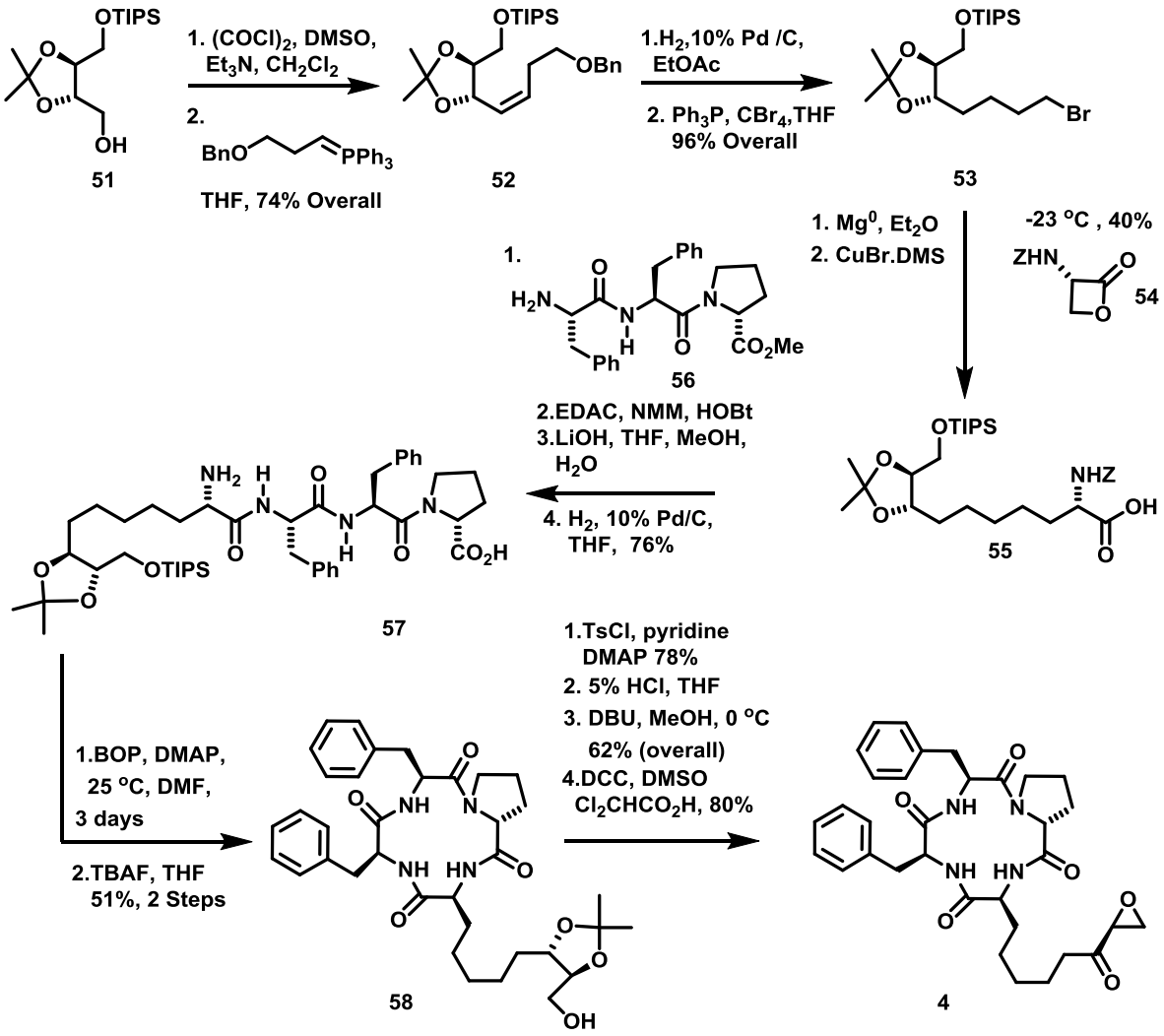
Scheme 6. Intramolecular nucleophilic displacement of the iodide group



Scheme 7. Synthesis of chlamydocin, 5 by Godfrey and co-workers

1.4.2 Synthesis of trapoxin

In 1996 Schreiber and co-workers[33] reported a synthesis of trapoxin **4**. In this synthesis, cyclization was achieved between the *N*-terminus residue of (2*S*, 9*S*)-2-amino-8-oxo-9, 10-epoxydecanoic acid (Aoe) and the C-terminus residue of (*R*)–proline. Hydrogenation and debenzylation of olefin **52** followed by bromination of the alcohol group gave **53**. Transmetalation of the Grignard reagent derived from **53** with CuBr.DMS followed by reaction with Z-serine β-lactone **54** gave acid **55**, which was coupled with tripeptide **56**. Finally the cyclization of **57** was achieved using BOP and DMAP in DMF. Removal of the TIPS protecting group with TBAF gave 51% yield of the pure cyclotetrapeptide alcohol **58** over two steps (Scheme 8).

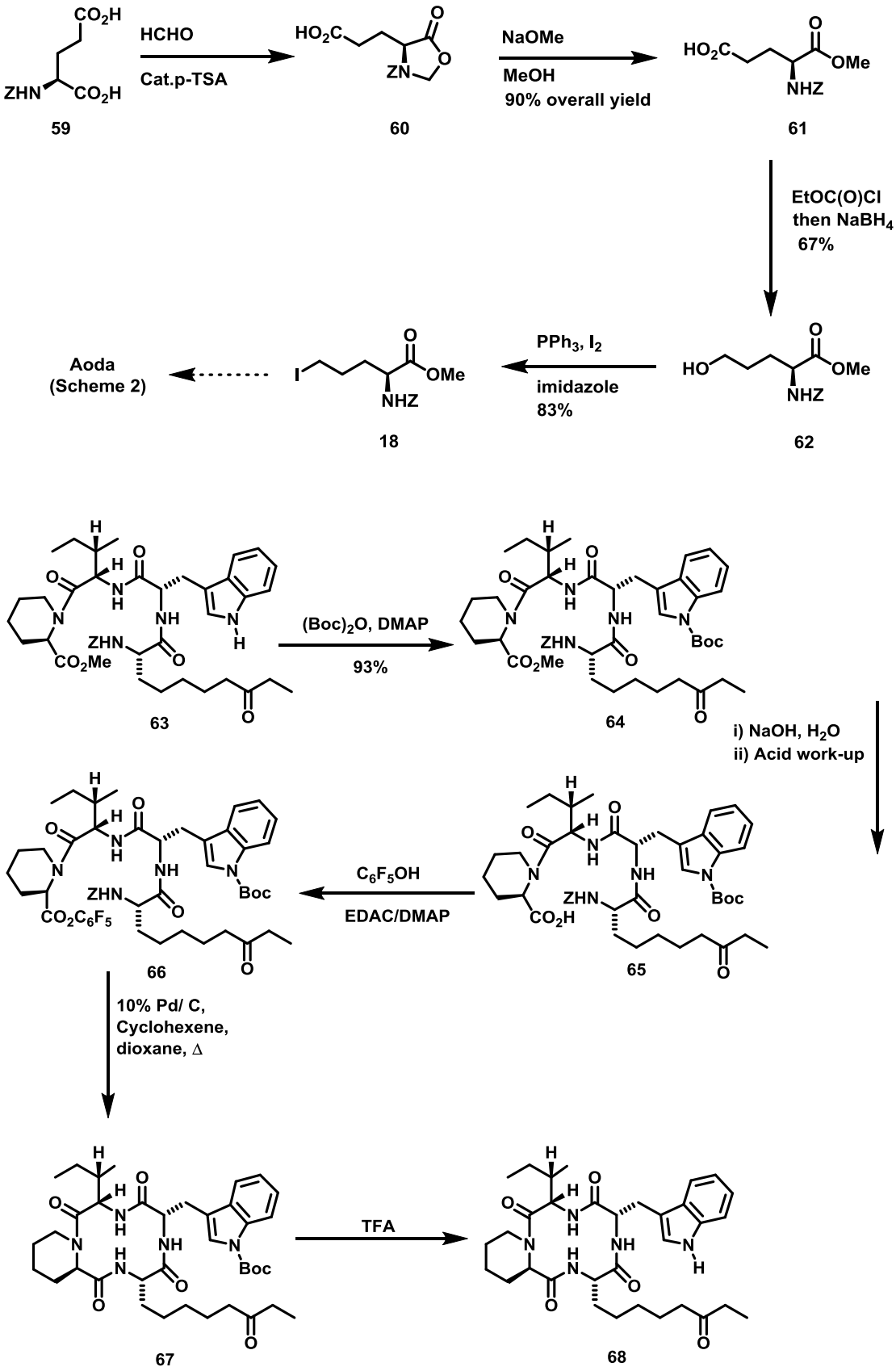


Scheme 8. Synthesis of trapoxin 4 by Schreiber and co-workers

The cyclic peptide **58** was converted into trapoxin by formation of the primary tosylate, treatment with aqueous HCl to remove the acetonide and then DBU/MeOH to give epoxide in 62% yield. Finally Moffatt oxidation gave the target compound **4**, 80% (Scheme 8).The same method was used in a total synthesis of chlamydocin; the main difference was the presence of an aminoisobutyric acid (Aib) residue adjacent to the (Aoe) residue rather than phenylalanine as in trapoxin.[33]

1.5 Synthesis of analogues of apicidin

In 2001 Singh and Mou[42] synthesized desmethoxy apicidin **68** starting from Z-*L*-glutamic acid **59**. Cyclization in the presence of formaldehyde, followed by reaction of **60** with methoxide gave 90% of monomethyl ester **61**. Reduction of **61** via the anhydride gave alcohol **62** which was further transformed to iodide **18** in 83% yield. The final reaction in this route is the radical addition of ethyl vinyl ketone **17** to **18** under conditions already shown in scheme 2 to make Aoda. For the synthesis of the cyclic tetrapeptide, cyclisation at the *R*-pip amide bond was chosen because recent report[51] screened cyclisation of other peptides incorporating *R*-pro with high yield. All coupling reactions were carried out using DCC/HOBt. After saponification and removal of the (Z) group, all attempts to cyclize failed, which the researchers ascribed to the present of the indole nitrogen. Therefore the indole was protected using a Boc group in 93% yield, followed by saponification of the methyl ester group of the pipecolic residue in compound **64**. The corresponding pentafluorophenyl ester **66** was formed from the linear tetrapeptide **65** using pentafluorophenol, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDAC) and a catalytic amount of DMAP and, finally, cyclization was achieved using the modified Schmidt protocol[52] to give **68** (Scheme 9).[42]

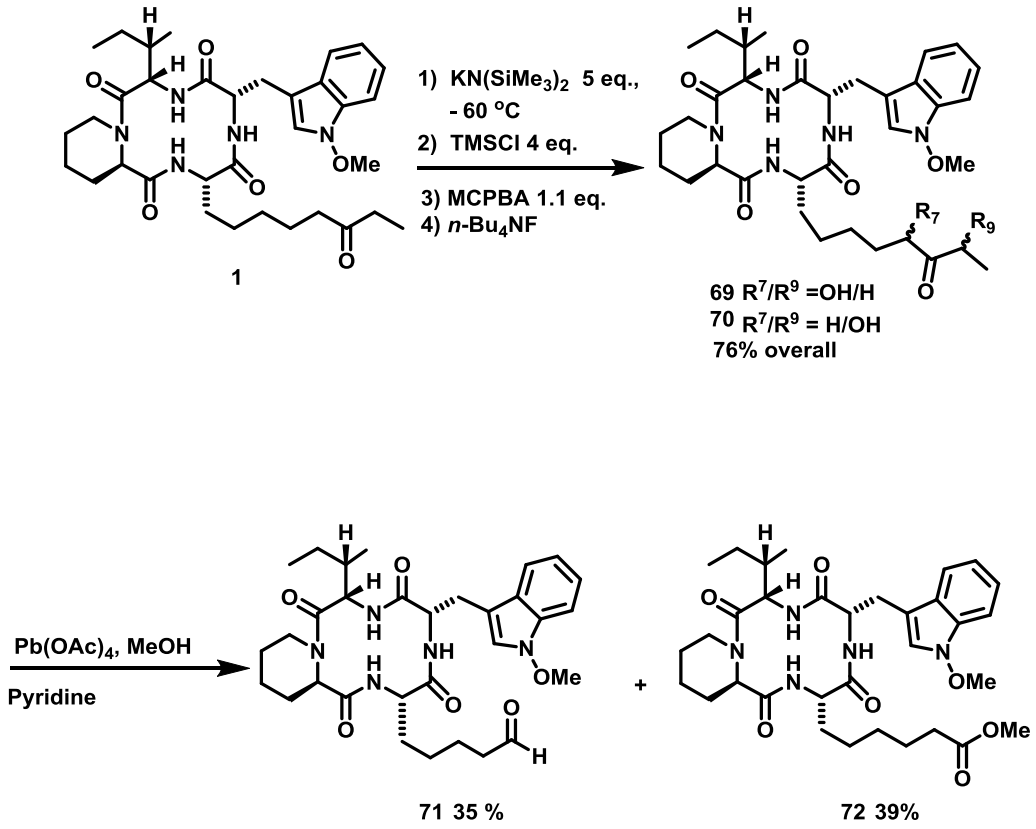


Scheme 9. Synthesis of analogues of apicidin

1.6 Chemistry of apicidin

1.6.1 Side chain reactions of Aoda 2

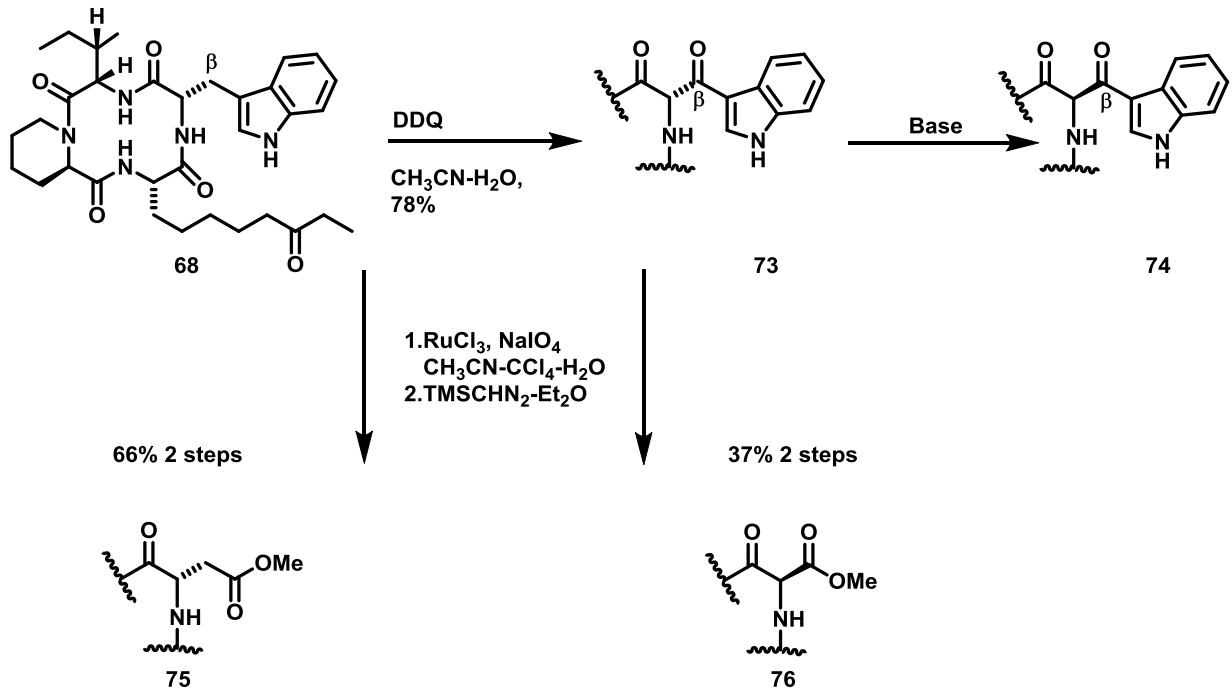
In 2000, Meinke and co-workers[53] reported that α-hydroxylation of the side chain ketone of natural apicidin **1** gave a mixture (1 : 1) of compounds **69** and **70**, which could be separated. The mixture was subjected to oxidative cleavage leading to the C7-aldehyde (**71**, 35%) and the corresponding C8-methyl ester (**72**, 39%) (Scheme 10).

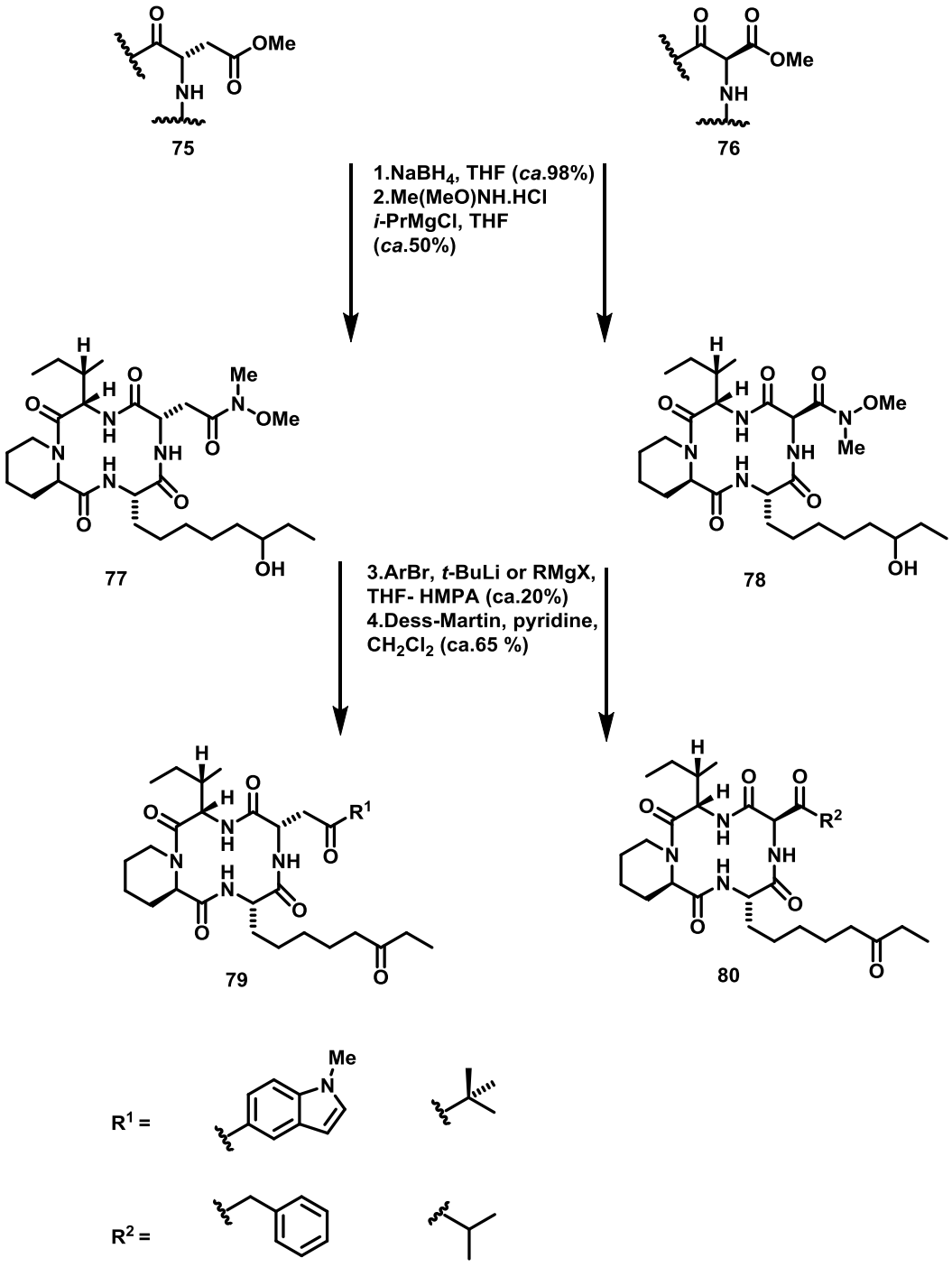


Scheme 10. Oxidative cleavage of side chain of apicidin

1.6.2 Tryptophan replacement in *N*-desmethoxy apicidin

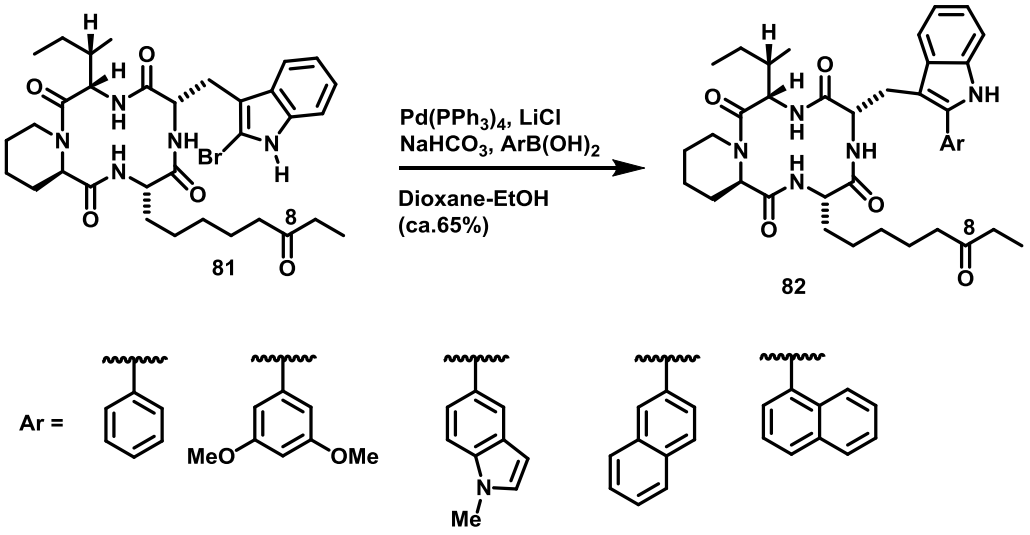
In 2000 Colletti and co-workers[36] reported oxidation of the β-position of tryptophan in compound **68**, the analogue of apicidin whose synthesis was described in scheme 9, to furnish β-oxo derivative **73**. Using Et_3N in CH_2Cl_2 or t-BuOK, t-BuOH-THF, **73** was quantitatively epimerized to give compound **74**. Oxidation of the indole in compound **68** using ruthenium tetraoxide [54-56] gave the methyl aspartate derivative **75** in good yield. Similar conditions were used with compound **73** to give methyl ester **76** (Scheme 11). Reduction of side chain C8 ketones of **75** and **76** gave their respective C8 alcohols and the methyl esters were then transformed to the corresponding *N*-methoxy-*N*-methyl amide **77** and **78**. This reduction was carried out to protect the ketone during subsequent organometallic addition. On treatment of Weinreb amides **77** and **78** with Grignard reagents or aryl lithiums, ketones **79**, **80** were obtained after oxidation of the side chain alcohol.[36]





Scheme 11. Ketone analogues of apicidin

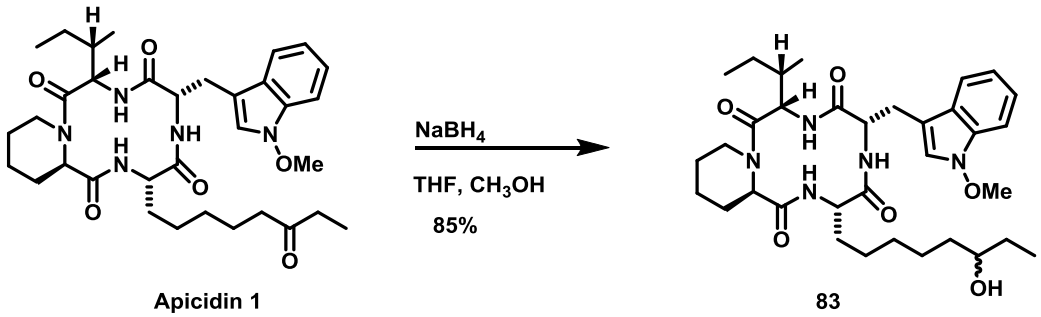
Bromination of the tryptophan unit was achieved using either pyridinium bromide perbromide, or NBS, to furnish the 2-bromoindole derivative **81** which was subsequently subjected to coupling with various aryl boronic acids under Suzuki conditions to give good yields of 2-arylindoles (Scheme 12).[36]



Scheme 12. 2-Arylindole analogs of apicidin and different aryl group on position 2 of indole

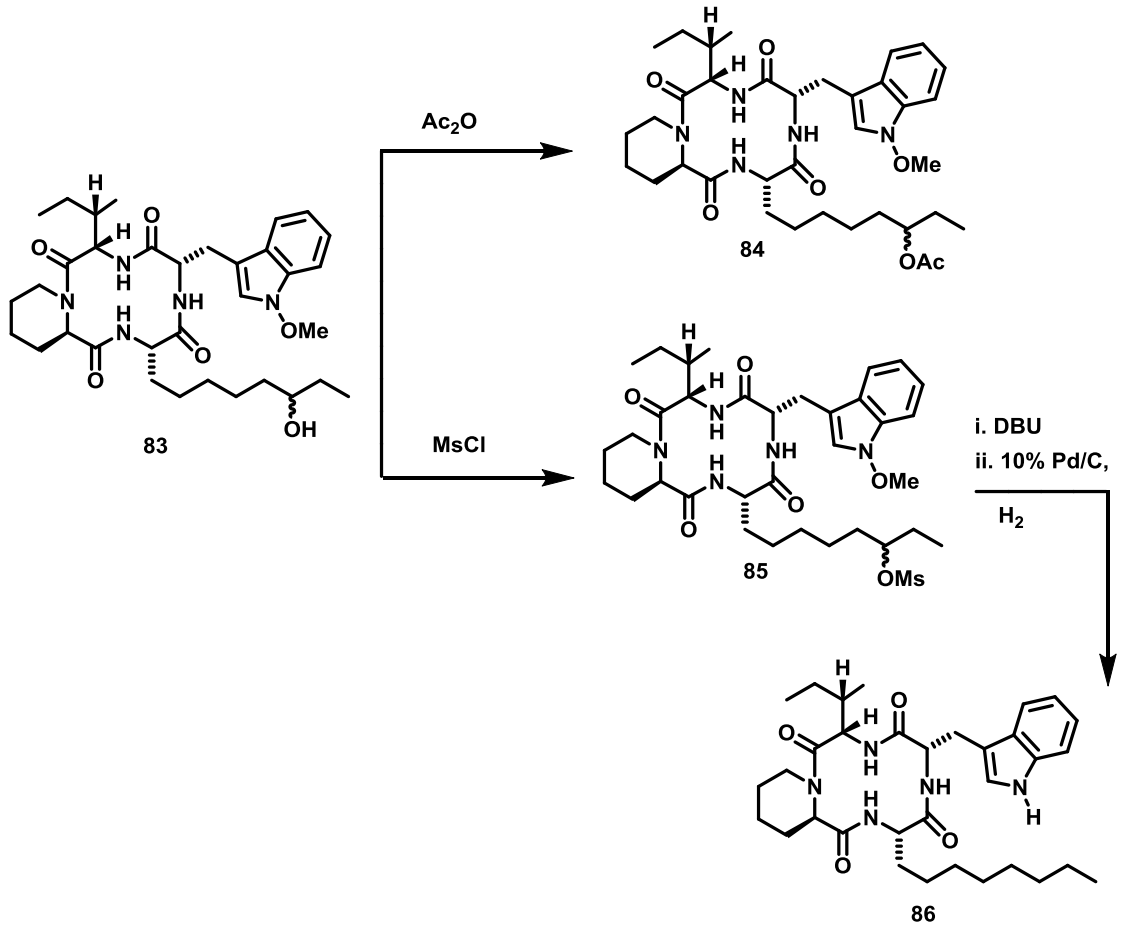
1.6.3 Further reactions of apicidin

In 2002 Singh and co-workers[57] reported that reduction of apicidin by $NaBH_4$ gave a (1 : 1) mixture of the inseparable diastereoisomeric alcohols **83** as shown in scheme 13.



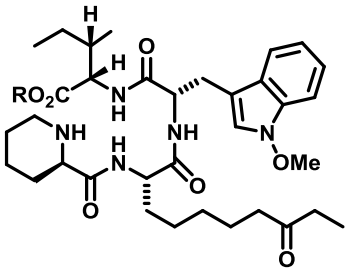
Scheme 13. Reduction of side chain of apicidin 1

Reaction of compound **83** with Ac_2O gave the acetates **84**. Mesylation of alcohol **83** by reaction with methanesulfonyl chloride followed by elimination with DBU gave a mixture of olefins, which was hydrogenated to give desmethoxy-deoxyapicidin **86** (Scheme 14).[57]



Scheme 14. Side chain modification of 83

Treatment of apicidin **1** with acidic ethanol gave the linear tetrapeptide **87** formed by selective cleavage of the amide bond between Pip and Ile. Hydrolysis of the ethyl ester **87** with LiOH furnished the acid **88** (Figure 7), but attempted recyclization using BOP reagent in DMF failed. This was ascribed to the steric effect of Ile because cyclization has been successfully achieved between Pro-NH and Phe-CO_2H in analogues of chlamydocin.[57]

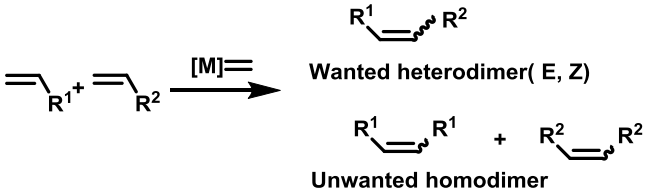


87 R = Et
88 R = H

Figure 7. Selective cleavage of the amide bond between Pip and Ile

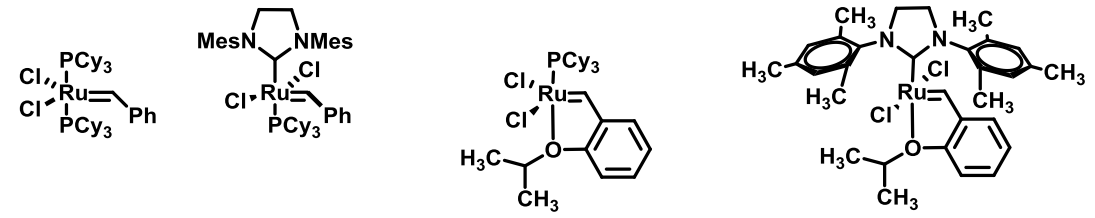
1.7 Olefin-Cross metathesis

Olefin cross metathesis (CM) is a potentially powerful synthetic method; however CM has been limited by the lack of predictability in product selectivity and stereoselectivity (Scheme 15). However by placing sterically large and electron-withdrawing groups near the reacting olefin there is the possibility to improve cross-metathesis (CM) product selectivity and stereoselectivity.[58-64]



Scheme 15. Cross metathesis product

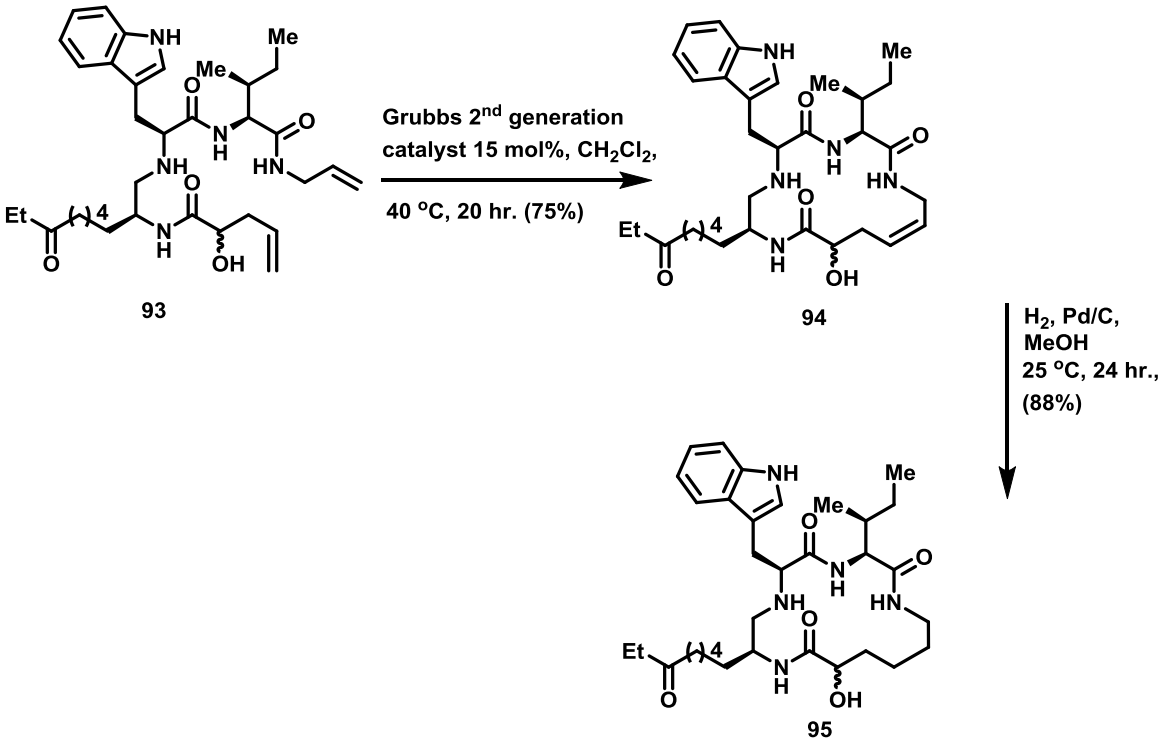
The first report using the first generation Grubbs catalyst in cross-metathesis appeared at the end of 1996.[65] Later Grubbs introduced *N*-heterocyclic carbenes (NHCs) Lewis basic ligands, in place of one phosphonium ligand in **89** producing the 2nd generation catalyst **90**. Subsequently, modified catalysts were developed including the Hoveyda Grubbs 1st generation catalyst **91** with an ortho-isoproxy group and the NHC-containing 2nd generation catalyst **92** (Figure 8).[66-68]



Grubbs 1st generation catalyst, 89 | **Grubbs 2nd generation catalyst, 90** | **Hoveyda-Grubbs catalyst 1st generation, 91** | **Hoveyda-Grubbs catalyst 2nd generation, 92**

Figure 8. Metathesis catalysts

The Grubbs 2nd generation catalyst has improved stability and activity in cross-metathesis and ring-closing metathesis (RCM) reactions. Macrocycle **94** was obtained successfully from ring closing metathesis reaction followed by hydrogenation to give **95** (Scheme 16). Compound **95** shows less biological activity as HDACi in HeLa cell compared to natural apicidin, which may be due to the fact that its core not rigid as apicidin **1**.[66,69]

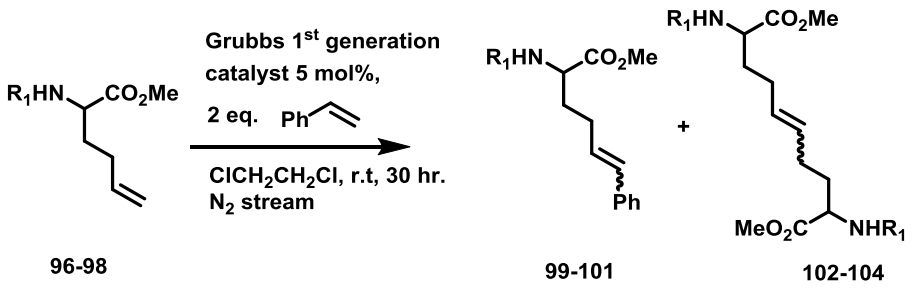


93 94 95

Scheme 16. Synthesis of apicidin analogues by RCM

1.7.1 Cross-metathesis reactions of unsaturated amino acids

In 1998 Gibson and co-workers[70] reported the cross-metathesis of homoallyl glycine derivatives with aryl- and alkyl-substituted alkenes. The amine and acid groups were protected to make sure that amino acid functionality did not interfere. Typical yields of the cross-metathesis products using styrene were around 50% with the mass balance being the self-metathesis products **102-104** (Scheme 17). For cross-metathesis the *trans* isomer predominated. As can be seen from the results (Table 1) the *N*-protecting group has little influence on the yield of desired products **99-101**.

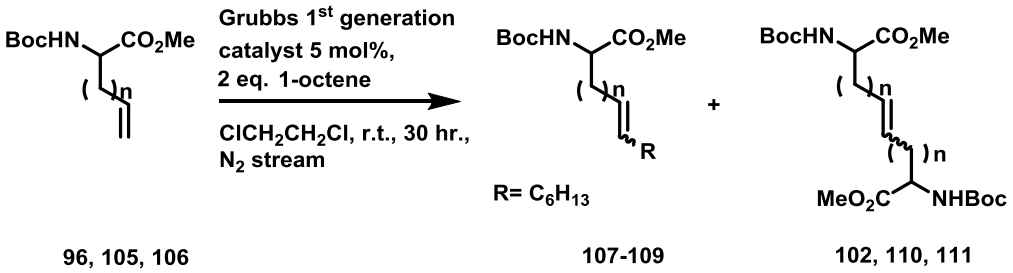


Scheme 17. Metathesis reaction by using Grubbs 1st generation catalyst

Table 1. Metathesis reaction product with using styrene

Entry	ST	R^1	Desired	Isolated yield %	Dimer	Isolated yield %
1	**96**	Boc	**99**	52	**102**	40
2	**97**	Phth	**100**	55	**103**	35
3	**98**	Ac	**101**	43	**104**	48

When the reaction was extended to 1-octene (Scheme 18), it is clear that by decreasing the number of carbon atoms in the amino acid chain, the yield of the product decreased (Table 2). The authors suggest that the most likely explanation is that the sterically bulky amino acid moiety is hindering the approach of the catalytic ruthenium species to the double bond of the substrate.[70]

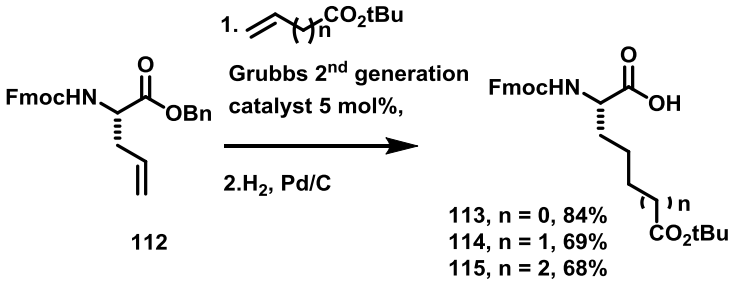


Scheme 18. Metathesis reaction using Grubbs 1st generation catalyst

Table 2. Metathesis reaction product with using oct-1-ene

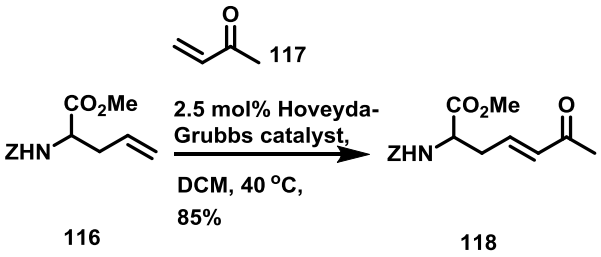
Entry	ST	n	Desired	Isolated yield %	Dimer	Isolated yield %
1	**96**	2	**107**	66	**102**	28
2	**105**	1	**108**	45	**110**	17
3	**106**	0	**109**	7	**111**	---

In 2005 Kennan and co-workers[71] reported the synthesis of analogues of glutamic acid **113-115** via olefin cross metathesis followed by hydrogenation (Scheme 19).



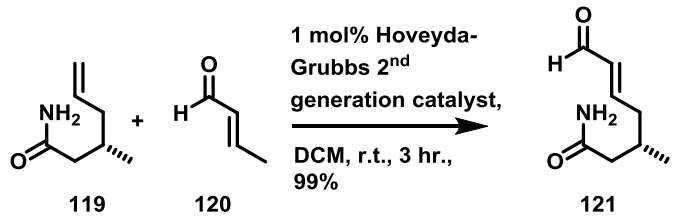
Scheme 19. Metathesis reaction using Grubbs 2nd generation catalyst

Also in 2005 Blechert and co-workers[72] reported a cross-metathesis reaction between protected racemic allyl glycine **116** and methyl vinyl ketone **117** using the phosphine free ruthenium catalyst (Hoveyda Grubbs 2nd generation catalyst) to give racemic compound **118** (Scheme 20).



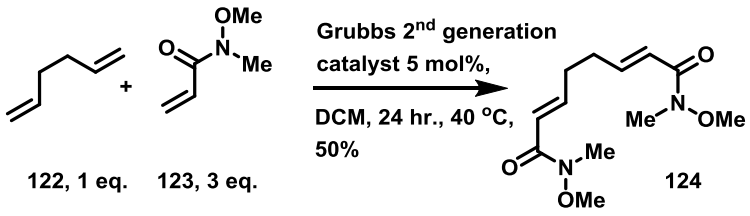
Scheme 20. Metathesis reaction using Hoveyda Grubbs 2nd generation catalyst

In 2013 Carter and co-worker[73] generated compound **121** in good yield by reaction of **119** and crotonaldehyde **120**, using Hoveyda-Grubbs 2nd generation catalyst and the product was shown to be stable in frozen benzene for an extended period (Scheme 21).



Scheme 21. Synthesis of enal

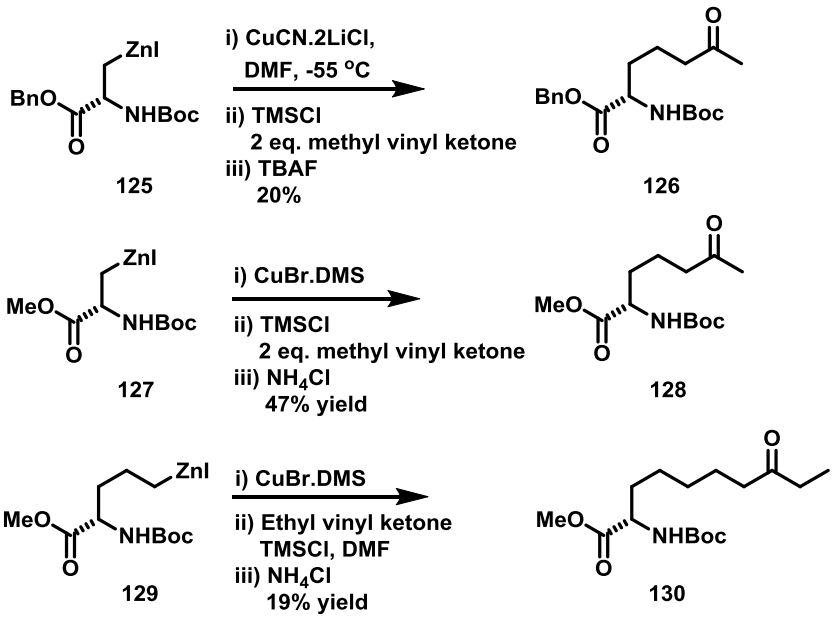
In 2013 Joseph and co-workers[74] prepared the symmetrical double Michael acceptor **124** by double cross-metathesis of 1,5-hexadiene **122** with Weinreb acrylamide **123** (Scheme 22).



Scheme 22. Two directional CM of compound 122

1.8 Jackson group chemistry

The zinc reagent **125** was converted into the corresponding zinc/copper reagent, using stoichiometric CuCN.2LiCl, and then treated with methyl vinyl ketone in the present of TMSCl to give the adduct **126** [75] (Scheme 23). Later, it was shown that catalytic amounts of copper bromide dimethyl sulfide could replace the stoichiometric amounts of copper cyanide. Copper catalyzed reaction of methyl and ethyl vinyl ketone with the serine and glutamic acid derived zinc reagents **127** and **129**, gave respectively, analogues of Aoda **128**, **130** unfortunately the yield of protected Aoda **130** was very poor.[76,77]



Scheme 23. Synthesis of Aoda and its analogues through Michael addition

1.9 Aims of the project

Cyclic peptides, specifically cyclic tetrapeptides, have a range of useful biological properties. We therefore propose to develop new synthetic approach to analogues of apicidin **1** a known histone deacetylase inhibitor, which shows in *vivo* activity against *plasmodium berhei* malaria in mice at low doses and is also known to inhibit proliferation of tumour cells.[78,79] The octan-6-one side-chain is structurally related to the side chains found in a series of other naturally occurring cyclic tetrapeptides, including HC-toxin, Trapoxin A, WF- 3161, Cly-2, and chlamydocin **5**. The significant difference is that in all these compounds, the functional group is a terminus epoxy ketone; illustrated by the structure of chlamydocin **5** (Figure 9). It is the epoxyketone that is responsible for the anti-proliferative activity.[33]

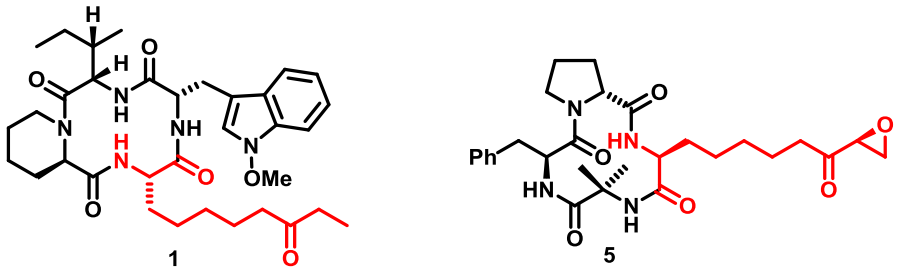


Figure 9. CTP with different functional group

Our route to cyclic tetrapeptides can follow one of two strategies, synthesis of the required amino acid analogues of Aoda or late stage modification of a tetrapeptide to produce the desired structure.

In the first strategy, Aoda and its analogues can in principle be made by reaction of organozinc reagents derived from serine, aspartic acid and glutamic acid with allyl chloride, followed by cross-metathesis with different unsaturated ketones using Grubbs catalyst and then hydrogenation (Figure 10). An alternative strategy could involve the conjugate addition of organozinc reagents to enones (Figure 11), as already mentioned in scheme 23.

We categorized the disconnection by designating the number of atoms in the side of the amino acid as “c”, and the number of atoms in the reaction partner as “m”, so in a process involving cross-metathesis the number of atoms in the side-chain of the product is given by “c” + “m” -2 (not as enene). In the conjugate addition reaction, the number of atom in the side chain of the product is simply “c” + “m”.

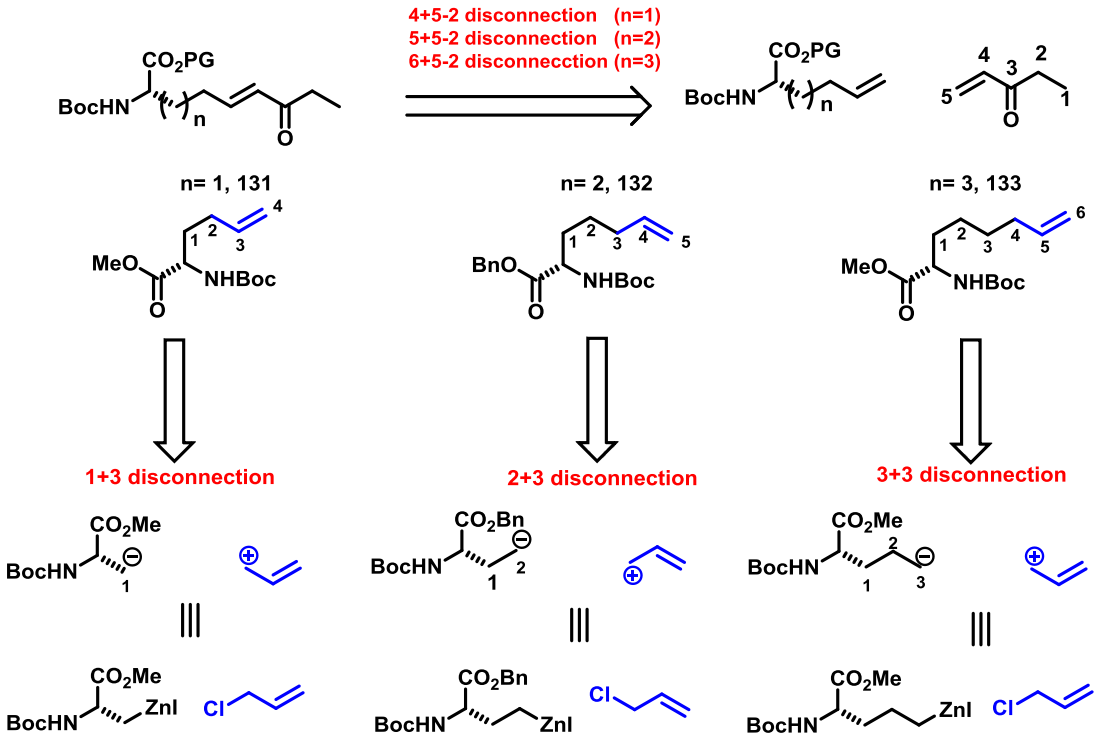


Figure 10. Retrosynthetic analysis using cross-metathesis

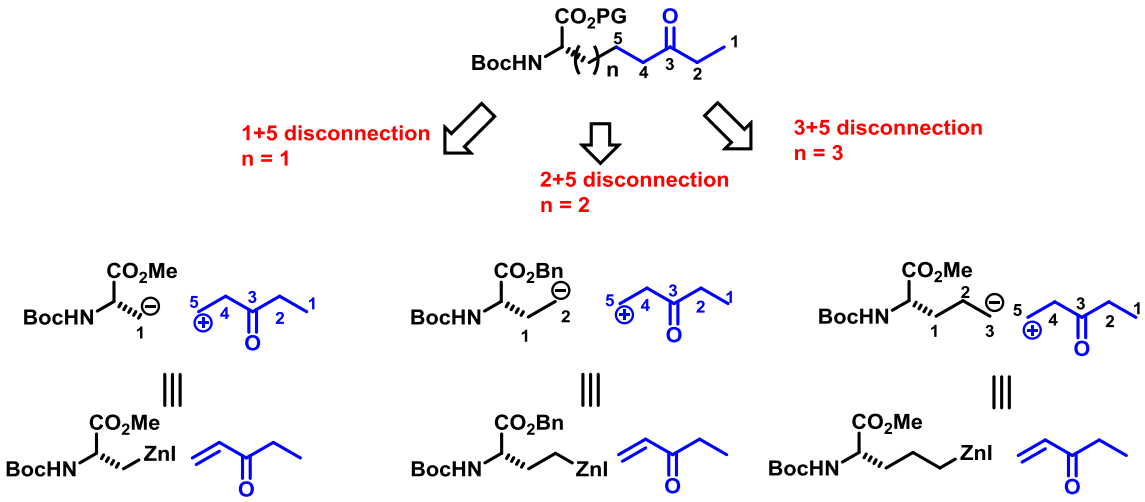
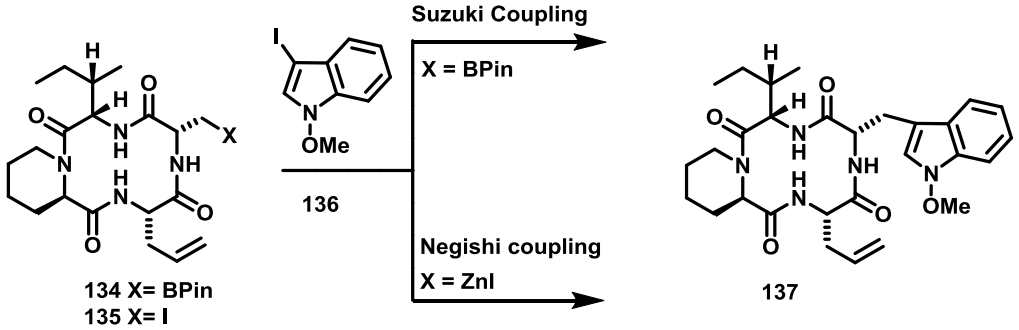


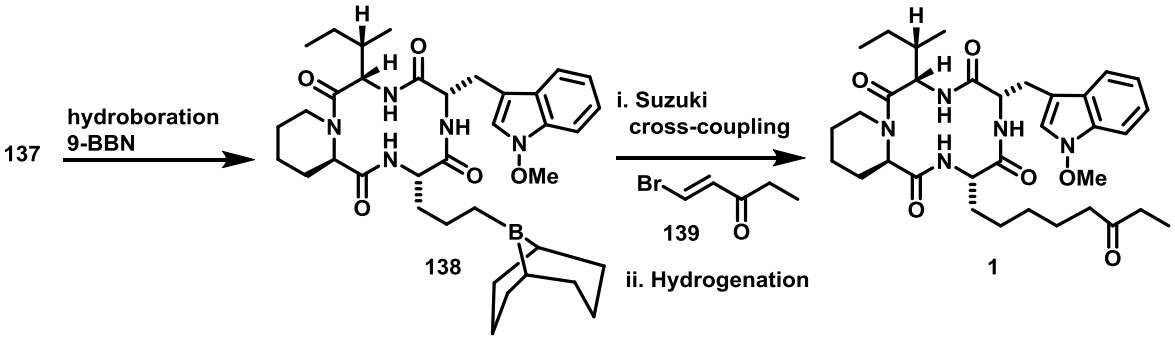
Figure 11. Retrosynthetic analysis for Aoda analogues via conjugate addition

One possible approach in the second strategy is the use of two non-natural side chains in a common cyclic tetrapeptide precursor. For example, cyclic tetrapeptide **134** could be used in a Suzuki coupling[80,81] with 2-iodo-1-methoxyindole derivatives **136** or the organozinc reagent could be made from **135**, followed by Negishi cross coupling[82] with **136** (Scheme 24).



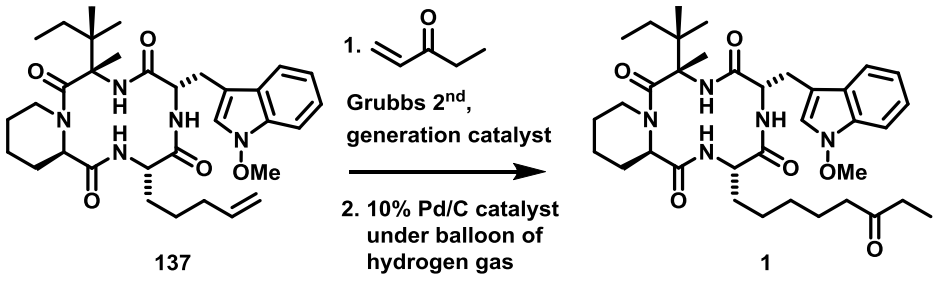
Scheme 24. Suzuki and Negishi coupling

Hydroboration/Suzuki cross-coupling[83] on the remaining alkene, using the bromo-enone **139** as an electrophile, followed by hydrogenation, would give the desired ketone **1** (Scheme 25).



Scheme 25. Hydroboration and Suzuki coupling of remaining alkene

In order to synthesis apicidin **1,** cross-metathesis is another possible approach to modify the side chain, via reaction of compound **137** with 1-penten -3-one using Grubbs 2nd generation catalyst, followed by hydrogenation of the enone (Scheme 26).



Scheme 26. Cross-metathesis of remaining alkene

Chapter 2: Analogues of Aoda

2.1 Introduction

The initial target was the synthesis of the unsaturated amino acids **131-133**, the required key intermediates for synthesis of the planned analogues of 2-amino-8-oxo-decanoic acid (Aoda), **2**. This required the synthesis of the corresponding amino acid derived iodides **140-142** (Figure 12).

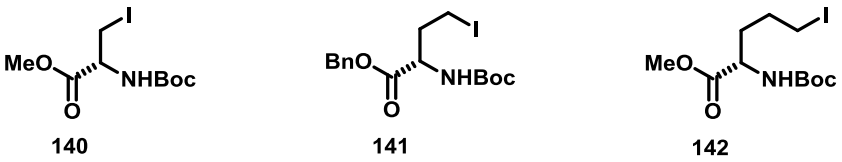


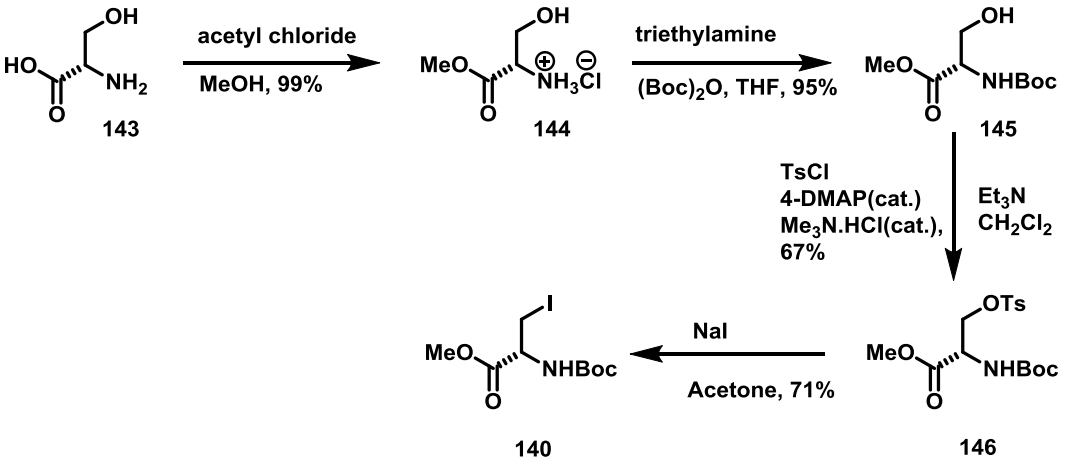
Figure 12. Key amino acid derived organozinc intermediates

2.2 Results and Discussion

2.2.1 Iodide synthesis

2.2.1.1 Synthesis of iodide 140

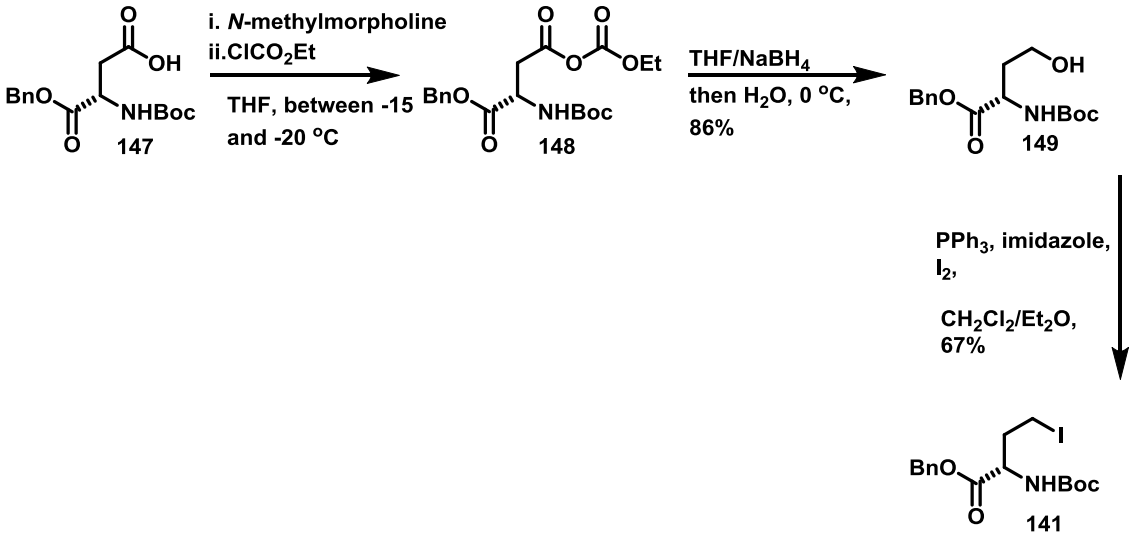
Iodide **140** was prepared by the literature method[84,85] shown (Scheme 27). The yields obtained were comparable to those previously reported.



Scheme 27. Preparation of iodide 140 from *S*-serine

2.2.1.2 Synthesis of iodide 141

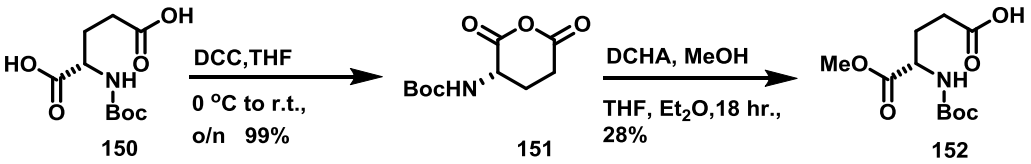
The key step to access iodide **141** is the chemoselective reduction of the carboxylic acid of Boc-*S*-aspartic acid 1-benzyl ester **147** to the corresponding primary alcohol **149**. Previous work in the Jackson group[86] had shown that this could be achieved in two steps via activation of the acid as an *N*-hydroxy-succinimide ester followed by reduction with $NaBH_4$, in 59% yield. A recent report[87] described an alternative method in which the mixed anhydride **148** was reduced using $NaBH_4$ to give alcohol **149**. The yield reported on small scale 0.33 mmol was 94%, and we achieved 86% on a larger scale (20 mmol) (Scheme 28). This latter method was preferred because of the shorter reaction time and better yield compared to activation via the *N*-hydroxy-succinimide ester. In the anhydride forming reaction it is important to maintain a temperature between -15 and -20 ^{o}C. Iodination of **149** under Appel conditions was achieved according to the literature[87] in good yield (67%, on a 23 mmol scale), compared to the literature yield (76%, on a 0.58 mmol scale).



Scheme 28. Preparation of iodide 141 from *S*-protected aspartic acid

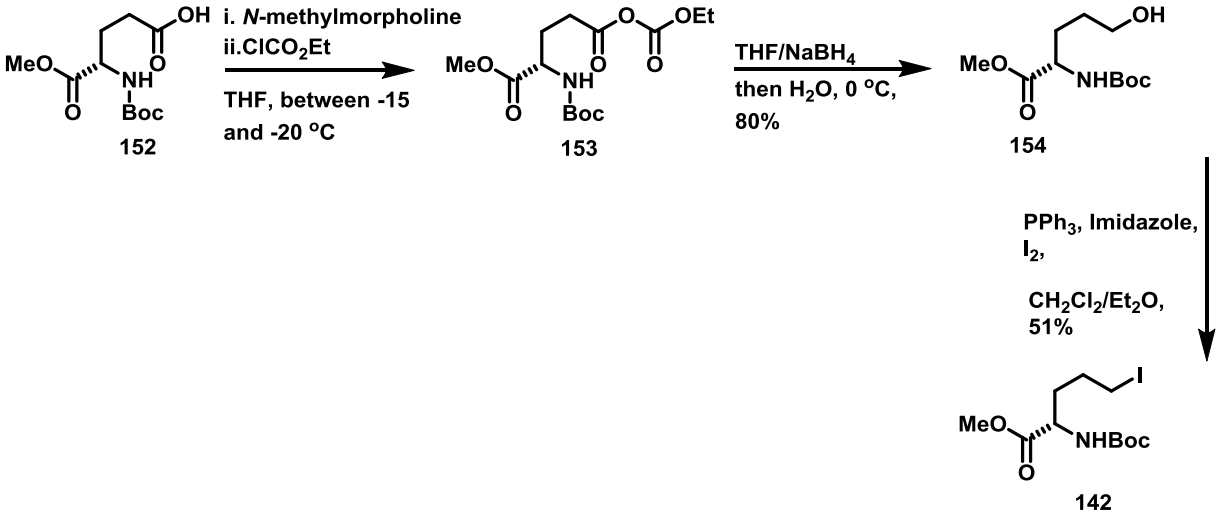
2.2.1.3 Synthesis of iodide 142

In order to prepare compound **142**, the carboxylic acid group at the α-position in glutamic acid **150** was protected as an ester. This can only be done by an indirect route, via ring-opening of the anhydride **151** (Scheme 29), according to a literature route.[88] Although the yield was low and not optimized, it allowed the preparation of sufficient material.



Scheme 29. Preparation of compound 152 via anhydride 151

Reduction of **152** to the alcohol **154** was carried out in the same way as previously described for **147**. Subsequent iodination of **154** was carried out to give compound **142** in reasonable yield (51% on a 21 mmol scale) (Scheme 30).[87]

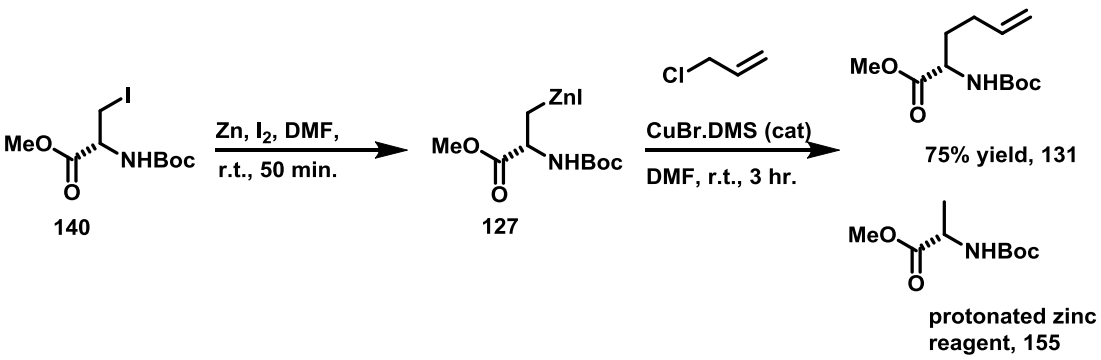


Scheme 30. Preparation of iodide 142 from *S*-protected glutamic acid

2.2.2 Allylation of protected amino acid

2.2.2.1 Synthesis of butenyl glycine 131 via zinc insertion

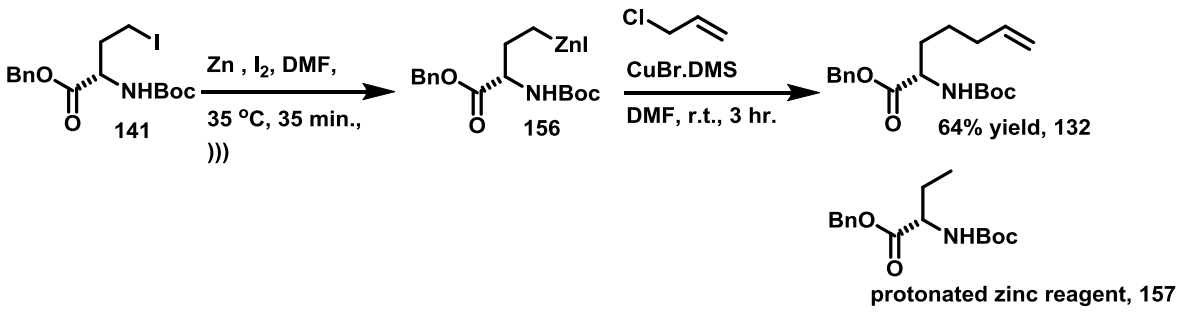
The zinc reagent **127** was prepared using prior Jackson group methodology[89] from protected iodoalanine **140**, and then converted into butenylglycine **131** 75% yield by copper-catalyzed allylation (Scheme 31). Previously in the Jackson group [90] the same allylation was achieved in 65% yield using stoichiometric amount of Cu(CN).2LiCl (1 eq.) instead of catalytic amount of CuBr.DMS.



Scheme 31. Preparation of protected butenyl glycine 131

2.2.2.2 Synthesis of pentenylglycine132 via zinc insertion

Zinc insertion into iodide **141** required more vigorous conditions, so the reaction was heated to 35 ^{o}C for 35 minutes under ultrasonication. The organozinc reagent **156** was added to a mixture of allyl chloride with CuBr.DMS catalyst at room temperature to give **132** in 64% yield after column chromatography and HPLC (Scheme 32), since column chromatography alone did not allow the isolation of a pure sample of **132**.



Scheme 32. Preparation of protected pentenyl glycine 132

Interestingly, the ^{1}H NMR spectrum of the product **132** showed a downfield shift of the NH signal on increasing the concentration (mmol/mL) (Figure 13), which is likely due to the influence of hydrogen bonding.

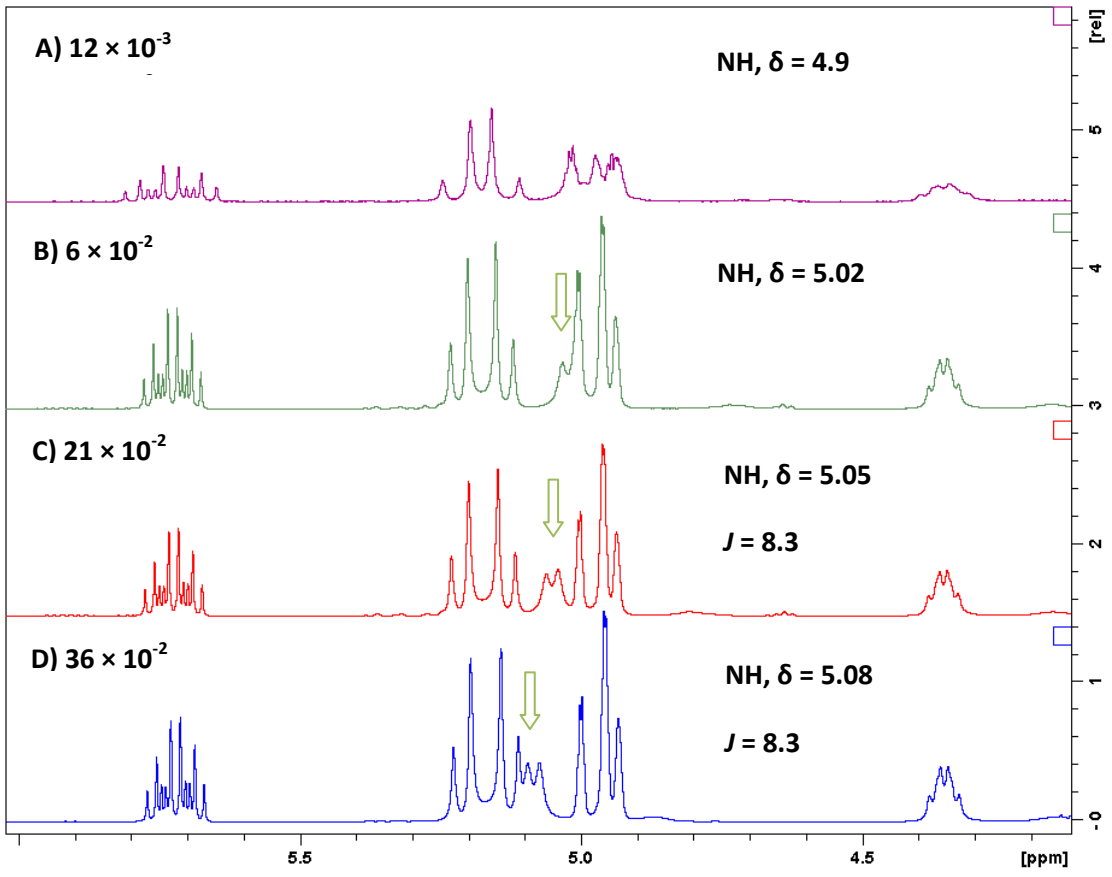
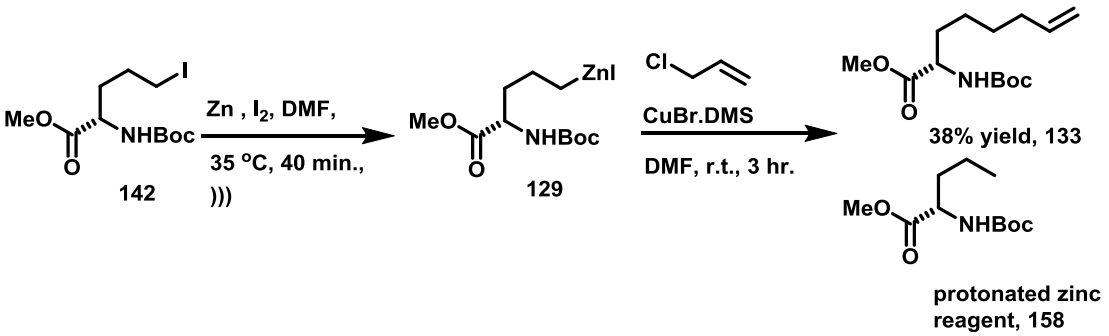


Figure 13. Influence of concentration on chemical shift of NH in compound 132 in $CDCl_3$

2.2.2.3 Synthesis of hexenylglycine 133 via zinc insertion

Copper catalyzed reaction of the organozinc reagent **129** with allyl chloride, under the same conditions as used for **156** gave a modest yield of **133** (38% on a 3 mmol scale) (Scheme 33). Under analogous conditions, without sonication, a very similar yield of **133** 37% was obtained; the mass balance was identified as the protonated zinc reagent. The obtained yield was congruent with that previously reported.[77]



Scheme 33. Preparation of protected hexenyl glycine 133

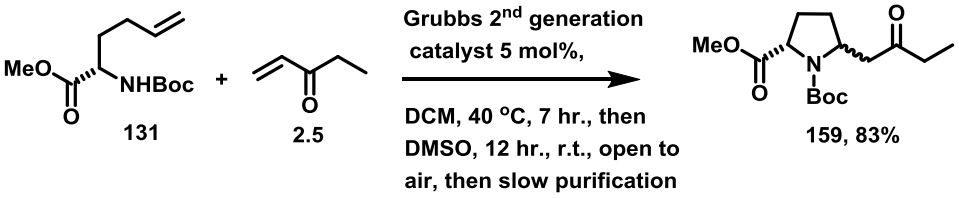
2.2.3 Cross-metathesis

With the three key starting materials **131-133** in hand, cross-metathesis with a selection of enones was investigated using general conditions reported in the literature[59]. All the reactions were carried out under positive pressure of nitrogen, and dry degases dichloromethane was used as the solvent.

2.2.3.1 Synthesis of 7-oxo amino acids

In an initial reaction, compound **131** was subjected to cross-metathesis with 1-penten-3-one in the presence of Grubbs 2nd generation catalyst (Scheme 34). Instead of the expected cross-metathesis product, the pyrrolidine **159** was isolated after column chromatography. Compound **159** had the same molecular mass, but quite different ^{13}C NMR spectrum from that expected for the target molecule (a peak corresponding to a saturated ketone appeared). In the ^{1}H NMR spectrum, the peak for the olefinic protons disappeared and IR confirmed the absence of an NH group. This suggested that, an intramolecular aza-Michael reaction had taken place, to form the pyrrolidine derivative **159**.

It is worth mentioning, that during purification a large amount of silica gel (133 gm for 0.4 mmol reacting product) was used and for an extended period (4.5 hr.). It therefore appeared that cyclisation occurred during purification. To minimize highly coloured ruthenium by product (in few cases) after 7 hr. reflux, DMSO was added to the crude reaction mixture then open to air stirred for 12 hr. at (r.t.).[91]



Scheme 34. Cross-metathesis and intramolecular aza-Michael reaction for compound 131

The initial ^{1}H NMR spectroscopy data showed two sets of signals. When the spectrum was recorded at higher temperature, it became clear that rotamers were present (Figure 14). This suggested that the cyclization had occurred with significant diastereoselectivity.

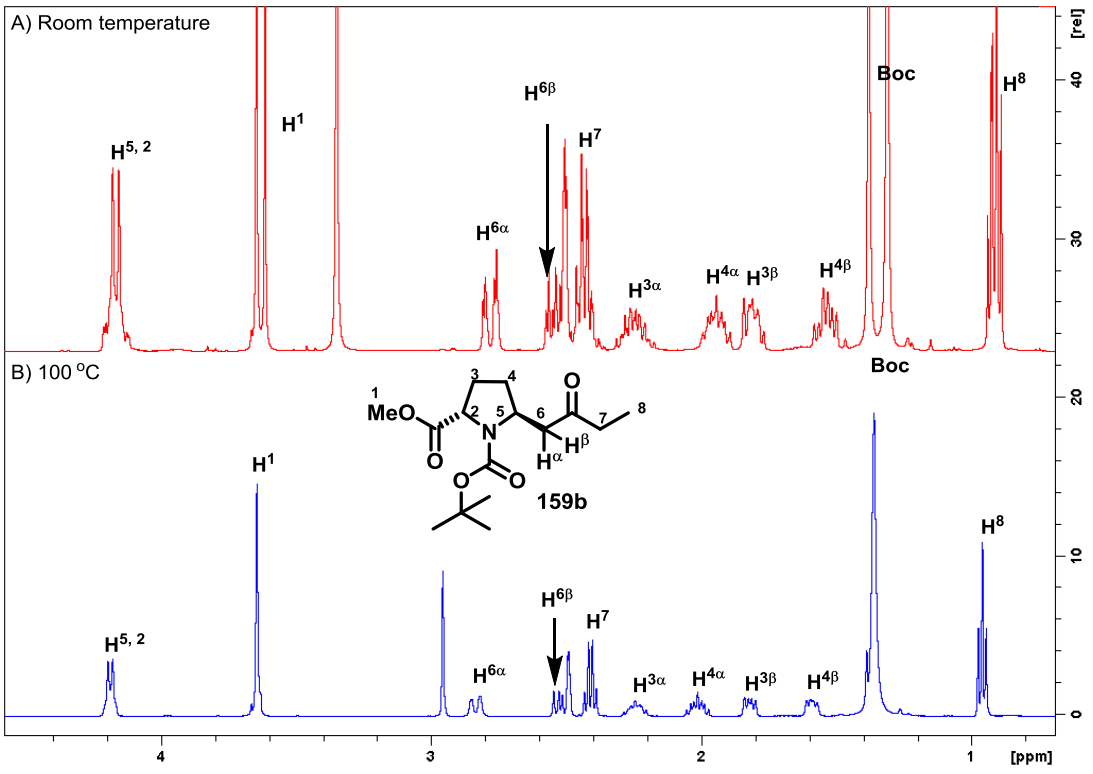


Figure 14. Rotameric behaviour of cross-metathesis product 159b, in DMSO.

Recrystallization of the sample of pyrrolidine **159b** gave crystals suitable for X-ray diffraction analysis. This showed that the product was indeed the pyrrolidine, as expected, and of *trans*-configuration (Figure 15). Since we were concerned that this stereoisomer may have preferentially crystallized, the ^{1}H NMR spectrum of the specific crystal used for X-ray analysis was recorded. Although the concentration was low, it is clear that the spectrum matched closely that of the bulk compound with its rotamer (Figure 16).

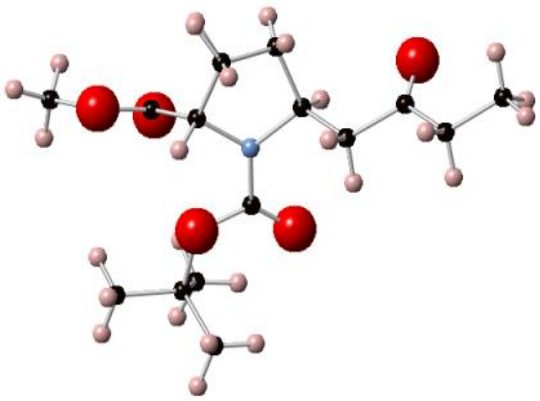


Figure 15. Crystal structure of compound 159b and it indicate *trans* configuration

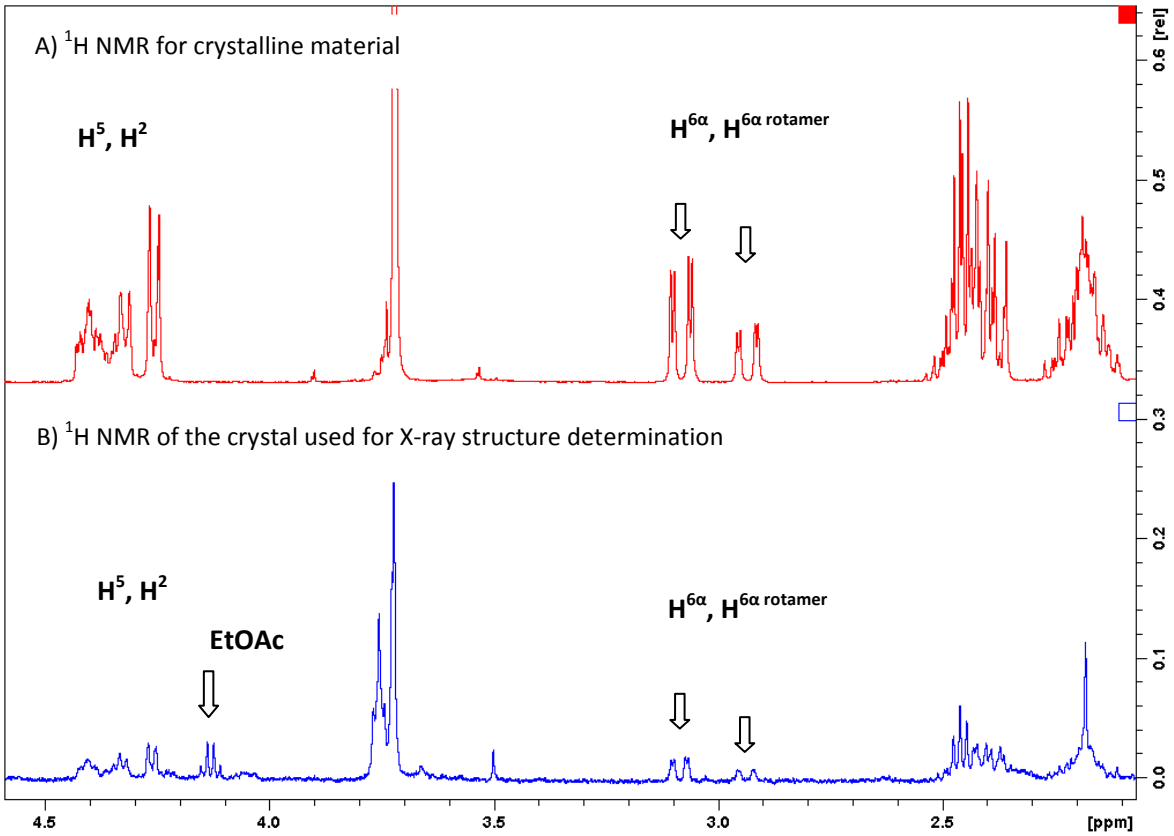
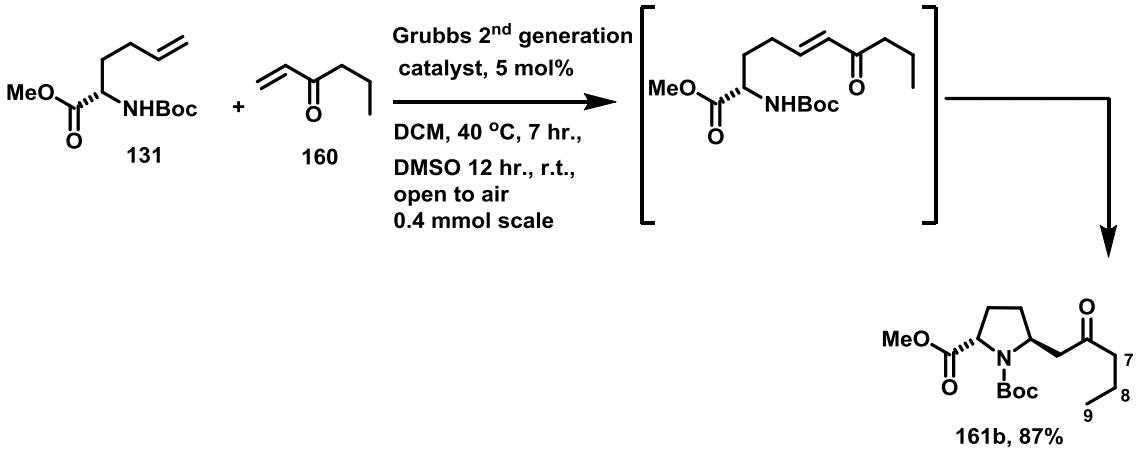


Figure 16. Comparison of ^{1}H NMR of compound 159b recorded at r.t.; *trans* configuration, $CDCl_3$

Cross-metathesis of **131** with 1-hexen-3-one **160**, followed by slow purification by column chromatography, gave the pyrrolidine **161b** (87%) (Scheme 35). Compound **161b** was assigned as the *trans* diastereoisomer by comparing its ^{1}H NMR spectrum with that of compound **159b** (Figure 17).



Scheme 35. Cross-metathesis and intramolecular aza-Michael reaction for compound 131

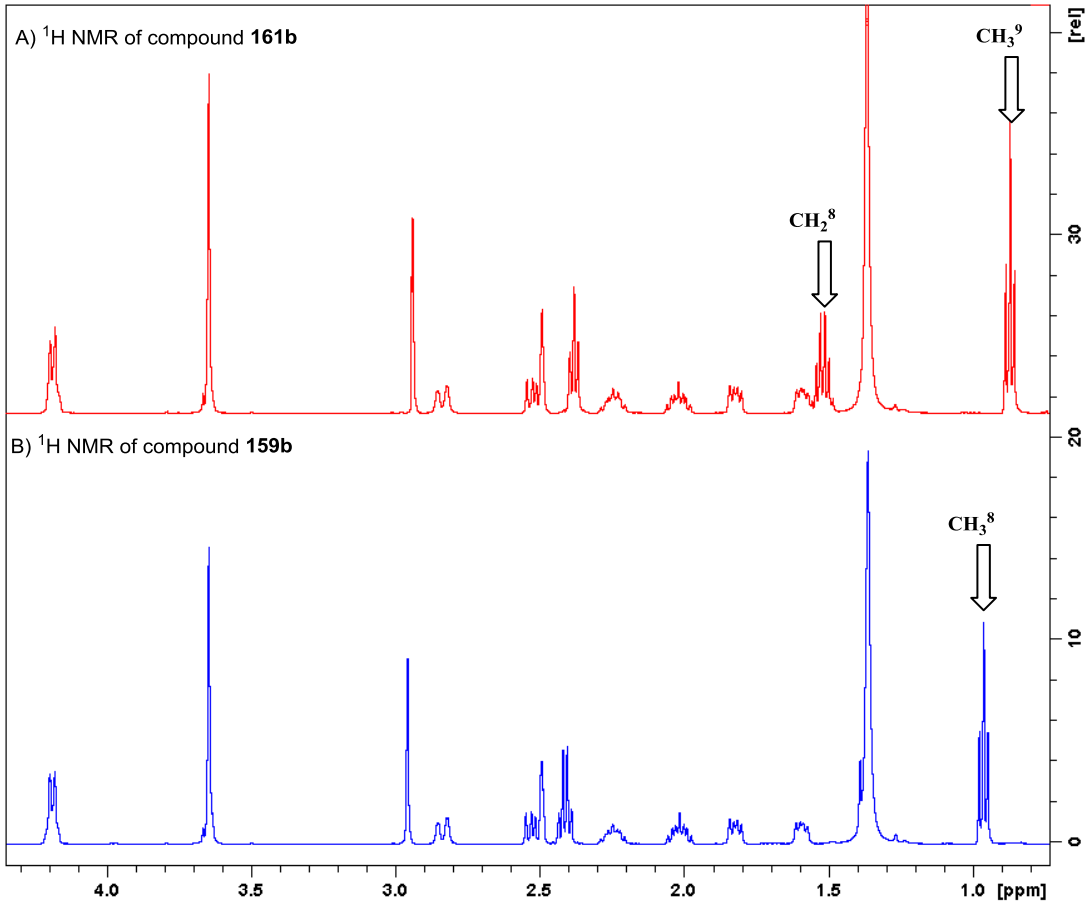
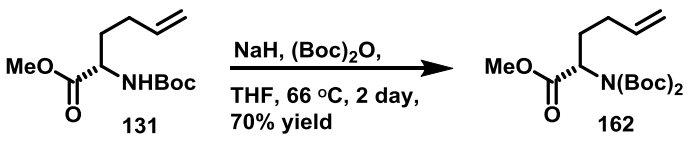


Figure 17. Comparison of ^{1}H NMR spectra of compound 161b with *trans* diastereoisomer 159b in DMSO 100 °C

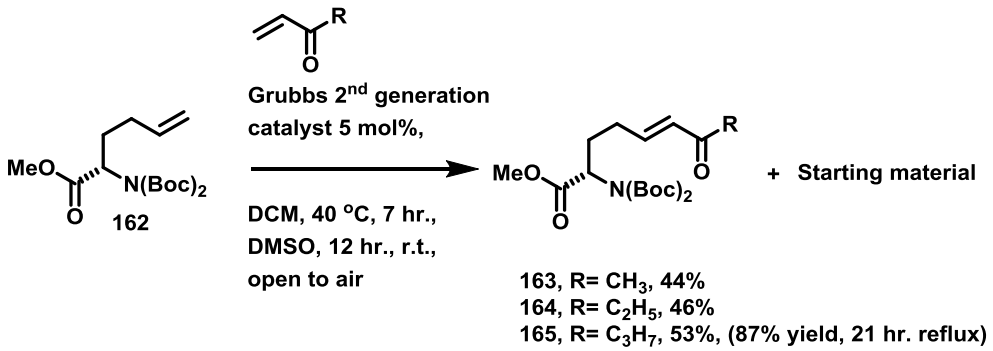
2.2.3.1.1 Double protection of amine

Since the initial cross-metathesis product appeared to be cyclizing during purification, we explored double protection of the amine. Compound **131** was therefore treated with di-^{t}butyl dicarbonate in the presence of strong base to give **162** [92] (scheme 36).



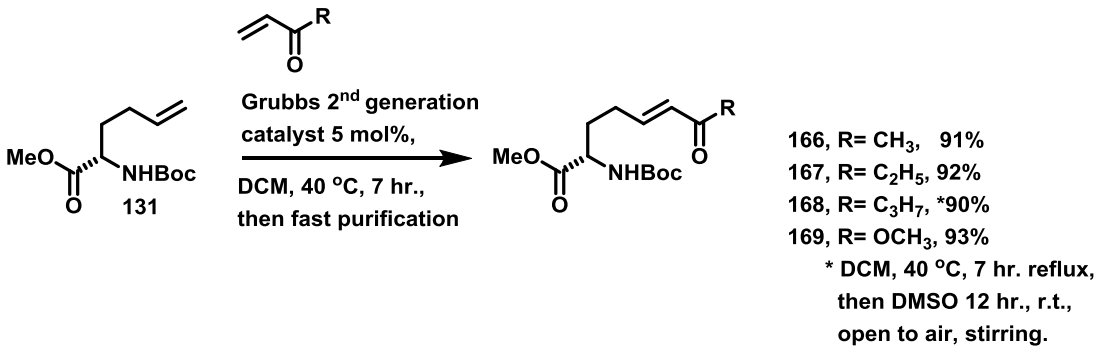
Scheme 36. Double protection of nitrogen atom in compound 131

Compound **162** was subjected to cross metathesis with different enones, in the precence of Grubbs 2nd generation catalyst. Modest yields of expected products **163-165** were obtained (Scheme 37). The low yield may be due to the absence of a free NH or the steric hindrance due to the presence of the second Boc group but the reactions were not optimized. While this did allow the isolation of the initially targeted enones, the introduction of additional step was not ideal.



Scheme 37. Cross-metathesis reaction of compound 162

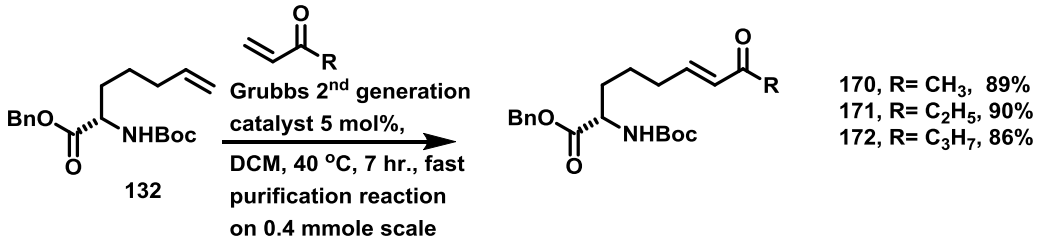
We decided to reinvestigate the use of the monoprotected derivative **131**. When the reaction was repeated purification was carried out using less silica gel (31 gm for 0.4 mmol reacting product) and more quickly (30 min), the desired cross-metathesis products **166-169** were isolated without any evidence of the pyrrolidines (Scheme 38), which suggested that it was indeed the prolonged exposure to silica gel that was inducing cyclization.



Scheme 38. Cross-metathesis reaction of compound 131, from mono *N*-protected serine

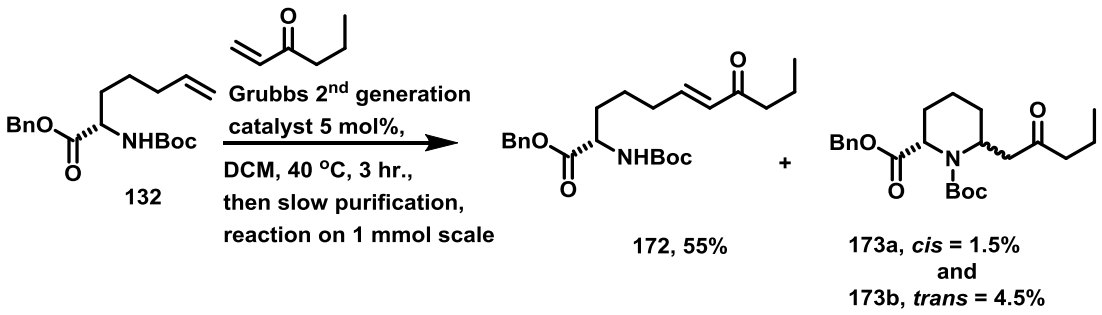
2.2.3.2 Synthesis of 8-oxo amino acids

Reaction of protected pentenyl glycine **132**, with 1-buten-3-one, 1-penten-3-one and 1-hexen-3-one in the presence of Grubbs 2nd generation catalyst, under standard cross metathesis reaction conditions, gave **170-172** each in over 80% yield.



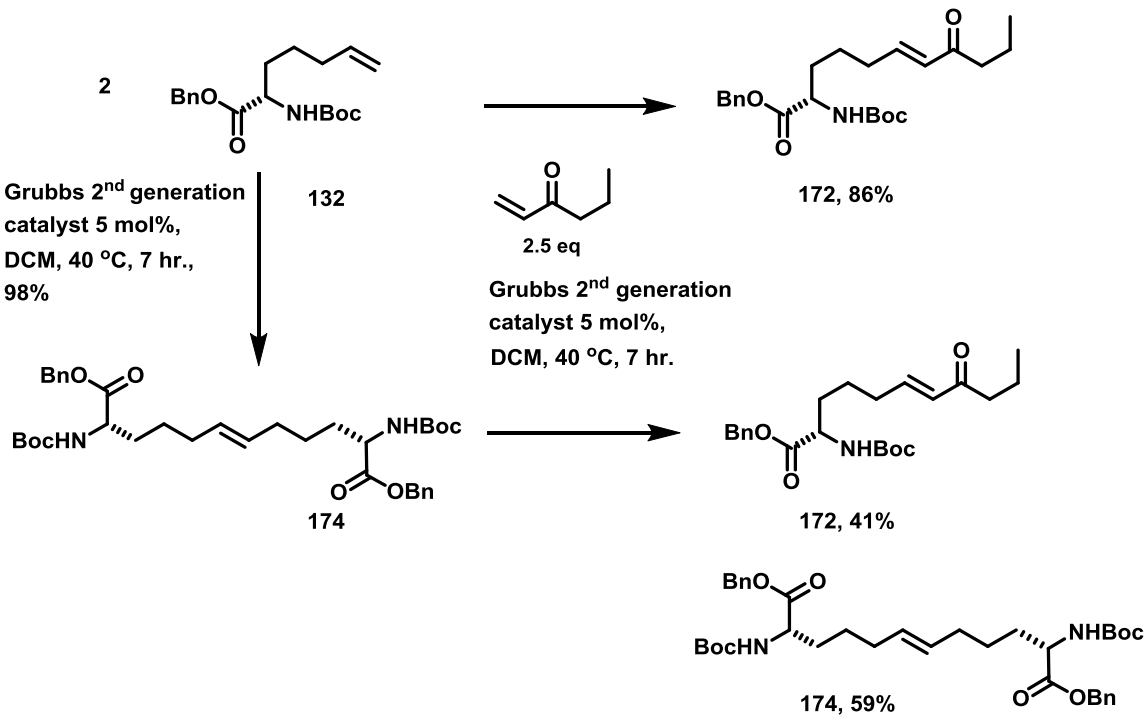
Scheme 39. Cross-metathesis reactions of compound 132, from protected aspartic acid

Interestingly, when the reaction with 1-hexen-3-one was repeated, and the product was purified slowly using a large silica gel column, small amounts of the cyclized products **173a/b** were isolated along with **172** (Scheme 40). After in-depth NMR analysis, the structure of these by products was assigned as the piperidine **173a/b**. It was not possible at this stage to identify which stereoisomer was cis and which was *trans*. Subsequently the configurations were assigned to the two isomeric piperidine derivatives **173a** (1.5%) and **173b** (4.5%) (*Vide infra*).



Scheme 40. CM and intramolecular aza-Michael reaction

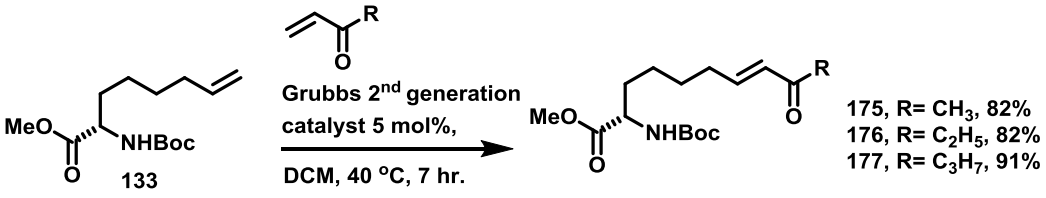
According to m/z (ES+) homodimers of **131-133** were found in the crude reaction mixture as expected. To better understand if the homodimer itself could be reacting with enone to generate products, another test reaction was carried out. When the alkene **132** was subjected to Grubbs 2nd generation catalyst in the absence of any enone, the homodimer **174** was isolated in excellent yield (98%). This was expected as, according to the literature,[59] terminal alkenes such as **132** are shown to undergo rapid homodimerization. Subjection of **174** to the standard cross-metathesis conditions with 1-hexen-3-one gave the expected product **172** (41%), together with recovered homodimer **174** (59%) (Scheme 41). Since the homodimer was not consumed under the reaction condition used for the initial cross-metathesis, we can conclude that it is not an intermediate in that process.



Scheme 41. Homodimer pathway

2.2.3.3 Synthesis of 9-oxo amino acids

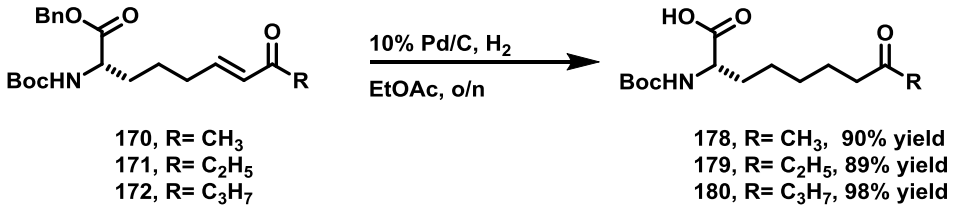
Cross-metathesis of protected hexenyl glycine **133** proceeded smoothly giving the expected enones, **175-177** (Scheme 42). No evidence for the formation of 7-membered ring-containing products was obtained, which is of course not surprising.



Scheme 42. Cross-metathesis reactions of protected hexenyl glycine with different enone

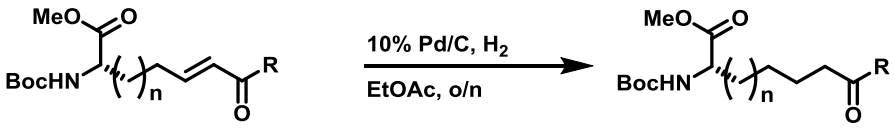
2.2.4 Hydrogenation of cross-metathesis products

With a range of enones available through cross-metathesis, the final step to prepare the target ω-oxoamino acids was hydrogenation. Hydrogenation was carried out at 1 atmosphere of H_2 and room temperature**,** in the presence of 10% Pd/C catalyst (Scheme 43 & 44) and the results are shown in table 3. In the case of the benzyl ester **170-172**, the reaction condition also resulted in the removal of the benzyl group.



Scheme 43. Hydrogenation of enone and removing of benzyl group

In case of the methyl ester **166-168** and **175-177**, the products were fully protected ω-oxo amino acids (Scheme 44).



Scheme 44. Hydrogenation of enone

Table 3. Yields for hydrogenation of cross-metathesis products 166-168 and 175-177

Entry	n	Substrate	R	Products	Yield %
1	1	**166**	CH_3	**181**	94
2	1	**167**	C_2H_5	**182**	99
3	1	**168**	C_3H_7	**183**	98
4	3	**175**	CH_3	**184**	94
5	3	**176**	C_2H_5	**185**	99
6	3	**177**	C_3H_7	**186**	99

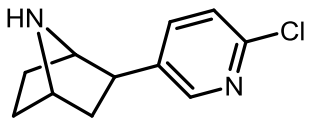
2.2.5 Conclusion

In conclusion we have shown that the cross-metathesis/hydrogenation, strategy is an efficient route for the synthesis of a wide range of Aoda analogues, and is arguably the most flexible route to this general class of compound. It was also established that cross-metathesis of the butenyl glycine derivative **131** can result in the formation of the pyrrolidines **159b** and **161b**, with high diastereoselectivity. Further investigation into this process is reported in chapter three.

Chapter 3: Synthesis of pyrrolidine & piperidine derivatives

3.1 Introduction:

As discussed in chapter two cross-metathesis of the three key starting materials **131-133** with a selection of enones gave the desired product, along with pyrrolidine by-products. Pyrrolidines, piperidines and their derivatives have emerged as important building blocks in the synthesis of natural products, biological active compounds and drug intermediates, for instance (-)- epibatidine (Figure 18).[93-100]

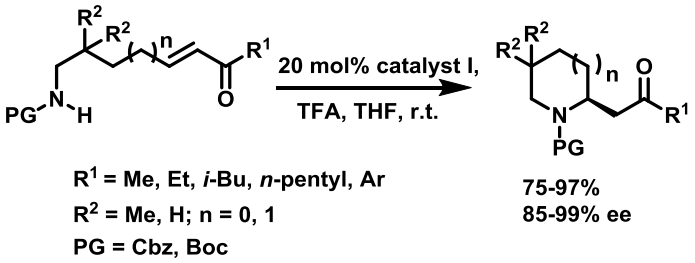


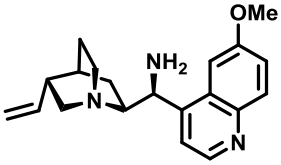
187

Figure 18. Isolated from skin extracts of Epipedobates tricolor

The preparation of enantiomerically pure compounds is essential in medicinal chemistry as shown by the fact that absolute configuration of many pharmaceutical constituents has a significant role on their biological activity.[101] With these factors in mind we decided to investigate further the stereoselective synthesis of pyrrolidine derivatives.

There are many examples in the literature of the use of the aza-Michael reaction to give pyrrolidines and piperidines. For example, intramolecular aza-Michael reaction of amino enones using a combination of the amine catalyst I and TFA as a co-catalyst gives *N*-heterocycles, in good yield and enantioselectivity[100] (Scheme 45).

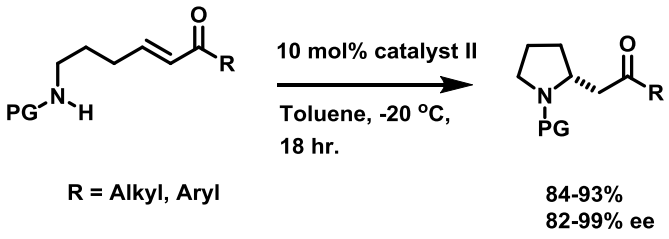


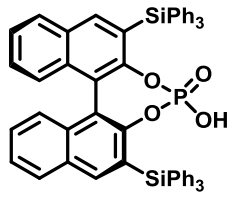


Catalyst I

Scheme 45. Synthesis of *N*-heterocycles using co-catalyst

Yo and co-workers found that a catalytic amount of TFA promoted cyclisation of amino enons, so they suggested that using a chiral Brønsted acid could induce enantioselectivity. After optimisation, it was found that use of the binaphthylphosphoric acid II in toluene at -20 ^{o}C induced cyclisation of a variety of enone carbamates to give 2-substituted pyrrolidines in good yield and with high enantioselectivity (Scheme 46).[102]

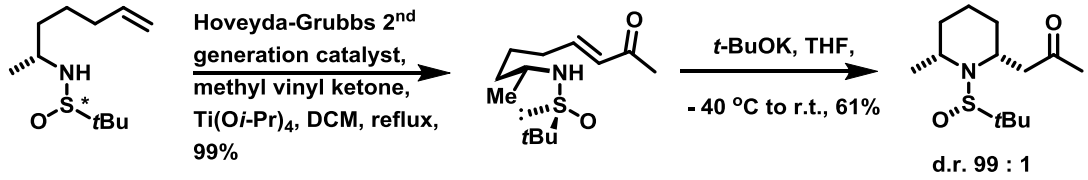




Catalyst II

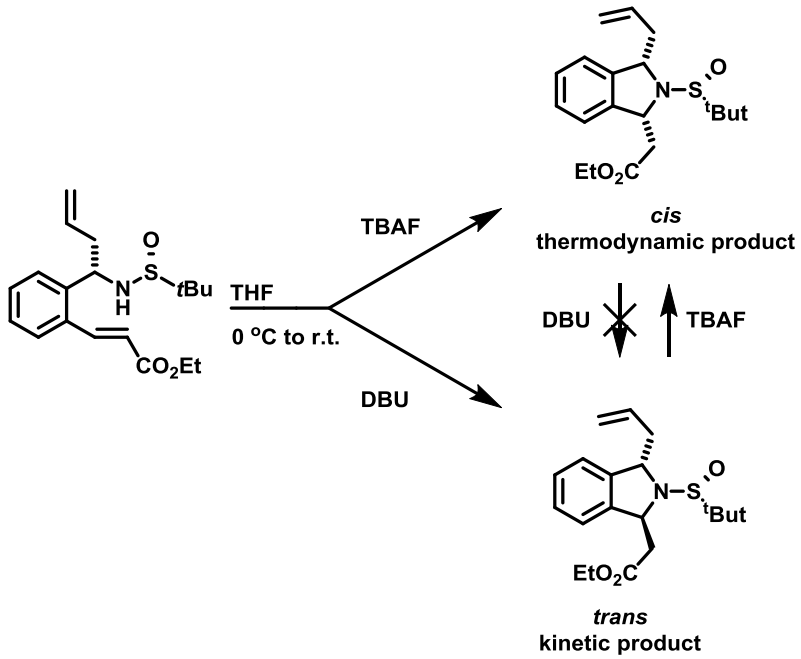
Scheme 46. Synthesis of pyrrolidines using phosphoric acid like a catalyst

Aza-Michael reaction can also be promoted using base. Cross-metathesis of the chiral sulfinamide with methyl vinyl ketone in the presence of Ti(O*i*-Pr)$_4$ followed by treatment with catalytic KO^{t}Bu, leads to the corresponding piperidine with high d.r. (Scheme 47).[99]



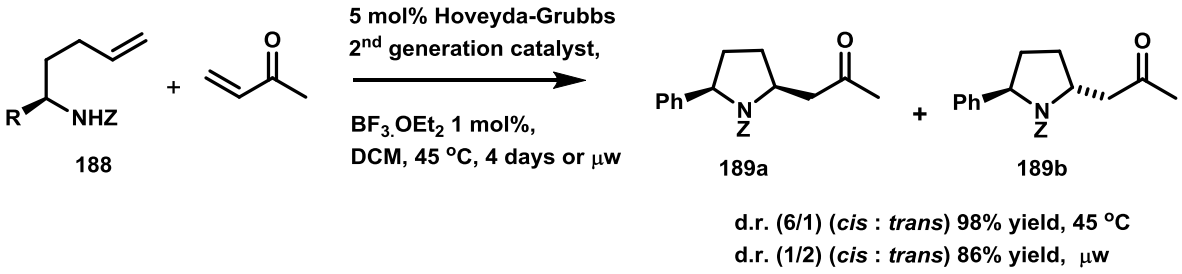
Scheme 47. Promote aza-Michael reaction by using base

A related cyclisation using tetrabutylammonium fluoride (TBAF) gave the *cis* product in good yield and as a single diastereoisomer, while use of DBU gave the *trans* product (Scheme 48). Later when the *trans* diastereoisomer was treated with TBAF complete epimerization to the *cis* product was observed. Then established that the *trans* product is formed under kinetic control, and the *cis* product is more stable.[103]



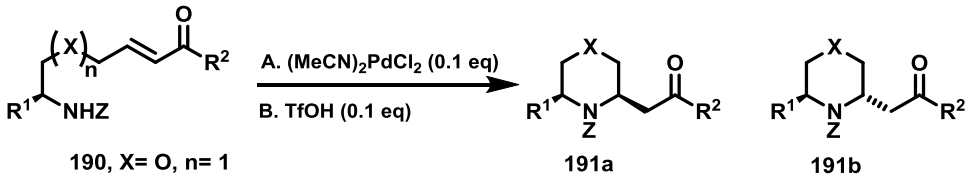
Scheme 48. Study on the thermodynamic/kinetic origin of *cis* and *trans*

In 2007, Fustero and co-workers[104] reported the formation of 2,5-disubstituted pyrrolidines and 2,6-disubstituted piperidines using a tandem cross metathesis intramolecular aza-Michael reaction, catalyzed by a Hoveyda-Grubbs 2nd generation catalyst in the presence of $BF_3.OEt_2$ and promoted either by microwave irradiation or heat. This process gave high yields under both thermal and microwave conditions (Scheme 49). This paper provides a clear precedent for the cyclization reported in chapter two to give **159**, **161** and **173a/b**, although the conditions for our cyclization are much milder.



Scheme 49. Fustero's tandem CM and cyclization reaction[104]

Subsequently Young *et al.*[105,106] reported the use of an achiral Pd^{2+} complex and different Brønsted acids to selectively obtain either the *cis* or *trans* isomer of chiral nitrogen-containing heterocycles using the aza-Michael reaction. Other researchers have also reported that transition metals can catalyze aza-Michael reactions.[11,107-109] When Pd^{2+} complexes are used, it believed that the reaction can involve two pathways: either Pd^{2+}, or a proton generated from hydrolysis of the transition metal complex, increases the Michael acceptor behavior of the enone.[11,107,109,110] Strong Brønsted acids are recognized for their catalysis of aza-Michael additions by protonation of the carbonyl of the enone, which increases its electrophilicity.[111] The initial conclusion drawn was that the diastereoselectivity of reactions to generate either *cis* or *trans* 3,5-disubstituted morpholines can be switched with different achiral catalysts (Scheme 50) (Table 4).



Scheme 50. General Scheme for the Diastereoselective Synthesis of Morpholines X= O, n= 1

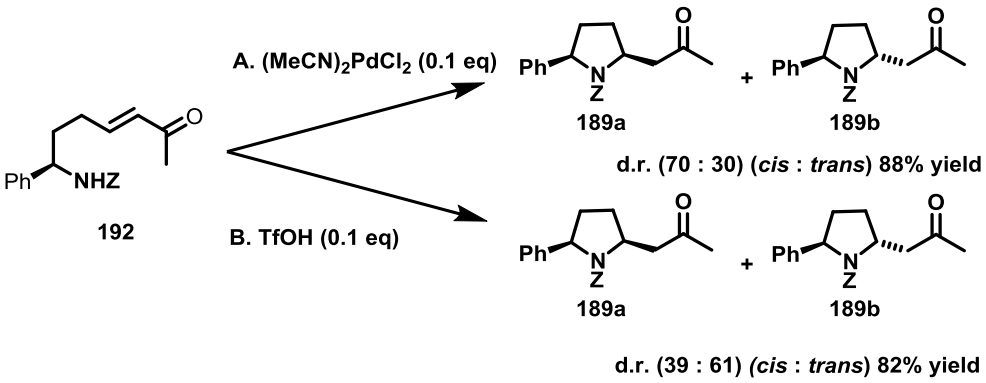
It was also found by Young *et al.*[105] that introduction of bulkier groups as the R^1 substituent resulted in slightly better diastereoselectivity than when they were present at the R^2 position. This is due to the fact that the R^1 substituent is closer to the point of ring formation, therefore allowing it to have more of an impact on the stereochemical outcome (Scheme 50). In the case of R^1 = CO_2Me, the *cis*-isomer was the major product under both sets of conditions. When R^1 = ^{i}Pr and Cy, use of Pd^{2+} gave preferentially the *cis*-isomer, but use of T*f*OH gave the *trans*-isomer (Scheme 46).

Table 4. Diastereoselectivity comparison between $Pd(MeCN)_2Cl_2$ and TfOH -catalyst[105]

R^1	R^2	conditions	Yield(%)	d.r. (*cis* : *trans*)
CO_2Me	CH_3	A	91	62 : 38
		B	93	76 : 24
^{i}Pr	C_2H_5	A	90	94 : 6
		B	85	14 : 86
Cy	CH_3	A	92	91 : 9
		B	95	11 : 89

A. $(MeCN)_2PdCl_2$ (0.1 eq), B. TfOH (0.1 eq)

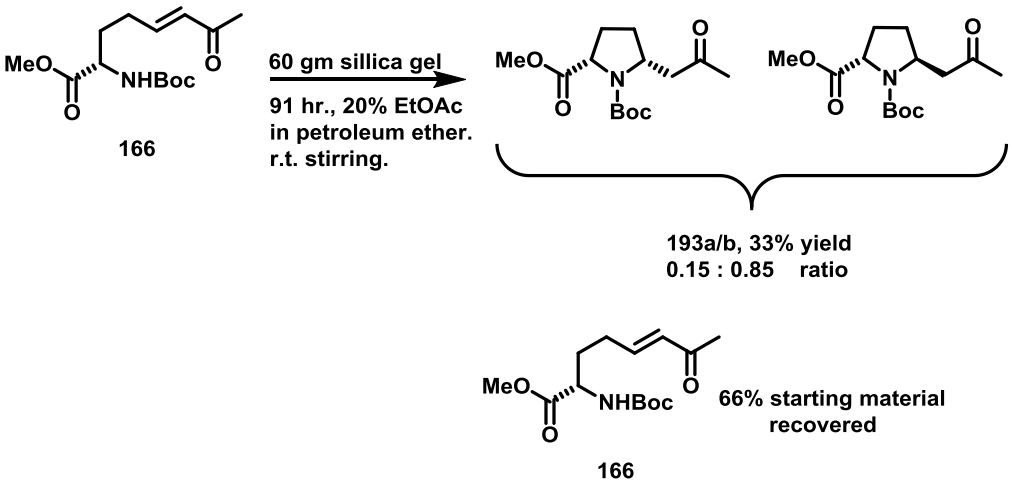
When the same conditions were applied by Young *et al*.[105] to the substrate **192** excellent conversions were recorded, with moderate diasteroselectivity (Scheme 51). Using Pd^{2+}, the major product was *cis*, while using T*f*OH the majore product was *trans*.



Scheme 51. Young *at el*. preparation 2,5-disubstituted pyrrolidines

3.2 Results and Discussion

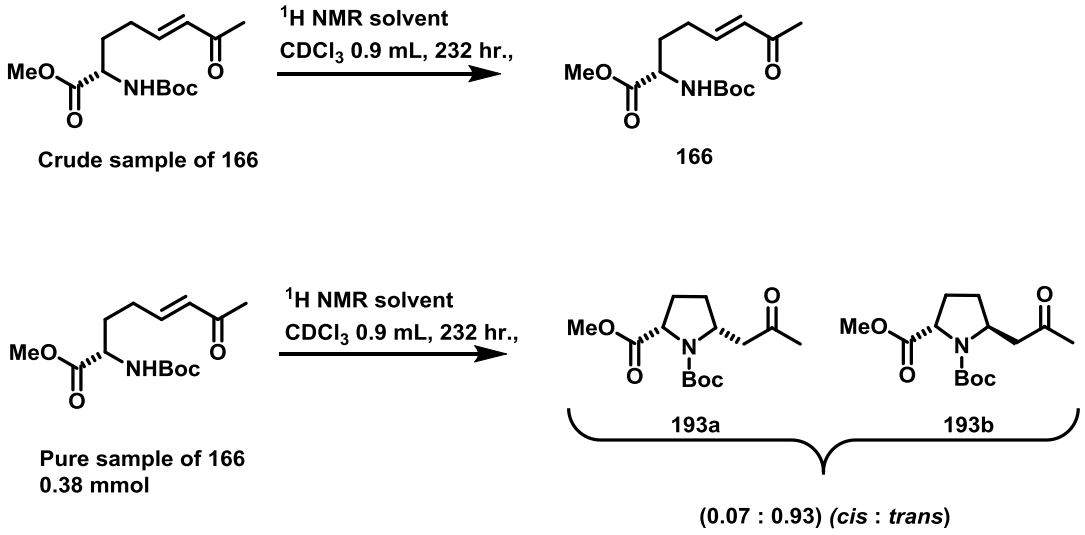
Our initial goal was to investigate cyclization more thoroughly when the cyclisation that we had observed occurred to give pyrrolidines. Since we had found conditions to prepare the enones **166-168**, we initially stirred **166** with excess silica gel in the same solvent used for the purification. After purification of the product by chromatography, a substantial amount of starting material (**166**, 66%) was recovered, along with an inseparable mixture of the pyrrolidines **193a** and **193b** (0.15 : 0.85) in 33% combined isolated yield (Scheme 52). The ratio of **193a** to **193b** was determined from ^{1}H NMR spectroscopy. This result was quite distinct from the very high levels of diastereoselectivity observed previously (Chapter 2), and cast doubt on our pervious interpretation that the highly diastereoselective cyclisation was promoted by chromatography.



Scheme 52. Cyclization of pure cross-metathesis product 166 using SiO_2

Given the literature precedent that the aza-Michael reaction can be catalysed by Brønsted acids, we considered that the cyclisation might be promoted by the NMR solvent, $CDCl_3$. A crude sample of **166** (20 mg) appeared to be stable in $CDCl_3$ (0.9 mL) for 232 hr. However, when a purified sample of **166** (109 mg, 0.38 mmol) was subjected to the same condition, quantitative cyclisation to give the pyrrolidine **193a/b** (0.07 : 0.93) was observed (Scheme 53). Our conclusion therefore is that the cyclisation that we had observed previously (Chapter 2) was occurring after the

NMR sample had been prepared, and before the spectrum was run; typically this delay was 2-18 hours. This means that a pure sample of enone **166** cyclised on standing in $CDCl_3$, but a crude sample is stable.

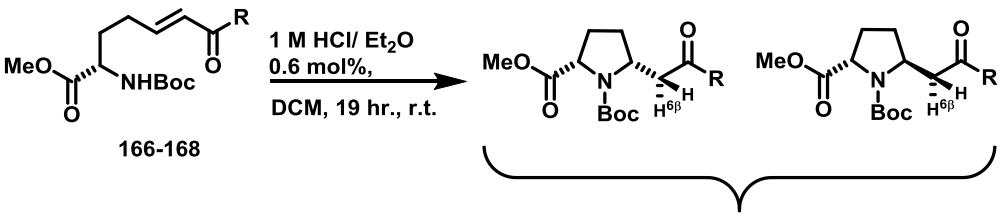


Scheme 53. Cyclization process in $CDCl_3$ with time at room temperature

3.2.1 Catalyst-induced cyclisation of cross-metathesis products

3.2.1.1 Synthesis of pyrrolidine derivatives

Interestingly in two cases when a pure sample of enone was allowed to stand in $CDCl_3$, no cyclisation was observed. Deliberate addition of HCl/Et_2O then promoted cyclisation. In order to avoid the variability associated with different samples of $CDCl_3$, it therefore appeared appropriate to investigate the use of HCl/Et_2O for cyclisation. The enones **166**-**168** were separately treated with HCl/Et_2O in dichloromethane (Scheme 54) and the results are shown in table 5. The diastereoisomer ratio for compound **193a/b** could be determined by ^1H NMR, but not for **159a/b** or **161a/b**. In this case GC was used, but it was established (entry 2) that both methods gave the same result for **193a/b**. Comparison of the ^1H NMR spectrum of the product obtained by cyclization of **166** with that already determined for **159b** and **161b**, allowed the assignment of the stereochemistry of **193b** as trans.



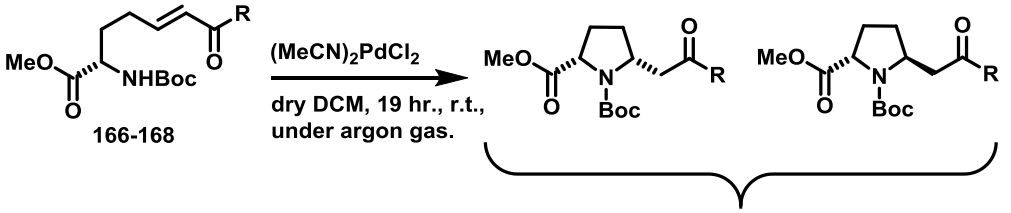
Scheme 54. Acid catalyzed cyclization process

Table 5. Obtained result of diastereoselective synthesis to 5-member ring

entry	Substrate	R	Product	conversion	*d.r. (*cis* : *trans*)
1	**166**	CH_3	**193a/b**	98.8 (1.2% SM)	0.06 : 0.94[a]
2	**166**	CH_3	**193a/b**	Complete	0.04 : 0.96[a,b]
3	**167**	C_2H_5	**159a/b**	Complete	0.02 : 0.98[b]
4	**168**	C_3H_7	**161a/b**	96 (4% SM)	0.02 : 0.98[b]

[a] Ratio determined with ^1H NMR, [b] ratio determined with GC, * NMR of crude reaction mixture

When compounds **166**-**168** were subjected to cyclization in the presence of a Pd^{2+} catalyst, the corresponding 2,5-pyrrolidine derivatives were formed (Scheme 55). In each case, a mixture of diastereoisomers was formed, and the ratio was determined by GC.



Scheme 55. Cyclization of pure cross-metathesis product by using Pd^{2+} catalyst

The results show that the diastereoselectivity of the cyclization was in each case low. In the case of **166**, the major diastereoisomer was the *cis*-compound **193a**, but in the case of the two homologues **167**, **168** there was either no diastereoselectivity or a very slight preference for the *trans* diastereosomer (Table 6).

Table 6. Obtained result of 5-member ring by using Pd^{2+}

R	Catalyst (mol%)	Product	conversion	*d.r. (*cis* : *trans*)
CH_3	25	**193a/b**	Complete	0.66 : 0.34[b]
C_2H_5	11	**159a/b**	//	0.5 : 0.5[b]
C_3H_7	11	**161a/b**	//	0.45 : 0.55[b]

[b] Ratio determined with GC, * NMR of crude reaction mixture

Figure 19 shows a possible mechanism to account for the cyclisation reaction using either Pd^{2+} or Brønsted acid catalyst.

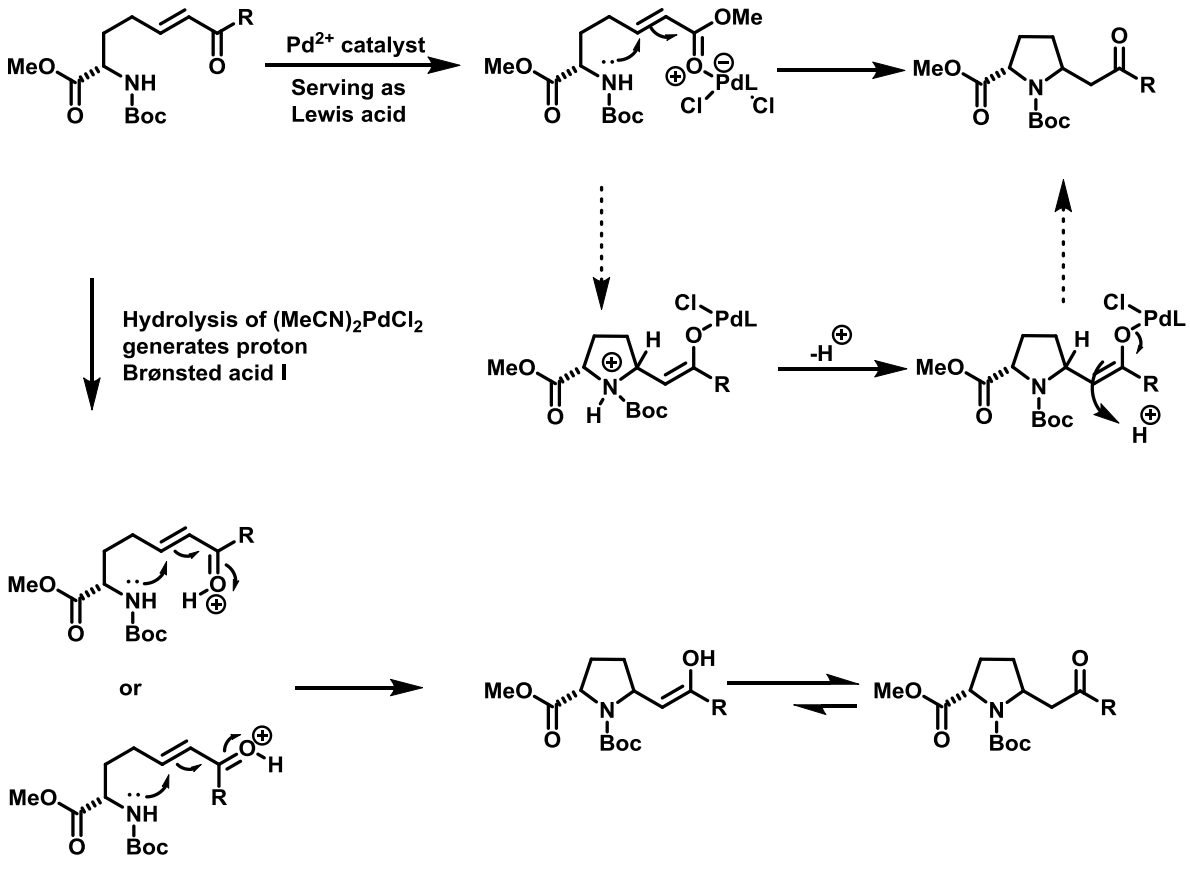
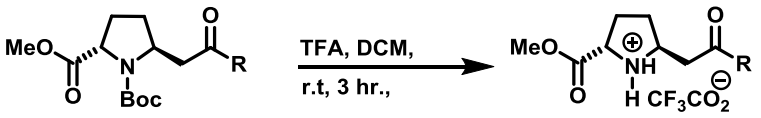


Figure 19. General cyclization mechanism

Boc deprotection for each of the compounds **193**, **159**, **161** using trifluoroacetic acid (Scheme 56) gave **194**-**196**. Fortunately the trifluoroacetate salts **194**-**196** were crystalline, and their structures are determined to be of *trans* configuration by X-ray diffraction (Figure 20). Since the structure of **159b** had already been established as the *trans*-pyrrolidine, this demonstrated that the deprotection reaction had proceeded without influencing the stereochemistry this means that the assignments already made by comparison of the ^{1}H NMR spectra of **161b** and **193b** with **159b** are confirmed.



When R = CH_3, 193b
R = C_2H_5, 159b
R = C_3H_7, 161b

R = CH_3, 194
R = C_2H_5, 195
R = C_3H_7, 196

Scheme 56. Boc deprotection to pyrrolidine

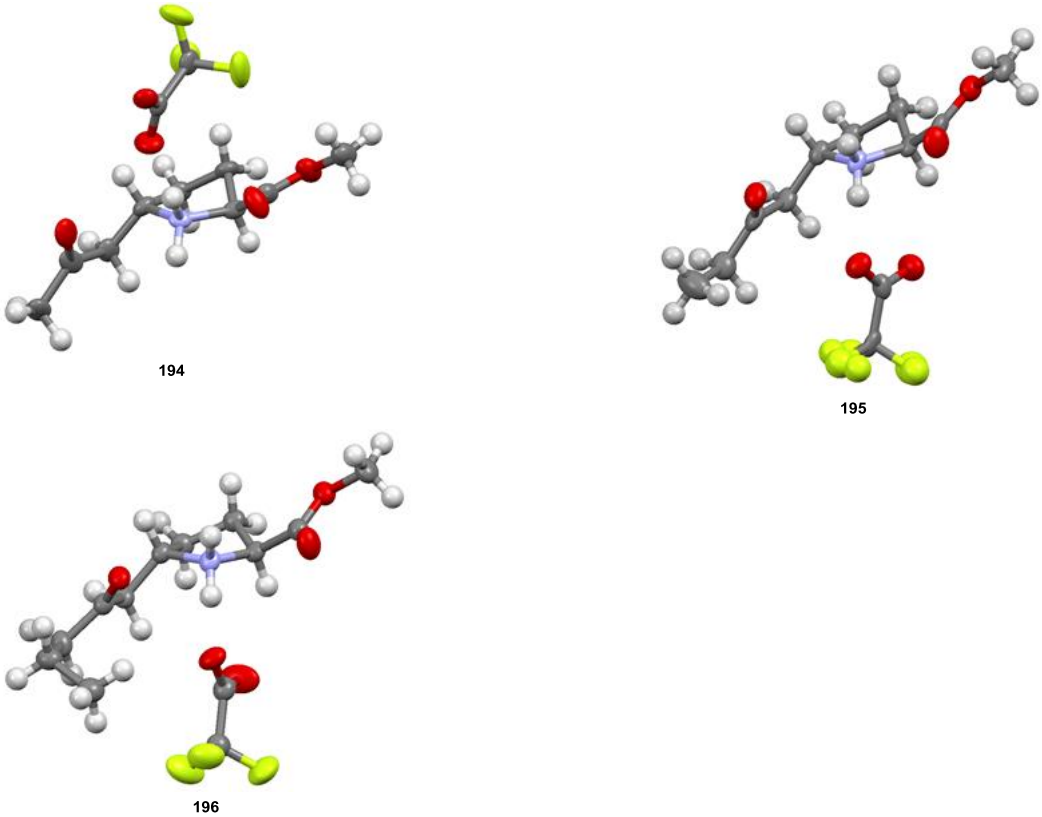
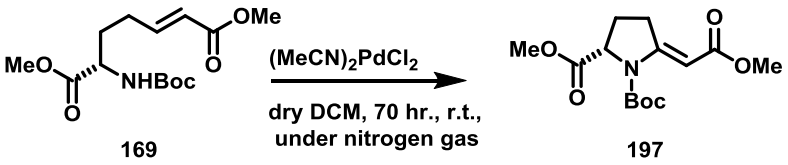


Figure 20. Crystallography shown trans configuration

When cross-metathesis product **169** (Scheme 38, p42) was treated with bis(acetonitrile)dichloropalladium(II) in dry DCM, the isolated product was identified as the vinylogous amide **197** (Scheme 57), whose structure was established by X-ray diffraction analysis (Figure 21).



Scheme 57. Oxidative cyclization of 169

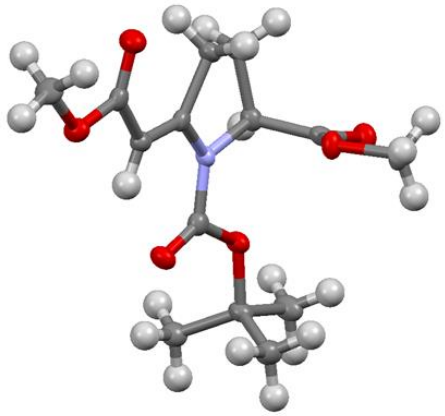


Figure 21. X-ray structure for compound 197

In the initial experiment three equal portions of catalyst (each 27 mole%) were added over a period of 70 hours. The product **197** was isolated in moderate yield (44%). When the reaction was repeated, but using less catalyst (11 mol%), the yield of **197** dropped to (9%). This suggested that the reaction may in fact be stoichiometric in Pd^{2+}. In an effort to re-oxidize the Pd^{2+} the reaction was conducted again (11 mol% Pd^{2+}) open to the air, but the yield of **197** dropped further. Finally, use of a stoichiometric amount of Pd^{2+} gave the product **197** in much higher yield (68%) (Table 7). When the crude compound or even a pure sample of compound **169** was dissolved in $CDCl_3$, no cyclization was observed.

Table 7. Cyclisation of using Pd^{2+}

entry	Catalyst (mol%)	Yield
1	81, added in 3 equal portions	44
2	11	9
3	11, open to the air	4.3
4	Stoichiometric amount	68

A suggested reaction mechanism showing cyclization followed β–hydride elimination is shown in figure 22. In principle, other appropriate oxidants might be capable of re oxidizing Pd^0 to Pd^{2+}, but this was not further explored.

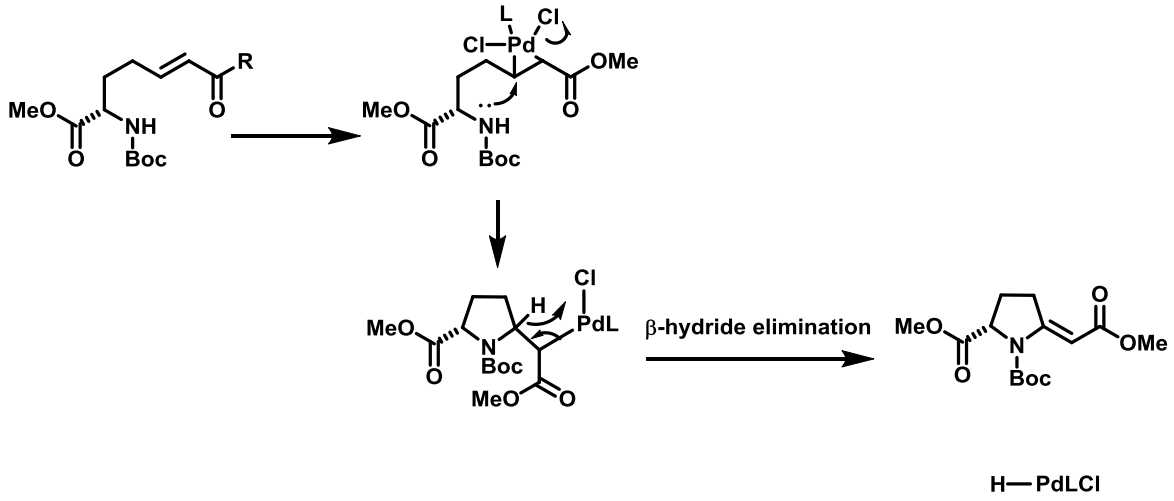
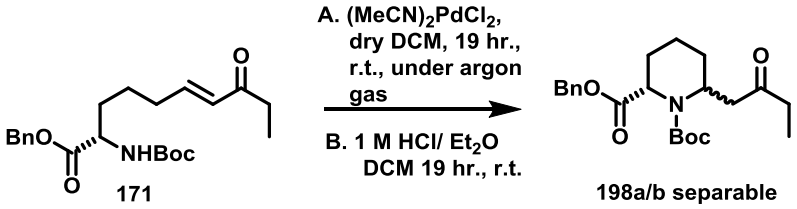


Figure 22. Cyclization & β- hydride elimination mechanism

3.2.1.2 Synthesis of piperidine derivatives

Treatment of the ethyl enone **171** with either $(MeCN)_2PdCl_2$ or HCl/Et_2O each resulted in cyclisation to give a separable mixture of the piperidines **198a** and **198b**. In each case the major isomer was the same (Scheme 58).



Scheme 58. Cyclization of cross-metathesis product by using Pd^{2+} and Brønsted- acid catalysts

Table 8. Obtained results to make compound 198 when different eq. of Pd^{2+} catalyst was used

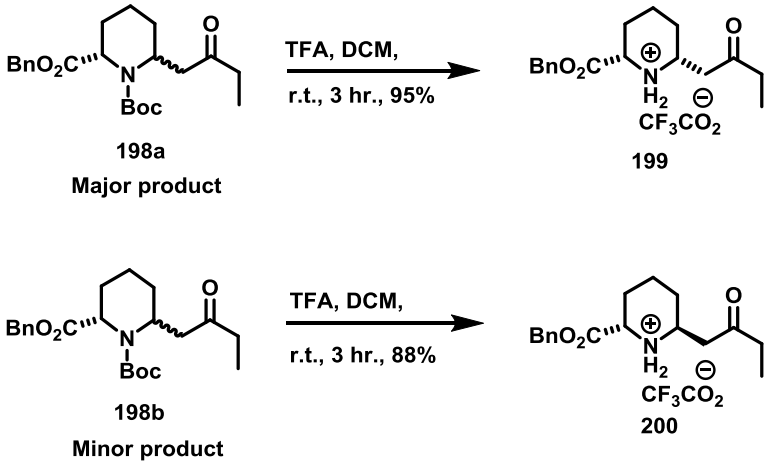
Reaction Conditions	Catalyst (mol%)	Yield%	*d.r. (*cis* : *trans*)
A	125	99	0.74 : 0.26
A	36	*Q	0.74 : 0.26
A	14	*91 (9% SM)	0.66 : 0.34
A	12	92 (4% SM)	0.7 : 0.3
B	0.6	20 (79.5% SM)	0.83 : 0.17

A : $(MeCN)_2PdCl_2$ mol%, dry DCM, 19 hr., r.t., under argon gas

B : 1 M HCl/Et_2O dry DCM 20 hr., r.t.

Q = quantitative, SM = starting material, * NMR of the crude reaction mixture

In order to elucidate the relative stereochemistry of products from the aza-Michael reaction, both purified diastereoisomers **198a** and **198b** were separately subjected to deprotection using trifluoroacetic acid (Scheme 59). Fortunately the trifluoroacetate salt **199** was crystalline, and its structure was determined to be *cis* by X-ray diffraction (Figure 23). Therefore the second diastereoisomers **200** as is **198b** *trans*.



Scheme 59. *N*-Boc deprotection of 198a/198b

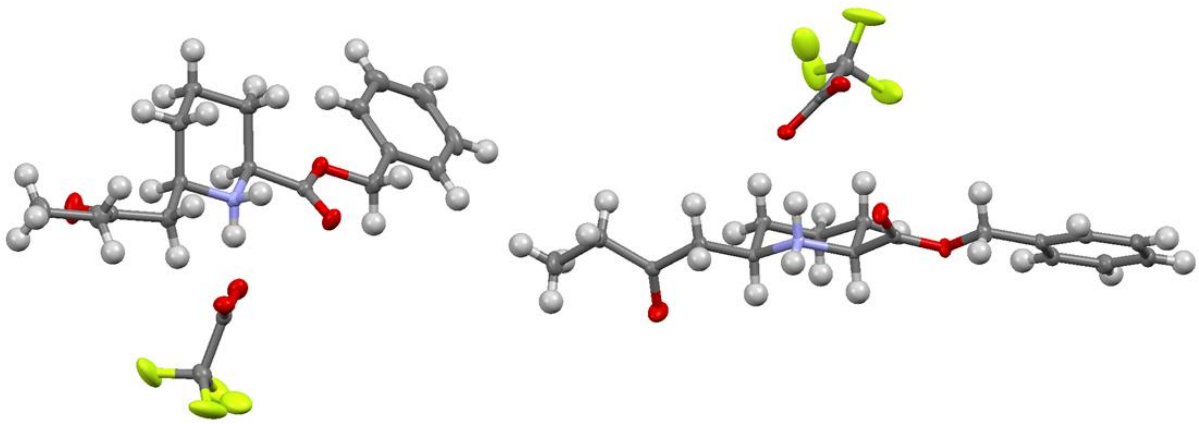
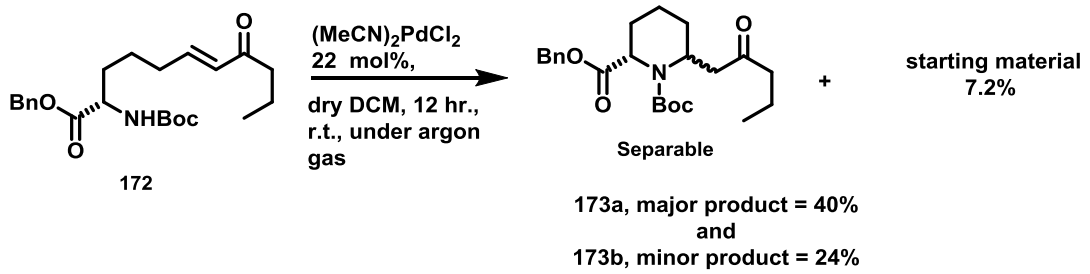


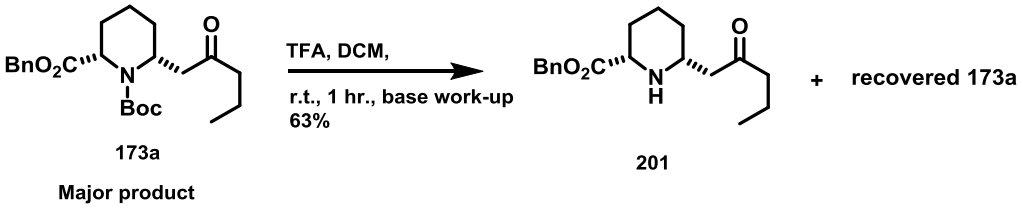
Figure 23. X-ray structure of compound 199

When compound **172** was subjected to cyclization conditions in the presence of Pd^{2+} catalyst (catalyst added in two equal portions each 11 mol% during 12 hr.) it gave two separable diastereoisomers **173a/b** (Scheme 60). NMR of the crude reaction mixture suggested that the isomers **173a** and **173b** had been formed in (0.62 : 0.38) ratio, and the isolated yields of **173a** and **173b** were 40% and 24% respectively. The close correlation between the diastereoisomers ratio determined from the NMR of the crude product, and the isolated yields, is very satisfactory.



Scheme 60. Cyclization of cross-metathesis product by using Pd^{2+} catalyst

When Boc deprotection was carried out for **173a** (1 hr.) followed by basic work-up it gave a mixture of **201** and **173a**, which suggests that the reaction time was not sufficient**.** When the reaction time for deprotection of **173b** was extended (from one hour to three hours) compound **202** was produced in excellent yield (Scheme 62). On the basis of the close precedent provided by the ethyl ketone **198a**, we tentatively assigned *cis* stereochemistry to the major product.



Scheme 61. *N*-Boc deprotection of 173a and neutralization

NOE studies were then performed to establish the relative stereochemistry. When H^3 in compound **201** was irradiated, H^7 was enhanced (C); irradiation of proton H^7 produced the expected nOe for proton H^3 (Figures 24) (B), therefore nOe suggests compound **201** is the *cis* diastereoisomer.

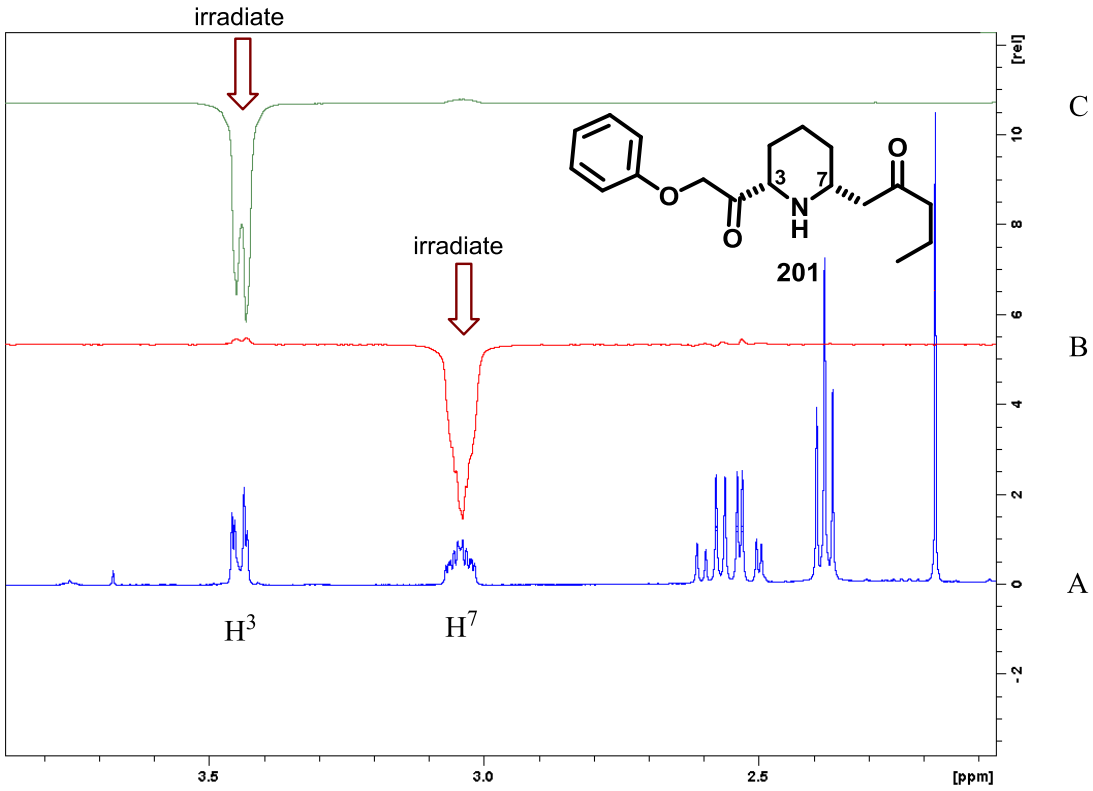
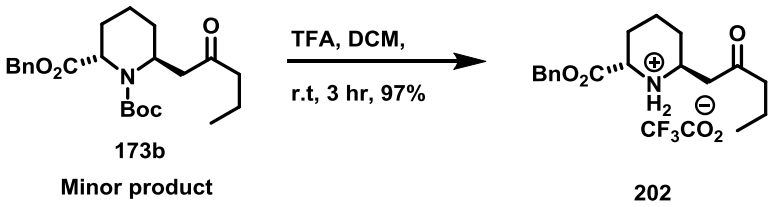


Figure 24. NOE when H^3 and H^7 in compound 201 irradiated



Scheme 62. *N*-Boc deprotection of 173b

Since **201** was established to be of *cis*-configuration the implication is that **202** is the *trans*-isomer. This was confirmed by the close similarity of the ^{1}H NMR spectra for compounds **202** and **200** (Figure 25). The configuration of **200** had already been determined be *trans*, on the basis that the structure of the stereoisomer **199** was determined as *cis* by X-ray crystallography.

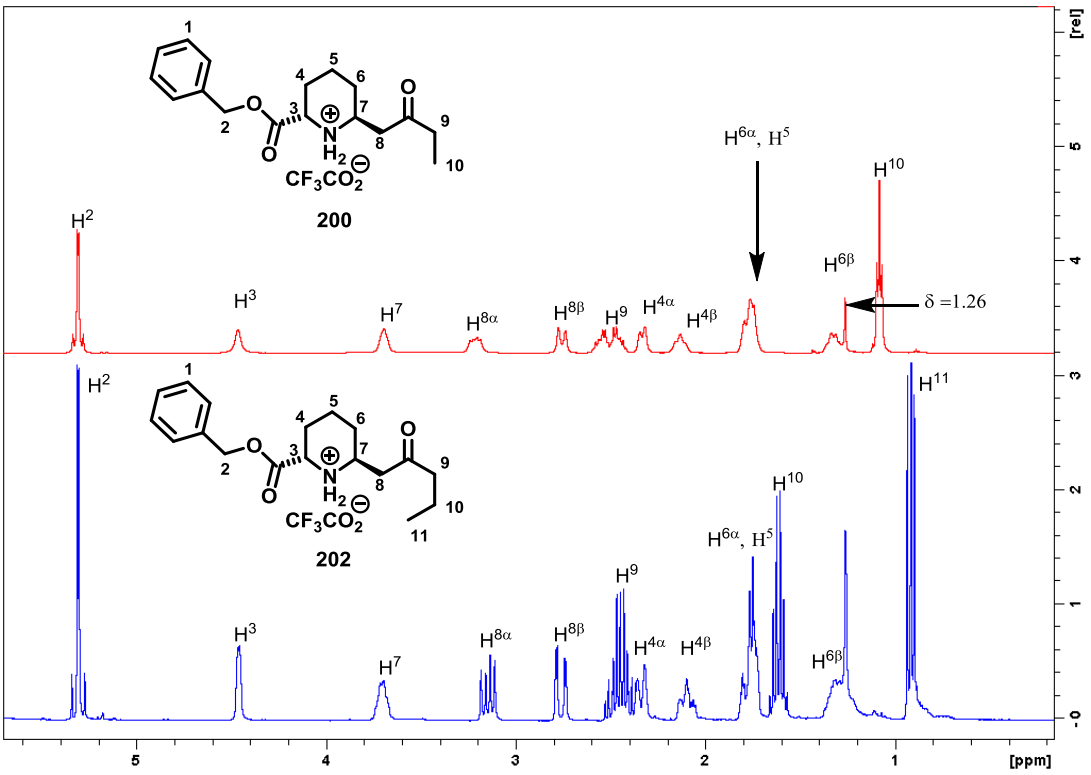
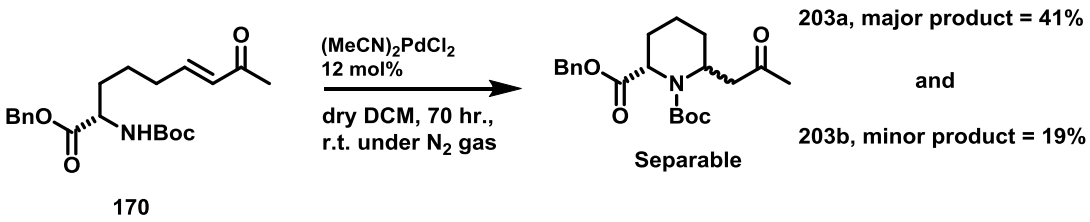


Figure 25. ^{1}H NMR comparison of compounds 200 and 202 to show *trans* configuration

Treatment of the enone **170** with a catalytic amount of $(MeCN)_2PdCl_2$ under conditions shown in scheme 63 gave a separable mixture of two diastereoisomeric piperidines **203a** and **203b**. NMR of the crude reaction mixture suggested that the isomers **203a** and **203b** had been formed in a (0.74 : 0.26) ratio; the isolated yields of **203a** and **203b** were 41% and 19% respectively. These were assigned as the *cis* and *trans* isomers on the basis of the close precedent provided by the ethyl ketone **198a/b**.



Scheme 63. Cyclization of cross-metathesis product by using Pd^{2+} catalyst

When nOe studies were conducted on each of the separated isomers **203a** and **203b**, no enhancements were observed. While this is the expected outcome for the *trans*-isomer, it suggests that the *cis*-isomer exist in a conformation in which the 2- and 6- substituents exist in an axial conformation, therefore the two protons H^3 and H^7 are each equatorial, and therefore distant from each other (Figure 26).

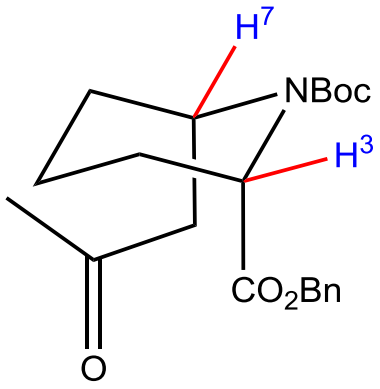


Figure 26. Suggested conformation of diastereoisomer 203a

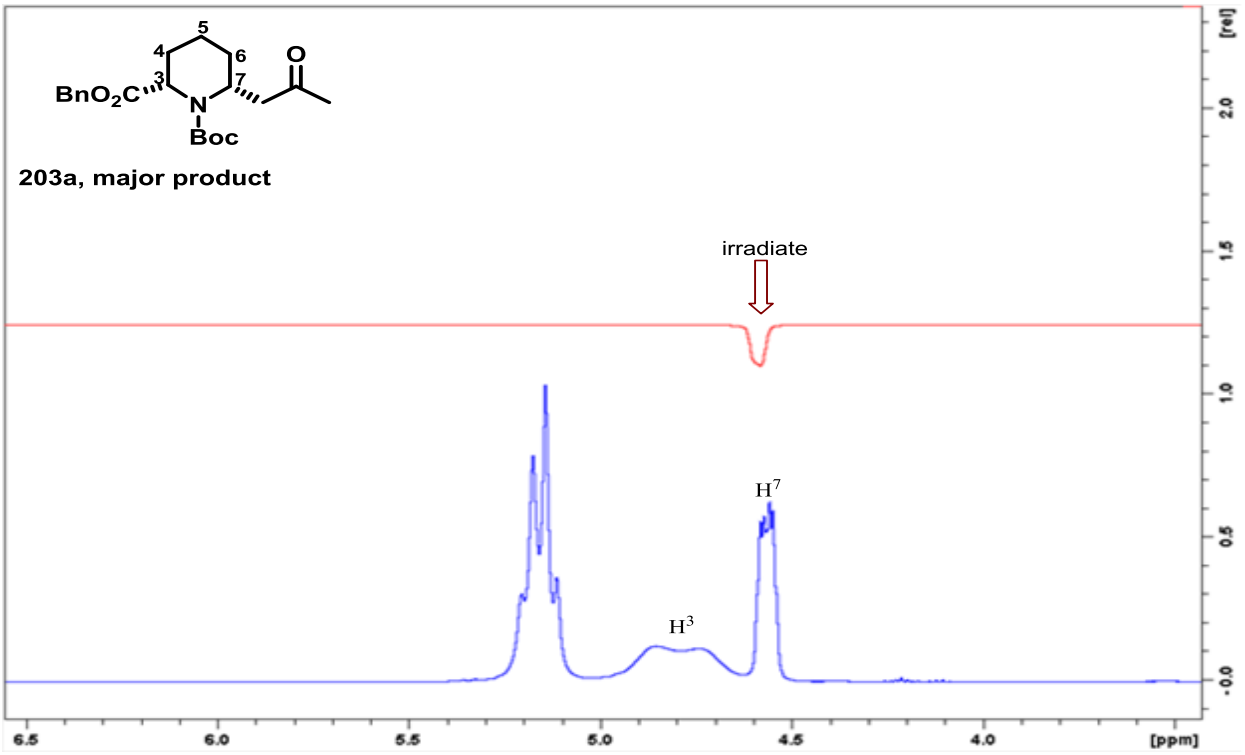


Figure 27. NOE when H^7 in compound 203a irradiated

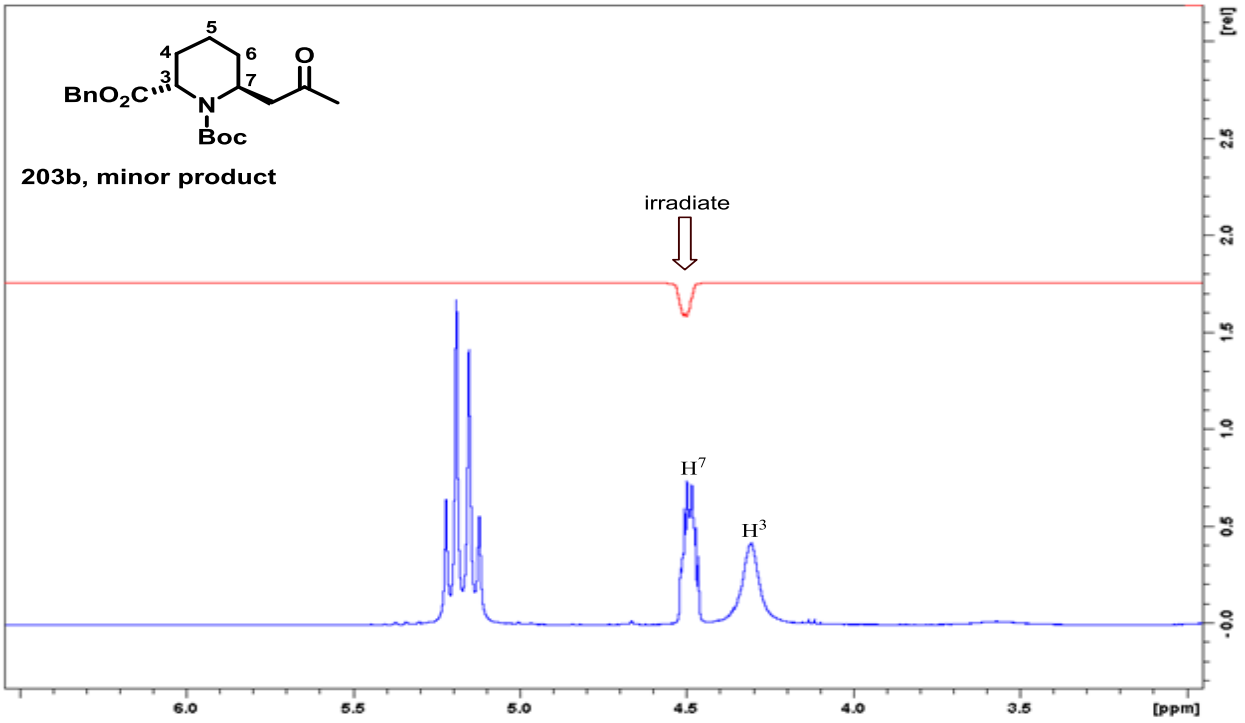
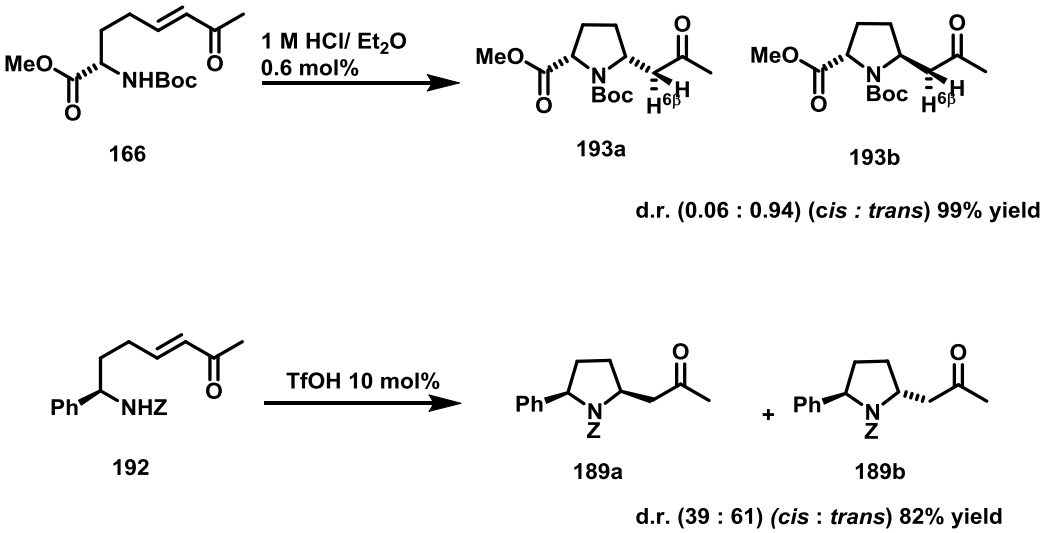


Figure 28. NOE when H^7 in compound 203b irradiated

3.2.2 Conclusion:

We have identified a highly stereoselective cyclisation of Boc-protected amino acids, containing an enone in the side chain using HCl/Et_2O leading to *trans* 2,5-pyrrolidines in excellent yields and high diastereoselectivity; the Boc group results in much higher trans selectivity compared to the *Z*-protected analogues reported by Young *et al*.[105] It is possible to whether the different is due to the CO_2Me or Boc-protection.



Scheme 64. d.r for two analogues with different protecting group

Chapter 4: Synthetic Approaches to Cyclic Tetrapeptides

4.1 Introduction:

With efficient routes both to the ω-oxoamino acids and a clear understanding of cross-metathesis and hydrogenation reactions needed for the side-chain of apicidin and its analogues, attention turned to making the target molecule (Figure 29).

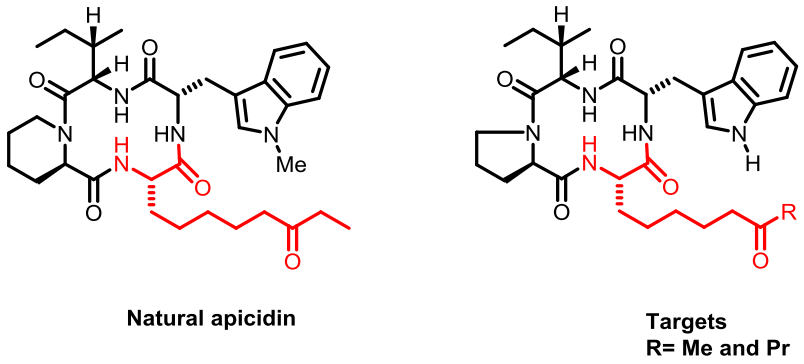


Figure 29. Natural apicidin and target molecule

Solid-phase synthesis is a method in which the growing molecules are bound on an insoluble resin. This method was initially developed in the 1963 in order to synthesize peptides.[112] In chapter one the synthesis of linear tetrapeptides (**49**, **57**, **66**) were described as precursors to chlamydocin **5**,[45] trapoxin **4**[33] and analogues of natural apicidin **68**.[42] These peptides (Figure 30) were made by classical solution phase synthesis (SPS).

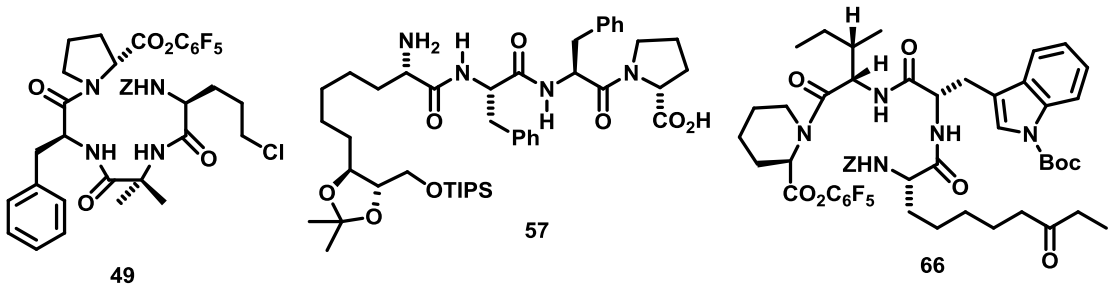
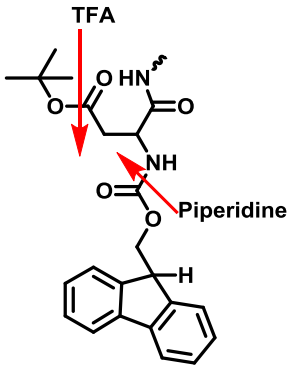


Figure 30. Linear tetrapeptides made by SPS

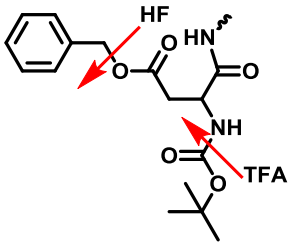
One characteristic advantage of SPPS is that it enables the use of an excess of reagents, in relation to the resin, to force the reaction to completion ensuring high yields in each step. Secondly, the work-up only includes filtration and washing to remove the excess of reagents and other soluble by-products.[113] There is no need to purify the growing peptide sequence by chromatography until after it is cleaved from the resin, and therefore peptide synthesis can be automated. The downside to SPPS is that the resin is not soluble in most NMR solvents; therefore it makes analysis of peptides on solid phase difficult, so only after cleavage from the resin is characterization possible. Qualitative tests such as a ninhydrin test can show the success of the coupling by showing whether free amine group remain. In solution phase, intermediate purification is needed after each coupling to remove by-products and this has an impact on yield due to mechanical loss, and increasing the cost.[114]

Since SPPS is more efficient we decided to use this approach to prepare the linear tetrapeptide precursor rather than solution phase synthesis.

In addition there are two general approaches to SPPS- Fmoc and Boc (Figure 31); protecting group strategy (Boc or Fmoc) is important as it has a direct impact on cleavage conditions.[114] In this project Fmoc protection method was preferred over Boc method due to ease of cleavage of the peptide from the resin.



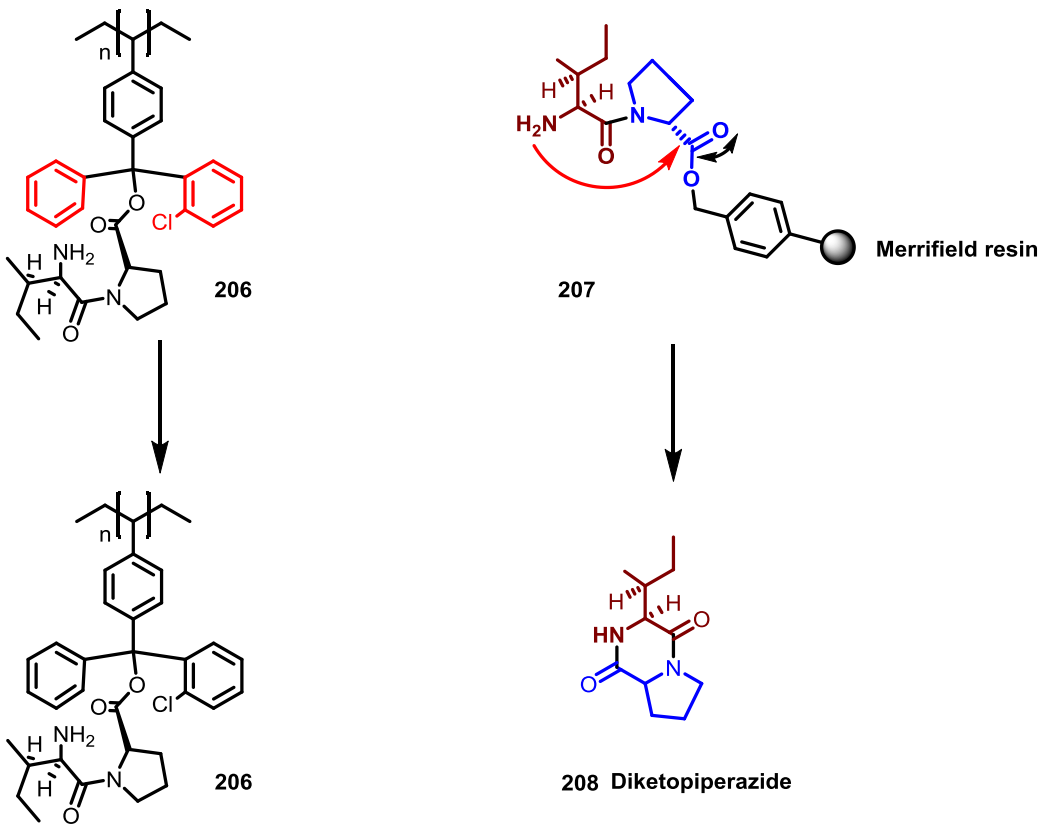
Fmoc
204



Boc
205

Figure 31. Deprotection and cleavage using Fmoc and Boc strategies[115]

The most important factor for resin selection is stability and loading capacity. The 2-chlorotrityl chloride resin (2-ClTrt-Cl) resin was chosen because it is stable under the basic conditions used for Fmoc deprotection, and mild acidic conditions are required for release of the peptide sequence. The resin can help prevent formation of diketopiperazide during attachment of the first two amino acid, due to the steric bulk of 2-ClTrt-Cl group (Scheme 65) and also minimizes racemization during attachment of the first amino acid[113]. Finally DMF is chosen as the solvent since it causes the resin to swell, allowing access to the growing peptide chain for the introduction each new residue.



Scheme 65. Steric bulk to prevent diketopiperazide

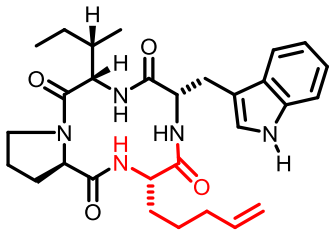
Ring strain is one of the major difficulties in making cyclic tetrapeptides, with its rigid twelve atom backbones. Due to strong conjugation in peptide bonds, the *trans* conformation is favoured which makes it difficult for the amino and carboxyl termini to be in close geometric proximity to react, leading to tough activation conditions and long reaction times.[17,114] Literature precedent[116] suggests that slow cyclization rates enhance side reactions such as oligomerization. Turn inducing elements in the linear peptide are important in making small head to tail CTP,[17] so in this project *R*-proline was used since it is known to induce β-hairpin structures thus making the peptide cyclisation easer.

In chapter one, the synthesis of the cyclic tetrapeptide **5** and **4** was described *via* formation of a bond between the *C*-terminus of the *R*-proline residue and the *N*-terminus of the Aoe residue in the linear tetrapeptide **49** and **57**. Therefore in this project cyclisation between the *C*-terminus of the *R*-proline residue and the *N*-terminus of pentenyl glycine **209** was chosen.

4.2 Results and Discussion

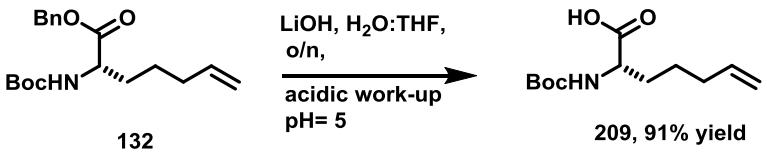
4.2.1 On resin linear tetrapeptide synthesis

The key precursor for our planned peptide modification was the cyclic tetrapeptide **210**. Three of the four residues were commercially available, and the fourth was easily prepared by hydrolysis of **132** (Scheme 66) using LiOH in (H_2O : THF) to give compound **209** in 91% yield.



210

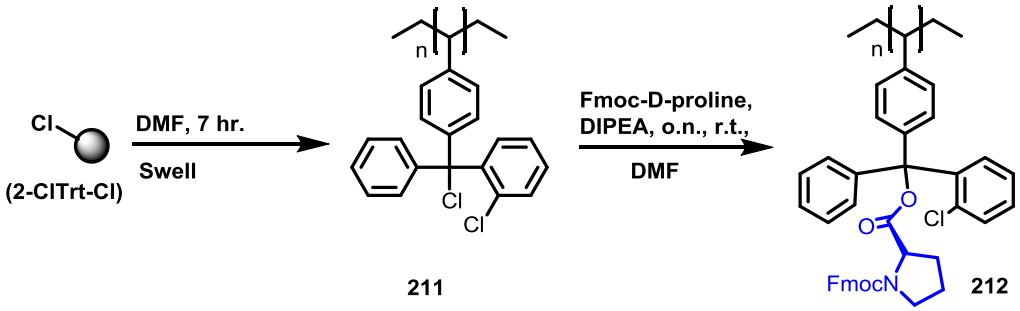
Figure 32. Cyclic tetrapeptide



132 **209, 91% yield**

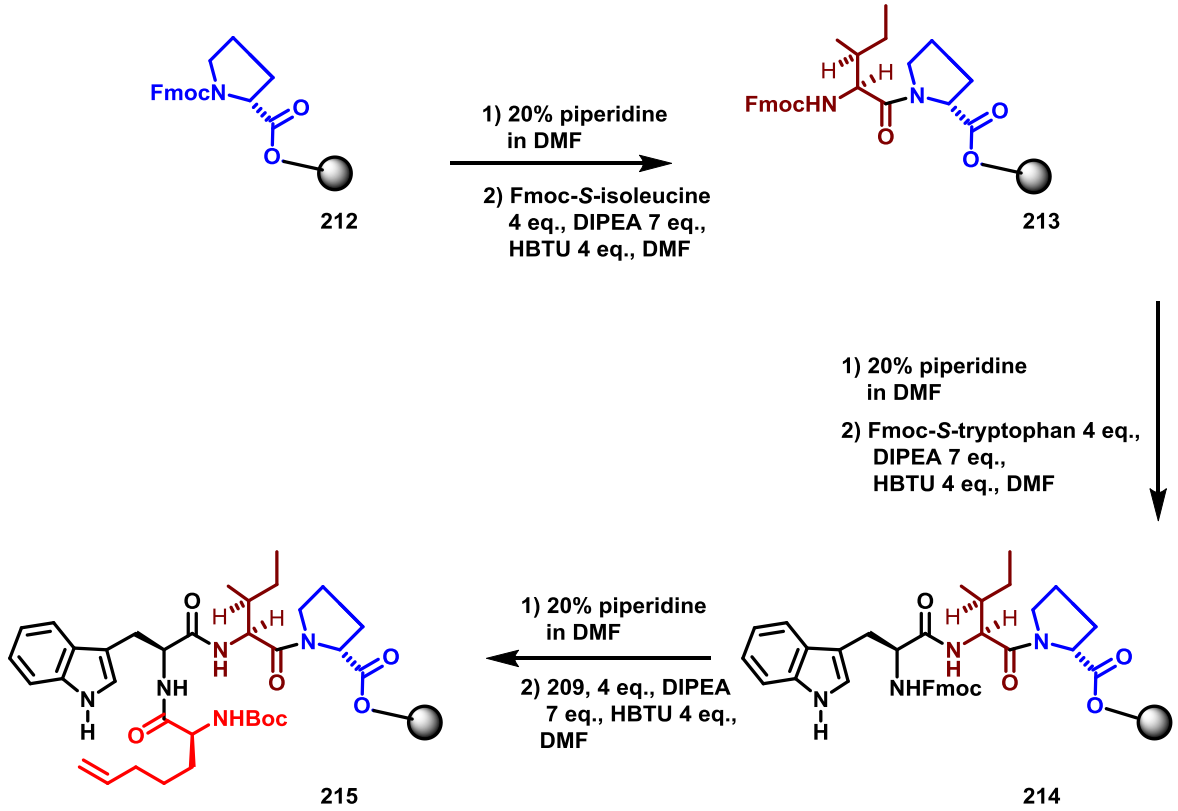
Scheme 66. Hydrolysis of ester group

The first step involves attachment of Fmoc-*R*-proline 4 eq. to the 2-ClTrt-Cl resin **211** using *N*,*N*-diisopropylethylamine to neutralise the HCl produced. The solution was allowed to drain from the resin, which was then washed with mixture of (DCM/ MeOH/DIPEA), followed by DCM and DMF (Scheme 67). Fmoc deprotection of the residue in compound **212** was carried out using piperidine (25% in DMF) to generate the free *N*-amino end group of the residue.



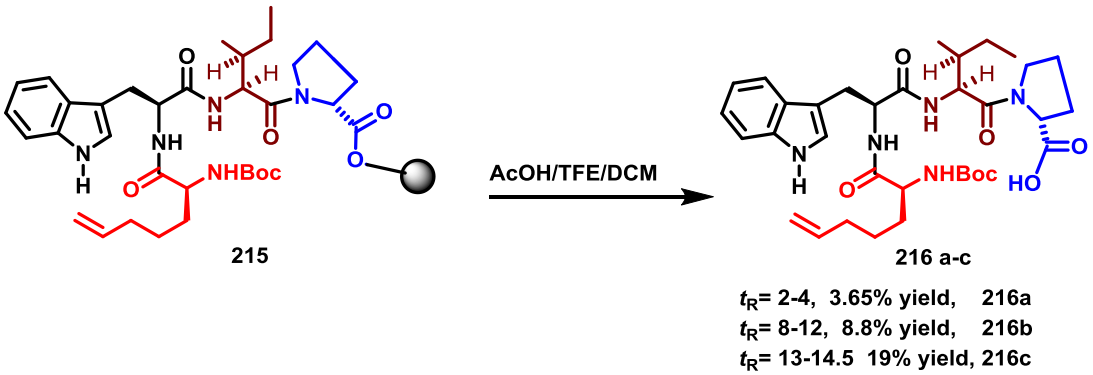
Scheme 67. Attaching first amino acid to the solid support

Then subsequent additions to complete the required peptide sequence were carried out using Fmoc-*S*-isoleucine, Fmoc-*S*-tryptophan and compound **209** under the same conditions, each time using 4 eq. of the protected amino acid. After each coupling and Fmoc deprotection, washing was carried out with a series of solvents that allow the polymeric support to swell so any by-products that may have formed during the coupling reaction were removed. HBTU was used as the coupling agent, since it is claimed to minimize racemization, also DIPEA used as a base.



Scheme 68. Growing amino acids on resin to make required sequence

Finally, the desired sequence was cleaved from the polymeric support using a mixture of (AcOH/TFE/DCM) (2 : 2 : 6) containing a structure enhancing solvent TFE to increase β-hairpin conformation.[117] The crude peptide solution was allowed to drain from the resin which was then washed with cleavage mixture, combined solution concentrated under reduced pressure to give compound **216** in 31% overall yield after HPLC. Since we have four amino acids in the sequence one can assume an average 83% yield for each step (Scheme 69).

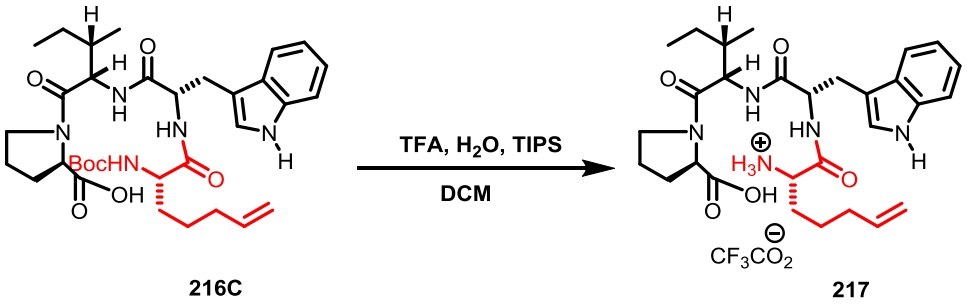


Scheme 69. Detaching the peptide from the resin

The crude product **216** was purified by HPLC and the isolated fractions of retention times, t_R= (2-4) = **216a**, t_R= (8-12) = **216b** and t_R= (13-14.5) = **216c**. According to m/z (ES+) found the same M.wt to them; **216a** = 662.3507, **216b** = 662.3515 and **216c** = 640.3710 , but **216a/216b** ionized by Na^+ and **216c** ionised by H^+, of the three compound isolated, **216c** was the highest yield also it was purest according to ^{13}C NMR and it was therefore chosen as the cyclisation substrate.

m/z (ES+) data and NMR suggested isolation of three isomers of identical molecular weight however, since HBTU was used (a coupling reagent designed to avoid racemization) might hope that the diastereoisomer did not result from use of this reagent.

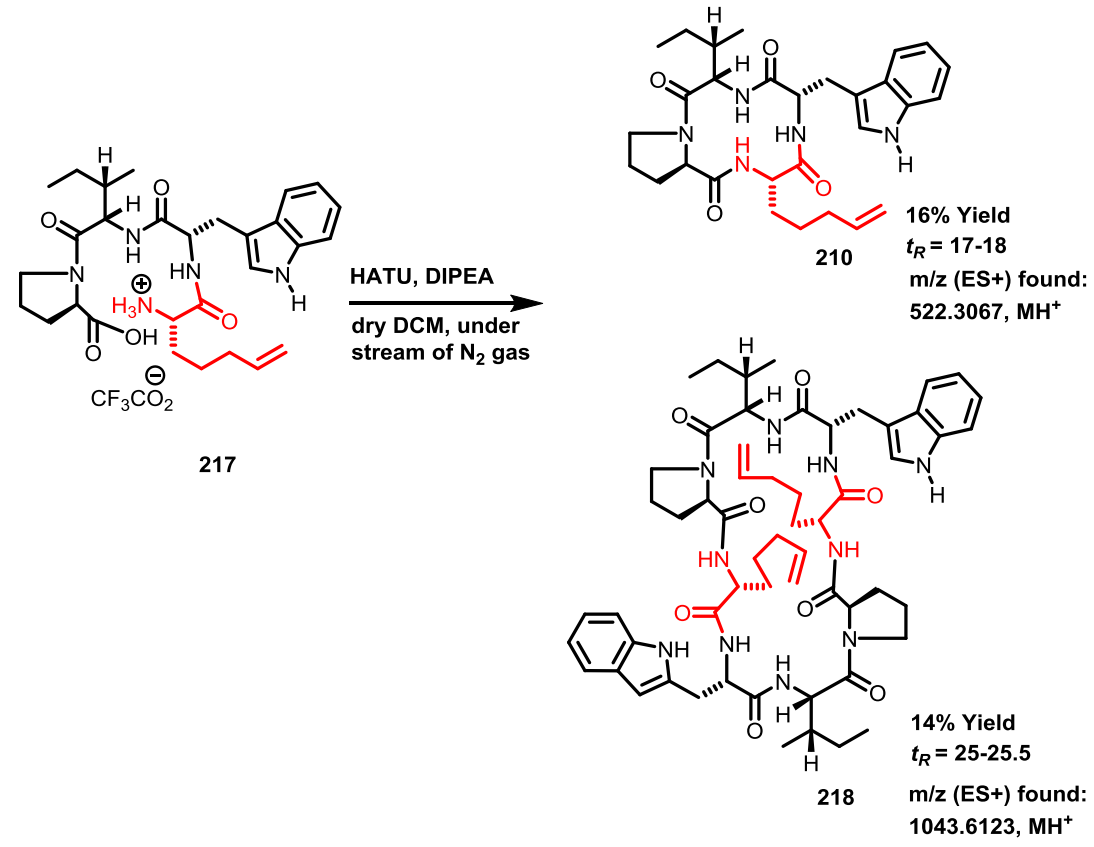
Treating *R*-Pro-Ile-Trip-Boc-α-pentenyl glycine **216c** with moderate strength acid, trifluoroacetic acid (TFA) in DCM gave **217** in quantitative yield (Scheme 70); in the presence of TIPS scavenger to avoid electrophilic aromatic substitution on the indole ring via *tert*-butyl (^{t}Bu) cation that is produced during the Boc deprotection of **216c**.



216C **217**

Scheme 70. Boc-deprotection

Cyclization of **217** was achieved between the *C*-terminus of the *R*-proline residue and *N*-terminus of the α-pentenyl glycine residue, via head-to-tail condensation by dropwise addition of LTP **217** to offer pseudo dilution to avoid homo dimerization. After purification of the crude product by HPLC beside the target molecule **210** (16%), **218** (14%) was also isolated (Scheme 71) with no sign of homodimers in the crude reaction mixture even after checking the HPLC fractions by m/z (ES+). However, the presence of **218** is evidence that dimerisation does happen, which then undergoes a cyclisation.



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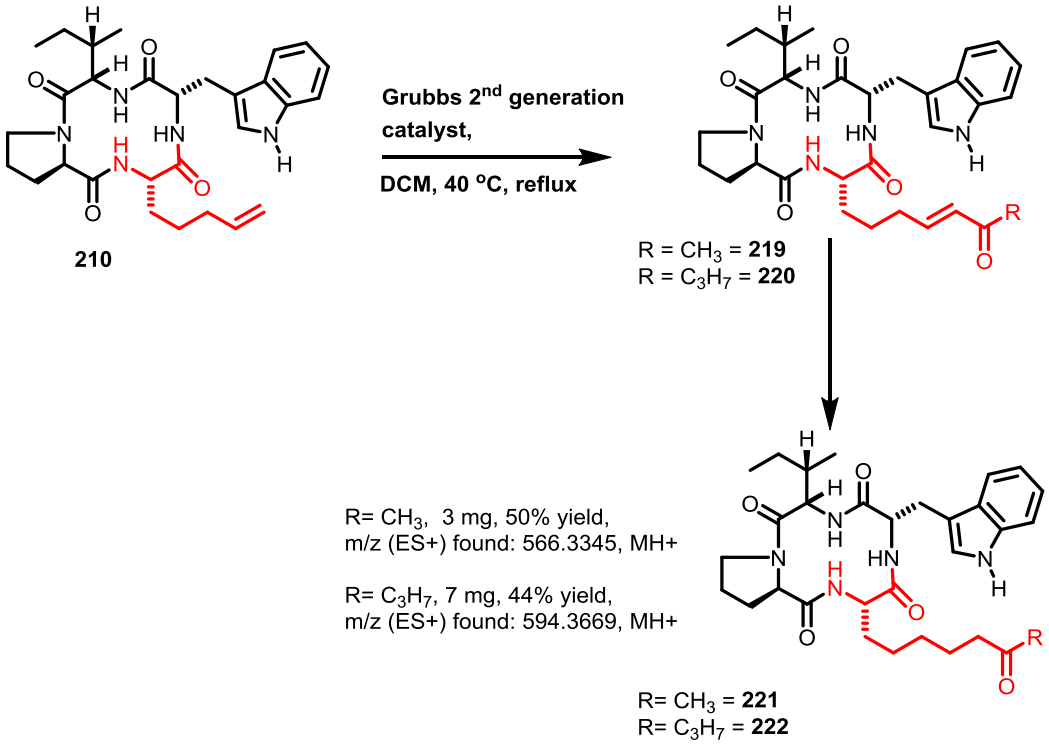
Scheme 71. Peptide cyclization

Compound **210** was subjected separately to cross metathesis reaction in two different batches with 1-hexen-3-one and 1-buten-3-one. Rather than isolating the presumed enones **219** and **220** the crude products were each subjected to hydrogenation. This allowed the isolation of the two cyclic peptides **221** (Figure 33) and **222** (Figure 34) in reasonable yields (Scheme 72).



Scheme 72. Cross-metathesis of cyclic tetrapeptide & hydrogenation

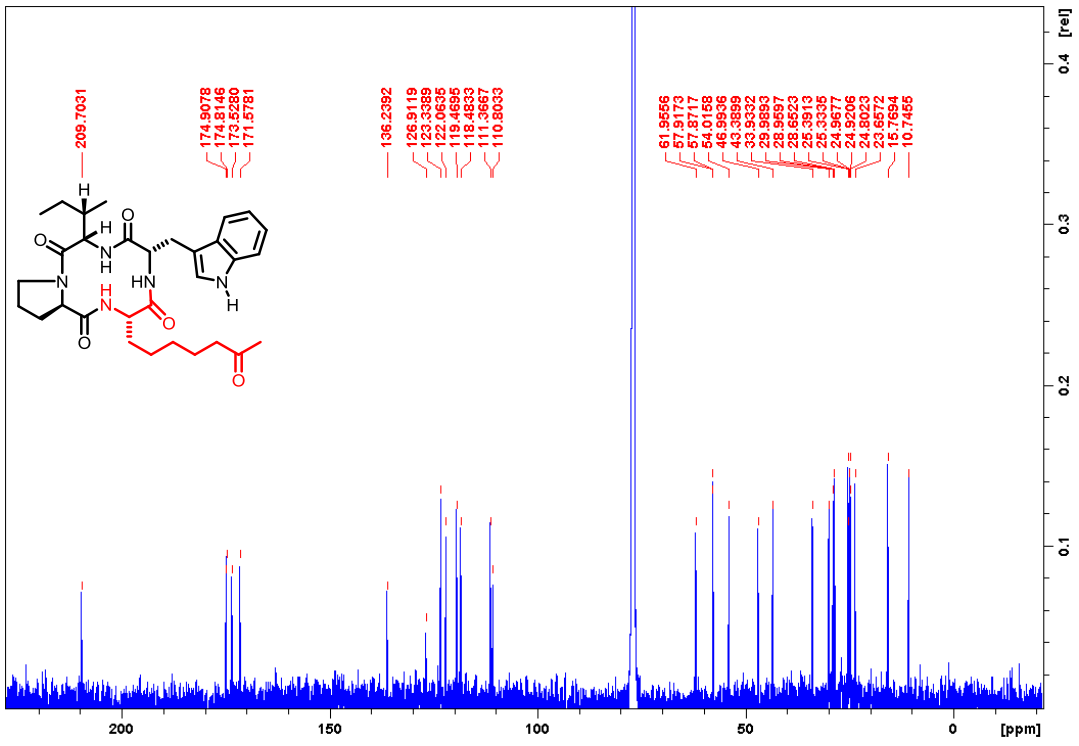


Figure 33. ^{13}C for compound 221

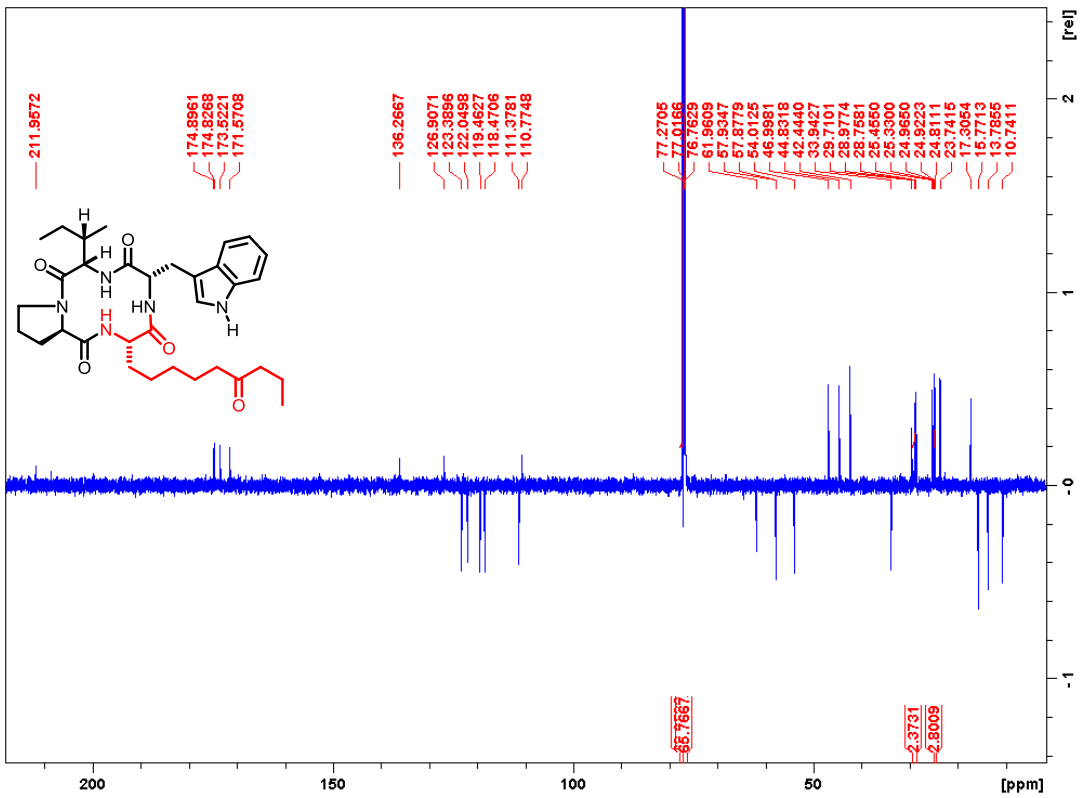
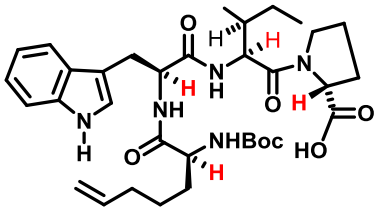


Figure 34. ^{13}C for compound 222

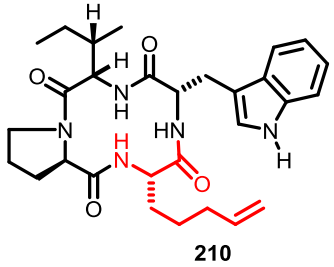
4.2.2 Conclusion:

During the synthesis of linear tetrapeptides epimerization was identified as a problem in chain elongation on 2-ClTrt-Cl resin, with three isomers **216a-c** isolated. Cyclisation of the linear tetrapeptide **216c** using HATU as coupling agent gave the cyclic tetrapeptide **210** (16%), together with the cyclic octapeptide **218** (14%). Successful modification of the side chain of the CTP **210** was achieved via cross-metathesis/hydrogenation to give compounds **221** 50% yield and **222** (44%) yield, respectively.

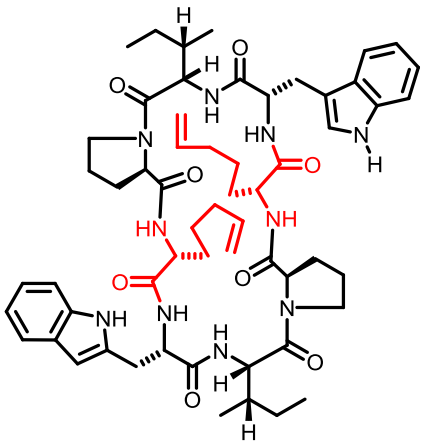


216 a-c

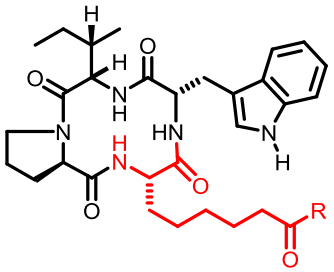
t_R= 2-4, 3.65% yield, 216a
t_R= 8-12, 8.8% yield, 216b
t_R= 13-14.5 20% yield, 216c



210



218

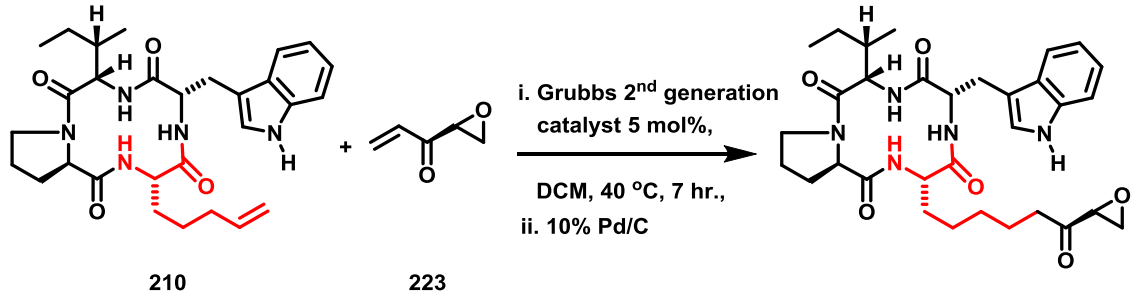


R= CH_3 = **221**
R= C_3H_7 = **222**

Figure 35. Some of the made compounds in this project

Future work

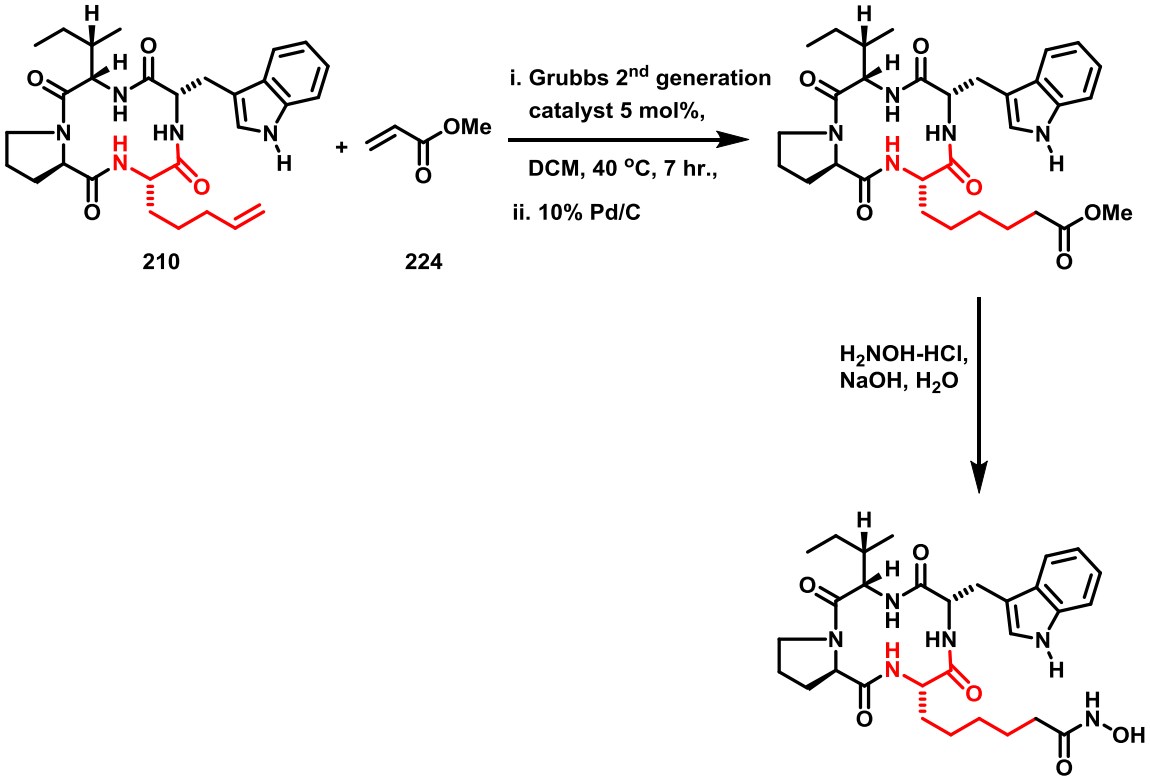
Our next target is making analogues of apicidin which incorporate α-epoxyketone and hydroxamic acid side chains. A possible route to introduce the epoxy ketone could be cross-metathesis/hydrogenation reaction between CTP **210** and the epoxyenone **223** (Scheme 73).



Scheme 73. Analogue of apicidin including α-epoxyketone

As already described in chapter one introduction of a hydroxamic acid group in the side chain to generate analogues of chlamydocin gave compound **10** with HDACi activity, so it would be interesting also to incorporate a hydroxamic acid in apicidin.

In order to make the hydroxamic acid, cross-metathesis/hydrogenation of **210** with methyl acrylate **224**, followed by conversion of the methyl ester to the hydroxamic acid seems viable (Scheme 74).



Scheme 74. Analogue of apicidin including hydroxamic acid

Chapter 5: Experimental

5.1 General

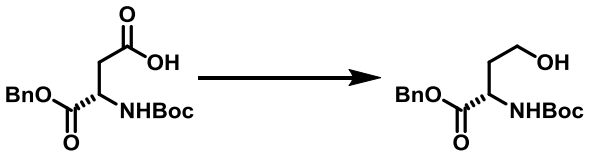
All moisture/air sensitive reactions were carried out under a nitrogen or argon atmosphere. All reagents used were purchased from commercial sources or prepared and purified accordingly by literature procedures. Iodide **140** was prepared by the literature method.[84,85] Compounds **147** and **152** were purchased from Sigma-Aldrich, or made by the literature method.[88,118] furthermore Fmoc-*R*-proline, Fmoc-*S*-isoleucine and Fmoc-*S*-tryptophan were purchased from Novabiochem VWR. Solvents used were HPLC grade; all other dry solvents were obtained from the in-house Grubbs dry solvent system (model: SPS-200-6). Solvent evaporation under reduced pressure was performed using a Büchi rotary evaporator. Organic extracts were dried over $MgSO_4$ or Na_2SO_4. Purification by column chromatography was performed using silica gel for flash chromatography. Thin layer chromatography was performed using pre-coated plates, and compounds visualised by UV light (254 nm), ninhydrin solution (5% in MeOH).

NMR spectra were recorded using Bruker AC 400 or Av III 400 or Bruker DRX 500. Coupling constants are given in Hertz to the nearest 0.1 Hz and were rationalized. ^{13}C NMR spectra were recorded at 100 MHz or 125 MHz. Optical rotations were measured on a Perkin Elmer 241 automatic polarimeter at λ 589 nm (Na, D-line) with a path length of 1 dm at 23 ^{o}C, the concentration is given in 10 mg/ml. Infrared spectra were recorded on a Perkin Elmer Paragon 100 FTIR spectrophotometer ($\boldsymbol{\nu}_{max}$ in cm^{-1}) as liquid films on sodium chloride plates. GC were recorded using column: Phenomenex ZB-5 (0.25 mm i.d. × 30 m., film thickness =250 µm.), oven temperature = 145 ^{o}C isothermal; carrier gas: H_2 at 1.4 mL/min.; injection: 250 ^{o}C/split = 34.7:1; detection: FID at 300 ^{o}C.

5.2 General Procedure A: Reduction of carboxylic acid via anhydride

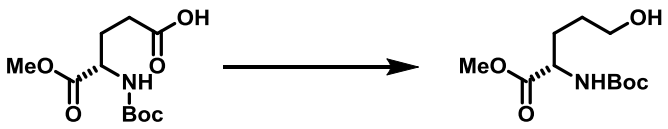
A two-necked round bottomed flask was charged with a magnetic stirrer bar then fitted with a dropping funnel equipped with a three-way tap on top and rubber septum on the other neck of the flask. It was flame-dried under vacuum and backfilled with nitrogen three times. The flask was allowed to cool, before adding *N*-Boc amino acid (1.0 eq.) by rapid removal and replacement of the dropping funnel under a stream of nitrogen; then dry THF was added and the solution of *N*-Boc amino acid cooled to -15 ^{o}C to -20 ^{o}C. After 5 minutes, *N*-methylmorpholine (1.1 eq.) was added by syringe followed by dropwise addition of EtOCOCl (1.2 eq.) in THF and the reaction stirred for 3 hr. at -15 ^{o}C to -20 ^{o}C. Then the reaction mixture was removed from the cooling bath and stirred for 20 minutes; then *N*-methylmorpholine hydrochloride was removed by filtration and washed with THF. The combined filtrate and washings were cooled to 0 ^{o}C and $NaBH_4$ (specified in each experimental) was added immediately followed by drop wise addition of H_2O (1 mL/1 mmol substrate). The reaction mixture was stirred at 0 ^{o}C for (1 hr.). The reaction was quenched by addition of saturated aqueous solution NH_4Cl (4 mL/1 mmol substrate) and stirring was continued for 40 minutes at 0 ^{o}C. The reaction mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. Purification was carried out by eluting over SiO_2 with 20-40% EtOAc in petroleum ether.

Benzyl (2*S*)-2-([(tertbutoxy)carbonyl]amino)-4-hydroxybutanoate (149)[87]



General procedure **A** using **147** (6.4 g, 20 mmol, 1.0 eq.) in dry THF (60 mL) *N*-methylmorpholine (2.4 mL, 22 mmol, 1.1 eq.), EtOCOCl (2.3 mL, 24 mmol, 1.2 eq.) in THF (20 mL), $NaBH_4$ (1.3 g, 34 mmol, 1.7 eq.) and H_2O (20 mL), and then quenched by addition saturated aqueous solution NH_4Cl gave benzyl (2*S*)-2-([(tertbutoxy)carbonyl]amino)-4-hydroxybutanoate **149** as a white solid (5.4 g, 17.4 mmol, 86%). M.p./52-54 °C, $[\alpha]_D$ -40.0 (c 0.1, MeOH), lit.[87]: $[\alpha]_D$ -40.2 (c 0.1, MeOH); R_f = 0.17 (30% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.32-7.41 (5 H, m, Ph), 5.40 (1 H, br.d, *J* = 6.8 Hz, NH), 5.21 (1 H, d, *J* = 12.2 Hz, Ph**CH**^{A}H^{B}CO_2), 5.17 (1 H, d, *J* = 12.2 Hz, Ph**C**H^{A}**H**^{B}CO_2), 4.50-4.59 (1 H, m, α–**CH**), 3.59 -3.75 (2 H, m, CH_2**CH_2**OH), 2.12-2.24 (1 H, m, α-CH**CH**^{A}H^{B}CH_2), 1.57-1.67 (1 H, m, α-CH**C**H^{A}**H**^{B}CH_2), 1.45 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 172.7, 156.4, 135.2, 128.6, 128.5, 128.3, 80.5, 67.3, 58.3, 50.7, 36.0, 28.3; ***m/z*** **(ES+)** found: 310.1660, $C_{16}H_{24}NO_5$ requires MH+ 310.1654.

Methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-5-hydroxypentanoate (154)[87]

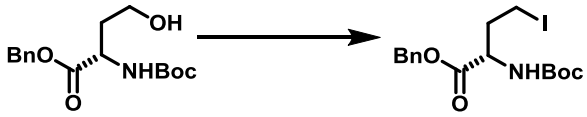


General procedure **A** using **152** (2.6 g, 10 mmol, 1.0 eq.) in dry THF (40 mL), *N*-methylmorpholine (1.2 mL, 11 mmol, 1.1 eq.), EtOCOCl (1.14 mL, 12 mmol, 1.2 eq.) in THF (10 mL), $NaBH_4$ (0.57 g, 15 mmol, 1.5 eq.) and H_2O (10 mL), and then quenched by addition saturated aqueous solution NH_4Cl gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-5-hydroxypentanoate **154** as an oil (1.97 g, 8 mmol, 80%). $[\alpha]_D$ +8.0 (c 1.0, $CHCl_3$), lit.[119] : $[\alpha]_D$ +6.8 (c 0.98, $CHCl_3$); R_f = 0.1 (30% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.32 (1 H, br.d, *J* = 7.8, NH), 4.22-4.31 (1 H, m, α-**CH**), 3.68 (3 H, s, O**CH_3**), 3.59 (2 H, t, *J* = 6.1, **CH_2**OH), 1.78-1.89 (1 H , m, **CH^AH^B**), 1.63-1.74 (1 H ,m, **CH^AH^B**), 1.56 (2 H, quintet, *J* = 6.7 Hz, CH_2**CH_2**CH_2), 1.41 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 173.3, 155.6, 79.9, 61.7, 53.2, 52.2, 29.3, 28.3, 28.2; **IR** (cm^{-1}), 3356, 1735, 1710, 1523, 1172; ***m/z*** **(ES+)** found: 248.1499, $C_{11}H_{22}NO_5$ requires MH+ 248.1498.

5.3 General procedure B: Iodination of hydroxyl groups

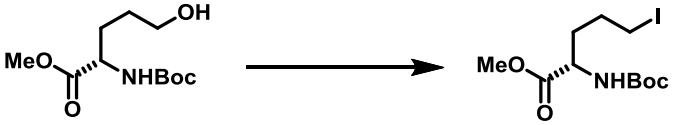
A two-necked round bottomed flask was charged with a magnetic stirrer bar then fitted with a three-way tap and rubber septum on the other neck of the flask. It was flame-dried under vacuum and backfilled with nitrogen three times. The flask was allowed to cool, before adding dry DCM (2 mL/1 mmol of substrate) by syringe; then triphenylphosphine (1.7 eq.) and imidazole (1.9 eq.) were added and stirred under nitrogen. The solution was cooled to 0 ^{o}C and then iodine (2.1 eq.) was added in three equal portions over 40 minutes then removed from the cooling bath and allowed to warm for 10 minutes and then the flask returned to ice-bath. A solution of amino alcohol (1 eq.) in dry CH_2Cl_2 (1 mL/1 mmol of substrate) was added slowly by syringe. After stirring for 7 hr. at 0 ^{o}C the reaction mixture was diluted with Et_2O and stirring was continued at room temperature for (9 hr.). The residue was washed with aqueous solution of $Na_2S_2O_3$ and brine then extracted with EtOAc and dried over Na_2SO_4. The solvent was removed under reduced pressure to give the crude product as yellow oil. Finally, the crude product was purified by silica gel column chromatography using a gradient of 20-30% EtOAc in petroleum ether.

Benzyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-4-iodobutanoate (141)[87]



General procedure **B** using triphenylphosphine (10.1 g, 38.4 mmol, 1.7 eq.), imidazole (2.9 g, 43 mmol, 1.9 eq.), iodine (12 g, 47.5 mmol, 2.1 eq.) and **149** (7 g, 22.6 mmol, 1 eq.) gave benzyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-4-iodobutanoate **141** as a white solid (6.35 g, 15.2 mmol, 67%). M.p./55-56 °C, $[\alpha]_D$ -30.0 (c 0.1, MeOH), lit.[87]: $[\alpha]_D$ -32.4 (c 0.1, MeOH); R_f = 0.42 (20% EtOAc in petroleumether); **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm: 7.33-7.41 (5 H, m, Ph), 5.22 (1 H, d , *J* = 12.2, Hz, PhC**H**^{A}H^{B}CO$_2$), 5.16 (1 H, d, *J* = 12.2 Hz, PhCH^A**H**^{B}CO$_2$), 5.09 (1 H, d, *J* =7.5 Hz, **NH**), 4.29-4.45 (1 H, m, α-**CH**), 3.08-3.19 (2H, m, CH$_2$**CH$_2$I**), 2.32-2.53 (1 H, m, CH$_2$**CH**^{A}H^{B}α-CH), 2.09-2.29 (1 H, m, CH$_2$**C**H^A**H**^{B}α-CH), 1.45 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 171.4, 155.3, 135.1, 128.7, 128.6, 128.3, 80.3, 67.4, 54.4, 37.0, 28.3; **IR** (cm^{-1}), 3374, 1742, 1720, 1502, 1168; ***m/z* (ES+)** found: 420.0667, $C_{16}H_{23}NO_4I$ requires MH+ 420.0672, one carbon signal missing in ^{13}C spectrum.

Methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-5-iodopentanoate (142)[87]

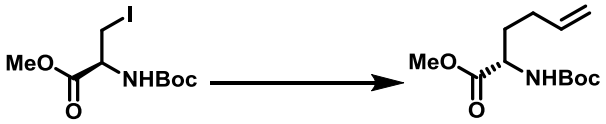


General procedure **B** using triphenylphosphine (9.27 g, 35.36 mmol, 1.7 eq.), imidazole (2.7 g, 39.5 mmol, 1.9 eq.), I_2 (11.1 g, 43.68 mmol, 2.1 eq.) and **154** (5.16 g, 20.8 mmol, 1.0 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-5-iodopentanoate **142** as an oil (3.8 g, 10.63 mmol, 51%). $[\alpha]_D$ +22.7 (c 1.1, $CHCl_3$); R_f = 0.4 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.08 (1 H, br.d, *J* = 8.1, **NH**), 4.26-4.35 (1 H, m, α-**CH**), 3.74 (3 H, s, O**CH$_3$**), 3.12-3.23 (2 H, m, **CH$_2$I**), 1.78-1.98 (3 H, m, **CH**^{A}H^B**CH$_2$**CH$_2$), 1.65-1.76 (1 H, m, **C**H^A**H**^{B}), 1.43 (9 H, s, ^{t}BuO); **^{13}C NMR** (400 MHz, $CDCl_3$) δ ppm: 172.9, 155.3, 80.1, 52.5, 52.4, 33.7, 29.3, 28.3, 5.4.

5.4 General Procedure C: Allylation reactions

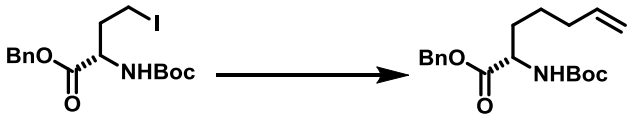
A two-necked round bottomed flask was charged with a magnetic stirrer bar then fitted with a rubber septum and three-way tap. The flask was flame-dried under vacuum and backfilled with nitrogen three times. Zinc dust (2.5 eq. relative to alkyl iodide generally) was added, flame-dried and again evacuated and backfilled with nitrogen three times, with continuous stirring. The flask was allowed to cool, dry DMF (1 mL/1 mmol of alkyl iodide) was added via syringe, and the heterogeneous mixture stirred vigorously. Iodine (0.2 eq. relative to alkyl iodide generally) was added by rapid removal and replacement of the three-way tap under a stream of nitrogen, turning the solvent yellow. The mixture was stirred for 1-2 minutes, until the solvent had become a colourless. The alkyl iodide (1.0 mmol) was added by rapid removal and replacement of the three-way tap under a stream of nitrogen (in the case of compound **142** was dissolved in DMF and added by syringe via rubber septum). The mixture was stirred and an exotherm was observed stirring continued for a further 50 minutes at r.t. or 35-40 minutes at 35 ^{o}C with sonication; these details are specified with each example. The solid zinc dust was allowed to settle before transferring the solution containing the zinc reagent into a new reaction vessel via syringe. During the activation period, a separate two-necked round bottomed flask fitted with a magnetic stirrer bar, a rubber septum and three-way tap; was flame-dried under vacuum and backfilled with nitrogen three times. It was allowed to cool, CuBr.DMS (0.1 eq. relative to alkyl iodide) was added and gently heated then evacuated and backfilled with nitrogen until the CuBr.DMS changed appearance from a grey-brown to light green powder. The flask was allowed to cool, before adding dry DMF (0.6 mL/1 mmol of alkyl iodide) and allyl chloride (1.4 eq. relative to alkyl iodide generally) via syringe. The mixture was stirred at room temperature for about 5 minutes, at which point the organozinc reagent was added dropwise via syringe, and was stirred at room temperature for (3 hr.). The crude reaction mixture was directly applied to SiO_2 column, using a gradiant of 20-30% EtOAc in petroleum ether.

Methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)hex-5-enoate (131)[89]



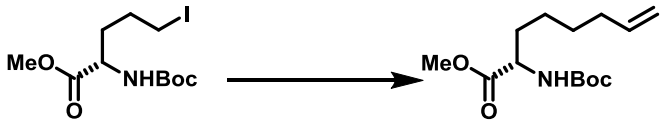
General procedure **C** using zinc dust (1.95 g, 30 mmol, 2.5 eq.), iodine (0.6 g, 2.4 mmol, 0.2 eq.), **140** (3.94 g, 12 mmol, 1 eq.), CuBr.DMS (0.246 g, 1.2 mmol, 0.1 eq.), and allyl chloride (1.36 mL, 16.8 mmol, 1.4 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)hex-5-enoate **131** (2.2 g, 9 mmol, 75 %) as a colourless oil. Zinc insertion took 50 minutes at (r.t.). $[\alpha]_D$ -17.0 (c 1.0, MeOH), lit.[120]: $[\alpha]_D$ -20.7 (c 0.97, MeOH); R_f = 0.57 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.80 (1 H, ddt, *J* = 16.9, 10.3 and 6.6 Hz, CH_2**CH**=CH_2), 4.98-5.08 (3 H, m, **NH**, C=C**H_2**), 4.28-4.37 (1 H, m, α-**CH**), 3.74 (3 H, s, CO_2**CH_3**), 2.06-2.17 (2 H, m, **CH_2**C=C), 1.85-1.96 (1 H, m, **CH^A**H^B), 1.65-1.77 (1 H, m, **C**H^A**H^B**), 1.44 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 173.3, 155.3, 136.9, 115.7, 79.8, 52.9, 52.3, 31.9, 29.5, 28.3; **IR** (cm^{-1}), 3370, 1742, 1712, 1516, 1451, 1365, 1254, 1168; ***m/z* (ES+)** found: 244.1549, $C_{12}H_{22}NO_4$ requires MH+ 244.1549.

Benzyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)hept-6-enoate (132)[89]



General procedure **C** using zinc dust (1.17 g, 18 mmol, 3 eq.), iodine (0.335 g, 1.32 mmol, 0.22 eq.), **141** (2.5 g, 6 mmol, 1 eq.), CuBr.DMS (0.123 g, 0.6 mmol, 0.1 eq.), and allyl chloride (0.7 mL, 8.4 mmol, 1.4 eq.). Purification by column chromatography (20% EtOAc in petroleum ether) afforded the mixture of compound **132** and protonated zinc reagent **157** which was further purified by HPLC (XBridge Prep OBD C18 5µm 19 mmid x 250 mm), using 30 : 70 water/acetonitrile, at a flow rate of 17 mL.min^{-1} and UV detection at 210 nm, room temperature. The HPLC analysis showed a main peak t_R = (8-10) that was identified as the target molecule benzyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)hept-6-enoate **132** (1.28 g, 3.8 mmol, 64%) as a colourless oil. Zinc insertion took 35 minutes with sonication at 35 ^{o}C. $[\alpha]_D$ -4.0 (c 1, $CHCl_3$), R_f = 0.54 (20% EtOAc in petroleum ether). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.31-7.40 (5 H, m, Ph), 5.72 (1 H, ddt, *J* = 16.9, 10.3 and 6.7 Hz, CH_2**CH**=CH_2), 5.22 (1 H, d, *J* = 12.4 Hz, Ph**CH^{A}**H^{B}CO_2), 5.13 (1 H, d, *J* = 12.4 Hz, Ph**C**H^{A}**H^{B}**CO_2), 5.05 (1 H, d, *J* = 8.3 Hz, **NH**), 5.02-4.92 (2 H, m, CH=**CH$_2$**), 4.30-4.39 (1 H, m, α-**CH**), 1.96-2.12 (2 H, m, CH_2**CH_2**CH), 1.75-1.88 (1 H, m, **CH^{A}**H^{B}), 1.58-1.69 (1 H, m, **C**H^{A}**H^{B}**), 1.31-1.52 (11 H, m, CH_2**CH_2**CH_2, ^{t}BuO).**^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 172.7, 155.4, 137.9, 135.5, 128.6, 128.4, 128.2, 115.1, 79.8, 66.9, 53.4, 33.1, 32.1, 28.3, 24.4; **IR** (cm^{-1}), 3368, 1745, 1712, 1634, 1501, 1366, 1253, 1165, 1001, 912; ***m/z*** **(ES+)** found: 334.2028, $C_{19}H_{28}NO_4$ requires MH+ 334.2018.

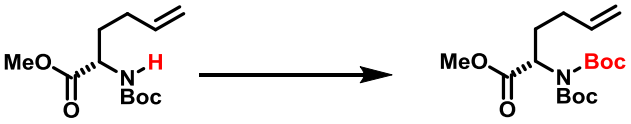
Methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)oct-7-enoate (133)[89]



General procedure **C** using zinc dust (487.5 mg, 7.5 mmol, 2.5 eq.), iodine (152.3 mg, 0.6 mmol, 0.2 eq.), **142** (1100 mg, 3 mmol, 1 eq.), CuBr.DMS (61.5 mg, 0.3 mmol, 0.1 eq.), and allyl chloride (320 µl, 3.9 mmol, 1.3 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)oct-7-enoate **133** (311 mg, 1.15 mmol, 38%) as a colourless oil. Zinc insertion took 40 minutes with sonication at 35 ^{o}C. $[\alpha]_D$ -17.6 (c 1.25, MeOH); R_f = 0.52 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.77 (1 H, ddt, *J* = 16.9, 10.1 and 6.8 Hz, CH_2**CH**=CH_2), 4.9-5.05 (3 H, m, CH=**CH_2**, **NH**), 4.25-4.33 (1 H, m, α-**CH**), 3.73 (3 H, s, O**CH_3**), 1.99-2.1 (2 H, m, CH_2**CH_2**CH=), 1.69-1.86 (1 H, m, **CH^A**CH^B), 1.53-1.68 (1 H, m, CH^A**CH^B**), 1.24-1.51 (4 H, m, C-**CH_2CH_2**-C), 1.44 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 173.4, 155.3, 138.5, 114.6, 79.8, 53.3, 52.2, 33.4, 32.6, 28.4, 28.3, 24.6; **IR** (cm^{-1}), 3370, 1745, 1720, 1509, 1168; ***m/z* (ES+)** found: 272.1850, $C_{14}H_{26}NO_4$ requires MH+ 272.1862.

5.5 Double protection to nitrogen of allylated products

Methyl (2*S*)-2-(bis[(tert-butoxy)carbonyl]amino)hex-5-enoate (162) [92]

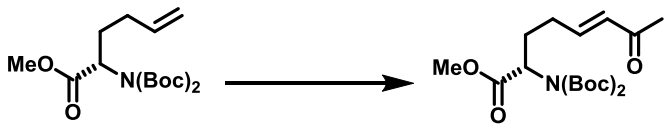


A mixture of mono Boc protected nitrogen of allylated product **131** (660 mg, 2.7 mmol, 1 eq.), sodium hydride (60% in oil*, 97 mg, 4 mmol, 1.5 eq.), and di-*tert*-butyl-dicarbonate (982 mg, 4.5 mmol, 1.66 eq.) in dry THF (25 mL) was stirred for 2 days at reflux 66 ^{o}C. The reaction mixture allowed to cool, water was used to quench the reaction and the organic mixture extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with $NaHCO_3$ (2 M), dried over sodium sulfate and solvent removed under reduce pressure. The crude product purify on silica gel column chromatography using gradient of 15-25% EtOAc in petroleum ether; gave methyl (2*S*)-2-(bis[(tert-butoxy)carbonyl]amino)hex-5-enoate **162** (650 mg, 1.89 mmol, 70%) as an oil. R_f = 0.63 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.80 (1 H, ddt, *J* = 17.0, 10.3 and 6.5 Hz, CH_2**CH**=CH_2), 5.04 (1 H, dq, *J* = 17.0 and 1.4 Hz, =**CH**H), 4.98 (1 H, br.dq, *J* = 10.3 and 1.5 Hz, =**C**H**H**), 4.87 (1 H, dd, *J* = 9.3 and 5.1, α-**CH**), 3.71 (3 H, s, O**CH$_3$**), 2.17-2.28 (1H, m, CH**CH**^{A}H^BCH_2), 2.12 (2 H, q, *J* = 7.1 Hz, CH_2**CH$_2$**CH), 1.91-2.03 (1 H, m, CH**C**H^A**H^B**CH_2), 1.49 (18 H, s, 2 ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 171.4, 152.1, 137.4, 115.4, 83.1, 57.5, 52.2, 30.3, 29.3, 27.9; **IR** (cm^{-1}), 1748, 1705, 1458, 1371, 1251, 1128; **m/z (ES+)** found: 344.2067, $C_{17}H_{30}NO_6$ requires MH+ 344.2073. * washed with dry THF before using.

5.6 General procedure D: Cross metathesis of unsaturated side chain amino acid

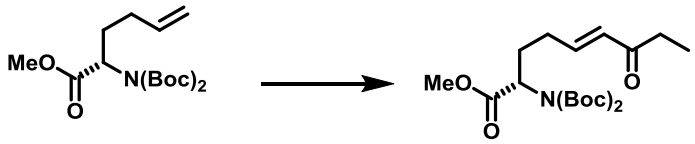
A two-necked round bottomed flask was charged with a magnetic stirrer bar then fitted with a condenser equipped with a three-way tap on top and rubber septum on the other neck of the flask. It was flame-dried under vacuum and backfilled with nitrogen three times. The flask was allowed to cool, before adding allylic amino acid **131-133** and enone in dry degassed DCM (2 mL) via syringe followed by adding Grubbs 2nd generation catalyst (5 mol% relative to substrate) in dry DCM (1 mL) were added by syringe. The reaction is heated at reflux for 7 hr., then the reaction mixture was concentrated and the residue was purified by column chromatography using a gradient of 15-35% EtOAc in petroleum ether.

Methyl (2*S*,5*E*)-2-(bis[(tert-butoxy)carbonyl]amino)-7-oxooct-5-enoate (163)



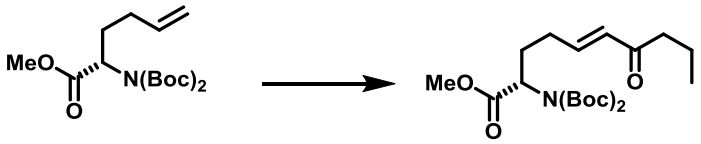
General procedure **D** using **162** (137.4 mg, 0.4 mmol, 1 eq.), vinyl methyl ketone (100 µl, 1.2 mmol, 3 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave methyl (2*S*,5*E*)-2-(bis[(tert-butoxy)carbonyl]amino)-7-oxooct-5-enoate **163** (69 mg, 0.18 mmol, 44%) as an oil. $[\alpha]_D$ -33.0 (c 0.73, $CHCl_3$); R_f = 0.2 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm spectrum broadened due to rotomers: 6.72-6.82 (1 H, m, CH_2**CH**=CH), 6.07 (1 H, d, *J* = 15.9 Hz, CH=**CH**CO), 4.84-4.89 (1 H, m, α–**CH**), 3.71 (1/2 H, O**CH_3**), 3.70 (1/2 H, O**CH_3**), 2.25–2.36 (3 H, m, CH_2**CH_2**CH=, α-CH**CH^A**$H^B$$CH_2$), 2.23 (1/2 H, CO**$CH_3$**), 2.22 (1/2 H, CO**$CH_3$**), 1.98-2.13 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.48 (9 H, s, ^{t}BuO), 1.47 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 198.4, 170.9, 152.1, 146.7, 131.8, 83.4, 57.5, 52.3, 29.2, 28.6, 27.9, 26.8; **IR** (cm^{-1}), 1747, 1697, 1678, 1366, 1252, 1144; **m/z (ES+)** found: 386.2170, $C_{19}H_{32}NO_7$ requires MH+ 386.2179.

Methyl (2S,5E)-2-(bis[(tert-butoxy)carbonyl]amino)-7-oxonon-5-enoate (164)



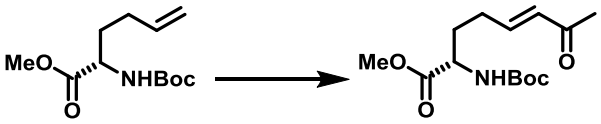
General procedure **D** using **162** (137.4 mg, 0.4 mmol, 1 eq.), vinyl ethyl ketone (120 µl, 1.2 mmol, 3 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave methyl (2S,5E)-2-(bis[(tert-butoxy)carbonyl]amino)-7-oxonon-5-enoate **164** (75 mg, 0.18 mmol, 46%) as an oil. $[\alpha]_D$ -37.0 (c 1, $CHCl_3$); R_f = 0.38 (20% EtOAc in petroleum ether); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 6.81 (1 H, dt, *J* = 15.9 and 6.6 Hz, CH_2**CH**=CH), 6.12 (1 H, d, *J* = 15.9 Hz CH=**CH**CO), 4.85-4.89 (1 H, m, α–**CH**), 3.72 (3 H, s, O**CH_3**), 2.56 (2 H, q, *J* = 7.3 Hz, CO**CH_2**CH_3), 2.24–2.35 (3 H, m, CH_2**CH_2**CH=, α-CH**CH**^{A}H^BCH_2), 1.99-2.12 (1 H, m, α-CH**C**H^A**H**^{B}CH_2), 1.49 (18 H, s, 2 ^{t}BuO), 1.09 (3 H, t, *J* = 7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 200.9, 170.9, 152.1, 145.2, 130.6, 83.3, 57.5, 52.2, 33.2, 29.1, 28.6, 27.9, 8.0; **IR** (cm^{-1}), 1745, 1700, 1368, 1258, 1116; **m/z (ES+)** found: 400.2316, $C_{20}H_{34}NO_7$ requires MH+ 400.2335.

Methyl (2*S*,5*E*)-2-(bis[(tert-butoxy)carbonyl]amino)-7-oxodec-5-enoate (165)



General procedure **D** using **162** (137.4 mg, 0.4 mmol, 1 eq.), vinyl propyl ketone (140 µl, 1.2 mmol, 3 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol %) in CH_2Cl_2 (3 mL) gave methyl (2*S*,5*E*)-2-(bis[(tert-butoxy)carbonyl]amino)-7-oxodec-5-enoate **165** (88 mg, 0.21 mmol, 53%) as an oil. $[\alpha]_D$ -32.0 (c 1, $CHCl_3$); R_f = 0.4 (20 EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 6.80 (1 H, dt, *J* = 15.9 and 6.6 Hz, CH_2**CH**=CH), 6.11 (1 H, d, *J* = 15.9 Hz, CH=**CH**CO), 4.84-4.9 (1 H, m, α–**CH**), 3.71 (3 H, s, O**CH_3**), 2.51 (2 H, t, *J* = 7.3 Hz, CO**CH_2**CH_2), 2.22–2.35 (3 H, m, CH_2**CH_2**CH=, α-CH**CH^A**H^BCH_2), 1.98-2.13 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.62 (2 H, sextet, *J* = 7.4 Hz, CH_2**CH_2**CH_3), 1.49 (18 H, s, 2 ^{t}BuO), 0.92 (3 H, t, *J* = 7.4 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 200.5, 170.9, 152.1, 145.4, 130.9, 83.4, 57.5, 52.3, 41.9, 29.1, 28.6, 27.9, 17.6, 13.8; **IR** (cm^{-1}), 1752, 1697, 1371, 1251, 1142; ***m/z* (ES+)** found: 436.2329, $C_{21}H_{35}NO_7Na$ requires MNa+ 436.2311. Homodimer of **162** was detected in the crude reaction mixture, ***m/z* (ES+)** found: 681.3608, $C_{32}H_{54}N_2O_{12}Na$ requires MNa+ 681.3574.

Methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxooct-5-enoate (166)

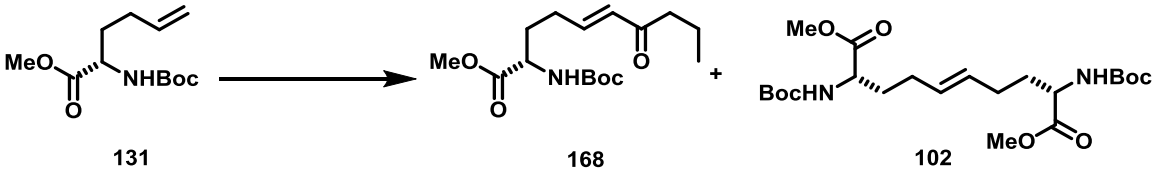


General procedure **D** using **131** (97 mg, 0.4 mmol, 1 eq.), vinyl methyl ketone (100 µl, 1.2 mmol, 3 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave methyl (2*S*, 5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxooct-5-enoate **166** (104 mg, 0.36 mmol, 91%) as an oil. $[\alpha]_D$ +40.0 (c 0.4, $CHCl_3$); R_f = 0.13 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 6.77 (1 H, dt, *J* = 16.0 and 6.7 Hz, CH_2**CH**=CH), 6.09 (1 H, br.d, *J* = 16.0 Hz, CH=**CH**CO), 5.08 (1 H, d, *J* = 8.1 Hz, **NH**), 4.30-4.42 (1H, m, α–**CH**), 3.76 (3 H, s, O**CH_3**), 2.19–2.39 (2 H, m, CH_2**CH_2**CH=), 2.25 (3 H, s, CO**CH_3**), 1.92-2.11 (1 H, m, α-CH**CH^A**$H^B$$CH_2$), 1.74-1.86 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.45 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 198.3, 172.7, 155.3, 146.1, 131.9, 80.1, 52.8, 52.4, 31.3, 28.3, 28.2, 26.9; **IR** (cm^{-1}), 3359, 1749, 1715, 1673, 1518, 1450, 1371, 1253, 1166; ***m/z*** **(ES+)** found: 286.1648, $C_{14}H_{24}NO_5$ requires MH+ 286.1654.

Methyl (2*S*, 5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxonon-5-enoate (167)

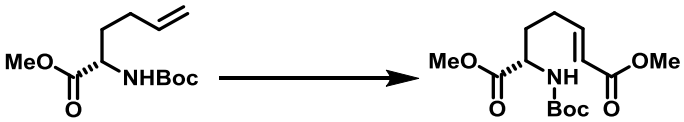
General procedure **D** using **131** (97 mg, 0.4 mmol, 1 eq.), vinyl ethyl ketone (120 µl, 1.2 mmol, 3 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave methyl (2*S*, 5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxonon-5-enoate **167** (110 mg, 0.37 mmol, 92%) as an oil. $[\alpha]_D$ +36.4 (c 0.55, $CHCl_3$); R_f = 0.17 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 6.76 (1 H, dt, *J* = 15.8 and 6.8 Hz, CH_2**CH**=CH), 6.09 (1 H, d, *J* = 15.8 Hz, CH=**CH**CO), 5.13 (1 H, d, *J* = 7.6 Hz, **NH**), 4.25-4.36 (1 H, m, α–**CH**), 3.72 (3 H, s, O**CH_3**), 2.53 (2 H, q, *J* = 7.3 Hz, CO**CH_2**CH_3), 2.17–2.35 (2 H, m, CH_2**CH_2**CH=), 1.88-2.06 (1 H, m, α-CH**CH^A**H^BCH_2), 1.71-1.82 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.41 (9 H, s, ^{t}BuO), 1.06 (3 H, t, *J* = 7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 200.7, 172.8, 155.3, 144.6, 130.7, 80.0, 52.9, 52.4, 33.4, 31.3, 28.3, 28.2, 8.0; **IR** (cm^{-1}), 3346, 1747, 1708, 1669, 1512, 1448, 1363, 1167; ***m/z* (ES+)** found: 300.1733, $C_{15}H_{26}NO_5$ requires MH+ 300.3670.

Methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxodec-5-enoate (168)



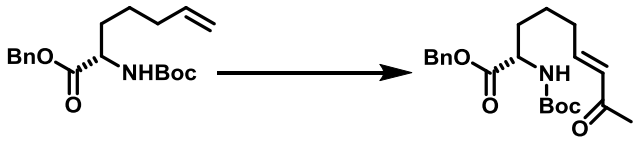
General procedure **D** using **131** (97 mg, 0.4 mmol, 1 eq.), vinyl propyl ketone (117 µl, 1 mmol, 2.5 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mmol%) in CH_2Cl_2 (3 mL) gave methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxodec-5-enoate **168** (113 mg, 0.36 mmol, 90%) as an oil. $[\alpha]_D$ +30.7 (c 0.88, $CHCl_3$); R_f = 0.17 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 6.78 (1 H, dt, *J* = 15.8 and 6.8 Hz, CH_2**CH**=CH), 6.11 (1 H, d, *J* = 15.8 Hz, CH=**CH**CO), 5.08 (1 H, d, *J* =7.6 Hz, **NH**), 4.27-4.39 (1 H, m, α–**CH**), 3.75 (3 H, s, O**CH_3**), 2.5 (2 H, t, *J* = 7.3 Hz, CO**CH_2**CH_2), 2.19–2.37 (2 H, m, CH_2**CH_2**CH=), 1.91-2.08 (1 H, m, α-CH**CH^A**H^BCH_2), 1.69-1.85 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.63 (2 H, sextet, *J* = 7.4 Hz, CH_2**CH_2**CH_3), 1.44 (9 H, s, ^{t}BuO), 0.93 (3 H, t, *J* = 7.5 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 200.4, 172.8, 155.3, 144.7, 130.9, 80.1, 52.9, 52.4, 42.2, 31.3, 28.3, 28.2, 17.6, 13.8; **IR** (cm^{-1}), 3359, 1750, 1714, 1675, 1521, 1448, 1366, 1249, 1167; ***m/z* (ES+)** found: 314.1889, $C_{16}H_{28}NO_5$ requires MH+ 314.3940. Homodimer **102** was detected in the crude reaction mixture, ***m/z* (ES+)** found: 459.2704, $C_{22}H_{39}N_2O_8$ requires MH+ 459.2706.

1, 7-Dimethyl (2*E*,6*S*)-6-([(tert-butoxy)carbonyl]amino)hept-2-enedioate (169)[121]



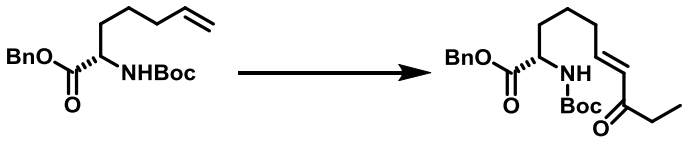
General procedure D using **131** (243 mg, 1 mmol, 1 eq.), methyl acrylate (271 µL, 3 mmol, 3 eq.) and Grubbs 2nd generation catalyst (42 mg, 0.05 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave 1, 7-dimethyl (2*E*,6*S*)-6-([(tert-butoxy)carbonyl]amino)hept-2-enedioate **169** (282 mg, 0.94 mmol, 93% yield) as an oil. $[\alpha]_D$ +28.0 (c 1.25, $CHCl_3$); R_f = 0.25 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 6.92 (1 H, dt, *J* = 15.5 and 6.9 Hz, CH_2**CH**=CH), 5.84 (1 H, d, *J* = 15.5 Hz, CH=**CH**CO), 5.10 (1 H, d, *J* = 7.8 Hz, **NH**), 4.25-4.39 (1 H, m, α–**CH**), 3.74 (3 H, s, O**CH_3**), 3.71 (3 H, s, O**CH_3**), 2.17–2.36 (2 H, m, CH_2**CH_2**CH=), 1.92-2.05 (1 H, m, α-CH**CH^AH^B**CH_2), 1.70-1.82 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.43 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 172.8, 166.8, 155.3, 147.3, 121.8, 80.1, 52.8, 52.4, 51.5, 31.2, 28.3, 28.0; **IR** (cm^{-1}), 3364, 1715, 1658, 1524, 1440, 1369, 1211, 1165; ***m/z* (ES+)** found: 302.1604, $C_{14}H_{24}NO_6$ requires MH+ 302.1604.

Benzyl (2*S*,6*E*)-2-([(tert-butoxy)carbonyl]amino)-8-oxonon-6-enoate (170)



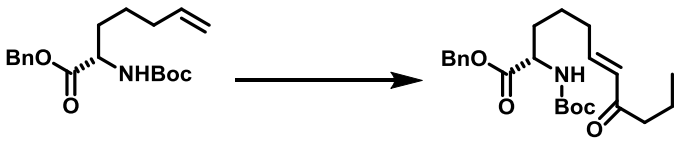
General procedure **D** using **132** (133.4 mg, 0.4 mmol, 1 eq.), vinyl methyl ketone (100 µl, 1.2 mmol, 3 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave benzyl (2*S*,6*E*)-2-([(tert-butoxy)carbonyl]amino)-8-oxonon-6-enoate **170** (134 mg, 0.36 mmol, 89%) as an oil. $[\alpha]_D$ -22.0 (c 0.91, MeOH); R_f = 0.32 (30% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.32-7.42 (5 H, m, Ph), 6.7 (1 H, dt, *J* = 16.0 and 6.9 Hz, CH_2**CH**=CH), 6.04 (1 H, d, *J* = 16.0 Hz, CH=**CH**CO), 5.22 (1 H, d, *J* = 12.2 Hz, Ph**CH^A**H^{B}OCO), 5.14 (1 H, d, *J* = 12.2, Hz, Ph**C**H^A**H^B**OCO), 5.05 (1 H, d, *J* = 8.1 Hz, **NH**), 4.33-4.41 (1 H, m, α–**CH**), 2.13-2.31 (5 H, m, CH_2**CH_2**CH=, CO**CH_3**), 1.77-1.91 (1 H, m, α-CH**CH^A**H^BCH_2), 1.59-1.72 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.37-1.57 (2 H, m, CH^{A}CH^B**CH_2**CH_2), 1.44 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 198.5, 172.5, 155.4, 147.1, 135.3, 131.6, 128.6, 128.5, 128.3, 79.9, 67.1, 53.2, 32.3, 31.7, 28.3, 26.9, 23.7; **IR** (cm^{-1}), 3342, 1715, 1694, 1673, 1625, 1499, 1364, 1250, 1157; ***m/z* (ES+)** found: 376.2109, $C_{21}H_{30}NO_5$ requires MH+ 376.2124.

Benzyl (2*S*,6*E*)-2-([(tert-butoxy)carbonyl]amino)-8-oxodec-6-enoate (171)



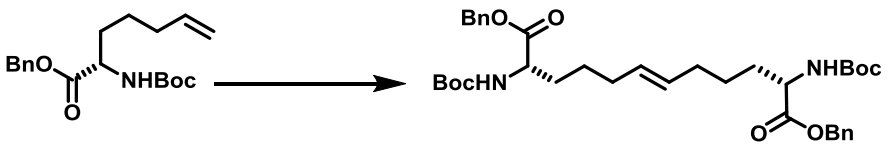
General procedure **D** using **132** (134 mg, 0.4 mmol, 1 eq.), vinyl ethyl ketone (200 µl, 2 mmol, 5 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave benzyl (2*S*,6*E*)-2-([(tert-butoxy)carbonyl]amino)-8-oxodec-6-enoate **171** (141 mg, 0.36 mmol, 90%) as an oil. $[\alpha]_D$ -19.5 (c 0.77, MeOH); R_f = 0.18 in (15% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.30-7.39 (5 H, m, Ph), 6.72 (1 H, dt, *J* = 16.0 and 6.8 Hz, CH_2**CH**=CH), 6.05 (1 H, br.d, *J* = 16.0 Hz, CH=**CH**CO), 5.21 (1 H, d, *J* = 12.2 Hz, Ph**CH^A**H^{B}OCO), 5.13 (1 H, d, *J* = 12.2 Hz, PhCH^A**H^B**OCO), 5.08 (1 H, d, *J* = 8.3 Hz, **NH**), 4.30-4.40 (1 H, m, α–**CH**), 2.52 (2 H, q, *J* = 7.3 Hz, CO**CH$_2$**CH$_3$), 2.11-2.27 (2 H, m, CH_2**CH$_2$**CH=), 1.77-1.89 (1 H, m, α-CH**CH^A**H^{B}CH$_2$), 1.58-1.71 (1 H, m, α-CH**C**H^A**H^B**CH$_2$), 1.36-1.56 (11 H, m, CH^{A}CH^B**CH$_2$**CH$_2$, ^{t}BuO), 1.10 (3 H, t, *J* = 7.3 Hz, CH_2**CH$_3$**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 200.8, 172.4, 155.4, 145.6, 135.3, 130.4, 128.6, 128.4, 128.3, 79.8, 67.1, 53.2, 33.3, 32.2, 31.7, 28.3, 23.7, 8.1; **IR** (cm^{-1}), 3362, 1741, 1696, 1673, 1629, 1499, 1248, 1159; ***m/z* (ES+)** found: 390.2283, $C_{22}H_{32}NO_5$ requires MH+ 390.2280.

Benzyl (2*S*,6*E*)-2-([(tert-butoxy)carbonyl]amino)-8-oxoundec-6-enoate (172)



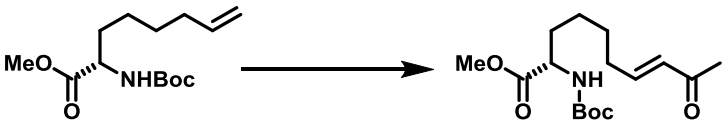
General procedure **D** using **132** (133.4 mg, 0.4 mmol, 1 eq.), vinyl propyl ketone (117 µl, 1 mmol, 2.5 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 gave benzyl (2*S*,6*E*)-2-([(tert-butoxy)carbonyl]amino)-8-oxoundec-6-enoate **172** (140 mg, 0.35 mmol, 86%) as an oil. $[\alpha]_D$ -30.0 (c 0.1, MeOH); R_f = 0.22 (15% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.33-7.41 (5 H, m, Ph), 6.73 (1 H, dt, *J* = 16.0 and 6.8 Hz, CH_2**CH**=CH), 6.06 (1 H, br.d, *J* = 16.0, CH=**CH**CO), 5.22 (1 H, d, *J* = 12.2 Hz, Ph**CH^A**H^{B}O), 5.14 (1 H, d, *J* = 12.2 Hz, Ph**C**H^A**H^B**O), 5.04 (1 H, br.d, *J* = 8.3 Hz, **NH**), 4.32-4.42 (1 H, m, α–**CH**), 2.48 (2 H, t, *J* = 7.32 Hz, CO**CH$_2$**CH$_2$), 2.13-2.27 (2 H, m, CH$_2$**CH$_2$**CH=), 1.78-1.91 (1 H, m, **CH^A**CH^B), 1.57-1.71 (3 H, m, **C**H^A**H^B**, CH$_2$**CH$_2$**CH$_3$), 1.37-1.56 (11 H, m, CH^{A}H^B**CH$_2$**CH$_2$, ^{t}BuO), 0.93 (3 H, t, *J* = 7.5 Hz, CH$_2$**CH$_3$**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 200.6, 172.5, 155.5, 145.7, 135.3, 130.7, 128.6, 128.5, 128.3, 80.2, 67.1, 53.2, 42.1, 32.3, 31.7, 28.3, 23.7, 17.6, 13.8; **IR** (cm^{-1}), 3357, 1747, 1712, 1675, 1629, 1499, 1457, 1365, 1256, 1162; ***m/z* (ES+)** found: 404.2426, $C_{23}H_{34}NO_5$ requires MH+ 404.2437.

1,12-Dibenzyl (2*S*,6*E*,11*S*)-2,11-bis(([(tert-butoxy) carbonyl] amino))dodec-6-enedioate (174)



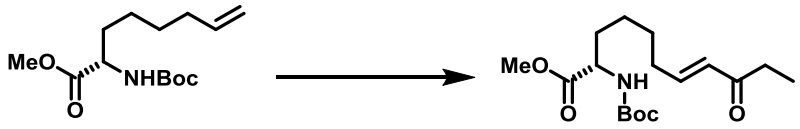
The title compound (presumed to be E, but this was not confirmed) was synthesised using general procedure **D**, with benzyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)hept-6-enoate **132** (110 mg, 0.33 mmol, 1 eq.) as the starting material and grubbs 2nd generation catalyst (14 mg, 0.016 mmol, 5 mmol%), gave compound **174** (104 mg, 0.163 mmol, 98%) as an oil. $[\alpha]_D$ -1.3 (c 1.5, $CHCl_3$); R_f = 0.3 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz,$CDCl_3$) δ ppm: 7.29-7.40 (10 H, m, 2 Ph), 5.25-5.36 (2 H, m, **CH=CH**), 5.21 (2 H, d, *J* = 12.5 Hz, Ph**CH^A**H^BCO_2), 5.12 (2 H, d, *J* = 12.5 Hz, Ph**C**H^A**H^B**CO_2), 4.99-5.01 (2 H, m, **NH**), 4.27-4.43 (2 H, m, α-**CH**), 1.87-2.08 (4 H, m, CH_2**CH_2**CH), 1.72-1.86 (2 H, m, α-CH**CH^A**H^BCH_2), 1.54-1.71 (2 H, m, α-CH**C**H^A**H^B**CH_2), 1.19-1.52 (4 H, m, CH_2**CH_2**CH_2), 1.44 (18 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 172.8, 155.4, 135.5, 130.1, 128.6, 128.4, 128.3, 79.8, 66.9, 53.5, 32.5, 31.9, 28.3, 25.0; **IR** (cm^{-1}), 3370, 1745, 1717, 1501, 1459, 1250, 1163; **m/z (ES+)** found: 639.3638, $C_{36}H_{51}N_2O_8$ requires MH+ 639.3645.

Methyl (2*S*,7*E*)-2-([(tert-butoxy)carbonyl]amino)-9-oxodec-7-enoate (175)



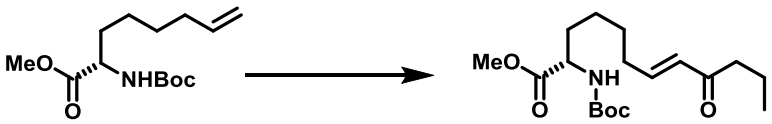
General procedure **D** using **133** (108.5 mg, 0.4 mmol, 1 eq.), vinyl methyl ketone (85 µl, 1 mmol, 2.5 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave methyl (2*S*,7*E*)-2-([(tert-butoxy)carbonyl]amino)-9-oxodec-7-enoate **175** (103 mg, 0.33 mmol, 82%) as an oil. $[\alpha]_D$ +20.0 (c 0.95, $CHCl_3$); R_f = 0.25 (30% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 6.76 (1 H, dt, *J* = 16.0 and 6.9 Hz, CH_2**CH**=CH), 6.05 (1 H, d, *J* = 16.0 Hz, CH=**CH**CO), 5.04 (1H, br.d, *J* = 8.1 Hz, **NH**), 4.24-4.34 (1 H, m, α–**CH**), 3.73 (3 H, s, O**CH_3**), 2.17-2.26 (2 H, m, CH_2**CH_2**CH=), 2.23 (3 H, s, CO**CH_3**), 1.75-1.87 (1 H, m, α-CH**CH^A**$H^B$$CH_2$), 1.56-1.68 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.29-1.55 (4 H, m, CH^ACH^B**CH_2CH_2**), 1.43 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 198.6, 173.2, 155.3, 147.7, 131.5, 79.9, 53.2, 52.3, 32.5, 32.1, 28.3, 27.6, 26.9, 24.8; **IR** (cm^{-1}), 3356, 1749, 1717, 1674, 1523, 1441, 1369, 1258, 1164; **m/z (ES+)** found: 314.1956, $C_{16}H_{28}NO_5$ requires MH+ 314.1967, Homodimer of **133** was detected in the crude reaction mixture, **m/z (ES+)** found: 515.3350, $C_{16}H_{28}NO_5$ requires MH+ 515.3332.

Methyl (2*S*, 7*E*)-2-([(tert-butoxy)carbonyl]amino)-9-oxoundec-7-enoate (176)



General procedure **D** using **133** (108.5 mg, 0.4 mmol, 1 eq.), vinyl ethyl ketone (100 µl, 1 mmol, 2.5 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol %) in CH_2Cl_2 (3 mL) gave methyl (2*S*,7*E*)-2-([(tert-butoxy)carbonyl]amino)-9-oxoundec-7-enoate **176** (108 mg, 0.33 mmol, 82%) as an oil. $[\alpha]_D$ +23.7 (c 0.93, $CHCl_3$); R_f = 0.22 (20% EtOAc in petroleum ether); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 6.79 (1 H, dt, *J* = 16.0 and 6.9 Hz, CH_2**CH**=CH), 6.08 (1 H, br.d, *J* = 16.0 Hz, CH=**CH**CO), 5.02 (1 H, br.d, *J* = 7.5 Hz, **NH**), 4.25-4.34 (1 H, m, α–**CH**), 3.74 (3 H, s, O**CH_3**), 2.55 (2 H, q, *J* = 7.3 Hz, CO**CH_2**CH_3), 2.21 (2 H, dq, *J* = 1.3 and 7.2 Hz, CH_2**CH_2**CH=), 1.73-1.87 (1 H, m, α-CH**CH^A**H^BCH_2), 1.58-1.68 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.28-1.55 (4 H, m, CH^{A}CH^B**$CH_2$$CH_2$**$CH_2$), 1.44 (9 H, s, ^{t}BuO), 1.09 (3 H, t, *J* =7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 201.0, 173.2, 155.3, 146.2, 130.2, 79.9, 53.2, 52.2, 33.2, 32.5, 32.1, 28.3, 27.6, 24.8, 8.1; **IR** (cm^{-1}), 3357, 1746, 1715, 1634, 1674, 1514, 1460, 1167; **m/z (ES+)** found: 328.2111, $C_{17}H_{30}NO_5$ requires MH+ 328.2124.

Methyl (2*S*,7*E*)-2-([(tert-butoxy)carbonyl]amino)-9-oxododec-7-enoate (177)

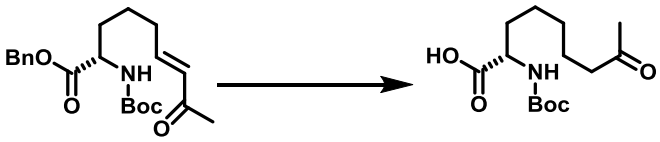


General procedure **D** using **133** (108.5 mg, 0.4 mmol, 1 eq.), vinyl propyl ketone (117 µl, 1 mmol, 2.5 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL) gave methyl (2*S*,7*E*)-2-([(tert-butoxy)carbonyl]amino)-9-oxododec-7-enoate **177** (125.4 mg, 0.37 mmol, 91%) as an oil. $[\alpha]_D$ +13.2 (c 0.38, $CHCl_3$); R_f = 0.29 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 6.77 (1 H, dt, *J* = 16.0 and 6.9 Hz, CH_2**CH**=CH), 6.07 (1 H, br.d, *J* = 16.0 Hz, CH=**CH**CO), 5.04 (1 H, br. d, *J* = 8.3 Hz, **NH**), 4.24-4.32 (1 H, m, α–**CH**), 3.72 (3 H, s, O**CH_3**), 2.49 (2 H, t, *J* = 7.3 Hz, CO**CH_2**CH_2), 2.19 (2 H, dq, *J* = 7.2 and 1.3 Hz, CH_2**CH_2**CH=), 1.74-1.85 (1 H, m, α-CH**CH^A**H^BCH_2), 1.56-1.67 (3 H, m, α-CH**C**H^A**H^B**CH_2, CH_2**CH_2**CH_3), 1.27-1.54 (4 H, m, CH^{A}CH^B**CH_2CH_2**CH_2), 1.42 (9 H, s, ^{t}BuO), 0.92 (3 H, t, *J* = 7.5 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 200.7, 173.3, 155.3, 146.4, 130.5, 79.9, 53.2, 52.2, 42.1, 32.5, 32.1, 28.3, 27.6, 24.8, 17.7, 13.8; **IR** (cm^{-1}), 3357, 1750, 1718, 1671, 1516, 1437, 1369, 1247, 1167; ***m/z* (ES+)** found: 342.2269, $C_{18}H_{32}NO_5$ requires MH+ 342.2280.

5.7 General Procedure E: Hydrogenation of cross-metathesis product

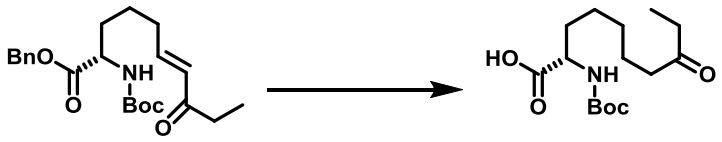
A two-necked round bottomed flask with magnetic stirrer bar was fitted with a rubber septum and three-way tap, and was flame-dried under vacuum and back-filled with nitrogen three times. The flask was allowed to cool, palladium on carbon catalyst (10% w/w) (1 eq.) but in case of compounds [**178-180**] (1.5 eq.) was added to the flask which was evacuated and back-filled with nitrogen three times. Then nitrogen gas line replaced with balloon of hydrogen gas, cross-metathesis product (1 eq.) was added to the flask as a solution in ethyl acetate (7 mL) via syringe. The flask was evacuated until the reaction mixture began to boil, and then back-filled with hydrogen gas. This procedure was repeated three more times, and the reaction stirred at room temperature for 1 day. To remove the catalyst the mixture was eluted through Celite® and then washed with EtOAc. The filtrate and washings were combined then the solvent removed under reduced pressure. No further purification was required.

(2*S*)-2-([(tert-butoxy)carbonyl]amino)-8-oxononanoic acid (178)[122]



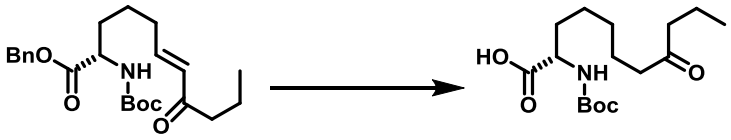
General procedure **E** using **170** (50 mg, 0.133 mmol, 1 eq.) gave (2*S*)-2-([(tert-butoxy)carbonyl]amino)-8-oxononanoic acid **178** (34 mg, 0.12 mmol 90%) as a colourless oil. $[\alpha]_D$ +5.0 (c 1.0, $CHCl_3$); R_f = 0.17 (EtOAc : petroleum ether : acetic acid, 5 : 5 : 0.1 mL); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.02 (1 H, d, *J* = 7.8 Hz, α–CH**NH**CO), 4.25-4.35 (1 H, m, α–**CH**), 2.44 (2 H, t, *J* = 7.3 Hz, CH_2**CH_2**CO), 2.14 (3 H, s, CO**CH_3**), 1.79-1.93 (1 H, m, α-CH**C**$H^A$$H^B$$CH_2$), 1.63-1.74 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.58 (2 H, quintet, *J* = 7.5 Hz, CH_2**CH_2**CCO), 1.27-1.48 (4 H, m, CH^ACH^B**CH_2CH_2**), 1.45 (9 H, s, ^{t}BuO); **^{13}C NMR** (400 MHz, $CDCl_3$) δ ppm: 209.6, 176.9, 155.6, 80.1, 53.3, 43.5, 32.3, 29.8, 28.6, 28.3, 25.0, 23.4. **IR** (cm^{-1}), 3340, 1730, 1700, 1658, 1520, 1390, 1368, 1252, 1167; **m/z (ES+)** found: 288.1806, $C_{14}H_{26}NO_5$ requires MH+ 288.1811

(2*S*)-2-([(tert-butoxy)carbonyl]amino)-8-oxodecanoic acid (179)[122]



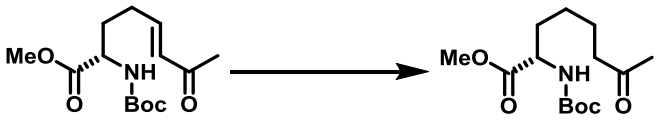
General procedure **E** using **171** (52 mg, 0.134 mmol, 1 eq.) gave (2*S*)-2-([(tert-butoxy)carbonyl]amino)-8-oxodecanoic acid **179** (35 mg, 0.12 mmol, 89%) as a colourless oil. $[\alpha]_D$ -37.2 (c 0.94, $CHCl_3$); R_f = 0.28 (EtOAc : petroleum ether : acetic acid, 5 : 5 : 0.1 mL); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.02 (1 H, d, *J* = 8.0 Hz, α–CH**NH**CO), 4.24-4.35 (1 H, m, α–**CH**), 2.42 (2 H, q, *J* = 7.3 Hz, CO**CH_2**CH_3), 2.41 (2 H, t, *J* = 7.3 Hz, CH_2**CH_2**CO), 1.79-1.92 (1 H, m, α-CH**CH^A**H^BCH_2), 1.63-1.74 (1 H, m, α-CH**C**H^A**H^B**CH_2), 1.58 (2 H, quintet, *J* = 7.3 Hz, CH_2**CH_2**CCO), 1.27-1.48 (4 H, m, CH^{A}CH^B**CH_2CH_2**), 1.45 (9 H, s, ^{t}BuO), 1.05 (3 H, t, *J* = 7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 212.1, 176.9, 155.6, 80.2, 53.3, 42.1, 35.9, 32.2, 28.7, 28.3, 25.1, 23.5, 7.8; **IR** (cm^{-1}), 3322, 1735, 1713, 1681, 1510, 1460, 1395, 1249, 1166; **m/z (ES+)** found: 302.1953, $C_{15}H_{28}NO_5$ requires MH+ 302.1967.

(2*S*)-2-([(tert-butoxy)carbonyl]amino)-8-oxoundecanoic acid (180)



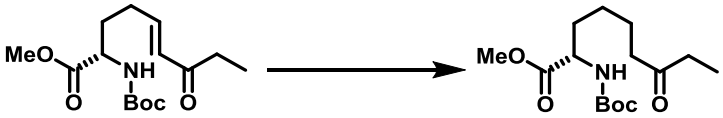
General procedure **E** using **172** (82 mg, 0.2 mmol, 1 eq.) gave (2*S*)-2-([(tert-butoxy)carbonyl]amino)-8-oxoundecanoic acid **180** (62 mg, 0.196 mmol, 98%) as a colourless oil. $[\alpha]_D$ -16.0 (c 0.5, $CHCl_3$); R_f = 0.25 (EtOAc : petroleum ether : acetic acid, 5 : 5 : 0.1 mL); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.03 (1 H, d, *J* = 8.1 Hz, α–CH**NH**CO), 4.24-4.35 (1 H, m, α–**CH**), 2.40 (2 H, t, *J* = 7.8 Hz, CH_2CO**CH_2**), 2.38 (2 H, t, *J* = 7.5 Hz, **CH_2**$COCH_2$), 1.77-1.93 (1 H, m, α-CH**CH^A**H^BCH_2), 1.52-1.74 (5 H, m, α-CHCH^A**CH^B**CH_2, CH_2**CH_2**CCO, CH_2**CH_2**CH_3), 1.24-1.51 (4 H, m, CH^{A}CH^B**CH_2CH_2**), 1.45 (9 H, s, ^{t}BuO), 0.91 (3 H, t, *J* = 7.4 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 211.6, 176.9, 155.6, 80.2, 53.3, 44.7, 42.5, 32.2, 28.7, 28.3, 25.1, 23.4, 17.3, 13.7; **IR** (cm^{-1}), 3346, 1717, 1701, 1688, 1511, 1453, 1366, 1243, 1159; **m/z (ES+)** found: 316.2119, $C_{16}H_{30}NO_5$ requires MH+ 316.2124.

Methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-7-oxooctanoate (181)



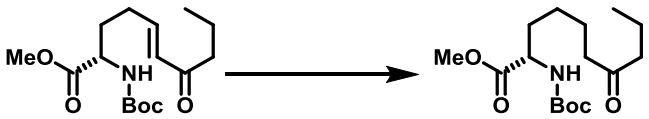
General procedure **E** using **166** (105 mg, 0.368 mmol, 1 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-7-oxooctanoate **181** (100 mg, 0.348 mmol, 94%) as a colourless oil. $[\alpha]_D$ +15.8 (c 0.95, $CHCl_3$); R_f = 0.31 (40% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.02 (1 H, d, *J* = 8.1 Hz, α–CH**NH**CO), 4.23-4.34 (1 H, m, α–**CH**), 3.74 (3 H, s, O**CH_3**), 2.43 (2 H, t, *J* = 7.2 Hz, CH_2**CH_2**CO), 2.13 (3 H, s, CO**CH_3**), 1.73-1.87 (1 H, m, α-CH**CH^A**$H^B$$CH_2$), 1.52-1.68 (3 H, m, α-CH**C**H^A**H^B**CH_2, CH_2**CH_2**CCO), 1.44 (9 H, s, ^{t}BuO), 1.22-1.40 (2 H, m, $CH^A$$CH^B$**$CH_2$**$CH_2$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 208.6, 173.3, 155.4, 79.8, 53.3, 52.3, 43.3, 32.5, 29.8, 28.3, 24.8, 23.2; **IR** (cm^{-1}), 3370, 1749, 1715, 1516, 1439, 1364, 1250, 1169; **m/z (ES+)** found: 310.1618, $C_{14}H_{25}NO_5Na$ requires MNa+ 310.1630.

Methyl(2*S*)-2-([(tert-butoxy)carbonyl]amino)-7-oxononanoate (182)



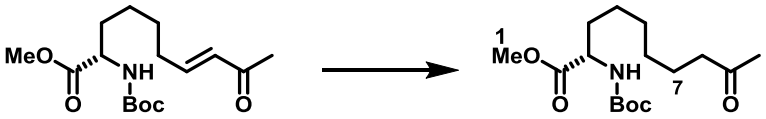
General procedure **E** using **167** (58 mg, 0.194 mmol, 1 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-7-oxononanoate **182** (58 mg, 0.193 mmol, 99%) as a colourless oil. $[\alpha]_D$ +16.0 (c 1, $CHCl_3$); R_f = 0.22 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.03 (1 H, d, *J* = 8.1 Hz, α–CH**NH**CO), 4.21-4.31 (1 H, m, α–**CH**), 3.70 (3 H, s, O**CH_3**), 2.34-2.43 (4 H, m, CH_2**CH_2**CO**CH_2**), 1.69-1.84 (1 H, m, α-CH**CH^A**$H^B$$CH_2$), 1.50-1.66 (3 H, m, α-CH**C**H^A**H^B**CH_2, CH_2**CH_2**CCO), 1.42 (9 H, s, ^{t}BuO), 1.22-1.37 (2 H, m, $CH^A$$CH^B$**$CH_2$**$CH_2$), 1.02 (3 H, t, *J* = 7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 211.3, 173.3, 155.4, 79.8, 53.3, 52.2, 41.9, 35.9, 32.6, 28.3, 24.9, 23.3, 7.8; **IR** (cm^{-1}), 3360, 1751, 1712, 1519, 1455, 1370, 1256, 1167; **m/z (ES+)** found: 302.1889, $C_{15}H_{28}NO_5$ requires MH+ 302.3830.

Methyl(2*S*)-2-([(tert-butoxy)carbonyl]amino)-7-oxodecanoate (183)



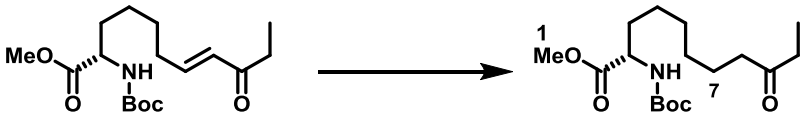
General procedure **E** using **168** (89 mg, 0.284 mmol, 1 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-7-oxodecanoate **183** (88 mg, 0.279 mmol, 98%) as colourless oil. $[\alpha]_D$ +15.0 (c 2, $CHCl_3$); R_f = 0.46 (30% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.01 (1 H, d, *J* = 8.1 Hz, α–CH**NH**CO), 4.23-4.34 (1 H, m, α–**CH**), 3.73 (3 H, s, -O**CH_3**), 2.39 (2 H, t, *J* = 7.3 Hz, CO**CH_2**), 2.36 (2 H, t, *J* = 7.3 Hz, **CH_2**CO), 1.71-1.87 (1 H, m, α-CH**CH^A**$H^B$$CH_2$), 1.51-1.67 (5 H, mα-CH**C**H^A**H^B**CH_2, CH_2**CH_2**CCO, CH_2**CH_2**CH_3), 1.44 (9 H, s, ^{t}BuO), 1.24-1.39 (2 H, m, $CH^A$$CH^B$**$CH_2$**$CH_2$), 0.91 (3 H, t, *J* = 7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 210.8, 173.3, 155.4, 79.8, 53.3, 52.2, 44.7, 42.3, 32.6, 28.3, 24.9, 23.3, 17.3, 13.7; **IR** (cm^{-1}), 3370, 1749, 1715, 1514, 1454, 1367, 1250, 1170; **m/z (ES+)** found: 316.2134, $C_{16}H_{30}NO_5$ requires MH+ 316.2124.

Methyl(2*S*)-2-([(tert-butoxy)carbonyl]amino)-9-oxodecanoate (184)



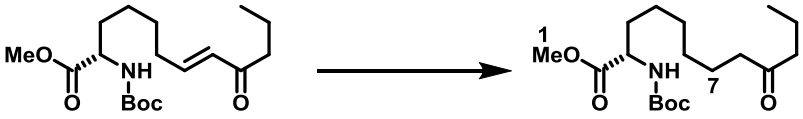
General procedure **E** using **175** (50 mg, 0.159 mmol, 1 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-9-oxodecanoate **184** (47.5 mg, 0.15 mmol, 94%) as a colourless oil. $[\alpha]_D$ +10.9 (c 1.1, $CHCl_3$); R_f = 0.17 (20% EtOAc in petroleum ether); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 4.99 (1 H, br.d, *J* = 7.7 Hz, α–CH**NH**CO), 4.22-4.32 (1 H, m, α–**CH**), 3.72 (3 H, s, O**CH_3**), 2.39 (2 H, t, *J* = 7.4 Hz, CH_2**CH_2**CO), 2.12 (3 H , s, CO**CH_3**), 1.69-1.82 (1 H, m, α-CH**CH^A**$H^B$$CH_2$), 1.51-1.65 (3 H, m, α-CH**C**H^A**H^B**CH_2, **CH_2**^{7}), 1.43 (9 H, s, ^{t}BuO), 1.22-1.37 (6 H, m, $CH^A$$H^B$**$CH_2CH_2CH_2$**); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 209.1, 173.4, 155.3, 79.8, 53.3, 52.1, 43.6, 32.6, 29.8, 28.9, 28.8, 28.3, 25.0, 23.6; **IR** (cm^{-1}), 3365, 1745, 1712, 1516, 1438, 1370, 1249, 1164; **m/z (ES+)** found: 316.2137, $C_{16}H_{30}NO_5$ requires MH+ 316.2124.

Methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-9-oxoundecanoate (185)



General procedure **E** using **176** (49 mg, 0.15 mmol, 1 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-9-oxoundecanoate **185** (49 mg, 0.148 mmol, 99%) as a colourless oil. $[\alpha]_D$ +19.3 (c 0.68, $CHCl_3$); R_f = 0.27 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.00 (1 H, br.d, *J* = 8.1 Hz, α–CH**NH**CO), 4.22-4.31 (1 H, m, α–**CH**), 3.72 (3 H, s, O**CH_3**), 2.34-2.44 (4 H, m, CH_2**CH_2**CO**CH_2**CH_3), 1.69-1.82 (1 H, m, α-CH**CH^A**$H^B$$CH_2$), 1.49-1.65 (3 H, m, α-CH**C**H^A**H^B**CH_2, **CH_2^{7}**), 1.43 (9 H, s, ^{t}BuO), 1.20-1.37 (6 H, m, $CH^A$$CH^B$**$CH_2CH_2CH_2$**), 1.03 (3 H, t, *J* = 7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 211.7, 173.4, 155.3, 79.8, 53.3, 52.2, 42.2, 35.8, 32.7, 28.9 (2 C), 28.3, 25.1, 23.7, 7.8; **IR** (cm^{-1}), 3367, 1746, 1711, 1704, 1518, 1455, 1364, 1168; **m/z (ES+)** found: 330.2290, $C_{17}H_{32}NO_5$ requires MH+ 330.2280.

Methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-9-oxododecanoate (186)



General procedure **E** using **177** (44 mg, 0.129 mmol, 1 eq.) gave methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)-9-oxododecanoate **186** (44 mg, 0.128 mmol, 99%) as a colourless oil. $[\alpha]_D$ +14.1 (c 0.85, $CHCl_3$); R_f = 0.57 (30% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.00 (1 H, br.d, *J* = 8.3 Hz, α–CH**NH**CO), 4.23-4.33 (1 H, m, α–**CH**), 3.73 (3 H, s, O**CH_3**), 2.37 (2 H, t, *J* = 7.3, CO**CH_2**CH_2), 2.36 (2 H, t, *J* = 7.3, CH_2**CH_2**CO), 1.69-1.85 (1 H, m, α-CH**CH^A**$H^B$$CH_2$), 1.49-1.67 (5 H, m, α-CH**C**H^A**H^B**CH_2, **CH_2^{7}**, CH_2**CH_2**CH_3), 1.44 (9 H, s, ^{t}BuO), 1.21-1.37 (6 H, m, $CH^A$$CH^B$**$CH_2CH_2CH_2$**), 0.90 (3 H, t, *J* = 7.5 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 211.4, 173.4, 155.4, 79.8, 53.4, 52.2, 44.7, 42.7, 32.7, 28.9 (2 C), 28.3, 25.1, 23.6, 17.3, 13.7; **IR** (cm^{-1}), 3370, 1749, 1713, 1692, 1520, 1462, 1247, 1172; **m/z (ES+)** found: 344.2437, $C_{18}H_{34}NO_5$ requires MH+ 344.2437.

5.8 General procedure F: Intramolecular aza-Michael reaction

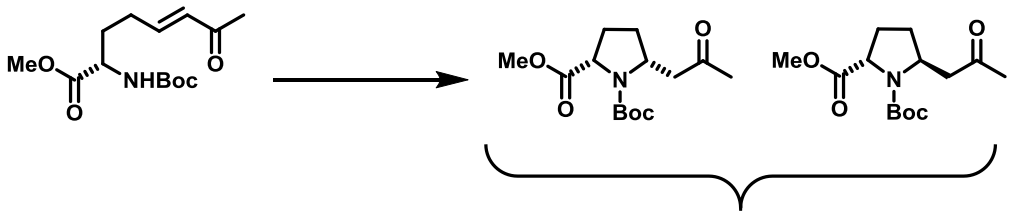
Method A. The cross-metathesis product (1 eq.) was dissolved in DCM (2 mL) and a solution of HCl in Et_2O (1 M, 0.6 mol%) was added. After 19 hr. stirring the resulting solution was concentrated under reduced pressure to give pure diastereoselective product.

Method B. A two-necked round bottomed flask with magnetic stirrer bar was fitted with rubber septum and three-way tap. The flask was flame-dried under vacuum and backfilled with nitrogen three times. The flask allowed to cool, $(MeCN)_2PdCl_2$ (specified in each experimental) was added by rapid removal and replacement of the three-way tap under a stream of nitrogen. Then nitrogen gas line replaced with balloon of argon gas, then cross-metathesis product (1 eq.) in dry DCM (2 mL) was added by syringe, and stirring was continued for an additional (T hr.). The reaction mixture was then filtered using diethyl ether through a pad of silica to remove the catalyst. The resulting filtrate and washings were combined and then concentrated under reduced presure. In the case of **173a/b**, **198a/b** and **203a/b** crude residue was purified by flash silica gel chromatography using a gradient of 15-30% EtOAc in petroleum ether.

5.9 General procedure G: Boc deprotection of amino acids

The *N*-Boc protected compound was dissolved in DCM (4 mL). Neat TFA (50 eq.) relative to the substrate was added and the reaction followed by TLC. The solvent removed under reduced pressure and an aqueous solution of NaOH 1 M was used to make pH 12, then EtOAc used to extract the organic compounds**.**

1-Tert-butyl 2-methyl (2*S*,5*R*)-5-(2-oxopropyl)pyrrolidine-1,2-dicarboxylate (193a) & 1-tert-butyl 2-methyl (2*S*,5*S*)-5-(2-oxopropyl)pyrrolidine-1,2-dicarboxylate (193b)

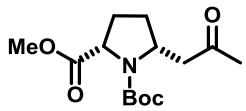


General procedure **F-A** using methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxooct-5-enoate **166** (32 mg, 0.112 mmol, 1 eq.) and 1 M HCl/Et_2O (6.6×10^{-4} mmol, 0.66 µL, 0.6 mol%) in DCM (2 mL), after 19 hr. stirring at r.t. gave the title compounds **193a/b** (32 mg, 0.11 mmol, 100%), as an oil, in a ratio of (0.04 *cis* **193a** : 0.96 *trans* **193b**) based on GC and ^{1}H NMR of the crude product in $CDCl_3$ see next page. $[\alpha]_D$ -54.0 (c 1, $CHCl_3$); R_f = 0.19 (20% EtOAc in petroleum ether); **^{1}H NMR** (500 MHz, DMSO 100 ^oC) δ ppm: 4.14-4.23 (2 H, m, CH^AH^B**CH**CH_2, CO**CH**CH_2), 3.65 (3 H, s, O**CH_3**), 2.85 (1 H, br.d, *J* = 16.2 Hz, CH**CH^A**H^BCO), 2.53 (1 H, dd, *J* = 16.2 and 9.7 Hz, CH**C**H^A**H^B**CO), 2.17-2.29 (1 H, m, COCHC**H^A**$H^B$$CH_2$), 2.09 (3 H, s, CO**$CH_3$**), 1.95-2.06 (1 H, m, CH_2**CH^A**$H^B$$CHCH_2$), 1.78-1.85 (1 H, m, $COCHCH^A$**H^B**CH_2), 1.55-1.64 (1 H, m, CH_2CH^A**H^B**$CHCH_2$), 1.36 (9 H, s, ^{t}BuO); **^{13}C NMR** (125 MHz, DMSO) δ ppm: 207.5 (207.4), 173.5 (173.0), 153.1 (153.4), 79.5 (79.8), 59.4 (59.1), 54.1 (53.9), 52.3 (52.2), 47.2 (48.1), 30.6 (30.7), 28.6 (29.3), 28.3 (28.4), 27.9 (27.1); **IR** (cm^{-1}), 1752, 1703, 1396, 1210, 1165, 1126; **m/z (ES+)** found: 286.1661, $C_{14}H_{24}NO_5$ requires MH+ 286.1654. In **^{13}C NMR** due to the rotamers, major isomer was reported and minor isomer signals are in parentheses, and it's clean as a mixture of two diastereoisomers.

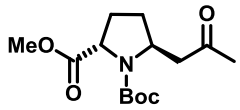
General procedure **F-B** using methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxooct-5-enoate **166** (40 mg, 0.14 mmol, 1 eq.) and $(CH_3CN)_2PdCl_2$ (9 mg, 0.035 mmol, 0.25 eq.) in dry DCM (2 mL), after 19 hr. stirring at r.t. gave the title compounds **193a/b** (40 mg, 100%), as an oil, in a ratio of (0.66 *cis* : 0.34 *trans*) based on GC of the crude product.

Different procedure:

Methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxooct-5-enoate **166** (84 mg, 0.29 mmol, 1 eq.) was added to silica gel (60 gm) in (20% EtOAc in petroleum ether) and stirred at room temperature for (90 hr.). Chromatography gave a mixture of **193a/b** (28 mg, 0.098 mmol, 33%), in a ratio of (0.15 *cis* : 0.85 *trans*) using a gradients of 20-30% EtOAc in petroleum ether.

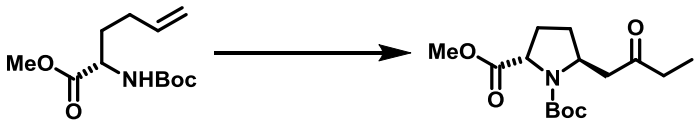


Significant signal for *cis* isomer in side mixture of two diastereoisomer when ^{1}H NMR solvent was $CDCl_3$; 2.49-2.72 (1H, m, $CHCH^{A}\mathbf{H}^{B}CO$).



Significant signal for *trans* isomer in side mixture of two diastereoisomer when ^{1}H NMR solvent was $CDCl_3$; 2.36-2.47 (1H, m, $CHCH^{A}\mathbf{H}^{B}CO$).

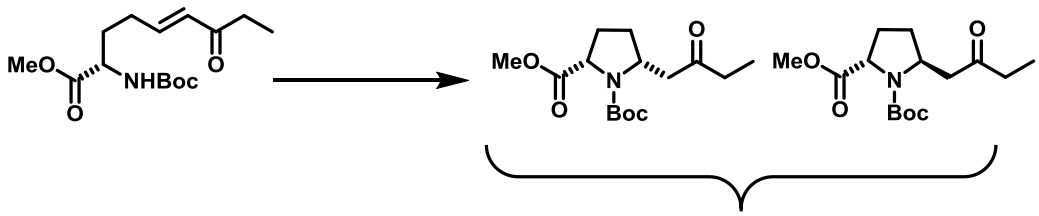
1-Tert-butyl 2-methyl (2*S*,5*S*)-5-(2-oxobutyl)pyrrolidine-1,2-dicarboxylate (159b)



General procedure **D** using methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)hex-5-enoate **131** (97 mg, 0.4 mmol, 1 eq.), vinyl ethyl ketone (100 µl, 1 mmol, 2.5 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL). Chromatography gave the title compound **159b** (100 mg, 0.33 mmol, 83%) as an oil, then NMR sample was allowed to stand in $CDCl_3$ and that all the subsequent characterization was from that material.

M.p./ 52-54 ^{o}C; $[\alpha]_D$ -50.5 (c 0.46, $CHCl_3$); R_f = 0.22 (20% EtOAc in petroleum ether); **^{1}H NMR** (500 MHz, DMSO 100 ^{o}C) δ ppm: 4.22-4.16 (2 H, m, CH_2**CH**CH^AH^B, CO**CH**CH_2), 3.65 (3 H, s, O**CH_3**), 2.84 (1 H, br.dd, *J* = 16.0 and 3 Hz, CH**CH^A**H^BCO), 2.52 (1 H, dd, *J* = 16.0 and 9.2 Hz, CH**C**H^A**H^B**CO), 2.41 (2 H, q, *J* = 7.4 Hz, CO**CH_2**CH_3), 2.19-2.31 (1 H, m, COCH**CH^A**$H^B$$CH_2$), 1.96-2.08 (1 H, m, CH_2**CH^A**$H^B$$CHCH_2$), 1.78-1.85 (1 H, m, COCH **C**H^A**H^B**CH_2), 1.55-1.63 (1 H, m, CH_2**C**H^A**H^B**$CHCH_2$), 1.36 (9 H, s, ^{t}BuO), 0.96 (3 H, t, *J* = 7.3, CH_2**CH_3**); **^{13}C NMR** (125 MHz, DMSO) δ ppm: 209.9 (209.8), 173.5 (173.0), 153.1 (153.4), 79.5 (79.7), 59.4 (59.1), 54.2 (54.0), 52.3 (52.2), 46.0 (46.7), 35.8 (35.9), 28.6 (29.3), 28.3 (28.4), 27.9 (27.1), 7.94 (7.98); **IR** (cm^{-1}), 1745, 1705, 1396, 1366, 1212, 1175, 1123; **m/z (ES+)** found: 300.1818, $C_{15}H_{26}NO_5$ requires MH+ 300.1811. Homodimer of **131** was detected in the crude reaction mixture, **m/z (ES+)** found: 459.2697, $C_{22}H_{39}N_2O_8$ requires MH+ 459.2706. In **^{13}C NMR** due to the rotamers, major isomer was reported and minor isomer signals are in parentheses.

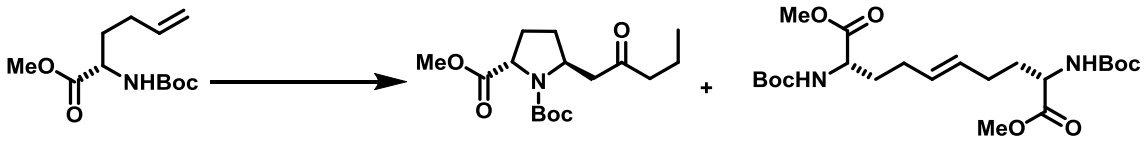
1-Tert-butyl 2-methyl (2*S*,5*R*)-5-(2-oxobutyl)pyrrolidine-1,2-dicarboxylate (159a) & 1-tert-butyl 2-methyl (2*S*,5*S*)-5-(2-oxobutyl)pyrrolidine-1,2-dicarboxylate (159b)



General procedure **F-A** using methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxonon-5-enoate **167** (30 mg, 0.1 mmol, 1 eq.) and 1 M HCl/Et_2O (6×10^{-4} mmol, 0.6 µL, 0.6 mol%) in DCM (2 mL), after 19 hr. stirring at r.t. gave the title compounds **159a/b** (30 mg, 100%), in a ratio of (0.02 *cis* : 0.98 *trans*) based on GC of the crude product.

General procedure **F-B** using of methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxonon-5-enoate **167** (183 mg, 0.612 mmol, 1 eq.) and $(CH_3CN)_2PdCl_2$ (18 mg, 0.07 mmol, 0.11 eq.) in dry DCM (2 mL), after 19 hr. stirring at r.t. gave the title compounds **159a/b** (182 mg, 0.61 mmol, 99%), in the ratio of (0.5 *cis* : 0.5 *trans*), based on GC of the crude product.

1-Tert-butyl 2-methyl (2*S*,5*S*)-5-(2-oxopentyl)pyrrolidine-1,2-dicarboxylate (161b) & 1,10-dimethyl (2*S*,5*E*,9*S*)-2,9-bis(([[(tert-butoxy)carbonyl]amino))dec-5-enedioate (102)

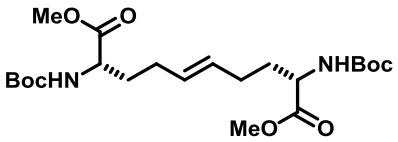


General procedure **D** using methyl (2*S*)-2-([(tert-butoxy)carbonyl]amino)hex-5-enoate **131** (97 mg, 0.4 mmol, 1 eq.), vinyl propyl ketone (117 µl, 1 mmol, 2.5 eq.) and Grubbs 2nd generation catalyst (16 mg, 0.02 mmol, 5 mol%) in CH_2Cl_2 (3 mL). Chromatography gave the title compounds **161b** (110 mg, 0.351 mmol, 87%) as an oil and homodimer **102** (7 mg, 0.015 mmol, 7.6%). Then NMR sample was allowed to stand in $CDCl_3$ and that all the subsequent characterization was from that material.

1-Tert-butyl 2-methyl (2*S*,5*S*)-5-(2-oxopentyl)pyrrolidine-1,2-dicarboxylate (161b)

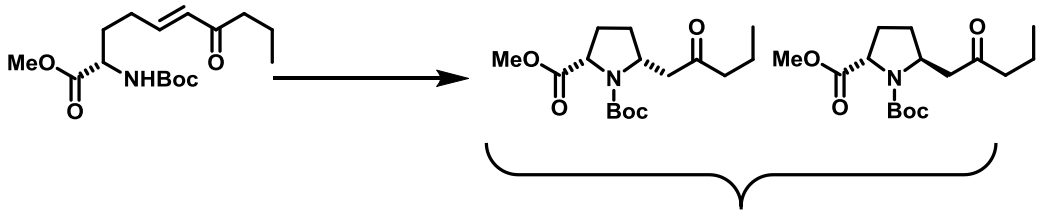
$[\alpha]_D$ -56.0 (c 1.25, $CHCl_3$); R_f = 0.18 (15% EtOAc in petroleum ether); **^{1}H NMR** (500 MHz, DMSO 100 ^oC) δ ppm: 4.15-4.22 (2 H, m, CH_2**CH**CH^AH^B, CO**CH**CH_2), 3.65 (3 H, s, O**CH_3**), 2.84 (1 H, br.d, *J* = 16.1 Hz, CH**CH^A**H^{B}CO), 2.52 (1 H, dd, *J* = 16.1 and 9.5 Hz, CH**C**H^A**H^B**CO), 2.38 (2 H, t, *J* = 7.3 Hz, CO**CH_2**CH_2), 2.19-2.30 (1 H, m, COCH**CH^A**H^BCH_2), 1.96-2.07 (1 H, m, CH_2**CH^A**H^B$CHCH_2$), 1.78-1.86 (1 H, m, COCH**C**H^A**H^B**CH_2), 1.56-1.64 (1 H, m, CH_2**C**H^A**H^B**$CHCH_2$), 1.52 (2 H, sextet, *J* = 7.3 Hz, CH_2**CH_2**CH_3), 1.36 (9 H, s, ^{t}BuO), 0.87 (3 H, t, *J* =7.4 Hz, CH_2**CH_3**); **^{13}C NMR** (125 MHz, DMSO) δ ppm: 209.1 (209.0), 173.0 (172.6), 152.6 (152.9), 79.1 (79.3), 58.9 (58.7), 53.7 (53.5), 51.8 (51.7), 45.8 (46.6), 44.2 (44.3), 28.2 (28.8), 27.8 (27.9), 27.5 (26.6), 16.5 (16.6), 13.5 (13.4); **IR** (cm^{-1}), 1751, 1699, 1392, 1258, 1085, 1020, 795; **m/z (ES+)** found: 314.1982, $C_{16}H_{28}NO_5$ requires MH+ 314.1967. In **^{13}C NMR** due to the rotamers, major isomer was reported and minor isomer signals are in parentheses.

1,10-Dimethyl(2*S*,5*E*,9*S*)-2,9-bis(([(tert-butoxy)carbonyl]amino))dec-5-enedioate (102)



R_f = 0.07 (15% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 5.37-5.48 (2 H, m, two olefinic proton), 5.01 (2 H, d, *J* = 8.8 Hz, 2 **NH**), 4.25-4.35 (2 H, m, 2 α-**CH**), 3.74 (6 H, s, 2 CO_2**CH_3**), 1.97-2.14 (4 H, m, **CH_2**CH=CH), 1.81-1.93 (2 H, m, **CH^A**H^B), 1.49-1.76 (2 H, m, **C**H^A**H^B**), 1.45 (18 H, s, 2 ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 173.3, 155.3, 137.3, 79.8, 52.9, 52.2, 32.4, 29.6, 28.3; **m/z (ES+)** found: 459.2718, $C_{22}H_{39}N_2O_8$ requires MH+ 459.2706.

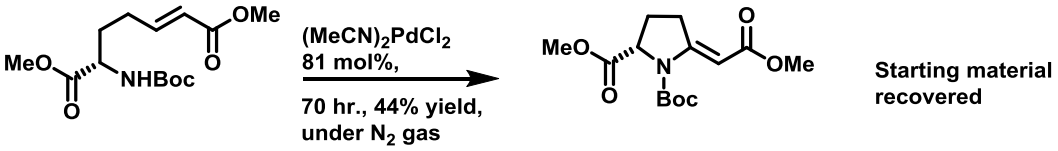
1-Tert-butyl 2-methyl (2*S*,5*R*)-5-(2-oxopentyl)pyrrolidine-1,2-dicarboxylate (161a) & 1-tert-butyl 2-methyl (2*S*,5*S*)-5-(2-oxopentyl)pyrrolidine-1,2-dicarboxylate (161b)



General procedure **F-A** using methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxodec-5-enoate **168** (49 mg, 0.16 mmol, 1 eq.) and 1 M HCl/Et_2O (9×10^{-4} mmol, 0.9 µL, 0.6 mol%) in DCM (2 mL), after 19 hr. stirring at r.t. gave the title compounds **161a/b** (49 mg, 0.16 mmol, 100%), as an oil, in a ratio of (0.02 *cis* : 0.98 *trans*) based on GC of the crude product.

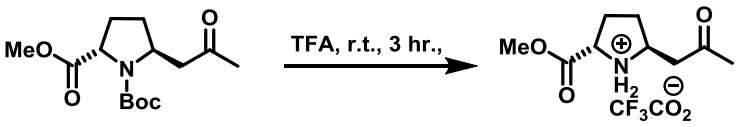
General procedure **F-B** using methyl (2*S*,5*E*)-2-([(tert-butoxy)carbonyl]amino)-7-oxodec-5-enoate **168** (178 mg, 0.57 mmol, 1 eq.) and $(CH_3CN)_2PdCl_2$ (16 mg, 0.062 mmol, 0.11 eq.) in dry DCM (2 mL), after 19 hr. stirring at r.t. gave the title compounds **161a/b** (177 mg, 0.565 mmol, 99%), as oil, in a ratio of (0.45 *cis* : 0.55 *trans*), based on GC of the crude product, and it's so clean as a mixture of two diastereoisomers.

1-Tert-butyl-2-methyl(2*S*,5*E*)-5-(2-methoxy-2-oxoethylidene)pyrrolidine-1,2-dicarboxylate (197)



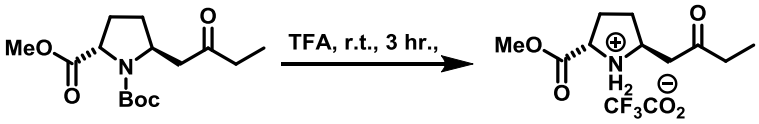
General procedure **F-B** using **169** (90 mg, 0.3 mmol, 1 eq.) and three equal portions of the $(MeCN)_2PdCl_2$ (each 21 mg, 0.08 mmol, 27 mol%) was added, during 70 hr.; chromatography gave 1-tert-butyl 2-methyl (2*S*,5*E*)-5-2-methoxy-2-oxoethylidene) pyrrolidine-1,2-dicarboxylate **197** as a solid (40 mg, 0.132 mmol, 44%). M.p./ 63-67 ^{o}C; $[\alpha]_D$ +16.0 (c 0.25, $CHCl_3$); R_f = 0.32 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 6.53 (1 H, br.s, C=**CH**CO), 4.59 (1 H, dd, *J* = 9.3 and 3.2 Hz,CO**CH**CH_2), 3.74 (3 H, s, O**CH_3**), 3.65 (3 H, s, O**CH_3**), 3.39 (1 H, dddd, *J* = 18.1, 9.1, 3.6 and 1.7 Hz, $CH_2C\mathbf{H}^{A}H^{B}C$), 2.96–3.09 (1 H, m, $CH_2\mathbf{C}H^{A}\mathbf{H}^{B}C$), 2.14-2.27 (1 H, m, $CH\mathbf{CH}^{A}H^{B}CH_2$), 1.96-2.05 (1 H, m, $CH\mathbf{C}H^{A}\mathbf{H}^{B}CH_2$), 1.46 (9 H, s,^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 172.2, 169.1, 156.7, 151.1, 96.5, 82.9, 62.1, 52.4, 50.8, 30.2, 27.9, 25.4; **IR** (cm^{-1}), 1753, 1733, 1623, 1438, 1380, 1136; **m/z (ES+)** found: 300.1434, $C_{14}H_{22}NO_6$ requires MH+ 300.1447.

(2*S*,5*S*)-2-(methoxycarbonyl)-5-(2-oxopropyl)pyrrolidin-1-ium trifluoroacetate (194)



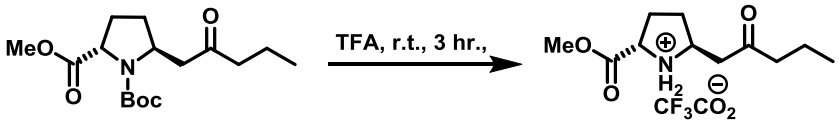
General procedure **G** using **193** (88 mg, 0.31 mmol, 1 eq.) and TFA (1.2 mL, 15.5 mmol, 50 eq.) gave (2*S*, 5*S*)-2-(methoxycarbonyl)-5-(2-oxopropyl)pyrrolidin-1-ium trifluoroacetate **194** (94 mg, 0.31 mmol, 100%) as solid. $[\alpha]_D$ -10.0 (c 1, $CHCl_3$); R_f = 0.075 (20% $CHCl_3$, 30% petroleum ether and 50% acetonitrile), **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 4.48-4.75 (1 H, m, CO**CH**CH_2), 3.94-4.08 (1 H, m, CH_2**CH**CH^AH^B), 3.86 (3 H, s, O**CH_3**), 3.18-3.37 (1 H, m, CH**CH^A**H^BCO), 2.93-3.13 (1 H, m, CH**C**H^A**H^B**CO), 2.53-2.67 (1 H, m, COCH**CH^A**$H^B$$CH_2$), 2.19-2.36 (1 H, m, CH_2**CH^A**$H^B$$CHCH_2$), 2.22 (3 H, s, CO**$CH_3$**), 2.04-2.18 (1 H, m, COCH**C**H^A**H^B**CH_2), 1.85-2.00 (1 H, m, CH_2CH^A**H^B**$CHCH_2$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 206.8, 169.7, 58.8, 57.1, 53.7, 44.1, 29.8, 29.5, 27.8; **IR** (cm^{-1}), 3425, 1751, 1715, 1676, 1445, 1366, 1245, 1185; **m/z (ES+)** found: 186.1125, $C_9H_{16}NO_3^+$ requires M+ 186.1130.

(2*S*,5*S*)-2-(methoxycarbonyl)-5-(2-oxobutyl)pyrrolidin-1-ium trifluoroacetate (195)



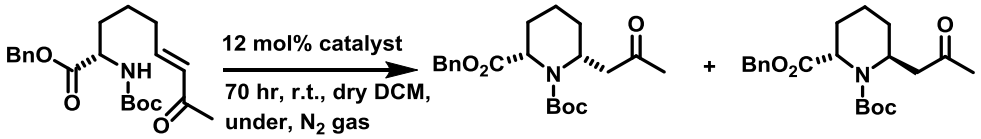
General procedure **G** using **159** (43 mg, 0.144 mmol, 1 eq.) and TFA (0.55 mL, 7.2 mmol, 50 eq.) gave (2*S*,5*S*)-2-(methoxycarbonyl)-5-(2-oxobutyl)pyrrolidin-1-ium trifluoroacetate **195** (45 mg, 0.143 mmol, 99% yield) as solid. $[\alpha]_D$ -6.1 (c 1.15, $CHCl_3$); R_f = 0.125 (20% $CHCl_3$, 30% petroleum ether and 50% acetonitrile); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 4.55-4.80 (1 H, m, CO**CH**CH_2), 3.92-4.04 (1 H, m, CH_2**CH**CH^AH^B), 3.88 (3 H, s, O**CH_3**), 3.22-3.38 (1 H, m, CH**CH^A**H^BCO), 2.93-3.08 (1 H, m, CH**C**H^A**H^B**CO), 2.60-2.72 (1 H, m, COCH**CH^A**$H^B$$CH_2$), 2.41-2.59 (2 H, m, CO**CH_2**CH_3), 2.21-2.35 (1 H, m, CH_2**CH^A**$H^B$$CHCH_2$), 2.05-2.18 (1 H, m, COCH**C**H^A**H^B**CH_2), 1.91-2.05 (1 H, m, CH_2**C**H^A**H^B**$CHCH_2$), 1.08 (3 H, t, *J* = 7.1 Hz, $COCH_2$**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 209.2, 169.7, 58.7, 56.9, 53.7, 43.1, 35.7, 30.1, 27.9, 7.3; **IR** (cm^{-1}), 1752, 1715, 1674, 1446, 1246, 1203, 1176; **m/z (ES+)** found: 200.1293, $C_{10}H_{18}NO_3^+$ requires M+ 200.1287.

(2*S*, 5*S*)-2-(methoxycarbonyl)-5-(2-oxopentyl) pyrrolidin-1-ium trifluoroacetate (196)



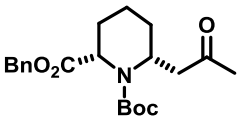
General procedure **G** using **161** (98 mg, 0.313 mmol, 1 eq.) and TFA (1.2 mL, 15.6 mmol, 50 eq.) gave (2*S*, 5*S*)-2-(methoxycarbonyl)-5-(2-oxopentyl) pyrrolidin-1-ium trifluoroacetate **196** (100 mg, 0.31 mmol, 99% yield) as a solid. $[\alpha]_D$ -2.0 (c 1, $CHCl_3$); R_f = 0.15 (20% $CHCl_3$, 30% petroleum ether and 50% acetonitrile); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 4.62 (1 H, t, *J* = 7.8 Hz CO**CH**CH_2), 3.89-4.04 (1 H, m, CH_2**CH**CH^AH^B), 3.87 (3 H, s, O**CH_3**), 3.29 (1 H, dd, *J* = 18.7 and 9.1 Hz, CH**CH^A**H^BCO), 2.97 (1 H, dd, *J* = 18.7 and 3.8 Hz, CH**C**H^A**H^B**CO), 2.56-2.67 (1 H, m, COCH**CH^A**$H^B$$CH_2$), 2.35-2.55 (2 H, m, CO**CH_2**CH_3), 2.20-2.34 (1 H, m, CH_2**CH^A**$H^B$$CHCH_2$), 2.03-2.16 (1 H, m, COCH **C**H^A**H^B**CH_2), 1.87-2.02 (1 H, m, CH_2**C**H^A**H^B**$CHCH_2$), 1.62 (2 H, sextet, *J* = 7.3 Hz, CH_2**CH_2**CH_3), 0.92 (3 H, t, *J* = 7.3 Hz, $COCH_2$**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 208.9, 169.7, 58.8, 57.2, 53.8, 44.4, 43.2, 29.9, 27.8, 16.8, 13.4; **IR** (cm^{-1}), 1749, 1715, 1674, 1446, 1246, 1203, 1176; **m/z (ES+)** found: 214.1439, $C_{11}H_{20}NO_3^+$ requires M+ 214.1443.

2-Benzyl 1-tert-butyl (2*S*,6*R*)-6-(2-oxopropyl)piperidine-1,2-dicarboxylate (203a) & 2-Benzyl 1-tert-butyl (2*S*,6*S*)-6-(2-oxopropyl)piperidine-1,2-dicarboxylate (203b)



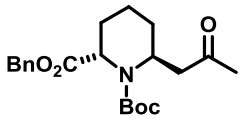
General procedure **F-B** using **170** (130 mg, 0.35 mmol, 1 eq.) and $(CH_3CN)_2PdCl_2$ catalyst (11 mg, 0.042 mmol, 0.12 eq.) in dry DCM . Chromatography gave **203a** (54.5 mg, 0.145 mmol, 41%), and **203b** (25 mg, 0.06 mmol, 19%). The ratio of (0.74 *cis* : 0.26 *trans*) was determined by ^{1}H NMR of the crude product.

2-Benzyl-1-tert-butyl (2*S*,6*R*)-6-(2-oxopropyl)piperidine-1,2-dicarboxylate (203a)



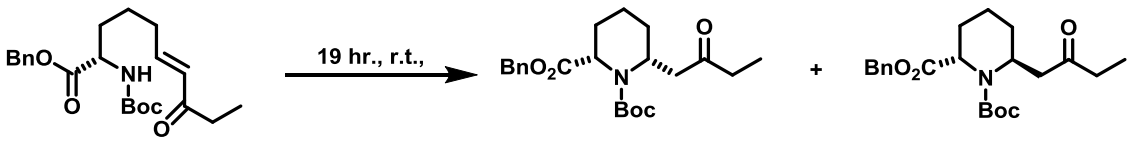
$[\alpha]_D$ -53.3 (c 1.8, $CHCl_3$); R_f = 0.27 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.30-7.40 (5 H, m, Ph), 5.19 (1 H, d, *J* = 12.0 Hz, Ph**C**H^{A}H^{B}O), 5.12 (1 H, d, *J* = 12.0 Hz, Ph**C**H^A**H^B**O), 4.63-4.97 (1 H, m, CO**C**HCH$_2$), 4.53 -4.62 (1 H, m, CH$_2$**C**HCH$_2$), 2.47-2.85 (2 H, m, CH**CH^{A}H^B**CO), 2.26 (1 H, d, *J* = 1.72 Hz, CH$_2$CH**CH^A**H^B), 2.04 (3 H, s, CO**CH$_3$**), 1.18-1.73 (14 H, m, **CH$_2$CH$_2$C**H^A**H^B**, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 207.3, 172.8, 155.1, 135.5, 128.6, 128.4, 127.9, 80.3, 66.8, 53.4, 52.7, 46.3, 30.2, 28.2, 27.5, 26.2, 15.5; **IR** (cm^{-1}), 1741, 1712, 1696, 1366, 1171, 1073; **m/z (ES+)** found: 376.2114, $C_{21}H_{30}NO_5$ requires MH+ 376.2124.

2-Benzyl-1-tert-butyl(2*S*,6*S*)-6-(2-oxopropyl)piperidine-1,2-dicarboxylate (203b)



$[\alpha]_D$ -3.1 (c 0.65, $CHCl_3$); R_f = 0.16 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.29-7.37 (5 H, m, Ph), 5.21 (1 H, d, *J* = 12.0 Hz, Ph**CH**^{A}H^{B}O), 5.13 (1 H, d, *J* =12.0 Hz, Ph**C**H^A**H**^{B}O), 4.45-4.53 (1 H, m, CH_2**CH**CH_2), 4.22-4.39 (1 H, m, CO**CH**CH_2), 2.50-2.93 (2 H, m, CH**CH**^{A}**H**^{B}CO), 2.17 (3 H, s, CO**CH$_3$**), 2.00-2.11 (1 H, m, **CH**^{A}H^BCH_2CH_2), 1.89-1.99 (1 H, m, **C**H^A**H**^{B}CH_2CH_2), 1.76-1.89 (1 H, m, CH_2CH_2**CH**^{A}H^B), 1.48-1.65 (3 H, m, CH_2**CH$_2$C**H^A**H**^{B}), 1.39 (9 H, s, ^{t}BuO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 206.7, 172.7, 155.3, 135.7, 128.5, 128.4, 128.2, 80.6, 66.7, 54.3, 47.9, 47.7, 30.1, 28.2, 24.8 (2 C), 14.7; **IR** (cm^{-1}), 1745, 1696, 1366, 1256, 1168; **m/z (ES+)** found: 376.2114, $C_{21}H_{30}NO_5$ requires MH+ 376.2124.

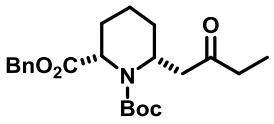
2-Benzyl 1-tert-butyl (2*S*,6*R*)-6-(2-oxobutyl)piperidine-1,2-dicarboxylate (198a) & 2-Benzyl 1-tert-butyl (2*S*,6*S*)-6-(2-oxobutyl)piperidine-1,2-dicarboxylate (198b)



General procedure **F-A** using **171** (181 mg, 0.465 mmol, 1 eq.) and 1 M HCl/Et_2O as a catalyst (3×10^{-3} mmol, 3 µl, 0.6 mol%.) in DCM (2 mL), after 19 hr. stirring at r.t.; chromatography gave **198a** (30 mg, 0.077 mmol, 16.6%) as a colourless oil, and **198b** (6.5 mg, 0.017 mmol, 3.6%) as a colourless oil, also starting material (142 mg, 0.365 mmol, 78.5%) recovered. The ratio of (0.83 *cis* : 0.17 *trans*) was determined by ^{1}H NMR of the crude product.

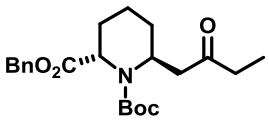
General procedure **F-B** using **171** (108 mg, 0.277 mmol, 1 eq.) and $(CH_3CN)_2PdCl_2$ catalyst (8.5 mg, 0.033 mmol, 0.12 eq.) in dry DCM (2 mL), after 19 hr. stirring at r.t.; chromatography gave **198a** (68 mg, 0.175 mmol, 63%) as white solid, and **198b** (31 mg, 0.079 mmol, 29%) as a colourless oil, also starting material **171** (4.3 mg, 0.11 mmol, 4%) recovered. The ratio of (0.7 *cis* : 0.3 *trans*) was determined by ^{1}H NMR of the crude product.

2-Benzyl 1-tert-butyl (2*S*,6*R*)-6-(2-oxobutyl)piperidine-1,2-dicarboxylate (198a)



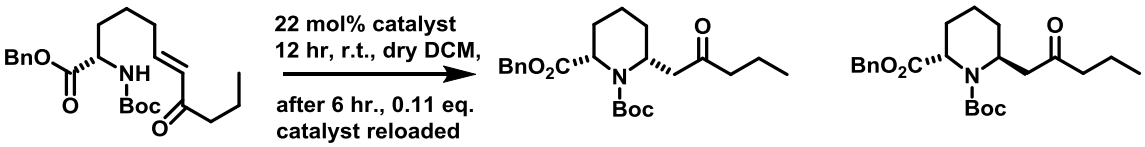
$[\alpha]_D$ -57.0 (c 0.65, $CHCl_3$); R_f = 0.44 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.29-7.41 (5 H, m, Ph), 5.19 (1 H, d, *J* = 12.0 Hz, Ph**CH^A**H^{B}O), 5.14 (1 H, d, *J* = 12.0 Hz, Ph**C**H^A**H^B**O), 4.66-4.99 (1 H, m, CO**CH**CH_2), 4.53-4.62 (1 H, m, CH_2**CH**CH_2), 2.62-2.85 (1 H, m, CH**CH^A**H^{B}CO), 2.47-2.61 (1 H, m, CH**C**H^A**H^B**CO), 2.16-2.46 (3 H, m, CO**CH_2**CH_3, , CH_2CH_2**CH^A**H^B), 1.28-1.75 (14 H, m, **CH_2CH_2C**H^A**H^B**, ^{t}BuO), 0.99 (3 H, t, *J* = 7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 210.0, 172.8, 155.1, 135.5, 128.6, 128.4 (2 C), 80.3, 66.8, 53.1, 46.6, 44.8, 36.2, 28.3, 27.6, 26.2, 15.6, 7.7; **IR** (cm^{-1}), 1748, 1715, 1698, 1375, 1178, 1078; **m/z (ES+)** found: 390.2297, $C_{22}H_{32}NO_5$ requires MH+ 390.2280.

2-Benzyl 1-tert-butyl (2*S*,6*S*)-6-(2-oxobutyl)piperidine-1,2-dicarboxylate (198b)



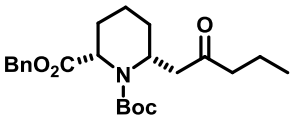
$[\alpha]_D$ -4.0 (c 0.5, $CHCl_3$); R_f = 0.31 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.29-7.37 (5 H, m, Ph), 5.2 (1 H, d, *J* = 12.0 Hz, Ph**CH^A**H^{B}O), 5.14 (1 H, d, *J* = 12.0 Hz, Ph**C**H^A**H^B**O), 4.45-4.53 (1 H, m, CH_2**CH**CH_2), 4.23-4.39 (1 H, m, CO**CH**CH_2), 2.53-2.83 (2 H, m, CH**CH^{A}H^B**CO), 2.47 (2 H, q, *J* = 7.3 Hz, CO**CH_2**CH_3), 1.91-2.12 (2 H, m, **CH_2**CH_2CH_2), 1.76-1.89 (1 H, m, CH_2CH_2**CH^A**H^B), 1.48-1.66 (3 H, m, CH_2**CH_2C**H^A**H^B**), 1.40 (9 H, s, ^{t}BuO), 1.05 (3 H, t, *J* = 7.3 Hz, CH_2**CH_3**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 209.3, 172.7, 155.3, 135.8, 128.5, 128.22 (2 C), 80.5, 66.7, 54.3, 48.1, 46.3, 36.1, 28.3, 24.9 (2 C), 14.8, 7.7; **IR** (cm^{-1}), 1745, 1696, 1391, 1369, 1170, 1109; **m/z (ES+)** found: 390.2287, $C_{22}H_{32}NO_5$ requires MH+ 390.2280.

2-Benzyl 1-tert-butyl (2*S*,6*R*)-6-(2-oxopentyl)piperidine-1,2-dicarboxylate (173a) & 2-Benzyl 1-tert-butyl (2*S*,6*S*)-6-(2-oxopentyl)piperidine-1,2-dicarboxylate (173b)



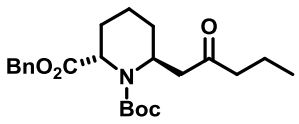
General procedure **F-B** using **172** (110 mg, 0.273 mmol, 1 eq.) and two equal portions of $(CH_3CN)_2PdCl_2$ (each 7.8 mg, 0.03 mmol, 11 mol%) in dry DCM (2 mL), after 12 hr. stirring at r.t.; chromatography gave **173a** (44 mg, 0.109 mmole, 40%) as a colourless oil and **173b** (26.7 mg, 0.066 mmol, 24%) as a colourless oil. The ratio of (0.62 *cis* : 0.38 *trans*) was determined by ^{1}H NMR of the crude product.

2-Benzyl 1-tert-butyl (2*S*,6*R*)-6-(2-oxopentyl)piperidine-1,2-dicarboxylate (173a)



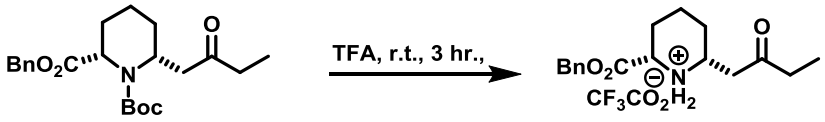
$[\alpha]_D$ -50.0 (c 1.5, $CHCl_3$); R_f = 0.4 (20% EtOAc in petroleum ether); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 7.30-7.40 (5 H, m, Ph), 5.19 (1 H, d, *J* = 12.0 Hz, Ph**C**H^{A}H^{B}O), 5.14 (1 H, d, *J* = 12.0 Hz, Ph**C**H^A**H**^{B}O), 4.82 (1 H, br.s, CO**C**HCH$_2$), 4.53-4.63 (1 H, m, CH$_2$**C**HCH$_2$), 2.46-2.78 (2 H, m, CO**C**H^A**H**^{B}CH), 2.16-2.36 (3 H, m, CO**C**H$_2$CH$_2$, CH$_2$CH$_2$**C**H^{A}H^B), 1.27-1.76 (16 H, m, CH$_2$**C**H$_2$CH$_3$, **C**H$_2$**C**H$_2$**C**H^A**H**^{B}, ^{t}BuO), 0.87 (3 H, t, *J* = 7.5 Hz, CH$_2$**C**H$_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 209.6, 172.8, 155.2, 135.5, 128.6, 128.4 (2 C), 80.3, 66.8, 53.3, 52.8, 46.4, 44.9, 28.3, 27.5, 26.2, 17.2, 15.6, 13.6; **IR** (cm^{-1}), 1745, 1693, 1393, 1370, 1344, 1174, 1096; **m/z (ES+)** found: 404.2417, $C_{23}H_{34}NO_5$ requires MH+ 404.2437.

2-Benzyl 1-tert-butyl (2*S*,6*S*)-6-(2-oxopentyl)piperidine-1,2-dicarboxylate (173b)



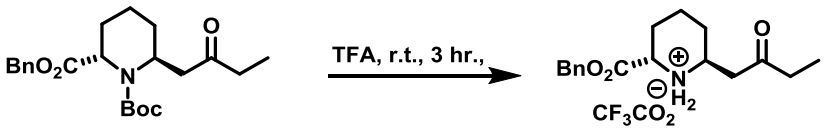
$[\alpha]_D$ +1.1 (c 0.95, $CHCl_3$); R_f = 0.33 (20% EtOAc in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.28-7.39 (5 H, m, Ph), 5.21 (1 H, d, *J* = 12.0 Hz, Ph**CH**^{A}H^{B}CO), 5.13 (1 H, d, *J* = 12.0 Hz, Ph**C**H^A**H**^{B}CO), 4.44-4.54 (1 H, m, CH_2**CH**CH^{A}H^B), 4.19-4.38 (1 H, m, CO**CH**CH_2), 2.52-2.85 (2 H, m, CH**CH**^{A}**H**^{B}CO), 2.42 (2 H, t, *J* = 6.8 Hz, CO**CH$_2$**CH_2), 1.88-2.09 (2 H, m, **CH$_2$**CH_2CH_2), 1.74-1.87 (1 H, m, CH_2CH_2**CH**^{A}H^B), 1.48-1.69 (5 H, m, CH_2**CH$_2$**CH_3, CH_2**CH$_2$C**H^A**H**^{B}), 1.39 (9 H, s, ^{t}BuO), 0.90 (3 H, t, *J* = 7.5 Hz, CH_2**CH$_3$**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 208.9, 172.7, 155.3, 135.8, 128.5, 128.2 (2 C), 80.5, 66.7, 54.3, 48.0, 46.5, 44.8, 28.3, 24.9, 24.8, 17.1, 14.8, 13.7; **IR** (cm^{-1}), 1745, 1696, 1454, 1393, 1370, 1174; **m/z (ES+)** found: 404.2422, $C_{23}H_{34}NO_5$ requires MH+ 404.2437.

(2*S*, 6*R*)-2-[(benzyloxy)carbonyl]-6-(2-oxobutyl)piperidin-1-ium trifluoroacetate (199)



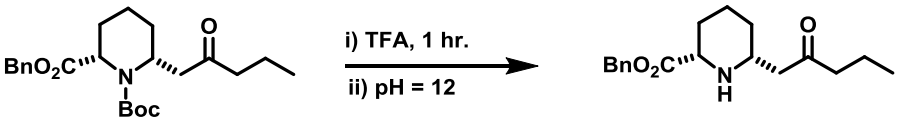
General procedure **G** using **198a** (97 mg, 0.24 mmol, 1 eq.), TFA (1.7 ml, 22.8 mmol, 95 eq.), H_2O (50 µl) and TIPS (20 µl). The crude product was purified by preparative HPLC (XBridge Prep OBD C18 5µm 19 mmid x 250 mm), using gradient from 95 : 5 to 5 : 95 water/acetonitrile, (H_2O contain 0.1% TFA) in 40 minutes, at a flow rate of 17 mL. min^{-1} and UV detection at 210 nm. The HPLC analysis showed a main peak t_R = (15-16) that was identified as the *cis* diastereoisomer, gave (2*S*,6*R*)-2-[(benzyloxy)carbonyl]-6-(2-oxobutyl)piperidin-1-ium trifluoroacetate **199** (95 mg, 0.23 mmol, 95%). M.p./ 127-138 ^oC; $[\alpha]_D$ -20.0, (c 0.25, $CHCl_3$); R_f = 0.83 (40% MeOH in EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 7.27-7.41 (5 H, m, Ph), 5.21 (1 H, d, *J* = 12.0 Hz, Ph**CH^A**H^{B}O), 5.16 (1 H, d, *J* = 12.0 Hz, Ph**C**H^A**H^B**O), 3.92 (1 H, br.d, *J* = 12.0 Hz, CO**CH**CH_2), 3.54-3.64 (1 H, m, CH_2**CH**CH_2), 3.26 (1 H, br.d, *J* = 17.6 Hz, CH**CH^A**H^{B}CO), 2.89-3.02 (1 H, m, CH**C**H^A**H^B**CO), 2.37-2.57 (2 H, m, CO**CH_2**CH_3), 2.28 (1 H, br.d, *J* = 13.5 Hz, **CH^A**H^BCH_2CH_2), 1.80-2.04 (3 H, m, **C**H^A**H^{B}CH^A**H^B**CH^A**H^B), 1.57-1.72 (2 H, m, CH_2, **C**H^A**H^{B}C**H^A**H^B**), 1.01 (3 H, t, *J* = 7.1 Hz, CH_2**CH_3**); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 209.1, 168.4, 134.3, 128.7 (2 C), 128.1, 68.0, 58.0, 53.6, 44.1, 36.2, 27.5, 25.8, 22.4, 7.3; **IR** (cm^{-1}), 1746, 1665, 1659, 1425, 1303, 1203; **m/z (ES+)** found: 290.1762, $C_{17}H_{24}NO_3^+$ requires M+ 290.1756.

(2*S*,6*S*)-2-[(benzyloxy)carbonyl]-6-(2-oxobutyl) piperidin-1-ium trifluoroacetate (200)



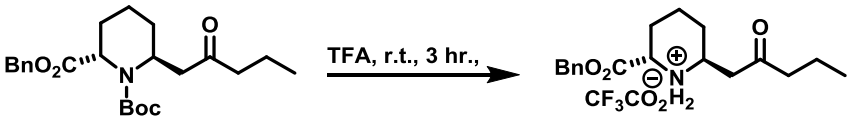
General procedure **G** using **198b** (10 mg, 0.026 mmol, 1 eq.) and TFA (99 µl, 1.3 mmol, 50 eq.). The crude product was purified by preparative HPLC (XBridge Prep OBD C18 5µm 19 mmid x 250 mm), using gradient from 95 : 5 to 5 : 95 water/acetonitrile, (H_2O contain 0.1% TFA) in 40 minutes, at a flow rate of 17 mL. min^{-1} and UV detection at 210 nm. The HPLC analysis showed a main peak t_R = (16-18) that was identified as the *trans* diastereoisomer (2*S*, 6*S*)-2-[(benzyloxy)carbonyl]-6-(2-oxobutyl) piperidin-1-ium trifluoroacetate **200** (9 mg, 0.023 mmol, 88%). $[\alpha]_D$ +24.0 (c 0.25, $CHCl_3$); R_f = 0.73 (40% MeOH in EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 7.38 (5 H, s, Ph), 5.32 (1 H, d, *J* = 12.0 Hz, PhC**H**^{A}H^{B}O), 5.29 (1 H, d, *J* = 12.0 Hz, PhCH^{A}**H**^{B}O), 4.42-4.53 (1 H, m, CO**CH**CH_2), 3.65-3.75 (1 H, m, CH_2**CH**CH_2), 3.13-3.29 (1 H, m, CH**CH**^{A}H^{B}CO), 2.71-2.82 (1 H, m, CH**C**H^{A}**H**^{B}CO), 2.40-2.63 (2 H, m, CO**CH_2**CH_3), 2.29-2.38 (1 H, m, **CH**^{A}H^{B}CH_2CH_2), 2.13 (1 H, t, *J* = 12.2 Hz, **C**H^{A}**H**^{B}CH_2CH_2), 1.69-1.84 (3 H, m, CH_2**CH_2**,**CH**^{A}H^{B}), 1.27-1.39 (1 H, m, CH_2CH_2,**C**H^{A}**H**^{B}), 1.08 (3 H, t, *J* = 7.0 Hz, CH_2**CH_3**); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 210.8, 168.2, 134.3, 128.9, 128.7, 128.6, 68.5, 54.6, 50.5, 43.6, 36.1, 27.6, 23.8, 19.2, 7.3; **IR** (cm^{-1}), 3284, 1752, 1690, 1678, 1409, 1344, 1200, 1135; **m/z (ES+)** found: 290.1751, $C_{17}H_{24}NO_3^+$ requires M+ 290.1756.

Benzyl (2*S*,6*R*)-6-(2-oxopentyl)piperidine-2-carboxylate (201)



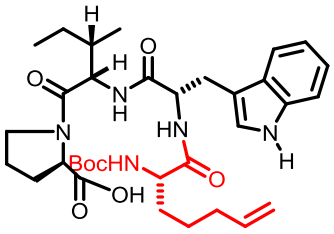
General procedure **G** using **173a** (8.5 mg, 0.021 mmol, 1 eq.) and TFA (77 µl, 1 mmol, 50 eq.), the reaction stirred for 1 hr. at room temperature. Then the solvent removed under reduced pressure, followed by base work-up by using 1 M NaOH, making pH = 12, after extraction with EtOAc (50 × 3 mL) gave benzyl (2*S*,6*R*)-6-(2-oxopentyl)piperidine-2-carboxylate **201** (4 mg, 0.0132 mmol, 63%) and also starting material **173a** (4 mg, 0.009 mmol). Purification was carried out by eluting over SiO_2 with 30-70% EtOAc in petroleum ether. $[\alpha]_D$ -40.0 (c 0.3, $CHCl_3$); R_f = 0.37 (30% petroleum ether in EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 7.29-7.38 (5 H, m, Ph), 5.19 (1 H, d, *J* = 12.3 Hz, Ph**CH^A**H^{B}O), 5.13 (1 H, d, *J* = 12.3 Hz, Ph**C**H^A**H^B**O), 3.42-3.47 (1 H, m, CO**CH**CH_2), 3.01-3.08 (1 H, m, CH_2**CH**CH_2), 2.59 (1 H, dd, *J* = 17.6 and 7.9 Hz, CH**CH^A**H^{B}CO), 2.52 (1 H, dd, *J* = 17.6 and 4.6 Hz, CH**C**H^A**H^B**CO), 2.38 (2 H, t, *J* = 7.3 Hz, CO**CH$_2$**CH_2), 2.01-2.07 (1 H, m, C**H^A**H^BCH_2CH_2), 1.84-1.91 (1 H, m, CH_2**CH^A**H^BCH_2), 1.60 (2 H, sextet, *J* = 7.4 Hz, CH_2**CH$_2$**CH_3), 1.53-1.59 (1 H, m, CH_2CH_2**CH^A**H^B), 1.36-1.49 (2 H, m, **C**H^A**H^{B}C**H^A**H^B**CH_2), 1.08-1.19 (1 H, m, CH_2CH_2**C**H^A**H^B**), 0.91 (3 H, t, *J* = 7.5 Hz, CH_2**CH$_3$**); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 210.2, 172.9, 135.7, 128.5, 128.2, 128.1, 66.4, 58.9, 51.8, 49.3, 45.4, 31.5, 28.8, 24.2, 17.2, 13.7; **IR** (cm^{-1}), 3582, 3336, 1741, 1711, 1454, 1386, 1193; **m/z (ES+)** found: 304.1899, $C_{18}H_{26}NO_3$ requires MH+ 304.1913.

((2*S*, 6*S*)-2-[(benzyloxy)carbonyl]-6-(2-oxopentyl)piperidin-1-ium trifluoroacetate (202)



General procedure **G** using **173b** (13 mg, 0.032 mmol, 1 eq.) and TFA (122 µl, 1.6 mmol, 50 eq.) to give ((2*S*, 6*S*)-2-[(benzyloxy)carbonyl]-6-(2-oxopentyl)piperidin-1-ium trifluoroacetate **202** (13 mg, 0.031 mmol, 97%). $[\alpha]_D$ +26.7 (c 0.6, $CHCl_3$); R_f = 0.23 (40% MeOH in petroleum ether); **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm: 7.36-7.40 (5 H, m, Ph), 5.33 (1 H, d, *J* = 12.0 Hz, Ph**CH**^{A}H^{B}O), 5.28 (1 H, d, *J* = 12.0 Hz, Ph**C**H^{A}**H**^{B}O), 4.43-4.51 (1 H, m, CO**CH**CH_2), 3.64-3.76 (1 H, m, CH_2**CH**CH_2), 3.15 (1 H, dd, *J* = 19.0 and 9.8 Hz CH**CH**^{A}H^{B}CO), 2.76 (1 H, dd, *J* = 19.0 and 3.5 Hz CH**C**H^{A}**H**^{B}CO), 2.37-2.54 (2 H, m, CO**CH$_2$**CH_2), 2.28-2.38 (1 H, m, **CH**^{A}H^{B}CH_2CH_2), 2.04-2.16 (1 H, m, **C**H^{A}**H**^{B}CH_2CH_2), 1.69-1.83 (3 H, m, CH_2**CH$_2$CH**^{A}H^{B}), 1.62 (2 H, sextet, *J* = 7.3 Hz, CH_2**CH$_2$**CH_3), 1.15-1.39 (1 H, m, CH_2CH_2**C**H^{A}**H**^{B}), 0.92 (3 H, t, *J* = 7.5, CH_2**CH$_3$**); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 210.3, 168.1, 134.3, 129.0, 128.8, 128.6, 68.5, 54.6, 50.5, 44.7, 43.9, 27.5, 23.9, 19.1, 16.8, 13.5; **IR** (cm^{-1}), 3414, 1746, 1678, 1591, 1415, 1206, 1185, 1131; **m/z (ES+)** found: 304.1923, $C_{18}H_{26}NO_3^+$ requires M+ 304.1913.

(2*R*)-1-[(2*S*,3*S*)-2-[(2*S*)-2-[(2*S*)-2-([(tert-butoxy)carbonyl]amino) hept-6-enamido]-3-(1H-indol-3-yl)propanamido]-3- methylpenta-noyl]pyrrolidine-2-carboxylic acid (216c)



A) **Resin loading**:

The 2-ClTrt-Cl resin 1.42 mmol/g (211 mg, 0.3 mmol, 1 eq.) and DMF (3 mL) were placed in varian bond elut reservoir 20 mL on orbital shaker, for 7 hr., to get the resin swollen followed by washing with DCM (2 × 3 mL). A solution of Fmoc-*R*-pro-OH (405 mg, 1.2 mmol, 4 eq.) and DIPEA (315 µl, 1.8 mmol, 6 eq.) in dry DCM (3 mL) was added and the mixture was stirred overnight. Then the solution was allowed to drain from the resin which was washed with (DCM/MeOH/DIPEA) (3 × 3 mL) (17 : 2 : 1) (7.65 : 0.9 : 0.45) mL and then with: DCM (3 × 3 mL), DMF (2 × 3 mL) and DCM (2 × 3 mL), (1.5 min each).

B) **Fmoc deprotection**:

Fmoc-*R*-pro-*O*-2ClTrt-resin swollen in DMF (3 mL) for 1 hr., the Fmoc protecting group was carried out using piperidine (25%) in DMF, (0.75 : 2.25), (3 mL, 1 × 5 min), (3 mL, 1 × 30 minutes); and then washings with DMF (2 × 3 mL), DCM (2 × 3 mL) and DMF (2 × 3 mL) (1.5 minutes each).

C) **Peptide synthesis protocol**:

The reaction was achieved by a coupling using HBTU in DMF as coupling reagent: DIPEA (367 µL, 2.1 mmol, 7 eq.) was added to Fmoc-*S*-isoleucine (424 mg, 1.2 mmol, 4 eq.) in DMF (3 mL), and stirred for (1/2 minutes) then HBTU (455 mg, 1.2 mmol, 4 eq.) was added and stirred for (5 minutes), the solution was loaded to the resin and stirred on orbital shaker for (3 hr.), then washing with: DCM (3 × 3 mL), DMF (2 × 3 mL) and DCM (2 × 3 mL) (1.5 minutes each). After each peptide coupling Fmoc de protection was done as in previous page mentioned.

2^{nd} peptide coupling solution of Fmoc-*S*-tryptophan (512 mg, 1.2 mmol, 4 eq.), DMF (3 mL), DIPEA (367 µL, 2.1 mmol, 7 eq.) and HBTU (455 mg, 1.2 mmol, 4 eq.) was loaded, to the resin, followed by washing and Fmoc deprotection.

3^{rd} coupling *N*-Boc-α-pent-4-enyl glycine (291.7 mg, 1.2 mmol, 4 eq.), DMF (3 mL), DIPEA (367 µL, 2.1 mmol, 7 eq.) and HBTU (455 mg, 1.2 mmol, 4 eq.) was loaded to the resin, 2^{nd} and 3^{rd} coupling were done in similar way to Fmoc-*S*-isoleucine.

D) **Cleavage (2-ClTrt-Cl)**:

The dried peptide resin was treated with cleavage mixture (AcOH/TFE/DCM) (2 : 2 : 6) (10 µL × 1 mg of resin) for (2 hr.), under stirring. Then the solution was filtered off and the resin washed with neat cleavage mixture (2 ×3 mL) 1.5 minutes each. Then the combined solution concentrated under reduced pressure followed by adding hexane (15 times volume) to remove acetic acid as an azeotrope, the filtrate was concentrated and lyophilized, to give crude product (111 mg). It was purified by preparative HPLC (XBridge Prep OBD C18 5µm 19 mmid x 250 mm), using 50 : 50 water/acetonitrile, at a flow rate of 17 mL. min^{-1} and UV detection at 254 nm. The HPLC analysis showed three main peaks t_R= (2-4), (8-12) and (13-14.5) that were identified by electrospray mass spectrometry as the linear Boc tetrapeptides; **216a** t_R= (2-4) (7 mg, 0.011 mmol, 3.6% yield), **216b** t_R= (8-12) (17 mg, 0.027 mmol, 8.8% yield) and **216c** t_R= (13-14.5) (37 mg, 0.058 mmol, 19% yield).

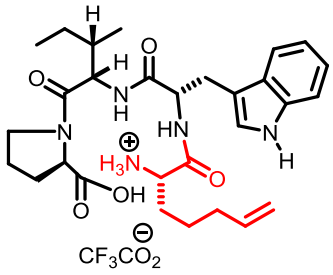
Boc-LTP (216c)

White solid m.p./127-137 ^{o}C; $[\alpha]_D$ -16.8 (c 1.25, CH_3CN); R_f = 0.83 (40% CH_3CN in H_2O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 8.65 (1 H, br.s), 7.32-7.81 (2 H, m), 7.21 (1 H, d, *J* = 6.7 Hz), 7.05 (1 H, t, *J* = 7.2 Hz), 6.86-7.02 (3 H, m), 5.81-5.65 (1 H, m), 5.04-4.88 (3 H, m), 4.73-4.85 (1 H, m), 4.59-4.72 (1 H, m), 4.4 (1 H, s), 4.00-4.22 (1 H, m), 3.64-3.99 (1 H, m), 3.37-3.62 (1 H, m), 2.96-3.33 (1 H, m), 2.30-1.64 (8 H, m), 1.62-1.18 (14 H, m), 0.96-1.15 (1 H, m), 0.75-0.93 (6 H, 2 **CH_3**); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 174.2, 172.3, 171.6, 170.6, 155.6, 138.1, 136.1, 127.8, 124.5, 121.5, 119.2, 118.5, 114.9, 111.4, 109.1, 80.0, 59.5 (2 C), 55.0, 54.7, 54.1, 47.8, 37.5, 33.2, 32.3, 28.9, 28.3, 24.8, 24.7, 24.5, 15.3, 10.9; **IR** (cm^{-1}), 3418, 3297, 3073, 2971, 1694, 1668, 1633, 1554, 1455, 1239, 1164; **m/z (ES+)** found: 640.3710, $C_{34}H_{50}N_5O_7$ requires MH+ 640.3710.

Boc-LTP (216a)

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 9.65 (1 H, br.s), 7.75 (1 H, d, *J* = 6.0 Hz), 7.36 (1 H, d, *J* = 7.8 Hz), 7.03-7.18 (3 H, m), 6.42 (1 H, br.s), 5.68-5.81 (1 H, m), 5.06-5.21 (1 H, m), 4.88-5.04 (2 H, m), 4.58-4.71 (2 H, m), 4.24-4.36 (1 H, m), 4.07-4.21 (1 H, m), 3.72-3.89 (1 H, m), 3.46-3.62 (2 H, m), 3.04-3.28 (2 H, m), 2.86-2.98 (2 H, m), 1.97-2.16 (6 H, m), 1.74-1.94 (3 H, m), 1.49-1.72 (5 H, m), 1.33-1.47 (12 H, m), 0.94- 1.05 (1 H, m), 0.74- 0.88 (6 H, m); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 177.2, 171.8, 170.8, 170.2, 155.6, 138.1, 136.4, 127.6, 125.1, 121.5, 119.1, 118.8, 114.9, 111.5, 109.3, 79.9, 61.4, 55.0, 54.6, 54.2, 47.8, 44.5, 37.7, 33.3, 32.4, 30.9, 29.5, 28.6, 28.3, 24.7 (2 C), 24.3, 22.7, 22.5, 15.3, 11.1; **m/z (ES+)** found: 662.3507, $C_{34}H_{49}N_5O_7Na$ requires MNa+ 662.3530. Extra peaks observed (6 protons and 4 carbon atoms) in aliphatic region and assigned to an unknown impurity.

e) (1*S*)-1-([(1*S*)-1-([(2*S*,3*S*)-1-[(2*R*)-2-carboxypyrrolidin-1-yl]-3-methyl-1-oxopentan-2-yl]carbamoyl)-2-(1H-indol-3-yl)ethyl]carbamoyl)hex-5-en-1-aminium trifluoroacetate (217)

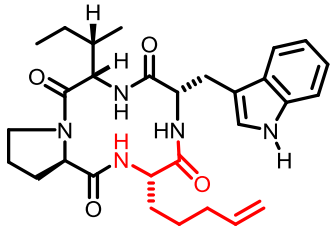


R-Pro-Ile-Trip- *N*-Boc-α-pentenyl glycine **216c** (22 mg, 0.034 mmol, 1 eq.) in DCM (3 mL) treated with TFA/H_2O/TIPS (93/5/2) (130 : 7 : 3) µL, under stirring for 7 hr., to give the crude linear peptide **217** (24 mg), it was sufficiently pure to use without purification; **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 175.1, 172.7, 169.7, 168.1, 136.5, 135.9, 126.9, 124.8, 121.9, 118.9, 118.2, 115.8, 112.7, 108.4, 59.9, 54.8, 53.9, 47.9, 37.9, 32.7, 31.9, 30.4, 28.8, 27.9, 24.8, 23.7, 23.1, 15.1, 10.8; **m/z (ES+)** found: 540.3162, $C_{29}H_{42}N_5O_5^+$ requires M+ 540.3186.

(3*S*,6*S*,9*S*,14a*R*)-9-[(2*S*)-butan-2-yl]-6-(1H-indol-3-ylmethyl)-3-(pent-4-en-1-yl)-tetradecahydropyrrolo[1,2-a]1,4,7,10- tetraazacyclododecane-1,4,7,10-tetrone (210) & (3*S*,6*S*,9*R*,12*R*,18*S*,21*S*,24*R*,27*R*)-3,18-bis[(2*S*)-butan-2-yl]-6-(1H-indol-2-ylmethyl)-21-(1H-indol-3-ylmethyl)-9,24-bis(pent-4-en-1-yl)-1,4,7,10,16,19,22,25-octaazatricyclo[25.3.0.0^{12},^{16}]triacontan-2,5,8,11,17,20,23,26-octone (218)

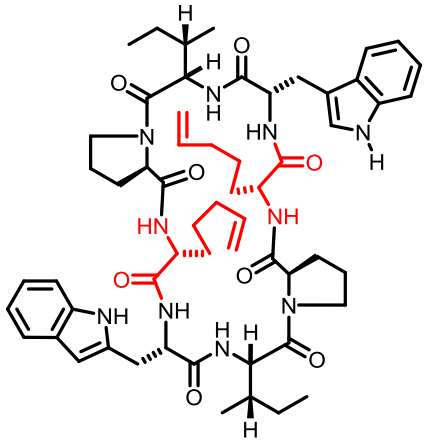
To HATU (28 mg, 0.072 mmol, 2 eq.) and DIPEA (16 µL, 0.093 mmol, 2.5 eq.) in dry DCM (500 mL) under nitrogen gas, *R*-Pro-Ile-Trip-*N*-α-pentenyl glycine (24 mg, 0.036 mmol, 1 eq.), was added dropwise in period of (1 hr.) at (0 ^{o}C) then outside the cooling bath stirring continued for (3 hr). Later the solvent removed under reduced pressure then the crude was purified by preparative HPLC (XBridge Prep OBD C18 5µm 19 mmid x 250 mm), using gradient from 95 : 5 to 5 : 95 water/acetonitrile, at a flow rate of 17 mL. min^{-1} and UV detection at 254 nm. The HPLC analysis showed two main peaks t_R = (17-18) and (25-25.5) that were identified by electrospray mass spectrometry as the cyclic tetrapeptide **210** t_R = (17-18) (3 mg, 0.006 mmol, 16% Yield), and cyclic octapeptide **218** t_R = (25-25.5) (2.6 mg, 0.0025 mmol, 14%).

(3*S*,6*S*,9*S*,14a*R*)-9-[(2*S*)-butan-2-yl]-6-(1H-indol-3-ylmethyl)-3-(pent-4-en-1-yl)-tetradecahydropyrrolo[1,2-a]1,4,7,10- tetraazacyclododecane-1,4,7,10-tetrone t_R = (17-18) (210)



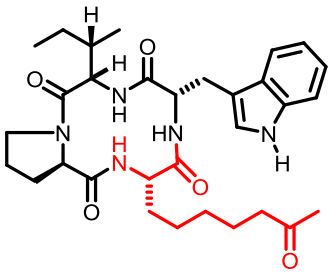
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 8.03 (1 H, br.s, **NH**), 7.61 (1 H, d, *J* = 7.9 Hz, aromatic proton), 7.34-7.39 (2 H, m, aromatic proton & **NH**), 7.19 (1 H, dt, *J* = 0.7 and 7.5 Hz, aromatic proton), 7.09-7.17 (2 H, m, aromatic proton & **NH**), 7.05 (1 H, d, *J* = 2.1 Hz, aromatic proton), 6.36 (1 H, d, *J* = 5.8 Hz, **NH**), 5.76 (1 H, ddt, *J* = 16.8, 10.2 and 6.6 Hz, sp^2**CH**), 4.96-5.04 (2 H, m, sp^2**CH**), 4.76 (1 H, dd, *J* = 7.8 and 1.6 Hz, α-**CH**), 4.55 (1 H, t, *J* = 10.7 Hz, α-**CH**), 4.13-4.20 (1 H, m, *J* = 8.4 Hz, α-**CH**), 3.95-4.03 (1 H, m, α-**CH**), 3.82-3.94 (2 H, m, each **H** belong to two different **CH_2**), 3.58 (1 H, dd, *J* = 14.7 and 6.9 Hz, **CH_2**), 3.51 (1 H, q, *J* = 8.4 Hz, **CH_2**), 2.34-2.42 (1 H, m, **CH_2**), 2.21-2.33 (1 H, m, **CH_2**), 1.98-2.11 (3 H, m, **CH_2** & α-**CH**), 1.88-1.98 (1 H, m), 1.49-1.88 (4 H, m, **2CH_2**), 1.24-1.39 (2 H, m), 1.09-1.22 (1 H, m), 0.91 (3 H, t, *J* = 7.6 Hz), 0.88 (3 H, d, *J* = 6.7 Hz); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 175.0, 174.7, 173.5, 171.6, 138.1, 136.1, 132.4, 127.0, 122.9, 122.2, 119.6, 118.6, 115.0, 111.2, 62.1, 57.8 (2 C), 54.0, 47.0, 33.8, 33.3, 31.2, 28.6, 25.3, 24.9, 24.8, 24.7, 15.7, 10.7; **IR** (cm^{-1}), 3444, 3359, 3045, 2934, 1731, 1650, 1620, 1103; **m/z (ES+)** found: 522.3067, $C_{29}H_{40}N_5O_4$ requires MH+ 522.3080.

(3*S*,6*S*,9*R*,12*R*,18*S*,21*S*,24*R*,27*R*)-3,18-bis[(2*S*)-butan-2-yl]-6-(1H-indol-2-ylmethyl)-21-(1H-indol-3-ylmethyl)-9,24-bis(pent-4-en-1-yl)-1,4,7,10,16,19,22,25-octaazatricyclo[25.3.0.0^{12},^{16}]triacontan-2,5,8,11,17,20,23,26-octone t_R = (25-25.5) (218)



^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 8.29 (2 H, s), 7.64-8.07 (4 H, br.m), 7.18-7.37 (6 H, m), 7.13 (2 H, t, *J* = 7.5 Hz), 6.91-7.03 (2 H, m), 6.65 (2 H, br.s), 5.76 (2 H, ddt, *J* = 16.7, 10.1 and 6.7 Hz), 4.55-5.14 (8 H, m), 4.13-4.51 (4 H, m), 2.69-3.72 (8 H, m), 2.25-2-40 (2 H, m), 1.19-2.18 (22 H, m), 0.98-1.13 (2 H, m), 0.75-0.97 (12 H, m); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 172.5, 172.4, 171.8, 171.5, 137.8, 135.8, 127.2, 121.9, 121.6, 119.3, 118.6, 115.3, 110.9, 110.8, 59.2 (2 C), 56.2, 53.7, 46.8, 35.8, 33.2, 31.4, 26.6, 26.5, 25.3, 24.6, 24.4, 15.6, 11.2; **m/z (ES+)** found: 1043.6123, $C_{58}H_{79}N_{10}O_8$ requires MH+ 1043.6082.

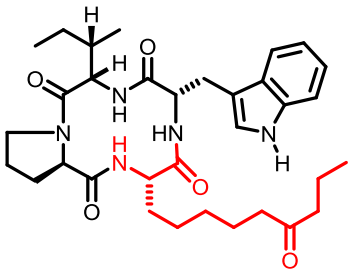
(3*S*,6*S*,9*S*,14a*R*)-9-[(2*S*)-butan-2-yl]-6-(1H-indol-3-ylmethyl)-3-(6-oxoheptyl)-tetradecahydropyrrolo[1,2-a]1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrone (221)



General procedure **D** using **210** (5 mg, 0.01 mmol, 1 eq.), vinyl methyl ketone (25 µl, 0.3 mmol, 30 eq.) and Grubbs 2nd generation catalyst (19 mg, 0.022 mmol, 2.2 eq.) in dry degases DCM (3 mL) then after overnight reflux the solvent removed under reduced pressure. The crude reaction mixture submitted to hydrogenation by adding 10% pd/C (10 mg, 10 eq.) in EtOAc (4 mL) (24 hr.), under balloon of hydrogen gas. The crude product was purified by preparative HPLC (XBridge Prep OBD C18 5µm 19 mmid x 250 mm), using 70 : 30 water/acetonitrile, at a flow rate of 17 mL. min^{-1} and UV detection at 254 nm. The HPLC analysis showed a main peak at t_R = (17.5-20) that was identified as apicidin **1** analogue, **221** (3 mg, 0.005 mmol, 50% yield over two steps). $[\alpha]_D$ -86.7 (c 0.15, CH_3CN); R_f = 0.53 in (2 acetone: 1 hexane) mL; **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 8.49 (1 H, s, **NH**), 7.6 (1 H, d, *J* = 7.5 Hz, aromatic proton), 7.35-7.42 (2 H, m, aromatic proton & **NH**), 7.19 (1 H, t, *J* = 7.5 Hz, aromatic proton), 7.08-7.16 (2 H, m, aromatic proton & **NH**), 7.07 (1 H, d, *J* = 2.1 Hz, aromatic proton), 6.26 (1 H, d, *J* = 5.6 Hz, **NH**), 4.76 (1 H, dd, *J* = 7.7 and 1.5 Hz, α-CH), 4.6 (1 H, t, *J* = 10.5 Hz, α-CH), 4.08-4.17 (1 H, m, α-CH), 3.96-4.03 (1 H, m, α-CH), 3.86-3.95 (2 H, m, for two different CH_2 aliphatic), 3.47-3.57 (2 H, m, for two different CH_2 aliphatic), 2.38-2.47 (2 H, m, aliphatic proton), 2.22-2.38 (2 H, m, aliphatic proton), 2.16 (3 H, s, aliphatic proton), 2.01-2.10 (1 H, m, α-CH), 1.89-2.00 (3 H, m, two different aliphatic CH_2), 1.77-1.88 (3 H, m, two different aliphatic CH_2), 1.47-1.73 (3 H, m, two different aliphatic CH_2), 1.09-1.41 (3 H, m, two different aliphatic CH_2), 0.93 (3 H, t, *J* = 7.27 Hz, CH_3), 0.88 (3 H, d, *J* = 6.8 Hz, CH_3); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 209.7, 174.9, 174.8, 173.5, 171.6, 136.2, 126.9, 123.3, 122.1, 119.5, 118.5, 111.4, 110.8, 61.9, 57.9, 57.8, 54.0, 46.9, 43.4, 33.9, 29.9, 28.9,

28.6, 25.4, 25.3, 24.9 (2 C), 24.8, 23.6, 15.7, 10.7; **IR** (cm^{-1}), 3339, 3279, 3056, 2959, 1700, 1664, 1617, 1524, 1444, 1241; **m/z (ES+)** found: 566.3345, $C_{31}H_{44}N_5O_5$ requires MH+ 566.3342.

(3*S*,6*S*,9*S*,14a*R*)-9-[(2*S*)-butan-2-yl]-6-(1H-indol-3-ylmethyl)-3-(6-oxononyl)-tetradecahydropyrrolo[1,2-a]1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrone (222)



General procedure **D** using **210** (14 mg, 0.027 mmol, 1 eq.), vinyl propyl ketone (95 µl, 0.81 mmol, 30 eq.) and Grubbs 2nd generation catalyst (57 mg, 0.067 mmol, 2.5 eq.) in dry degases DCM (3 mL), then after overnight reflux the solvent removed under reduced pressure. The crude reaction mixture submitted to hydrogenation by adding 10% pd/C (28 mg, 10 eq.) in EtOAc (4 mL) (24 hr.), under balloon of hydrogen gas. The crude product was purified by preparative HPLC (XBridge Prep OBD C18 5µm 19 mmid x 250 mm), using gradient from 95 : 5 to 5 : 95 water/acetonitrile in 40 minutes, at a flow rate of 17 mL. min^{-1} and UV detection at 254 nm. The HPLC analysis showed a main peak t_R = (22.5-23.5) that was identified as the apicidin **1** analogue, **222** (7 mg, 0.012 mmol, 44% yield over two steps). $[\alpha]_D$ -35.0 (c 0.2, $CHCl_3$); R_f = 0.55 in (2 Acetone: 1 hexane); **^{1}H NMR** (500 MHz, $CDCl_3$) δ ppm: 8.53 (1 H, s, **NH**), 7.6 (1 H, d, *J* = 7.5 Hz, aromatic proton), 7.34-7.42 (2 H, m, aromatic proton & **NH**), 7.19 (1 H, t, *J* = 7.4 Hz, aromatic proton), 7.08-7.15 (2 H, m, aromatic proton & **NH**), 7.07 (1 H, d, *J* = 1.7 Hz, aromatic proton), 6.19 (1 H, d, *J* = 7.7 Hz, **NH**), 4.76 (1 H, br.d, *J* = 6.9 Hz, α-**CH**), 4.6 (1 H, t, *J* = 10.5 Hz, α-**CH**), 4.08-4.16 (1 H, m, α-**CH**), 3.96-4.03 (1 H, m, α-H), 3.85-3.96 (2 H, m, for two different **CH_2** aliphatic), 3.46-3.57 (2 H, m, for two different **CH_2** aliphatic), 2.15-2.46 (6 H, m, 3 **CH_2** aliphatic proton), 2.01-2.12 (1 H, m, α-**CH**), 1.89-1.98 (1 H, m, aliphatic **CH_2**), 1.77-1.88 (2 H, m, 2 different aliphatic **CH_2**), 1.47-1.72 (6 H, m, 4 different aliphatic **CH_2**), 1.11-1.36

(5 H, m, 3 different aliphatic **CH_2**), 0.94 (3 H, t, *J* = 7.4 Hz, **CH_3**), 0.93 (3 H, t, *J* = 7.3 Hz, **CH_3**), 0.86-0.91 (3 H, m, **CH_3**); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 211.9, 174.9, 174.8, 173.5, 171.6, 136.3, 126.9, 123.4, 122.1, 119.5, 118.5, 111.4, 110.7, 61.9, 57.9, 57.8, 54.1, 47.0, 44.8, 42.4, 33.9, 28.9, 28.7, 25.5, 25.3, 24.9 (2 C), 24.8, 23.7, 17.3, 15.7, 13.8, 10.7; **IR** (cm^{-1}), 3336, 3309, 3274, 3013, 2966, 1662, 1618, 1523, 1443, 1263; **m/z (ES+)** found: 594.3669, $C_{33}H_{48}N_5O_5$ requires MH+ 594.3655.

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7. Appendix

Crystal structure data

Crystal structure of compound **159b** and it indicate *trans* configuration.

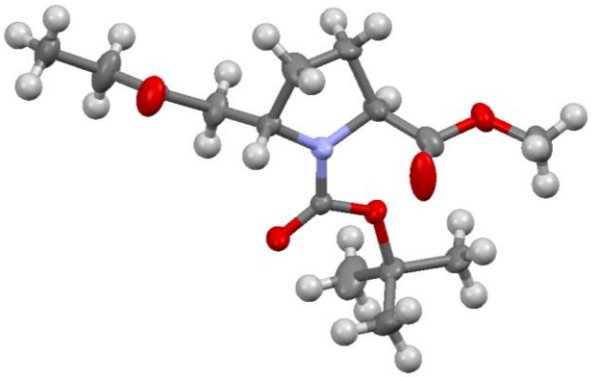

Table 1. Crystal data and structure refinement for compound **159b**.

Empirical formula	$C_{15}H_{25}NO_5$	
Formula weight	299.36	
Temperature	97(2) K	
Wavelength	0.71073 Å	
Crystal system	Tetragonal	
Space group	P4(3)2(1)2	
Unit cell dimensions	a = 8.8171(6) Å	α= 90°.
	b = 8.8171(6) Å	β= 90°.
	c = 42.310(3) Å	γ = 90°.
Volume	3289.3(4) $Å^3$	
Z	8	
Density (calculated)	1.209 Mg/m^3	
Absorption coefficient	0.090 mm^{-1}	
F(000)	1296	
Crystal size	0.32 x 0.10 x 0.10 mm^3	
Theta range for data collection	1.93 to 27.73°.	
Index ranges	-11<=h<=11, -11<=k<=11, -55<=l<=55	
Reflections collected	32165	
Independent reflections	32165 [R(int) = 0.0000]	
Completeness to theta = 25.00°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9911 and 0.9718	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	32165 / 119 / 216	
Goodness-of-fit on F^2	1.131	
Final R indices [I>2sigma(I)]	R1 = 0.0555, wR2 = 0.1267	
R indices (all data)	R1 = 0.0615, wR2 = 0.1300	
Absolute structure parameter	?	
Largest diff. peak and hole	0.293 and -0.273 e.$Å^{-3}$	

Crystal structure of compound **194** and it indicate *trans* configuration.

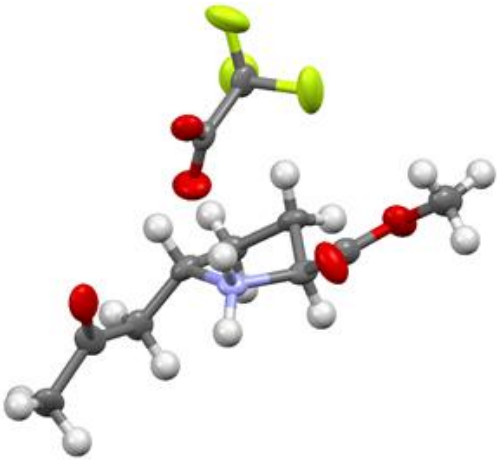

Table 6. Crystal data and structure refinement for compound **194**

Empirical formula	$C_{11}H_{16}F_3NO_5$	
Formula weight	299.25	
Temperature	97(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21	
Unit cell dimensions	a = 8.9295(10) Å	α= 90°.
	b = 14.3156(15) Å	β= 93.139(4)°.
	c = 21.898(2) Å	γ = 90°.
Volume	2795.1(5) $Å^3$	
Z	8	
Density (calculated)	1.422 Mg/m^3	
Absorption coefficient	0.135 mm^{-1}	
F(000)	1248	
Crystal size	0.180 x 0.120 x 0.030 mm^3	
Theta range for data collection	0.931 to 24.998°.	
Index ranges	-8<=h<=10, -17<=k<=17, -26<=l<=25	
Reflections collected	31198	
Independent reflections	9761 [R(int) = 0.1042]	
Completeness to theta = 25.000°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.99 and 0.92	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	9761 / 181 / 729	
Goodness-of-fit on F^2	1.080	
Final R indices [I>2sigma(I)]	R1 = 0.0924, wR2 = 0.2193	
R indices (all data)	R1 = 0.1451, wR2 = 0.2483	
Absolute structure parameter	?	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.452 and -0.416 $e.Å^{-3}$	

Crystal structure of compound **195** and it indicate *trans* configuration.

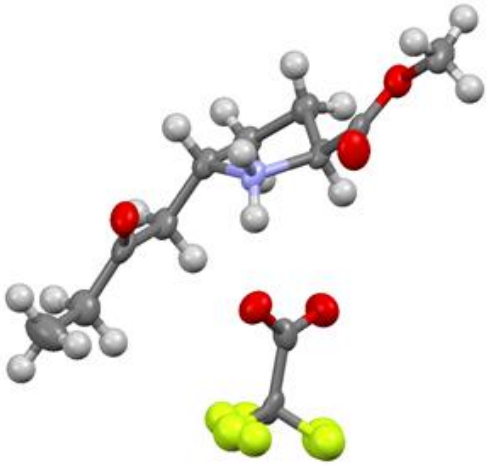

Table 1. Crystal data and structure refinement for **195**.

Empirical formula	$C_{12}\ H_{18}\ F_3\ N\ O_5$	
Formula weight	313.27	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 6.9029(3) Å	α= 90°.
	b = 9.9051(5) Å	β= 90°.
	c = 21.6344(11) Å	γ = 90°.
Volume	1479.23(12) $Å^3$	
Z	4	
Density (calculated)	1.407 Mg/m^3	
Absorption coefficient	0.131 mm^{-1}	
F(000)	656	
Crystal size	0.30 x 0.20 x 0.05 mm^3	
Theta range for data collection	2.26 to 27.41°.	
Index ranges	-8<=h<=8, -12<=k<=12, -27<=l<=27	
Reflections collected	23146	
Independent reflections	3338 [R(int) = 0.0778]	
Completeness to theta = 27.41°	99.6 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9935 and 0.9617	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3338 / 18 / 190	
Goodness-of-fit on F^2	1.520	
Final R indices [I>2sigma(I)]	R1 = 0.0863, wR2 = 0.2209	
R indices (all data)	R1 = 0.1254, wR2 = 0.2401	
Absolute structure parameter	0(2)	
Largest diff. peak and hole	0.722 and -0.588 $e.Å^{-3}$	

Crystal structure of compound **196** and it indicate *trans* configuration.

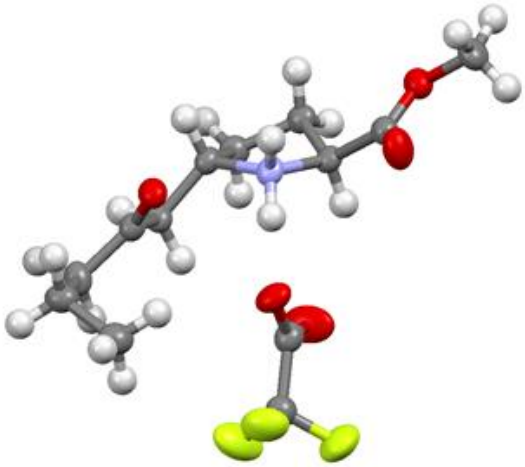

Table 1. Crystal data and structure refinement for compound **196**.

Empirical formula	$C_{13}H_{20}F_3NO_5$	
Formula weight	327.30	
Temperature	97(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 9.7482(14) Å	α= 90°.
	b = 22.448(3) Å	β= 90°.
	c = 7.0961(12) Å	γ = 90°.
Volume	1552.8(4) $Å^3$	
Z	4	
Density (calculated)	1.400 Mg/m^3	
Absorption coefficient	0.128 mm^{-1}	
F(000)	688	
Crystal size	0.320 x 0.290 x 0.100 mm^3	
Theta range for data collection	1.814 to 26.371°.	
Index ranges	-12<=h<=12, -28<=k<=27, -8<=l<=8	
Reflections collected	23033	
Independent reflections	3153 [R(int) = 0.1232]	
Completeness to theta = 25.000°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.98 and 0.87	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3153 / 49 / 201	
Goodness-of-fit on F^2	1.045	
Final R indices [I>2sigma(I)]	R1 = 0.0882, wR2 = 0.2271	
R indices (all data)	R1 = 0.1214, wR2 = 0.2506	
Absolute structure parameter	?	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.489 and -0.434 $e.Å^{-3}$	

Crystal structure of compound **197**.

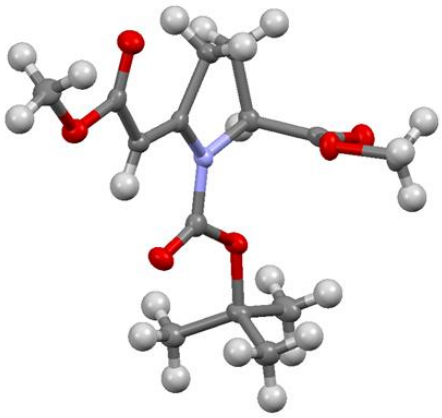

Table 1. Crystal data and structure refinement for compound **197**.

Empirical formula	$C_{14}H_{21}NO_6$	
Formula weight	299.32	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 5.8377(13) Å	α= 90°.
	b = 15.109(3) Å	β= 90°.
	c = 16.996(4) Å	γ = 90°.
Volume	1499.1(6) $Å^3$	
Z	4	
Density (calculated)	1.326 Mg/m^3	
Absorption coefficient	0.104 mm^{-1}	
F(000)	640	
Crystal size	0.120 x 0.040 x 0.020 mm^3	
Theta range for data collection	1.803 to 27.405°.	
Index ranges	-7<=h<=7, -19<=k<=19, -19<=l<=21	
Reflections collected	20198	
Independent reflections	3307 [R(int) = 0.1170]	
Completeness to theta = 25.242°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9979 and 0.9877	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3307 / 0 / 196	
Goodness-of-fit on F^2	1.033	
Final R indices [I>2sigma(I)]	R1 = 0.0585, wR2 = 0.1179	
R indices (all data)	R1 = 0.1245, wR2 = 0.1448	
Absolute structure parameter	?	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.243 and -0.331 $e.Å^{-3}$	

Crystal structure of compound **199** and it indicate *cis* configuration.

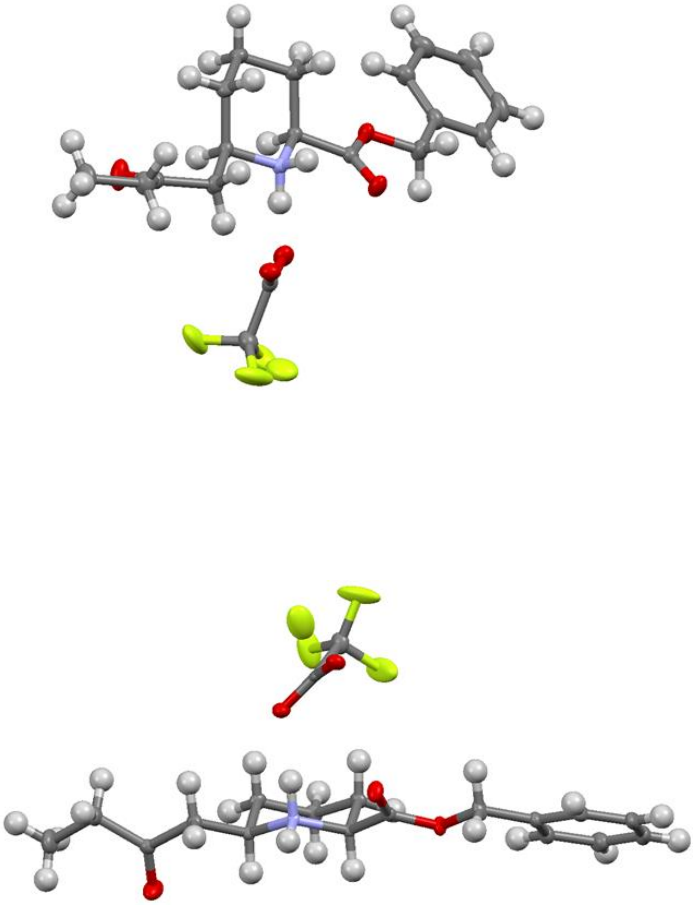

Table 1. Crystal data and structure refinement for compound **199**.

Empirical formula	$C_{19}H_{24}F_3NO_5$	
Formula weight	403.39	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 8.2038(3) Å	α= 90°.
	b = 10.8504(4) Å	β= 90°.
	c = 22.9281(9) Å	γ = 90°.
Volume	2040.93(13) $Å^3$	
Z	4	
Density (calculated)	1.313 Mg/m^3	
Absorption coefficient	0.962 mm^{-1}	
F(000)	848	
Crystal size	0.320 x 0.320 x 0.290 mm^3	
Theta range for data collection	3.856 to 66.554°.	
Index ranges	-9<=h<=9, -12<=k<=12, -26<=l<=25	
Reflections collected	9563	
Independent reflections	3511 [R(int) = 0.0213]	
Completeness to theta = 67.679°	95.8 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3511 / 19 / 264	
Goodness-of-fit on F^2	0.912	
Final R indices [I>2sigma(I)]	R1 = 0.0354, wR2 = 0.0930	
R indices (all data)	R1 = 0.0365, wR2 = 0.0958	
Absolute structure parameter	0.03(3)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.200 and -0.411 $e.Å^{-3}$	