Université de Montréal

Développement de méthodes de synthèse pour les acides aminés indolizidin-9-ones substitués et utilisation des acides aminés azabicycloalcanes en tant que mimes peptidiques.

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Cette thèse intitulée :

Développement de méthodes de synthèse pour les acides aminés indolizidin-9-ones substitués et utilisation des acides aminés azabicycloalcanes en tant que mimes peptidiques.

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Résumé.

Cette thèse relate le développement d'une méthodologie de synthèse pour les acides 8-amino-1-azabicyclo[4.3.0]nonan-9-one-2-carboxyliques (acides aminés indolizidin-9-ones) substitués. Elle relate également dans ce cadre, l'étude des différents processus intéressants observés tels les stéréo et régiosélectivités, les épimérisations ou les éliminations. Les travaux de synthèse peptidique ont porté sur l'utilisation des acides aminés azabicycloalcanes pour étudier les relations entre structure et activité.

Le contenu de cette thèse présente tout d'abord une revue de la littérature sur les voies de synthèse et les utilisations des acides aminés azabicycloalcanes. Cette revue permet de visionner les différents types d'azabicycloalcanes existant, au niveau de la taille des cycles, de la présence d'une ou plusieurs chaînes latérales ou de la présence d'un hétéroatome supplémentaire dans la structure bicyclique. Elle permet également de comparer les différentes méthodes de synthèse utilisées pour la formation des structures bicycliques comme pour l'introduction des chaînes latérales. Elle montre aussi les applications de ce type de composé dans le développement de médicament ou l'étude d'enzymes ou de récepteurs.

Ayant comme objectif l'introduction de substituant varié sur l'acide aminé indolizidin-9-one, des essaies de réactivité de la cétone α,β-insaturée intermédiaire ont été effectués à l'aide d'une série de nucléophile. Des stéréosélectivités et des régiosélectivités intéressantes ont été observées lors de l'addition de réactifs de Grignard sur la fonction énone. Ceci nous a conduit à étudier plus spécifiquement ces phénomènes.

La synthèse des diastéréoisomères de l'acide aminé 4-phényl-indolizidin-9-one (4-Ph-I^9aa) a été effectuée. Ceci a été réalisé pour chacun des diastéréoisomères obtenus après introduction d'un substituant phényle par addition conjuguée. L'un de ces diastéréoisomères conduit directement à un seul diastéréoisomère du 4-Ph-I^9aa. L'autre diastéréoisomère conduit, en revanche, à un mélange de trois diastéréoisomères du 4-Ph-I^9aa en passant par

une série de processus non désirés tel l'élimination d'une des fonctions amines et l'épimérisation des centres chiraux acides aminés.

Afin d'étudier la relation entre structure et activité des antagonistes analogues de la partie *C*-terminale du « calcitonin gene-related peptide » (CGRP), une série de peptide a été synthétisée sur support solide. Pour cela, 3 types d'acide aminé azabicycloalcane, l'I^2aa, l'I^9aa et le 4-Ph-I^9aa ont été incorporés à la séquence peptidique. Six nouveaux undécapeptides et 7 nonapeptides ont ainsi été préparés.

Enfin, à partir d'un peptide antagoniste de « opioid receptor-like 1 » (ORL1) développé précédemment dans notre groupe, une étude structure / activité a été menée. Cette étude a porté sur l'importance des groupes fonctionnels et de la séquence du peptide. Elle a conduit à la mise au point d'un nouveau peptide antagoniste de ORL1 sélectif vis-à-vis des autres récepteurs opioides.

Ces résultats permettront de mieux comprendre et appréhender les problèmes pouvant survenir lors de l'introduction, à venir, de chaînes latérales sur les cétones linéaires précurseurs des acides aminés quinolizidinone, pyrroloazepinone et pyrrolizidinone. Ces travaux ouvrent la voie au développement d'une gamme de mime peptidique substitué de manière similaire. Ceci, afin de comparer l'influence de la variation des angles dièdres du repliement grâce aux différentes tailles de cycle. Ils ouvrent également la voie à la comparaison de l'orientation des chaînes latérales en utilisant différents diastéréoisomères d'un même mime.

Mots Clés : Acides aminés azabicycloalcanes, contraintes conformationnelles, structure peptidique secondaire, dipeptide, activité biologique, ligand de récepteurs.

Abstract.

This thesis reports the development of a methodology for the synthesis of substituted 8-amino-1-azabicyclo[4.3.0]nonan-9-one-2-carboxylic acids (indolizidin-9-one amino acids). It presents the different process involved in the observed selectivity, epimerization and elimination. The peptide synthesis work involved the use of azabicycloalkane amino acids to study structure / activity relationship.

First, a review focuses on recent syntheses of azabicyclo[X.Y.0]alkanone amino acids and their investigation in biologically active peptide mimics. These heterocyclic amino acids are challenging synthetic targets and useful tools for studying structure / activity relationships of native peptide ligands. They have been employed to increase potency and stability of conformationally rigid enzyme inhibitors and receptor ligands. Since last reviewed in 1997, activity in their synthesis and application has increased significantly and access is now available to a wider diversity of these peptide mimics.

Secondary, conjugate addition of aryl Grignard reagents to (2*S*, 5*E*, 8*S*)-di-*tert*-butyl-4-oxo-2,8-bis-[*N*-(PhF)amino]non-5-enedioate in THF proceeded with complete chemoselectivity and >9:1 stereoselectivity to provide predominantly (2*S*,6*R*,8*S*)-6-aryl 4-oxo-2,8-diaminoazelates. This remarkable asymmetric induction in the conjugate addition of Grignard reagents was further studied to understand the different process involved.

Four methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo [4.3.0]nonane carboxylates (**11**, 4-Ph-I^{9}aa-OMe) were synthesized from (2*S*,8*S*,5*E*)-di-*tert*-butyl-4-oxo-5-ene-2,8-bis[*N*-(PhF)amino]azelate [(5*E*)-**7**, PhF = 9-(9-phenylfluorenyl)] via a seven-step process featuring a conjugate addition / reductive amination / lactam cyclization sequence. Various nucleophiles were used in the conjugate addition reactions on enone (5*E*)-**7** as a general route for making α,ω-diaminoazelates possessing different substituents in good yield albeit low diastereoselectivity except in the case of aryl Grignard reagents (9/1 to 15/1 drs). (6*S*)- and (6*R*)-6-Phenylazelates were separated by

chromatography and diastereoselective precipitation and independently transformed into 4-Ph-I^9aa-OMe. From (6*S*)- 6-phenylazelates, (2*S*,4*R*,6*R*,8*S*)-4-Ph-I^9aa-OMe was prepared selectively in 51% yield. Reductive amination of (6*R*)-6-phenylazelates provided the desired pipecolates along with desamino compound, which was minimized by performing the hydrogenation in the presence of ammonium acetate. Subsequent ester exchange, lactam cyclization and amine protection provided three products (2*R*,4*S*,6*S*,8*R*)-, (2*R*,4*S*,6*S*,8*S*)-, and (2*S*,4*S*,6*R*,8*S*)-4-Ph-I^9aa-OMe in 10, 6, and 6% yields, respectively, from (6*R*)-6-phenylazelates. Ester hydrolysis of (2*S*,4*R*,6*R*,8*S*)- 4-Ph-I^9aa-OMe furnished 4-phenyl indolizidin-9-one *N*-(Boc)amino acid as a novel constrained Ala-Phe dipeptide surrogate for studying conformation-activity relationships of biologically active peptides.

The structure / activity relationship of *C*-terminal CGRP analogs antagonist was studied by synthesizing a series of peptide on solid support. These peptides included three azabicycloalkane amino acids, I^2aa, I^9aa and 4-Ph-I^9aa. Six new undecapeptides and seven new nonapeptides were prepared and are currently under investigation for biological activity.

Finally, a series of ORL1 antagonists' hexapeptides including Qaa were made to study the structure / activity relationship. This study leads to the development of a new selective ORL1 antagonist.

Keywords. conformational constraint; azabicycloalkane amino acid; mimicry; peptide secondary structure; dipeptide; biological activity; enzyme inhibitor; receptor ligand.

Note.

Je désire préciser explicitement ma contribution à cette thèse de PhD dans le but d'éviter un questionnement inopportun et une confusion de la part du lecteur quant à ma contribution aux niveaux scientifique et intellectuel, de la conception des stratégies de synthèse, du travail au laboratoire et de la rédaction des articles.

Pour les chapitres 4 à 6, j'ai effectué tous les travaux en laboratoire et rédigé toutes les parties expérimentales. Pour l'ensemble de la thèse, j'ai rédigé les articles et les différents chapitres qui ont été révisés par le professeur William D. Lubell.

Le chapitre 6 décrit l'introduction du sujet et la synthèse des peptides sous forme d'article. Les tests biologiques sont actuellement en cours dans les laboratoires du professeur Bouvier au département de biochimie de l'Université de Montréal. La partie description des résultats n'est donc pas disponible pour l'instant mais sera fournie dès que possible.

Pour le chapitre 7, les travaux de laboratoire ont été effectués en commun entre moi-même et le docteur Van Cauwenberghe (Vrije Universiteit Brussel). La synthèse de l'acide aminé quinolizidinone (*N*-(Boc)-Qaa-*t*-Bu) a été effectuée en parallèle par chacun de nous dans les laboratoires du professeur Lubell à l'Université de Montréal. J'ai effectué la conversion du *N*-(Boc)-Qaa-*t*-Bu en acide libre, *N*-(Boc)-Qaa-OH. La synthèse des peptides sur support solide a été effectuée en commun dans les laboratoires du professeur Tourwé à la Vrije Universiteit Brussel, en Belgique. La synthèse de l'agent de gualylation, *N*,*N*'-bis(Boc)-1-guanylpyrazole ainsi que l'étape de libération du peptide par HF ont été effectuées sous la supervision du Dr. Van Cauwenberghe. La purification et la caractérisation des peptides ont été faites à Bruxelles par le docteur Van Cauwenberghe de même que la rédaction de l'article relié mis en annexe (Annexe 2). Le chapitre 7 est inspiré de cet article auquel ont été ajoutés des données expérimentales et des éléments de comparaison avec des composés d'activités similaires. Les tests biologiques ont été effectués par le Dr. Becker dans les laboratoires du professeur Simonin à l'Université Louis Pasteur de Strasbourg.

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Liste des abréviations :

[α] : rotation spécifique

ACE : enzyme de conversion de l'angiotensine

AcOEt : acétate d'éthyle

Acides aminés, codes unilettres et trilettres : les acides aminés D sont représentés par des minuscules en code unilettre et sont précédés de D- pour le code trilettre.

G = Gly = glycine

A = Ala = alanine

V = Val = valine

L = Leu = leucine

I = Ile = isoleucine

Cha = cyclohexylalanine

S = Ser = serine

T = Thr = thréonine

F = Phe = phénylalanine

*p*ClPhe = *para*-chlorophénylalanine

Y = Tyr = tyrosine

W = Trp = tryptophane

H = His = histidine

R = Arg = arginine

Cit = citruline

K = Lys = lysine

Orn = ornithine

C = Cys = cysteine

M = Met = methionine

D = Asp = acide aspartique

E = Glu = acide glutamique

N = Asn = asparagine

Q = Gln = glutamine

P = Pro = proline

BINOL : 1,1'-binaphthalene-2,2'-diol
Boc : *tert*-butoxycarbonyle
BTD : Beta Turn Dipeptide, acide (3*S*,6*S*,9*R*)-2-oxo-3-amino-7-thia-1-aza-bicyclo[4.3.0]nonane-9-carboxylique
n-Bu : *normal*-butyle
t-Bu : *tert*-butyle
°C : degré Celsius
Cbz : benzyloxycarbonyle
CCM : chromatographie sur couche mince (= TLC)
CD : dichroïsme circulaire
CGRP : protéine reliée au gène de la calcitonine
CRLR : Calcitonin receptor-like receptor
COD : cyclooctadiène
D. : décomposition
DBU : 1,8-diazabicyclo[5.4.0]undec-7-ene
DCM : dichlorométhane
DHQD : déhydroquinidine
DIBAl-H : di-*iso*-butylaluminium hydride
DIC : di-*iso*-propylcarbodiimide
DMAP : diméthylaminopyridine
DMS : sulfure de diméthyle
DMSO : diméthyle sulfoxide
DNA : acide déoxyribonucléique
dr = rd = ratio diastéréoisomérique
EC_{50} = concentration effective à 50%
EDC : 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide
ee : excès énantiomérique
Et : éthyle
FAB+: fast atom bombing positive
Fmoc-OSu : *N*-(9-Fluorenylmethoxycarbonyloxy) succinimide
FTIR : infra rouge à transformé de Fourier
GPCR : récepteurs couplés à la protéine-G

Haic : acide 5-amino-1,2,4,5,6,7-tetrahydro-azepino[3,2,1-hi]indole-4-one-2-carboxylique

HATU : *N*-[dimethylamino)-1*H*-1,2,3,-triazolo[4,5-β] pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide

HMPA : hexaméthylphosphoramide

HOBt : *N*-hydroxybenzotriazole

I^2aa : acide aminé indolizidin-2-one ou acide 2-oxo-3-amino-1-aza-bicyclo[4.3.0]nonane-9-carboxylique

I^9aa : acide aminé indolizidin-9-one ou acide 9-oxo-8-amino-1-aza-bicyclo[4.3.0]nonane-2-carboxylique

IBTM : Indol-Based Turn-Mimic, acide (2S,5S, 11βR)-2-amino-3-oxohexahydroindolizino[8,7-b]indole-5-carboxylique

IC_{50} : concentration d'inhibition à 50%

IIDQ : 1-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline

IR : infra rouge

K*i* = constante d'inhibition

LCMS : Liquid Chromatography Mass Spectrometry

M: molaire (mole/l)

MBHA : résine 4-méthylbenzhydrylamine

Me : méthyle

MHz : méga hertz

mmol: millimole

N : normal

NMR : nuclear magnetic resonance (voir RMN)

nOe : nuclear Overhauser effect

Oic : acide octahydroindole-2-carboxylique

ORL1 : opioid receptor-like 1

Paa: acide aminé pyrrolizin-2-one ou acide 2-oxo-3-amino-1-aza-bicyclo[3.3.0]octane-8-carboxylique

Ph : phényle

PhF : phénylfluorènyle

4-Ph-I^9aa : acide aminé 4-phényl-indolizidin-9-one

PhtN : phthalimide
ppm : partie par million
P.R. : pas de réaction
Pr : propyle
i-Pr : *iso*-propyle
PyBroP : hexafluorophosphate de bromotripyrrolidinophosphonium
Qaa: acide aminé quinolizidin-2-one ou acide 2-oxo-3-amino-1-aza-bicyclo[4.4.0]decane-10-carboxylique
RAMP1 : Receptor-Activity-Modifying Proteins 1
RMN : Résonance Magnétique Nucléaire
Abréviations liées à la RMN : (en anglais)
s & br s: singulet et singulet large
d & br d: doublet et doublet large
t & br t: triplet et triplet large
q : quadruplet
m & br m : multiplet et multiplet large
DEPT135 : **D**istortion-less **E**nhancement by **P**olarization **T**ransfer at 135°
COSY : COrrelated SpectroscopY
NOESY : Nuclear Overhauser Effect 2D SpectroscopY
TADDOL : (4*R*,5*R*)-2,2-dimethyl-α,α,α',α'-tetra(naphth-2-yl)-1,3-dioxolane-4,5-dimethanol
TBAF : fluorure de tributylamine
TBDMSCl : chlorure de *tert*-butyl-diméthylsilyle
TBTU : tétrafluoroborate de *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium
TFA : acide trifluoroacétique
THF : tétrahydrofurane
TIC MS : Spectrométrie de masse : courrant ionique total
TMSCl : chlorotriméthyle silane
TPfTU : tétrafluoroborate de *O*-pentafluorophenyl-1,1,3,3-tétraméthyluronium
^{t}R : temps de rétention (chromatographie sur couche mince)
UV: ultra violet

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Je tiens tout d'abord à remercier le professeur William D. Lubell pour m'avoir accepté dans son groupe de recherche, pour sa confiance et la liberté de recherche qu'il m'a laissé ainsi que pour sa patience à nous apprendre à rédiger des articles scientifiques rigoureux.

Je tiens à remercier les membres du groupe passés et présents pour leur amitié, leur support et leur aide. Les nommer tous serait trop long mais je pense en particulier à Francis Gosselin et Laurent Bélec de qui j'ai énormément appris dans le laboratoire.

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À Christian et Martine,
et en mémoire de Pierre.

Chapitre 1.

Introduction.

1.1. Le peptidomimétisme.

La très grande diversité et l'importante activité biologique des peptides naturels et non naturels en font des cibles particulièrement intéressantes pour le développement de nouveaux médicaments. Cependant, l'utilisation des structures peptidiques en temps qu'agents thérapeutiques présente un certain nombre de problèmes.[1] Par exemple, les peptides naturels possèdent une faible bio-disponibilité due à leurs dégradations par digestions enzymatiques et à bas pH. Ils présentent une durée d'action relativement courte. En effet, les temps de demi-vie sont de l'ordre de la seconde ou de la minute pour les petits peptides telles que la calcitonine (32 acides aminés (aa), $t_{1/2}$=1-3 min), qui a une action sur le métabolisme de Ca et P, la cyclosporine (11 aa, $t_{1/2}$=6 min), qui est un immunosuppresseur ou la vasopressine (9 aa, $t_{1/2}$=5-15 min), qui a des effets sur la mémoire. Ce temps de demi-vie peut aller jusqu'à l'heure pour les gros peptides tels que l'insuline (51 aa, $t_{1/2}$=6-14 h), qui est utilisée pour le traitement du diabète ou les interférons α, β et γ (20-160 aa, $t_{1/2}$=0.5-16 h), qui peuvent être utilisés comme antiviraux (α), comme traitements contre la sclérose en plaque (β) ou comme anti-infectieux (γ). Tous ces éléments constituent un frein considérable quant à leurs utilisations. Des analogues comportant de légères ou de profondes modifications à la structure peptidique ont donc été développés afin de pallier à ces problèmes. Il s'agit du peptidomimétisme et du développement des 'prodrugs'.

Dans ce cadre, les diverses modifications apportées aux peptides visent à augmenter leur résistance aux peptidases et aux protéases ainsi que leur sélectivité et leur activité biologique. Elles visent également à diminuer leur toxicité et leurs effets secondaires. Le développement d'un médicament à partir d'un peptide parent naturel (ou obtenu par chimie combinatoire) passe donc par toute une série d'étapes.

Ces étapes débutent généralement par l'étude systématique de chaque résidu et de leur chaîne latérale par la comparaison avec d'autres acides aminés naturels et non-naturels similaires (Cha, Cit, Orn, etc). D'autres modifications passent par le remplacement itératif d3e chaque acide aminé par l'alanine, la proline ou les acides aminés de la série D. Ces modifications apportent des informations sur les pharmacophores importants mais également sur la relation structure / activité. Des modifications plus spécifiques et importantes peuvent ensuite être apportées. Ainsi, afin d'empêcher certains ponts hydrogènes ou de stabiliser une structure tridimensionnelle (hélice, feuillet ou repliement), il est possible d'utiliser différents 'outils' comme la *N*-méthylation, la C^{α}-méthylation,[2] les déhydro acides aminés,[3] les β-acides aminés,[4,5] les azapeptides,[6] les depsipeptides[7,8] ou des acides aminés contraints tels que les analogues de prolines,[9] les lactames de Freidinger,[10,11] les azabicycloalcanes[12,13] ou des polypeptides cycliques[14] (Figure 1). La chaîne peptidique peut également être remplacée par des éléments pseudopeptidiques voir non-peptidiques (aliphatiques, hétérocycliques ou saccharidiques).[15] Ceux-ci servent à diriger spatialement les pharmacophores et/ou à induire une conformation au reste de la chaîne peptidique. Les composés s'éloignent alors de plus en plus d'une structure peptidique pour se rapprocher de la structure de composés

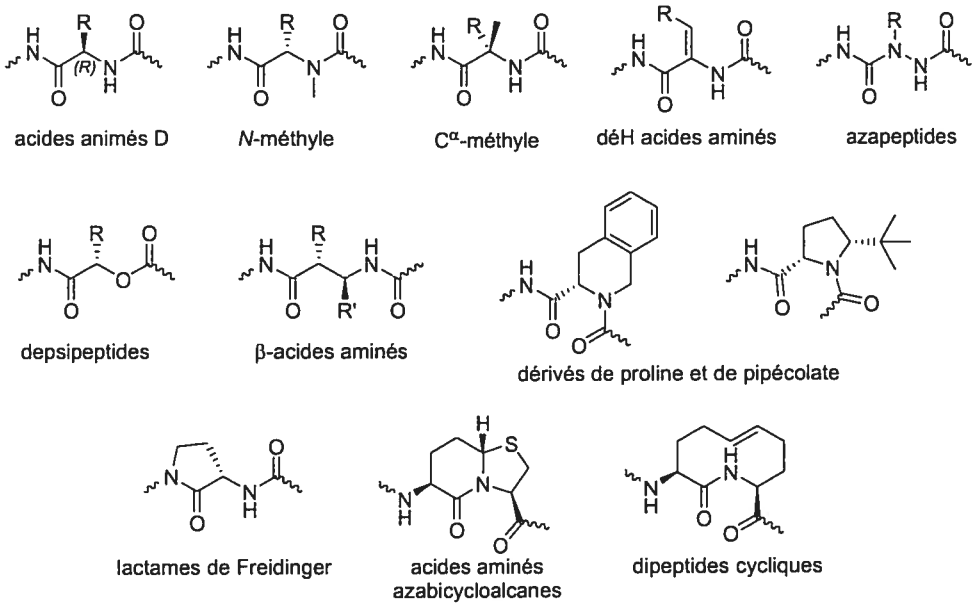
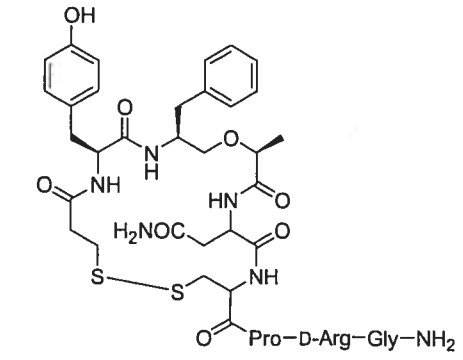
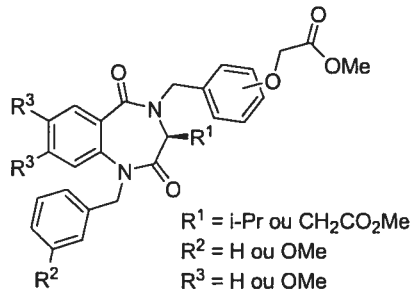


FIGURE 1 Modifications de la chaîne peptidique utilisées pour stabiliser et étudier la conformation active du peptide.

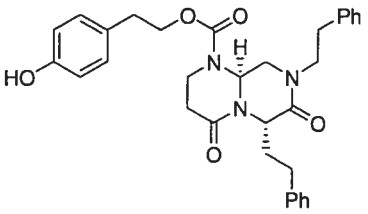
'drug-like' à faibles poids moléculaire, résistant et contenant généralement moins de centres chiraux tels que les composés **1**,[16] **2**,[17] **3**,[18] **4**[19] et **5**[20,21] (Figure 2).



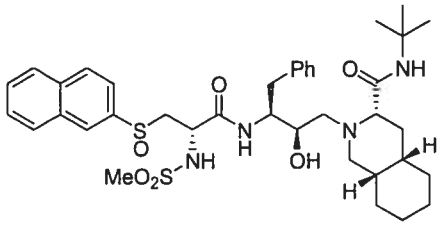
1 : Analogue de desmopressine (c[Cys-Tyr-Phe-Gln-Asn-Cys]-Pro-D-Arg-Gly-NH_2) contenant un mime de repliement γ.



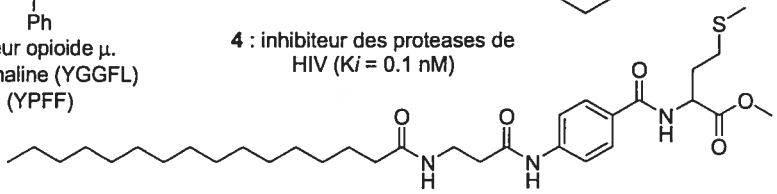
2 : Librairie de 16 membres mise au point pour le développement d'antagoniste du recepteur de l'endotheline.



3 : agonist du recepteur opioide μ.
Agoniste de Leu-enkephaline (YGGFL) et endomorphine (YPFF)



4 : inhibiteur des proteases de HIV (K*i* = 0.1 nM)



5 : FTase inhibitor (IC_{50} = 3.7 μM)
Analogue de farnesylpyrophosphate et de la séquence CVIM

FIGURE 2 Exemples de modification pseudopeptidique ou non peptidique dans différents composés.

Dans ce cadre, ce projet de recherche vise à développer de nouveaux outils pour le peptidomimétisme et plus spécifiquement des acides aminés contraints (azabicycloalcanes) mimes de repliement β.

1.2. Les mimes de repliement β.

Un repliement β est une structure secondaire tétrapeptidique pour laquelle la distance entre les C^{α}_{i} et C^{α}_{i+3} est inférieure ou égale à 7 Å. À cette distance, un pont hydrogène à 10 membres peut exister entre CO_i et NH_{i+3} (Figure 3). Les différents types de repliements sont caractérisés par leurs angles

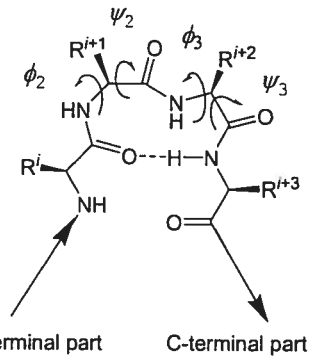


FIGURE 3 Structure d'un repliement β

TABLEAU 1 Valeur des angles dièdres des différents repliements β

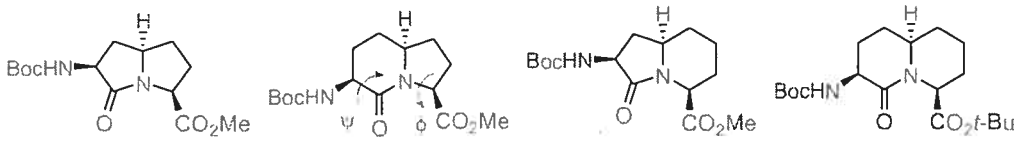
	Type	ϕ_2	ψ_2	ϕ_3	ψ_3
	I	-60	-30	-90	0
	I'	60	30	90	0
	II	-60	120	80	0
	II'	60	-120	-80	0
	VIII	-60	-30	-120	120
proline (i+2) *cis*	VIa1	-60	120	-90	0
	VIa2	-120	120	-60	0
	VIb	-135	135	-75	160
	IV	repliements n'appartenant pas aux autres catégories			

dièdres ψ_2, ϕ_2, ψ_3 et ϕ_3 et sont classés comme type I, I', II, II', III, III', IV, V, V', VI, et VIII (Tableau 1).[22,23,24,25] Les repliements β font partie intégrante des structures tridimensionnelles des peptides et sont nécessaires à leur activité. Cependant, la multitude de conformations existantes pour la chaîne peptidique ainsi que de l'orientation très variable des chaînes latérales, les rend complexes à mimer.

Différents types de mimes peptidiques ont été développés pour induire un repliement β, soit par l'insertion d'une contrainte (lactames de Freidinger,[11] diazépines[26] ou analogues de proline[27]), soit en mimant un type de repliement spécifique par une structure plus rigide (acides aminés azabicycloalcanes, Figure 1).[12,13] Les études conformationelles[28] ainsi que les exemples d'utilisation[29] de ces mimes de repliement β ont mis en évidence l'utilité et l'efficacité de ces contraintes. Ces mimes permettent, en effet, d'induire ou de stabiliser un repliement dans des structures peptidiques cycliques ou acycliques ainsi qu'à maintenir ou augmenter l'activité vis-à-vis des peptides parents. Ils donnent, de plus, l'occasion d'étudier la conformation peptidique active.

La recherche sur le développement de nouveaux mimes de repliement est en constante évolution. Elle permet la mise au point d'acides aminés contraints aux valeurs d'angles dièdres variées et mimant les chaînes latérales dans différentes conformations. Ceci afin de mieux adopter les conformations actives des peptides.

Notre groupe de recherche a ainsi développé une série d'acides aminés azabicycloalcanes, mimes de repliement β. La mesure de leurs angles dièdres par diffraction des rayons X[30,31,32,33] a montré des changements significatifs dans les valeurs de ces angles avec les différentes tailles de cycle (Figure 4).[13]



Paa ψ= -149, ϕ= -45 | I²aa ψ= -176, ϕ= -78 | I⁹aa ψ= -141, ϕ= -34 | Qaa ψ= -163, ϕ= 48

FIGURE 4 Différents acides aminés azabicycloalcanes développés dans le groupe Lubell et leurs angles dièdres mesurés par diffraction des rayons X.

1.3. Applications et développements.

Il existe différents styles de peptides intéressants pour l'étude de la relation structure / activité à l'aide des acides aminés azabicycloalcanes. En effet, si la structure tridimensionnelle active des peptides est très bien définie par les études spectroscopiques, l'étude de ces peptides permet alors de développer des acides aminés azabicycloalcanes spécifiques dans le but d'augmenter l'activité, la sélectivité et/ou la stabilité du peptide. Ils peuvent permettre également de tester l'efficacité de l'azabicycloalcane à mimer un type de repliement. Au contraire, si la structure tridimensionnelle du peptide est aléatoire ou variable, l'utilisation des acides aminés azabicycloalcanes sert à confirmer la présence ou non d'un repliement important pour l'activité. Ils permettent également de définir le type de repliement.

Une étude préliminaire a été effectuée sur la partie *C*-terminal du « Calcitonin Gene Related Peptide » (CGRP, Figure 5). Le CGRP est un peptide naturel vasodilatateur, aux fonctions exactes et aux récepteurs mal connus. L'étude a porté sur le développement d'analogue antagoniste du CGRP en utilisant un des mimes de repliement β, l'acide aminé indolizidin-2-one (I^2aa).[34] La structure tridimensionnelle du CGRP est constituée d'une boucle disulfure *N*-terminale (positions 2 à 7) suivie d'une partie *C*-terminale linéaire, elle même constituée d'une hélice (positions 8 à 20) et d'une partie aléatoire

hα-CGRP	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-NH_2
[D^{31}, P^{34}, P^{35}]$CGRP_{27-37}$	FVPT**D**VG**PF**AF-NH_2
[I^2aa^{31-32}, P^{34}, P^{35}]$CGRP_{27-37}$	FVPT**I^2aa**G**PF**AF-NH_2
[I^2aa^{31-32}, P^{34}, P^{35}]$CGRP_{29-37}$	PT**I^2aa**G**PF**AF-NH_2
[D^{31}, I^2aa^{33-34}, P^{35}]$CGRP_{27-37}$	FVPT**DVI^{2}aaF**AF-NH_2
[D^{31}, I^2aa^{33-34}, P^{35}]$CGRP_{29-37}$	PT**DVI^{2}aaF**AF-NH_2
[D^{31}, I^2aa^{34-35}]$CGRP_{27-37}$	FVPT**DVGI^{2}aa**AF-NH_2
[D^{31}, I^2aa^{34-35}]$CGRP_{29-37}$	PT**DVGI^{2}aa**AF-NH_2

FIGURE 5 Structure du hαCGRP et d'antagonistes du CGRP.

(positions 20 à 37). Chacune de ces deux parties, cyclique et linéaire, prisent séparément, conduit respectivement à des analogues agonistes (partie cyclique) et à des analogues antagonistes (partie linéaire).[35,36,37,38,39,40] À partir de l'undécapeptide [D^{31}, P^{34}, F^{35}]$CGRP_{27-37}$,[41] le plus petit antagoniste dérivé de la partie *C*-terminale du CGRP, différents analogues de 9 ou 11 acides aminés incorporant l'I^2aa aux positions 31-32, 33-34 et 34-35 ont été préparés afin de confirmer la présence de repliement dans la structure active du peptide (Figure 5). Ces analogues, notamment les nonapeptides, ont conservé une activité antagoniste malgré l'ablation des chaînes latérales de l'acide aspartique 31 et de la phénylalanine 35 qui étaient apparues comme jouant un rôle important dans l'activité antagoniste du peptide [D^{31}, P^{34}, F^{35}]$CGRP_{27-37}$.[41] Suite à ces résultats encourageants et à la volonté de développer de nouveaux mimes de repliement substitués, une étude plus approfondie a été décidée. Celle-ci mettra en œuvre trois mimes différents. Deux existent déjà, ce sont l'I^2aa et l'acide aminé indolizidin-9-one (I^9aa). Le troisième, un analogue substitué de l'I^9aa est à développer. Ce dernier acide aminé devra posséder une chaîne latérale phényle pouvant mimer une séquence Pro-Phe ou Ala-Phe aux positions 34-35 et 36-37. Il est également intéressant dans le cadre du développement de cet acide aminé, d'étudier les différentes possibilités de substitution de l'I^9aa par la voie de synthèse choisie (Voir chapitre 3).

Le contenu de cette thèse présente tout d'abord une revue de la littérature sur les voies de synthèses et les utilisations des acides aminés

azabicycloalcanes (chapitre 2). Cette revue permet de visionner les différents types d'azabicycloalcanes existant, au niveau de la taille des cycles, de la présence d'une ou plusieurs chaînes latérales ou de la présence d'un hétéroatome supplémentaire dans la structure bicyclique. Elle permet également de comparer les différentes méthodes de synthèse utilisées pour la formation des structures bicycliques comme pour l'introduction des chaînes latérales. Elle montre aussi les applications récentes de ce type de composés dans le développement de médicaments, l'étude d'enzymes et de récepteurs.

Le projet de cette thèse est de développer des méthodes de substitution de l'I^9aa à partir de l'intermédiaire linéaire utilisé pour la synthèse de l'I^9aa. Le chapitre 3 présente la méthode de substitution choisie pour l'introduction de la chaîne latérale.

Le chapitre 4 reporte les essais de réactivité de l'addition conjuguée d'une série de nucléophile sur la cétone α,β-insaturée intermédiaire. La stéréosélectivité et la régiosélectivité de l'addition de réactifs de Grignard et de cuprates ainsi que les interactions induisant leurs sélectivités sont discutées dans l'article 2.

La synthèse des différents diastéréoisomères de l'acide aminé 4-phényl-indolizidin-9-one est ensuite rapportée dans le chapitre 5. Une brève communication (article 3) rapporte les résultats préliminaires obtenus et l'article 4 décrit en détail les processus de cyclisation, ainsi que les diastéréoisomères obtenus pour chacun des diastéréoisomères de la cétone linéaire.

Le chapitre 6 décrit la synthèse sur support solide de peptides analogues de la partie *C*-terminale de CGRP pour l'étude de la relation structure / activité à partir de leur activité antagoniste. Ces peptides contiennent chacun un des 3 types d'acides aminés azabicycloalcanes, I^2aa, I^9aa ou 4-Ph-I^9aa.

Enfin, le chapitre 7, décrit les résultats obtenus lors de l'étude de la relation structure / activité effectuée sur un hexapeptide antagoniste de ORL1 (opioid receptor-like 1) contenant l'acide aminé quinolizidinone (Qaa).

1.4. Conclusion.

Les acides aminés azabicycloalcanes ont depuis leurs premières synthèses et applications démontré leur utilité dans le domaine de la chimie peptidique, du développement de médicament et de l'étude des structures peptidiques actives. Les structures tridimensionnelles actives des peptides et notamment celle des repliements sont extrêmement variées. Le mime de telles structures à partir de peptides contraints rigides tels que les acides aminés azabicycloalcanes demande donc le développement de nombreux composés aux conformations variées.

Ce projet vise donc, dans le cadre du mimétisme et des sciences peptidiques, à montrer le développement d'un nouvel outil aux propriétés (angles dièdres, orientation de la chaîne latérale) uniques. À l'utiliser pour l'étude de peptides et notamment, dans une étude structure / activité d'un peptide en comparaison directe avec son analogue non-substitué pour montrer son utilité et sa capacité à mimer une séquence peptidique (Xaa-Phe) tout en induisant un repliement β de type II'.

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Chapitre 2.

Article 1.

Cluzeau, J.; Lubell, W.D. 'Design, Synthesis and Application of Azabicyclo[X.Y.0] alkanone Amino Acids as Constrained Dipeptide Surrogates and Peptide Mimics' revue soumise à *Biopolymers: Peptide Science* **2004**.

Design, Synthesis and Application of Azabicyclo[X.Y.0]alkanone Amino Acids as Constrained Dipeptide Surrogates and Peptide Mimics[††]

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2.1. Abstract.

Azabicyclo[X.Y.0]alkanone amino acids are challenging synthetic targets and useful tools for studying structure / activity relationships of native peptide ligands. They have been employed to make more potent and stable conformationally rigid enzyme inhibitors and receptor ligands. Since last reviewed in 1997, activity in their synthesis and application has increased significantly and access is now available to a wider diversity of these peptide mimics. This review focuses on recent syntheses of these heterocyclic amino acids and their investigation in biologically active peptide mimics.

Keywords: *conformational constraint; azacycloalkane amino acid; mimicry; peptide secondary structure; dipeptide; biological activity; enzyme inhibitor; receptor ligand.*

2.2. Introduction.

Azabicyclo[X.Y.0]alkanone amino acids have proven to be effective tools for restraining backbone conformation and orienting the display of pharmacophores in peptide science research. Since last reviewed in 1997,[1] activity in the synthesis and application of azabicyclo[X.Y.0]alkanone amino acids has increased significantly such that another review of the subject is warranted at this time. As discussed previously,[2] the heterocycle framework restricts the geometry of the peptide backbone by a combination of structural constraints as well as steric interactions such that the flexibility about five dihedral angles is inhibited by these fused bicycles. Conformational analyses of azabicycloalkanone amino acids possessing an L,L-configuration along the backbone have shown their effectiveness at mimicking the shape of a type II' β-turn,[2,3] as demonstrated by studies using X-ray diffractometry, IR and NMR spectroscopy[4,5,6] and computation.[4,7] Geometry is, however, influenced significantly by the stereochemistry at the ring fusion centre, the presence of side-chain substituents on the heterocycle and particularly, the heterocycle ring size, such that a spectrum of turn conformations may be studied by employing a series of azabicyclo[X.Y.0]alkanone amino acids. Employment of sets of related azabicycloalkane amino acids to study the relationship of turn conformation and biological potency and specificity has thus proven effective for enhancing the activity and for improving understanding of the active turn geometry of native peptides. Furthermore, bicycles possessing alternative backbone stereochemistry have also been exploited for mimicry of alternative conformations.

In light of other reviews and specific articles that have addressed the conformational aspects of azabicyclo[X.Y.0]alkanone amino acids,[2,3] this review focuses primarily on the syntheses of these heterocyclic amino acids that have been reported since the 1997 review. In addition, the later part of this review describes their application in investigations of biologically active peptides and peptide receptors. Finally, a graphical listing is provided featuring the structures of recently prepared azabicyclo[X.Y.0]alkanone amino acid analogs.

In discussing the syntheses and applications of these constrained dipeptide surrogates, this review illustrates the diverse methodology for accessing and employing such structures to facilitate efforts in peptide mimicry and medicinal chemistry.

2.3. Synthesis of azabicycloalkanone amino acids.

2.3.1. Synthesis of pyrrolizidin-2-one amino acids (Paa).

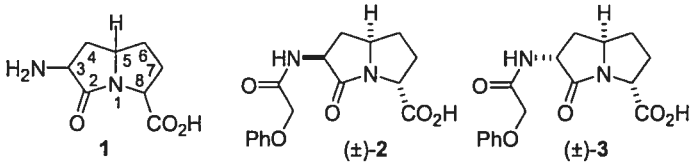
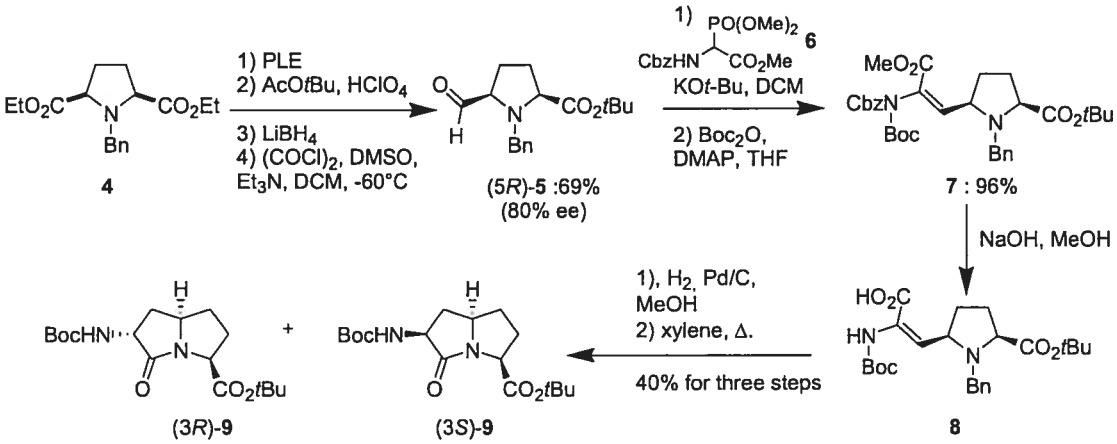


FIGURE 1 General structure for pyrrolizidinone amino acids and examples of previously reviewed analogs.

Pyrrolizidin-2-one amino acid **1**[8] possesses the peptide backbone constrained within a fused 5,5-bicyclic structure. The synthesis of racemic analogs of **1** (**2** and **3**) have been previously reviewed (FIGURE 1).[1]

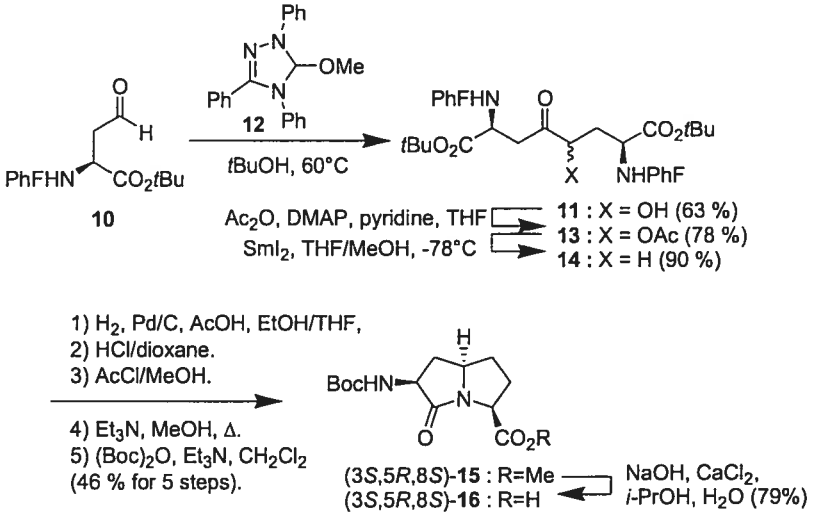
An enantiomerically enriched protected form of **1** was synthesized from *N*-benzyl 2,5-di(ethylcarboxylate)-pyrrolidine **4** (SCHEME 1).[9] Diester **4** was desymmetrized with pig liver esterase (PLE) to afford the monoacid in 95% yield and 80% ee which was then converted to the aldehyde **5** in 73% yield.[10] Horner-Wadsworth-Emmons olefination of aldehyde (5*R*)-**5** with glycine-derived phosphonate **6** gave dehydro amino ester **7**. The Cbz group was



SCHEME 1 Synthesis of pyrrolizidin-2-one *N*-(Boc)amino esters **9** from pyrrolidine **4**.[9,10]

exchanged for a Boc group in two steps by acylation of the carbamate with Boc_2O and DMAP in THF, followed by treatment with NaOH in MeOH, which consequently caused ester hydrolysis to form amino acid **8**. Hydrogenation with palladium-on-carbon effected double bond reduction and benzyl group removal to provide an amino acid, that was subjected to lactam cyclization in xylene at reflux to give a separable 1:1 mixture of the (3*S*)- and (3*R*)-pyrrolizidin-2-one amino esters **9** in 26% yield from diester **4**.

Enantiopure *N*-(Boc)amino pyrrolizidinone acid **16** was later synthesized from α-*tert*-butyl *N*-(PhF)-aspartate β-aldehyde **10** (SCHEME 2, PhF = 9-(9-phenylfluorenyl)),[11] which can be prepared on 15 g scale from aspartic acid in 4 steps.[12] Acyloin condensation of aldehyde **10** gave α-hydroxy ketone **11**. The most efficient conditions employed 5-methoxy-4,5-dihydro-1,3,4-triphenyl-1*H*-1,2,4-triazole **12**[13] as catalyst in *t*BuOH at 80°C and provided α-hydroxy ketone **11** in 63% yield. Alcohol acetylation and samarium iodide reductive acetate cleavage furnished linear ketone **14**. Reductive amination, ester group exchange, lactam cyclization and amine protection then provided (3*S*,5*R*,8*S*)-*N*-(Boc)amino pyrrolizidin-2-one ester **15** as a single diastereomer in 46% yield and 5 steps from **14**. For use in peptide synthesis, *N*-(Boc)amino pyrrolizidin-2-one acid **16**, was prepared by hydrolysis of methyl ester **15** using NaOH and $CaCl_2$ in *i*-PrOH/H_2O.[14]



SCHEME 2. Synthesis of *N*-(Boc)pyrolizidin-2-one amino acid **16** from aspartate β-aldehyde **10**.[11]

2.3.2. Synthesis of substituted pyrrolizidin-2-one amino acids.

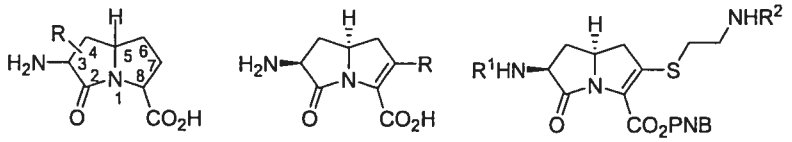
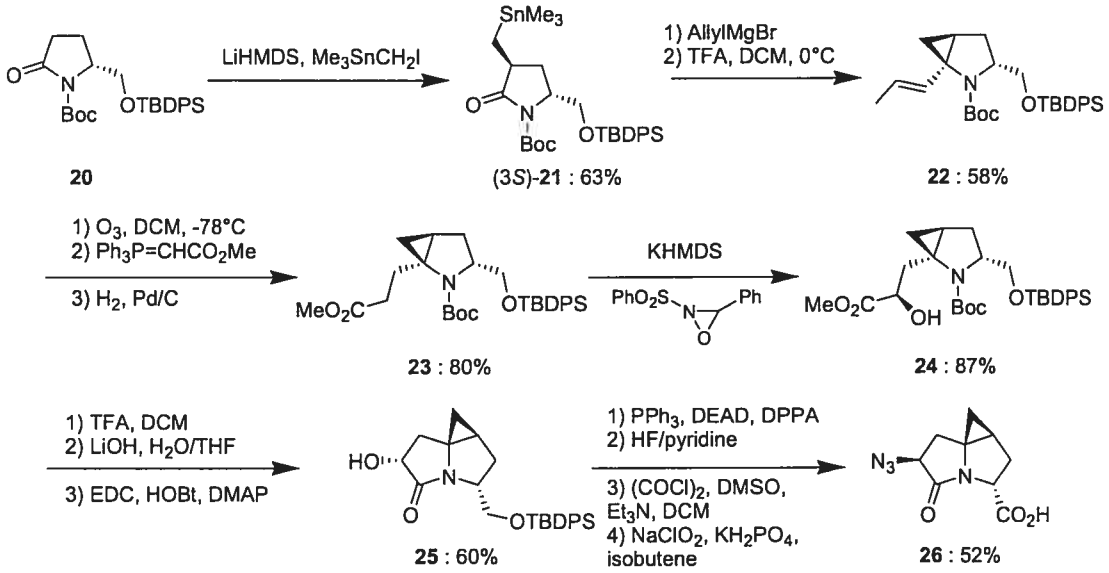


FIGURE 2 General structure for substituted pyrrolizidinone amino acid and examples of previously reviewed analogs.

7-Substituted-7,8-dehydropyrrolizidinone amino acids **18** and **19** have been previously reviewed (FIGURE 2).[1]

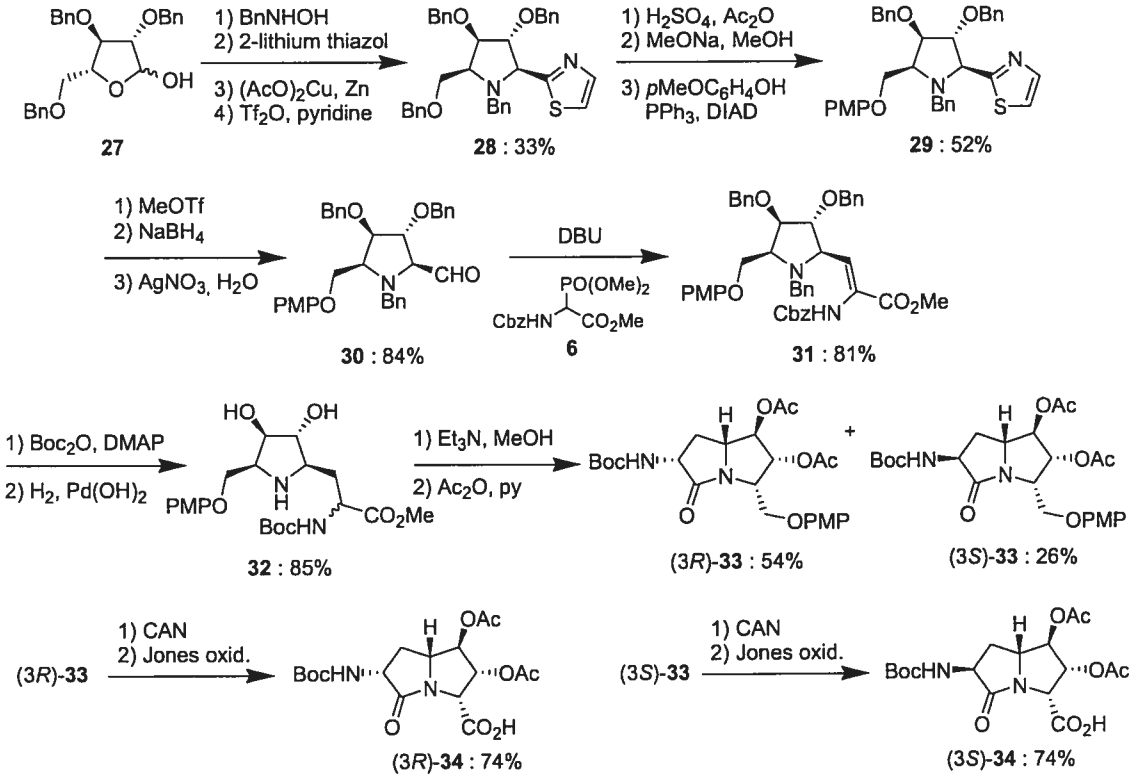
Tricyclic pyrrolizidinone **26** was prepared as a single diastereoisomer from pyroglutamate (SCHEME 3).[15] Lactam **20**[16] was alkylated with trimethylstannylmethyl iodide to give (3*S*)-**21** as the major isomer in 63% yield. Reaction with allylmagnesium bromide afforded a hemiaminal intermediate which after treatment with TFA, lead to bicycle **22** in 58% yield. Cyclopropane formation was suggested to occur via the formation of an iminium ion intermediate onto which the organostannane cyclized. Ozonolysis of the double bond, Horner-Wadsworth-Emmons reaction on the resulting aldehyde and olefin reduction by hydrogenation with Pd/C gave ester **23**, which was submitted to a stereoselective enolate α-hydroxylation using Davis reagent (2-benzensulfonyl-3-phenyloxaziridine)[17] to provide hydroxyester **24** as a



SCHEME 3 Synthesis of tricyclic pyrrolizidin-2-one azido acid **26**.[15]

single diastereomer in 25% overall yield from **20**. Cleavage of the Boc group with TFA and methyl ester hydrolysis with LiOH, followed by lactam formation using EDC, HOBt and DMAP gave tricycle **25**. The alcohol was then replaced by an azide with stereo-inversion under Mitsunobu conditions and the silyloxymethyl group was deprotected and oxidized in two steps to furnish substituted azido pyrrolizidin-2-one acid **26** in 52% overall yield from alcohol **25**.

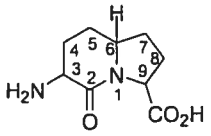
Polyhydroxylated *N*-(Boc)amino pyrrolizidin-2-one acids **34** have been made from pentofuranose derivatives (SCHEME 4).[18] Thiazole **28** was prepared on multigram scale from tribenzyl pentofuranose **27** in 4 steps by nitrone formation, addition of lithium thiazol, dehydroxylation, trifluoromethanesulfonate formation and S_N2 displacement in 33% overall yield.[19] The primary benzyl ether was selectively deprotected, and transformed into a *para*-methoxyphenyl (PMP) ether under Mitsunobu conditions. The thiazole was converted to aldehyde **30** by a three steps protocol involving *N*-methylation, reduction and hydrolysis in 84% yield.[20] Horner-Wadsworth-



SCHEME 4 Synthesis of 6,7-dihydroxylated pyrrolizidin-2-one *N*-(Boc)amino acids **34** from pentafuranose **27**.[18,19]

Emmons olefination of aldehyde **30** with glycine-derived phosphonate **6** gave dehydro amino ester **31** which was protected as its *N*-(Boc)-*N*-(Cbz)amino ester. Hydrogenation in the presence of $Pd(OH)_2$ deprotected the three benzyl groups and the Cbz group and reduced the olefin to give a diastereomeric mixture of amino esters **32**. Lactam formation under alkaline conditions and acetylation of the secondary alcohols furnished a separable 2:1 mixture of bicycles (3*R*)- and (3*S*)-**33** in 54% and 26% yield from **32**. Finally, selective deprotection of the PMP group with cerium ammonium nitrate (CAN) and oxidation of the primary alcohol to the acid furnished (3*R*,5*R*,6*R*,7*R*,8*R*)- and (3*S*,5*R*,6*R*,7*R*,8*R*)-**34** in 12% and 5% overall yields from thiazole **28**.

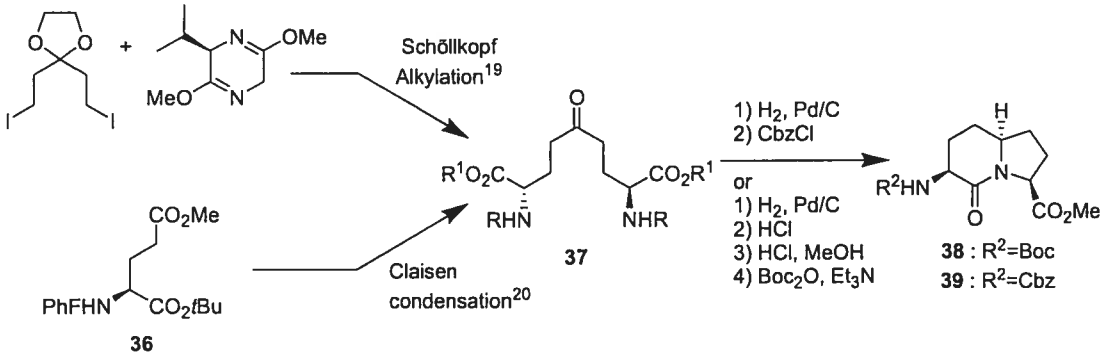
2.3.3. Synthesis of indolizidin-2-one amino acids (I^2aa).



35

One of the most studied azabicycloalkane amino acids has been indolizidin-2-one dipeptide surrogate **35**, onto which substituents have been added at the 3 to 8-positions inclusively.

Since the synthesis of the parent indolizidinone system was reviewed in 1997 (SCHEME 5),[1] six methods have been reported for preparing this azabicycloalkane amino acid. The original syntheses involved formation of linear keto diaminoazelates **37** by Schöllkopf alkylation[21] or Claisen

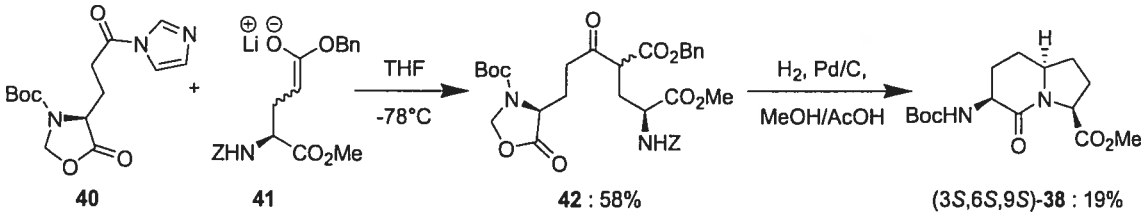


36

SCHEME 5 Original approaches for the synthesis of indolizidin-2-one amino esters **38** and **39**.

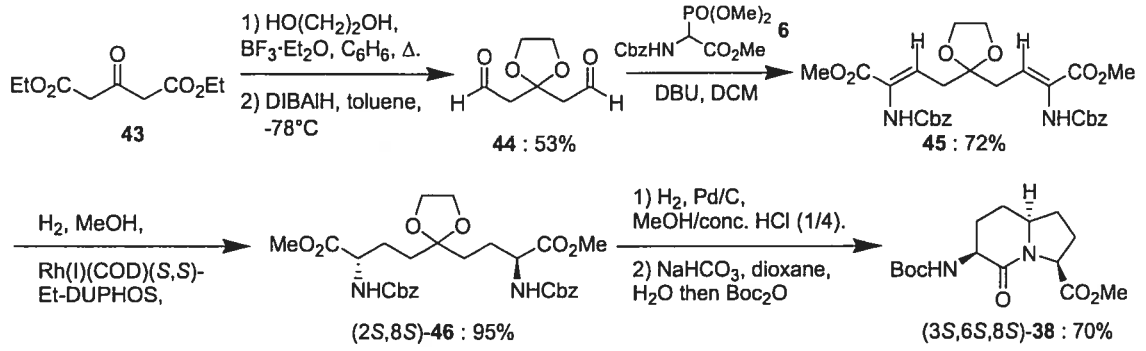
condensation,[22] followed by reductive amination and lactam cyclization to form respectively *N*-(Boc)- and *N*-(Cbz)-protected bicycles **38** and **39**.

Claisen condensation of glutamate-derived imidazolide oxazolidinone **40** with the lithium enolate of Cbz-Glu(OBn)-OMe **41** was later reported to give β-ketoester **42** (SCHEME 6),[23] that was converted to (3*S*,6*S*,9*S*)-methyl-*N*-(Boc)amino indolizidin-2-one ester **38** in 11% overall yield from **40** by a similar reductive amination / lactam cyclization sequence.



SCHEME 6 Synthesis of (3*S*,6*S*,9*S*)-indolizidin-2-one amino ester **38**.[23]

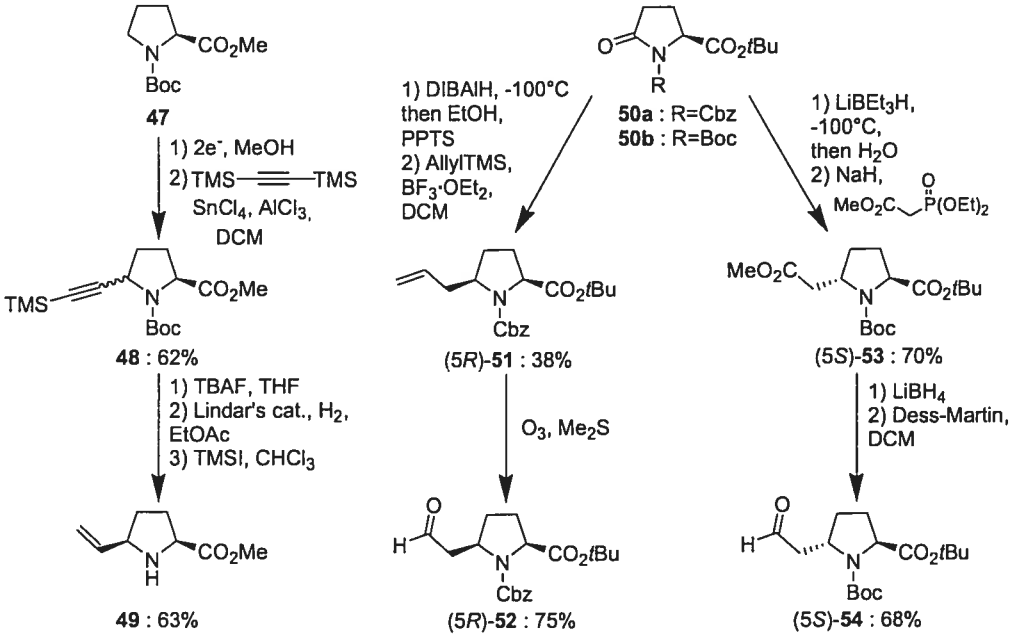
Similarly, a protected version of linear ketone **37** was synthesized by a double Horner-Wadsworth-Emmons reaction on dialdehyde **44** with glycine-derived phosphonate **6** followed by enantioselective reduction (SCHEME 7).[24] Hydrogenation with Rh(I)(COD)-Et-DUPHOS as catalyst gave either (2*S*,8*S*)- or (2*R*,8*R*)-**46** contingent on ligand configuration. Conversion of protected keto azelate **46** into the *N*-(Boc)amino indolizidin-2-one ester **38** proceeded by a reductive amination/lactam cyclization/amine protection sequence in 70% overall yield.



SCHEME 7 Synthesis of (3*S*,6*S*,9*S*)-indolizidin-2-one amino ester **38**.[24]

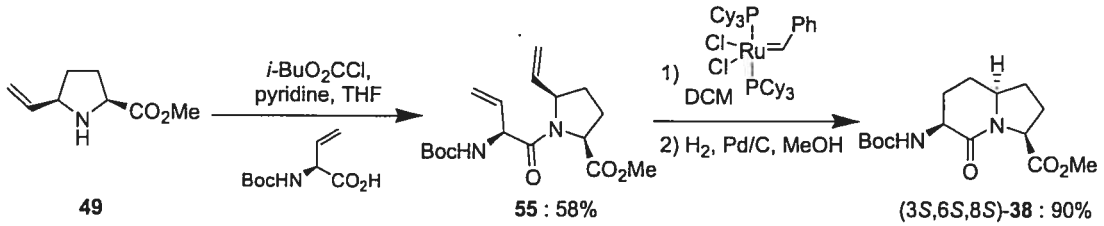
Alternative strategies for synthesizing indolizidin-2-one amino esters have involved annulation of pyroglutamic acid and proline (SCHEMES 8, 9 and 10).[10,25,26] Such routes begin by the synthesis of a suitably protected 5-

substituted proline. For example, *cis*-5-vinylproline (5*R*)-**49** was synthesized by anodic oxidation of *N*-(Boc)proline methyl ester **47**, bis(trimethylsilyl)acetylene addition to the resulting hemiacetal under Lewis acid conditions, desilylation, olefin reduction with Lindar's catalyst and Boc deprotection.[27] *Cis*-5-allylproline (5*R*)-**51** was similarly prepared from *N*-(Cbz)pyroglutamate **50a** by reduction to a hemiacetal that was reacted with allyltrimethylsilane[28] under Lewis acid conditions. *Cis*-Aldehyde (5*R*)-**52** was then obtained by ozonolysis of olefin (5*R*)-**51**.[9] *Trans*-aldehyde (5*S*)-**54** was made by reduction of *N*-(Boc)pyroglutamate **50b**, Horner-Wadsworth-Emmons olefination on the hemiacetal, methyl ester reduction, and alcohol oxidation with Dess-Martin reagent (SCHEME 8).[25]



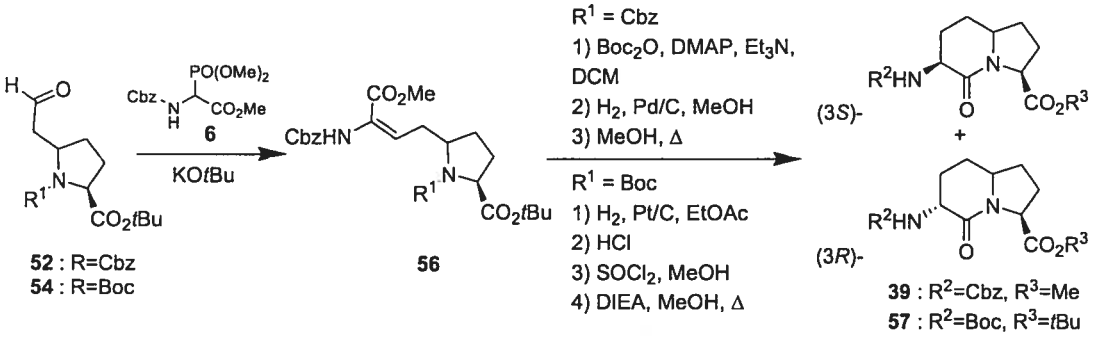
SCHEME 8 Synthesis of 5-substituted prolines **49**, (5*R*)-**52** and (5*S*)-**54**.[9,25,27,28]

Indolizidinone amino esters were then constructed from 5-substituted prolines **49**, (5*R*)-**52** and (5*S*)-**54** from routes featuring olefin metathesis and Horner-Wadsworth-Emmons olefination. For example, (3*S*,6*S*,9*S*)-*N*-(Boc)amino indolizidin-2-one methyl ester **38** was synthesized in 36% overall yield from *cis*-5-vinylproline (5*R*)-**49** by acylation with *N*-(Boc)vinylglycine via a mixed anhydride to form diene **55**, which was cyclized by ring closing metathesis and hydrogenated (SCHEME 9).[26]



SCHEME 9 Synthesis of *N*-(Boc)amino indolizidin-2-one methyl ester **38** by ring closing metathesis.[26]

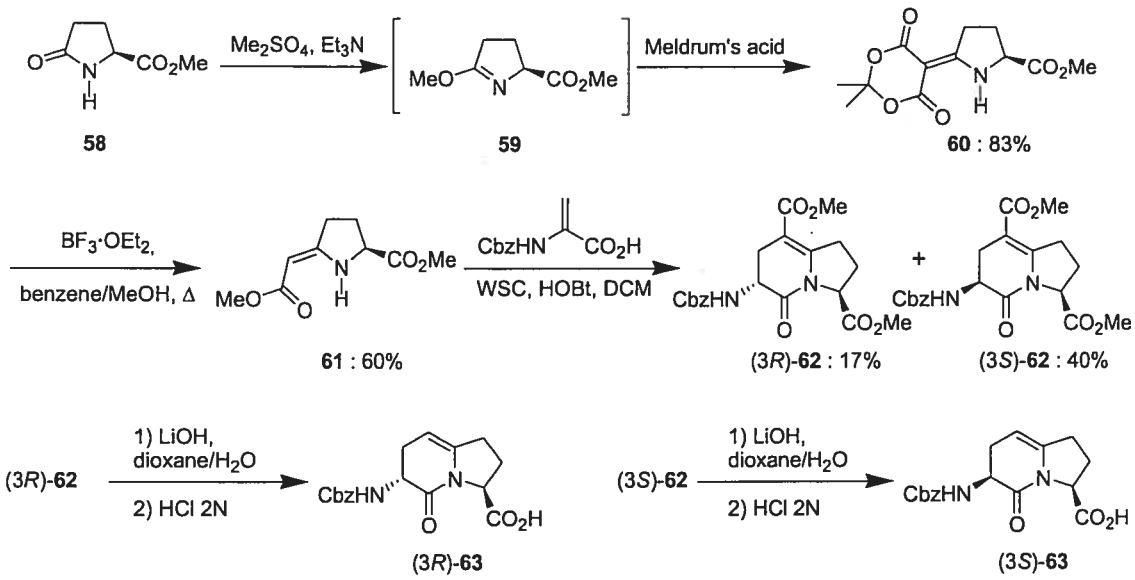
Alternatively, dehydro amino esters were synthesized by treating aldehydes (5*R*)-**52** and (5*S*)-**54** with glycine-derived phosphonate **6** (SCHEME 10).[10,25] Indolizidin-2-one amino esters **39** and **57** were synthesized respectively in 4 and 3 steps by olefin hydrogenation, protecting group shuffle and lactam cyclization. The diastereoselectivity of the double bond hydrogenation varied from 2:1 to 1:1.5 (3*R*/3*S*) contingent on the 5-position configuration, proline *N*-protecting group and catalyst. Diastereomeric mixtures of **39** and **57** were separated by chromatography and isolated in yields ranging from 10% to 20% overall from pyroglutamate **50**.



SCHEME 10 Synthesis of indolizidin-2-one amino esters from aldehydes **52** and **54**.[10,25]

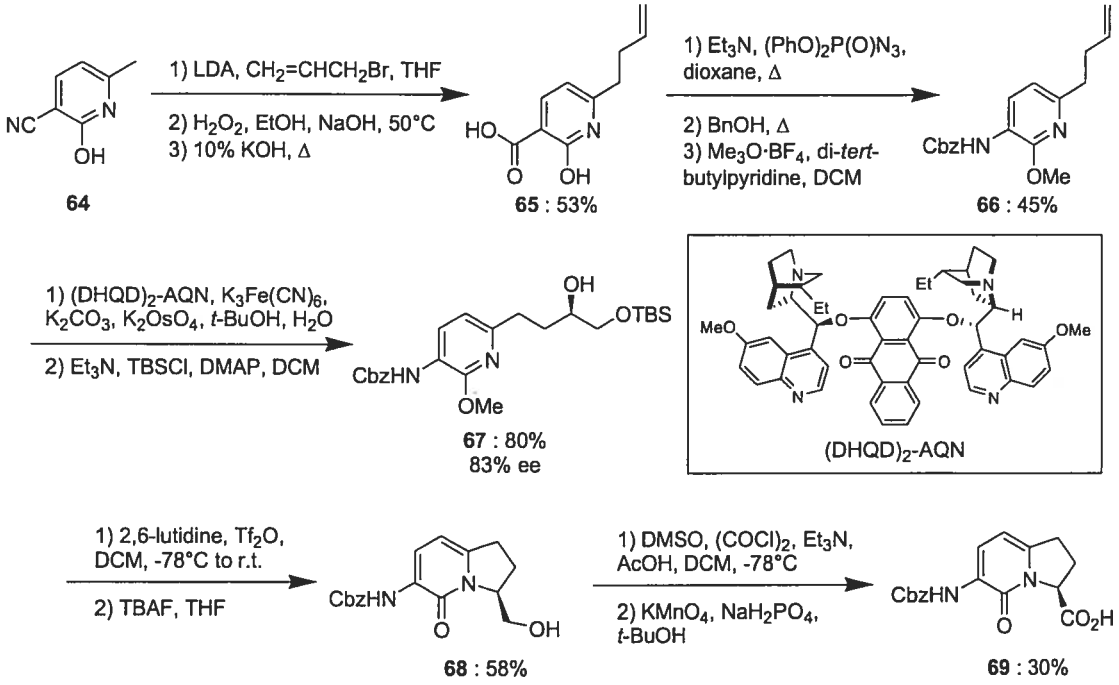
5,6-Dehydroindolizidin-2-one amino esters have also been synthesized from pyroglutamate by a route featuring conversion to *O*-methylimidate **59**, condensation with Meldrum's acid and decarboxylation with boron trifluoride in benzene/methanol to yield enaminoester **61** (SCHEME 11).[29] A one-pot Michael addition / lactam cyclization, by treating **61** with *N*-(Boc)dehydroalanine, 1-ethyl-3-(3'-dimethylamino-propyl)-carbodiimide (WSC) and HOBt furnished a separable mixture of dehydroindolizidinone diastereoisomers **62**. Saponification and acid induced decarboxylation gave the

corresponding *N*-(Cbz)amino dehydroindolizidin-2-one acids (3*S*)- and (3*R*)-**63** in 20% and 8% respective overall yields from pyroglutamate **58**.



SCHEME 11 Synthesis of 5,6-dehydroindolizidin-2-one amino acids **63**.[29]

Tetrahydroindolizinone amino acid **69** has been synthesized using an intramolecular *N*-alkylation of a substituted pyridine as key step from trisubstituted pyridine **64** (SCHEME 12).[30] Substituted nicotinic acid **65** was made by alkylation with allyl bromide and nitrile hydrolysis. Conversion of acid **65** to its corresponding azide using diphenylphosphoryl azide and Curtius rearrangement in the presence of benzyl alcohol provided the protected 3-amino-2-pyridinone which was O-alkylated to give 2-methoxy-3-*N*-(Cbz)amino pyridine **66**. The indolizinone **68** was obtained by a route featuring catalytic asymmetric dihydroxylation with (DHQD)2-AQN, selective protection of the primary alcohol, trifluoro-methanesulfonate formation with intramolecular N-alkylation and ether deprotection. Oxidation of the alcohol provided tetrahydroindolizinone amino acid **69** in 3% overall yield from trisubstituted pyridine **64**.



SCHEME 12 Synthesis of 2,7,8,9-tetrahydroindolizin-2-one *N*-(Cbz)amino acid **69**.[30]

2.3.4. Synthesis of substituted indolizidin-2-one amino acids

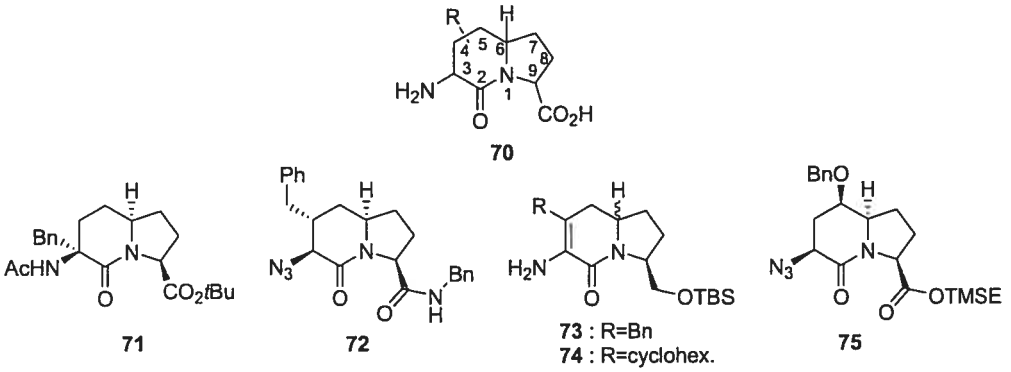
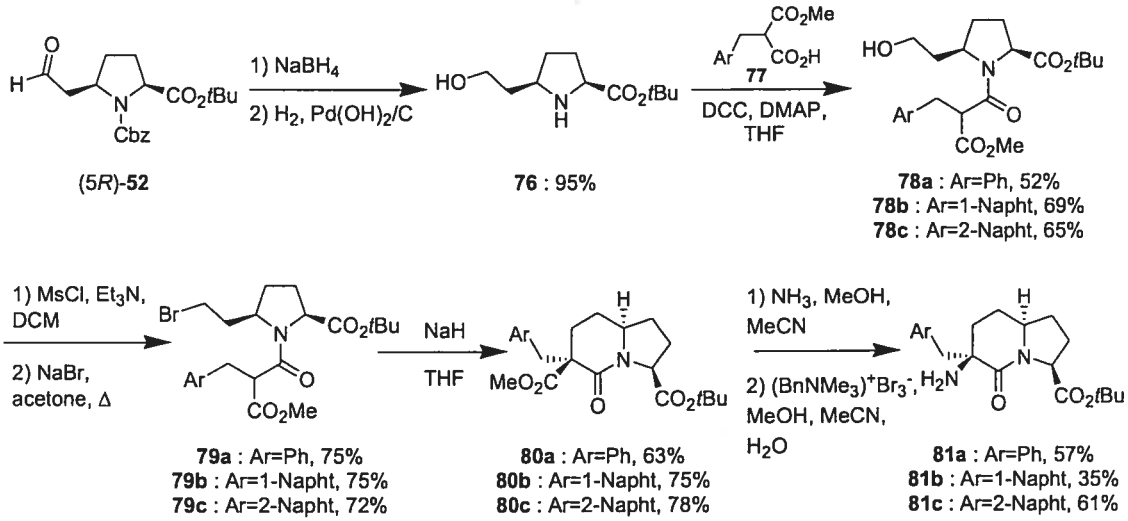


FIGURE 3 General structure for substituted indolizidinone amino acid and examples of previously reviewed analogs.

Substituents have been introduced at positions 3, 4 and 5 in indolizidin-2-ones **71**, **72** and **75**, as well as at position 4 in dehydroindolizidin-2-ones **73** and **74** as previously reviewed (FIGURE 3).[1]

3-Substituted indolizidin-2-one amino esters **81** have been recently synthesized from aldehyde (5*R*)-**52** by a route beginning with reduction and

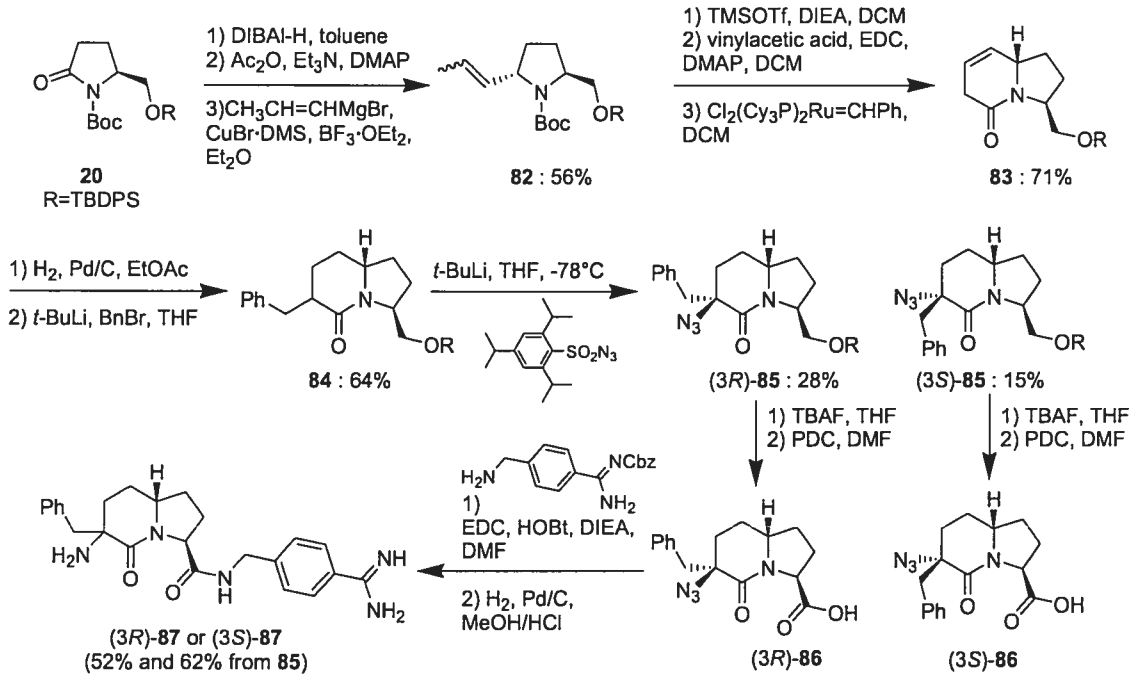
amine deprotection to give amino alcohol **76** (SCHEME 13).[31] The amine was then acylated with a series of malonate monoacid derivatives **77** to furnish malonamides **78** with different aryl substituents: phenyl (**78a**), 1-naphthyl (**78b**) and 2-naphthyl (**78c**). Methanesulfonation of the alcohol and displacement with sodium bromide furnished the corresponding bromides **79**. Finally, the 3-substituted indolizidin-2-one amino esters were obtained by a 3 step process featuring diastereoselective intramolecular alkylation under basic conditions to form diester **80**, selective methyl ester aminolysis and Hofmann rearrangement to provide the respective amino esters (3*R*)-**81a-c** in 15%, 15% and 24% respective overall yields from aldehyde (5*R*)-**52**.



SCHEME 13 Synthesis of 3-arylmethylindolizidin-2-one amino esters **81**.[31]

3-Benzyl indolizidin-2-one azido esters **86** have been synthesized from pyroglutamate-derived silyl ether **20** by a route featuring olefin metathesis (SCHEME 14).[32] Amide reduction and acetylation provided the *O*-acyl aminol which on reaction with propenyl magnesium bromide in the presence of cuprous bromide furnished 5-substituted prolinol silyl ether **82**. The formation of the bicyclic compound **83** was achieved by cleavage of the *N*-Boc group, *N*-acylation with vinylacetic acid and ring closing metathesis using the first generation of the Grubb's catalyst ($Cl_2(Cy_3P)_2Ru{=}CHPh$). 3-Benzyl indolizidinone **84** was then prepared by reduction of the double bond, enolization with *t*-BuLi and alkylation with benzyl bromide. A second

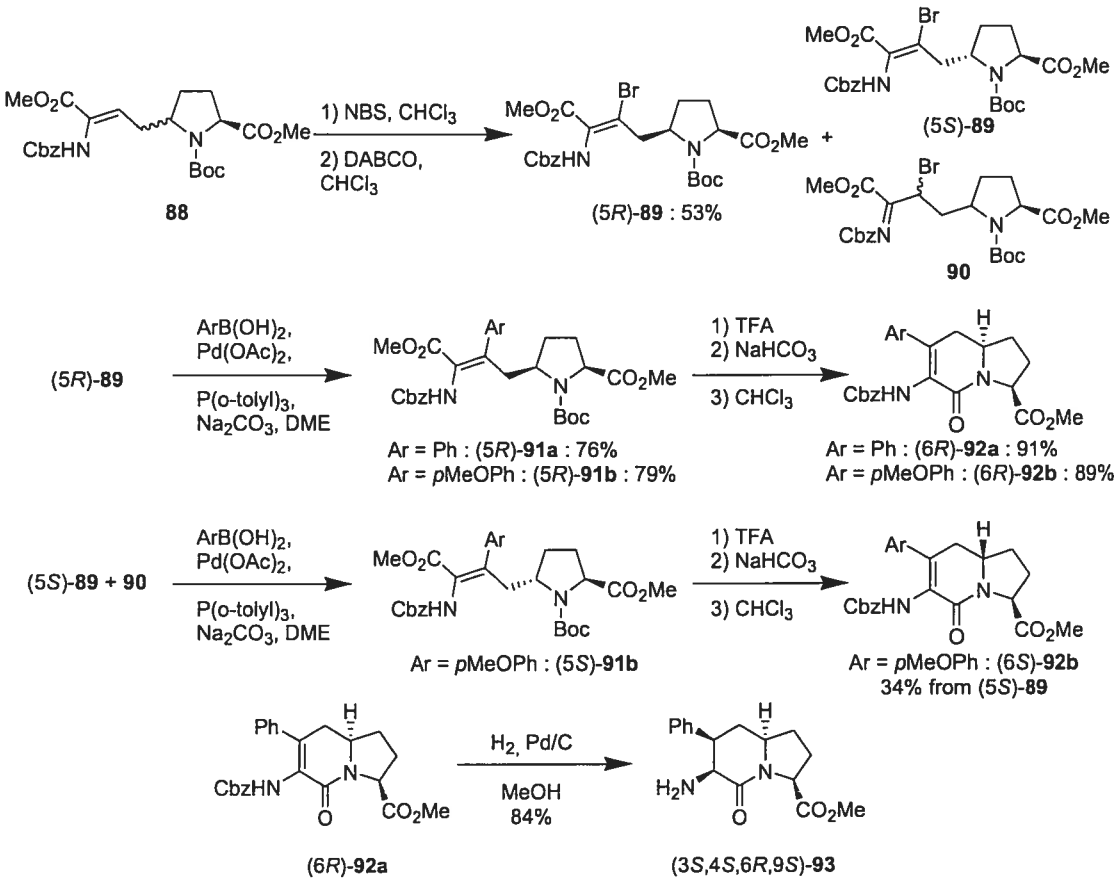
deprotonation with *t*-BuLi and reaction with trisyl azide (2,4,6-tri-*iso*-propylphenylsulfonylazide) gave a separable 2 : 1 mixture of azido ethers (3*R*)- and (3*S*)-**85** in 13% and 7% overall yield from prolinol ether **82**. Diastereomeric amino amides **87** were finally prepared by silyl ether cleavage with TBAF, alcohol oxidation with PDC, coupling of the crude acid **86** to *N*-(Cbz)-4-(aminomethyl) benzylamidine with EDC and HOBt, followed by hydrogenation, which removed the Cbz group and reduced the azide function.



SCHEME 14 Synthesis of 3-benzyl indolizidin-2-one amino amides **87**.[32]

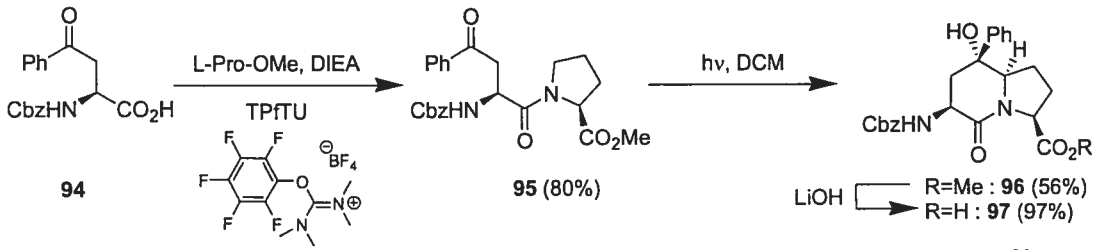
4-Phenyl indolizidin-2-one amino ester **93** has been synthesized by a route featuring bromination of dehydroamino esters **88** followed by cross coupling of aryl boronic acids to the resulting vinyl bromides **89** (SCHEME 15).[33] Protected (6*R*)-3,4-dehydro-indolizidin-2-one *N*-(Cbz)amino esters (6*R*)-**92** possessing 4-phenyl (**92a**) and 4-*para*-methoxy-phenyl (**92b**) substituents were produced by Boc group removal with TFA and lactam cyclization in 91% and 89% respective overall yields from (5*R*)-**91a** and (5*R*)-**91b**. The (6*S*)-epimer of **92b** was similarly prepared by a cross-coupling / lactam cyclization process in 34% yield from an inseparable mixture of vinyl bromide (5*S*)-**89** and α-bromo-imine intermediate **90**. (3*S*,4*S*,6*R*,9*S*)-4-Phenyl-indolizidin-2-one amino ester

93 was finally produced by a one-pot reduction of the double bond and Cbz deprotection using hydrogen and Pd/C.



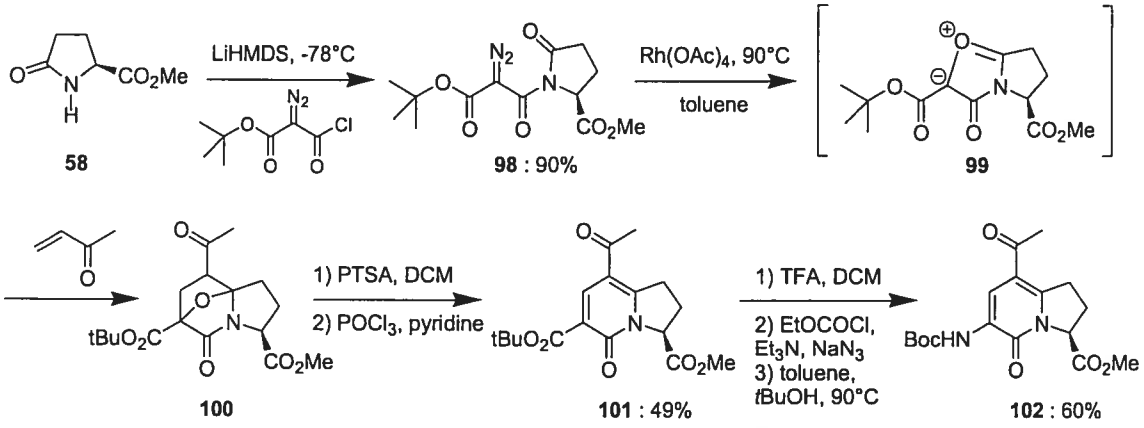
SCHEME 15 Synthesis of 3,4-dehydro-4-aryl- and 4-phenyl-indolizidin-2-one amino esters **92** and **93**.[33]

5-Hydroxyl-5-phenyl indolizidinone *N*-(Cbz)amino ester **96** has been synthesized from dipeptide **95**, derived from coupling L-β-benzoylalanine **94**[34,35] and L-proline methyl ester (SCHEME 16).[36] Photochemical cyclization of dipeptide **95** and methyl ester hydrolysis afforded the acid (3*S*,5*R*,6*R*,8*S*)-**97** in 43% overall yield from **94**.



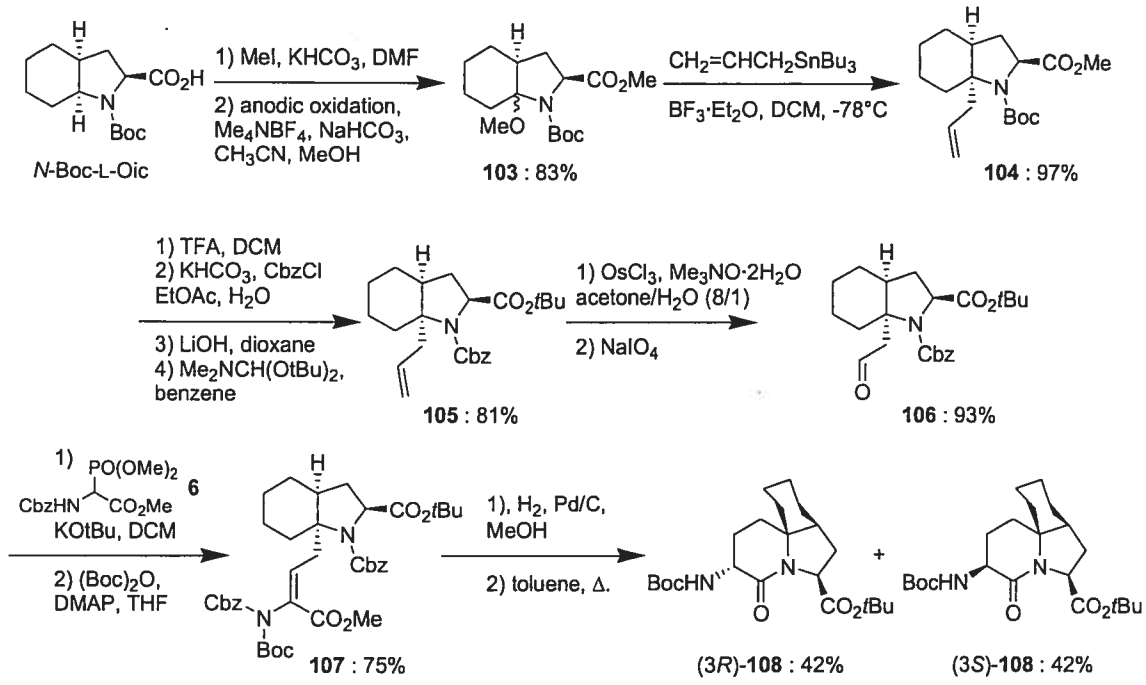
SCHEME 16 Synthesis of 5-hydroxyl-5-phenyl indolizidin-9-one *N*-(Boc)amino acid **97**.[36]

5-Acetyl tetrahydroindolizinone amino ester **102** has been synthesized using a [3+2]-cycloaddition as key synthetic step from pyroglutamate **58** (SCHEME 17).[37] Tricycle **100** was synthesized by acylation of **58** with *tert*-butyl diazomalonyl chloride to form the diazoimide **98**, which was decomposed with rhodium *tetra*-acetate to provide isomünchnone intermediate **99** that reacted with methyl vinylketone in a dipolar cycloaddition. Synthesis of *N*-(Boc)amino ester **102** was achieved in 29% yield from **100** by acidic ring opening and dehydration, which furnished tetrahydroindolizinone **101**, followed by cleavage of the *tert*-butyl ester and Curtius rearrangement of the acyl azide.



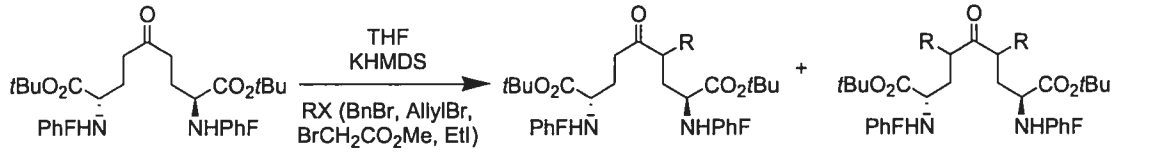
SCHEME 17 Synthesis of 5-acetyl-2,7,8,9-tetrahydroindolizin-2-one amino ester **102**.[37]

The introduction of an alkyl substituent at position 6, instead of the ring-fusion centre, was achieved in the synthesis of 6,7-cyclohexyl indolizidinone *N*-(Boc)amino esters **108** from commercial *N*-Boc-L-octahydroindole-2-carboxylic acid (*N*-Boc-L-Oic, SCHEME 18).[38] Electrochemical oxidation of *N*-Boc-L-Oic-OMe proceeded in an analogous fashion to the 5-position oxidation of *N*- (Boc)proline methyl ester **47** (SCHEME 8) providing *O*-methyl aminol **103**. Aldehyde **106** was obtained in 66% overall yield from *N*-Boc-L-Oic by treatment of **103** with allyl tributyltin, protecting group exchange and olefin oxidation. Horner-Wadsworth-Emmons olefination, reduction of the dehydro amino ester and lactam cyclization gave a separable 1:1 mixture of (3*S*)- and (3*R*)-**108** in 19% and 19% respective overall yields from *N*-Boc-L-Oic.

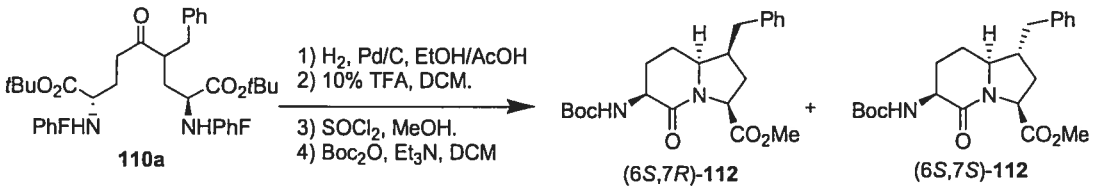


SCHEME 18 Synthesis of 6,7-cyclohexyl indolizidin-2-one *N*-(Boc)amino esters **108**.[38]

Several methods exist for adding substituents to the indolizidinone 7-position. For example, 7-benzyl indolizidinone amino acids have been synthesized by a route featuring alkylation of (2*S*,8*S*)-di-*tert*-butyl-5-oxo-2,8-di-[*N*-(PhF)amino]azelate **109** (SCHEME 19).[39] Ketone **109** was enolized selectively using KN(SiMe3)2 and reacted with a series of alkyl halides. (4*R*)-Di-*tert*-butyl-4-benzyl-5-oxo-2,8-di[*N*-(PhF)amino]azelate (4*R*)-**110a** was prepared and isolated from a 1:7 diastereomeric mixture after alkylation with benzyl bromide. Epimerization of the 1:7 mixture gave access to a separable 1.3:1 mixture of 4*S*/4*R* diastereomers.



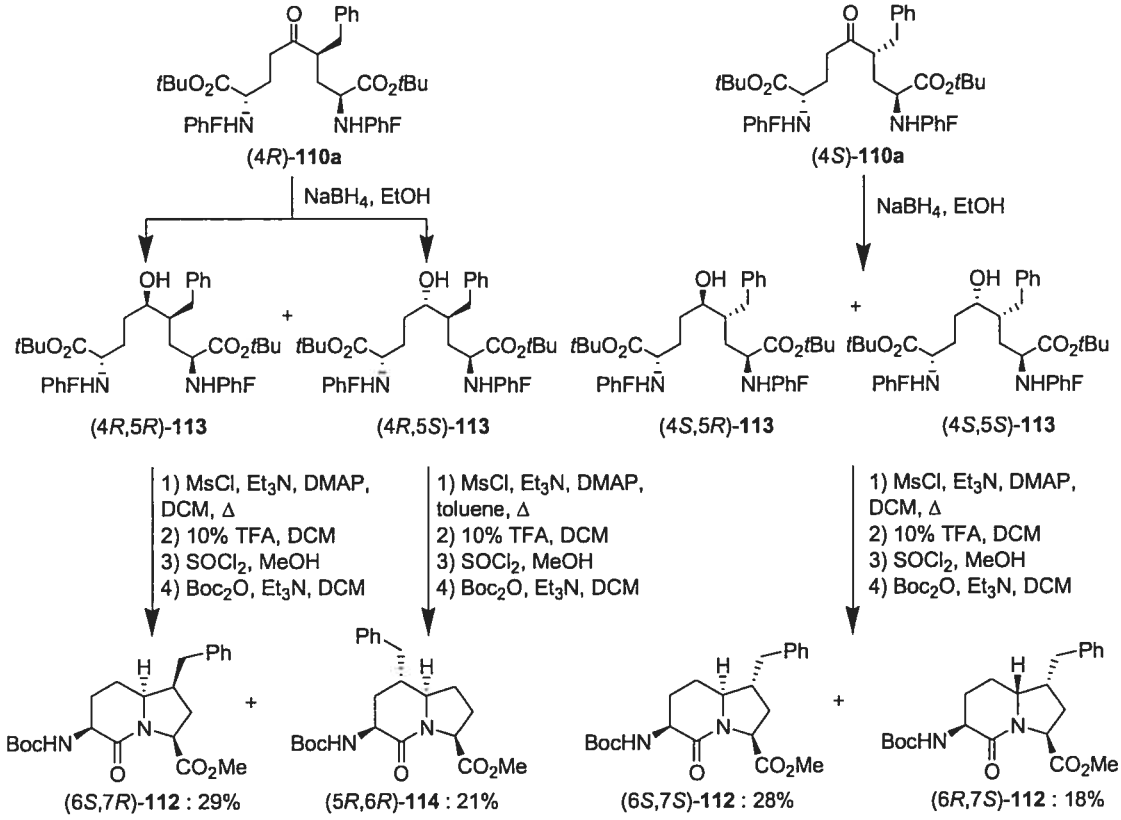
SCHEME 19 Synthesis of 4-substituted di-*tert*-butyl-5-oxo-2,8-di-[*N*-(PhF)amino]azelates **110**.[39]



substrate	H_2 (atm)	(6*S*,7*R*)-**112**	(6*S*,7*S*)-**112**
(4*R*)-**110a**	1	24%	36%
(4*R*)-**110a**	9	10%	53%
(4*S*)-**110a**	1	21%	31%
(4*S*)-**110a**	9	6%	49%

SCHEME 20 Synthesis of 7-benzyl indolizidin-2-one amino esters **112**.[39]

Conversion of 4-benzyl ketone **110a** into the corresponding 5- and 7-substituted indolizidinone amino acids was achieved by two methods. Reductive amination, lactam cyclization and amine protection converted both diastereomers of ketone **110a** to a separable mixture of (6*S*,7*R*)-**112** and (6*S*,7*S*)-**112** due to epimerization at the 7-position as a result of an imine-enamine tautomerization during the reductive amination (SCHEME 20). The

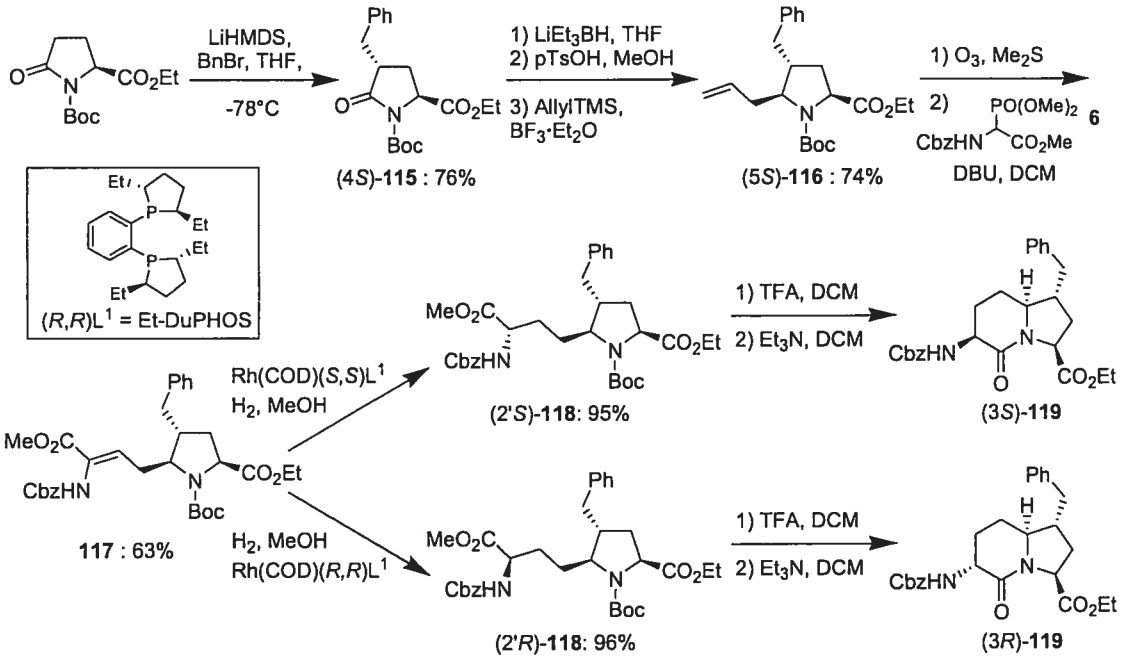


SCHEME 21 Synthesis of 5- and 7-benzyl indolizidin-2-one amino esters **114** and **112**.[39]

diastereomeric ratio of the two bicycles varied from 1.5:1 to 5:1 as the hydrogen pressure in the reductive amination was increased from 1 to 9 atm.

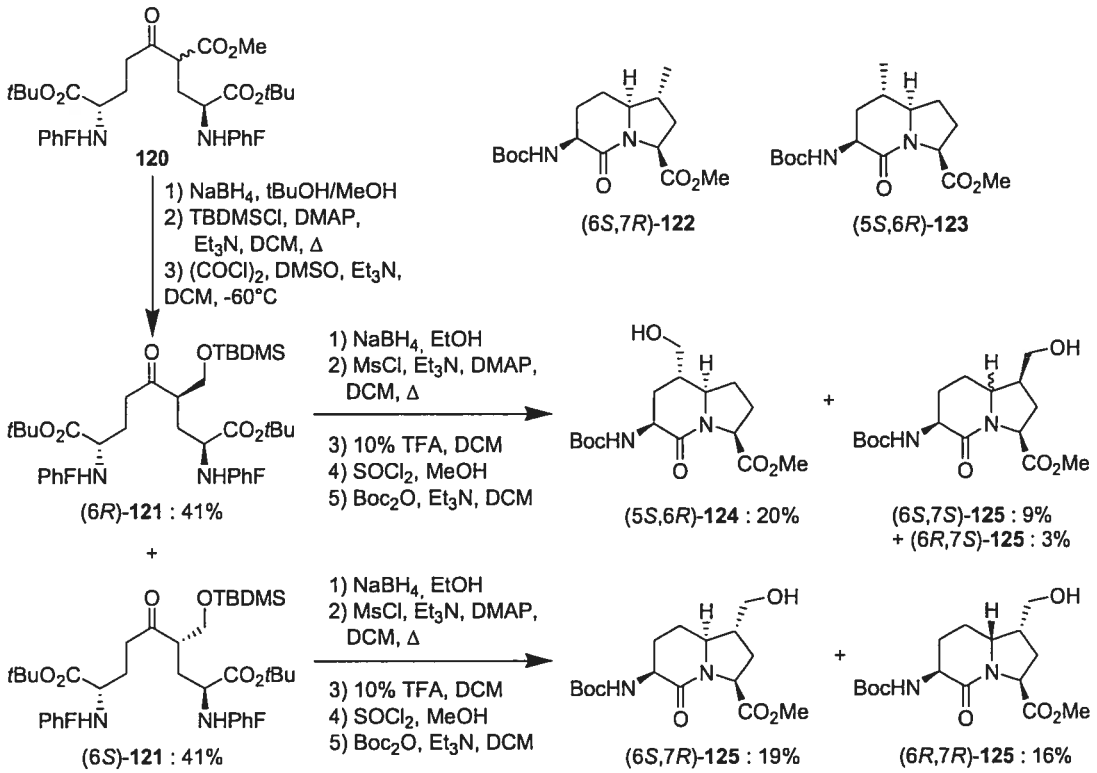
In the second method, reduction of ketone **110a**, activation of the resulting alcohol as a methanesulfonate, intramolecular nucleophilic displacement, lactam cyclization and amine protection provided one 5- and three 7-benzyl indolizidinones. Their formation was contingent upon the stereochemistry of the alcohol and benzyl group bearing carbons (SCHEME 21); (6*S*,7*R*)-**112**, (5*R*,6*R*)-**114**, (6*S*,7*S*)-**112** and (6*R*,7*S*)-**112** were obtained in 29%, 21%, 28% and 18% respective overall yields from ketone **110a**.

7-Benzyl indolizidinone *N*-(Cbz)amino esters **119** have also been synthesized from (4*S*)-benzyl pyroglutamate **115**, which was obtained from alkylation of ethyl *N*-(Boc)pyroglutamate (SCHEME 22).[40] As previously described for the synthesis of 5-allyl proline **51** from pyroglutamate (SCHEME 8), 4-benzyl-5-allyl proline **116** was prepared from **115** by a route involving imine alkylation with allyl trimethylsilane. Ozonolysis of olefin **116** and Horner-Wadsworth-Emmons olefination on the resulting aldehyde with glycine phosphonate **6** provided dehydroamino ester **117**. Stereoselective reduction of **117** was achieved using [Rh(I)(COD)(*S,S*)- or (*R,R*)-Et-DuPHOS]OTf [41] such that either (3*S*)- or (3*R*)-7-benzyl indolizidin-2-one amino ester **119** could be selectively prepared after Boc group removal and lactam cyclization.



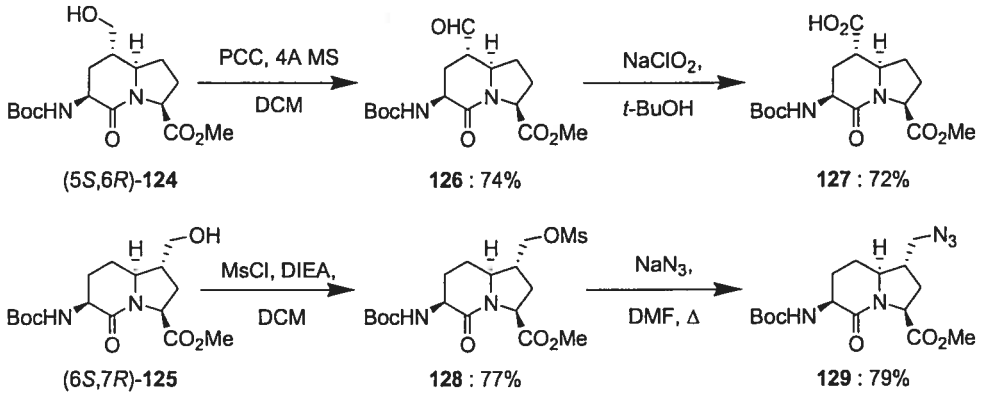
SCHEME 22 Synthesis of 7-benzyl indolizidin-2-one amino esters **119**.[40]

A series of 5- and 7-methyl and hydroxymethyl indolizidinone amino esters **122-125** has been prepared from di-*tert*-butyl-4-carboxymethyl-5-oxo-2,8-di[*N*-(PhF)amino]azelate **120**, which is derived from Claisen condensation of γ-methyl α-*tert*-butyl *N*-(PhF)glutamate **36** (SCHEME 23).[42] Separable silyloxymethyl ketones **121** were prepared by reduction of β-keto esters **120** to the corresponding diols, selective protection of the primary alcohol and re-oxidation of the secondary alcohol. Reductive amination of the diastereomeric mixture and lactam cyclization gave low yields of the hydroxymethyl indolizidinones (5*S*,6*R*)-**124** and (6*S*,7*S*)-**125**; instead, 5- and 7-methylindolizidin-2-one amino esters **122** and **123** were isolated as significant side-products resulting from loss of the silyloxy group via imine-enamine tautomerization, β-elimination and reduction. On the other hand, cyclization via ketone reduction and methanesulfonate displacement followed by lactam formation and amine protection provided selectively 5- and 7-hydroxymethyl indolizidinone amino esters (5*S*,6*R*)-**124**, (6*S*,7*S*)-**125**, (6*R*,7*S*)-**125**, (6*S*,7*R*)-**125** and (6*R*,7*R*)-**125** in 3% to 20% overall yields, contingent on the stereochemistry of the methanesulfonate- and silyloxymethyl-bearing carbons.



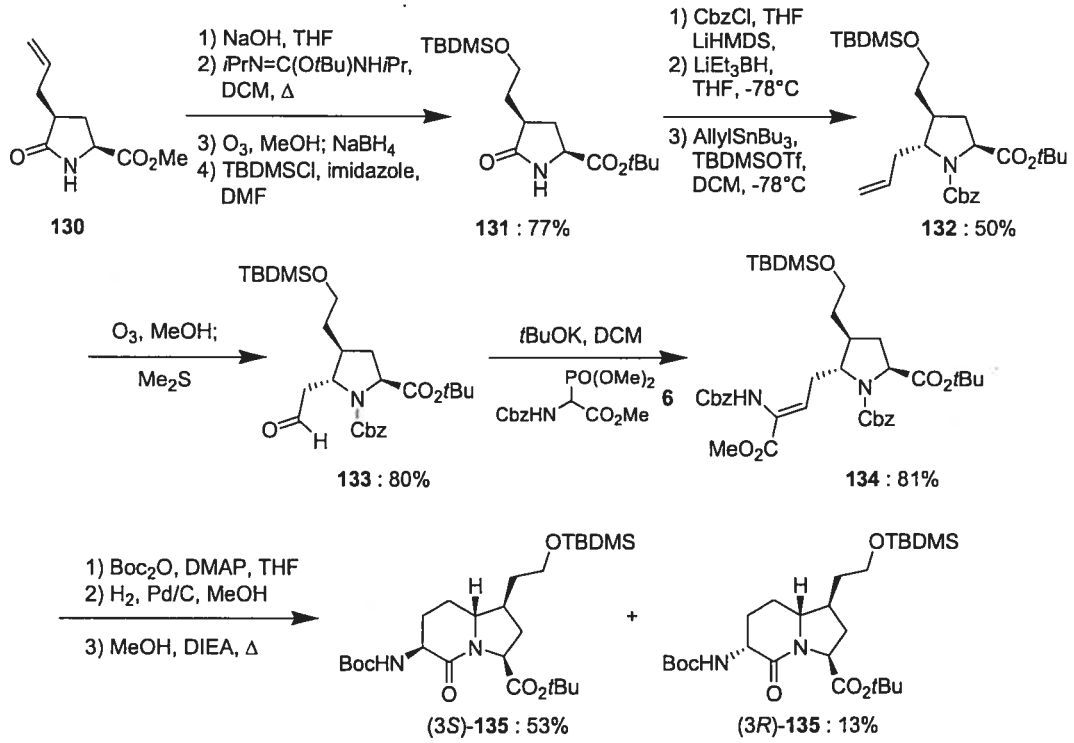
SCHEME 23 Synthesis of 5- and 7-hydroxymethylindolizidin-2-one amino esters **124** and **125**.[42]

The diversity of 5- and 7-position substituents was further expanded by oxidation of the 5-hydroxymethyl group to an acid, and by activation of the 7-hydroxymethyl group as its corresponding methanesulfonate **128** and displacement with azide anion, which furnished respectively orthogonally protected Glu-Pro and Ala-Lys surrogates **127** and **129** (SCHEME 24).



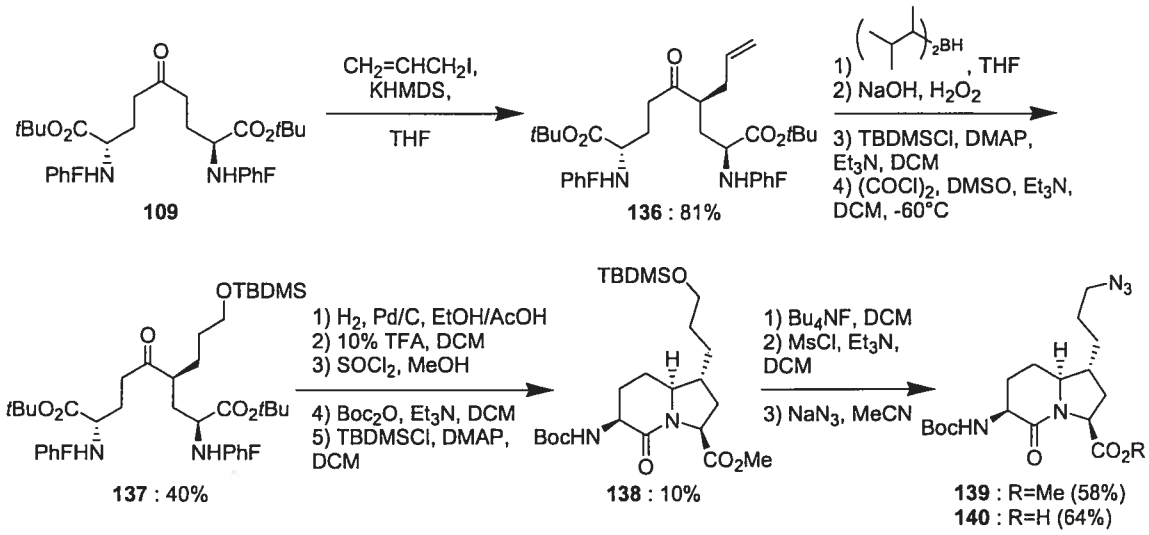
SCHEME 24 Synthesis of 5-carboxyl indolizidin-2-one *N*-(Boc)amino esters **127** and 7-azidomethyl indolizidin-2-one *N*-(Boc)amino esters **129**.[42]

7-Silyloxyethyl indolizidinone *N*-(Boc)amino esters **135** have been prepared by a Horner-Wadsworth-Emmons olefination / reduction sequence as previously described (cf. SCHEME 22) from 5-silyloxyethyl prolinate *tert*-butyl ester **131** (SCHEME 25),[43] which was in turn prepared from 4-allyl pyroglutamate methyl ester **130**[44] by a route featuring carboxylate saponification and esterification, ozonolysis, ozonide reduction and alcohol protection. After amide protection and reduction to the aminol, 4-silyloxyethyl-5-allyl proline **132** was prepared, using a similar method as described for **51** from pyroglutamate (cf. SCHEME 8), by imine alkylation with allyl tributyltin. Ozonolysis of olefin **132** followed by Horner-Wadsworth-Emmons addition of glycine phosphonate **6** onto aldehyde **133** provided dehydroamino ester **134**. 7-Silyloxyethyl indolizidinones were finally prepared as a separable 1:4 mixture of (3*R*)- and (3*S*)-**135** in 13% and 3% respective overall yields from pyroglutamate **130** by amine protection of dehydro amino ester **134**, hydrogenation which reduced the olefin and cleaved the Cbz group, and lactam cyclization.



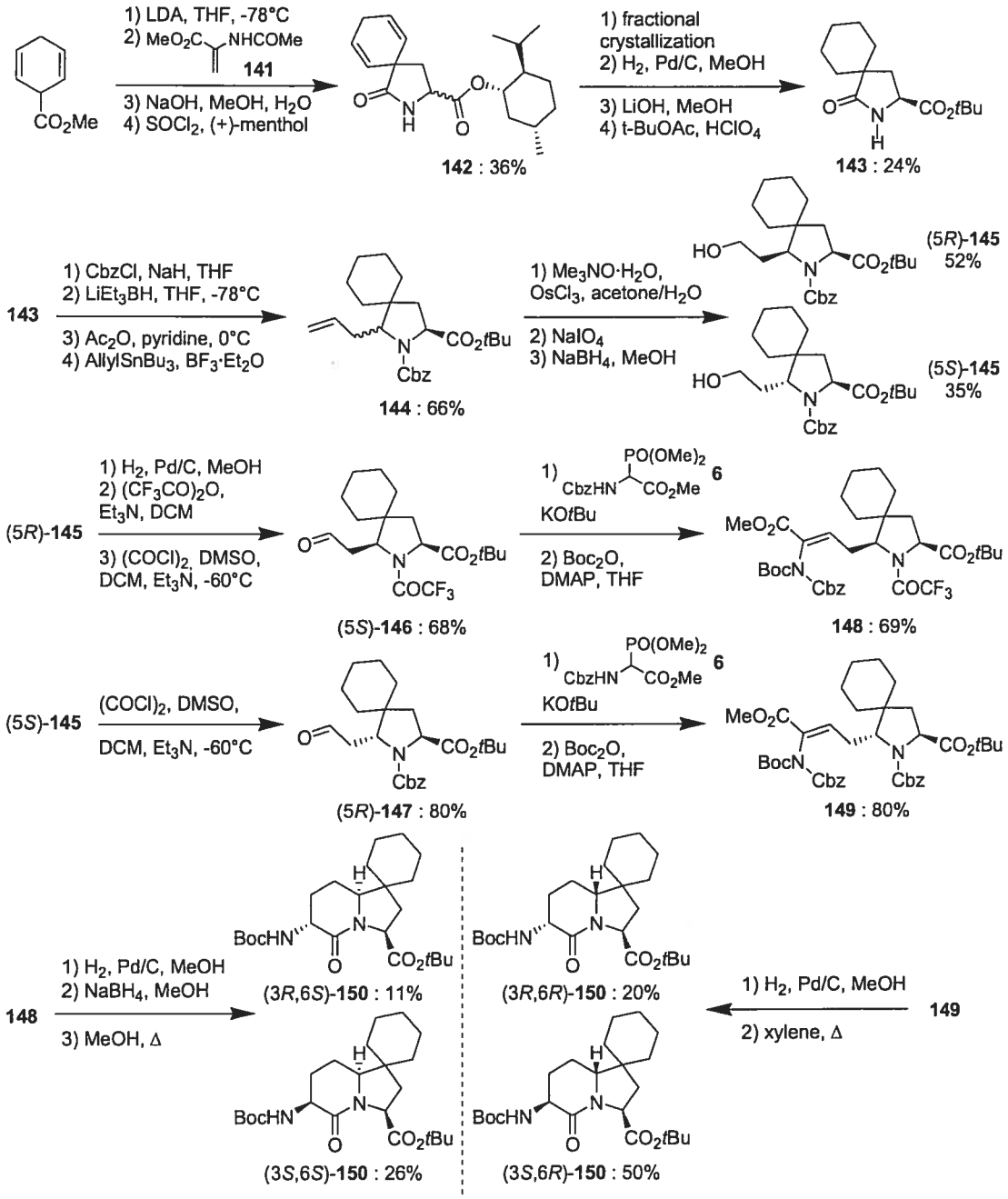
SCHEME 25 Synthesis of 7-silyloxyethyl indolizidin-2-one *N*-(Boc)amino esters **135**.[43,44]

7-Silyloxypropyl indolizidinone *N*-(Boc)amino ester **138** was synthesized by a route commencing with the stereoselective (d.r.=15:1) alkylation of ketone **109** with allyl iodide, which gave the (4*R*)-isomer **136** in 81% yield (SCHEME 26).[45] The corresponding silyloxypropyl ketone **137** was then prepared by a sequence involving hydroboration with disiamyl borane, sodium hydroxide and hydrogen peroxide, selective protection of the primary alcohol with TBDMSCl and oxidation of the secondary alcohol. Reductive amination, lactam cyclization and amine protection provided (6*S*,7*R*)-7-silyloxypropyl-indolizidin-2-one *N*-(Boc)amino ester (6*S*,7*R*)-**138** in 3% overall yield from ketone **109**. Deprotection of the silyl protecting group and displacement of the corresponding methanesulfonate with sodium azide provided orthogonally protected Ala-Lys dipeptide surrogate **139**. For use in peptide synthesis, 7-azidopropyl indolizidin-2-one *N*-(Boc)amino acid **140** was synthesized by methyl ester hydrolysis using LiOH in dioxane.



SCHEME 26 Synthesis of 7-(azidopropyl)indolizidin-2-one amino acid **140**.[45]

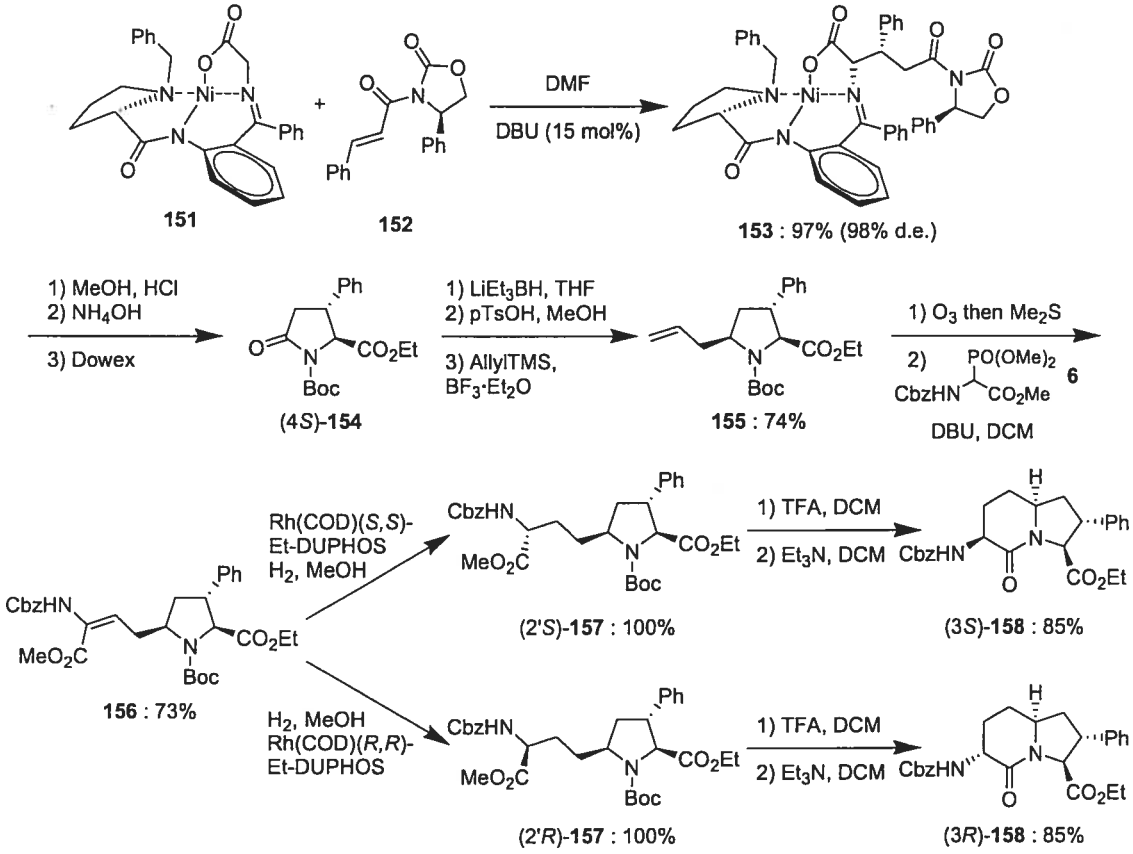
7-Spirocyclohexyl indolizidin-2-one *N*-(Boc)amino esters **150** have been synthesized from **143** by a route featuring an olefination / reduction / lactam cyclization sequence (SCHEME 27).[46] *tert*-Butyl γ-spirocyclohexyl pyroglutamate **143** was initially made in eight steps and 9% overall yield from methyl-1,4-dihydrobenzoate.[47] Employing a similar strategy as described for the synthesis of 4-hydroxyethyl proline **133** (Scheme 24), spirocycle **143** was converted into a separable mixture of diastereomeric γ,δ-disubstituted prolines **145** in 7 steps and 56% overall yield. Alcohol (5*R*)-**145** was converted to *N*-(trifluoroacetyl)proline aldehyde **146** by protecting group exchange and Swern oxidation. Horner-Wadsworth-Emmons olefination, olefin reduction and lactam cyclization gave a separable 1:2.5 mixture of 7-spirocyclohexyl indolizidin-2-one *N*-(Boc)amino esters (3*R*,6*S*)- and (3*S*,6*S*)-**150** in 11% and 26% respective overall yields from dehydroamino ester **148**. Alcohol (5*S*)-**145** was oxidized to *N*-(Cbz)proline aldehyde **147**, which was similarly converted to a separable 1:2.5 mixture of indolizidin-2-ones (3*R*,6*R*)-**150** and (3*S*,6*R*)-**150** in 20% and 50% respective overall yields from dehydro amino ester **149**.



SCHEME 27 Synthesis of 7-spirocyclohexyl indolizidin-2-one *N*-(Boc)amino esters **150**.[46,47]

8-Phenyl indolizidinone *N*-(Cbz)amino esters **158** were prepared from β-phenyl pyroglutamate **154**,[48] which was synthesized in 5 steps and 54% yield via the conjugate addition of the chiral glycine enolate of the Ni(II) complex of (*S*)-*o*-[*N*-(*N*-benzylprolyl)amino]benzophenone **151** onto (4*R*)-4-phenyl-*N*-(*E*-enoyl)oxazolidin-2-one **152** (SCHEME 28).[40] As in the synthesis of the parent

indolizidinone from 4-benzyl-5-allyl proline **116** (SCHEME 21), the 3-phenyl-5-allyl proline **155** was made from pyroglutamate **154** and converted to 8-phenyl indolizidin-2-one *N*-(Cbz)amino esters **158** by olefin ozonolysis, Horner-Wadsworth-Emmons olefination, stereoselective reduction and lactam cyclization.



SCHEME 28 Synthesis of 8-phenyl indolizidin-2-one *N*-(Cbz)amino esters **158**.[48]

2.3.5. Synthesis of multi-substituted indolizidin-2-one amino acids

Few examples of indolizidinone amino acid analogs possessing multiple substituents were previously reviewed, notably the synthesis of 5-benzyloxy-7-benzyl indolizidinone azido ester **160**.[1] Several new strategies have been developed over the past seven years (FIGURE 4).

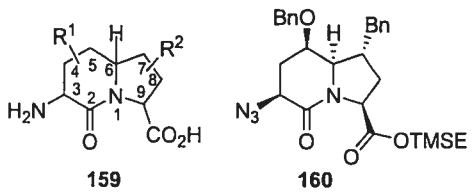
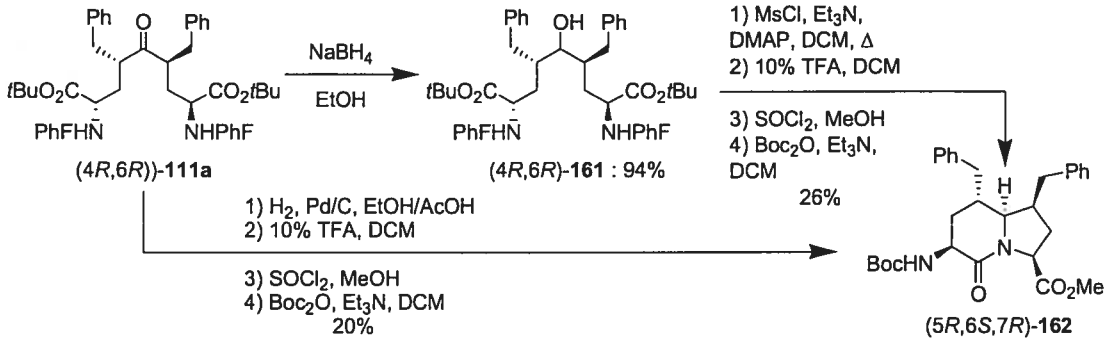


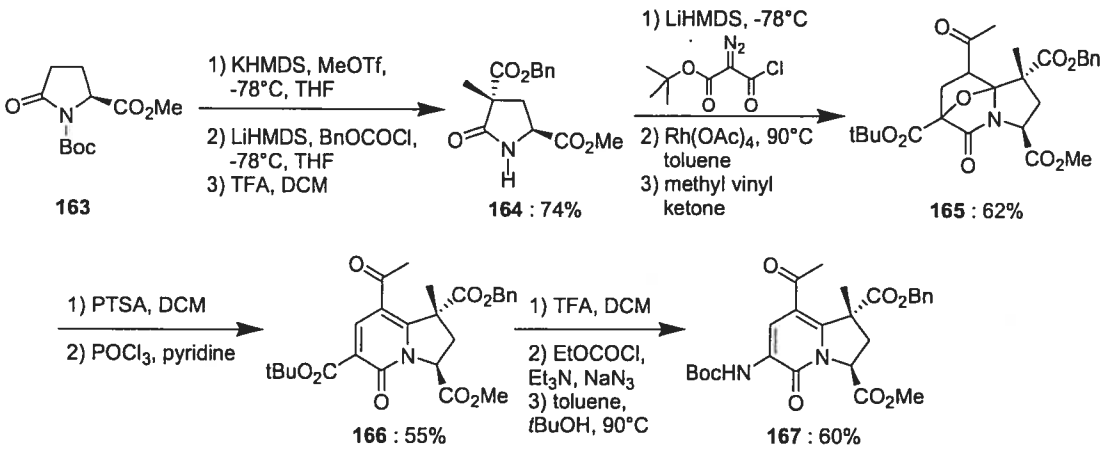
FIGURE 4 General structure for multi substituted indolizidinone amino acids and previously reviewed example **160**.

5,7-Dibenzyl indolidin-2-one *N*-(Boc)amino ester **162** has been synthesized from dialkylated ketone **111a** by a similar procedure as described previously for 5- and 7-benzyl indolizidinones **112** and **114** (SCHEMES 20 and 21).[39] Ketone **111a** was converted to a single diastereomer by either a reductive amination / lactam cyclization or a methanesulfonate displacement / lactam cyclization sequence in 20 and 25% respective overall yields from ketone **111a** (SCHEME 29).[39]



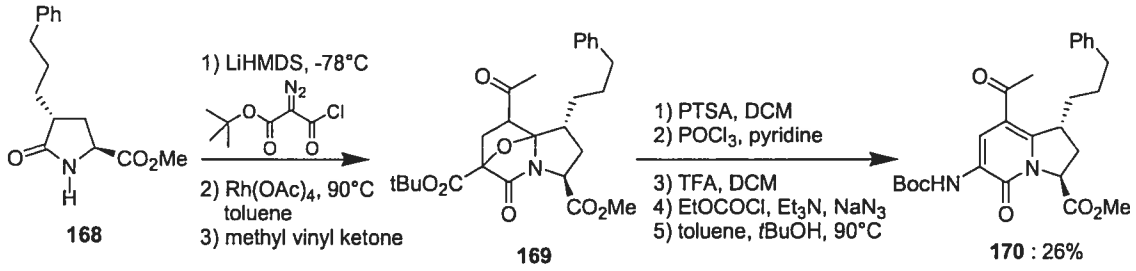
SCHEME 29 Synthesis of 5,7-benzyl indolizidin-2-one amino ester **162**.[39]

5-Acetyl-7-methyl-7-benzyloxycarbonyltetrahy-droindolizinone amino ester **167** has been synthesized using a similar [3+2]-cycloaddition step as used for the synthesis of tetrahydroindolizinone **102** (SCHEME 17), from pyroglutamate **163** (SCHEME 30).[37] γ,γ-Disubstituted pyroglutamate **164** was first synthesized by alkylation with methyl triflate, followed by acylation with benzyl chloroformate and Boc deprotection. Synthesis of 5,7,7-trisubstituted indolizinone amino ester **167** was achieved in 21% overall yield from **164** by a sequence featuring formation of a diazoimide and dipolar cycloaddition to produce tricycle **165**. Ring opening and dehydration gave triester **166**. The *tert*-butyl ester was selectively solvolyzed and subjected to a Curtius rearrangement to provide *N*-(Boc)amino diester **167**. 5-Acetyl-7-phenylpropyl



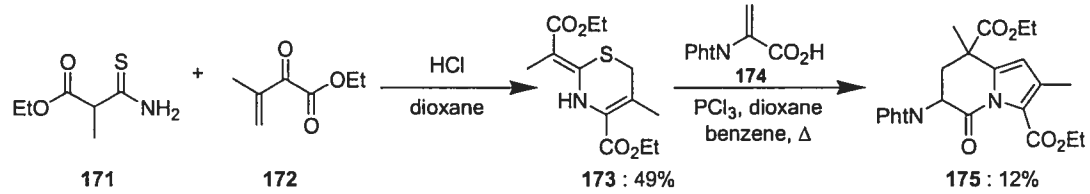
SCHEME 30 Synthesis of 5-acetyl-7-methyl-7-benzyloxycarbonyl-2,7,8,9-tetrahydroindolizin-2-one amino ester **167**.[37]

tetrahydroindolizinone amino ester **170** was similarly obtained in 26% overall yield from γ-phenylpropyl pyroglutamate **168** (SCHEME 31)[37] which was prepared by alkylation of pyroglutamate **156** with 3-phenyl-1-bromo-propane and Boc removal.



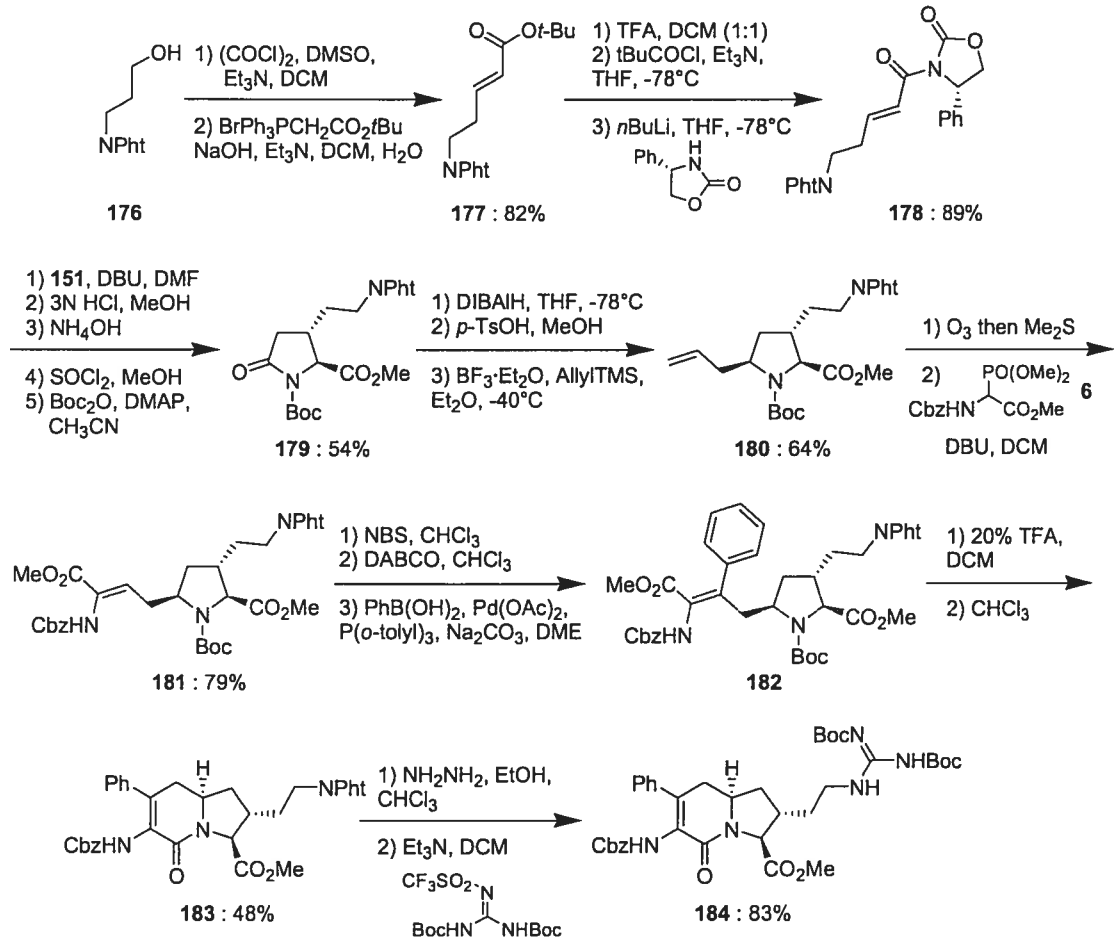
SCHEME 31 Synthesis of 5-acetyl-7-phenylpropyl-2,7,8,9-tetrahydroindolizin-2-one amino ester **170**.[37]

Racemic 5,8-dimethyl indolizinone *N*-phthalimido 5,9-dicarboxylate **175** was prepared in 12% by condensation of thiazine **173** with dehydroalanine **174** (SCHEME 32).[49] Thiazine **173** was first prepared by acidic condensation of thioamide **171** and vinyl keto ester **172** in 49% yield.[50]



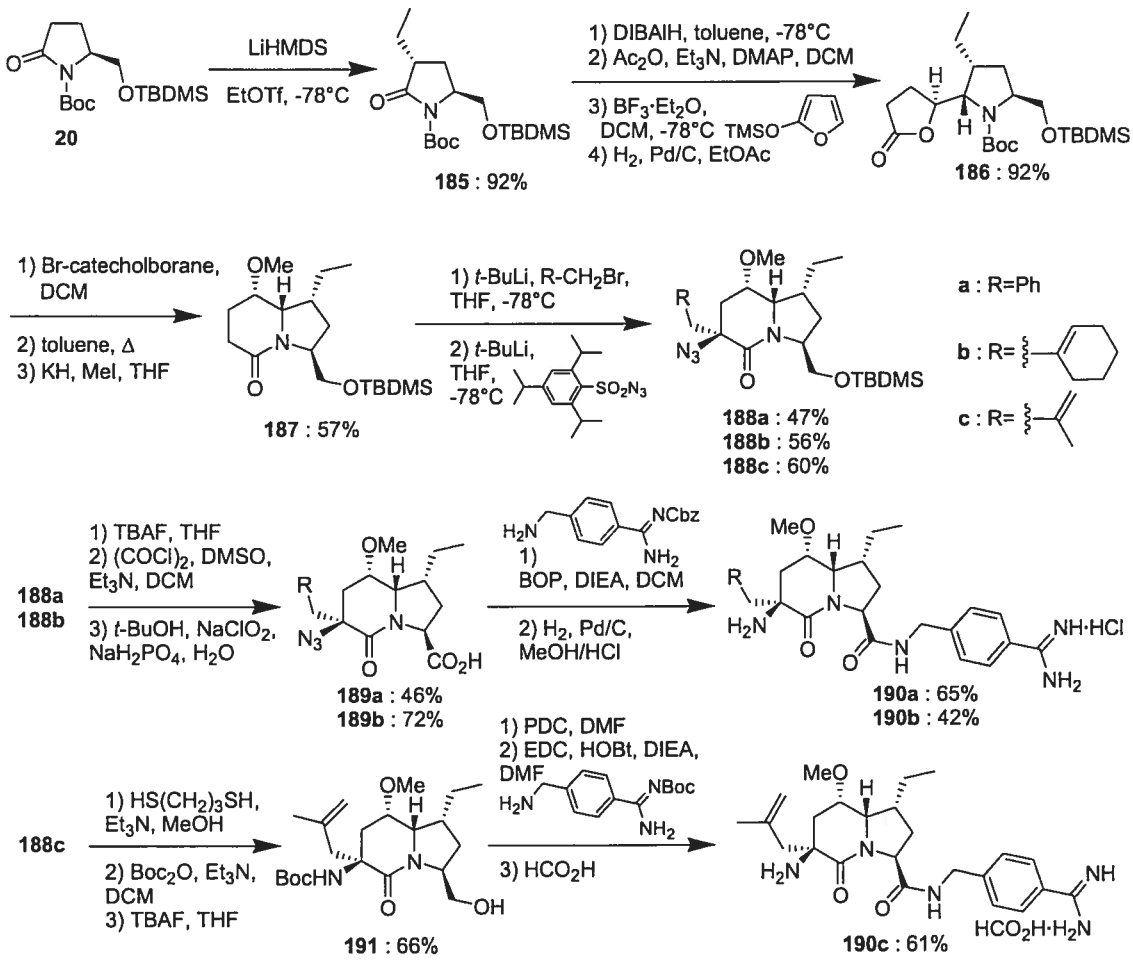
SCHEME 32 Synthesis of 5,8-dimethyl-2,3,4,5-tetrahydroindolizin-2-one phtalimido-5,9-dicarboxylate **175**.[49,50]

4-Phenyl-8-(*N,N'*-bis(Boc)-*N''*-ethylguanidine)-indolizidinone *N*-(Cbz)amino ester **184** was synthesized from substituted pyroglutamate **179**, which was in turn prepared by conjugate addition of chiral glycine equivalent **151** (SCHEME 28) and (4*S*)-*N*-(5-phthalimidopent-2-enoyl)-4-phenyl oxazolidinone **178** (SCHEME 33).[51] As in the synthesis of 4-phenyl indolizidinone **158** (SCHEME 28), the 3-phthalimidoethyl-5-allyl proline **180** was synthesized first from pyroglutamate **179** and then converted to phenyl dehydroamino ester **182** by olefin ozonolysis, H-W-E olefination, bromination and Suzuki cross-coupling. 4-Phenyl-8-(*N,N'*-bis(Boc)-*N''*-ethylgua-nidine)-3,4-dehydroindolizidinone *N*-(Cbz)amino ester **184** was then prepared in 40% overall yield from **182** by lactam cyclization, phthalimide deprotection and guanidinylation.



SCHEME 33 Synthesis of 4-phenyl-8-(*N,N'*-bis(Boc)-*N''*-ethylguanidine) indolizidin-2-one amino ester **184**.[51]

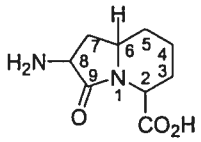
A series of 3,5,7-trisubstituted indolizidinone amino amides **190**[52] have been synthesized from pyroglutamate-derived silyl ether **20** (SCHEME 34).[53] Trisubstituted pyrrolidine **186** was synthesized from **20** by a route featuring 4-position alkylation with ethyl triflate and the addition of trimethylsilyloxyfuran at the 5-position via an iminium ion intermediate. Bicycle **187** was made from **186** by cleavage of the Boc group, migration of the lactone carbonyl to form the lactam and *O*-methylation. A series of 3-substituted bicycles **188a-c** were prepared as single diastereomers by sequential alkylations of the amide lithium enolate with various alkyl bromides and azidations with trisyl azide (2,4,6-tri-*iso*-propylbenzenesulfono azide). (3*S*,5*S*,6*S*,7*S*,8*S*)-3-Benzyl and (3*S*,5*S*,6*S*,7*S*,8*S*)-3-(cyclohex-1-enylmethyl)-5-methoxy-7-ethyl indolizidin-2-one azido acids **189a** and **189b** were obtained by silyl protecting group cleavage and oxidation to the acid in two steps. The acids were then converted



SCHEME 34 Synthesis of 3-alkyl-5-methoxy-7-ethyl indolizidin-2-one azido amides **190**.[52]

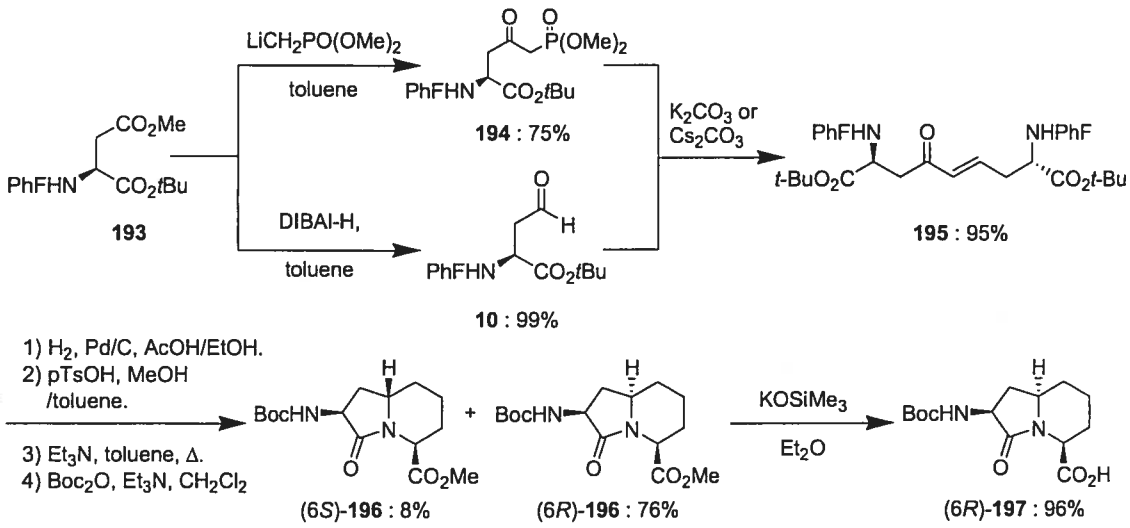
to amides **190a** and **190b** by coupling to *N*-(Cbz)-4-(aminomethyl)benzylamidine using BOP as reagent, followed by hydrogenation which, simultaneously, removed the Cbz group and reduced the azide function. 3-Methylpropenyl-5-methoxy-7-ethyl indolizidin-2-one *N*-(Boc)amino alcohol **191** was synthesized from azide **188c** by reducing the azide function with propanedithiol and triethylamine, protection of the free amine and deprotection of the silyl ether. Amide (3*S*,5*S*,6*S*,7*S*,8*S*)-**190c** was then made by oxidizing the alcohol to an acid, which was coupled to *N*-(Boc)-4-(aminomethyl)benzylamidine with EDC, and HOBt, followed by Boc group deprotection with formic acid.

2.3.6. Synthesis of indolizidin-9-one amino acids (I^9aa).



192

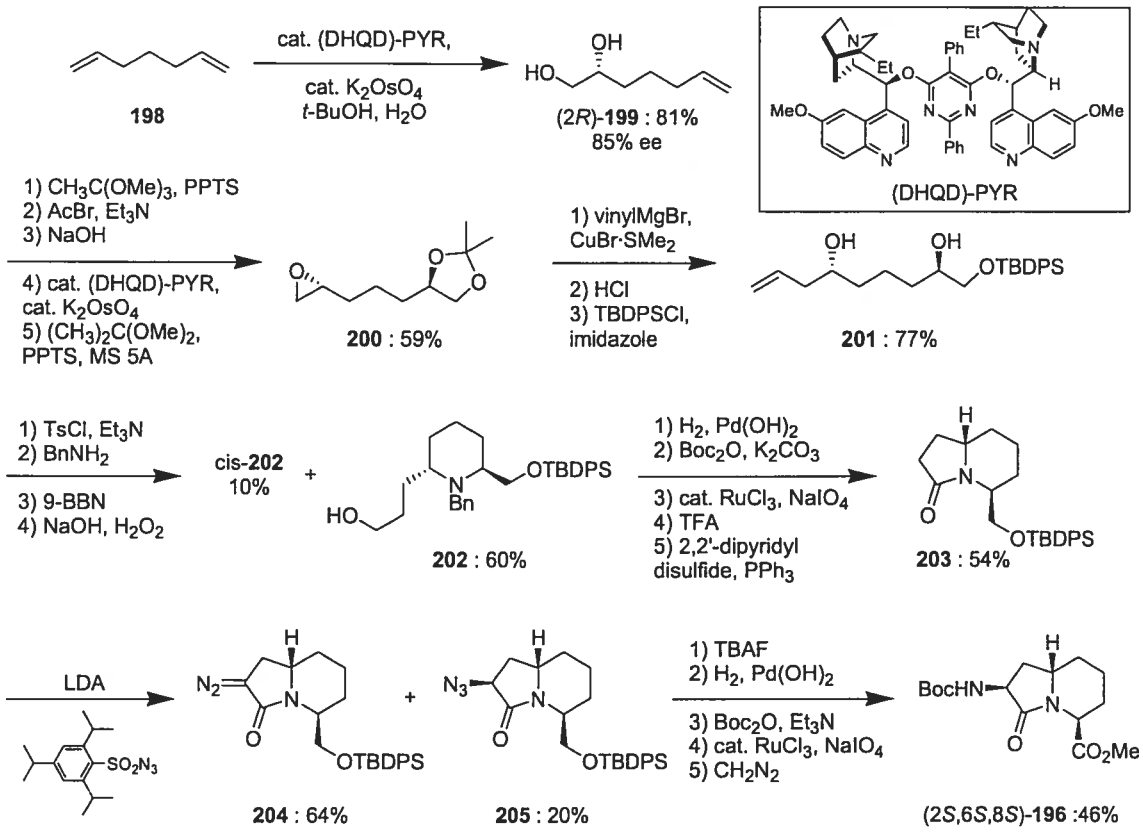
Indolidin-9-one amino acids (I^9aa), in which the peptide backbone is constrained within a 5,6-bicyclic structure, have received less attention than their 6,5-bicyclic counterpart I^2aa. The first synthesis of the unsubstituted I^9aa was made from β-methyl α-*tert*-butyl-*N*-(PhF)aspartate **193**, which was independently reduced to β-aldehyde **10** and reacted with lithium methyl dimethylphosphonate to form β-ketophosphonate **194** (SCHEME 35).[12] The Horner-Wadsworth-Emmons olefination between **10** and **194** gave linear enone **195**. *N*-(Boc)Amino indolizidin-9-one ester (I^9aa) **196** was obtained as a 9:1 mixture of (2*S*,6*R*,8*S*)- and (2*S*,6*S*,8*S*)-I^9aa **196** in 54% and 6% yields from aspartate **193** from enone **195** using a similar reductive amination / ester exchange / lactam cyclization / amine protection sequence as that employed in the preparation of its I^2aa counterpart (SCHEME 5). For use in peptide synthesis, methyl ester **196** was hydrolysed using $KOSi(CH_3)_3$ in ether to furnish *N*-(Boc)amino indolizidin-9-one acid **197** in 96% yield.



SCHEME 35 Synthesis of indolizidin-9-one *N*-(Boc)amino acid **197**.[12]

(2*S*,6*S*,8*S*)-Indolidin-9-one amino ester **196** has also been synthesized from 1,6-heptandiene **198** by a route featuring asymmetric dihydroxylations with K_2OsO_4 and (DHQD)-PYR as chiral ligand (SCHEME 36).[54] Diol (2*R*)-**199** was first prepared from diene **198** in 81% yield and 85% ee, and then converted to an epoxide using the Sharpless-Kolb protocol[55] prior to the second asymmetric dihydroxylation which afforded, after treatment with 2,2-dimethoxypropane, acetal **200** as an inseparable mixture of diastereomers with (2*R*,6*R*)-**200** as the major component. Diastereomerically pure (2*S*,6*S*)-piperidine **202** was obtained from **200** by a sequence featuring regioselective ring opening of the epoxide with vinylmagnesium bromide, deprotection of the acetal, selective protection of the primary alcohol, activation of the two secondary alcohols at the same time as toluenesulfonates which were displaced with benzyl amine and hydroboration of the olefin provided a separable 1:6 mixture of piperidines (2*S*,6*R*)- and (2*S*,6*S*)-**202**. Bicycle **203** was constructed by a route featuring protecting group exchange, oxidation of the primary alcohol to an acid, cleavage of the Boc group and lactam cyclization. Azidation of the lithium enolate of lactam **203** with trisyl azide furnished the desired azido indolizidinone **205** as the minor product in 20% yield, accompanied by the undesired diazo lactam **204** as major product. (2*S*,6*S*,8*S*)-*N*-(Boc)Amino indolizidin-9-one ester **196** was then obtained in 46% yield from azide **205** by silyl ether deprotection, azide reduction with hydrogen and $Pd(OH)_2$, amine

protection, alcohol oxidation to the acid and formation of the methyl ester with diazomethane.



SCHEME 36 Synthesis of *N*-(Boc)indolizidin-9-one amino ester (6*S*)-**196**.[54]

2.3.7. Synthesis of substituted indolizidin-9-one amino acids

The previously reviewed example of a substituted indolizidin-9-one amino acid, IBTM **207** was prepared by a route featuring the Pictet-Spengler condensation of L-Trp and L-Asp β-aldehyde (FIGURE 5).[1]

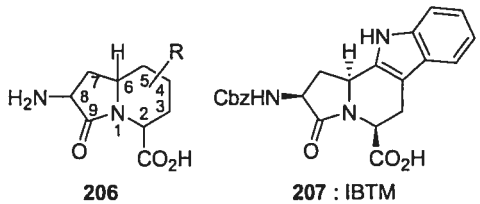
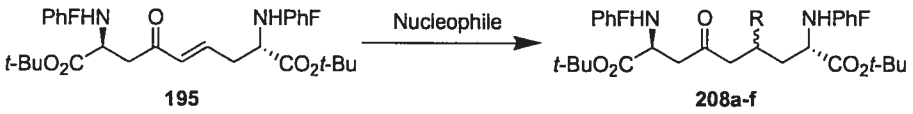


FIGURE 5 General structure for substituted indolizidin-9-one amino acids and IBTM

TABLE 1 Conjugate additions on enone **195**.



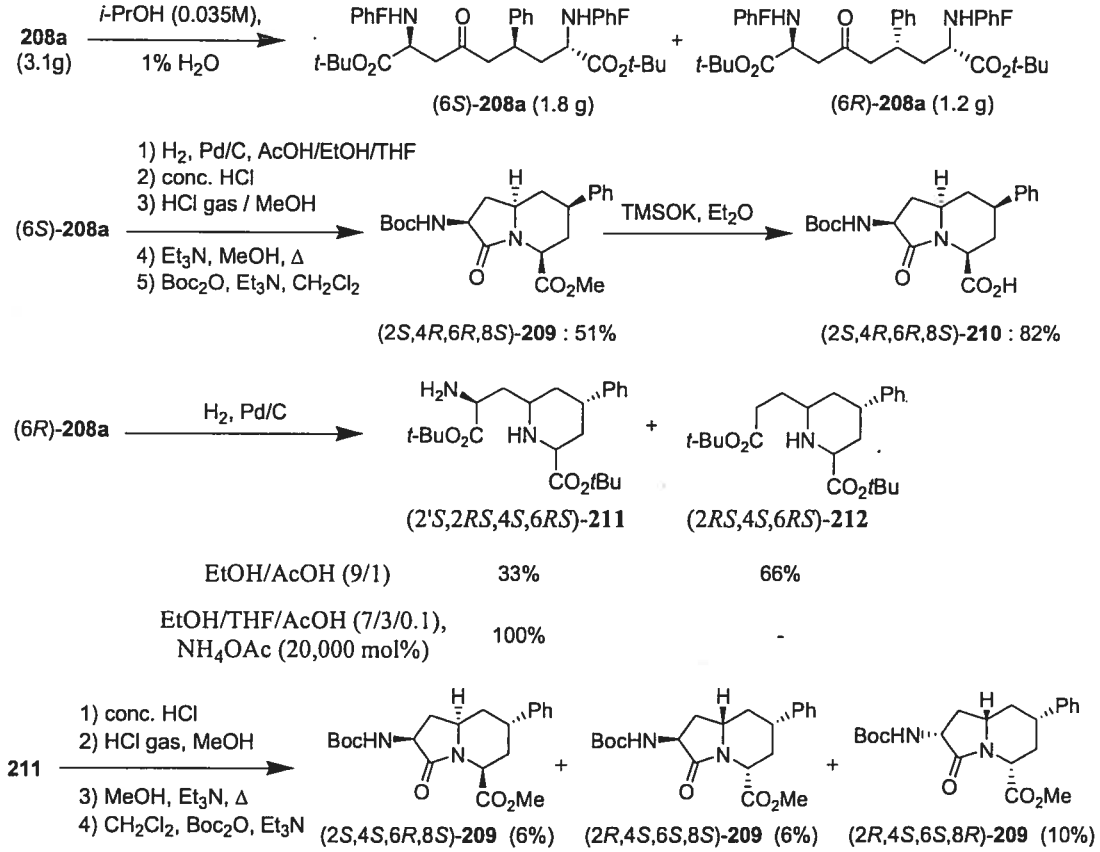
195 **208a-f**

Entry	Nucleophile	R	Yield	Product	d.r.
1	$Ph_2CuCN(MgBr)_2$	Ph	98	**208a**	1 : 2
2	$(p\text{-MeO-Ph})_2CuCN(MgBr)_2$	*p*-MeO-Ph	98	**208b**	1 : 2
3	$i\text{-Pr}_2CuCN(MgBr)_2$	*i*-Pr	80	**208c**	5 : 1
4	$PhMgBr / MgBr_2$	Ph	100[a]	**208a**	15 : 1
5	$i\text{-PrMgBr} / MgBr_2$	*i*-Pr	80[a]	**208c**	6.5 : 1
6	$MeNO_2$ / DBU	CH_2NO_2	94	**208d**	2 : 1
7	KCN / 18-crown-6	CN	68	**208e**	1 : 1
8	$MeO_2CCH_2CO_2Me$ / NaH	$CH(CO_2Me)_2$	77	**208f**	1 : 1

a: % conversion.

(2*S*,4*R*,6*R*,8*S*)-4-Phenyl indolizidin-9-one amino acid **210** has been synthesized by a route featuring a conjugate addition reaction onto enone **195** (SCHEME 37).[56] A variety of nucleophiles were successfully added to enone **195** to give ketones **208a-f** in good yields albeit with generally low diastereoselctivity (TABLE 1). Phenyl Grignard reagent in the presence of MgBr2 reacted with **195** in a notably diastereoselective 1,4-addition (15:1) to produce (6*R*)-**208a** as the major product. Alternatively, Ph2CuCN(MgBr)2 gave a 1:2 d.r. with (6*S*)-**208a** as the major product. Diastereoselective precipitation of a 1:2 mixture of ketone **208a** (3.1 grams) from *iso*-propanol / water gave 1.8 g of a product containing ketone (6*S*)-**208a** in 13/1 d.r. as well as a mother liquor containing 1.2 g of (6*R*)-**208a** in a 13/1 d.r.

(2*S*,4*R*,6*R*,8*S*)-*N*-(Boc)Amino 4-phenyl-indolizidin-9-one ester **209** was produced diastereselectively in 51% yield from the (6*S*)-isomer by a reductive amination / lactam cyclization sequence (SCHEME 37). Saponification of methyl ester **209** using potassium trimethylsilanolate gave 4-phenyl-indolizidin-9-one acid **210** for application in peptide synthesis. Reductive amination on ketone (6*R*)-**208a** under standard hydrogenation conditions (H_2, Pd/C, EtOH, AcOH) gave a separable 1:2 mixture of diamino pipecolate **211** and its desamino counterpart **212** which likely arose from an imine-enamine tautomerization followed by β-elimination of the amine.[56] Elimination product

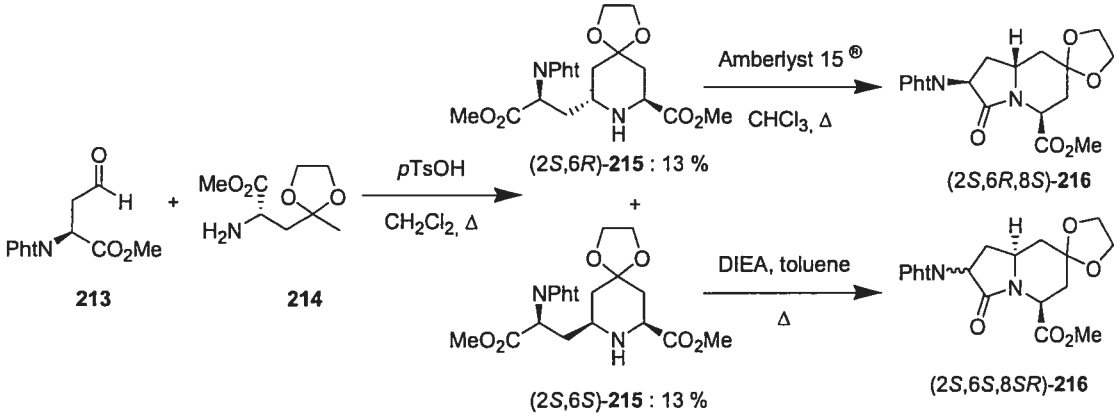


SCHEME 37 Synthesis of 4-phenyl-indolizidin-9-one *N*-(Boc)amino esters **209**.[56]

212 was minimized and practical conditions for obtaining the diamino pipecolate **211** were found by performing the hydrogenation in the presence of ammonium acetate in a mixture of ethanol / THF / acetic acid (7/3/0.1). Using these hydrogenation conditions in the reductive amination / lactam cyclization sequence, ketone (6*R*)-**208a** was converted to a separable 1.7:1:1 mixture of three diastereomers of *N*-(Boc)amino 4-phenyl indolizidin-9-one ester **209** (SCHEME 37). Expected (2*S*,4*S*,6*R*,8*S*)-**209** was isolated in 6% yield, accompanied by (2*R*,4*S*,6*S*,8*S*)-**209** and (2*R*,4*S*,6*S*,8*R*)-**209** in 6% and 10% respective yields due to epimerisation at the C2 and C8 centres during the reductive amination.

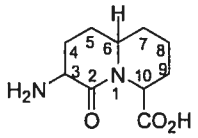
4-Spirodioxacyclopentyl indolizidin-9-one amino esters **216** have been synthesized by a route featuring an intramolecular Mannich reaction. *N*-(Phthalimido)aspartate β-aldehyde **213** was reacted with amino ketal **214** to furnish separable pipecolates (2*S*,6*R*)- and (2*S*,6*S*)-**215** in 13 % yield for each

diastereomer (SCHEME 38).[57] (2*S*,6*R*,8*S*)-Indolizidin-9-one amino ester **216** was quantitatively prepared from (6*R*)-**215** by lactam cyclization under acidic conditions. During the lactam cyclization of (6*S*)-pipecolate (6*S*)-**215** under basic conditions, epimerization occurred at the C8 centre to furnish a mixture of (2*S*,6*S*,8*S*)- and (2*S*,6*S*,8*R*)-4-spirodioxacyclopentyl indolizidin-9-one amino esters **216**.



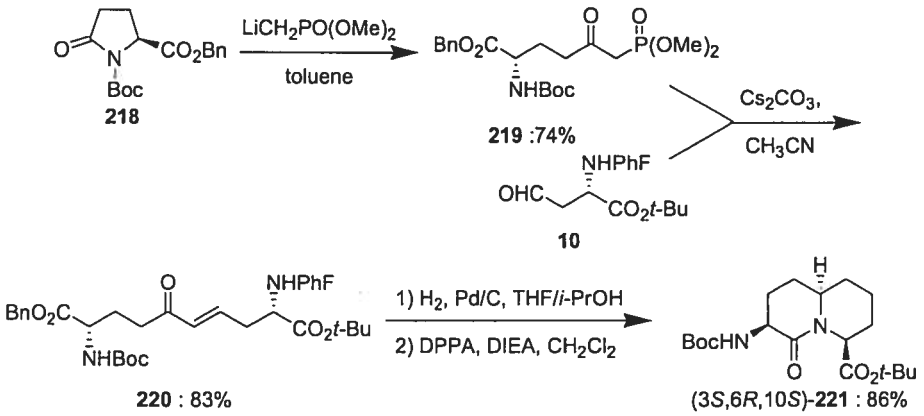
SCHEME 38 Synthesis of 4-spirodioxacyclopentyl-indolizidin-9-one *N*-(Pht)amino esters **216**.[57]

2.3.8. Synthesis of quinolizidin-2-one amino acids (Qaa)



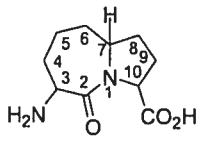
217

Quinolizidin-2-one amino acids (Qaa) constrain the peptide backbone within a fused 6,6-bicyclic structure. Quinolizidinone amino ester **221** was made from orthogonally protected linear enone **220**, which was prepared from the Horner-Wadsworth-Emmons olefination of α-*tert*-butyl-*N*-(PhF)aspartate-β-aldehyde **10** with pyroglutamate-derived β-ketophosphonate **219** (SCHEME 39).[58] Reductive amination and lactam cyclization gave selectively the (3*S*,6*R*,10*S*)-isomer of quinolizidinone amino ester **221** in 60% overall yield from pyroglutamate **218**.

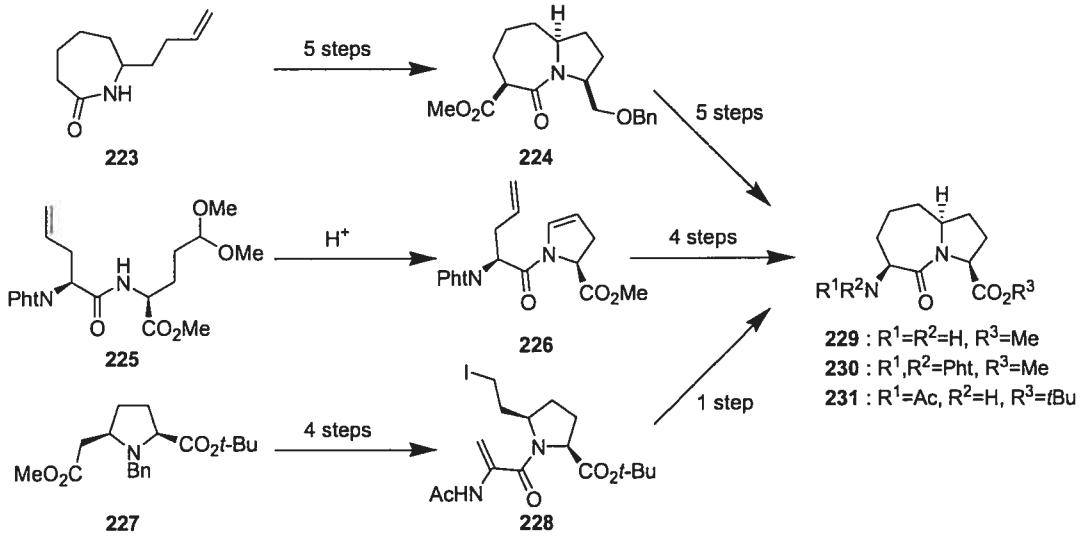


SCHEME 39 Synthesis of quinolizidin-2-one *N*-(Boc)amino ester **221**.[58]

2.3.9. Synthesis of pyrroloazepin-2-one amino acids

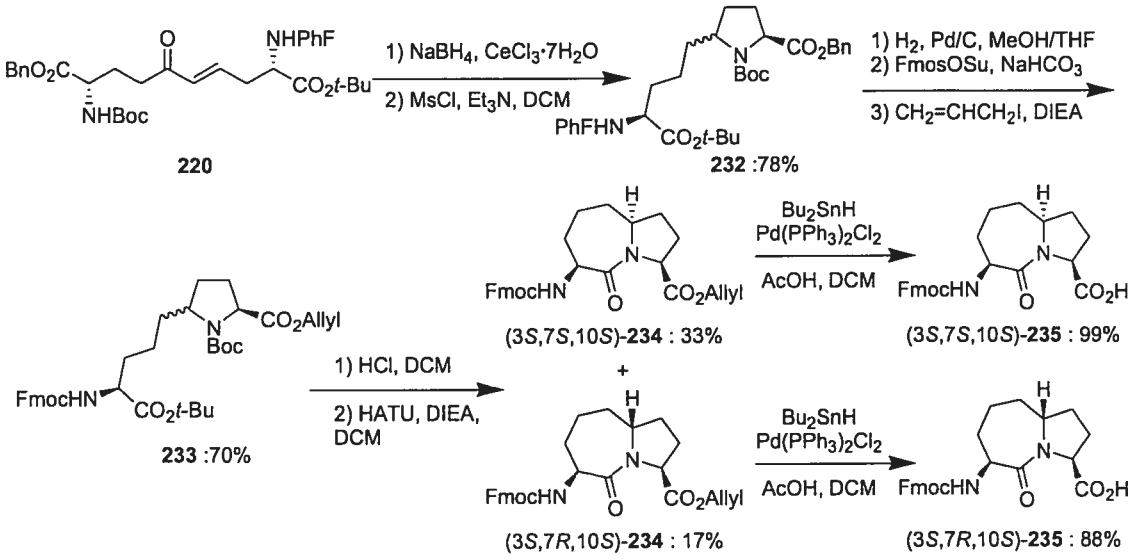


Pyrroloazepin-2-one amino acids constrain the peptide backbone within a fused 7,5-bicyclic structure. The three pyrroloazepinone amino esters **229-231** have been previously reported and reviewed (SCHEME 40),[1] including the synthesis of racemic ester **229** by annulation of the 5-membered ring onto 7-membered lactam **223**, intramolecular *N*-acyliminium ion alkylation using enamine **226**, and radical-mediated cyclization using bromide **228**.



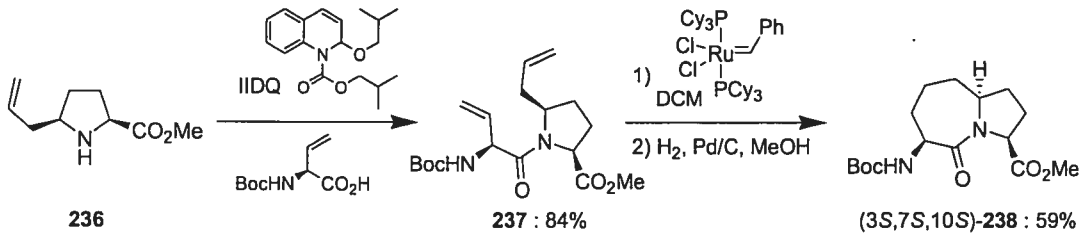
SCHEME 40 Early syntheses of pyrroloazepin-2-one amino esters **229**, **230** and **231**.

Pyrroloazepin-2-one *N*-(Fmoc)amino acids **235** were synthesized from the same linear enone intermediate **220**, as previously described for the synthesis of *N*-(Boc)amino quinolizidin-2-one ester **221** (SCHEME 41).[58] 5-Substituted proline **232** was prepared by Luche reduction of ketone **220**, using sodium borohydride and cerium trichloride, methanesulfonation and selective displacement by the *N*-(Boc)amine; the *N*-(PhF)amine was prevented from cyclization by the *E*-olefin geometry. After a protecting group shuffle, the Boc and *tert*-butyl groups were removed. Lactam formation was best accomplished using HATU (*N*-[dimethylamino)-1*H*-1,2,3,-triazolo[4,5-β]pyridin-1-ylmethylene]-*N*-methyl methanaminium hexafluorophosphate *N*-oxide) to give a separable 2:1 mixture of (3*S*,7*S*,10*S*)- and (3*S*,7*R*,10*S*)-*N*-(Fmoc)amino pyrroloazepin-2-one allyl esters **234** in 11% and 6% respective overall yields from pyroglutamate **218**. Allyl esters **234** were hydrolyzed using tributyltin hydride and $Pd(PPh_3)_2Cl_2$ to give (7*S*)- and (7*R*)-*N*-(Fmoc)amino acids **235** in 99% and 88% respective yields.



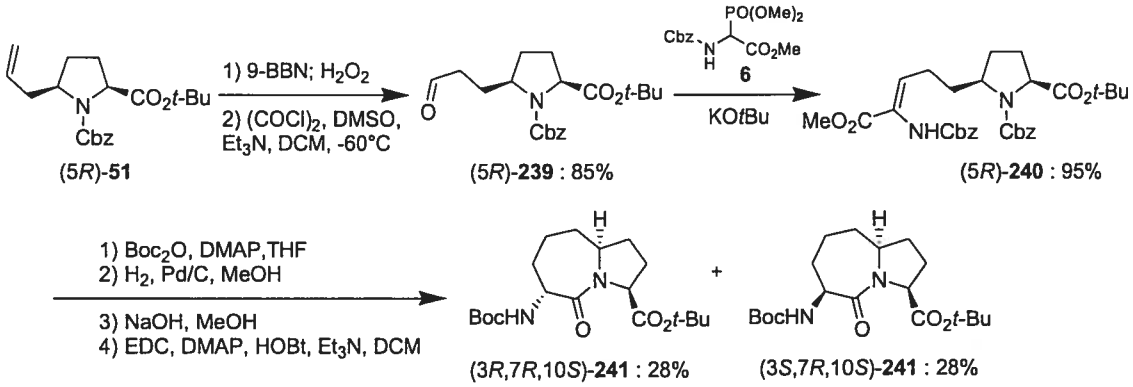
SCHEME 41 Synthesis of pyrroloazepin-2-one *N*-(Fmoc)amino acids **235**.[58]

Pyrroloazepin-2-one amino acid **238** was synthesized in 50% overall yield from 5-substituted proline **236** by a route featuring ring closing metathesis and hydrogenation (SCHEME 42), similar to the route described for the synthesis of indolizidin-2-one amino ester **38** (SCHEME 9).[26] The 7-membered ring was synthesized by acylation of *cis*-5-allylproline (5*R*)-**236** with *N*-



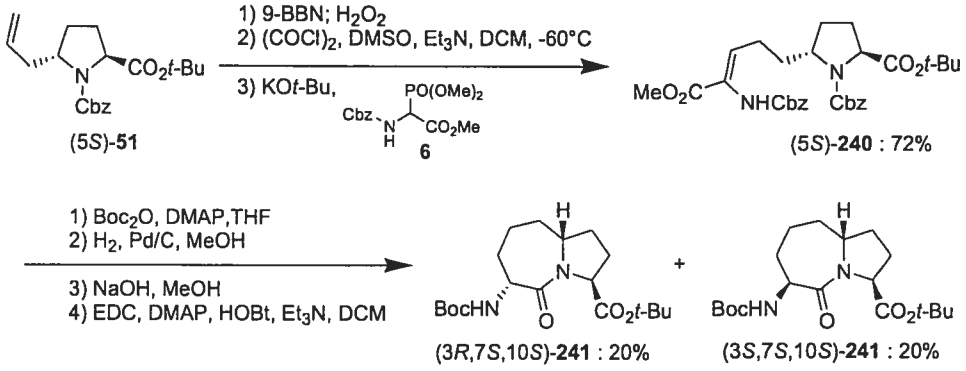
SCHEME 42 Synthesis of pyrroloazepin-2-one *N*-(Boc)amino ester **238**.[26]

(Boc)vinylglycine using IIDQ in THF, ring closing metathesis of the resulting diene **237** and hydrogenation.



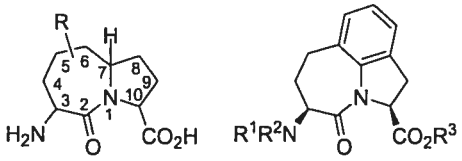
SCHEME 43 Synthesis of pyrroloazepin-2-one *N*-(Boc)amino esters (7*R*)-**241**.[9]

Pyrroloazepinone *N*-(Boc)amino esters **241** have also been synthesized by a route featuring a Horner-Wadsworth-Emmons reaction between glycine phosphonate **6** and proline aldehyde derivative **239** (SCHEME 43),[9] similar to approaches described for making pyrrolizidinone and indolizidin-2-one amino esters (SCHEMES 1 and 10). Hydroboration of *cis*-allyl proline (5*R*)-**51** and oxidation of the resulting alcohol gave aldehyde **239**. Olefination, hydrogenation, methyl ester hydrolysis and lactam cyclization furnished a separable 1:1 mixture of (3*S*,7*R*)- and (3*R*,7*R*)-pyrroloazepin-2-one amino esters **241** in 23% and 23% respective overall yields from (5*R*)-**51**. When the *trans*-allyl proline, (5*S*)-**51** was used in a similar sequence, a separable 1:1 mixture of (3*S*,7*S*)- and (3*R*,7*S*)-pyrroloazepin-2-one amino esters **241** was obtained in 28% overall yield from (5*S*)-**51** (SCHEME 44).[9]



SCHEME 44 Synthesis of pyrroloazepin-2-one *N*-(Boc)amino esters (7*S*)-**241**.[9]

2.3.10. Synthesis of substituted pyrroloazepin-2-one amino acids



242

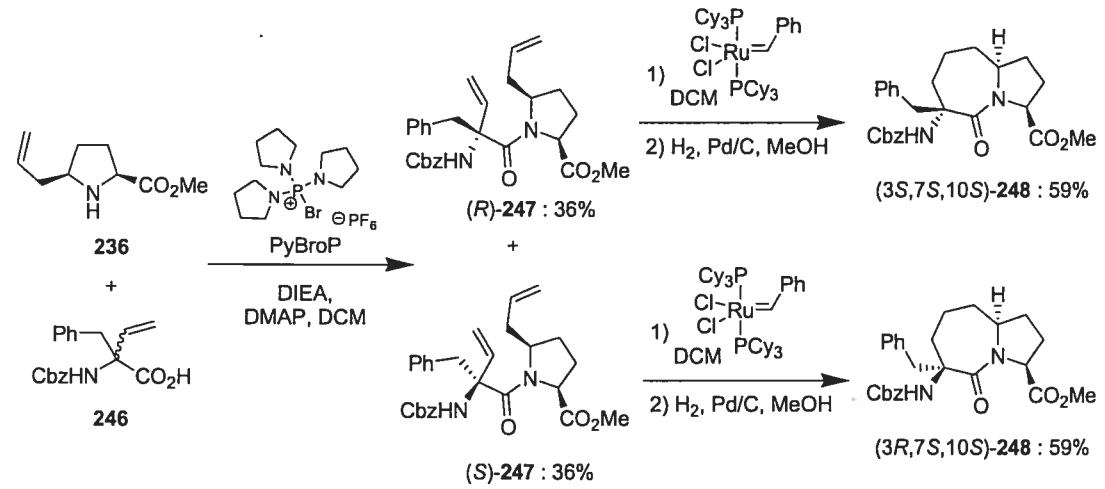
Haic

243 : R^1=R^2=Pht, R^3=Et
244 : R^1=Ac, R^2=H, R^3=Me
245 : R^1=$COCF_3$, R^2=H, R^3=Me

FIGURE 6 General structure for substituted pyrroazepinone amino acids and previously reviewed examples.

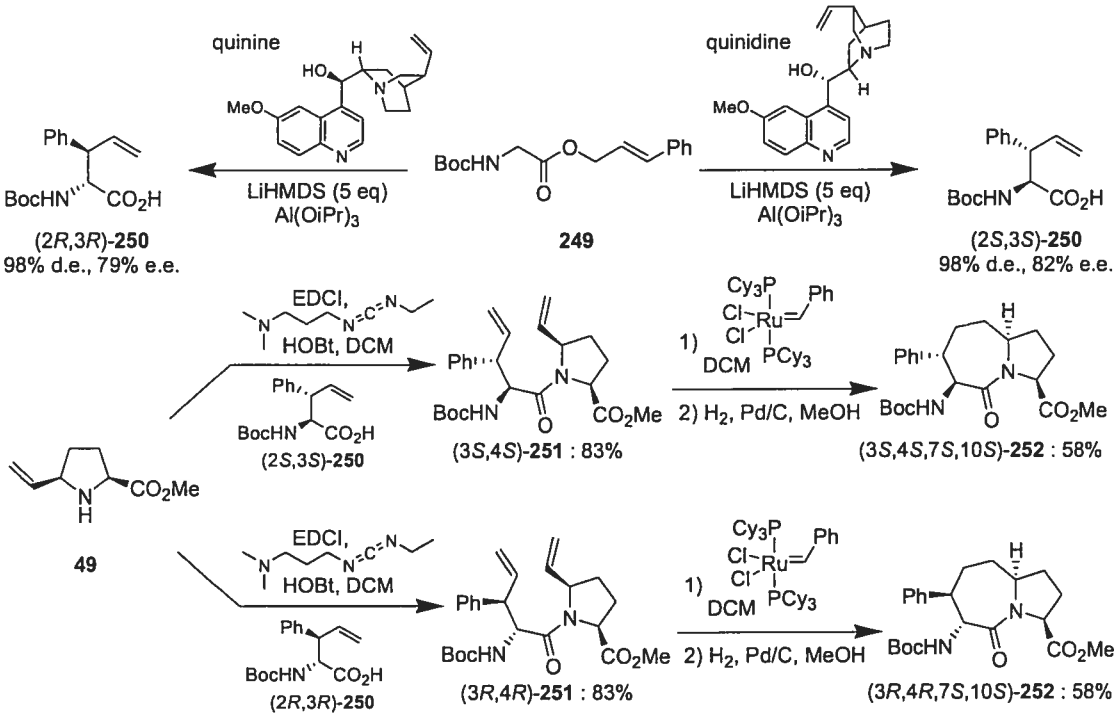
An example of a substituted pyrroloazepin-2-one amino acid previously reviewed, Haic, (5-amino-1,2,4,5,6,7-tetrahydro-azepino[3,2,1-hi]indole-4-one-2-carboxylic acid, **243 - 245**) has been prepared by routes featuring *N*-acylation and intramolecular Friedel-Crafts acylation of indoline-2-carboxylate with L-aspartic acid (FIGURE 6).[1]

3-Benzyl pyrroloazepin-2-one amino esters have been made from *cis*-5-allyl proline **236** by a similar olefin metathesis route as described for the preparation of the parent heterocycle **238** (SCHEME 42).[26] Racemic *N*-Cbz-α-vinyl-phenylalanine **246** was coupled to **236** to give a separable mixture of diastereomeric dienes **247**, that were each independently submitted to ring closing metathesis and reduction to provide either (3*S*,7*S*,10*S*)- or (3*R*,7*S*,10*S*)-3-benzyl pyrroloazepinone amino esters **248** (SCHEME 45).[59]



SCHEME 45 Synthesis of 3-benzyl pyrroloazepin-2-one *N*-(Cbz)amino esters **248**.[59]

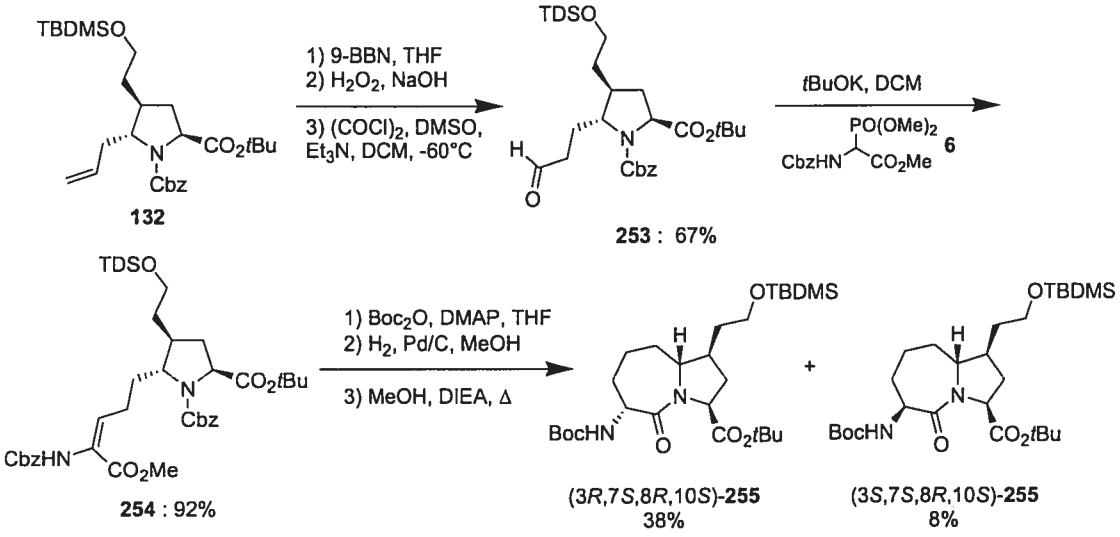
4-Phenyl pyrroloazepin-2-one amino esters (3*S*,4*S*,7*S*,10*S*)- and (3*R*,4*R*,7*S*,10*S*)-**252** have been prepared by a similar ring-closing metathesis process from 5-vinyl proline **49** (SCHEME 46).[26] Diastereomeric dienes **251** were synthesized by acylation of 5-vinyl proline **49** with either (2*S*,3*S*)- or (2*R*,3*R*)-3-(phenyl)allylglycine **250**, which were prepared by stereoselective Cope rearrangements of *N*-(Boc)glycine cinnamyl ester **249** using respectively quinidine or quinine as chiral ligand.[60] Ring closing metathesis and double



SCHEME 46 Synthesis of 4-phenyl pyrroloazepin-2-one *N*-(Boc)amino esters **252**.[26,60]

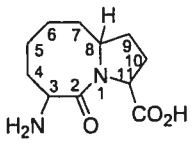
bond reduction furnished the 4-phenyl pyrroloazepin-2-one amino esters (3*S*,4*S*,7*S*,10*S*)- and (3*R*,4*R*,7*S*,10*S*)-**252** in 48% and 43% respective overall yields from **250**.

8-Substituted pyrrolazepinone amino esters **255** were synthesized from 4,5-dialkyl proline **132** by a route featuring a Horner-Wadsworth-Emmons olefination / reduction / lactam cyclization strategy (SCHEME 47).[43] In a similar manner as 5-allylproline **51** was converted to the parent heterocycle (SCHEME 44), (3*R*)- and (3*S*)-8-(*tert*-butyldimethylsilyloxyethyl) pyrroloazepin-2-one amino esters **255** were synthesized as a separable 5:1 mixture in 23% and 5% overall yields from **132**.



SCHEME 47 Synthesis of 8-(*tert*-butyldimethylsilyloxyethyl)pyrroloazepin-2-one *N*-(Boc)amino esters **255**.[43]

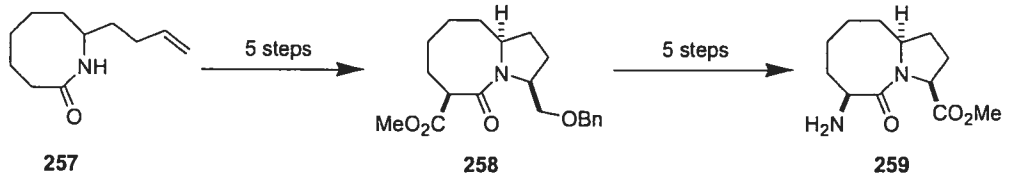
2.3.11. Synthesis of pyrroloazocin-2-one amino acids



256

The pyrroloazocin-2-one amino acids constrain the peptide backbone within a fused 8,5-bicyclic structure. One preparation of racemic pyrroloazocinone

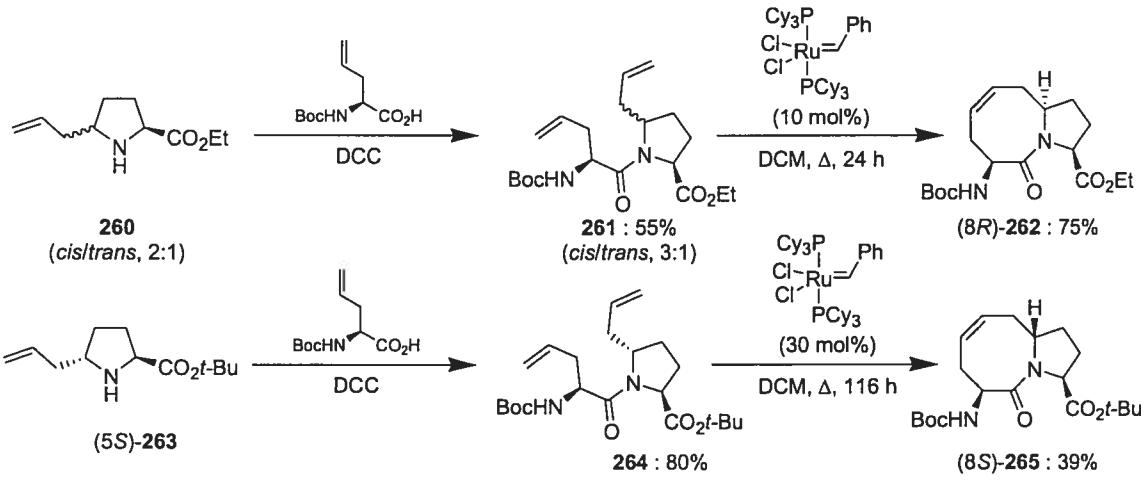
amino esters **259** was previously reviewed (SCHEME 48),[1] which featured a route involving annulation of the 5-membered ring onto 8-membered lactam **257**.



257 **258** **259**

SCHEME 48 Racemic synthesis of pyrroloazocin-2-one amino ester **259**.

4,5-Dehydro pyrroloazocin-2-one amino esters **262** and **265** were synthesized from 5-allyl prolines **260** and **263** by routes featuring proline acylation with *N*-(Boc)allylglycine and ring-closing metathesis, which provided (8*R*)-**262** in 41% overall yield from a 2:1 (*cis* / *trans*)-mixture of 5-allyl prolines **260**, and (8*S*)-**265** in 31% overall yield from *trans*-5-allyl proline (5*S*)-**263** (SCHEME 49).[61]



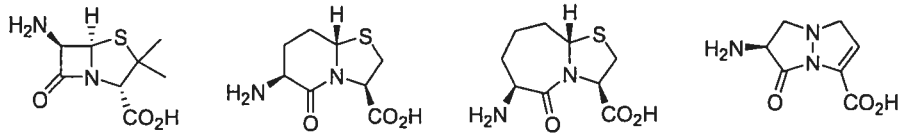
260 (*cis*/*trans*, 2:1) **261** : 55% (*cis*/*trans*, 3:1) (8*R*)-**262** : 75%

(5*S*)-**263** **264** : 80% (8*S*)-**265** : 39%

SCHEME 49 Synthesis of 4,5-dehydro pyrroloazocin-2-one amino esters **262** and **265**.[61]

2.4. Synthesis of thiaza-, oxaza- and diaza-bicycloalcanone amino acids.

Azabicyclo[X.Y.0]alkane amino acids with additional hetero atoms inside the heterocycle frame-work were the first examples of this general class of dipeptide surrogate to be synthesized for a number of reasons. Penicillanic acid



266 **267** : BTD **268** **269**

FIGURE 7 Early examples of azabicycloalkane amino acids with additional heteroatoms.

266, a fused 4,5-bicyclic system, has been an important target because of its existence in structures having antibiotic properties.[62,63] The facile formation of thiazolidine carboxylates from cysteine and an α-amino acid bearing an ω-aldehyde gave access to thiaindolizidinone and thiapyrroazepinone amino acids, such as **267** (BTD) and **268**, which were later used respectively as β-turn mimics and components of metalloprotease inhibitors (FIGURE 7).[64,65] Moreover, the positioning of a nitrogen instead of a carbon at the ring fusion in fused 5,5-systems gave rise to homologs of β-lactams, such as **269**, that were relatively easy to synthesize and exhibited effective antibacterial activity.[66] As time passed, efforts turned to prepare azabicyclo[X.Y.0]alkanone amino acids which possessed a single nitrogen in an all carbon frame. In principle, such analogs may offer advantages such as (1) greater conformational rigidity due to the additional gauche interactions created by replacement of a heteroatom with a methylene carbon, (2) additional sites for introducing side-chain functionality and (3) greater chemical stability from the removal of a potentially oxidizable sulphur and an acid labile masked aldehyde moiety. In practice, however, such advantages have yet to be demonstrated in head-to-head comparisons. Heteroatom-bearing bicyclic amino acids, such as the thiaindolizidinone amino acid BTD (**267**), are sold commercially and have been introduced into peptide libraries to explore potential bioactive turn conformations.[67] In addition, several effective new approaches have recently been reported for synthesizing oxa, thia and aza-varients of the previously described heterocyclic systems, which should find general use in programs in peptide science and medicinal chemistry.

2.4.1. Synthesis of substituted thia- and oxa-pyrrolizidin-8-one amino acids

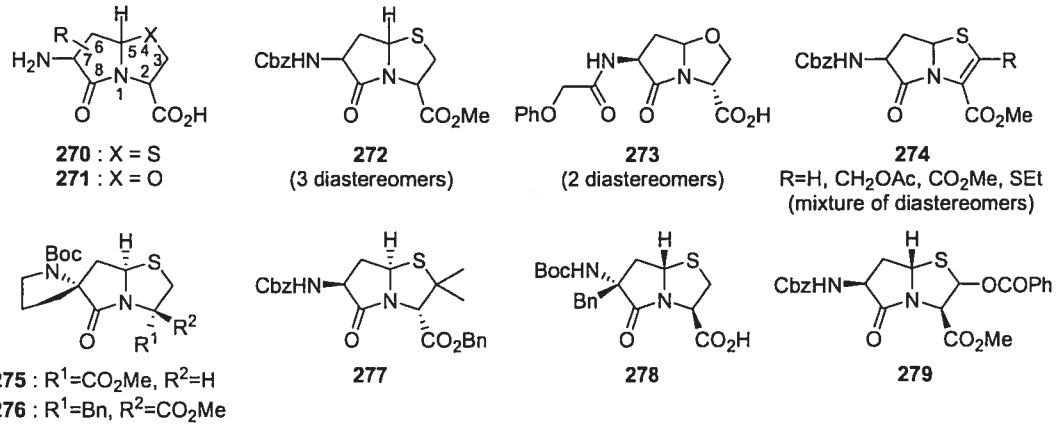
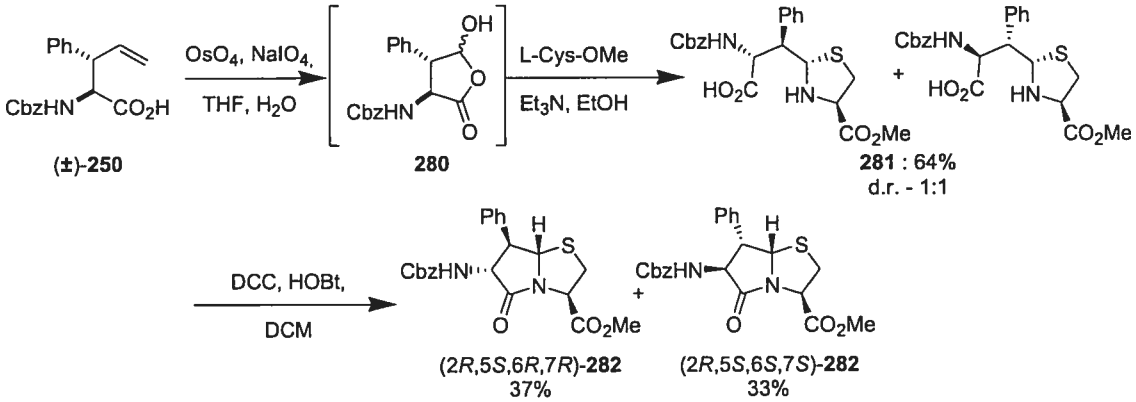


FIGURE 8 General structure for substituted thia- and oxa-pyrrolizidinone amino acids and previously reviewed examples.

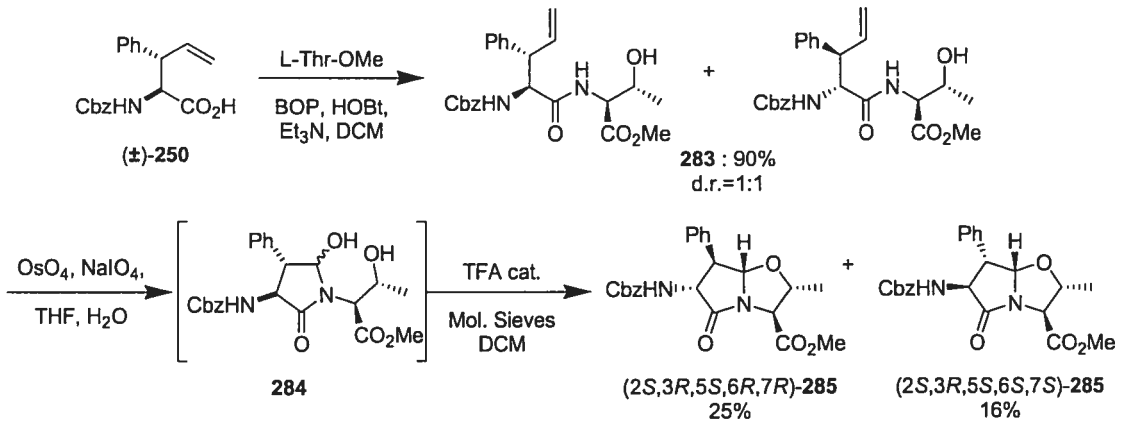
4-Thia- and 4-oxa-pyrrolizidin-8-one amino acids **272** and **273**, as well as various substituted analogs were reported earlier and reviewed (FIGURE 8).[1] They were synthesized by routes featuring condensation of aspartate β-aldehyde analogs with serine or cysteine derivatives.

6-Phenyl-4-thiapyrrolizidin-8-one *N*-(Cbz)amino esters **282** were synthesized from L-Cys-OMe and racemic 3-(phenyl)allylglycine **250** (SCHEME 50).[68] The hemiacetal intermediate **280** was produced by oxidative cleavage of the double bond of **250**, and condensed with cysteine methyl ester to give a 1:1 mixture of thiazolidine carboxylates **281**. Lactam cyclization furnished (2*R*,5*S*,6*R*,7*R*)- and (2*R*,5*S*,6*S*,7*S*)-**282** in 24% and 21% overall yields from **250**.



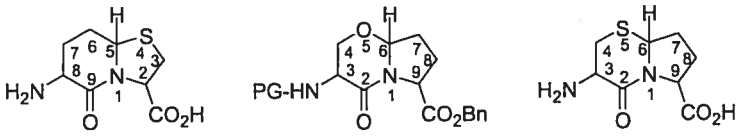
SCHEME 50 Synthesis of 6-phenyl-4-thiapyrrolizidin-8-one *N*-(Cbz)amino esters **282**.[68]

3-Methyl-6-phenyl-4-oxapyrrolizidin-8-one *N*-(Cbz)amino esters **285** were prepared starting from acylation of L-threonine with racemic 3-(phenyl)allylglycine **250** to furnish a 1:1 mixture of dipeptides **283**, which were converted to cyclic aminal intermediates **284** by oxidative cleavage of the double bond (SCHEME 51).[68] . Upon dehydration in the presence of acid, the oxazolidine ring was closed and (2*S*,3*R*,5*S*,6*R*,7*R*)- and (2*S*,3*R*,5*S*,6*S*,7*S*)-**285** were isolated in 22% and 14% respective overall yields from **250**.



SCHEME 51 Synthesis of 3-methyl-6-phenyl-4-oxapyrrolizidin-8-one *N*-(Cbz)amino esters **285**.[68]

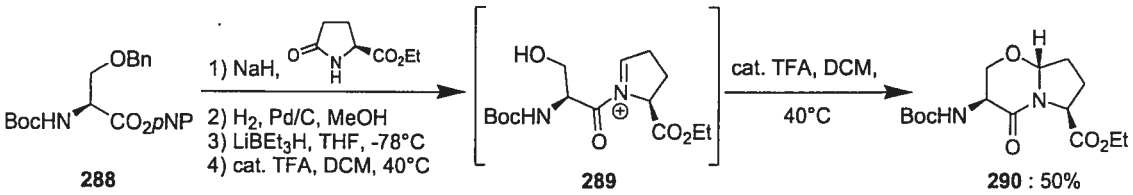
2.4.2. Synthesis of thia- and oxa-indolizidinone amino acids



267 : BTD **286** PG = Boc, Fmoc, Cbz **287**

FIGURE 9 Previously reviewed examples of 4-thia- and 5-oxa-indolizidinone amino carboxylates **267** and **286** and general structure of 5-thia-indolizidinone amino acid **287**

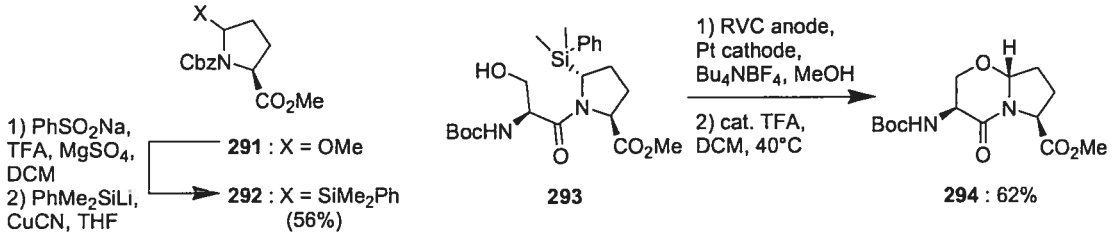
As previously reviewed, 5-thia-indolizidinone amino acid **267** (BTD), one of the first azabicycloalkane amino acids to be developed,[1,64] was prepared by condensation of glutamate γ-aldehyde and cysteine (FIGURE 9). 5-Oxa-indolizidinone amino esters **286** possessing an oxygen atom in the lactam ring were previously synthesized by routes featuring preparation of iminium dipeptide **289** which was cyclized *in situ* (SCHEME 52).[1]



SCHEME 52 Synthesis of 5-oxa-indolizidin-2-one *N*-(Boc)amino ester **290**.[69]

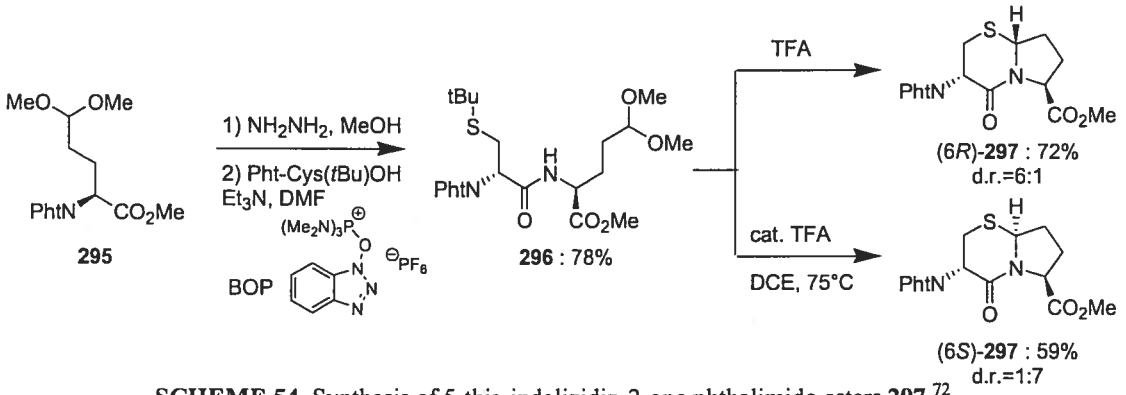
5-Oxa-indolizidinone amino ester **290** has more recently been prepared from ethyl pyroglutamate by a route consisting of acylation with *N*-(Boc)-*O*-benzyl serine *para*-nitrophenyl ester **288**, hydrogenolytic removal of the benzyl ether, selective reduction of the intracyclic amide and cyclization with a catalytic amount of TFA (SCHEME 52).[69]

5-(Phenyldimethylsilyl)proline **292** was prepared to facilitate 5-position oxidation and iminium ion cyclization in peptides, by addition of a silyl cuprate onto a proline iminium ion. Dipeptide **293** was then selectively oxidized electrochemically at the carbon silyl bond to form the methoxyaminal, which was cyclized under acidic conditions to give 5-oxaindolizidinone amino ester **294** (SCHEME 53).[70] Without the 5-position silyl substituent, attempts to prepare oxaindolizidinone by electrochemical oxidation of *N*-protected serinyl prolinate failed, resulting in considerable loss of formaldehyde to produce the corresponding glycinyl prolinate.[71]



SCHEME 53 Synthesis of 5-oxa-indolizidin-2-one *N*-(Boc)amino ester **294**.[70]

5-Thiaindolizidinone amino esters **297** possessing a sulphur atom in the lactam ring have been synthesized by coupling L-glutamate γ-dimethyl acetal **295** and D-*S*-(*tert*-butyl)-*N*-(phthalimido) cysteine to give dipeptide **296** (SCHEME 54).[72] Both, (6*R*)- and (6*S*)-thiaindolizidinone phthalimido esters **297** were obtained diastereoselectively (d.r. ≥ 6:1) in 72% and 59% yield by submitting dipeptide **296** to neat TFA at room temperature, or to catalytic TFA at 75°C, respectively.



SCHEME 54 Synthesis of 5-thia-indolizidin-2-one phthalimido esters **297**.[72]

2.4.3. Synthesis of substituted thia- and oxa-indolizidinone amino acids

A series of substituted 4-thia- and 5-oxa-indolizidinone amino acids were previously reported and reviewed (FIGURE 10).[1] Their syntheses featured condensations of glutamate γ-aldehyde with penicillamine and β-phenyl cysteine analogs for **301** and **302**, as well as electrochemical cyclization of α-methylserinyl-proline dipeptide for **303**.

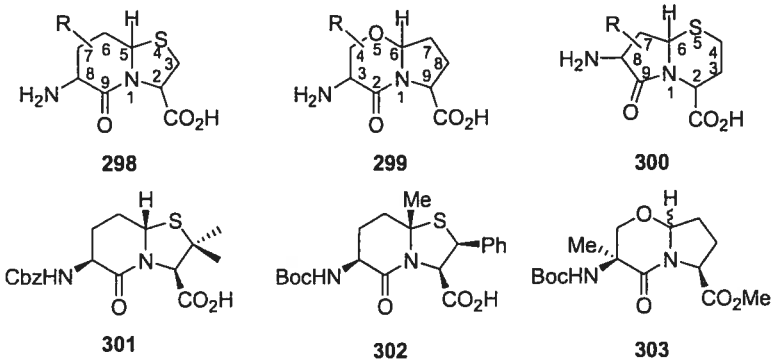
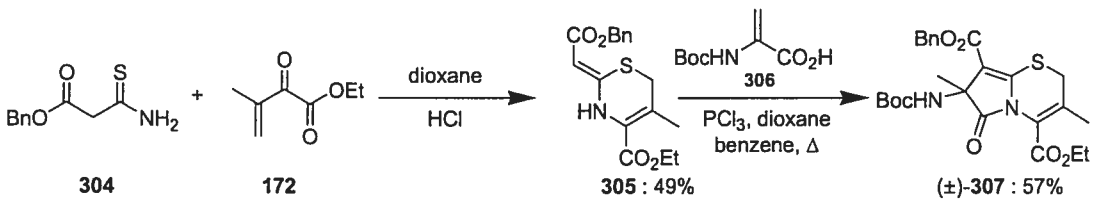


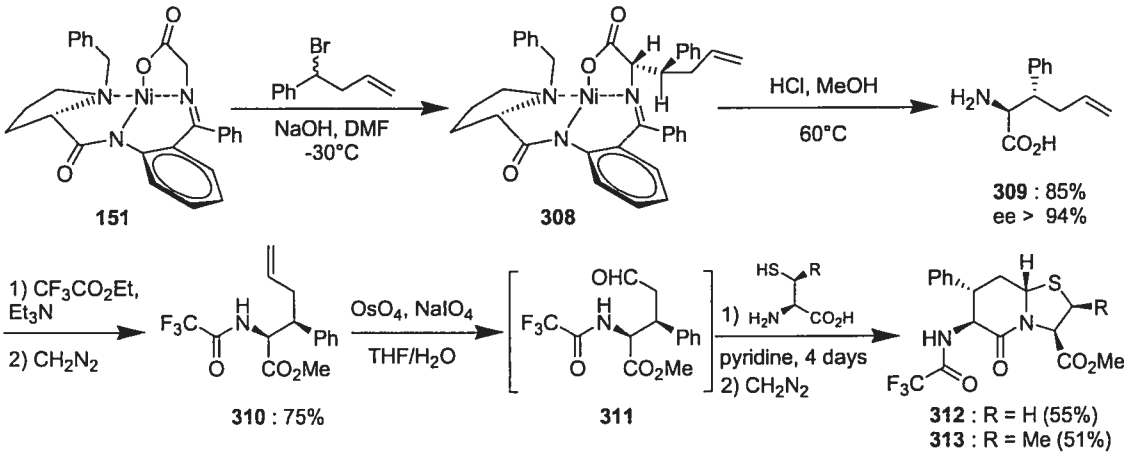
FIGURE 10 General structure for substituted thia- and oxa-indolizidinone amino acids and previously reviewed examples.

Using a similar strategy as employed for the synthesis of indolizinone **175** (SCHEME 32), condensation of thiazine **305** and *N*-(Boc)dehydroalanine **306** proceeded via an entirely different mechanism and better yield to give racemic 5-thia-indolizin-9-one amino diester (±)-**307** in 57% yield (SCHEME 55).[73]



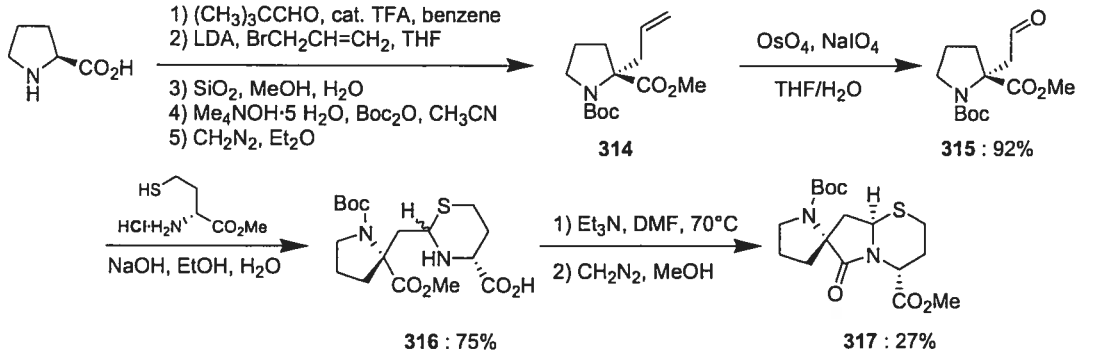
SCHEME 55 Synthesis of 3,8-dimethyl-4,5,8,9-tetrahydro-5-thia-indolizin-9-one *N*-(Boc)amino-3,7-dicarboxylate **307**.[73]

7-Phenyl-4-thiaindolizidin-9-one amino acid **312** and 3-methyl-7-phenyl-4-thiaindolizidin-9-one amino acid **313** were synthesized in 41% and 38% overall yields, respectively, from 2-amino-3-phenyl-hex-5-enoic acid **309** by routes featuring oxidative olefin cleavage and condensations with cysteine and β-methyl cysteine methyl esters to construct the thiazolidine prior to lactam formation (SCHEME 56).[74] Diastereoselective alkylation of chiral glycine equivalent **151** with 1-bromo-1-phenyl-but-3-ene afforded the common amino acid starting material **309** in 85% overall yield with ee up to 94% from **151**.



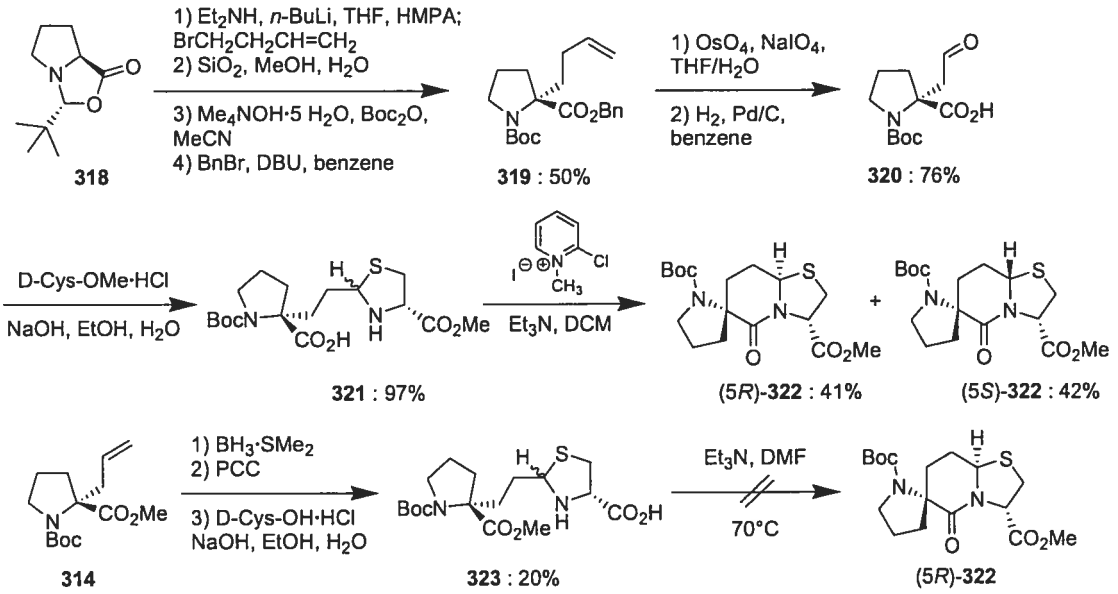
SCHEME 56 Syntheses of 7-phenyl- and 3-methyl-7-phenyl-4-thiaindolizidin-9-one amino esters **312** and **313**.[74]

8-(2-Spiropyrrolidinyl)-5-thiaindolizidin-9-one amino ester **317** was synthesized in 20% overall yield from 2-allyl proline **314**[75] by a route featuring olefin oxidation,[76] condensation of the resulting aldehyde with homocysteine and lactam cyclization (SCHEME 57).[77]



SCHEME 57 Synthesis of 8-(2-spiropyrrolidinyl)-5-thia-indolizidin-9-one *N*-(Boc)amino ester **317**.[75,76,77]

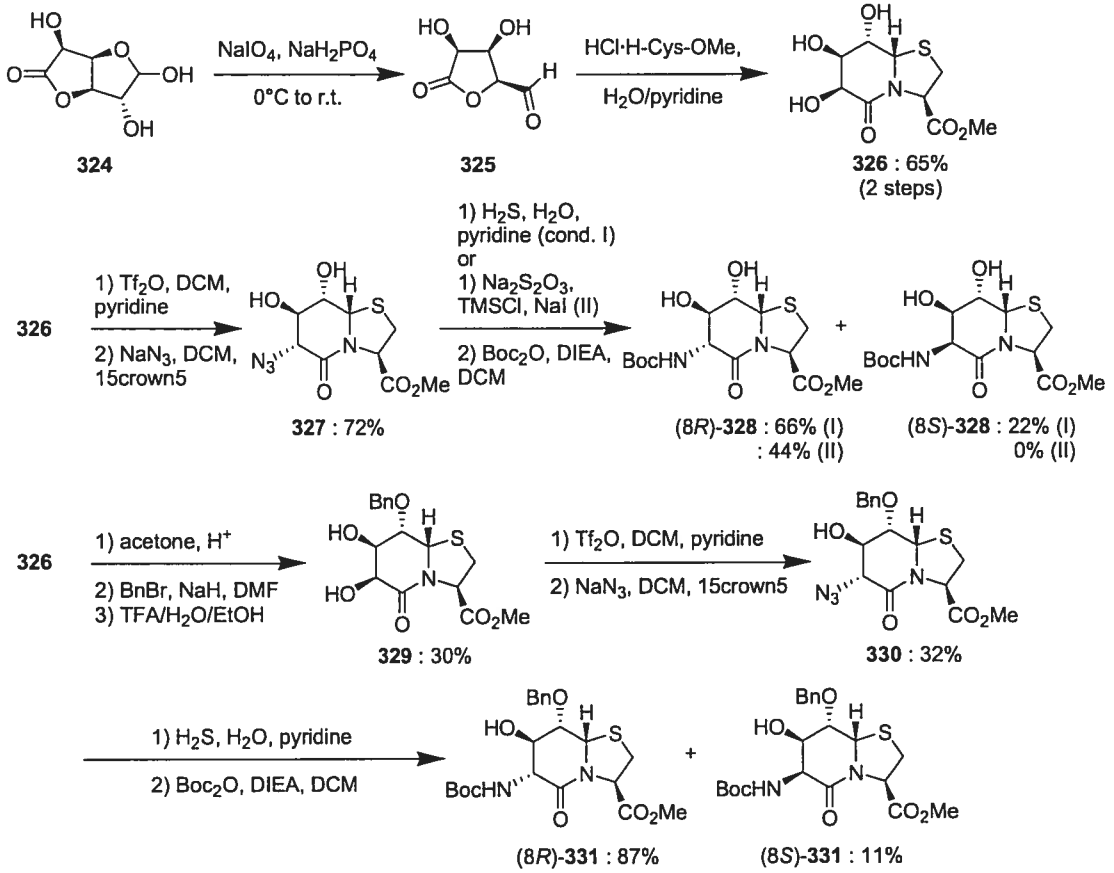
8-(2-Spiropyrrolidinyl)-4-thiaindolizidin-9-one amino esters **322** were synthesized as a separable 1:1 mixture of (5*R*)- and (5*S*)-isomers in 30% and 30% respective overall yields from 2-homoallyl proline **319** by a route featuring olefin oxidation, condensation of the resulting aldehyde **320** with D-cysteine methyl ester to form a thiazolidine and lactam cyclization using Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide,[78] SCHEME 58).[77] An attempt to synthesize **322** from 2-allyl prolinate **314** featured olefin hydroboration to the primary alcohol, oxidation to an aldehyde and condensation with D-cysteine to provide the thiazolidine methyl ester **323**. Lactam formation with ester **323** failed, however, to provide the desired



SCHEME 58 Synthesis of 8-(2-spiropyrrolidyl)-4-thiaindolizidin-9-one *N*-(Boc)amino esters **322**.[77]

tricyclic compound. Diastereoselective alkylation of proline-derived oxazolidinone **318** with bromo but-3-ene, hydrolysis and protection provided the starting 2-alkyl prolinate **319** in 50% yield.

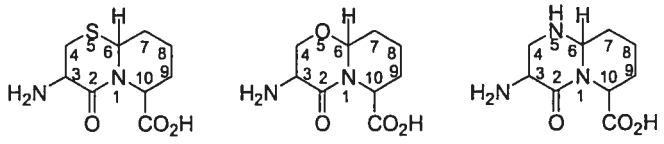
6,7-Dihydroxy-4-thiaindolizidin-9-one *N*-(Boc)amino esters (8*R*)- and (8*S*)-**328** were synthesized in 31% and 10% overall yields from D-glucorono-3,6-lactone **324** (SCHEME 59).[79] Periodate cleavage of diol **324** gave lactone aldehyde **325**, which was condensed with cysteine methyl ester to form the bicycle. Selective alcohol activation as a trifluoromethanesulphonate, azide displacement, azide reduction and Boc protection furnished amino ester **328**. The 6-hydroxyl group was also selectively converted to benzyl ether **329** by a three-step process from triol **326** involving cyclic acetal formation on the 7- and 8-hydroxyl groups, benzylation and acetal deprotection. 6-Benzyloxy-7-hydroxy-4-thiaindolizi-din-9-one *N*-(Boc)amino esters (8*R*)- and (8*S*)-**331** were



SCHEME 59 Syntheses of 6,7-dihydroxy and 6-benzyloxy-7-hydroxy-4-thiaindolizidin-9-one *N*-(Boc)amino esters **328** and **331**.[79]

then produced from **329** in 28% and 3% respective overall yields by a similar sequence as used to make the 6-hydroxy analog **328** described above.

2.4.4. Synthesis of thia-, oxa- and aza-quinolizidinone amino acids

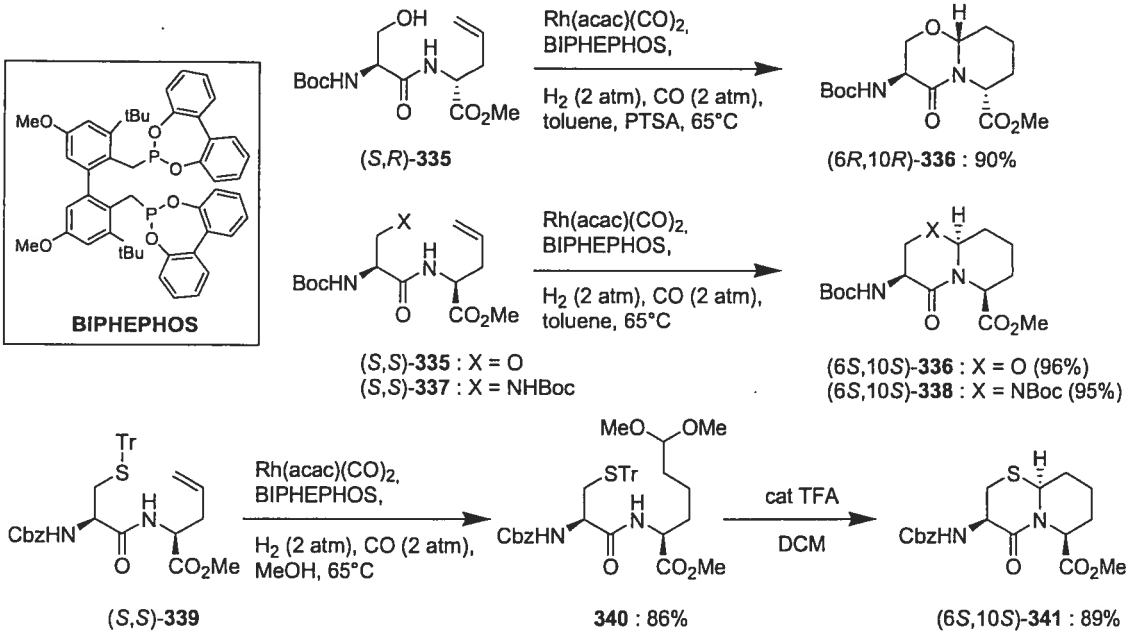


332 **333** **334**

FIGURE 11 Thia-, oxa- and aza-quinolizidinone amino acids.

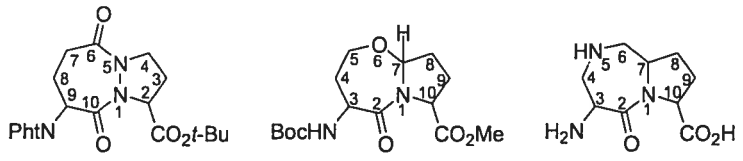
Since the synthesis of the first azabicyclo[4.4.0]alkanone amino acid, Qaa **217** (SCHEME 39), different analogs **332-334,** containing heteroatoms, have been prepared (FIGURE 11).

5-Oxaquinolizidinone amino esters **336** have been synthesized from Boc-serinyl-allylglycine methyl ester **335** by rhodium catalyzed cyclohydrocarbonylation (SCHEME 60).[80] Stereochemistry at the ring junction was contingent on the chirality at the allylglycine α-centre, such that (*S*,*S*)- and (*S*,*R*)-**335** furnished respectively (3*S*,6*S*,10*S*)- and (3*S*,6*R*,10*R*)-**336** in 96% and 90% respective yields. 5-*N*'-(Boc)Aza-quinolizidin-2-one *N*-(Boc)amino ester **338** and 5-thiaquinolizidinone amino ester **341** were synthesized by similar cyclohydrocarbonylation routes from *N*,*N*'-bis(Boc)diaminopropionyl-allylglycine methyl ester **337** and *S*-(trityl)-*N*-(Boc)cysteinyl-allylglycine methyl ester **339** in 95% and 76% yields, respectively.[80] In the later case, the aldehyde intermediate was trapped as dimethyl acetal **340** prior to deprotection of the thiol and cyclization with TFA (SCHEME 60).



SCHEME 60 Synthesis of 5-oxa, 5-*N*'-(Boc)aza- and 5-thia-quinolizidin-2-one *N*-(Boc)amino ester **336, 338** and **341**.[80]

2.4.5. Synthesis of aza- and oxa-pyrroazepinone amino acids

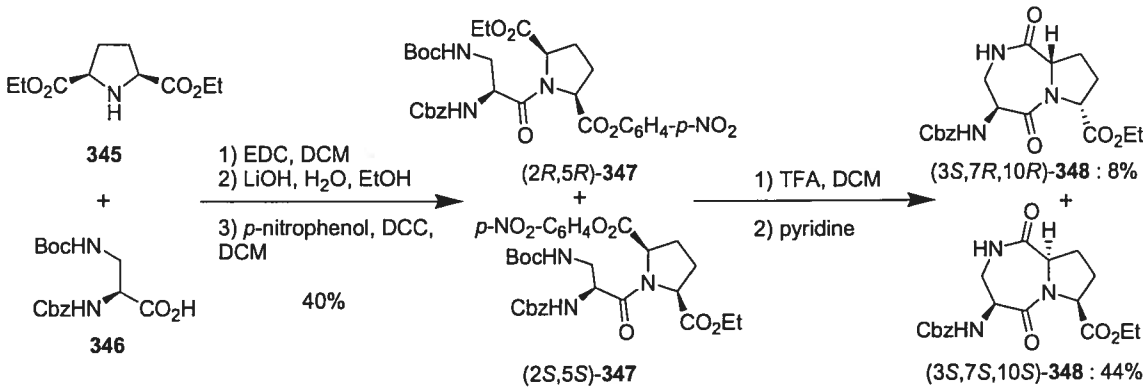


342 **343** **344**

FIGURE 12 Previously reviewed examples of 5-aza- and 6-oxa-pyrroloazepinone amino carboxylates **342** and **343** and general structure of 5-aza-pyrroloazepin-2-one amino acid **344**.

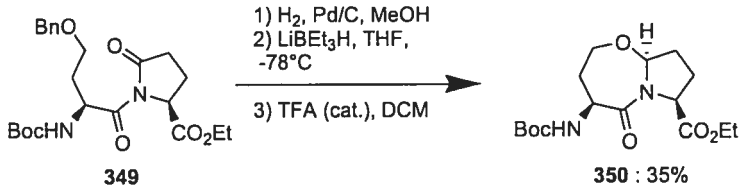
6-Aza-pyrroloazepinone amino ester **342** possessing a nitrogen atom at the ring junction, has been synthesized by a route featuring lactam cyclization of glutamyl pyrrazolidine-3-carboxylate dipeptide. 6-Oxa-pyrroloazepinone amino ester **343** possessing an oxygen atom in the lactam ring has been synthesized by a route featuring electrochemical oxidation of homoserinyl-proline dipeptide as reviewed (FIGURE 12).[1]

5-Aza-pyrroloazepin-2,6-dione *N*-(Cbz)amino esters **348** have been synthesized from *cis*-2,5-di(ethylcarboxylate) pyrrolidine **345** and α-*N*-(Cbz)-β-*N*-(Boc)diaminopropionic acid **346** by two successive amide bond formations (SCHEME 61).[81] Initially, dipeptide was formed by acylation of the pyrrolidine dicarboxylate with the protected diamino acid. Hydrolysis of one ester and carboxylate activation as a *para*-nitrophenyl ester furnished an inseparable mixture of diastereomeric dipeptides **347**. Removal of the Boc protecting group and lactam cyclization furnished a separable 1 : 5.5 mixture of (3*S*,7*R*,10*R*)-**348** and (3*S*,7*S*,10*S*)-**348** in 8% and 44% respective yields.



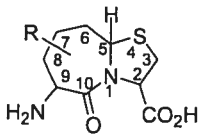
SCHEME 61 Synthesis of 5-aza-pyrroloazepin-2,6-dione *N*-(Cbz)amino esters **348**.[81]

More recently, 5-oxa-pyrroloazepinone amino ester **350** has been prepared from homoserinyl-pyroglutamate dipeptide **349** by a route featuring hydrogenolytic removal of the benzyl ether, selective reduction of the intracyclic amide and cyclization with a catalytic amount of TFA (SCHEME 62).[69]



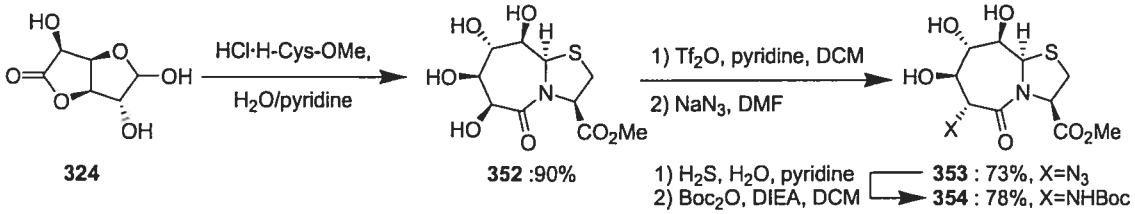
SCHEME 62 Synthesis of 6-oxa-pyrroloazepin-2-one *N*-(Boc)amino ester **350**.[69]

2.4.6. Synthesis of substituted thia-pyrrolizidinone amino acids



351

6,7,8-Trihydroxy-4-thiapyrroloazepin-10-one amino ester **354** has been synthesized from D-glucorono-3,6-lactone **324** (SCHEME 63) by a similar route as described for the synthesis of thiaindolizidinones **328** and **331** (SCHEME 59). Glucoronolactone **324** was condensed with L-cysteine methyl ester, which gave the poly-hydroxylated bicyclic ester **352**. Selective trifluoromethanesulfonation of the C-9 hydroxyl group and displacement with sodium azide provided azido ester **353**.[82,83] 6,7,8-Trihydroxy-4-thiapyrroloazepin-10-one *N*-(Boc)amino ester **354** was finally obtained by azide reduction and amine protection in 78% yield from **353**.



324 **352** :90%

SCHEME 63 Synthesis of 6,7,8-trihydroxy-4-thiapyrroloazepin-10-one *N*-(Boc)amino ester **354**.[82,83]

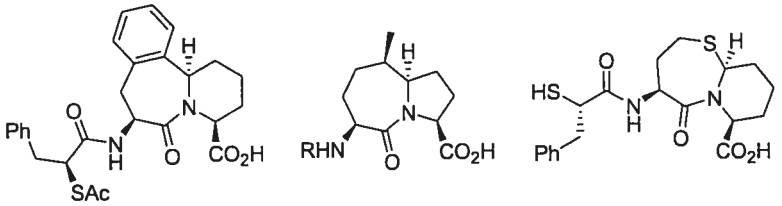
2.5. Biological applications.

As β-lactam antibiotic homologs, azabicloalkanone amino acids have generally exhibited relatively low antibiotic activity. Incorporation of BTD **267** into the peptide antibiotic Gramicidin S as β-turn mimic provided, however, analogs with similar antibacterial activity as the native peptide. Various other applications of azabicycloalkane amino acids have been previously reviewed, including their employment in enzyme inhibitors (ACE, metalloprotease), neuropeptide antagonists of G-protein coupled receptors (GPCRs : enkephalin, dopamine, TRH, LH-RF, neurokinin A) and other peptides (gramicidin S, cyclosporin A).[1]

Since these earlier efforts, the application of azabicycloalkanone amino acids has intensified in the study of biologically active peptides. Many new enzyme inhibitors and receptor agonists and antagonists have since been reported.[84-128]

2.5.1. Enzyme inhibition

Angiotensin converting enzyme (ACE), neutral endopeptidase (NEP) and endothelin converting enzyme (ECE) represent important zinc-metalloproteases, that play key roles in blood pressure regulation, body fluid homeostasis and cell growth. Three azabicycloalkane amino acid analogs, MDL 100,240, **355** (Aventis)[84,85], ER-40133, **356** (Eisai)[86,87] and Omapatrilat (Vanlev) **357** (Bristol-Myer-Squibb)[88, 89] are currently under investigation for treating angina, congestive heart failure, high blood pressure and hypertension (FIGURE 13).



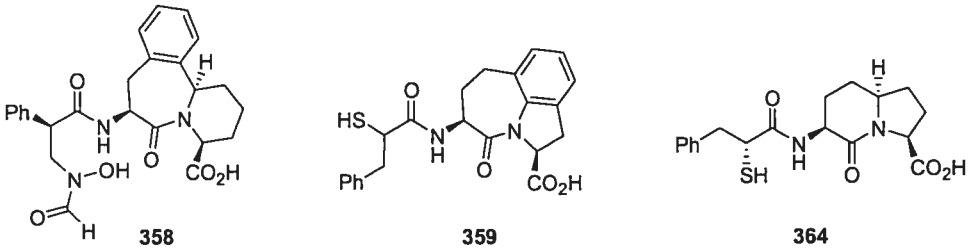
MDL 100,240 : **355**
ACE IC_{50} : 0.11 nM
NEP IC_{50} : 0.08 nM

ER-40133 : **356**

Omapatrilat : **357**
ACE IC_{50} : 6 nM
NEP IC_{50} : 9 nM

FIGURE 13 Azabicycloalkane analogs under evaluation as dual ACE and NEP inhibitors.

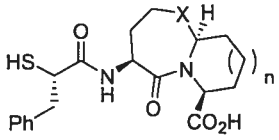
Several analogs of MDL 100,240 **355** and Omapatrilat **357** have been tested as ACE/NEP inhibitors (FIGURE 14). For example, the *N*-formyl hydroxylamine analog **358** exhibits low nM activity.[90] The Haic analog **359** exhibited relatively poor activity.[91] In Omapatrilat analogs **360-363**, the sulphur atom was replaced by oxygen and a methylene carbon, and the pipecolate ring was replaced by proline.[92] These compounds had comparable activity to Omapatrilat **357** against ACE and NEP; however, they exhibited shorter durations of action. of , The 6*R*- and 6*S*-diastereomers of I^2aa have also been used to make analogs, such as (2'*R*,3*S*,6*R*,9*S*)-**364**, which was found to be a selective ACE inhibitor (FIGURE 14).[93]



358
ACE IC_{50} : 3.4 nM
NEP IC_{50} : 1.8 nM

359
ACE IC_{50} : 48 nM
NEP IC_{50} : 40 nM

364
ACE IC_{50} : 0.87 nM
NEP IC_{50} : 512 nM



360-363

compound		ACE[a]	NEP[a]
360	X=O, n=1	8	9
361	X=O, n=0	7	26
362	X=CH_2, n=1	11	25
363	X=CH_2, n=0	5	18

[a] : IC_{50} (nM)

FIGURE 14 Various analogs tested for ACE and NEP inhibition.

Thrombin (FIIa), a trypsin-like serine protease, is the ultimate enzyme in the blood coagulation cascade. Thrombin along with other blood coagulation factors such as Factors VII and X (FVIIa and FXa) have been a major target for rational drug design efforts. Selectivity against thrombin, FVIIa and FXa relative to plasmin, a proteolytic enzyme involved in blood clot retraction, and to trypsin, another serine protease, is essential to avoid undesirable side effects.

A series of thrombin inhibitors have been developed using azabicycloalkane amino acids. Active compounds from these studies include 3-benzyl-6-aza- and 3-benzyl indolizidinone amino amides **365**, **366** and **367**,[94] 3-benzyl-5-methoxy-7-ethyl and 3-cyclohexenyl-5-methoxy-7-ethyl indolizidinone amino amides **190a** and **190b**,[52] *N*-(tetrahydroquinolinylsulfonyl)amino, *N*-(1-naphtyl-

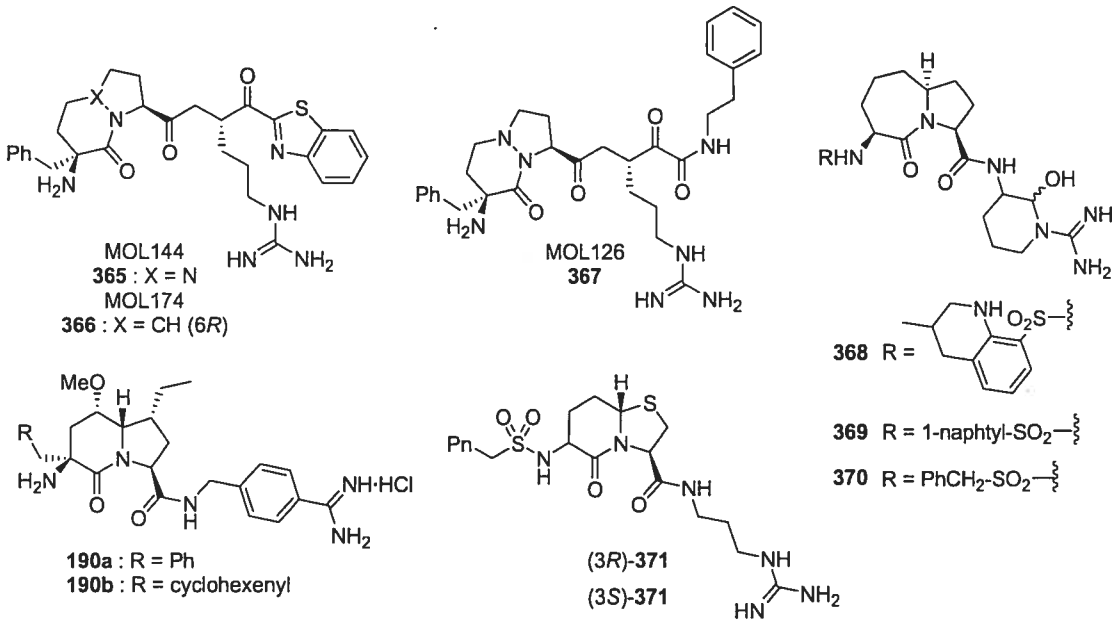


FIGURE 15 Various thrombin inhibitors containing azabicycloalkane amino acids.

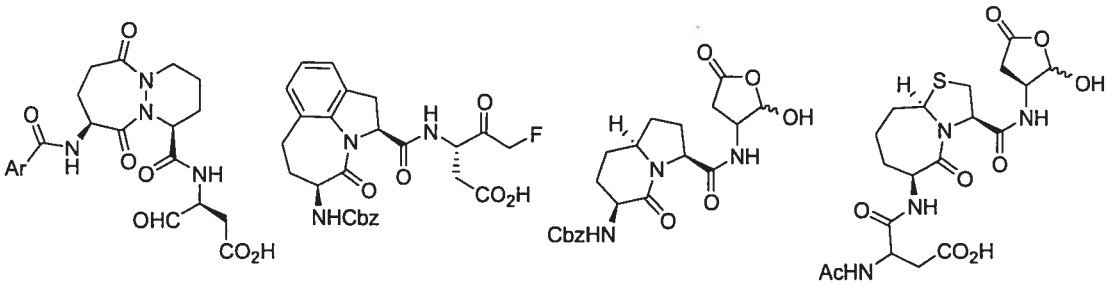
sulfonyl)amino and *N*-(benzylsulfonyl)amino pyrroloazepinone amides **368**, **369** and **370**,[95] as well as (3*R*)- and (3*S*)-4-thia-indolizidinone *N*-(benzyl-sulfonyl)amino amides **371**[96] (FIGURE 15). Although a direct comparison of the K*i* and IC_{50} values is not possible, the reported results for inhibition of coagulation factors (FIIa, FVIIa and FXa) and other serine proteases (trypsin and plasmin) are resumed in TABLE 2.

TABLE 2 Inhibition and selectivity of coagulation factors vs trypsin and plasmins.

	Compound	Thrombin (FIIa)	FVIIa	FXa	Trypsin	Plasmin	References
K*i*	**365**	0.65	270	19	0.640	415	89
	366	0.85	270	200	0.230	251	89
	367	0.071			0.027		89
	(3*S*)-**371**	111		>33700	7900		91
	(3*R*)-**371**	145		26000	5100		91
IC_{50}	**368**	18			100	1040	90
	369	110				10000	90
	370	110				10000	90
	190a	210	420			1100	50
	190b	170	4600			14100	50

K*i* (nM), IC_{50} (nM)

Caspase 1 / ICE cleaves substrate after an aspartate residue. In particular, it cleaves interleukin beta producing an active form that exerts proinflammatory effects. Caspase 3 has been identified as a mediator of apoptosis in mammalian cells. Overexpression of caspase 1 and 3 can induce apoptosis in fibroblasts. Inhibitors of caspases have been pursued for indications such as rheumatoid arthritis, Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and Parkinson's disease.



372 : Ar=*p*-C_6H_4-OCH_2CO_2H **374** : IDN5370 **375** **376**
373 : Ar=*o*-C_6H_4F

FIGURE 16 Caspase inhibitors based on azabicycloalkane amino acids

Several peptide mimics containing azabicycloalkane amino acids have been investigated as caspase 1 and 3 inhibitors (TABLE 3). For example, 6-aza-piperidinoazepinone-aspartate α-aldehydes **372** and **373** exhibited nanomolar activity (K*i* = 1 nM for caspase 1).[97] Similarly, Haic based inhibitor IDN5370 **374** (Idun/Novartis) exhibited nanomolar activity against both caspase 1 and 3 (K*i* = 1 nM for caspase 1 and K*i* = 10 nM for caspase 3).[98] *N*-(Cbz)Amino I^2aa-aspartate α-aldehyde **375** and *N*-(*N*'-acetyl-α-aspartyl)amino 4-thia-pyrroloazepinone-aspartate α-aldehyde **376** were respectively found to be selective inhibitors of caspase 1 (**375** : IC_{50} = 36 nM) and caspase 3 (**376** : IC_{50} = 18 nM, FIGURE 16).[99]

TABLE 3 Caspase inhibitors and selectivity of compounds **372** to **376**.

Compound	caspase-1	caspase-3
372[a]	1	
373[a]	1	
374[a]	1	10
375[b]	36	>10000
376[b]	10400	18

[a] K*i* (nM), [b] IC_{50} (nM)

To develop new drugs against diverse targets such as tumor, virus and autoimmune diseases, azabicycloalkanes have been employed to create peptide mimics that target enzymes involved in these processes. For example, from a series of 20 azabicycle peptide mimics, Haic analog **377** (Merck), exhibited the best inhibition of Granzyme B (K*i* = 7 nM) and stimulation of cytotoxic T lymphocyte (CTL) apoptosis (IC_{50} = 3.1 μM).[100] Tetrahydroindolizinone peptide mimic **378** (Pfizer) was developed as an irreversible Michael acceptor to inhibit human rhinovirus 3C protease (HRV 3CP) and appeared to be active against multiple HRV serotypes (EC_{50} = 37 to 162 nM).[101] Tetrapeptides **379** and **380** were designed as a β-turn mimetic inhibitors of farsenyl transferase (Ftase, IC_{50} = 5 and 7 μM respectively, FIGURE 17). Farnesyl transferase is an enzyme responsible for the activation of retrovirus-associated DNA sequences proteins (ras proteins) which are GTP-binding proteins playing a role in normal cellular growth and involved in 90% of pancreatic cancer and 40 % of colorectal cancers.[102]

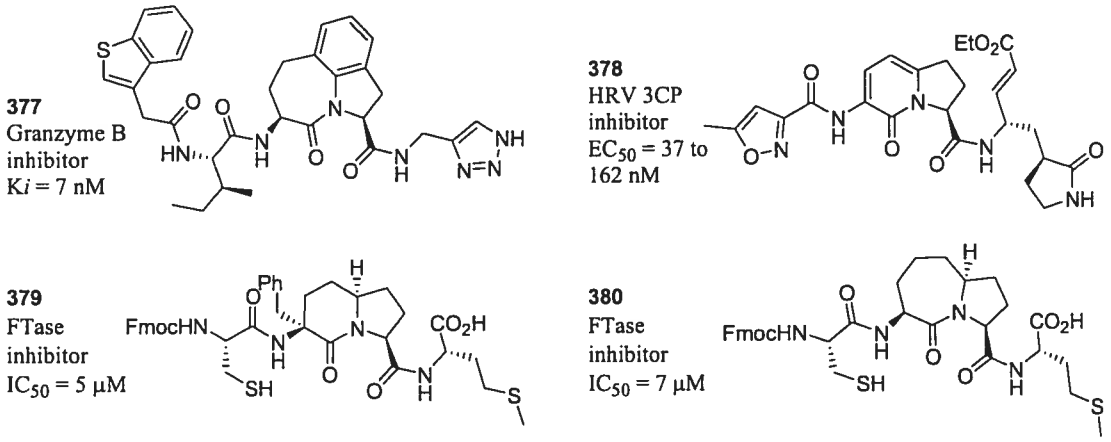


FIGURE 17 Enzyme inhibitors based on azabicycloalkane amino acids.

In addition, (3*S*)- and (3*R*)-*N*-(furan-2-carbonyl) IBTM analogs **381** were weak inhibitors against a series of matrix metalloproteinases (MMPs, FIGURE 18).[103]

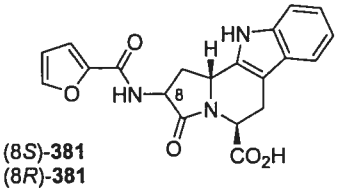


FIGURE 18 IBTM based MMPs inhibitors.

2.5.2. G-protein coupled receptor (GPCR) ligands

Various azabicycloalkane amino acid analogs have been synthesized as G-protein coupled receptor (GPCR) ligands to explore the receptor specificity and the structure / activity relationships of peptide ligands.

For example, constrained thyroliberin (TRH) tripeptide analogs **382** and **383** exhibited partial agonist activity (**382** : K*i* = 2 / 1.3 μM; **383** : K*i* = 1.5 μM) at the THR receptor (THR-R) without THR-R1 / THR-R2 subtype selectivity (FIGURE 19).[104,105] In a series of 26 tripeptides containing IBTM **207**, *N*-(Cbz)-IBTM-Phe **384** has shown antagonist activity (IC_{50} = 4.7 nM) at the cholecystokinin (CCK_1) receptor without activity at the CCK_2 receptor (FIGURE 19).[106,107,108] Dehydroindolizidinone tripeptide mimic **385** acted as a specific neorokinin (NK_1) receptor antagonist (K*i* = 79 nM) without activity at the NK_2 receptor (K*i* > 3000 nM; FIGURE 19).[109] Incorporation of BTD **267** into the calcitonin gene-related peptide (CGRP) antagonist, $CGRP_{8-37}$ (VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-NH_2) at positions 19-20, 33-34 and both 19-20 and 33-34 lead to the conservation of antagonist activity (pA_2 = 6.0 to 6.2), demonstrating the importance of turn conformations at these positions for antagonism.[110]

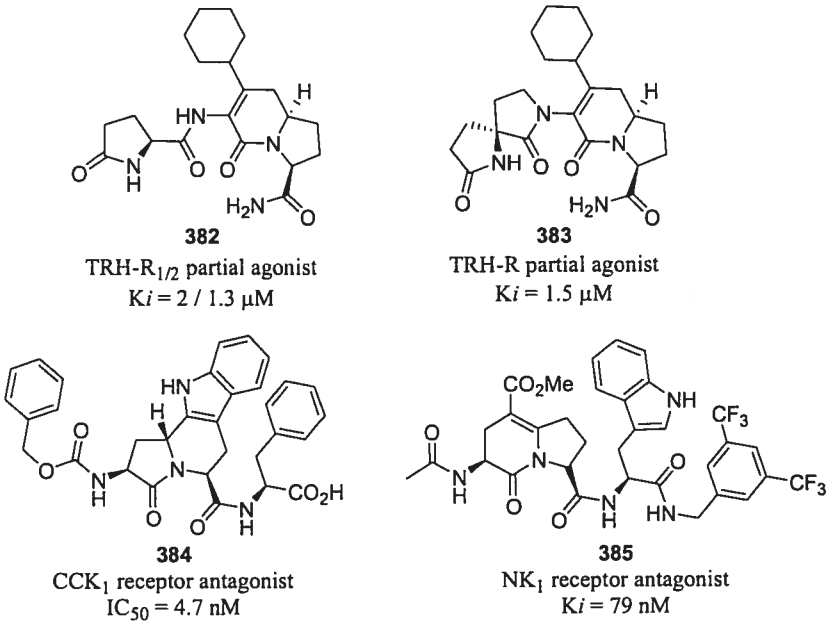


FIGURE 19 Neuropeptide analogs based on azabicycloalkane amino acids.

Leu-enkephalin analogs **386** containing the different diastereomers of 8-phenyl-4-thia-indolizidinone amino acid[111] as well as **387** containing I^9aa[112] were three order of magnitude less active in *in vivo* assays than Leu-enkephaline; however **387** reached 68% of the maximum signal obtained with morphine on the μ-receptor and was at least as long acting, suggesting that its conformation retained the requirements for interaction with the biological receptor (FIGURE 20).

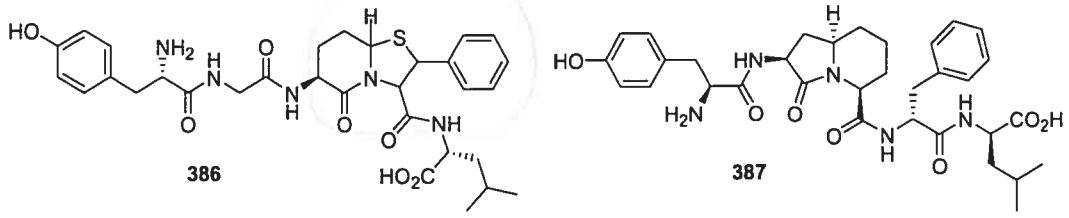
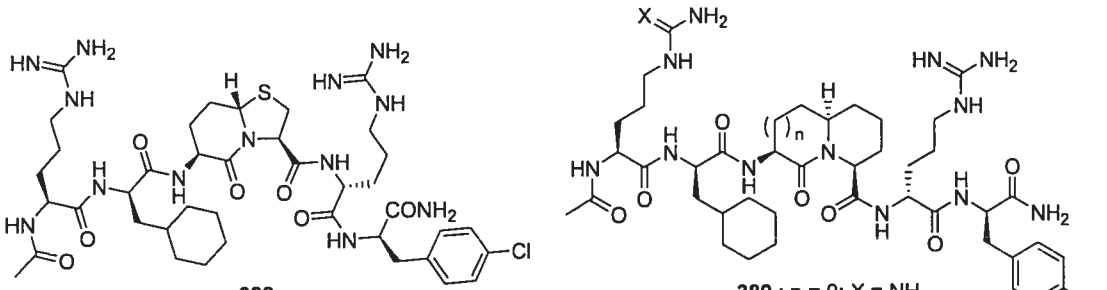


FIGURE 20 Leu-enkephalin analogs based on azabicycloalkane amino acids.

Screening of a combinatorial library of constrained peptides against the opioid receptor-like (ORL1) receptor identified thiaindolizidinone (BTD) analog **388** which exhibited 34 nM antagonist potency,[67] but poor selectivity against other opioid receptors (53, 222, 78 nM for μ, δ and κ receptors).[67,113,114] Systematic replacement of the central thiaindolizidinone with indolizidin-2-



388
Opioid receptor antagonist

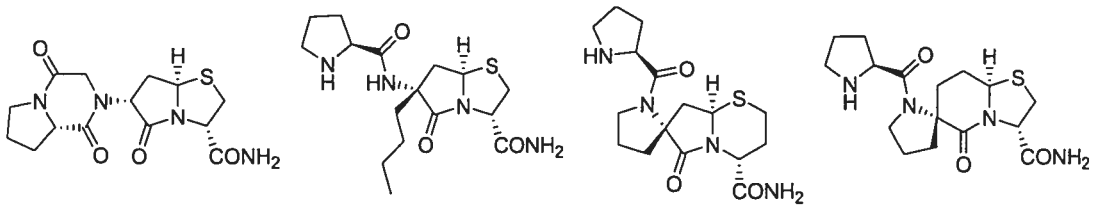
389 : n = 0; X = NH
390 : n = 1; X = NH
391 : n = 1; X = O
Opioid receptor antagonist

Pept	hORL	hMOR	hKOR	hDOR
388	34	53	78	222
389	44	75	190	>5000
390	27	90	105	>5000
391	72	2555	4270	>5000

Ki (nM)

FIGURE 21 ORL1 antagonists based on azabicycloalkane amino acids.

one, indolizidin-9-one and quinolizidinone amino acids provided analogs **389-391**. The I^2aa hexapeptide mimic **389** retained potency at the μ and ORL1 receptors. The quinolizidinone hexapeptide mimic **390** retained potency and exhibited improved selectivity for the ORL1 versus the other opioid receptors.[115] Subsequent replacement of the *N*-terminal Arg residue by citruline provided ORL1 specific antagonist **391** (72 , 2555, 4270 and >5000 nM for the ORL1, μ, κ and δ receptors, FIGURE 21).[116]

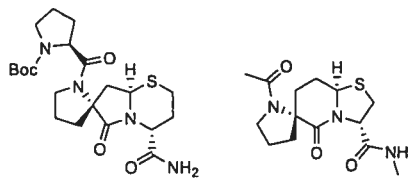


392 (+30% at 100 μg/kg) **393** (+56% at 0.1 μg/kg) **394** (+95% at 10 μg/kg) **395** (+88% at 1 μg/kg)

FIGURE 22 Constrained PLG analogs and their effect on apomorphine-induced rotational behaviour in 6-hydroxydopamine-lesioned rats.

L-Prolyl-L-leucylglycinamide (PLG) is a hypothalamic tripeptide from enzymatic degradation of oxytocin. PLG is able to modulate the dopamine D2-receptor by increasing dopamine D2 agonist affinity. To understand the structural requirement for this modulation, various constrained analogs of the tripeptide were made. For example, a series of bicyclic and tricyclic tripeptide analogs **392-395** were prepared and tested for their effect on apomorphine-induced rotational behaviour in 6-hydroxydopamine-lesioned rats. An increase of the modulation at lower doses was observed for **393** (+56% at 0.1 μg/kg), **394** (+95% at 10 μg/kg) and **395** (+88% at 1 μg/kg), relative to PLG (+30% at 1 mg/kg), suggesting a biologically active conformation in the receptor similar to that of the native peptide. The specific dihedral angles ϕ and ψ for the active conformation may reflect those measured in the X-ray structures of analogs **396** and **397** (FIGURE 22, TABLE 4).[77,117,118]

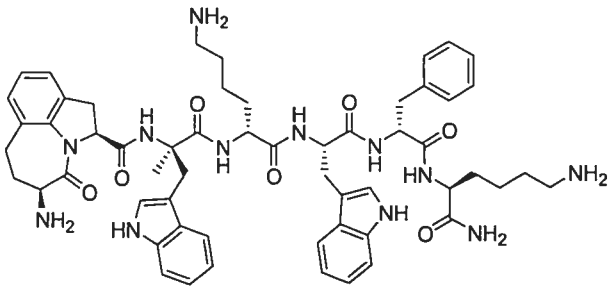
TABLE 4 Dihedral angle values from X-ray analysis of compounds **396** and **397**.



396 **397**

system	ϕ_2	ψ_2	ϕ_3	ψ_3
396	-48.9	134.1	116.0	-29.8
397	-56.0	133.7	97.3	-19.9
ideal type II β-turn	-60.0	120.0	80.0	0.0

A heptapeptide containing Haic, EP80317 (**398**, Neosystem) was identified to be a growth hormone secretagogue (GHS) and to have activity against H9c2 cells (IC $_{50}$ = 200 nM) and CALU-1 cells (IC_{50} = 54 nM, FIGURE 23).[119,120]

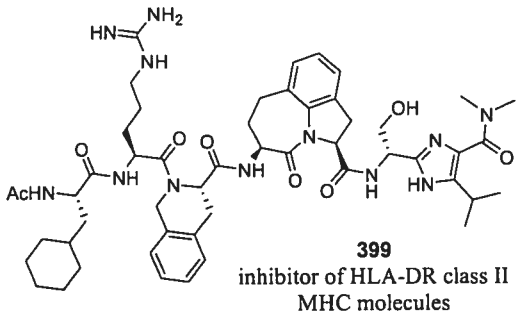


398 : EP80317
Growth hormone secretagogue

FIGURE 23 Growth hormone secretagogue (GHS) containing azabicycloalkane amino acid.

2.5.3. Immune system

D-Related human leukocyte antigens (HLA-DR) major histocompatibility complex (MHC) molecules are inherited peptide receptors expressed on the surface of animal cells and involved in immune response cascade. Inhibitors of MHC molecules have been pursued to treat autoimmune diseases such as rheumatoid arthritis or multiple sclerosis. For example, from libraries of heptapeptides and pseudoheptapeptides, the Haic containing pseudopeptide **399** was found to bind to MHC class II molecules and to be one of the most active inhibitors of DR1 and DR4 (IC_{50} = 405 and 68 nM respectively) and was resistant to degradation by cathepsin B (FIGURE 24).[121,122]



399
inhibitor of HLA-DR class II
MHC molecules
DR4 : IC_{50} = 68 nM
DR1 : IC_{50} = 405 nM

FIGURE 24 Inhibitor of antigen presentation by MHC class II molecule.

Integrins are a large family of α/β heterodimeric transmembrane glycoproteins that attach cells to extracellular matrix proteins. Although they are involved in fundamental cellular processes, they may also implicated in disease states such as tumors, immune and inflammatory disorders. The peptide sequence RGD (Arg-Gly-Asp) is well recognized by integrins and constrained RGD analogs may selectively inhibit interactions of integrin receptors with their native ligands. For example, cyclic thiaindolizidinone peptide **400** is suggested by ^{1}H NMR and computer simulations to adopt a γ-turn about the RGD residu and binds specifically to $\alpha_V\beta_3$ (50 nM) with eightfold weaker activity to $\alpha_V\beta_5$ (400 nM) and without activity to fibronectin and IIb/IIIa.[123] Systematic replacement of D-Phe-*N*-Me-Val in the cyclic pentapeptide c-(RGDf[*N*-Me]V)[124] with diastereomers of indolizidin-2-one and pyrroloazepinone dipeptide surrogates produced (3*R*,7*R*,10*S*)-pyrroloazepinone peptide **401** which was a potent, albeit non selective inhibitor of $\alpha_V\beta_3$ (IC_{50} = 3.7 nM, K*i* = 3 nM) and $\alpha_V\beta_5$ (IC_{50} = 1.4 nM).[125] Subsequent replacement of pyrroloazepinone by (3*R*,6*R*,9*S*)-3-benzyl-indolizidin-2-one provided $\alpha_V\beta_5$ (IC_{50} = 4.1 nM) specific inhibitor **402** (IC_{50} ratio $_{\alpha V\beta3/\alpha V\beta5}$ = 192, FIGURE 25).[126]

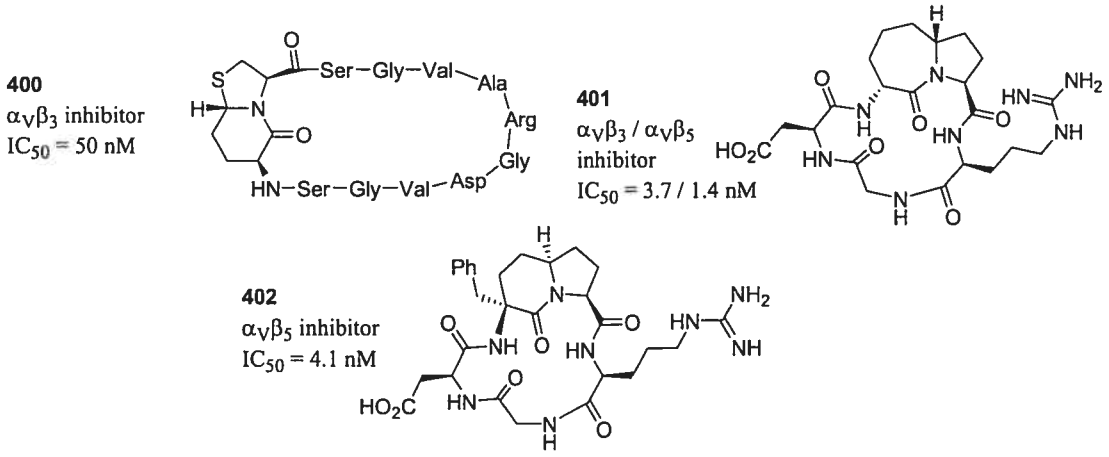
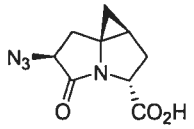


FIGURE 25 Inhibitors of integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$.

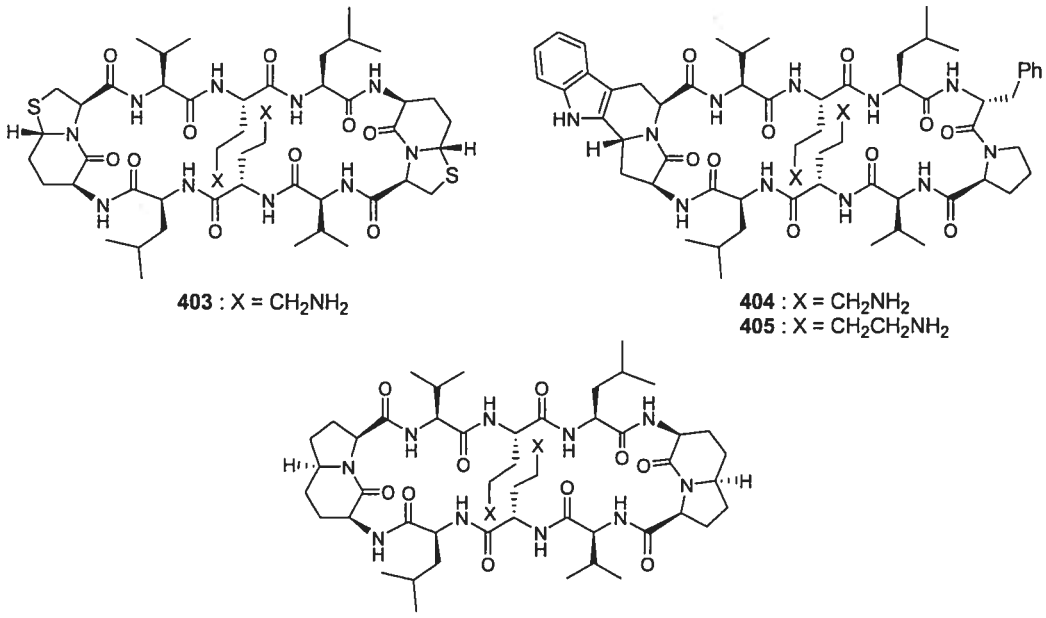
2.5.4. Antibiotics

Carbapenem mimic **26** did not show antibacterial activity; however, it improved the activity of ceftazidine against various bacteria.[15]



26

Gramicidin S (GS), a cyclic decapeptide containing two type II' β-turns, has been used as a model for examining the ability of azabicycloalkane amino acids to mimic a turn conformation. For example, introduction of BTD **267** into GS resulted in antibiotic analog **403** with similar activity as the parent peptide (FIGURE 26). More recently, a single IBTM **207** and two I^2aa **35** residues have been introduced in gramicidin S to furnish analogs **404-407** (FIGURE 26) which maintained similar conformations as the parent peptide as shown by CD and NMR data as well as antibiotic activity (minimal inhibitory concentration against *Staphylococcus Aureus*. GS : 4 μg/ml; **404** : 8 μg/ml; **405** : 16 μg/ml; **406** : 12.5 μg/ml; **407** : 12.5 μg/ml).[127,128]



403 : X = CH_2NH_2

404 : X = CH_2NH_2
405 : X = $CH_2CH_2NH_2$

406 : X = CH_2NH_2
407 : X = $CH_2CH_2NH_2$

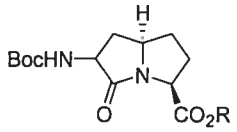
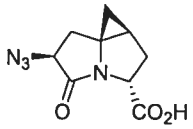
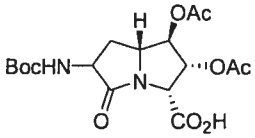
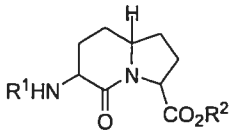
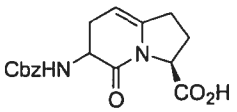
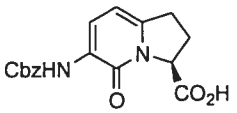
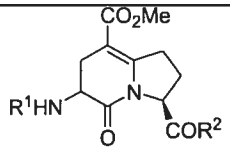
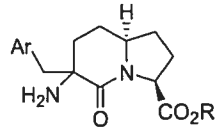
FIGURE 26 Analogs of gramicidin S (GS) containing IBTM and I^2aa.

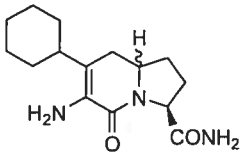
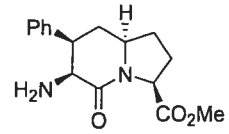
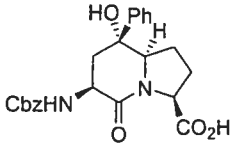
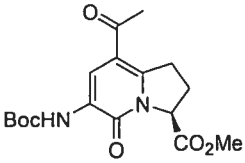
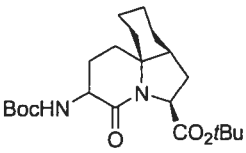
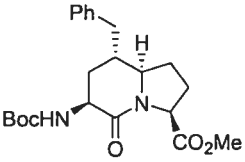
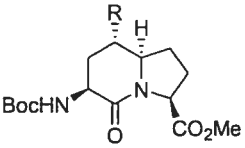
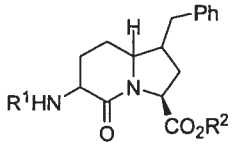
2.6. Conclusion.

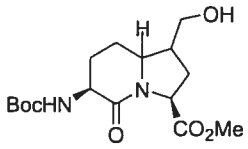
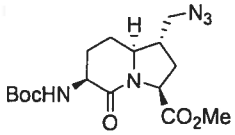
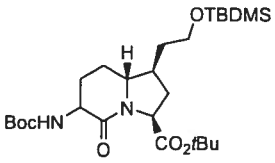
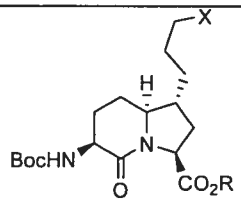
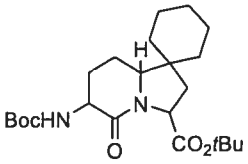
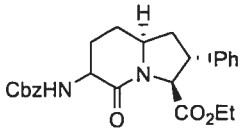
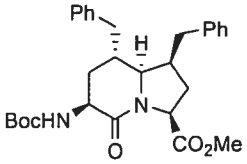
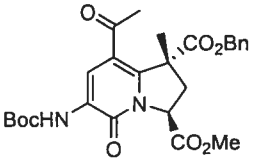
Since 1997, the synthesis of azabicycloalkanone amino acids of varying ring size, configuration and side chain diversity has furnished a variety of tools for studying the role of conformation in peptide-receptor biology. Many of the examples presented in this review have already been employed as dipeptide surrogates to investigate peptide conformation-activity relationships. Analogs exhibiting enhanced potency and selectivity have also been identified effectively by employing series of different azabicycloalkanone amino acids at the same region of a peptide to provide a spectrum of backbone conformations and side chain orientations. Multiple methods are presently effective for the synthesis of 5-alkylproline derived analogs such as the indolizidin-2-one and pyrroloazepinone systems. More recently, methodology has been developed for making other ring systems such as those possessing 6-alkylpipecolates such as the indolizidin-9-one and quinolizidinone systems. Future application of azabicycloalkanone amino acids in peptide science is thus expected to increase particularly as methodology for their synthesis improves and a greater number of these peptide mimics become available to a broader community of peptide scientists.

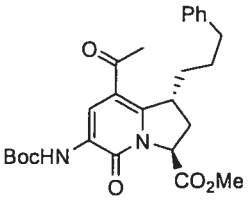
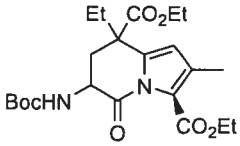
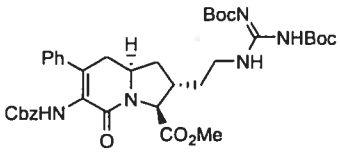
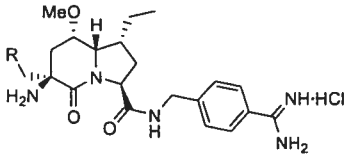
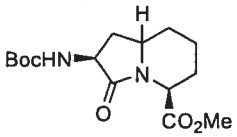
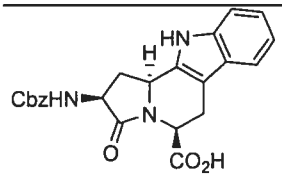
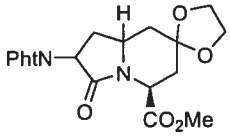
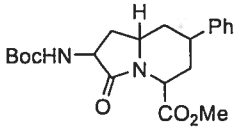
We are grateful to Dr. John Blankenship, Dr. Karl Hansford and Dr. Ramesh Kaul for their assistance in proof reading our earlier manuscript.

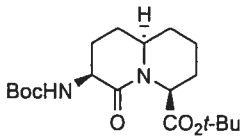
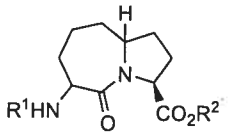
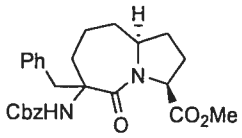
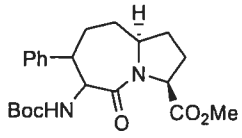
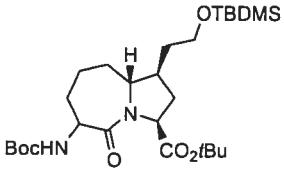
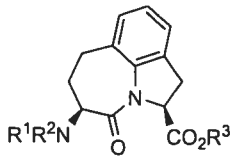
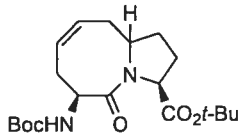
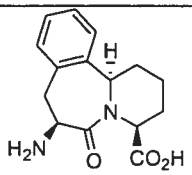
2.7. List of compounds.

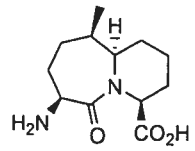
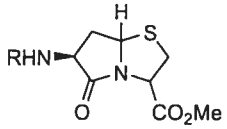
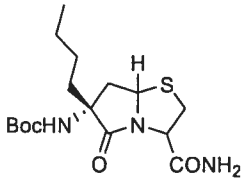
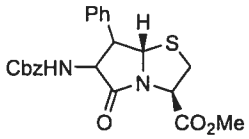
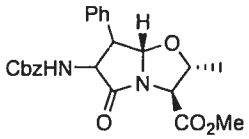
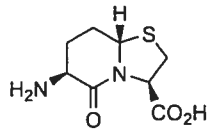
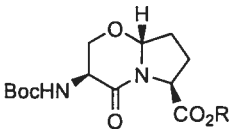
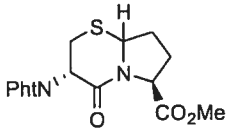
Structure	Compound	Synthesis	Applications (ref.)
	R = *t*-Bu ; (3*S*)-**9**, (3*R*)-**9** R = H ; (3*S*)-**16**	9 11	
	**26**	14	Antibiotic activity modulator : 14
	(3*S*)-**34**, (3*R*)-**34**	17	
	R^1 = Boc, R^2 = Me : **38** R^1 = Cbz, R^2 = Me : **39** R^1 = Boc, R^2 = *t*-Bu : **57**	21, 22, 24 22, 23 9	Caspase 1 / ICE inhibitors : 99 ORL1 antagonist : 115 Gramicidin S
	**63**	27	
	**69**	28	HRV 3CP inhibitor : 101
	R^1 = Cbz, R^2 = OH : **62** R^1 = COMe, R^2 = NHR : **384**	27 109	NK1 receptor antagonist : 109
	R = O*t*-Bu, Ar = phenyl : **81a** R = O*t*-Bu, Ar = 1-naphtyl : **81b** R = O*t*-Bu, Ar = 2-naphtyl : **81c** R = NHR', Ar = phenyl : **81**	29 29 29 32	FTAse inhibitors (Ar=Ph) : 102 thrombin inhibitor : 32, 94 $\alpha_v\beta_5$ inhibitor (Ar=Ph) : 126

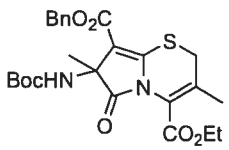
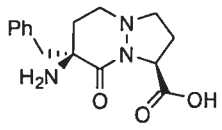
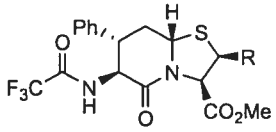
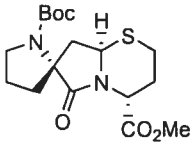
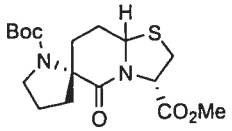
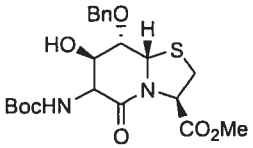
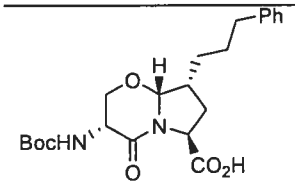
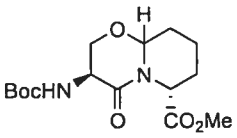
Structure	Compound	Synthesis	Applications (ref.)
	**406**	129	TRH receptor partial agonists : 104, 105
	**93**	33	
	**97**	36	
	**102**	37	
	**108**	38	
	**114**	39	
	R = CH_2OH : **124** R = CO_2H : **127**	42	
	R1 = Boc, R2 = Me : **112** R1 = Cbz, R2 = Et : **119**	39 40	

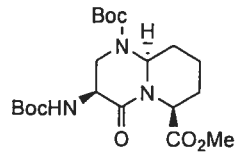
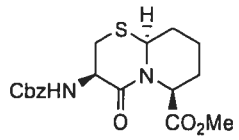
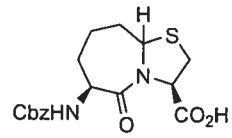
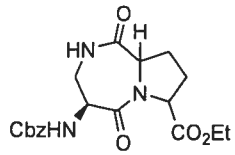
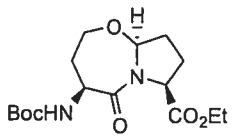
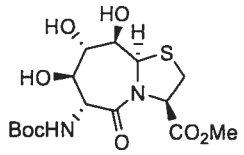
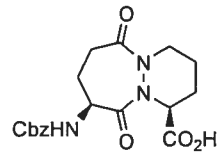
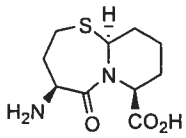
Structure	Compound	Synthesis	Applications (ref.)
	**125**	42	
	**129**	42	
	**135**	43	
	X = OTBDMS, R = Me : **138** X = N_3, R = H : **140**	45	
	**150**	46	
	**158**	40	Leu-enkephalin analog : 111
	**162**	39	
	**167**	37	

Structure	Compound	Synthesis	Applications (ref.)
	**170**	37	
	**175**	49	
	**184**	51	
	R=phenyl : **190a** R=cyclohexenyl : **190b** R=isopropenyl : **190c**	52,53	Thrombin inhibitors : 52,53
	(6*R*)-**196** (6*S*)-**196**	12 54	Leu-enkephalin analog : 112
	**207**	130	MMPs inhibitors : 103 CCK receptor antagonists : 106,107,108
	**216**	57	
	**210**	56	

Structure	Compound	Synthesis	Applications (ref.)
	**221**	58	ORL1 antagonist : 115, 116
	R^1 = Fmoc, R^2 = H : **235** R^1 = Boc, R^2 = Me : **238** R^1 = Boc, R^2 = *t*-Bu : **241**	58 26 9	thrombin inhibitor : 95 FTAse inhibitors : 102 $\alpha_V\beta_5$ and $\alpha_V\beta_3$ inhibitor : 125
	**248**	59	
	**252**	26	
	**255**	43	
	R^1=R^2=Pht, R^3=Et : **243** R^1=Ac, R^2=H, R^3=Me : **244** R^1=$COCF_3$, R^2=H, R^3=Me : **245**	131	ACE / NEP inhibitor : 91 Caspase 1 / ICE inhibitors : 98 Granzyme B inhibitor : 100 GH secretagogue : 119, 120 HLA-DR MHC : 121, 122
	**265**	72	
	**407**	132	ACE / NEP inhibitor : 84, 85, 90

Structure	Compound	Synthesis	Applications (ref.)
	**408**	133	ACE / NEP inhibitor : 86, 87
	**272**	134	PLG analogs : 117
	**366**	118	PLG analogs : 118
	**282**	68	
	**285**	68	
	**267**	64	thrombin inhibitor : 96 CGRP antagonists : 110 ORL1 antagonist : 67, 113, 114 $\alpha_V\beta_3$ inhibitor : 124
	R = Et : **290** R = Me : **294**	69 70	
	**297**	72	

Structure	Compound	Synthesis	Applications (ref.)
	**307**	73	
	**409**	135	thrombin inhibitor : 94
	R = Me : **313** R = H : **312**	74	
	**317**	77	PLG analogs : 77
	**319**	77, 136	PLG analogs : 77 SH3 domain binding : 136
	**331**	79	
	**410**	69	
	**336**	80	

Structure	Compound	Synthesis	Applications (ref.)
	**338**	80	
	**341**	80	
	**411**	137	Caspase 1 / ICE inhibitors : 99
	**348**	81	
	**350**	69	
	**354**	82, 83	
	**412**	138	Caspase 1 / ICE inhibitors : 97
	**413**	92	ACE / NEP inhibitor : 88, 89, 92

2.8. References.

† In the memory of professor Murray Goodman, an exceptional scientist and mentor, whose encouragement and enthusiasm for science will always be remembered.

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Chapitre 3.

Stratégie de synthèse pour la substitution de l'acide aminé indolizidin-9-one.

3.1. Stratégie.

La synthèse de l'acide aminé indolizidin-9-one non substitué ultérieurement développée dans le groupe part de l'α-*tert*-butyl-γ-méthyl-*N*-(PhF)aspartate **1**.[1] La fonction ester méthylique est ensuite, soit réduite pour donner l'aldéhyde **2**, soit alkylé par l'anion du méthyl(diméthyl)phosphonate pour donner le β-cétophosphonate **3**. La réaction de Horner-Wadsworth-Emmons entre **2** et **3** fourni le diamino azélate **4**. Celui-ci est ensuite soumis à la séquence amination réductrice / cyclisation de lactame / protection de l'amine pour donner l'I[9]aa (Schéma 1). La stratégie choisie pour la synthèse d'acides aminés indolizidin-9-ones substitués passe par la fonctionnalisation de l'intermédiaire linéaire diamino azélate **4** (Figure 1).

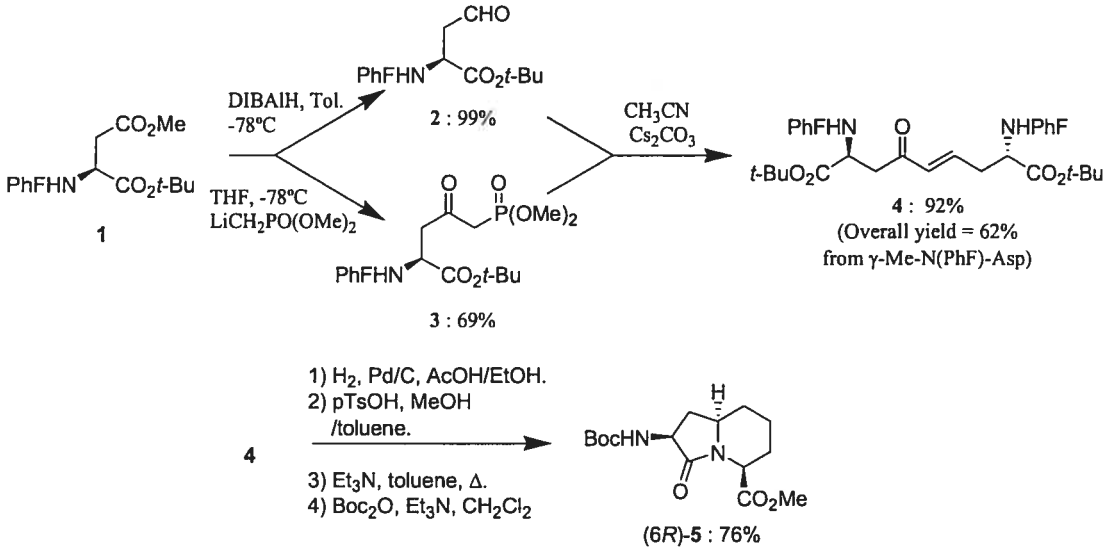

SCHÉMA 1. Synthèse de l'acide aminé indolizidin-9-one.

Parmi les stratégies possibles pour introduire un substituant, nous avons choisi d'étudier la réaction conjuguée sur la cétone α,β-insaturée (Figure 1). L'avantage de cette méthode est la régiosélectivité de la réaction pour la position 6. Un autre avantage est la très grande diversité de nucléophiles applicable à la réaction conjuguée. Les différents types de nucléophiles possibles sont résumés à la Figure 2.[2,3,4,5,6]

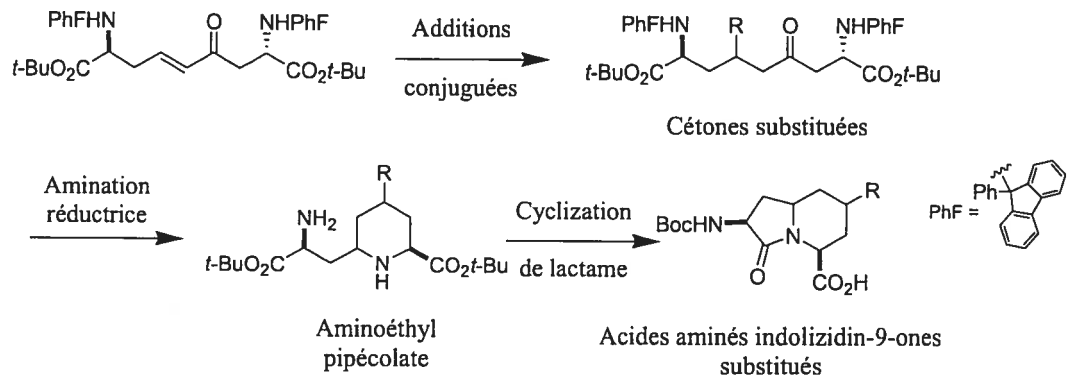

FIGURE 1. Stratégie générale pour la synthèse d'indolizidin-9-ones substitués.

La plupart de ces nucléophiles peuvent être utilisés sur l'énone **4**, tels que les anions dérivés de nitroalcanes, de malonates ou de glycines activées, les anions mous simples (CN, N_3, thiols) et les organocuivreux, les cuprates ou les réactifs organométalliques (Grignard, lithiens) catalysés par du cuivre. L'addition conjuguée de certains de ces nucléophiles sera décrite dans les chapitres 4 et 5. Certains nucléophiles ne pourront être utilisés car ils réagissent partiellement ou totalement sur la fonction cétone plutôt que sur l'oléfine. Les réactions qui utilisent des acides de Lewis forts, susceptibles d'induire une

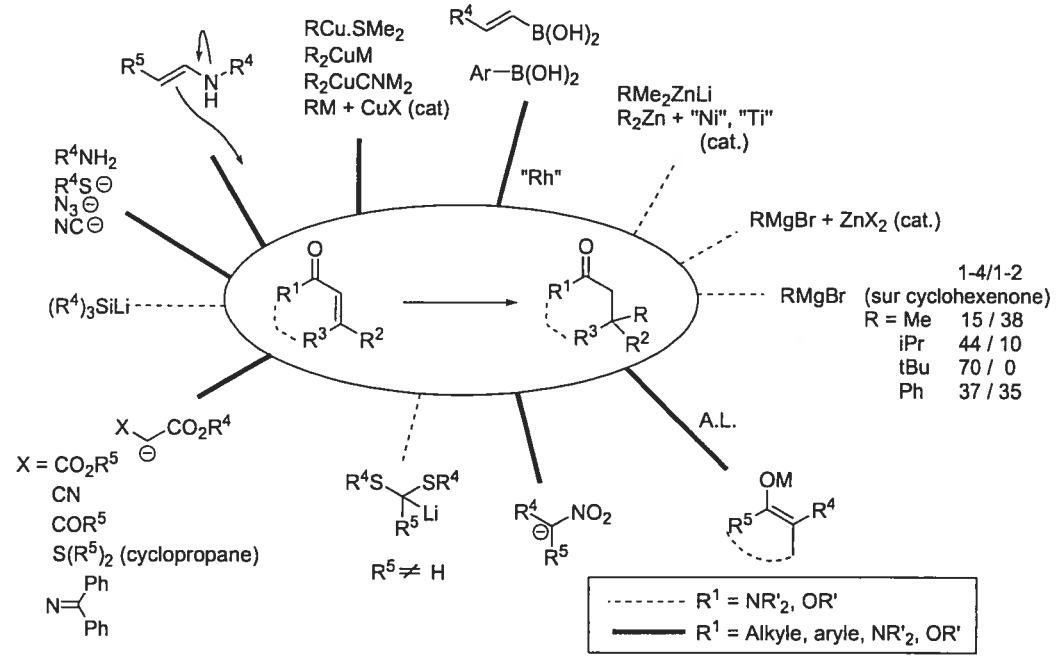

FIGURE 1 Différents nucléophiles connus pour réagir par addition conjuguée

déprotection des esters *tert*-butyliques ou des phénylfluorènylamines seront également à éviter.

3.2. Références.

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Chapitre 4.

Réactivité et sélectivité de l'addition conjuguée sur la cétone α,β-insaturée intermédiaire.

4.1. Introduction.

En vue de déterminer le potentiel et les limites de la voix de synthèse choisie pour introduire la chaîne latérale, différents essais d'additions conjuguées ont été effectués sur le diaminoazélate **1** à l'aide de nucléophiles mous tels que représentés à la Figure 1 et connus pour réagir de manière conjuguée.

FIGURE 1 Nucléophiles connus pour réagir de manière conjuguée.

4.2. Études préliminaires et essais.

L'anion du malonate de diméthyle réagit bien, pour donner à 77% le composé **2a** dans un rapport diastéréomérique (r.d.) de 1:1. Les diastéréoisomères observés par RMN du proton et du carbone 13 n'ont jamais pu être séparés par CCM ou par chromatographie flash. Les esters méthyliques pouvant réagir intramoléculairement avec les fonctions amines libres formées durant la réaction d'amination réductrice, ils doivent être modifié pour les rendre non-réactif. La méthode choisie passe par leur saponification, qui doit entraîner la décarboxylation d'une des deux fonctions acides. La fonction restante doit ensuite être réduite à l'alcool et protégé. La saponification est quantitative mais donne le diacide libre **4** plutôt que le mono acide **3**. Les différentes méthodes essayées pour forcer la décarboxylation sont le chauffage en milieu acide ou la réaction de Krapcho.[1] Elles n'ont conduit qu'à la décomposition du produit. L'addition de l'acide de Meldrum (2,2-diméthyl-[1,3]dioxane-4,6-dione) au lieu du malonate de diméthyle est un moyen de facilité la décarboxylation. Malheureusement, l'addition conjuguée de l'acide de Meldrum est très lente et ne conduit qu'à des traces du diamino azélate désiré.

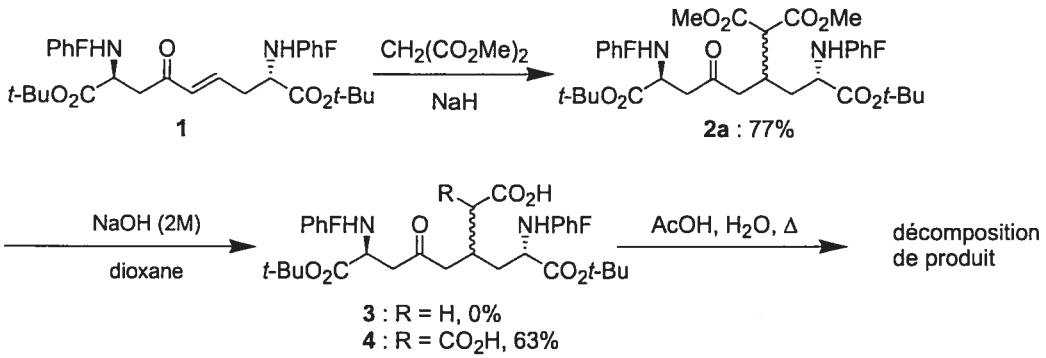


SCHÉMA 1 Synthèse du diamino azélate **2a** et modofication de la fonction malonate.

L'anion du nitrométhane réagit également très bien pour donner le composé **2b** dans un rendement de 99% et un r.d. de 2:1 (Schéma 2). Les diastéréoisomères observés par RMN du proton et du carbone 13 n'ont jamais pu être séparés par CCM ou par chromatographie flash. La fonction nitro devant être réduite puis protégée avant l'étape d'amination réductrice, différentes méthodes de réduction ont été tentées et sont présentées dans le tableau 1. Ces différents essais de réduction ont été effectués à partir du nitroalcool **5** obtenu sous forme d'un mélange de quatre diastéréoisomères par réduction de la cétone par NaBH4. Toutes ces méthodes n'ont conduit qu'au recouvrement du produit de départ ou à la décomposition du produit **5** par perte de phénylfluorènyle ou réduction, déprotection ou réaction intramoléculaire des esters *tert*-butyliques.

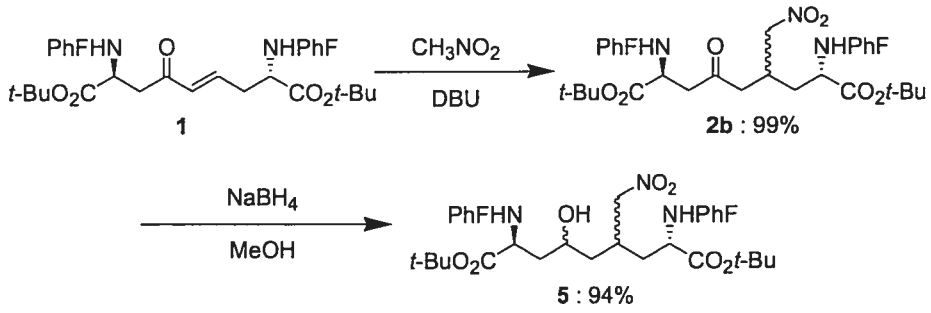


SCHÉMA 2 Synthèse des diamino azélates **2b** et **5**..

TABLEAU 1 Tentatives de réduction de la fonction nitro du composé **5** en amine.

Conditions de réduction de **5**	Résultats
H_2 (1 atm), Pd/C, AcOEt	Pas de réaction (P.R.)
H_2 (2.5 atm), Pd/C, AcOEt	Décomposition (D) : - PhF
H_2 (1 atm), Pt/C, AcOEt	P.R.
H_2 (1 atm), PtO_2, EtOH, $CHCl_3$	D : - PhF
$NaBH_4$, Pd/C, THF	D : - *t*-Bu
$HCO_2^-NH_4^+$, Pd/C, THF/MeOH	P.R.
H_2 (1 atm), Ni Raney, MeOH	D : - *t*-Bu
$NiCl_2$, $NaBH_4$, MeOH	D : - *t*-Bu
SmI_2, THF, MeOH	P.R.
$LiAlH_4$, THF, -78°C à -25°C	P.R.
$LiAlH_4$, THF, 0°C	D lente : - *t*-Bu

Le cyanure de potassium réagit beaucoup plus lentement que le malonate ou l'anion du nitrométhane pour donner le composé **2c** en 32% de rendement. La réaction peut néanmoins être poussée jusqu'à un rendement de 68% par l'ajout d'éther couronne 18-6 qui permet de dissocier le potassium du cyanure. Différentes méthodes de réduction de la fonction nitrile, tels que le DIBAl-H à différentes températures, le $BH_3{\cdot}SMe_2$ ou le nickel de Raney ont conduit, soit au recouvrement du produit de départ, soit à une décomposition par surréaction du produit.

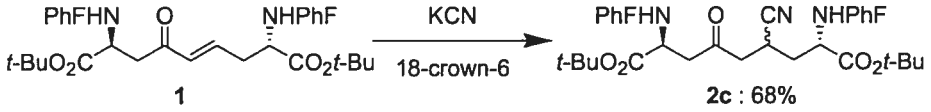


SCHÉMA 3 Synthèse des diamino azélates **2c**.

Différents analogues d'amines ont également été essayées tels que l'azoture de sodium ou le carbamate de méthyle catalysé par un métal. L'azoture de sodium ne réagit pas. Le carbamate de benzyle est décrit comme pouvant être additionné de manière conjuguée à toute une série de substrats en présence de différents métaux.[2,3,4,5] Dans notre cas, le groupe protecteur doit résister aux conditions d'hydrogénation, le carbamate de méthyle a donc été substitué au carbamate de benzyle. La réaction a été effectuée en présence de chlorure d'argent, de trichlorure de ruthénium, de nitrate de thallium, de triflate

d'ytterbium ou de trifluorure de bore. Malheureusement aucune réaction n'a été observée.

Les alkyles cuivreux, cuprates et magnésiens ont ensuite été essayés et les résultats obtenus sont décrits dans le chapitre 5 ci-après.

4.3. Conclusions.

L'introduction de carbanions activés semble relativement facile mais conduit à des mélanges de diastéréoisomères non séparables. La réduction sélective des groupes électroattracteurs introduits, nitro et nitrile, ainsi que la décarboxylation du malonate se sont avérées impossibles. Les problèmes de mauvaises diastéréosélectivités et de modifications des groupes fonctionnels introduits restent encore à être surmonter pour permettre l'utilisation de ces voies de synthèse pour l'introduction de chaînes latérales sur l'acide aminé indolizidin-9-one.

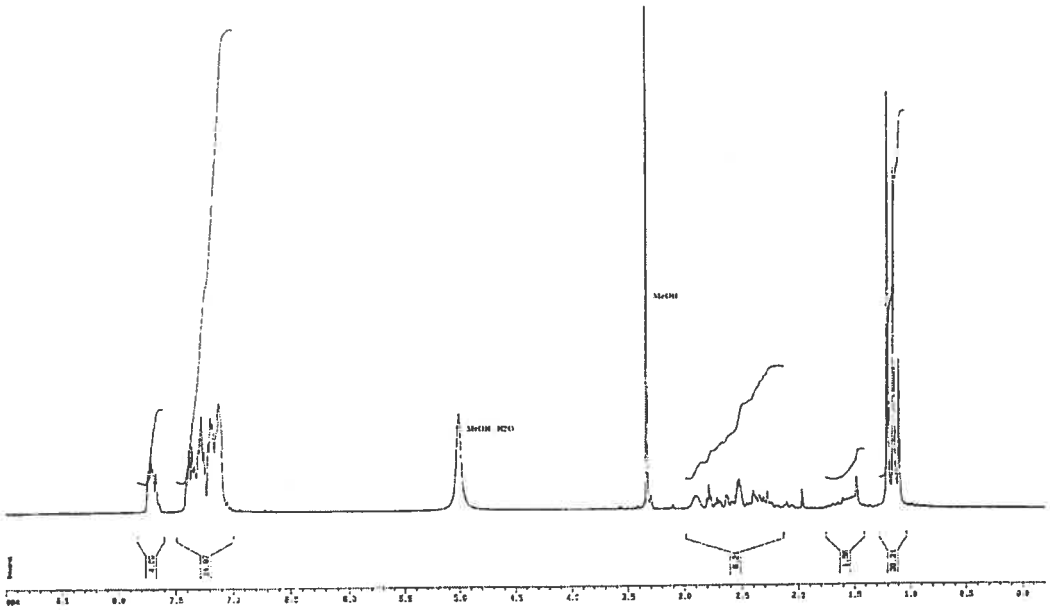
4.4. Partie expérimentale.

Les chromatographies flash ont été réalisées sur du gel de silice 230-400 mesh. Les RMN ^{1}H et ^{13}C ont été prises dans le $CDCl_3$, le C_6D_6 et le CD_3OD sur des appareils Bruker AMX300 et ARX400. Les spectres sont référencés sur le tétraméthylsilane pour le $CDCl_3$ et le C_6D_6 et sur le méthanol résiduel à 3.3 ppm pour le CD_3OD. Les signaux aromatiques en RMN ^{13}C ne sont pas rapportés dans le cas des composés contenant le groupe protecteur PhF. Pour les détails des composés **2a**, **2b** et **2c**, partie expérimentale et caractérisations, voir le matériel supplémentaire au chapitre 5 (Ch. 5.5.).

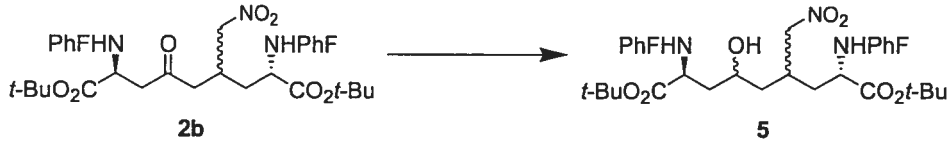
(2*S*,6*SR*,8*R*)-Di-*tert*-butyl-4-oxo-6-(dicarboxyméthyl)-2,8-bis[N-(PhF)amino]-azelate (3).

2a **4**

Une solution du diester **2a** (1.14 g, 1.2 mmol) dans le dioxane (3 ml) est traitée avec une solution de NaOH 2M (3 ml) puis est chauffée pour 2 h à reflux. La solution est reprise dans un mélange NaH_2PO_4 1 M / acétate d'éthyle. Les phases sont séparées et la phase organique est ré-extraite 3x avec acétate d'éthyle. Les phases organiques sont réunies, lavées avec de la saumure, séchées avec Na_2SO_4 puis évaporées. L'huile obtenue est purifiée par chromatographie flash avec un gradiant de 80 / 20 / 1 (hexane / AcOEt / AcOH) à 70 / 30 / 1 (hexane / AcOEt / AcOH) pour donner 4 (696 mg, 63%) comme un mélange de diastéréoisomères. ^{t}R=0.1 (80/20/1 : hexane/AcOEt/AcOH); RMN ^{1}H δ (CD_3OD) 7.77–7.61 (m, 4H), 7.42-7.20 (m, 22H), 2.90 (sl, 1H), 2.78 (t, 0.5H, *J*=7.0 Hz), 2.70 (m, 0.5H), 2.64-2.05 (m, 5.5H), 1.78-1.49 (m, 1.5H), 1.19 (s, 9H), 1.16 (s, 6H), 1.10 (s, 3H). Masse (FAB+) m/z 927.4 ($M + H^+$).

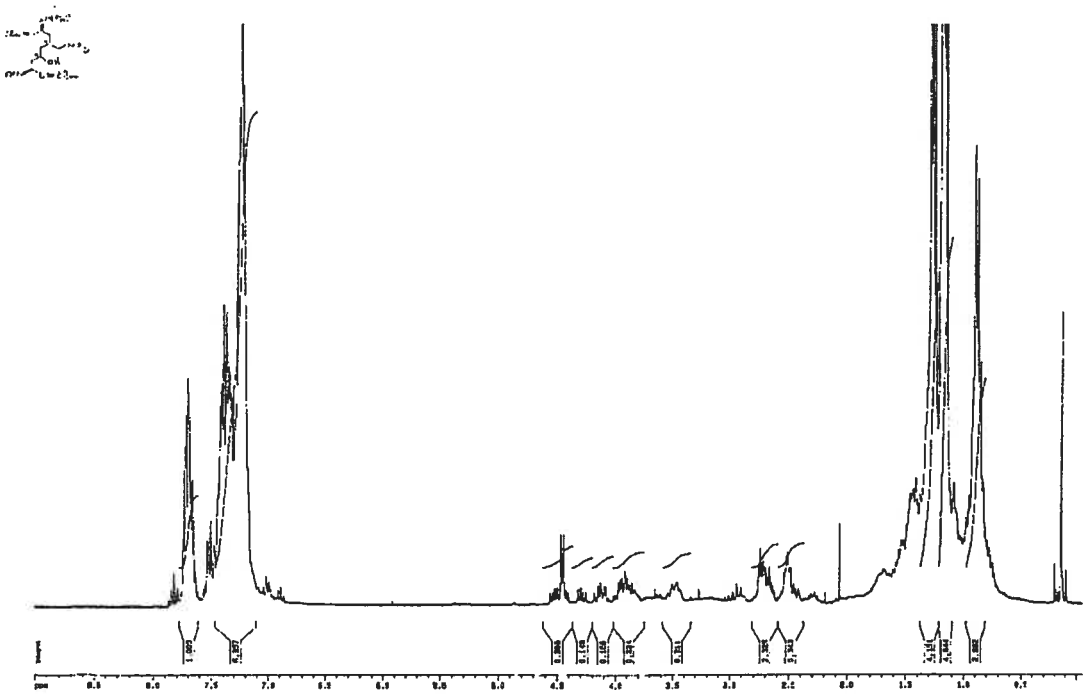


(2*S*,4*SR*,6*SR*,8*R*)-Di-*tert*-butyl-4-hydroxy-6-nitrométhyl-2,8-bis[N-



(PhF)amino]-azelate (5).

Une solution du diester **2b** (257 mg, 0.29 mmol) dans le méthanol sec (25 ml) est traitée avec $NaBH_4$ (44 mg, 400 mol%) puis agitée à température pièce pour une nuit. La réaction est neutralisée par l'ajout d'eau (15 ml) puis le méthanol est évaporé. La phase aqueuse est reprise dans un mélange AcOEt (60 ml), saumure (15 ml) et NaOH 1M (30 ml). Les phases sont séparées et la phase aqueuse est ré-extraite 3x avec de l'AcOEt (75 ml). Les phases organiques sont réunies, lavées avec de la saumure, séchées avec Na_2SO_4, filtrées puis évaporées. Le produit est rapidement purifié sur silice pour donner **5** (243 mg, 94%) sous forme d'une mousse blanche. ^tR=0.29 (80/20 Hexane/AcOEt); RMN ^1H très complexe car mélange de quatre diastéréoisomères (voir ci-dessous), Masse (FAB+) m/z 886.5 ($M + H^+$).



4.5. Références.

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Article 2.

Cluzeau, J.; Lubell, W.D. 'Remarkable Asymmetric Induction from Remote Stereocenters in Conjugate Addition Chemistry for the Synthesis of Alkyl-Branched α,ο-Diaminoazelates' publié dans *Israel Journal of Chemistry* **2001**, *41*, 271-281. dans un numéro spécial dédié aux professeurs H.B. Kagan, R. Noyori et B.K. Sharpless pour le prix de la Wolf Foundation 2001.

Remarkable Asymmetric Induction from Remote Stereocenters in Conjugate Addition Chemistry for the Synthesis of Alkyl-Branched α,ω-Diaminoazelates†

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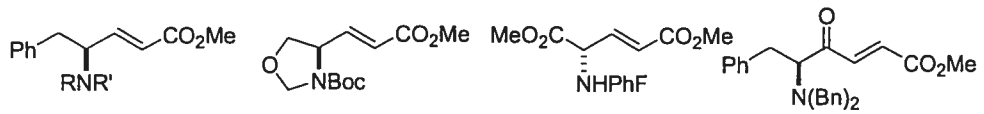
4.6. Abstract.

Conjugate addition of aryl Grignard reagents to (2*S*, 5*E*, 8*S*)-di-*tert*-butyl 4-oxo-2,8-bis-[*N*-(PhF)amino]non-5-enedioate (**6**, PhF = 9-(9-phenylfluorenyl)) in THF proceeded with complete chemoselectivity and >9:1 stereoselectivity to provide predominantly (2*S*,6*R*,8*S*)-6-aryl 4-oxo-2,8-diaminoazelates **7**. In the presence of magnesium dibromide, diastereoselectivity in the addition of PhMgBr to enone **6** was improved to 15:1 in favor of the 6*R*-isomer. Although lower chemoselectivity and stereoselectivity were obtained from the analogous reaction of **6** with *iso*-propyl magnesium bromide in the absence of $MgBr_2$, both were improved significantly when the addition reaction was performed in the presence of $MgBr_2$. In contrast, the corresponding higher-order cuprates reacted with low diastereoselectivity on **6** by a 1,4-addition pathway. In an attempt to identify the source of the high selectivity in the conjugate addition chemistry with **6** and Grignard reagents, the syntheses of enones **12** and **13** provided model systems in which one of the two amino carboxylate moieties of **6** were replaced by a branched alkyl substituent. Conjugate addition reactions on **12** and **13** demonstrated that chemoselectivity with Grignard reagents in the 1,4-addition reaction was contingent on the presence of an amino carboxylate moiety near the ketone of the enone system. Furthermore, because diastereoselectivity with Grignard reagents was significantly lower in additions to amino enones **12** and **13** relative to diamino enone **6**, the presence of both amino carboxylate moieties has been highlighted as an important factor for remarkable asymmetric induction in the conjugate addition of Grignard reagents.

4.7. Introduction

Conjugate addition (1,4-addition) reactions on α,β-unsaturated carbonyl compounds are among the most useful methods for making carbon-carbon and carbon-heteroatom bonds.[1] The development of stereocontrolled conjugate addition chemistry has thus been an active area of research from which successful enantioselective and diastereoselective methods have emerged.[1] For example, ligands derived from BINOL,[2] TADDOL[3] and oxazolines,[4] all have been successfully used in catalytic enantioselective conjugate addition reactions on cyclic and acyclic systems. Diastereoselective conjugate addition reactions have similarly been successful using enamide and enoate systems[1d] possessing chiral auxiliaries linked to the carboxylate, such as sultams,[5] carbohydrates,[6] oxazoline derivatives[7] and binaphtol.[8] Chiral auxiliaries positioned at the β-carbon of enones, such as sulfoxides[9] and *N*-(acyl)prolinol ethers,[10] have also directed conjugate additions with good diastereoselectivity.

A stereocenter present in the substrate can serve to direct the stereochemical outcome of the conjugate addition through steric interactions[11] or electronic chelation.[12-14] For example, in the three-component coupling synthesis of prostaglandins developed by Noyori and coworkers,[11d] the steric effect of a neighboring *tert*-butyldimethylsiloxy substituent causes the incoming organocopper reagent to add with complete stereoselectivity.[11c] On the other hand, a coordinating oxygen atom can be used to achieve diastereoselective induction. For example, 1,2-induction from a chiral acetal directed cuprate addition to furnish selectively a key intermediate in the synthesis of levuglandin E2.[13a] Benzyl ethers have similarly been shown to provide favorable 1,2-induction in diastereoselective conjugate additions of allylsilanes by Lewis acid chelation with $TiCl_4$.[13b]

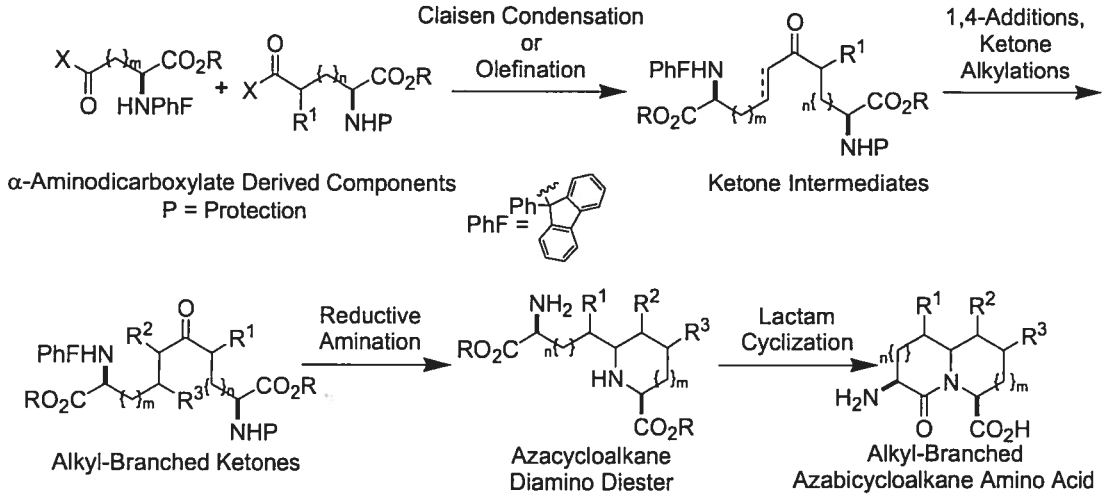


2a : R = R' = Bn
2b : R = Boc, R' = H

3 **4** **5**

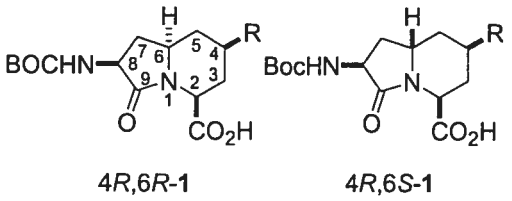
Figure 1. Representative amine-bearing α,β-unsaturated esters studied previously in conjugate chemistry

The directing effects of nitrogen substituents on the conjugate addition chemistry of organometallic reagents have been less well studied than those of their alkyl and oxygen counterparts.[14-19] Among the few acyclic examples,[19] nitrogen substituents at the γ-position of α,β-unsaturated esters have exhibited varying influences on the approach of the attacking nucleophile contingent on the amine protecting group.[14-18] Synthesized typically by olefination of their respective α-amino aldehyde counterpart,[20] the γ-amino α,β-unsaturated ester derivatives have reacted to provide product with varying stereoselectivity dependent upon the conditions of the conjugate addition chemistry. For example, Gilman reagents (R_2CuLi)[21] reacted diastereoselectively with phenylalaninal-derived γ-dibenzylamino α,β-unsaturated ester **2a** (Figure 1) to furnish predominant *syn*-products (11:1 to >19:1) in the presence of trimethylsilylchloride, yet failed to react in its absence.[14] On the other hand, when the same R_2CuLi reagents were employed with TMSCl on the related γ-*N*-(BOC)amino α,β-unsaturated ester **2b**, diastereoselectivity diminished (3:1 to 4:1 *syn:anti*).[14] High diastereoselectivity was recovered in this system on employment of the γ-*N*-benzyl-*N*-(BOC)amino α,β-unsaturated ester derivative.[15] Serinal-derived γ-*N*-(Boc)oxazolidino α,β-unsaturated ester **3** has reacted with both R_2CuLi and $Ar_2CuMgBr$ reagents in the presence of TMSCl to afford predominant *syn*-products from 1,4-addition with diastereoselectivity varying from 4:1 to >50:1 dependant on the nature of the cuprate reagent.[16] In an example more closely related to the present work, *N*-PhF-3,4-didehydroglutamate **4** reacted with Me_2CuLi in the absence of TMSCl to afford a 1:1 mixture of β-methyl glutamate derivatives, yet decomposed in the presence of TMSCl (PhF = 9-(9-phenylfluorenyl)).[17] These few examples demonstrate that successful 1,2-induction can afford predominant *syn*-product contingent on the conditions and substrate in conjugate addition reactions on *N*-protected γ-amino α,β-unsaturated ester systems. In a rare case of 1,4-induction by a nitrogen substituent, (5*S*)-benzyl 5-*N*,*N*-dibenzylamino-4-oxo-6-phenyl-hex-2-enoate **5** was found to react regioselectively with organocopper reagents (RCu) in the presence of diethylaluminum chloride to afford the 2-substituted adducts in moderate yields with high diastereoselectivity (9:1 to 16:1 *anti*:*syn*).[18]



SCHEME 1. General strategy for azabicyclo[X.Y.0]alkane amino acid synthesis

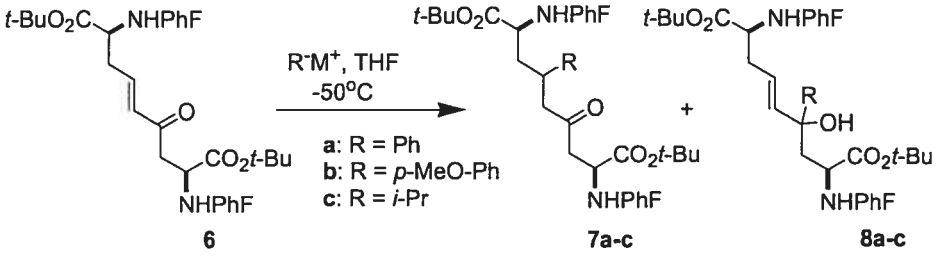
In the context of our research on peptide mimicry,[22] we became interested in studying the influence of *N*-(PhF)amino carboxylate moieties in the conjugate addition reactions of α,β-unsaturated ketones as a means for installing side-chain functional groups onto conformationally rigid dipeptide surrogates. Recently, we reported that the Horner-Emmons olefination of aspartate β-aldehyde **10** with β-ketophosphonates, derived from aspartate, glutamate and α-aminoadipate, furnished a series of linear enone intermediates for the synthesis of azabicyclo[X.Y.0]alkane amino acid analogs (Scheme 1).[23] In this process, exposure of such linear ketone intermediates to reductive aminations, methanesulfonate displacements and lactam cyclizations has afforded the spectrum of fused 5,6-, 6,6-, 6,5- and 7,5-ring systems with stereocontrol and capacity for appending side-chains onto the heterocycle.[22] For example, the olefination of aspartate β-aldehyde **10** with aspartate derived β-ketophosphonate **11** furnished α,ω-diaminoazelate **6** which after reductive amination, ester exchange, lactam cyclization and amine protection provide the fused 5,6-ring system, (2*S*,6*R*,8*S*)-indolizidin-9-one amino acid [I^9aa, (2*S*,6*R*,8*S*)-**1a**, (Figure 2)] in good overall yield.[23a] Applying I^9aa in the study of biologically active peptides, the (2*S*,6*R*,8*S*)-isomer has served as a β-turn mimic to examine the spatial requirements for activity at the different opioid receptor subtypes.[24]



4*R*,6*R*-**1** 4*R*,6*S*-**1**

Figure 2. 4-Substituted indolizidin-9-one amino acids **1**, **1a** (R = H) and **1b** (R = Ph).

In light of the importance of amino acid side-chains in recognition events involving β-turn geometry, we have begun to focus our attention on employing conjugate additions to enone **6** to synthesize effectively different substituted analogues of I^9aa **1**. The development of stereoselective conjugate addition chemistry on enone **6** was particularly desired in order to furnish specific diastereomers of the final 4-substituted indolizidin-9-one amino acid targets.



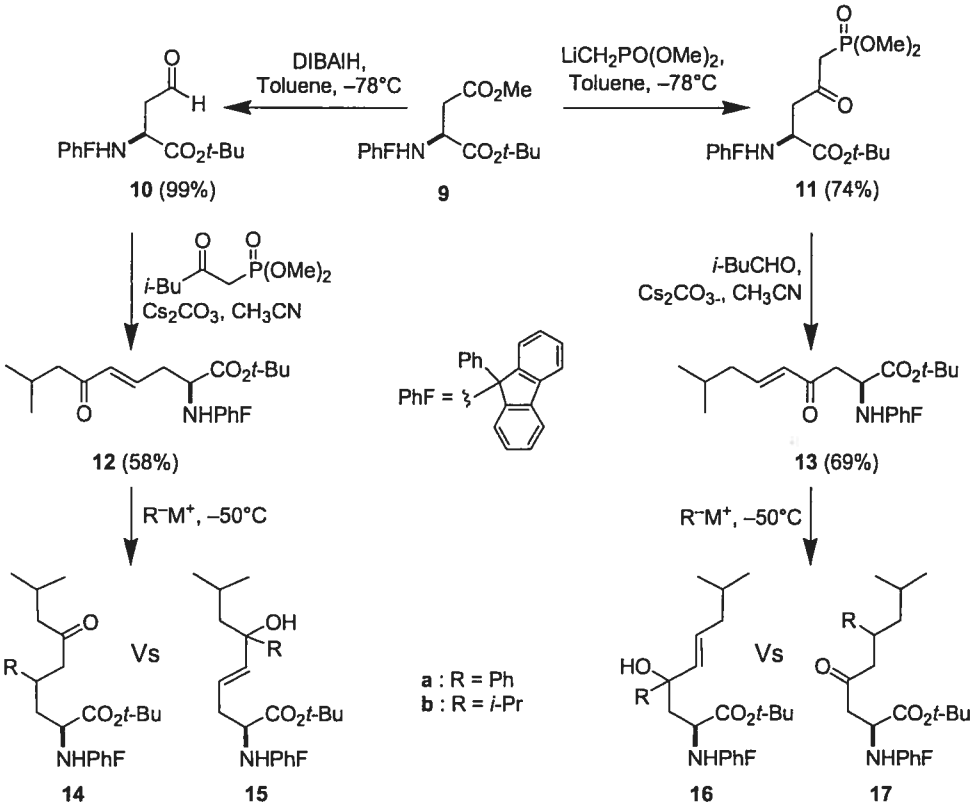
SCHEME 2. Organometallic additions to enone **6**.

In our preliminary investigations,[25] we noted that conjugate addition of the higher order cuprate $Ph_2CuCN(MgBr)_2$ to enone **6** proceeded in excellent yield to provide ketone **7a** as a mixture of diastereomers that could be separated by column chromatography (Scheme 2). The desired 4-phenyl indolizidin-9-one amino acid analogs (2*S*,4*R*,6*R*,8*S*)- and (2*S*,4*R*,6*S*,8*S*)-**1b** were subsequently prepared by treating the (4*S*)-diastereomer of 4-phenyl ketone **7a** under the same reductive amination / lactam cyclization protocol as used to furnish the parent I^9aa **1a** (Figure 2).[23a,25] In addition to establishing proof of concept for the addition of side-chains to I^9aa **1**, this sequence of experiments allowed us to establish the relative stereochemistry of the conjugate addition product **7a** by detailed two-dimensional NMR experiments on the final fused 5,6-ring system **1b**.[25] Pursuing this approach, we tried next PhMgBr in the

conjugate addition and were pleased to observe complete 1,4-regioselectivity and high diastereoselectivity [9:1 (6*S*)-:(6*R*)-**7a**]. This interesting result has now led us to investigate this conjugate addition chemistry with other organometallic reagents, to explore the influence of chelating metals on stereoselectivity and to study the effect of each nitrogen bearing stereocenter on the chemoselectivity and stereoselectivity of the addition reaction.

4.8. Results and Discussion.

Three enones **6**, **12** and **13** were synthesized in order to study the importance of each of the two amino carboxylate centers on the selectivity of the conjugate addition chemistry. As an inert, achiral replacement for each of the amino carboxylate moieties of **6**, the *iso*-butyl group was introduced into enones **12** and **13** in order to maintain the chain-length between the conjugated system and branching unit without introducing the additional coordinating and steric effects found in the parent system. As in the synthesis of α,ω-



SCHEME 3. Synthesis and organometallic additions to enones **12** and **13**.

diaminoazelate **6**, enones **12** and **13** were prepared from γ-methyl α-*tert*-butyl *N*-(PhF)aspartate **9** (Scheme 3). As previously described,[23a,26] aspartate β-aldehyde **10** was synthesized in quantitative yield by reduction of **9** with DIBAl-H in toluene at –78°C. β-Ketophosphonate **11** was prepared in 74% yield by acylation of the lithium anion of dimethylmethylphosphonate with **9**.[23a] Dimethyl-4-methyl-2-oxopentylphosphonate was similarly prepared in 97% yield by treating ethyl *iso*-valerate with an excess of the lithium anion of dimethylmethylphosphonate. Enone **12** was prepared by Horner-Emmons olefination of aldehyde **10** with dimethyl-4-methyl-2-oxopentylphosphonate in acetonitrile using $CsCO_3$ as base and gave a 9:1 *E*-:*Z*-isomeric mixture of **12** in 75% yield, from which pure *E*-**12** was obtained in 58% yield after purification by chromatography. Isomerically pure *E*-enone **13** was synthesized by olefination of *iso*-valeraldehyde with β-ketophosphonate **11** using similar conditions in 69% yield.

Table 1. Yields and diastereoselectivity from organometallic additions to enone **6**.

entry	organometallic reagents	mol%	isolated yield of **7** (%)	d.r. of **7**[a]	ratio (**7**:**8**)	yield of **8** (%)
1	$Ph_2CuCN(MgBr)_2$	200	98	3:2	1:0	-
2	$Ph_2CuCN(MgBr)_2$ / TMSCl	200 / 100	95	3:2	1:0	-
3	$Ph_2CuCNLi_2$	200	58	3:2	1:0	-
4	$PhCu{\cdot}SMe_2$	100	74	3:1	1:0	-
5	$(p\text{-MeO-Ph})_2CuCN(MgBr)_2$	200	98	2:1	1:0	-
6	$i\text{-Pr}_2CuCN(MgBr)_2$	200	80	5:1	1:0	-
7	*i*-PrMgBr / CuI (cat.)	300 / 10	83[b]	5:1	1:0	-
8	PhMgBr	300	86	1:9	1:0	-
9	PhMgBr	100	70[b]	1:9	1:0	-
10	*p*-MeO-PhMgBr	300	82	1:10	1:0	-
11	*i*-PrMgBr	300	71	2:1	2:1[a]	23[a,b]
12	*i*-PrMgBr	100	19[b]	3:1	2:1[a]	6[b]

[a]Diastereomeric ratios (d.r.) were determined by ^1H NMR spectroscopy of crude product in $CDCl_3$ by measuring *t*-Bu signals respectively at 1.16 and 1.12 ppm for **7a**, 1.22 and 1.17 ppm for **7b**, as well as in C_6D_6 by measuring methyl group doublets centered at 0.74 and 0.68 ppm for **7c**, and 0.56 and 0.43 ppm for **8c**. [b]NMR yield.

A series of organometallic additions were initially performed on enone **6** to study the conjugate addition chemistry for introducing alkyl side-chains onto azelate **7** (Table 1). Among the three different phenyl copper reagents

examined (entries 1-4), the higher order cuprate derived from the addition of phenyl magnesium bromide to CuCN provided complete 1,4-addition in excellent yield albeit with low diastereoselectivity [2:3 (6*R*)-:(6*S*)-**7b**]. Attempts to improve selectivity using the higher order cuprate derived from the addition of phenyl lithium to CuCN as well as the phenylcopper-DMS complex,[21] both resulted in lower yields with limited gain in diastereoselectivity. Furthermore, diastereoselectivity was not improved with the addition of trimethylsilylchloride to the cuprate reaction (entry 2).[27] Subsequently, higher order cuprates prepared from CuCN and *para*-methoxyphenyl and *iso*-propyl magnesium bromide, respectively were shown to react with enone **6** to provide 1,4-addition products **7** with low selectivity (entries 5 and 6). In the best case, *iso*-propyl cuprate provided ketone **7c** as a 5:1 ratio of diastereomers. Similar yield and selectivity were also achieved using *iso*-propyl magnesium bromide in the presence of catalytic CuI (entry 7).

In contrast to the low diastereoselectivity from aryl cuprate additions to **6**, the corresponding aryl Grignard reagents (entries 8-10) reacted with higher selectively favoring the opposite diastereomer of the 1,4-addition product **7** [9-10:1 (6*R*)-:(6*S*)-**7**].[28] Although the aryl Grignard reagents reacted completely by a 1,4-addition route, competitive 1,2-addition chemistry was observed in the case of *iso*-propyl Grignard which produced predominantly ketone **7c** with low diastereoselectivity contaminated with tertiary alcohol **8c** (entries 11 and 12, Table 1, Scheme 2).[29]

Table 2. Yields and diastereoselectivity from organometallic additions to enones **12** and **13**.

entry	Organometallic reagents (mol%)	Enone	Ketone	% Yield (d.r.)	Alcohol (% Yield)
1	Ph-MgBr (300)	**12**	**14a**	30 (3:1)	**15a** (41)
2	Ph-MgBr (300) / $MgBr_2$ (300)	**12**	**14a**	33 (2:1)[a]	**15a** (66)[a]
3	$Ph_2CuCN(MgBr)_2$ (200)	**12**	**14a**	29 (1:1)	-
4	*i*-$Pr_2CuCNMgBr_2$ (200)	**12**	**14b**	74 (1:1)	-
5	Ph-MgBr (300)	**13**	**16a**	99 (4:1)	-
6	Ph-MgBr (100)	**13**	**16a**	70 (3:1)	-
7	*i*-Pr-MgBr (300)	**13**	**16b**	82 (1:1)	-
8	$Ph_2CuCN(MgBr)_2$ (200)	**13**	**16a**	97 (1:1)	-
9	*i*-$Pr_2CuCNMgBr_2$ (200)	**13**	**16b**	78 (1:1)	-

[a]NMR yield

To further study the importance of each amino carboxylate moiety for chemoselectivity and diastereoselectivity in the conjugate addition reactions of Grignard reagents to enone **6**, we examined next addition chemistry on enones **12** and **13** using the higher order cuprate and the Grignard reagents produced from bromobenzene and *iso*-propyl bromide (Scheme 3, and Table 2). The chemoselectivity of the addition of Grignard reagents to enones **12** and **13** was significantly influenced by the relative position of the amino carboxylate moiety. With phenyl magnesium bromide, ε-keto amino ester **12** reacted by both 1,2- and 1,4-addition pathways producing nearly equal amounts of ketone **14a** and tertiary alcohol **15a** (Table 2, entry 1). On the other hand, γ-keto amino ester **13** reacted chemoselectively with phenyl magnesium bromide and *iso*-propyl magnesium bromide by 1,4-addition routes to afford respectively ketones **16a** and **16b** in 99% and 82% yields (Table 2, entries 5-7). Diastereoselectivity was diminished in the 1,4 additions to amino enones **12** and **13** relative to diamino enone **6**. For example, the 9:1 selectivity obtained from 1,4-addition of PhMgBr to **6** was reduced to 3-4:1 from additions to **12** and **13**. Similarly, the low (2:1) selectivity obtained with *i*-PrMgBr and **6** was lost in addition to **13**. As observed in the addition of methyl cuprate to *N*-PhF-3,4-didehydroglutamate **4**,[17] the higher order cuprates from PhBr and *i*-PrBr reacted chemoselectively with **12** and **13** to give respectively 1,4-addition products **14** and **16**, each as a 1:1 mixture of diastereomers (Table 2, entries 3, 4, 8 and 9).

Attempting to improve selectivity, we examined next the influence of added salts on the addition of Grignard reagents to enone **6**. Chelating metal salts have been shown to greatly influence the outcome of conjugate addition reactions.[1,9,10,12,13,18,30] For example, the chelating effect of $ZnBr_2$ has significantly enhanced diastereoselectivity in conjugate additions of diorganomagnesium and Grignard reagents to cycloalkenones.[9,10] As mentioned earlier, aluminum containing Lewis acids, such as dialkylaluminum chlorides have improved the regioselectivity and diastereoselectivity in conjugate additions to enones and enamides.[18,30]

Table 3. Additon of Grignard reagents to enone **6** in THF in the presence of added salt (300 mol%).[a]

RMgBr (mol%)	salt	%conv.	d.r. of **7**	ratio **7:8**	d.r. of **8**
i-Pr (300)	-	86	1.5 : 1	2 : 1	4 : 1
i-Pr (300)	NaI	27	2 : 1	1.5 : 1	3 : 1
i-Pr (300)	LiBr	57	2 : 1	4 : 1	3 : 1
i-Pr (300)	$ZnCl_2$	21	3 : 1	1 : 0	-
i-Pr (300)	$MgBr_2$	100	6.5 : 1	4 : 1	7.5 : 1
i-Pr (100)	$MgBr_2$	22	4.5 : 1	1 : 0	-
i-Pr (300)	$CeCl_3$	70	3.5 : 1	6 : 1	4 : 1
Ph (300)	$MgBr_2$	100	15 : 1	1 : 0	-

[a]Percent conversion and product ratios were calculated using ^{1}H NMR spectroscopy by measuring the diastereotopic methyl group doublets centred at 0.74 and 0.68 ppm for **7c** and 0.56 and 0.43 ppm for **8c** in C_6D_6, as well as the *tert*-butyl singlets at 1.16 and 1.12 ppm for **7a** in $CDCl_3$.

A series of metal salts was examined in the addition of *i*-PrMgBr to enone **6** in order to improve regioselectivity and diastereoselectivity (Table 3). Although many Lewis acids had been employed to enhance yield and selectivity in conjugate addition chemistry,[1] our selection was restricted by the strong potential for acid catalyzed deprotection of the *tert*-butyl ester and PhF-amino groups. As mentioned, the addition of TMSCl had no influence on the diastereoselectivity of the addition of higher order phenyl cuprate reagents to **6**. Similarly, monovalent salts, such as NaI and LiBr (300 mol%),[12a] exhibited little effect on the chemoselectivity and diastereoselectivity of the addition of *i*-PrMgBr (300 mol%) to **6**. Multivalent salts, such as $ZnCl_2$, $MgBr_2$ and $CeCl_3$, caused an improvement in 1,4- vs. 1,2-selectivity, which in certain cases may have been caused by the formation of alternative organometallic intermediates such as organozincate and organocerium reagents. Moreover, the multivalent salts helped to improve diastereoselectivity of the 1,4-addition reaction. In the best case, $MgBr_2$ (300 mol%) was found to improve diastereoselectivity in the conjugate addition to **6** up to 6.5:1 with *i*-PrMgBr and up to 15:1 with PhMgBr (Table 3). On the other hand, the presence of $MgBr_2$ had little influence on the conjugate addition of Grignard reagent to enone **12** and resulted in a slight drop in the reaction diastereoselectivity (Table 2, entry 2).

Intramolecular hydrogen-bonds between the acidic nitrogen proton and a remote carbonyl group have been suggested to be a stereocontrolling element in free-radical *C*-allylation reactions of β-, γ-, δ- and ε-amido esters and amides.[31] Investigating if similar intramolecular hydrogen-bonding could be a source of stereocontrol in the additions to **6**, we found no evidence of a six-membered hydrogen bond between the ketone and proximal amine NH in enones **6** and **13**. We observed no significant differences in the ^{1}H NMR signals of the NH protons in **12** and **13**, which came respectively at 3.18 and 3.28 ppm in $CDCl_3$, nor were unusual absorption bands detected for ketones **12** and **13**, which exhibited similar stretching bands at 1671 cm^{-1} in their FTIR spectra.

Alcohol deprotonation and subsequent anionic chelation of Grignard reagents has previously been suggested to account for their diastereoselective addition to enamide and enone systems.[12] To examine if deprotonation of the *N*-(PhF)amine preceded addition, 100 mol% of PhMgBr was added respectively to enones **6** and **13**. In both cases, exclusive 1,4-addition products were obtained in slightly lower yields than observed when 300 mol% of Grignard reagent had been employed (Table 1, entry 9 and Table 2, entry 6). These results suggested that PhMgBr was not causing amine deprotonation to any significant extent during the conjugate addition. On the other hand, treatment of enone **6** with only 100 mol% of *i*-PrMgBr resulted in a sharp drop in yield, which was indicative of this stronger base causing deprotonation of the PhF-amino groups prior to addition (Table 1, entry 12). Hence, the two Grignard reagents (PhMgBr and *i*-PrMgBr) may add to **6** by somewhat different mechanisms.

In summary, remarkable asymmetric induction has been observed in the 1,4-addition reaction of Grignard reagents to diamino enone **6**. By studying the simpler amino enone systems **12** and **13**, we have demonstrated the importance of having two amino carboxylate moieties in **6** for chemoselective and stereoselective conjugate addition. In particular, the presence of the *N*-(PhF)amino and *tert*-butyl ester groups near the carbonyl of enones **6** and **13** resulted in selective 1,4-conjugate addition chemistry. On the other hand,

positioning the same groups near the olefin of the enone **12** resulted in mixtures of 1,2- and 1,4-addition products. Although 1,3- and 1,5-asymmetric induction was respectively observed in additions of PhMgBr to amino enones **13** and **12**, the diastereoselectivity was low compared to the selectivity obtained in the conjugate addition on diamino enone **6**. Furthermore, the presence of $MgBr_2$ improved significantly chemoselectivity and diastereoselectivity in the additions of Grignard reagents to **6**, yet had little influence on the reactions of Grignard reagents with **12** and **13**.

The two amino carboxylate moieties appear to work cooperatively to direct chemoselective and diastereoselective additions of Grignard reagents to enone **6**. Chiral diamino reagents have previously been used as coordinating agents to control the stereochemical outcome of intermolecular reactions featuring organometallic reagents and prochiral substrates.[32,33] For example, enantioselective additions of Grignard reagents have been achieved using chiral bis-pyrrolidine ligands.[33] In light of such precedence for dual coordination of Grignard reagents by chiral diamines, one may suggest that stereoselective addition of Grignard reagents arises from a transition state involving chelation of the organometallic reagent by the two amines of enone **6**. Although such a detailed mechanism can not yet be presented to account for the remarkable asymmetric induction from the remote stereocenters in **6**, our systematic approach has highlighted the significance of two amino carboxylate moieties for high diastereoselectivity. Characterizing the requirements for achieving high selectivity in the construction of alkyl substituted α,ω-diamino azelates **7**, we have also made fundamental progress towards the stereoselective construction of substituted indolizidinone amino acids **1** for application as conformationally rigid surrogates that mimic the backbone and side-chains of natural peptides.

4.9. Experimental.

General: Unless otherwise noted, all reactions were performed under an atmosphere of dry argon. THF and Et_2O were distilled from sodium/benzophenone; toluene and bromobenzene were distilled from sodium; CH_2Cl_2 and $CHCl_3$ were distilled from P_2O_5; $(CH_3)_3SiCl$ (TMSCl), Et_3N and acetonitrile were distilled from CaH_2; methanol was distilled from Mg/I_2. Methyl sulfide (DMS) was degassed by saturation with argon bubbles prior to use. All organometallic reagents, except *n*-BuLi, were freshly prepared before use as described below. CuI was recrystallized as described in ref. 21. Chromatography was carried out using 230-400 mesh silica gel. Radial chromatography was performed on a Chromatotron™ using 1 and 2 mm thick plates of silica gel 60 PF-254. ^1H and ^{13}C NMR spectra were taken in $CDCl_3$, C_6D_6 and CD_3OD on Bruker AV300, ARX400, AV400 and DMX600, and are referenced to internal tetramethylsilane for $CDCl_3$ and C_6D_6 solutions and residual MeOH at 3.3 ppm for CD_3OD. Aromatic ^{13}C NMR signals are not reported in the case of compounds containing PhF groups.

Solutions of phenyl and *p*-methoxyphenyl magnesium bromide in THF were prepared from sonicated magnesium turnings (396 mg, 110 mol%) that were flame dried under an argon flow, cooled to room temperature and suspended in THF (29 mL). The respective arylbromide (15 mmol) was added to the magnesium and the suspension was heated to 50°C when a reflux was observed. The mixture was stirred for an additional 2 h and used directly as a theoretical 0.5M solution.

Solutions of *iso*-propyl magnesium bromide in THF were prepared from sonicated magnesium turnings (396 mg, 110 mol%) that were flame dried under an argon flow, cooled to room temperature and suspended in THF (4 mL). The suspension was heated at 50°C and treated with a solution of 2-bromopropane (1.36 mL, 15 mmol) and 1,2-dibromoethane (50 µL, 0.8 mol%) in THF (4 mL), stirred for 3 h, cooled to room temperature, diluted with THF (20 mL) and titrated by quenching 1 mL of the Grignard solution containing a 100 µL of a solution 2,2'-bipyridyl in toluene (0.013 M) using a solution of

menthol (78 mg, 0.5 mmol) in THF (1 mL). Solution concentrations varied between 0.3 M to 0.4 M.

General procedure for the addition of Grignard reagents to enones. A solution of enone (100 mol%) in THF (1 mL) was added dropwise to a solution of the Grignard reagent (300 mol%) at –48°C, stirred for 2 h and quenched with saturated NH_4Cl (1 mL). The resulting suspension was extracted with ether (3 x 4 mL) and the combined organic layers were washed with brine (5 mL), dried with Na_2SO_4 and concentrated on a rotary evaporator.

General procedure for the addition of higher order cuprate reagents to enones. Under an argon flow, CuCN (240 mol%) was gently flame dried, allowed to cool to room temperature, cooled further to –48°C, treated with a THF solution of the respective organometallic reagent [400 mol%: 0.66 M PhLi, 0.5 M PhMgBr, 1 M *p*-MeOPhMgBr and 0.5 M *i*-PrMgBr], stirred for 30 min, treated with a solution of enone (100 mol%) in THF, stirred for 2 h and quenched with saturated NH_4Cl (1 mL). The resulting suspension was extracted with ether (3 x 4 mL) and the combined organic layers were washed with brine (5 mL), dried with Na_2SO_4 and concentrated on a rotary evaporator.

General protocol for the addition of Grignard reagents in the presence of added salt. A solution of enone (100 mol%) in THF (1 mL) was added to a pre-dried sample of the salt (300 mol%) and stirred at room temperature for 30 minutes. The mixture was then added to a –48°C solution of Grignard reagent (300 mol%) in THF, stirred for 2.5 h, quenched with NH_4Cl and worked-up according to the protocols described above. The crude sample was examined by ^1H NMR spectroscopy in order to measure the product ratios.

(2*S*,6*S*,8*S*)- and (2*S*,6*R*,8*R*)-Di-*tert*-butyl-4-oxo-6-phenyl-2,8-bis[N-(PhF)amino]-azelate [(6*S*)- and (6*R*)-7a]. Enone **6** (0.8 g, 0.97 mmol) was reacted with $Ph_2CuCN(MgBr)_2$ in THF as described above and the residue obtained from extractive work-up was purified by flash chromatography using toluene / *iso*-octane / *iso*-propylether (45 / 45 / 10) as eluant. First to elute was (6*S*)-**7a** (495 mg, 57%): R_f = 0.17 (45/45/10 : toluene / *iso*-octane / *i*-Pr_2O); ^1H

NMR δ ($CDCl_3$) 7.69-7.63 (m, 4 H), 7.37-7.12 (m. 27 H), 3.26 (m, 1 H), 2.95 (br s, 1 H), 2.79 (br t, 1 H), 2.47 (m, 3 H), 2.35 (dd, 1 H), 2.21 (dd, 1 H), 1.73 (t, 2 H), 1.20 (s, 9 H), 1.16 (s, 9 H); ^{13}C NMR δ ($CDCl_3$) 206.1, 175.0, 173.2, 80.9, 80.7, 72.9, 72.8, 54.8, 52.9, 50.5, 48.2, 42.4, 37.5, 27.8, 27.6; MS (FAB+) m/z 901.2 ($M + H^+$); $[\alpha]^{20}_D = -136.1°$ (*c* = 0.018, $CHCl_3$). Next to elute was a mixed fraction of (6*S*)- : (6*R*)-**7a** (115 mg, 7%). Last to elute was (6*R*)-**7a** (312 mg, 36%): R_f = 0.12 (45/45/10 : toluene/*iso*-octane/*i*-Pr_2O); R_f = 0.31 (85/15, hexane/EtOAc); ^1H NMR δ ($CDCl_3$) 7.68-7.52 (m, 4 H), 7.44-7.05 (m. 27 H), 3.46 (t, 1H, *J* = 7.4 Hz), 3.15 (br s, 2 H), 2.73 (t, 1 H, *J* = 5.1 Hz), 2.47-2.35 (m, 3 H), 2.25-2.14 (m, 2 H), 1.79-1.64 (m, 2 H), 1.19 (s, 18 H); ^{13}C NMR δ ($CDCl_3$) 206.1, 175.0, 173.2, 80.9, 80.7, 72.9, 72.8, 54.2, 52.7, 49.4, 48.7, 42.7, 36.7, 27.9, 27.7. MS (FAB+) m/z 901.3 ($M + H^+$)

The reaction of enone **6** (0.1 g, 0.121 mmol) with PhMgBr in THF (0.49 mL, 300 mol%) as described above, followed by flash chromatography using 5% EtOAc in hexane as eluant, provided a 9 : 1 (6*R*)- : (6*S*)-**7a** mixture (94 mg, 86 %) as measured by integration of the triplets at 2.76 and 2.73 ppm in the ^1H NMR spectrum.

Addition of $PhCu_2 \cdot SMe_2$ to Enone 6. Under an argon flow, CuI (49 mg, 105 mol%) was suspended in DMS (0.1 mL), cooled to –48°C, diluted with DMS (1 mL) and treated dropwise with a freshly prepared solution of PhLi in Et_2O (0.66 M, 0.365 mL, 100 mol%, prepared as described in reference 34), when the yellow-orange solution turned to a dark-green. After stirring 30 min, the reaction mixture was cooled to –78°C, treated with a solution of enone **6** (0.2 g, 0.243 mmol) in DMS (0.5 mL), stirred for 2 h and quenched with saturated NH_4Cl solution (1 mL). The resulting suspension was extracted with ether (3 x 4 mL), and the combined organic layers were washed with brine (5 mL), dried with Na_2SO_4 and concentrated on a rotary evaporator. The residue was purified by flash chromatography using hexane/EtOAc (95/5) as eluant to give **7a** (162 mg, 74%) as a 1:3 mixture of (6*R*)-: (6*S*)-diastereomers.

(2*S*,6*R*,8*S*)-Di-*tert*-butyl-4-oxo-6-(4'-methoxyphenyl)-2,8-bis[*N*-(PhF)amino]-azelate (6*S*-7b). Enone **6** (0.1 g, 0.121 mmol) was reacted with *p*-methoxyphenyl magnesium bromide (300 mol%) in THF, and the residue

obtained after extractive work-up was purified by flash chromatography using hexane/EtOAc (95/5) as eluant to give **7b** (92 mg, 81%) which was determined to be a 10:1 mixture of (6*S*)- to (6*R*)-diastereomers on measuring the integrations of the diastereotopic triplets at 2.75 and 2.70 ppm. Major isomer (6*R*)-**7b**; R_f = 0.38 (80/20, hexane/EtOAc); ^{1}H NMR δ ($CDCl_3$) 7.65-7.55 (m, 4 H), 7.37-7.09 (m, 22 H), 6.92 (d, 2 H, *J* = 8.5 Hz), 6.70 (d, 2 H, *J* = 8.5 Hz), 3.73 (s, 3 H), 3.35 (m, 1 H), 3.13 (br s, 2 H), 2.69 (t, 1 H, *J* = 5.1 Hz), 2.35 (m, 3 H), 2.15 (m, 2 H), 1.65 (m, 2 H), 1.17 (s, 9 H), 1.16 (s, 9 H); ^{13}C NMR δ ($CDCl_3$) 206.2, 175.0, 173.2, 80.9, 80.6, 72.9, 72.8, 55.1, 54.1, 52.7, 49.6, 48.7, 43.1, 35.9, 27.8, 27.6; MS (FAB+) m/z 931.2 ($M + H^+$).

(2*S*,6*SR*,8*R*)-Di-*tert*-butyl-4-oxo-6-(4'-methoxyphenyl)-2,8-bis[*N*-(PhF)amino]-azelate (7b). Enone **6** (0.1 g, 0.121 mmol) was reacted with (p-$H_3COC_6H_4)_2CuCN(MgBr)_2$ in THF as described above, and the residue obtained after extractive work-up was purified by flash chromatography using hexane/EtOAc (95/5) as eluant to give **7b** (96 mg, 86%) which was determined to be a 1:2 mixture of (6*R*)- to (6*S*)-diastereomers on measuring the integrations of the diastereotopic triplets at 2.75 and 2.70 ppm; R_f = 0.40 (80/20, hexane/EtOAc). ^{1}H NMR δ ($CDCl_3$) signals for the major (6*S*)-isomer: 6.81 (d 2 H, *J* = 8.6 Hz), 6.70 (d, 2 H, *J* = 8.6 Hz), 3.73 (s, 3 H), 3.18 (m, 1H), 2.75 (t, 1 H, *J* = 5.1 Hz), 1.17 (s, 9 H), 1.13 (s, 9 H); ^{1}H NMR δ ($CDCl_3$) for the minor (6*R*)-isomer: 6.90 (d, 2 H, *J* = 8.5 Hz), 6.70 (d, 2 H, *J* = 8.5 Hz), 3.74 (s, 3 H), 3.33 (m, 1 H), 2.70 (t, 1 H, *J* = 5.1 Hz), 1.18 (s, 9 H), 1.16 (s, 9 H); MS (FAB+) m/z 931.2 ($M + H^+$).

(2*S*,6*SR*,8*S*)-Di-*tert*-butyl-4-oxo-6-*iso*-propyl-2,8-bis[*N*-(PhF)amino]-azelate (7c). Enone **6** (0.1 g, 0.121 mmol) was reacted with (i-Pr)$_2$CuCN(MgBr)$_2$ in THF as described above and the residue from extractive work-up was purified by flash chromatography using hexane/EtOAc (95/5) as eluant to give **7c** (84 mg, 80%), which was determined to be a 5:1 mixture of diastereomers on measuring the integrations of doublets at 0.76 and 0.67 ppm in the ^{1}H NMR spectrum in C_6D_6; R_f = 0.38 (85/15, hexane/EtOAc); ^{1}H NMR δ (C_6D_6) for major isomer: 7.64-7.31 (m, 13 H), 7.13-6.16 (m, 13 H), 3.7-3.2 (br s, 2 H), 3.06 (t, 1 H, *J* = 5 Hz), 2.75 (t, 1 H, *J* = 7 Hz), 2.40 (m, 3 H), 2.14 (dd, 1 H, *J* =

17.1, 7.2 Hz), 1.94 (dd, 1 H, J = 16.6, 6.4 Hz), 1.86 (m, 1 H), 1.59 (m, 1 H), 1.46 (m, 1 H), 1.25 (s, 9 H), 1.20 (s, 9 H), 0.76 (d, 3 H, J = 6.8 Hz), 0.73 (d, 3 H, J = 6.9 Hz); distinct signals for minor isomer included: 3.12 (t, 1 H, J = 5.0 Hz), 2.65 (dd, 1 H, J = 4.85, 16.40 Hz), 1.25 (s, 9 H), 1.16 (s, 9 H), 0.70 (d, 3 H, J = 7.0 Hz), 0.67 (d, 3 H, J = 6.8 Hz); MS (FAB+) m/z 867.2 ($M + H^+$).

When enone **6** (0.1 g, 0.121 mmol) was reacted with *i*-PrMgBr in THF as described above, purification of the residue by flash chromatography using hexane/EtOAc (95/5) as eluant gave **7c** (76 mg, 71%) as a 1:2 mixture of diastereomers as well as a 4:1 mixture of diastereomeric alcohols **8c** as ascertained on measurement of the methyl doublets at 0.60 and 0.39 ppm in the C_6D_6 spectrum of the crude product. Diastereomeric alcohols **8c** were isolated by chromatography using 5% EtOAc in hexane ^{1}H NMR δ ($CDCl_3$) 7.67 (m, 4 H), 7.38-7.16 (m, 22 H), 6.07 (m, 1 H), 6.00 (d, 1 H, J = 11.5 Hz), 3.25 (br m, 2 H), 2.96-2.73 (m, 2.5 H), 2.68 (m, 1 H), 2.63 (m, 1.5 H), 2.54 (m, 1 H), 2.39 (m, 1 H), 1.24, 1.20, 1.15, 1.11 (4 s, 18 H), 0.74 (major, d, 2 H, J = 6.7), 0.70 (major, d, 2 H, J = 6.7), 0.64 (minor, d, 1 H, J = 6.6), 0.59 (minor, d, 2 H, J = 6.5).

(2*S*,6*R*,8*S*)- and (2*S*,6*S*,8*S*)-Di-*tert*-butyl-4-oxo-6-*iso*-propyl-2,8-bis[*N*-(PhF)amino]-azelate (7c). Under an argon flow, CuI (23 mg, 10 mol%) was cooled to –48°C, treated with a solution of *i*-PrMgBr (0.38 M, 9.6 mL, 300 mol%) in THF, stirred for 15 min, treated dropwise over 10 min with a solution of enone **6** (1 g, 1.22 mmol) in THF (10 mL), stirred for 2 h and quenched with saturated NH_4Cl solution (4 mL). The resulting suspension was extracted with ether (3 x 5 mL), and the combined organic layers were washed with brine (10 mL), dried with Na_2SO_4 and concentrated on a rotary evaporator. The residue was purified by flash chromatography using hexane/EtOAc (95/5) as eluant. First to elute was a pure diastereomer of **7c** (582 mg, 55%): ^{1}H NMR δ ($CDCl_3$) 7.69-7.61 (m, 4 H), 7.40-7.16 (m, 22 H), 3.30 (br s, 1 H), 2.97 (br s, 1 H), 2.80 (br s, 1 H), 2.41 (m, 2 H), 2.27 (dd, 1 H, J = 5.4, 16.1 Hz), 2.14 (m, 2 H), 1.86 (m, 1 H), 1.72 (m, 1 H), 1.40 (m, 1 H), 1.24 (s, 9 H), 1.16 (s, 9 H), 1.11 (m, 1 H), 0.73 (m, 6 H); ^{13}C NMR δ ($CDCl_3$) 207.9, 175.7, 173.6, 81.2, 80.6, 73.3, 73.1, 54.1, 53.2, 48.5, 44.9, 37.0, 35.5, 28.2, 28.1, 28.0, 18.9, 18.5.

Next to elute was an 1:1 mixture of diastereomers **7c** contaminated with a third unknown product (435 mg).

(2*S*, 4*E*)-*tert*-Butyl 8-Methyl-6-oxo-2-[*N*-(PhF)amino]-non-4-enoate 12. *n*-Butyllithium (2.15 M, 23.25 mL, 250 mol%) was added dropwise to a solution of freshly distilled dimethylmethylphosphonate (5.4 mL, 250 mol%) in toluene (100 mL) at –78°C, stirred for 20 min, treated dropwise with ethyl isovalerate (3 mL, 20 mmol), stirred for 2 h, brought to room temperature and quenched with 1 M NaH_2PO_4 (50 mL). Brine (50 mL) was added to the reaction mixture. The phases were separated and the aqueous phase was extracted with EtOAc (5 x 25 mL). The combined organic layers were dried and concentrated. The crude product (6.78 g, 97%) was used in the next step without further purification and was composed of a 72:28 mixture of dimethylmethylphosphonate and dimethyl 4-methyl-2-oxo-pentyl-1-phosphonate: R_f = 0.31 (EtOAc); ^{1}H NMR δ ($CDCl_3$) 3.80 (s, 3 H), 3.78 (s, 3 H), 3.07 (d, 2 H, *J* = 22.8 Hz), 2.50 (d, 2 H, J = 6.9 Hz), 2.16 (m, 1 H), 0.93 (d, 6 H, *J* = 6.7 Hz).

A solution of crude 4-methyl-2-oxo-pentyl-1-phosphonate (4.04 g, 19.4 mmol) and aspartate β-aldehyde **10** (8.42 g, 105 mol%, prepared according to reference 26) in acetonitrile (140 mL) was treated with cesium carbonate (6.65 g, 105 mol%), stirred for 15 h, filtered and concentrated to a residue, that was purified by flash chromatography with hexane/EtOAc (95/5). First to elute was pure *Z*-**12** (320 mg, 3%). R_f = 0.67 (80/20 hexane/EtOAc); ^{1}H NMR δ ($CDCl_3$) 7.68-7.65 (m, 2 H), 7.41-7.18 (m, 11 H), 6.17 (d, 1 H, *J* =15.9), 6.12 (m, 1 H), 3.25 (br s, 1 H), 2.80 (m, 1 H), 2.65 (m, 2 H), 2.30 (d, 2H, *J*=7.3), 2.12 (n, 1 H, *J*=6.7), 1.16 (s, 9 H), 0.92 (d, 6 H, *J*=6.6); ^{13}C NMR δ ($CDCl_3$) 201.4, 174.3, 80.8, 72.9, 55.5, 53.2, 35.3, 27.8, 24.9, 22.6; Next to elute was a 1 to 3 ratio of *Z*-/ *E*-isomer mixture (1.32g, 14%) of **12** followed by *E*-**12** (5.59 g, 58%) as colorless oil: R_f = 0.37 (85/15 hexane/EtOAc); ^{1}H NMR δ ($CDCl_3$) 7.69-7.66 (m, 2 H), 7.41-7.18 (m, 11 H), 6.72 (dt, 1 H, *J* =15.9, 7.7 Hz), 6.02 (d, 1 H, *J* = 15.9 Hz), 3.18 (br s, 1 H), 2.67 (t, 1 H, *J* = 6.2 Hz), 2.40 (m, 2 H), 2.27 (m, 2 H), 2.18 (m, 1 H), 1.18 (s, 9 H), 0.96-0.93 (m, 6 H); ^{13}C NMR δ ($CDCl_3$) 200.3, 174.1, 81.3, 73.1, 55.7, 48.9, 39.1, 28.0, 25.2, 22.9; IR

(film/NaCl) 3308, 2957, 2870, 1724, 1695, 1629, 1671, 1153; MS (FAB+) m/z 496.0 ($M + H^+$).

(2*S*,5*E*)-*tert*-Butyl 8-Methyl-4-oxo-2-[*N*-(PhF)amino]-non-5-enoate 13. A solution of β-ketophosphonate **11** (2.14 g, 4 mmol, prepared according to ref 23a) and isovaleraldehyde (0.6 mL, 140 mol%) in acetonitrile (30 mL) was treated with cesium carbonate (1.37 g, 105 mol%), stirred for 15 h, filtered and concentrated to a residue, that was purified by flash chromatography with hexane/EtOAc (95/5) as eluant. Evaporation of the collected fractions gave **13** (1.357 g, 69%): R_f = 0.21 (90/10 hexane/EtOAc); ^{1}H NMR δ ($CDCl_3$) 7.68-7.65 (m, 2 H), 7.37-7.16 (m, 11 H), 6.55 (dt, 1 H, *J* = 15.9, 7.9 Hz), 5.91 (d, 1 H, *J* = 15.9 Hz), 3.28 (br s, 1 H), 2.91 (t, 1 H, 6.0 Hz), 2.62 (dd, 1 H, *J* = 14.9, 6.2 Hz), 2.54 (dd, 1 H, *J* = 14.9, 5.9 Hz), 2.04 (t, 2 H, *J* = 6.6 Hz), 1.72 (m, 1 H), 1.21 (s, 9 H), 0.91 (m, 6 H); ^{13}C NMR δ ($CDCl_3$) 198.0, 173.7, 81.3, 73.2, 54.0, 45.5, 42.0, 28.1, 22.6; IR (film/NaCl) 3310, 2957, 2930, 2870, 1724, 1696, 1627, 1671, 1155; MS (FAB+) m/z 496.3 ($M + H^+$).

(2*S*,4*RS*)-*tert*-Butyl 8-Methyl-4-phenyl-6-oxo-2-[*N*-(PhF)amino]-nonanoate (14a) and (2*S*,6*RS*)-*tert*-Butyl 8-Methyl-6-phenyl-6'-hydroxy-2-[*N*-(PhF)amino]-nonanoate (15a). Enone **12** (0.1 g, 0.203 mmol) was treated as described above with PhMgBr in THF. The residue, after extractive work-up, was purified by flash chromatography using hexane/EtOAc (95/5) as eluant. First to elute was **14a** (35 mg, 30%), which was ascertained to be a 1 to 3 mixture of diastereomers by measurement of the integrations of the *tert*-butyl singlets at 1.21 and 1.19 ppm: R_f = 0.30 (95/5, hexane/EtOAc); ^{1}H NMR δ for major isomer (C_6D_6) 7.65-7.22 (m, 7 H), 7.12-6.76 (m, 12 H), 3.76 (m, 1 H), 3.36 (br s, 1 H), 2.71 (t, 1 H, *J* = 7.0 Hz), 2.32-2.22 (m, 1 H), 2.08 (dd, 1H, *J* = 5.5, 16.6 Hz), 1.99-1.89 (m, 3 H), 1.80-1.66 (m, 2 H), 1.19 (s, 9 H), 0.71-0.66 (m, 6 H); distinct signals for the minor isomer were observed: 3.55 (m, 1 H), 2.78 (t, 1 H, *J* = 6.0 Hz), 1.21 (s, 9 H); ^{13}C NMR δ ($CDCl_3$) for major isomer 208.9, 175.1, 80.7, 72.8, 54.3, 52.5, 49.3, 42.7, 36.9, 27.9, 24.2, 22.5, 22.4. Next to elute was tertiary alcohol **15a** (48 mg, 41%), which was ascertained to be a 1:1 mixture of diastereomers by measurement of the integrations of the *tert*-butyl singlets at 1.10 and 1.09 ppm: ^{1}H NMR δ (C_6D_6) 7.52-7.00 (m, 18

H), 5.77 (m, 2 H), 3.37 (br s, 1 H), 2.86 (br s. 1 H), 2.29 (m, 2 H), 1.86-1.66 (m, 3 H), 1.45 (d, 1 H, J = 17.5 Hz), 1.10, 1.09 (2 s, 9 H), 0.98 (d, 3 H, J = 5.8 Hz), 0.81 (d, 3 H, J = 6.3 Hz); ^{13}C NMR δ ($CDCl_3$) 174.6, 174.5, 80.6, 77.1 (2), 73.0, 56.2, 56.1, 51.2, 51.1, 38.8, 38.6, 27.8, 24.5 (2), 24.2.

(2*S*,4*RS*)-*tert*-Butyl 8-Methyl-4-*iso*-propyl-6-oxo-2-[*N*-(PhF)amino]-nonanoate (14b). The reaction was performed as described above with enone **12** (0.1 g, 0.2 mmol) and *i*-$Pr_2CuCN(MgBr)_2$ in THF. The residue, after extraction, was purified by radial chromatography using hexane/EtOAc (95/5) as eluant and gave **14b** (80 mg, 74 %) which was shown to be a 1:1 mixture of diastereomers by measurement of the integrations of the *tert*-butyl singlets at 1.19 and 1.17 ppm: R_f = 0.45 (85/15, hexane/EtOAc); ^{1}H NMR δ (C_6D_6) 7.61-7.43 (m, 6 H), 7.08-7.00 (m, 7 H), 2.74 (m, 1 H), 2.40 (m, 0.6 H), 2.31 (m, 0.4 H), 2.18-1.94 (m, 3 H), 1.88 (m, 2 H), 1.62 (m, 1 H), 1.45 (m, 1 H), 1.19 (s, 5.4 H), 1.17 (s, 3.6 H), 0.86-0.81 (m, 6 H), 0.75-0.68 (m, 6 H); ^{13}C NMR δ (C_6D_6) 209.0, 208.8, 176.0, 80.7, 80.5, 78.0, 73.9, 55.3, 55.0, 52.6, 52.4, 44.8, 44.7, 38.8, 38.2, 36.0, 35.9, 29.6, 29.0, 28.4, 24.9, 23.1, 22.7, 19.7, 19.2, 18.9, 18.5.

When the reaction was performed as described with enone **12** (0.116 g, 0.235 mmol) and $Ph_2CuCN(MgBr)_2$ in THF The residue, after extraction, was purified by flash chromatography using hexane/EtOAc (95/5) as eluant and gave **14a** (103 mg, 77%) as 1:1 mixture of diastereomers.

(2*S*,6*RS*)-*tert*-Butyl 8-Methyl-6-phenyl-4-oxo-2-[*N*-(PhF)amino]nonanoate (16a). Enone **13** (0.1 g, 0.203 mmol) was treated with PhMgBr in THF as described above, and the residue was purified by flash chromatography using hexane/EtOAc (95/5) as eluant to yield **16a** (99 mg, 85%) which was shown to be a 3:1 mixture of diastereomers upon examination of the integrations of the *tert*-butyl singlets at 1.18 and 1.13 ppm ; R_f = 0.23 (95/5, hexane/EtOAc); ^{1}H NMR δ (C_6D_6) 7.51-6.95 (m, 18 H), 3.35 (br m, 1 H), 3.02 (t, 1 H, J = 5.1 Hz), 2.40-2.28 (m, 4 H), 1.39-1.31 (m, 3 H), 1.18 (s, 6.6 H, major), 1.13 (s, 2.2 H, minor), 0.90 (m, 3 H), 0.79 (m, 3 H); ^{13}C NMR δ ($CDCl_3$) 207.1, 207.0, 173.4, 81.2, 73.2, 73.1, 53.1, 53.0, 51.0, 49.0, 48.7, 45.9, 45.8, 38.9, 27.8, 25.5, 23.7, 21.8; MS (FAB+) m/z 574.0 ($M + H^+$).

When the reaction was performed as described with enone **13** (0.116 g, 0.235 mmol) and $Ph_2CuCN(MgBr)_2$ in THF_2 after extractive work-up, the residue was purified by radial chromatography using hexane/EtOAc (95/5) as eluant to give **16a** (103 mg, 77%) as a 1:1 mixture of diastereomers.

(2*S*,6*RS*)-*tert*-Butyl 8-Methyl-6-*iso*-propyl-4-oxo-2-[*N*-(PhF)amino]nonanoate (16b). Enone **13** (0.1 g, 0.203 mmol) was treated with *i*-$Pr_2CuCN(MgBr)_2$ in THF as described above, and the residue was purified by radial chromatography using hexane/EtOAc (95/5) as eluant to give **16b** (85 mg, 78 %) as 2:1 mixture of diastereomers as indicated by measurement of the integrations of the methyl group doublets at 0.81 and 0.77 ppm in the ^1H NMR spectrum: ^1H NMR δ (C_6D_6) 7.54-7.39 (m, 6 H), 7.16-6.95 (m, 7 H), 3.08 (q, 1 H, J = 5.3 Hz), 2.45 (t, 2 H, J = 4.8), 2.17-2.06 (m, 2 H), 2.01-1.93 (m, 1 H), 1.69 (m, 1 H), 1.48 (m, 1 H), 1.22 (s, 9 H), 1.11-0.95 (m, 2 H), 0.93-0.88 (m, 6 H), 0.81 (d, 1.93 H, J = 6.9, minor), 0.77 (d, 3.75 H, J = 6.9, major); ^{13}C NMR δ (C_6D_6) 207.2, 207.1, 173.5 (2), 80.8, 73.7, 53.7, 48.9, 48.8, 45.4, 45.3, 41.3, 41.1, 37.0 (2), 30.0 (2), 28.0, 25.9, 25.8, 23.5, 22.9, 19.5 (2), 18.8, 18.7.

When the reaction was performed as described with enone **13** (0.1 g, 0.203 mmol) and *i*-PrMgBr in THF, purification by radial chromatography using hexane/EtOAc (95/5) as eluant gave ketone **16b** (89 mg, 82 %) as a 2:1 mixture of diastereomers: R_f = 0.42 (90/10, hexane/EtOAc).

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4.10. References.

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Chapitre 5.

Synthèse des acides amines 4-phényl-indolizidin-9-ones

Article 3.

Cluzeau, J.; Lubell, W.D. 'Mimicry of the Backbone Side-Chain Geometry of Peptide Turn: Synthesis of Novel 4-Substituted Indolizidin-9-one Amino Acids' communication publié dans *Peptides: Chem., Structure and Biology*, M. Lebel and R. Houghten, Eds; ESCOM, Leiden, The Netherlands, 2001 p. 597-598.

Mimicry of the Backbone Side-Chain Geometry of Peptide Turn: Synthesis of Novel 4-Substituted Indolizidin-9-one Amino Acids

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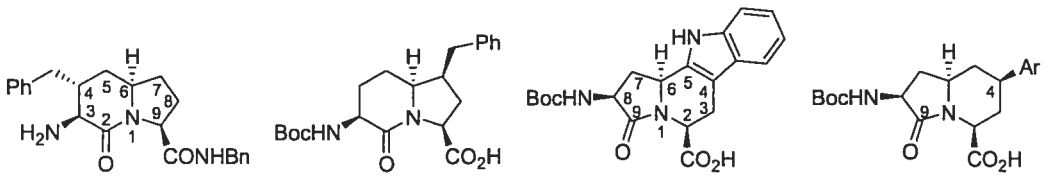
5.1. Introduction.

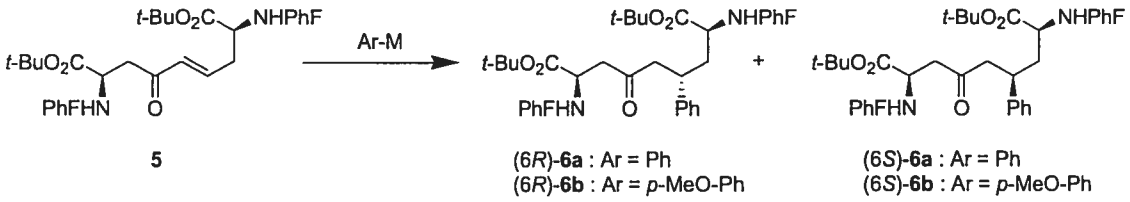
Indolizidinone amino acids have served as β-turn mimics for exploring conformation-activity relationships in natural peptides.[1] Focus has been particularly placed on mimicry of peptide containing aromatic residues within turn region because of their importance in various recognition events. For example, 4- and 7-benzyl indolizidin-2-one amino acid analogs **1** and **2** have been respectively used to synthesized ligands of the tachykinin and opioid receptors.[2,3] Similarly, indolizidin-9-one amino acid **3** has served in the synthesis of potent gramicidin S antibiotic peptides.[4] Interested in expanding the variety of indolizidinone amino acids possessing aromatic side-chains, we have now developed a new means for synthesizing 4-aryl-indolizidin-9-one amino acids **4**.



1[2] **2**[3] **3**[4] **4a** : Ar = Ph **4b** : Ar = *p*-MeO-Ph

FIGURE 1. Example of arylindolizidinone amino acids.

5.2. Result and Discussion.



5

(6*R*)-**6a** : Ar = Ph
(6*R*)-**6b** : Ar = *p*-MeO-Ph

(6*S*)-**6a** : Ar = Ph
(6*S*)-**6b** : Ar = *p*-MeO-Ph

SCHEME 1. Conjugate addition on azelate **5**.

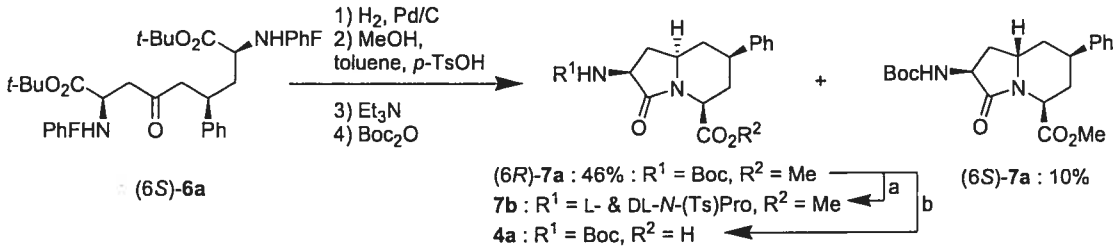
Azelate **5** was synthesized from aspartic acid in 6 steps and 62% overall yield on 15g scale by a route featuring the Horner-Wadsworth-Emmons olefination of β-aldehyde and β-ketophosphonate components from α-tert-butyl-γ-methyl *N*-(PhF)aspartate.[5] Conjugate addition reaction on enone **5** were explored using aromatic organometallic reagents to provide 6-arylazelates **6a** and **6b** (Table 1).[6] Aryl Grignard reagents furnished predominantly product with 6*R* stereochemistry in high yield and good selectivity. On the other hand, higher-order cuprates added in high yield with a preference for producing the 6*S* diastereomer albeit with lower diastereoselectivity. Separation of a 1.5 to 1 mixture of 6*S* and 6*R* diastereomers was accomplished using a ternary eluant composed of toluene / *iso*-octane / *iso*-propyl ether (45/45/10) and gave 57% of (6*S*)-**6a** and 36% of (6*R*)-**6a**.

TABLE 1. Selectivity of 1,4-addition reactions on enone **5**.

Ar-M	Solvent	Yields (%)	Product	Ratio (6*R*/6*S*)
PhMgBr	Et_2O/THF	96%	**6a**	9/1
$Ph_2CuCN(MgBr)_2$	THF	98%	**6a**	1/1.5
(*p*-MeOPh)MgBr	THF	82%	**6b**	9/1
$(p\text{-MeOPh})_2CuCN(MgBr)_2$	THF	98%	**6b**	1.6/1

4-phenylindolizidin-9-one **7a** was synthesized using our reductive amination / lactam cyclization protocol on (6*S*)-**6a**. Treatment of (6*S*)-**6a** in 9:1 EtOH:AcOH with Pd/C under 7 atm of H_2 caused cleavage of the PhF groups, intramolecular imine formation and reduction of the protonated imine to furnish a disubstituted pipecolate intermediate. Removal of the *tert*-butyl ester with concurrent reprotection as methyl ester was accomplished using *p*-TsOH in 10:1 toluene:MeOH. Arylindolizidinones (6*R*)-**7a** and (6*S*)-**7a** were finally

isolated in 46% and 10% respective overall yields after lactam cyclization with *p*-TsOH:Et3N in toluene, amine protection with Boc2O and Et3N in DCM and purification by chromatography on silica gel (hexane / *i*-Pr2O / *i*-PrOH, 85/10/5).



SCHEME 2. Synthesis of 4-aryl indolizidin-9-one amino esters **7**: (a) i. TFA, DCM. ii. L- and DL-*N*-(Ts)Pro-Cl (93%, 2 steps). (b) TMSOK, Et_2O (98%)

The relative stereochemistry of the (6*R*)- and (6*S*)-**7a** as well as their linear ketone counterparts were assigned based on 1D and 2D (COSY, NOESY) ^{1}H NMR data. In the spectra of (6*R*)-**7a**, nOe were observed between H^6 and both H^8 and H^4. In the case of (6*S*)-**7a**, no similar nOe was observed which was compatible with the coupling constant data and the convex configuration. The enantiomeric purity of (6*R*)-**7a** was evaluated after conversion to diastereomeric *N*-(*p*-Ts)proline amide **7b**. By measuring the diastereomeric methyl ester singlets at 3.66 and 3.64 ppm in C_6D_6 using ^{1}H NMR spectroscopy, amides **7b** were shown to be of 99% de. *N*-(Boc)amino acid **4a** was synthesized by hydrolysis of ester (6*R*)-**7a** using TMSOK in Et2O in 98%.

Acknowledgment: This research was supported by NSERC (Canada) and by the Ministère de l'Éducation du Québec.

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Article 4.

Cluzeau, J.; Lubell, W.D. 'Conformationally Constrained Dipeptide Surrogates with Aromatic Side-Chains: Synthesis of 4-Aryl Indolizidin-9-one Amino Acids by Conjugate Addition to a Common α,ω-Diaminoazelate Enone Intermediate' publié dans *Journal of Organic Chemistry* **2004**, *69*, 1504-1512.

Conformationally Constrained Dipeptide Surrogates with Aromatic Side-Chains: Synthesis of 4-Aryl Indolizidin-9-one Amino Acids by Conjugate Addition to a Common α,ω-Diaminoazelate Enone Intermediate

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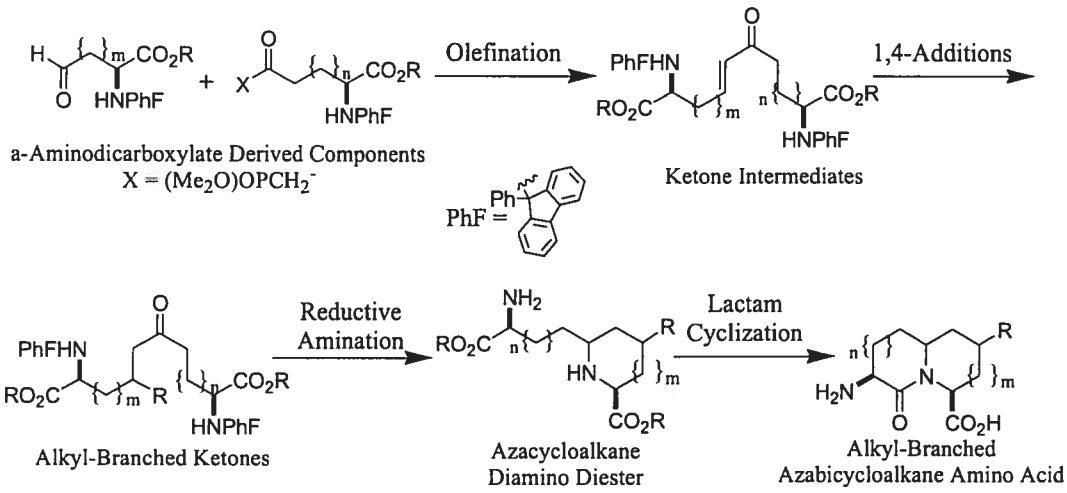
5.4. Abstract.

Four methyl 9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo [4.3.0]nonane carboxylates (**11**, 4-Ph-I^{9}aa-OMe) were synthesized from (2*S*,8*S*,5*E*)-di-*tert*-butyl-4-oxo-5-ene-2,8-bis[*N*-(PhF)amino]azelate [(5*E*)-**7**, PhF = 9-(9-phenylfluorenyl)] via a seven-step process featuring a conjugate addition/reductive amination/lactam cyclization sequence. Various nucleophiles were used in the conjugate addition reactions on enone (5*E*)-**7** as a general route for making α,ω-diaminoazelates possessing different substituents in good yield albeit low diastereoselectivity except in the case of aryl Grignard reagents (9/1 to 15/1 drs). 6-Phenylazelates (6*S*)-**8d** and (6*R*)-**8d** were separated by chromatography and diastereoselective precipitation and independently transformed into 4-Ph-I^{9}aa-OMe. From (6*S*)-**8d**, (2*S*,4*R*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe **11** was prepared selectively in 51% yield. Reductive amination of (6*R*)-**8d** provided the desired pipecolates **9** along with desamino compound **10**, which was minimized by performing the hydrogenation in the presence of ammonium acetate. Subsequent ester exchange, lactam cyclization, and amine protection provided three products (2*R*,4*S*,6*S*,8*R*)-, (2*R*,4*S*,6*S*,8*S*)-, and (2*S*,4*S*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe **11** in 10, 6, and 6% yields, respectively, from (6*R*)-**8d**. Ester hydrolysis of (2*S*,4*R*,6*R*,8*S*)-**11** furnished 4-phenyl indolizidin-9-one *N*-(Boc)amino acid **3** as a novel constrained Ala-Phe dipeptide surrogate for studying conformation-activity relationships of biologically active peptides.

5.5. Introduction.

Turn motifs play important roles in the recognition and activity of biologically relevant peptides and proteins.[1] Conformationally constrained mimics of peptide turns have thus become important synthesis targets because of their utility for studying the relationship between structure and function as well as their potential to improve the pharmacological properties of the native peptide.[2] Among turn mimics,[3] azabicyclo[X.Y.0]alkane amino acids have proven to be useful for constraining peptide backbone and side-chain geometry for studying structure-activity relationships.[4-9]



SCHEME 1. General strategy for azabicyclo[X.Y.0]alkane amino acid synthesis

In the context of our research on peptide mimicry, we have employed different azabicyclo[X.Y.0]alkane amino acids in structure-activity studies of various peptides.[10-12] For example, introduction of indolizidin-9-one amino acid **1** (I^9aa) into a Leu-enkephalin (YGGFL) analogue as a rigid surrogate of its Gly-Gly residue produced an active mimic that exhibited greater duration of action relative to the parent peptide.[10] The circular dichroism spectrum of the indolizidin-9-one Leu-enkephalin analogue also exhibited a curve shape characteristic of a turn conformation in support of the importance of a turn about the glycine residues for activity of the native peptide.

Approaches for installing side-chain functionality at the different ring-positions on the indolizidin-9-one amino acid may furnish improved dipeptide surrogates for peptide mimicry. Although methodology is abundant for adding side-chain functionality to the 3-,[5e,i,x] 4-,[5a,b,d,l,s,v] 5-,[5c,6a,b] 6-,[5o] 7-,[5o,p,q,u,y,6a,b,c] and 8-positions[5u,v] of the related fused 6,5-ring system, indolizidin-2-one amino acid **2** (I^2aa), few methods have been reported for adding side-chains onto its fused 5,6-ring counterpart, indolizidin-9-one **1** (Figure 1).[7b,c] For example, we have previously reported on three effective routes for appending a series of aliphatic as well as a set of heteroatomic side chains onto the 5- and 7-positions of indolizidin-2-one amino acid, using a common β-keto ester starting material obtained from the Claisen condensation of α-*tert*-butyl γ-methyl *N*-(PhF)glutamate (Scheme 1).[6] On the other hand, Boc-IBTM **4** (Figure 1), a condensation product of the Asp-Trp dipeptide represents a rare example of an indolizidin-9-one amino acid possessing side-chain functionality.7b Replacement of the D-Phe-Pro residue of gramicidin S (*c*-[D-Phe-Pro-Val-Orn-Leu]2) with IBTM has produced analogues with potency similar to the parent antibiotic,13 contingent on the indolizidin-9-one ring-fusion stereochemistry. The promising biological activity of the enkephalin analogue (Tyr-I^9aa-Phe-Leu) and antibiotic peptide analogue (*c*-[IBTM-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu]), possessing indolizidin-9-one amino acids **1** and **4**, illustrates their effectiveness as mimics of secondary structure and suggests similar potential for peptide mimicry with related analogues possessing side-chain ring-substituents.

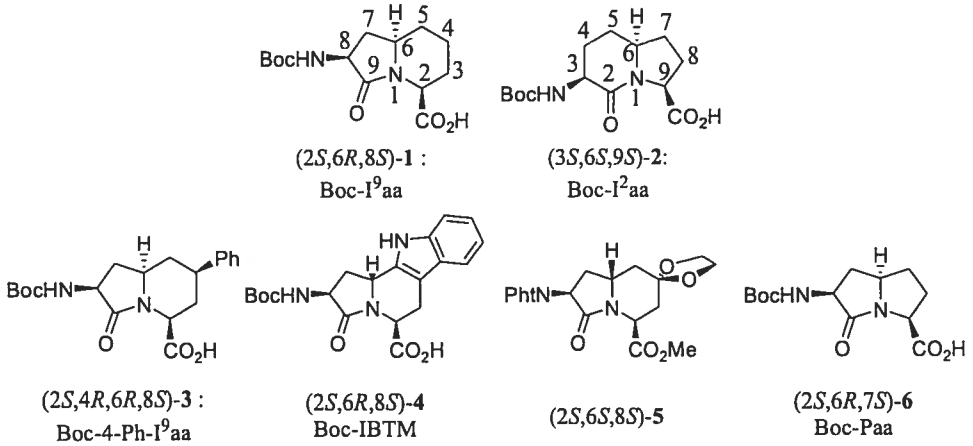
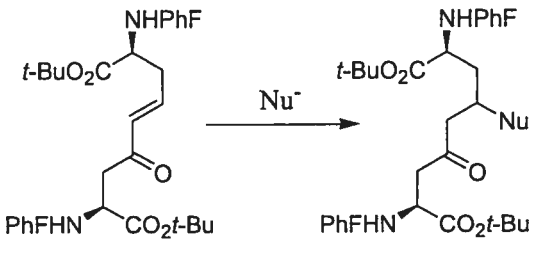


Figure 1. Representative azabicylo[X,Y,0] alkanone amino acids.[4,7a-c,9]

In our general strategy for constructing indolizidin-9-one amino acid systems, linear α,ω-diaminoazelate enone (5*E*)-**7** was converted to the heterocycle via a reductive amination/lactam cyclization sequence (Scheme 1).[7a] Conjugate addition (1,4-addition) reactions on enone (5*E*)-**7** have now provided a series of α,ω-diaminoazelate ketones **8** for conversion to a variety of substituted indolizidin-9-ones.[14] Conditions for the conversion of ketones **8** into 4-aryl indolizidin-9-one amino acids are now presented by the syntheses of constrained Ala-Phe dipeptide surrogate **3** (*N*-Boc-4-Ph-I[9]aa). In particular, we have developed an effective six-step synthesis of (2*S*,4*R*,6*R*,8*S*)-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane carboxylic acid **3** from (5*E*)-**7** with 42% overall yield.

5.6. Results and Discussion.

Conjugate addition reactions on enone (5*E*)-**7** were studied using various nucleophiles as a general route for making α,ω-diaminoazelates possessing different substituents (Scheme 2). Various nucleophiles reacted effectively on enone (5*E*)-**7** in good yield albeit with low diastereoselectivity. For example, methylmalonate, cyanide, and nitromethane all were introduced onto ketone (5*E*)-**7** in good yield and from 1:1 to 2:1 diastereoselectivity (entries 1-3). Different alkyl and aryl substituents were introduced as organocopper species, namely, higher order cyano cuprates, aryl copper reagents, and Grignard reagents in the presence of catalytic amounts of copper. These various conditions employing copper reagents gave generally good yield and low diastereoselectivity (2/1 to 5/1, entries 4-8), with limited influence of the copper species on the diastereomeric ratio observed for the final ketone.[14]



SCHEME 2.
Synthesis of 6-substituted diaminoazelate **8**.

Table 1. Conjugate addition on enone **7**.

entry	nucleophile	Product [%Yield, d.r.]
1	$MeO_2CCH_2CO_2Me$ / NaH	**8a** [77, 1 :1]
2	KCN / 18Crown6	**8b** [68, 1 :1]
3	CH_3NO_2 / DBU	**8c** [94, 2 :1]
4	$Ph_2CuCN(MgBr)_2$	**8d** [98, 1 :2]
5	$PhCu.S(CH_3)_2$	**8d** [74, 1 :3]
6	$(p(CH_3O)\text{-}C_6H_4)_2CuCN(MgBr)_2$	**8e** [98 1:2]
7	$i\text{-}Pr_2CuCN(MgBr)_2$	**8f** [80, 5 :1]
8	*i*-PrMgBr / CuI (cat)	**8d** [83, 5 :1]
9	PhMgBr	**8d** [86, 9 :1]
10	PhMgBr / $MgBr_2$	**8d** [100[a], 15 :1]
11	$p(CH_3O)\text{-}C_6H_4\text{-}MgBr$	**8e** [82, 10 :1]
12	*i*-PrMgBr	**8f** [71[b], 3 :1]
13	*i*-PrMgBr / $MgBr_2$	**8f** [80[a], 6 :1]

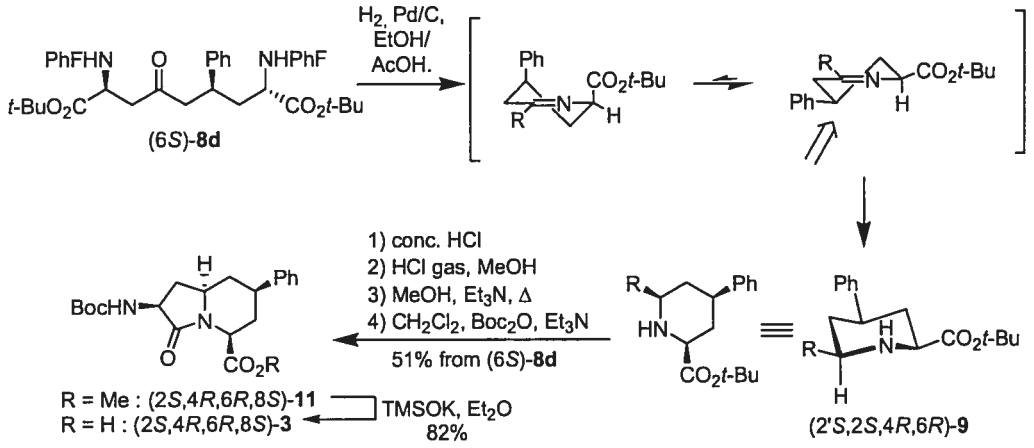
a: % conversion. b: 23% of 1,2 adduct was also isolated.

Remarkable regio- and diastereoselectivity was observed when ordinary aryl Grignard reagents were added to ketone **7**.[14] For example, phenylmagnesium bromide added selectively in a 1,4 manner to provide a 9:1 ratio in favor of (6*R*)-**8d** (entry 9). Furthermore, the addition of metal salts to the reaction mixture was shown to give a significant improvement in the diastereoselectivity. For example, addition of magnesium bromide prior to reaction with the Grignard reagent increased the diastereoselectivity of the phenyl and *iso*-propyl products (6*R*)-**8d** and (6*R*)-**8f** up to ratios of 15:1 and 6.5:1, respectively (entries 10 and 13).

Diastereomeric mixtures from the conjugate addition were generally difficult to separate using chromatography. However, in the case of the phenyl analogue, a ternary eluant (45/45/10, *iso*-octane / toluene / *iso*-propyl ether) was developed that provided baseline separation of the diastereomers on 100 mg scale. Later, we found that 1.8 g of (6*S*)-**8d** could be precipitated diastereoselectively from 3.1 g of a 2/1 mixture of (6*S*)- and (6*R*)-**8d** in a 0.035 M solution of *i*-PrOH containing 1% H_2O. This method provided each isomer

in a diastereomeric ratio of 13 to 1. Diastereomerically enriched samples (13:1 and 1:13) of (6*S*)- and (6*R*)-**8d** were used in subsequent reductive amination/lactam cyclization sequences described below. Stereochemical assignment at the 6-position of compound **8d** was made on the basis of assignments for (2*S*,4*R*,6*R*,8*S*)-4-phenylindolizidin-9-one **11**, from cyclization of (6*S*)-**8d**, as characterized below.

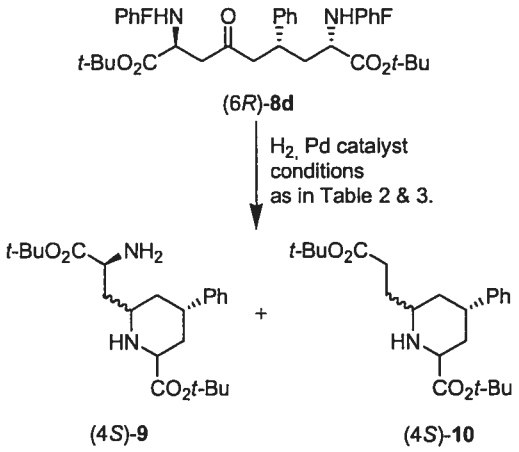
(2*S*,6*R*,8*S*)-Di-*tert*-butyl-4-oxo-6-phenyl-2,8-bis[*N*-(Ph-F)amino]azelate [(6*S*)-**8d** (13:1 dr)] was converted to (2*S*,4*R*,6*R*,8*S*)-methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane carboxylate (2*S*,4*R*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe, **11**] in 51% overall yield by our reductive amination/lactam cyclization protocol (Scheme 3).[7a] Hydrogenation using 10% palladium-on-carbon in a mixture of EtOH and AcOH (9/1) provided the trisubstituted pipecolate (2*S*□,2*S*,4*R*,6*R*)-**9** as a single diastereoisomer as determined by ^{1}H and ^{13}C NMR spectroscopy. The *tert*-butyl esters were then removed using concentrated HCl and replaced by methyl esters using HCl gas in MeOH, as followed by ^{1}H NMR spectroscopy by monitoring the methyl ester singlets at 3.90 and 3.84 ppm, as well as by TLC. Lactam cyclization was performed by treating the diester in MeOH with Et3N at reflux until NMR spectroscopy showed replacement of the two methyl ester singlets by a new singlet at 3.86 ppm (approximately 24 h). The amine was then protected with Boc2O and Et3N in CH_2Cl_2. Chromatography of the final residue provided (2*S*,4*R*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe **11** in 51% overall yield from ketone (6*S*)-**8d**.[15] Ester



SCHEME 3. Synthesis of 4-Ph-I^{9}aa-OMe (2*S*,4*R*,6*R*,8*S*)-**11** via reductive amination of (6*S*)-**8d**.

(2*S*,4*R*,6*R*,8*S*)-**11** was then converted to the corresponding *N*-(Boc)amino acid (2*S*,4*R*,6*R*,8*S*)-**3**, suitable for application in peptide synthesis, using potassium trimethylsilanolate in Et_2O for 1 h at room temperature (Scheme 3).

(2*S*,6*R*,8*S*)-Di-*tert*-butyl-4-oxo-6-phenyl-2,8-bis[*N*-(PhF)amino]azelate [(6*R*)-**8d** (13:1 dr)] was initially hydrogenated using the same conditions as in the synthesis of pipecolate (2*S*□,2*S*,4*R*,6*R*)-**9** from ketone (6*S*)-**8d**. The desired pipecolates **9** and their desamino counterparts **10** were isolated as mixtures of diastereomers in 27 and 56% respective yields (Scheme 4). Loss of the amine function was presumed to arrive from imine to enamine tautomerization followed by *β*-elimination of ammonium ion to form the α,*β*-unsaturated imine, which underwent subsequent reduction.[16] In related hydrogenation processes, we have reported *â*-elimination of nitrogen from a *β*-amino imine intermediate4 and of oxygen from *β*-hydroxy, *β*-silyloxy, and *β*-acetoxy imine intermediates.[6b,16] To prevent *β*-elimination, different proton sources, catalysts, and reaction temperatures were studied. For example, four forms of Pd catalyst were examined at 9 atm of H_2 in 9/1 EtOH/AcOH (Table 2). Their effect on the elimination process under these conditions was significant, and the ratio of pipecolate **9** to *β*-elimination product **10** changed from 1:1 with wet Pd/C (10% w/w) up to 1:4 using "eggshell" Pd/C (20 wt %).



SCHEME 4 : Hydrogenation of (6*R*)-**8d** proceeded with β-elimination.

Table 2. Influence of the Pd Catalyst on the Hydrogenation of (6*R*)-**8d**

Entry	Catalyst[a]	d.r. of **9** (6*S* / 6*R*)	Ratio **9** vs **10**	d.r. of **10**
1	Pd/C 10% wet	1/3	1/1	1/3
2	Pd/C 10%	1/1	1/1	1/2
3[b]	$Pd(OH)_2$/ C 20%	1/1	1/2[c]	1/2[d]
4	Pd/C 20% eggshell	3/1	1/4	3/1

a. Hydrogenation was performed in EtOH/AcOH (9/1) under 9 atm of H_2. b. When MeOH/EtOAC (7/3) was employed, the ratio for **9** was 1/4 and for **10** was 1/4. c. At 0°C a 1/4 ratio was obtained. d. At 50°C a 9/1 ratio was obtained.

In our synthesis of pyrrolizidinone amino acid **6**, a similar loss of amine due to a *β*-elimination process was inhibited significantly by diminishing the quantity of acid employed in the hydrogenation with Pd/C as catalyst.4 In the case of (6*R*)-**8d**, no reaction was observed when using only 100 mol % AcOH, and a 1:1 ratio of **9** and **10** was obtained when using 100 mol % of pyridinium *para*-toluenesulfonate (Table 3, entries 2 and 3).

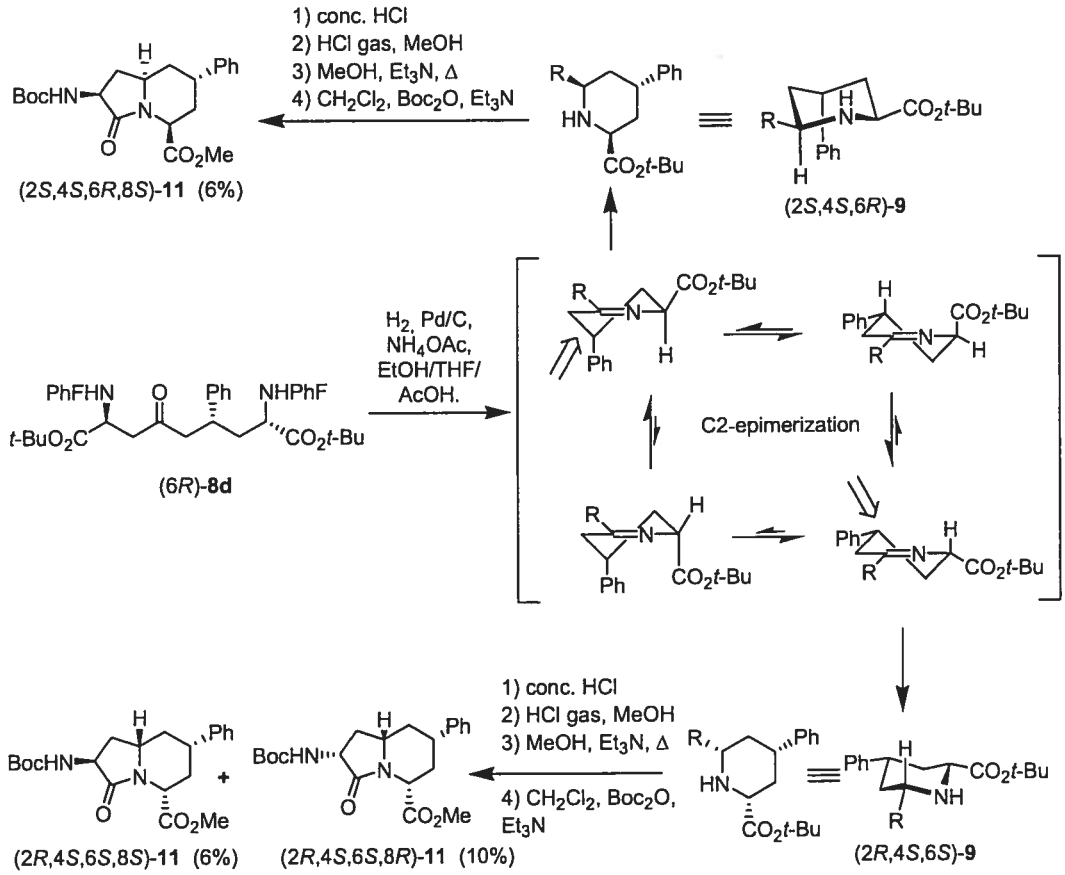
Practical conditions for obtaining the diamino compound **9** were found when the reaction mixture was saturated with ammonium acetate (entry 4), which according to Le Chatelier's law was expected to suppress the *β*-elimination product. In the absence of added AcOH, this saturation technique was not always reproducible (entries 4 and 5), such that a protocol was developed in which the ammonium acetate was first suspended in and dried by evaporation from toluene and then left to sit under high vacuum overnight. The resulting white crystalline solid was then employed in the hydrogenation of (6*R*)-**8d** using Pd/C in a solvent system of EtOH / THF / AcOH (7/3/0.1) for 48 h, which provided selectively the pipecolates **9**. Desamino compound **10** was not detectable by ^{1}H and ^{13}C NMR spectroscopy of the crude product, yet was visible when concentrated samples were spotted on TLC.

Table 3. Influence of the Proton Source on the Hydrogenation of (6*R*)-**8d**.

Entry	Catalyst	Solvent	Proton source	d.r. of **9** (6*S* / 6*R*)	Ratio **9** vs **10**	d.r. of **10**
1	Pd/C 10%	EtOH	AcOH (excess)	1/1	1/2	2/1
2	Pd/C 10%	EtOH/THF (8/2)	AcOH (1 eq)	-	n.r.	-
3	Pd/C 10%	EtOH/THF (8/2)	PPTS (1 eq)	1/4	1/1	1/4
4	Pd/C 10%	EtOH/THF (7/3)	NH_4OAc (200 eq)	4/1	6/1	1/1
5	Pd/C 10%	EtOH/THF (7/3)	NH_4OAc (dry, 200 eq)[a]	-	n.r.	-
6	Pd/C 10%	EtOH/THF (7/3)	NH_4OAc (dry, 200 eq)[a] AcOH (1%/solv)	3/1	>100/1	-
7	$Pd(OH)_2/C$ 20%	MeOH/EtOAc (7/3)	NH_4OAc (200 eq)	3/1	4/1	1/1

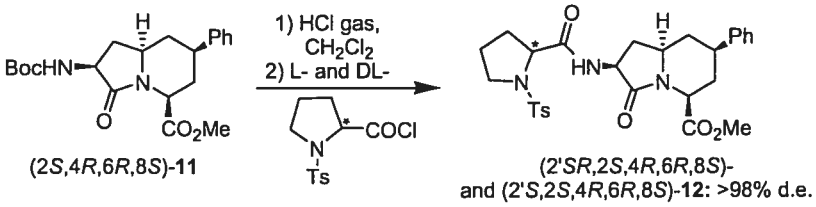
a. Ammonium acetate was dried as discussed in the experimental section.

As described above for the synthesis of (2*S*,4*R*,6*R*,8*S*)-**11**, the *tert*-butyl esters of pipecolates **9** were removed using concentrated HCl and replaced with methyl esters employing HCl gas in MeOH (Scheme 5). Because the ^{1}H NMR spectrum was too complex, formation of methyl esters was best monitored by TLC. The lactam cyclization was then performed in MeOH at reflux with Et3N for 48 h followed by amine protection using Et3N and (Boc)2O in CH_2Cl_2 for 12 h. After two chromatographies, eluting first with 60/40 hexane/EtOAc, followed by 85/10/5 hexane / *i*-Pr2O / *i*-PrOH, three diastereomeric products, (2*R*,4*S*,6*S*,8*S*)-, (2*R*,4*S*,6*R*,8*S*)-, and (2*R*,4*S*,6*S*,8*R*)-4-Ph-I^{9}aa **11** were isolated in 6, 6, and 10% overall yields, respectively, from (6*R*)-**8d**.



SCHEME 5. Synthesis of (4*S*)-4-Ph-I^{9}aa-OMe **11** via reductive amination of (6*R*)-**8d**.

Enantiomeric Purity and Stereochemical Assignment of Indolizidines 11. The configurational integrity of the 4-phenyl-indolizidin-9-one amino acid was evaluated by the preparation and spectral analysis of diastereomeric *N*-(*p*-toluenesulfonyl)prolinamides **12** using (2*S*,4*R*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe **11**, which was obtained from the hydrogenation/lactam cyclization sequence using (6*S*)-**8d**. After cleavage of the Boc protecting group using HCl gas, the resulting hydrochloride salt was treated with Et_3N and coupled respectively to L- and DL-*N*-(*p*-tolylsulfonyl)prolyl chloride in CH_2Cl_2 (Scheme 6). Observation of the diastereomeric methyl ester singlets at 3.75 and 3.73 ppm by 400 MHz ^1H NMR spectroscopy in C_6D_6 during incremental additions of the diastereomeric mixture demonstrated prolylamide (2'*S*)-**12** to be of >98% diastereomeric excess. Hence, (2*S*,4*R*,6*R*,8*S*)-4-Ph-I9aa-OMe **11** as well as its corresponding acid (2*S*,4*R*,6*R*,8*S*)-**3** are presumed to be of similarly high enantiomeric purity.



SCHEME 6. Enantiomeric Purity of (2*S*,4*R*,6*R*,8*S*)-**11**

The relative stereochemistry of the different diastereomers of **11** was established using one- and two dimensional NMR experiments (Figure 2). The majority of the NMR signals were well resolved at distinct chemical shifts (Table 4). All protons were assigned using COSY and HMQC experiments, which established their linear sequence around the bicycle from the carbamate NH to the C-2 proton. Diastereomeric protons cis and trans to the carboxylate at C-2 are designated β and α, respectively. The assignments of the α and β protons at C-3, C-5, and C-7 were made using their signal multiplicity and coupling constants and supported by NOE observed in the NOESY experiments.

In the ^{1}H NMR spectrum of (2*S*,4*R*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe **11** in C_6D_6, the protons at C-2, C-3β, C-4, C-5β, and C-6 all exhibited large coupling constants (10.4-12.5 Hz) indicative of their axial position on the pipecolate, which adopted a chair conformation (Figure 2). For example, the C-2 proton was observed as a doublet of doublets and shared, respectively, vicinal coupling constants of 3.4 and 11.2 Hz with the C-3R and C-3$\hat{a}$ protons, corresponding to dihedral angles of ±60° and ±160°.[17] The pseudoaxial conformation of the C-7β proton in the five-member lactam was assigned on the basis of observation of an apparent quadruplet, due to similar vicinal couplings with the pseudoaxial C-8 proton and the axial C-6 proton as well as a geminal coupling with the C-7α proton. The concave shape of the bicyclic system and the (4*R*)-stereochemistry, which positions the C-2, C-4, C-6, and C-8 protons all on the same face, were supported by NOESY experiments. Characteristic NOE correlations were observed between the three piperidine axial protons at C-2, C-4, and C-6. In addition, NOE was measured between the C-6 and C-8 protons on one face as well as between the axial C-3β and C-5β protons on the other face.

In the ^{1}H NMR spectrum of (2*R*,4*S*,6*S*,8*S*)-4-Ph-I^{9}aa-OMe **11**, the first of the two minor isomers from the cyclization of (6*R*)-**8d**, the C-2, C-3β, C-4, and C-5β protons all exhibited large coupling constants in $CDCl_3$ (11.9 to 13.4 Hz), indicative of their axial position on the pipecolate, which adopted a chair conformation. For example, the C-4 proton was observed as a triplet of triplets with coupling constants of 3.3 and 12.4 Hz corresponding to dihedral angles close to (61° with both the C-3R and C-5R protons and close to ±175° with both of the C-3β and C-5β protons.[16] With the (4*S*)-phenyl substituent sitting equatorial, the data suggested epimerization at C-2 and 2*R*,4*S*,6*S* stereochemistry for the pipecolate ring. The 2*R*,4*S*,6*S*,8*S* configuration was confirmed using NOESY experiments in which NOEs were observed between the axial C-2, C-4, and C-6 protons, confirming complete equatorial substitution about the piperidine cycle. Additional NOE between the C-5β and C-8 protons on the opposite face established the 8*S* stereochemistry.

Table 4. ^{1}H NMR data for *N*-(Boc)-4-Phenyl-indolizidin-9-one amino esters **11**

	2	3α	3β	4	5α	5β	6	7α	7β	8	NH	Me	*t*-Bu
(2*S*,4*R*,6*R*,8*S*)-**11** (C_6D_6, 400 MHz)	3.48dd 3.4, 11.2	1.70d 12.5	1.80q 12.3	2.08t 11.9	1.31d 9.6	1.00q 11.7	2.34t 10.4	2.25br s	1.11q 11.3	4.16m	5.00br d 5.2	3.62	1.42
(2*R*,4*S*,6*S*,8*S*)-**11** ($CDCl_3$, 600 MHz)	3.88dd 3.5, 12.0	2.10d 13.4	1.89q 12.6	2.80tt 3.3, 12.4	2.06d 12.9	1.52q 12.3	3.74m	2.24t 6.9		4.17m	5.01br s	3.80	1.44
(2*S*,4*S*,6*R*,8*S*)-**11** (C_6D_6, 400 MHz)	4.26t 5.0	1.93ddd 3.7, 5.9, 9.6	1.56m	2.77br t	1.56m	1.82ddd 9.7, 12.3, 13.9	2.91m	2.42m	1.16q 11.0	4.35m	5.12d 6.0	3.37	1.43

In the ^{1}H NMR spectrum of the second minor isomer from cyclization of (6*R*)-**8d**, (2*S*,4*S*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe **11**, in C_6D_6, the coupling constant pattern for protons in the piperidine cycle differed from that of a chair conformation. For example, the C-2 proton exhibited a small 5 Hz coupling constant and appeared as a triplet, suggesting an axial position for the ester function. The C-5β proton was observed as a double doublet of doublets (ddd) with three large coupling constants (9.7, 12.3, 13.9 Hz), indicative of a pseudoequatorial position for the phenyl ring. The C-7β proton exhibited an 11.0 Hz coupling constant and quadruplet multiplicity indicative of a trans relationship with the C-8 and C-6 protons. The C-6 stereocenter was assigned the 6*R* relative configuration because, in the NOESY spectrum, correlations were observed between the C-8 and C-6 protons and between the C-6 and C-2 protons, in agreement with the 2*S*,6*R*,8*S* relative stereochemistry. Additional NOEs were observed between the C-7β proton and both the carbamate NH and the C-5$\hat{a}$ protons, as well as between ortho aromatic protons and both C-2 and C-6 protons, which helped to confirm the stereochemistry of (2*S*,4*S*,6*R*,8*S*)-**11**.

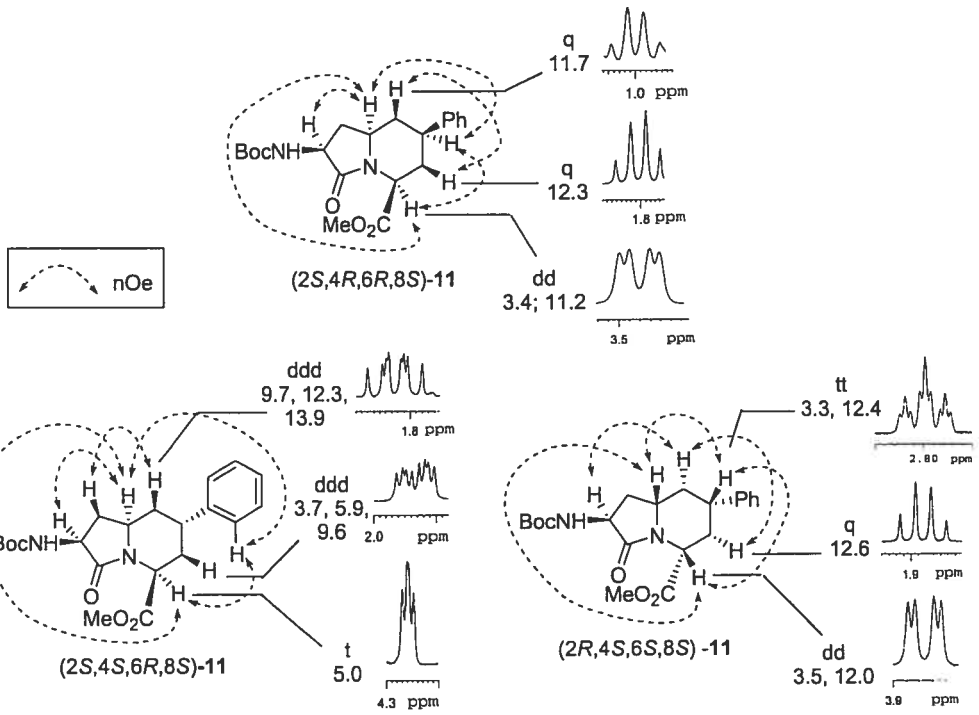
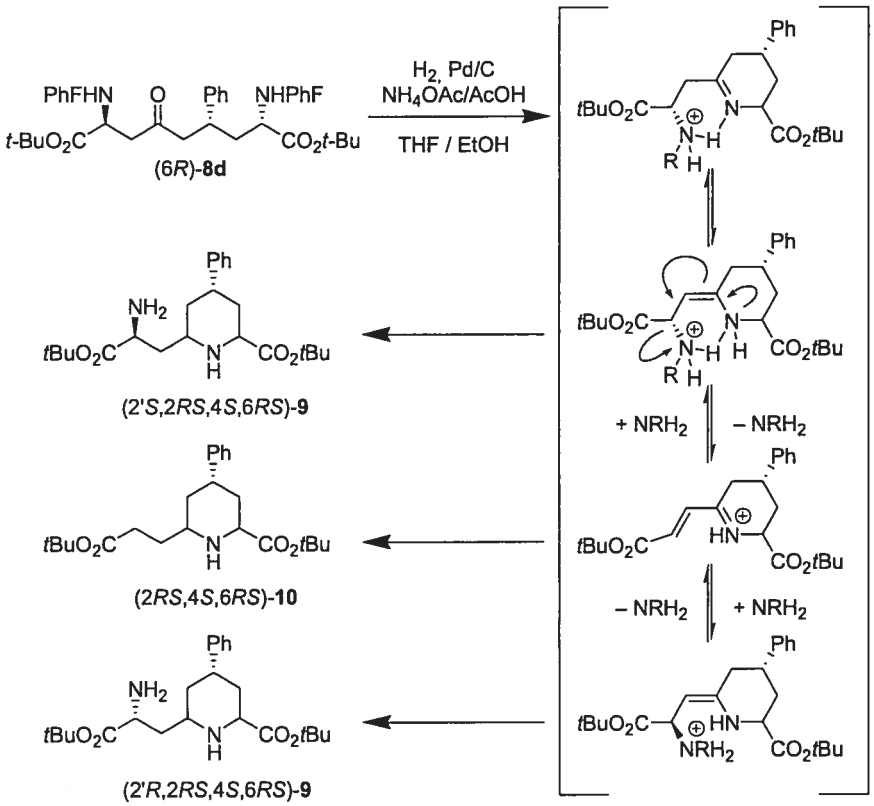


Figure 2. nOe correlations observed in the NOESY NMR spectra of **11**.

The diverse NMR data confirmed the presence of (2*S*,4*R*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe **11**, which might come from residual (6*S*)-**8d** in the hydrogenation of (6*R*)-**8d**; however, the mass of isolated product exceeded expectations. The presence of its enantiomer, (2*R*,4*S*,6*S*,8*R*)-**11**, coming from epimerization at both the C-2 and C-8 stereocenters during transformation of (6*R*)-**8d** was validated by coupling L-*N*-(*p*-tolylsulfonyl)-prolyl chloride to the isolated sample of (2*S*,4*R*,6*R*,8*S*)-4-Ph-I^{9}aa-OMe **11**; the amide products **12** were shown to be of only 33% diastereomeric excess (66:33 dr).

Stereoselection and Epimerization. In the synthesis of the parent indolizidin-9-one system, I^{9}aa **1**, the six membered ring iminium ion was preferentially reduced on the face opposite the ester, leading predominantly to the concave diastereomer (9:1).[7a] In the synthesis of (4*R*)-4-Ph-I^{9}aa, the phenyl substituent works in harmony with the ester function to favor their pseudoequatorial orientation in the iminium ion such that attack occurs exclusively to give the concave 6*R* diastereoisomer (Scheme 3). In the case of



SCHEME 7. Hydrogenation of (6*R*)-**8d** may proceed with β-elimination as well as C-2 and C-8 epimerization

the (4*R*)-phenyl isomer, the phenyl and carboxylate substituents battle to avoid pseudoaxial orientations in the iminium ion, such that two conformers exist in equilibrium, with a likely preference for the conformer having the phenyl group positioned equatorially (Scheme 5). Hydrogenation of the more stable iminium ion favored formation of the piperidine in a chair conformer having the C-4 and C-6 substituents positioned equatorially, which was transformed into (2*R*,4*S*,6*S*,8*R*)- and (2*R*,4*S*,6*S*,8*S*)-4-Ph-I[9]aa-OMe **11**.18 Reduction of the less stable iminium ion having the phenyl group positioned pseudoaxially favored a piperidine in a chair conformer with the C-2 and C-6 substituents positioned equatorially, which furnished (2*S*,4*S*,6*R*,8*S*)-4-Ph-I[9]aa-OMe **11**.

Epimerization of one and both α-amino carboxylate centers of azelate (6*R*)-**8d** occurred during the synthesis of (2*R*,4*S*,6*S*,8*S*)- and (2*R*,4*S*,6*S*,8*R*)-4-Ph-I9aa-OMe **11**, respectively. Epimerization of different stereocenters has been sometimes reported in the syntheses of mono- and bicyclic amino acids. In particular, the α-proton of cyclic amino acids has been removed under basic conditions[7c,19,20] contingent on the nitrogen protecting group. Imine derivatives of α-amino acids are particularly sensitive to α-epimerization even in the absence of base[21] and as iminium ions in acidic conditions.[19] To the best of our knowledge, no α-epimerization of iminium ion intermediates has been previously reported in applications of reductive aminations to form substituted prolines and pipecolates using catalytic hydrogenation under mildly acidic conditions. Although a detailed assessment of the source of C-2 epimerization was not possible because the pipecolate diastereomers were inseparable, a driving force for epimerization during hydrogenation of the iminium ion would be the formation of the more stable intermediate having both substituents positioned pseudoequatorially (Scheme 5). A less likely alternative would involve C-2 epimerization by selective enolization of an α-ammonium carboxylate after hydrogenation during cleavage of the *tert*-butyl esters and formation of the methyl esters under acidic conditions. Epimerization at C-8 was only observed in the product from hydrogenation of (6*R*)-**8d** in the presence of excess NH4OAc. Application of NH4OAc to prevent formation of desamino pipecolate **10** apparently increased the potential for conjugate addition of ammonia to the unsaturated iminium ion, product from *β*-

elimination, prior to reduction of the iminium ion intermediate (Scheme 7). The preferred formation of (2*R*,4*S*,6*S*,8*R*)-4-Ph-I[9]aa-OMe **11** instead of product epimerized only at C-8 remains inexplicable with our present data.

5.7. Conclusion.

Toward a general approach for constructing 4-substituted indolizidin-9-one amino acids, conjugate addition reactions on enone **7** were studied using various nucleophiles, and a versatile route has been developed for making 6-substituted α,ω-diaminoazelates in good yield and diastereomeric ratios from 1:1 to 15:1. 6-Phenylsubstituted diaminoazelates, (6*S*)- and (6*R*)-**8d**, were obtained in high diastereoselectivity (13:1 dr) by selective precipitation from a 0.035 M solution of *i*-PrOH/H_2O (99/1). Enantiopure (2*S*,4*R*,6*R*,8*S*)-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane carboxylic acid ((2*S*,4*R*,6*R*,8*S*)-**3**) was synthesized from (6*S*)-**8d** in six steps and 42% overall yield via a reductive amination/ lactam cyclization sequence. On the other hand, reductive amination of (6*R*)-**8d** was accompanied by β-elimination leading to desamino analogue **10**. Although β-elimination could be avoided by performing the hydrogenation in the presence of excess ammonium acetate, epimerisation during the conversion of (6*R*)-**8d** to **9** was detected at both the C-2 and C-8 stereocenters and three products, (2*R*,4*S*,6*S*,8*R*)-, (2*R*,4*S*,6*S*,8*S*)-, and (2*S*,4*S*,6*R*,8*S*)-**11**, were finally isolated from the lactam cyclization protocol. A novel synthesis methodology for preparing 4-substituted indolizidin-9-one amino acids has thus been demonstrated by the synthesis of 4-Ph-I[9]aa **3**, a new constrained Ala-Phe dipeptide surrogate for examining conformation-activity relationships of biologically active peptides.

5.8. Experimental Section.

(2*S*,6*S*,8*S*)- and (2*S*,6*R*,8*S*)-Di-*tert*-butyl-4-oxo-6-phenyl-2,8-bis[*N*-(PhF)amino]-azelate [(6*S*)- and (6*R*)-8d]. Under an argon flow, CuCN (1.25 g, 240 mol %) was gently flame dried, allowed to cool to room temperature, cooled further to -48 °C, treated with a 0.5 M solution of PhMgBr in THF (48.5 mL, 400 mol %), stirred for 30 min, treated with a solution of enone (5*E*)-7[7a] (4

g, 4.8 mmol) in THF (50 mL), stirred for 2 h, and quenched with saturated NH4Cl (20 mL). The resulting suspension was extracted with ether (3 x 20 mL), and the combined organic layers were washed with brine (30 mL), dried with Na_2SO_4, and concentrated on a rotary evaporator. The residue obtained was purified by column chromatography using hexane/EtOAc (90/10) as an eluant to give **8d** (3.2 g, 72%) as a 2/1 mixture of (6*S*)-/(6*R*)-**8d** as measured by the *tert*-butyl singlets at 1.16-1.12 and 1.16 ppm in the ^{1}H NMR spectrum. The mixture was dissolved in *i*-PrOH (100 mL, 0.035 M), treated with 1 mL of water, and stirred for 18 h. The mother liquor was decanted and evaporated to give a 1/13 mixture of [(6*S*)-/(6*R*)-**8d**,1.1 g, 25%]: R^f 0.12 (45/45/10 toluene / *iso*-octane / *i*-Pr_2O); *Rf* 0.31 (85/15, hexane/EtOAc); ^{1}H NMR δ ($CDCl_3$) 7.68-7.52 (m, 4 H), 7.44-7.05 (m. 27 H), 3.46 (t, 1H, *J* = 7.4 Hz), 3.15 (br s, 2 H), 2.73 (t, 1 H, *J* = 5.1 Hz), 2.47-2.35 (m, 3 H), 2.25-2.14 (m, 2 H), 1.79-1.64 (m, 2 H), 1.19 (s, 18 H); ^{13}C NMR δ ($CDCl_3$) 206.1, 175.0, 173.2, 80.9, 80.7, 72.9, 72.8, 54.2, 52.7, 49.4, 48.7, 42.7, 36.7, 27.9, 27.7; MS (FAB+) *m/z* 901.3 (M + H+). The dry white precipitate was a 13/1 mixture of [(6*S*)-/(6*R*)-**8d**,1.8 g, 42%]: R^f 0.17 (45/45/10 toluene / *iso*-octane / *i*-Pr_2O); ^{1}H NMR δ ($CDCl_3$) 7.69-7.63 (m, 4 H), 7.37-7.12 (m. 27 H), 3.26 (m, 1 H), 2.95 (br s, 1 H), 2.79 (br t, 1 H), 2.47 (m, 3 H), 2.35 (dd, 1 H, *J* = 16.2, 5.7 Hz), 2.21 (dd, 1 H, *J* = 16.2 Hz, 4.6 Hz), 1.73 (t, 2 H, *J* = 6.5 Hz), 1.20 (s, 9 H), 1.16 (s, 9 H); ^{13}C NMR δ ($CDCl_3$) 206.1, 175.0, 173.2, 80.9, 80.7, 72.9, 72.8, 54.8, 52.9, 50.5, 48.2, 42.4, 37.5, 27.8, 27.6; MS (FAB+) *m/z* 901.2 (M + H+); $[\alpha]_D^{20}$ -136.1 (*c* 0.018, $CHCl_3$).

(2*S*,4*R*,6*R*,8*S*)-Methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4,3,0] nonane Carboxylate [(2*S*,4*R*,6*R*,8*S*)-11]. Ketone (6*S*)-**8d** (1.75 g, 1.94 mol) was dissolved in a solution of THF (36 mL), EtOH 95% (126 mL), and AcOH (18 mL) and treated with palladium-on-carbon 10% (175 mg, 10 wt %). The vessel containing the suspension was filled, vented, and refilled three times with hydrogen. After stirring for 24 h under 9 atm of H_2, the suspension was filtered through Celite and concentrated on a rotary evaporator. The residue was partitioned between saturated $NaHCO_3$ (50 mL) and $CHCl_3$ / *i*-PrOH (4/1, 50 mL). The aqueous phase was extracted (3 x 20 mL) with $CHCl_3$ / *i*-PrOH (4/1). The organic phases were combined, dried with MgSO4,

and concentrated under vacuum to give the free amine as a colorless oil: TLC R^f 0.1 (90/9/1 hexane / *i*-PrOH / Et3N); ^{1}H NMR (CDCl3) δ 7.33-7.28 (m, 2H), 7.22 (m, 3H), 3.58 (br s, 1H), 3.42 (d, 1H, J = 11.0 Hz), 2.95 (br s, 1H), 2.73 (t, 1H, J = 12.2 Hz), 2.19 (d, 1H, J = 12.7 Hz), 2.07 (br s, 2H), 1.90 (m, 1H), 1.82 (d, 1H, J = 12.7 Hz), 1.61 (m, 1H), 1.52 (q, 1H, J = 12.0 Hz), 1.46 and 1.45 (2s, 18H), 1.43-1.33 (m, 2H); ^{13}C NMR δ 175.5, 172.2, 145.4, 128.4, 126.7, 126.3, 81.0 (2C), 59.3, 52.5, 51.9, 42.8, 40.8, 39.9, 36.7, 27.9 (2C). The oil was dissolved in 12 N HCl (30 mL) and stirred for 15 min, at which point ^{1}H NMR spectroscopy in CD3OD showed the disappearance of the *tert*-butyl singlets at 1.55 and 1.51 ppm. Evaporation of the volatiles under vacuum gave the free diamino acid as the HCl salt: TLC R^f 0.05 (4/1/1 *n*-BuOH / H2O / AcOH); ^{1}H NMR (CD3OD) δ 7.36-7.23 (m, 5H), 4.26- 4.22 (m, 2H), 3.88 (m, 1H), 3.10 (tt, 1H, J = 12.7, 2.9 Hz), 2.48 (m, 2H), 2.31 (d, 1H, J = 13.7 Hz), 2.21-2.15 (m, 2H), 1.90 (q, 1H, J = 13.7 Hz), 1.77 (q, 1H, J = 13.8 Hz); 13C NMR (CD3-OD) ä 171.0, 170.8, 144.4, 130.0, 128.3, 128.0, 59.0, 55.4, 50.7, 41.2, 36.0, 35.0, 33.9. The crude white solid was dissolved in methanol (65 mL) and treated with bubbles of HCl gas until complete disappearance of starting material was observed by TLC (4/1/1 *n*-BuOH/H2O/AcOH), approximately 1 h. The volatiles were removed under vacuum to give the diester as a white solid. TLC *Rf* 0.35 (4/1/1 *n*-BuOH/H2O/AcOH); 1H NMR (CD3OD) ä 7.32-7.23 (m, 5H), 4.47-4.28 (m, 2H), 3.90 (s, 3H), 3.85 (m, 4H), 3.16 (m, 1H), 2.60 (m, 1H), 2.42 (m, 1H), 2.30 (m, 2H), 2.05-1.85 (m, 2H); ^{13}C NMR δ 170.8, 170.0, 144.4, 130.0, 128.4, 128.0, 58.9, 55.3, 54.5, 54.1, 50.7, 40.9, 35.8, 35.0, 33.8. The solid was dissolved in MeOH (45 mL), treated with Et3N (0.54 mL, 200 mol %), and heated at reflux for 48 h at which point ^{1}H NMR spectroscopy in CD3OD of an aliquot showed complete disappearance of the methyl singlets at 3.90 and 3.84 ppm and appearance of a new methyl singlet at 3.71 ppm. After removal of the volatiles under vaccum, the crude oil was dissolved in CH2Cl2 (45 mL), treated with Et3N (2.7 mL, 1000 mol %) and Boc2O (0.846 g, 200 mol %), stirred for 15 h, and concentrated on a rotary evaporator. Purification by column chromatography was performed using an eluant of hexane / EtOAc (70/30) as an eluant. First to elute was (2*R*,4*S*,6*S*,8*S*)-methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4,3,0]nonane carboxylate [(2*R*,4*S*,6*S*,8*S*)-**11**, 22mg, 3%]: TLC R^f 0.1 (85/10/5 hexanes / *i*-Pr2O / *i*-PrOH).

Next to elute was (2*S*,4*R*,6*R*,8*S*)-methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane carboxylate [(2*S*,4*R*,6*R*,8*S*)-**11**, 340 mg, 51%]: TLC R^f 0.13 (70/30 Hex / EtOAc); $[\alpha]^{20}_D$ -36.1; ^{13}C NMR (C_6D_6) δ 171.7 (2 C), 170.8 (C), 144.6 (C), 129.0 (2 CH), 128.5 (CH), 127.1 (2 CH), 79.4 (C), 56.4 (CH), 53.9 (CH), 52.6 (CH), 52.3 (CH), 40.6 (CH2), 38.5 (2CH2), 36.2 (2 CH3), 28.6; MS (FAB+) *m/z* 389.1 (M + H+); HRMS calcd for $C_{21}H_{28}N_2O_5$ (MH+) 389.2077, found 389.2098.

(4*S*)-Methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane carboxylate [(4*S*)-11]. Ketone (6*R*)-**8d** (900 mg, 1 mmol) was dissolved in THF (15 mL) and treated with a solution of NH_4OAc (suspended in and dried by evaporation from toluene, and then left to sit under high vacuum overnight, 4.27 g, 5500 mol %) in 95% EtOH (35 mL) followed by AcOH (0.5 mL) and Pd/C 10% (90 mg, 10 wt %). The vessel containing the suspension was filled, vented, and refilled three times with hydrogen. After stirring for 24 h under 9 atm of H_2, the suspension was then filtered through Celite and concentrated on a rotary evaporator to give **9**: TLC R^f 0.1 (90/10 hexane / *i*-PrOH). Distinct signals for the major isomer of (4*S*,6*S*)-**9**: ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 3H), 7.20 (m, 2H), 3.40 (d, 2H, J = 10.3 Hz), 2.91 (m, 1H), 2.71 (t, 1H, J = 11.9 Hz), 2.20 (d, 1H, J = 12.8 Hz), 1.87 (m, 3H), 1.70-1.50 (m, 2H), 1.45 (s, 18H), 1.36 (q, 1H, J = 12.0 Hz); ^{13}C NMR ($CDCl_3$) δ 175.4, 172.1, 145.7, 128.5 (2C), 126.9 (2C), 125.8, 81.1 (2C), 59.5, 55.1, 54.2, 42.8, 41.1, 39.6, 36.4, 28.0 (6 CH_3); MS (FAB+) *m/z* 405.2 (M + H+). As described above for the synthesis of (2*S*,4*R*,6*R*,8*S*)-**11**, ester exchange, lactam formation, and *N*-protection were performed to provide a residue that was purified by column chromatography using hexane/ EtOAc (70/30) as an eluant, which gave (2*R*,4*S*,6*S*,8*S*)-methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane carboxylate [(2*R*,4*S*,6*S*,8*S*)-**11**, 18 mg, 6%]: TLC R^f 0.25 (85/10/5 hexane / *i*-Pr_2O / *i*-PrOH); ^{13}C NMR (CDCl3) *ä* 170.3, 155.9, 129.0, 128.0, 127.1, 126.9, 80.2, 56.8, 56.5, 52.7, 51.5, 41.4, 38.9, 35.1, 33.6, 28.5; MS (FAB+) *m/z* 389.2 (M + H+). HRMS calcd for $C_{21}H_{28}N_2O_5$ (MH+) 389.2077, found 389.2085. A second fraction was collected containing a mixture of two compounds that were separated by column chromatography using hexane / *i*-Pr_2O / *i*-PrOH (85/10/5) as an eluant. First to elute was (2*S*,4*S*,6*R*,8*S*)-methyl-9-oxo-8-(*N*-(Boc)-amino)-

4-phenyl-1-azabicyclo[4.3.0]-nonane carboxylate [(2*S*,4*S*,6*R*,8*S*)-**11**, 17 mg, 6%]: TLC R^f 0.22 (85/10/5 hexane / *i*-Pr2O / *i*-PrOH); ^{13}C NMR (C_6D_6) δ 171.8, 171.7, 156.3, 146.3, 79.3, 63.9, 52.4, 52.1, 48.4, 38.2, 36.9, 34.7, 31.6, 28.6; MS (FAB+) *m/z* 389.1 (M + H+); HRMS calcd for $C_{21}H_{28}N_2O_5$ (MH+) 389.2077, found 389.2093. Second to elute was (2*R*,4*S*,6*S*,8*R*)-methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl- 1-azabicyclo[4.3.0]nonane carboxylate [(2*R*,4*S*,6*S*,8*R*)-**11**, 28 mg, 10%]: TLC R^f 0.17 (85/10/5 hexane / *i*-Pr2O / *i*-PrOH); $[\alpha]_D^{20}$ +0.8. The NMR spectral data were identical with those of (2*S*,4*R*,6*R*,8*S*)-**11**. The enantiomeric purity of (2*R*,4*S*,6*S*,8*R*)-**11** was assessed by the preparation of diastereomeric prolyl amides (2□*S*,2*R*,4*S*,6*S*,8*R*)-**12** as described below and measured at 33% ee.

Enantiomeric Purity of (2*S*,4*R*,6*R*,8*S*)-Methyl-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane Carboxylate 11. Compound (2*S*,4*R*,6*R*,8*S*)-**11** (28 mg, 72 *i*mol) was dissolved in CH_2Cl_2 (5 mL) and treated with HCl gas for 1 h. The volatiles were removed under reduced pressure, and the product was dissolved in CH_2Cl_2 (3 mL), treated with DIEA (9 *i*L, 140 mol %) and either L- or DL-*N*-(*p*-toluenesulfonyl)prolyl chloride (10 mg, 100 mol %), stirred for 6 h at r.t., diluted with CH_2Cl_2 (3 mL), and washed sequentially with 10% citric acid (3 mL), 1 N NaOH (3 mL), and brine (3 mL), dried (MgSO4), filtered, and concentrated. In the case of the DL-diastereomer, 18 mg (93%) of (2□*RS*,2*S*,4*R*,6*R*,8*S*)-methyl-9-oxo-8-(*N*-(*p*-toluenesulfonyl)-prolinamido)-4-phenyl-1-azabicyclo[4,3,0]nonane carboxylate **12** was obtained as a 1:1 mixture of diastereoisomers as determined by measuring the signals of the methyl ester singlets at 3.75 and 3.73 ppm. For the (2'*S*)-diastereoisomer, 12 mg (62%) of (2'*S*,2*S*,4*R*,6*R*,8*S*)-methyl-9-oxo-8-(*N*-(*p*-toluenesulfonyl) prolinamido)-4-phenyl-1-azabicyclo[4.3.0]nonane carboxylate **12** was obtained: ^{1}H NMR (C_6D_6) δ 7.69 (d, 2H, *J* = 8.2 Hz), 7.57 (d, 1H, *J* = 6.5 Hz), 7.13-7.04 (m, 3H), 6.86 (d, 2H, *J* = 8.4 Hz), 6.80 (d, 2H, *J* = 8.0 Hz), 4.36 (m, 1H), 4.19 (dd, 1H, *J* = 3.0, 8.6 Hz), 3.66 (s, 3H), 3.53 (dd, 1H, *J* = 4.0, 11.0 Hz), 3.27 (m, 1H), 2.90 (m, 1H), 2.48 (m, 1H), 2.34 (m, 1H), 2.18-2.07 (m, 2H), 1.89 (s, 3H), 1.85 (m, 1H), 1.70 (dd, 1H, *J* = 1.5, 13.2 Hz), 1.67-1.52 (m, 2H), 1.33 (d, 1H, *J* = 11.1 Hz), 1.20-1.11 (m, 2H), 1.02 (m, 1H). The limits of detection were determined by observation of the diastereomeric singlets at 3.75

and 3.73 ppm in a 400 MHz 1H NMR spectrum of (2'*S*)-**12** in C6D6 during incremental additions of the diastereomeric mixture (2□*RS*)-**12**, which demonstrated (2'*S*)-**12** to be of >98% diastereomeric purity. Distinct signals for (2'*R*)-**12** include: ^{1}H NMR (C_6D_6) *ä* 7.63 (d, 2H, *J* = 8.2 Hz), 7.47 (d, 1H, *J* = 8.2 Hz), 6.88 (d, 2H, *J* = 7.5 Hz)), 6.74 (d, 2H, *J* = 8.0 Hz), 4.78 (m, 1H), 3.64 (s, 3H), 1.85 (s, 3H).

(2*S*,4*R*,6*R*,8*S*)-9-Oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane Carboxylic Acid [(2*S*,4*R*,6*R*,8*S*)-3]. Ester (2*S*,4*R*,6*R*,8*S*)-**11** (519 mg, 1.34 mmol) in Et_2O (40 mL) was treated with potassium trimethylsilanolate (257 mg, 150 mol %), stirred for 2 h, quenched with water (1 mL), and evaporated to a residue that was partitioned between $CHCl_3$ (50 mL) and 10% citric acid solution (50 mL). The phases were separated, and brine (25 mL) was added to the aqueous phase, which was extracted with $CHCl_3$ (4 x 25 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated to provide (2*S*,4*R*,6*R*,8*S*)-9-oxo-8-(*N*-(Boc)-amino)-4-phenyl-1-azabicyclo[4.3.0]nonane carboxylic acid [(2*S*,4*R*,6*R*,8*S*)-**3**, 410 mg, 82%]: ^{1}H NMR (C_6D_6) δ 9.84 (br s, 1H), 7.15 (m, 2H), 7.07 (m, 1H), 6.94 (m, 2H), 5.81 (d, 1H, *J* = 5.6 Hz), 5.10 (d, 1H, *J* = 5.6 Hz), 4.40 (br s, 1H), 3.58 (m, 1H), 2.66 (br t, 1H, *J* = 11.6 Hz), 2.43 (m, 2H), 1.57-1.48 (m, 3H), 1.45 (s, 9H), 1.08 (q, 1H, *J* = 11.8 Hz); ^{13}C (C_6D_6) δ 174.0, 173.2, 156.7, 145.1, 129.1, 127.5, 127.1, 80.0, 53.3, 52.5, 52.4, 39.3, 38.8, 35.2, 33.7, 28.8; MS (FAB+) *m/z* 375.1 (M + H+); HRMS calcd for $C_{20}H_{26}N_2O_5$ (MH+) 375.1920, found 375.1915.

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5.9. Supplementary data.

General: Unless otherwise noted, all reactions were performed under an atmosphere of dry argon. THF and Et_2O were distilled from sodium/benzophenone; toluene and bromobenzene from sodium; CH_2Cl_2 and $CHCl_3$ from P_2O_5; $(CH_3)_3SiCl$ (TMSCl) and Et_3N from CaH_2. The procedures for making compounds 8d, 8e and 8f (Table 1) have been described in reference 14. Chromatography was carried out using 230-400 mesh silica gel. ^1H and ^{13}C NMR spectra were taken in $CDCl_3$, C_6D_6 and CD_3OD on 300, 400 or 600 MHz spectrometers, and are referenced to internal tetramethylsilane for $CDCl_3$ and C_6D_6 solutions and on MeOH at 3.31 ppm in CD3OD. Aromatic ^{13}C NMR signals are not reported in the case of compounds containing PhF groups.

(2*S*,6*SR*,8*R*)-Di-*tert*-butyl-4-oxo-6-nitromethyl-2,8-bis[N-(PhF)amino]-azelate (8c). A solution of enone (5*E*)-**7** (0.5 g, 0.6 mmol) in acetonitrile (5 ml) was treated with DBU (108 µl, 120 mol%) and nitromethane (40 µl, 120 mol%) and stirred for 18 h. The volatiles were removed under vacuum and the solid was dissolved in CH_2Cl_2 (20 ml), washed with 10% citric acid (10 ml) and brine (10 ml), dried with Na_2SO_4 and concentrated on a rotary evaporator. Chromatography was performed using hexane / EtOAc (90 / 10) as eluant and gave a 2:1 diastereomeric mixture of **8c** (525 mg, 99 %): TLC R^f 0.24 (85/15 hexane / EtOAc); Major isomer: ^1H NMR δ 7.70-7.62 (m, 8H), 7.47-7.16 (m, 44H), 4.05 (dd, 1H, J = 4.5, 12.1 Hz), 3.80 (dd, 1H, J = 6.2, 12.2 Hz), 3.21 (br s, 2H), 2.84 (t, 1H, J = 5.2 Hz), 2.54-2.15 (m, 4H), 1.61-1.30 (m, 2H), 1.25 (s, 9H), 1.17 (s, 9H). Distinct signals for the minor isomer include: δ 4.47 (t, 2H, J = 6.2 Hz), 2.94 (br s, 2H), 2.78 (t, 1H, J = 5.0 Hz), 1.93 (dd, 1H, J = 4.0 Hz, J = 8.7 Hz), 1.26 (s, 9H), 1.16 (s, 9H). MS (FAB+) m/z 884.5 (M + H+).

(2*S*,6*SR*,8*R*)-Di-*tert*-butyl-4-oxo-6-cyano-2,8-bis[*N*-(PhF)amino]-azelate (8b). A solution of enone (5*E*)-**7** (0.5 g, 0.6 mmol) in THF (20 ml) was treated with 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6, 24 mg, 15 mol%) and KCN (195 mg, 500 mol%). The solution was heated at reflux and stirred for 24 h. The volatiles were removed by rotary evaporation and the resulting solid was dissolved in CH_2Cl_2 (20 ml), washed with water (10 ml) and brine

(10 ml), dried with Na_2SO_4 and concentrated on a rotary evaporator. Purification by column chromatography using hexane / EtOAc (90 / 10) as eluant gave a 1:1 diastereomeric mixture of **8b** (336 mg, 67 %): TLC R^f 0.28 (90/10 hexane / EtOAc); ^{1}H NMR δ 7.63-7.56 (m, 8 H), 7.37-7.10 (m, 44 H), 3.36 (m, 1H), 3.15 (m, 2H), 3.00 (m, 1H), 2.80 (t, 2H, *J* = 5.2 Hz), 2.58-2.20 (m, 6H), 1.94 (d, 1H, *J* = 5.0 Hz), 1.71 (m, 1H), 1.52 (m, 1H), 1.45-1.39 (m, 2H), 1.19 (s, 9H), 1.18 (s, 9H), 1.13 (s, 9H), 1.10 (s, 9H). ^{13}C NMR δ 206.5, 173.9, 173.6, 121.7, 120.8, 81.6, 72.7, 54.0, 53.6, 53.0, 43.9, 37.0, 27.7, 27.6, 27.5, 22.6, 22.1. MS (FAB+) m/z 850 (M + H+).

(2*S*,6*SR*,8*R*)-Di-*tert*-butyl-4-oxo-6-(bis-methoxycarbonyl-methyl)-2,8-bis[N-(PhF)amino] -azelate (8a). A solution of enone (5*E*)-**7** (0.5 g, 0.6 mmol) in THF (5 ml) and hexamethylphosphoramide (0.5 ml) was treated with NaH (26.4 mg, 110 mol%) and dimethylmalonate (75 µl, 110 mol%), stirred for 6 h, treated with saturated NH_4Cl solution (10 ml), and extracted with EtOAc (3×10 ml). The combined organic phase was washed with saturated NH_4Cl solution (5×10 ml), dried with Na_2SO_4 and concentrated on a rotary evaporator. Purification by column chromatography using hexane / EtOAc (85 / 15) gave a 1:1 diastereomeric mixture of **8a** (441 mg, 77 %): TLC Rf 0.29 (80/20 hexane / EtOAc); ^{1}H NMR δ 7.68-7.61 (m, 8H), 7.42-7.16 (m, 44H), 3.93 (d, 1H, *J* = 4.1 Hz), 3.76 (s, 3H), 3.68 (s, 3H), 3.66 (s, 3H), 3.62 (s, 3H), 3.25 (d, 1H, *J* = 4.0 Hz), 2.98 (br m, 4H), 2.87 (m, 1H), 2.77 (m, 1H), 2.63 (dd, 1H, *J* = 4.4, 17.8 Hz), 2.53-2.30 (m, 7H), 2.22 (dd, 1H, *J* = 5.1, 16.2 Hz), 2.06 (dd, 1H, *J* = 4.9, 17.8 Hz), 1.64-1.33 (m, 6H), 1.25 (s, 9H), 1.23 (s, 9H), 1.16 (s, 9H), 1.13 (s, 9H). ^{13}C NMR δ 206.6, 175.0, 174.8, 169.3, 169.2, 169.0, 168.9, 81.1, 81.0, 80.8, 80.6, 73.0, 72.7, 64.3, 60.4, 54.0, 53.9, 53.1, 53.0, 52.6, 52.3, 52.2, 52.1, 52.0, 47.7, 47.6, 45.3, 44.5, 30.6, 30.0, 29.7, 27.8, 27.7, 14.2, 13.7. MS (FAB+) m/z 956.4 (M + H+).

***tert*-Butyl-6-(3'-*tert*-butyl-propionate)-4-phenyl-piperidine-2-carboxylate (10).** Ketone (6*R*)-**8d** (0.05 g, 55 µmol) was dissolved in MeOH (7 ml) and EtOAc (3 ml), treated with palladium-hydroxide-on-carbon 20 wt% (5 mg, 10 wt%). The reaction vessel was filled, vented and refilled three times with hydrogen. After stirring for 24 h under 9 atm of H_2, the suspension was then

filtered through Celite™ and concentrated on a rotary evaporator. The solid was partitioned between saturated NaHCO3 and 4/1 $CHCl_3$ / *i*-PrOH. The aqueous phase was extracted with 4/1 CHCl3 / *i*-PrOH (3x5 ml). The organic phases were combined, dried with MgSO4 and concentrate under vacuum. Purification by column chromatography using hexane / *i*-PrOH (90/10) as eluant gave compound (4*S*,6*R*)-**9** (1/4 d.r.) and (4*S*)-**10** (1/4 d.r.) in a 1 / 1 ratio. (4*S*,6*R*)-*tert*-Butyl-6-(3'-*tert*-butyl-2-amino-propionate)-4-phenyl-piperidine-2-carboxylate (4*S*,6*R*)-**9**, 7 mg, 30 %]: TLC R^f 0.1 (90/10 hexane/*i*-PrOH); distinct signals for major isomer: ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 3H), 7.20 (m, 2H), 3.54-3.47 (m, 2H), 3.33 (br s, 1H), 2.98 (t, 1H, *J* = 7.85 Hz), 2.70 (q, 1H, *J* = 6.99 Hz), 2.47 (d, 1H, *J* = 13.5 Hz), 2.14 (d, 1H, *J* = 13.8 Hz), 1.92-1.55 (m, 6H), 1.47 (s, 9H), 1.41 (s, 9H). ^{13}C NMR (CDCl3) δ 175.5 (C), 172.9 (C), 143.4 (C), 128.4 (2 CH), 127.7 (2 CH), 125.7 (CH), 81.0 (2 C), 54.9 (CH), 52.2 (CH), 47.9 (CH), 41.1 (CH2), 36.7 (CH2), 35.3 (CH), 33.1 (CH2), 28.0 (3 CH3), 27.9 (3 CH3). MS (FAB+) m/z 405.2 (M + H+). First to elute was *tert*-butyl-6-(3'-*tert*butyl-propionate)-4-phenyl-piperidine-2-carboxylate (**10**, 7 mg, 30 %): TLC Rf 0.28 (90/10 hexane/*i*-PrOH); distinct signal for major isomer, assigned to the 2*S*,4*S*,6*R* stereochemistry on analogy with **9**: ^{1}H NMR (CDCl3) δ 7.32 (m, 3H), 7.22 (m, 2H), 3.45 (dd, 1H, *J* = 2.7, 11.4 Hz), 3.31 (br s, 1H), 2.79 (m, 1H), 2.45 (d, 1H, *J* = 13.7 Hz), 2.30 (m, 2H), 2.18 (d, 1H, *J* = 13.7 Hz), 1.92 (m, 2H), 1.71 (q, 2H, *J* = 7.3 Hz), 1.47 (s, 9H), 1.41 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 172.8 (2 C), 128.2 (2 CH), 127.7 (2 C), 125.7 (CH), 81.1 (CH), 80.2 (C), 55.1 (CH), 50.6 (CH), 35.7 (CH), 35.1 (CH_2), 33.2 (CH_2), 32.2 (2 CH_2), 28.0 (6 CH_3). MS (FAB+) m/z 390.1 (M + H+). The 2*S*,4*S*,6*S* diastereoisomer of compound **10** was obtained as the major product from hydrogenation of ketone (6*R*)-**8d** (0.05 g, 55 μmol) in EtOH (9 ml) and AcOH (1 ml), using palladiumhydroxide-on-carbon 20% (5 mg, 10 wt%), which gave a 1 / 2 mixture of (4*S*,6*S*)-**9** (2/1 d.r.) and 10 (9/1 d.r.): (2*S*,4*S*,6*S*)-*tert*-butyl-6-(3'-*tert*-butyl-propionate)-4-phenylpiperidine-2-carboxylate [(2*S*,4*S*,6*S*)-**10**, 10 mg, 47 %]: TLC R^f 0.28 (90/10 hexane / *i*-PrOH); distinct signals for the major isomer: ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 3H), 7.20 (m, 2H), 3.38 (dd, 1H, *J* = 2.3, 11.5 Hz), 2.69 (m, 2H), 2.33 (m, 2H), 2.17 (d, 1H, *J* = 12.7Hz), 1.84 (d, 1H, *J* = 12.7 Hz), 1.80-1.68 (m, 3H), 1.51 (q, 2H, *J* = 12.2 Hz), 1.45 (s, 9H), 1.43 (s, 9H), 1.27 (q, 1H, *J* = 12.1 Hz). ^{13}C NMR (CDCl3) δ 172.8 (C), 172.3 (C),

145.6 (C), 128.5 (2 CH), 126.9 (2 CH), 126.4 (CH), 81.2 (C), 80.3 (C), 59.5 (CH), 55.4 (CH), 42.8 (CH), 39.3 (CH_2), 36.7 (CH_2), 32.0 (CH_2), 31.9 (CH_2), 28.1 (3 CH_3), 28.0 (3 CH_3). MS (FAB+) m/z 390.1 (M + H+).

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Chapitre 6.

Utilisation des acides aminés azabicycloalcanes pour l'étude des antagonistes analogues de CGRP sur le récepteur CGRP1.

Article 5.

Cluzeau, J.; Lubell, W.D. 'The Use of Turn-Inducing Amino Acids to Explore the Conformational Requirements for Antagonist Potency of $CGRP_{27-37}$ Analogs at the Human Calcitonin Gene-Related Peptide 1 Receptor' article en attente des résultats biologiques pour soumission au *Journal of Medicinal Chemistry*.

The Use of Turn-Inducing Amino Acids to Explore the Conformational Requirements for Antagonist Potency of $CGRP_{27-37}$ Analogs at the Human Calcitonin Gene-Related Peptide 1 Receptor

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6.1. Abstract.

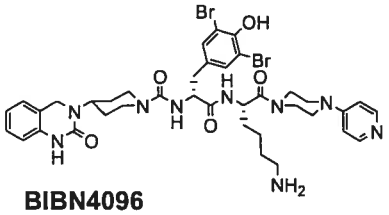
Calcitonin gene-related peptide antagonists exhibit potential for the treatment and prevention of disease states such as non-insulin dependant diabetes mellitus, migraine headache, pain and inflammation. To gain insight into the spatial requirements for CGRP antagonism, we have employed three β-turn mimics to constrain the peptide backbone and side-chain geometry of the antagonist *C*-terminal peptides $[D^{31},P^{34},F^{35}]CGRP_{27-37}$ **5** and $[D^{31},P^{34},F^{35}]CGRP_{29-37}$ **12**. A combination of (3*S*,6*S*,9*S*)-2-oxo-3-(*N*-(Fmoc)-amino)indolizidin-2-one amino acid (Fmoc-I^2aa, **1**), (2*S*,6*S*,8*S*)-9-oxo-8-(*N*-(Fmoc)-amino)indolizidin-9-one amino acid (Fmoc-I^9aa, **2**) and (2*S*,4*R*,6*R*,8*S*)-9-oxo-8-(*N*-(Fmoc)-amino)-4-phenyl-indolizidin-9-one amino acid (Fmoc-4-Ph-I^9aa, **3**) was used to explore the importance of turn structures and to study the side-chain orientations of the Phe residues. Six nonapeptides and six undecapeptides were made incorporating the I^2aa, I^9aa and 4-Ph-I^9aa in positions 31-32, 34-35 and 36-37.

6.2. Introduction.

Calcitonin Gene-Related Peptide (CGRP, Figure 1) is a 37 amino acid peptide produced in two forms (α and β) by an alternative splicing of calcitonin mRNA.[1] Both α and β human CGRP are present in the central and peripheral nervous systems, and although they differ by three amino acids, they produce similar biological activity.[1,2,3] As the most potent endogen vasodilator known, CGRP exhibits effects on blood pressure, the cardiovascular system, pregnancy, the central nervous system, skeletal muscles, calcium metabolism, insulin regulation and gastric secretion.[4]

CGRP apparently binds to two different receptors.[5,6,7] The more well studied, CGRP1 receptor is composed of three different proteins: the CRL (Calcitonin Receptor-Like) receptor, a seven transmembrane domain receptor, the Receptor Component Protein (RCP), which acts as a chaperone protein and the Receptor-Activity-Modifying Proteins (RAMPs), on which depends the specificity of the receptor, as either a CGRP receptor (RCP/CLR/RAMP1), an

hα-CGRP	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-NH_2
hα-CGRP$_{8-37}$	VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-NH_2
[But-Cys^{18}]CGRP$_{19-37}$	*t*-Bu CGGVVKNNFVPTNVGSKAF-NH_2
[BTD$^{19-20}$]CGRP$_{27-37}$	VTHRLAGLLSR(**BTD**)GVVKNNFVPTNVGSKAF-NH_2
[BTD$^{33-34}$]CGRP$_{27-37}$	VTHRLAGLLSRSGGVVKNNFVPTNV(**BTD**)KAF-NH_2
[BTD$^{19-20}$, BTD$^{33-34}$]CGRP$_{27-37}$	VTHRLAGLLSR(**BTD**)GVVKNNFVPTNV(**BTD**)KAF-NH_2
hα-CGRP$_{27-37}$	FVPTNVGSKAF-NH_2
[D^{31}, P^{34}, P^{35}]CGRP$_{27-37}$	FVPT**D**VG**PF**AF-NH_2



BIBN4096

FIGURE 1 Structure of hαCGRP, and peptidic and non-peptidic CGRP antagonists.

Adrenomedulin (AM) receptor (RCP/CLR/RAMP2) or as a non-selective CGRP/AM receptor (RCP/CLR/RAMP3).[8,9,10] The second receptor, $CGRP_2$ receptor is present in sexual body parts such as the uterus[11,12,13] and the vas deferens,[14,15] and is less sensitive to $CGRP_{8-37}$ antagonism.[10] The concentration of CGRP is greater in woman, dependant on sex steroid hormones, and augmented during pregnancy, labour and postpartum when CGRP regulates placenta blood flow and uterine contractility.[6]

The dose-dependant dilation effects of CGRP suggested potential for application of CGRP agonists in clinic to treat severe hypertension, cerebrovascular vasospasm and Raynaud's phenomenon; however, drawbacks including lack of efficacy and tachycardia have limited their development.[16,17,18] CGRP antagonists remain attractive candidates for treating a variety of indications, including, non-insulin dependant diabetes mellitus, migraine headache, pain inflammation and morphine induced analgesia. For example, a dipeptide analog, BIBN4096 has recently entered into clinical trials for the treatment of migraine.[19]

The structure of CGRP has been shown by NMR and CD spectroscopy to comprise an amino-terminal disulfide-bonded loop containing residues 2-7 that nucleates an α-helical region between residues 8 and 18 which unfolds, via a turn about residues 19-21, into a less ordered sequence that may adopt turn conformations.[20,21,22,23,24,25] Initial examination of CGRP by NMR spectroscopy in 1:1 trifluoroethanol : water identified the presence of a turn-like conformation about residues 19-21.[8] Examination of CGRP in DMSO by NMR spectroscopy and computational analysis provided further evidence to suggest a γ-turn between the amide carbonyl of Ser^{19} and the amide NH of Gly^{21}.[12] Spectroscopic analysis of the *C*-terminal [But-Cys^{18}]h$CGRP_{19-37}$ fragment in DMSO also revealed a turn conformation in the Ser^{19}-Gly^{21} region as well as indicated a second type I β-turn in the region of Asn^{31}-Val^{32}-Gly^{33}.[13] Although this second turn was not observed in spectroscopic studies of the parent CGRP peptide,[12] a comparison with an *N*-terminal fragment of CGRP and two different agonists, both constrained by a disulfide ring, one comprised at the *C*-

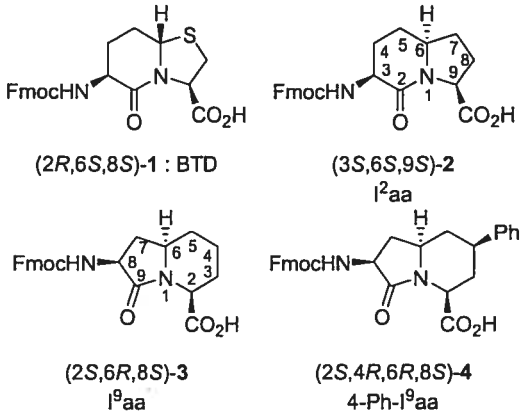


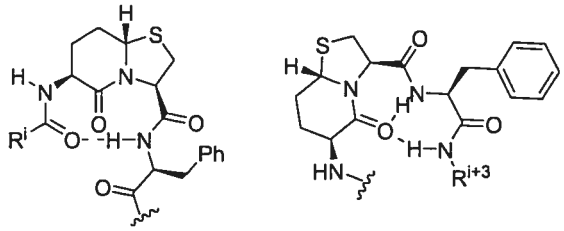
Figure 2. Conformationaly restricted amino acids

and the other at the *N*-terminal fragment, indicated that the Val^{32}-Gly^{33}-Ser^{34} sequence may adopt an inverse γ-turn.[26,27]

The analysis of abridged CGRP analogs has shown that *N*-terminal fragments usually activate the receptor and that the *C*-terminal fragments act as antagonists of CGRP action.[5,6] For example, modification of the selective antagonist $[Y^0]CGRP_{28-37}$ furnished recently $[D^{31},P^{34},F^{35}]CGRP_{27-37}$ which exhibited a 100-fold increase in affinity.[28,29,30] Examination of $[D^{31},P^{34},F^{35}]CGRP_{27-37}$ and related analogs by CD spectroscopy revealed that increases in ordered structure correlated with high binding affinity and suggested that replacement of Ser^{34} by proline to reinforce a turn already present in the unmodified segment.[31] Studies of analogs of $CGRP_{27-37}$ indicated that Thr^{30}, Val^{32} and Phe^{37} play critical roles for biological activity. Computational and NMR spectroscopy studies on two of these analogs have also suggested the participation of Thr^{30} in a turn conformation.[32] Two alternative turn conformations have also been proposed for antagonist activity of *C*-terminus fragments: a possible γ-turn around Pro^{34} in $[D^{31},P^{34},F^{35}]CGRP_{27-37}$ and a left-handed helical turn from V^{32} to A^{36} in $[D^{31},A^{34},F^{35}]CGRP_{27-37}$. More recently, the introduction of the turn inducing thiaindolizidinone amino acid (BTD, Figure 2) at position 19,20 and 33,34 of the antagonist $h\alpha CGRP_{8-37}$ produced constrained analogs that exhibited similar antagonist activity (pA_2 = 6.0 to 6.2) as the parent peptide ($pA_{2\ (CGRP27-37)}$ = 6.0).[10] This observation has led to a suggestion that the active conformer possesses two β-bends at positions 18-21 and 32-35.

6.3. Design and Synthesis of CGRP antagonists.

Our approach towards understanding the conformational requirements peptide based CGRP antagonists has employed three indolizidinone amino acid surrogates to modify the potent undecapeptide $[D^{31},P^{34},F^{35}]CGRP_{27-37}$. Indolizidin-2- and 9-one *N*-(Fmoc)amino acids (Fmoc-I^2aa and Fmoc-I^9aa) were placed at positions 31-32 and 34-35 to compare the influence of backbone geometry on activity. Study of *N*-acetamido indolizidinone *N'*-methylamide[33] and cyclo[Asp-I^2aa-Asp-I^2aa][34] using NMR spectroscopy and modeling techniques as well as the X-ray structure of (3*S*, 6*S*, 9*S*)-indolizidinone *N*-(Boc)amino methyl ester,[35] all have indicated that I^2aa may adopt the *i* / *i* + 1 positions as well as the *i* + 1 / *i* + 2 positions of a β-turn and the *i* / *i* + 1 positions of a γ-turn. Similar preferences for both the *i* / *i*+1 positions and the *i*+1 / *i*+2 positions of β-turn conformations have also been observed when the thiaindolizidinone amino acid (BTD, Figure 3) was introduced respectively into cyclic hexapeptide mimics of tendamistat[36] and into the antibiotic cyclic peptide gramicidin S.[37] The introduction of BTD at positions 33-34 of $CGRP_{8-37}$ indicated the importance of a turn conformation for antagonist activity.[15] In peptide **5**, Pro^{34} may likely situate itself at the *i* / *i* + 1 positions of γ- and β-turns as well as the *i* + 1 / *i* + 2 positions of β-turns. In lights of success with BTD as a surrogate of Gly-Pro at position 33-34, the Pro-Phe residues at the 34-35 position were replaced with I^2aa and I^9aa to examine further proline positioning in the turn region. For comparison with turn inducing amino acids, (2*S*,4*R*,6*R*,8*S*)-4-phenyl-indolizidin-9-one *N*-(Fmoc)amino acid (Fmoc-4-Ph-



I^2aa at residues i+1 and *i*+2 of β-turn

I^2aa at residues i and *i*+1 of γ- and β-turn

Figure 3. Positioning azabicycloalkane amino acids in peptide turns.

I^9aa) was introduced at position 34-35 to mimic the Phe side chain. In addition, the influence of the *C*-terminal aromatic side-chain geometry on activity was studied by replacing the Ala-Phe at the position 36-37 by Fmoc-4-Ph-I^9aa.

Chemistry. Peptide synthesis was performed on BHA resin with an amino caproic acid spacer and Rink linker to furnish the *C*-terminal amides. Peptide elongation employed a Fmoc strategy[38] with TBTU as coupling agent[39] in combination with HOBt in DMF. At the ninth amino acid residue (Pro), the resin was split in two portions: one cleaved to furnish the nonapeptides, the second elongated and cleaved to give the undecapeptides.

Bioassays. Biological activity was determined using « CatchPoint™ Cyclic-AMP Fluorescent Assay Kit » from Molecular Devices using CRLR / RAMP1 as the receptor.

6.4. Results/Discussions.

The importance of turn conformations in the region 31-32 and 34-35 of [D^{31},P^{34},F^{35}]$CGRP_{27-37}$ was investigated using I^2aa **2**, I^9aa **3** and 4-Ph-I^9aa **4** as rigid dipeptide surrogates (Table 1). The biological data for peptides are under investigation.

Table 1. $[D^{31},P^{34},F^{35}]CGRP_{27-37}$ and azabicycloalkane-containing peptides analogs.

	Peptides[a]	Calcd $(M+H^+)$	MS $(M+H^+)$	HPLC (min)[b]	HPLC (min)[c]	Purity (%)
5	$[D^{31},P^{34},F^{35}]CGRP_{27-37}$	1195.6	1196.0	18.4	22.7	>98
6	$[I^{2}aa^{31-32},P^{34},F^{35}]CGRP_{27-37}$	1161.7	1162.1	17.8	22.4	86
7	$[I^{9}aa^{31-32},P^{34},F^{35}]CGRP_{27-37}$	1161.7	1162.0	18.6	22.5	87
8	$[D^{31},I^{2}aa^{34-35}]CGRP_{27-37}$	1131.6	1131.8	12.6	19.9	91
9	$[D^{31},I^{9}aa^{34-35}]CGRP_{27-37}$	1131.6	1131.8	13.6	20.1	86
10	$[D^{31},Xaa^{34-35}]CGRP_{27-37}$	1207.6	1207.4	20.1	23.1	>99
11	$[D^{31},P^{34},F^{35},Xaa^{36-37}]CGRP_{27-37}$	1233.6	1233.7	21.0	23.8	92
12	$[D^{31},P^{34},F^{35}]CGRP_{29-37}$	949.5	949.5	15.1	21.5	>99
13	$[I^{2}aa^{31-32},P^{34},F^{35}]CGRP_{29-37}$	915.5	915.7	14.5	20.6	>99
14	$[I^{9}aa^{31-32},P^{34},F^{35}]CGRP_{29-37}$	915.5	915.7	15.0	20.8	>98
15	$[D^{31},I^{2}aa^{34-35}]CGRP_{29-37}$	885.4	885.3	8.2	17.7	91
16	$[D^{31},I^{9}aa^{34-35}]CGRP_{29-37}$	885.4	885.3	9.4	17.8/18.1	60/30[d]
17	$[D^{31},Xaa^{34-35}]CGRP_{29-37}$	961.5	961.7	16.6	21.7	>99
18	$[D^{31},P^{34},F^{35},Xaa^{36-37}]CGRP_{29-37}$	987.5	987.7	17.0	22.5	>99

(a) Xaa = 4-Ph-I^{9}aa. (b) Eluant #1: water / acetonitrile from 80/20 to 50/50 in 30 min, 50/50 to 10/90 in 5 min. (c) Eluant #2: water / MeOH from 80/20 for 5 min, 80/20 to 20/80 in 20 min, 20/80 to 10/90 in 5 min. (d) Two signals (2:1 ratio) corresponding to the same mass were observed, which may correspond to either a conformational or a configurational mixture.

6.5. Experimental.

According to our reported method, (3*S*,6*S*,9*S*)-indolizidin-2-one *N*-(Boc)amino acid (Boc-I^{2}aa) was prepared from L-glutamic acid as inexpensive chiral adduct in 23% overall yield,[35] (2*S*,6*R*,8*S*)-indolizidin-9-one *N*-(Boc)amino acid (Boc-I^{9}aa) and (2*S*,4*R*,6*R*,8*S*)-4-phenyl-indolizidin-9-one *N*-(Boc)amino acid (Boc-4-Ph-I^{9}aa) were prepared from L-aspartic acid in 28% and 8% respective overall yield.[40,41] Subsequent conversion to their *N*-Fmoc amino acid counterparts was performed as described below. Solvents (DMF, CH_2Cl_2 and Et_2O) were dried using a GlassContour™ solvent dispensing system. *N,N*-Diisopropylethylamine (DIEA) was successively distilled from ninhydrin and CaH_2 and was kept under argon atmosphere. Solid-phase synthesis was performed in SPE tubes (6, 12 and 60 mL) using vortex agitation.

(2*S*,4*R*,6*R*,8*S*)-4-Phenyl-indolizidin-9-one *N*-(Fmoc)amino acid (Fmoc-4-Ph-I^{9}aa, 3). (2*S*,4*R*,6*R*,8*S*)-9-Oxo-8-(*N*-(Boc)-amino)-4-phenyl-indolizidin-9-one amino acid (400 mg, 1.07 mmol) was dissolved in a 1:1 solution of TFA/DCM and stirred for 2 h. The volatiles were evaporated and the residue was dissolved in and evaporated from toluene twice. The amino acid was treated with DIEA (0.74 mL, 400 mol%) and Fmoc-OSu (720 mg, 200 mol%) in dioxane (10 mL) for 16 h. The solution was evaporated to a residue that was partitioned between $CHCl_3$ (50 mL) and 10% citric acid solution (50 mL). The phases were separated, and brine (25 mL) was added to the aqueous phase, which was extracted with $CHCl_3$ (4 x 25 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography using $CHCl_3$ / MeOH / AcOH (94 / 5 / 1) as eluant to provided (2*S*,4*R*,6*R*,8*S*)-9-oxo-8-(*N*-(Fmoc)-amino)-4-phenyl-indolizidin-9-one amino acid **3** (330 mg, 63%). ^{1}H NMR ($CHCl_3$) δ 7.67 (d, 2H, *J* = 7.5 Hz), 7.51 (d, 2H, *J* = 7.4 Hz), 7.31 (t, 2H, *J* = 7.4 Hz), 7.24-7.18 (m, 4H), 7.13 (t, 1H, *J* = 7.2 Hz), 7.06 (d, 2H, *J* = 7.4 Hz), 5.61 (d, 1H, *J* = 5.3 Hz), 4.91 (d, 1H, *J* = 5.2 Hz), 4.30 (m, 2H), 4.14 (t, 1H, *J* = 6.8 Hz), 3.77 (s, 1H), 2.70 (m, 2H), 2.40 (d, 1H, *J* = 13.3 Hz), 2.02 (d, 1H, *J* = 12.6 Hz), 1.81 (m, 1H), 1.58 (q, 1H, *J* = 10.7 Hz), 1.58 (q, 1H, *J* = 12.4 Hz); ^{13}C NMR ($CHCl_3$) δ 173.2, 172.5, 156.4, 143.7, 141.3, 128.7, 127.7, 127.0, 126.8, 126.6, 125.1, 119.9, 67.0, 52.7,

52.0, 51.4, 47.0, 39.4, 38.1, 35.1, 33.1; $[\alpha]^{20}_{D}$. –14.3° (c 0.006, $CHCl_3$/MeOH: 1/1); MS (ESI) *m/z* 497.3 ($M + H^+$).

Peptide Synthesis. Peptides were synthesized on a 0.16 mmol scale on Rink linker with an amino caproic spacer attached to BHA (0.4 mmol/g) resin to obtain *C*-terminal amides after resin cleavage and deprotection. Side chains of threonine and aspartic acid were respectively protected as *tert*-butyl ether and ester. At the ninth amino acid residue (Pro), the resin was spit in two parts. One part (0.08 mmol) was deprotected with piperidine and cleaved from the resin using neat TFA. The second part (0.08 mmol) was elongated by two more amino acids and cleaved. Peptides were assembled by stepwise addition of *N*-Fmoc amino acids following a typical Fmoc strategy[38] monitored by colorimetric Kaiser's test.[42] Natural amino acids (300 mol%) were coupled using TBTU (300 mol%) in combination with HOBt (300 mol%) and DIEA (600 mol%) in DMF (10mL/g of resin) for two hours. Indolizidinone amino acids were submitted twice to the coupling condition (100 mol% and 50 mol%) using TBTU (100 mol% and 50 mol%), HOBt (100 mol% and 50 mol%) and DIEA (200 mol% and 100 mol%). Final, Fmoc deprotection was followed by treatment with neat TFA for 45 min to cleave the resin and deprotect amino acid side chains. The resin was washed twice with TFA, the TFA layers were combined and treated with excess Et_2O to precipitate peptides. The peptides were removed by centrifugation and the solution was evaporated to an oil, that was precipitated with ether. The combined white solids were dried under vacuum, dissolved in 50% water / 50% acetonitrile and purified on semi-preparative HPLC / MSQ using an Alltech C18 column (25cm x 22 mm), a 20 mL/min flow rate and a gradient of water : acetonitrile containing 0.1% of TFA. Purification was made using semi-preparative LC-MS and furnished peptides in 23% average overall yields.

6.6. References.

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Chapitre 7.

Études des relations entre structures et activités pour des peptides antagonistes du récepteur ORL1.

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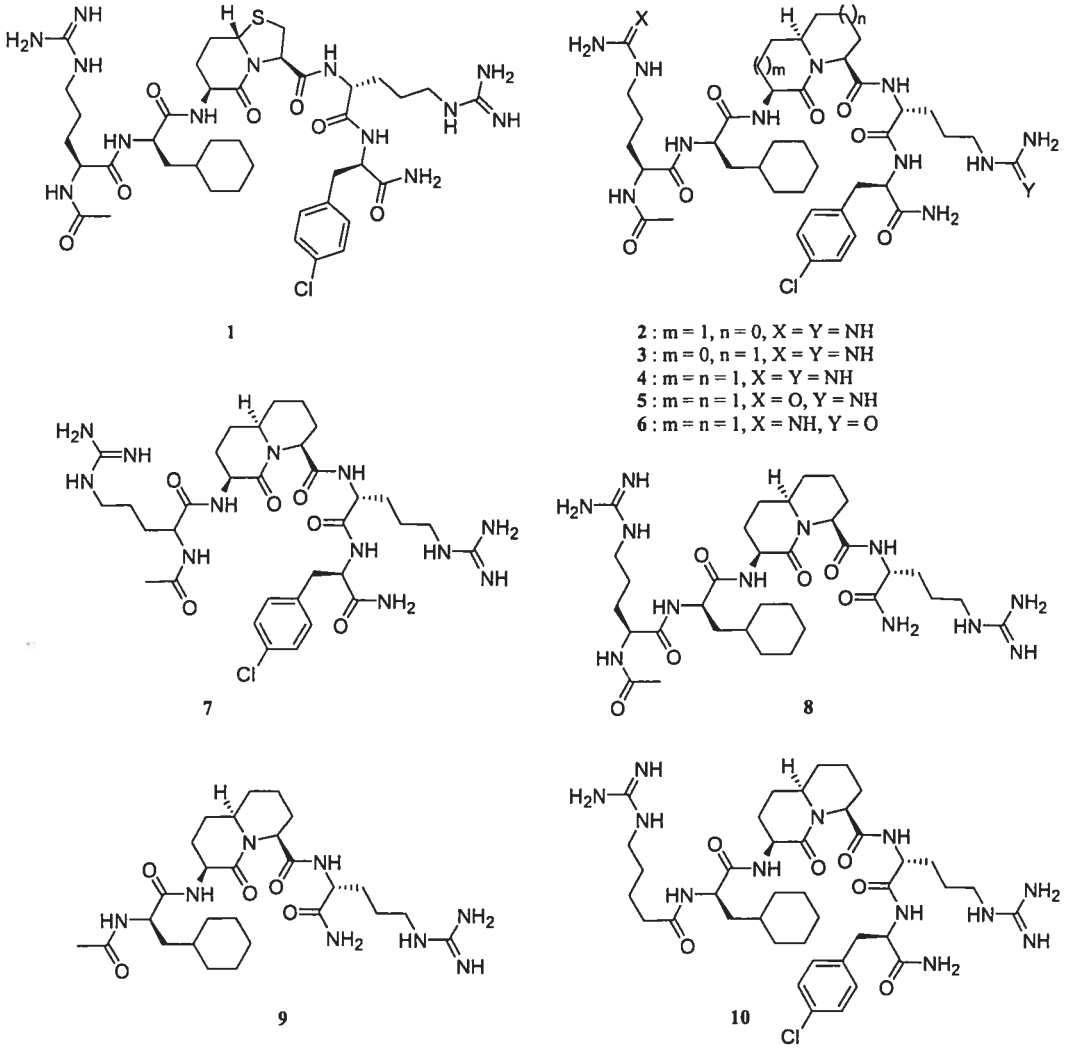
7.1. Introduction.

Le récepteur « opioid receptor-like 1 » (ORL1) fait parti de la famille des récepteurs couplés aux protéines G (GPCR). Il est relié structurellement aux récepteurs opioïdes μ, δ et κ avec environ 60% d'homologie.[1-4] Il est ainsi parfois appelé aussi récepteur opioïde 4 (OP4). Les ligands des récepteurs opioïdes n'ont cependant que peu d'affinité pour ce récepteur, dont le ligand naturel est l'heptadécapeptide nociceptine/orphanine FQ.[5,6] Les fonctions exactes de ORL1 sont encore mal définies bien que nociceptine/orphanine FQ présente de nombreuses activités. Les différents effets observés sont la



2 : m = 1, n = 0, X = Y = NH
3 : m = 0, n = 1, X = Y = NH
4 : m = n = 1, X = Y = NH
5 : m = n = 1, X = O, Y = NH
6 : m = n = 1, X = NH, Y = O

FIGURE 1 Structure du peptide III-BTD **1** et des peptides contenant les acides aminés azabicyclo[X.Y.0]alcanes **2** à **10**.

modulation de la douleur et des réflexes locomoteurs, un effet anxiolytique ou de stimulation de la faim.[7,8]

La recherche par chimie combinatoire d'antagonistes peptidiques de ORL1 dans le but de faciliter la compréhension des fonctions du récepteur à conduit au développement de l'hexapeptide **1** (Ac-Arg-D-Cha-BTD-D-Arg-D-*p*ClPhe-NH_2).[9] Ce peptide possède une bonne activité sur ORL1 (K*i* = 34 nM) mais n'est pas sélectif vis-à-vis des autres récepteurs opioïdes. Une étude, subséquente de remplacements systématiques de l'acide aminé azabicycloalcane BTD par les acides aminés I^2aa, I^9aa et Qaa a été effectuée.[10] Elle a démontré que les peptides contenant I^2aa (peptide **2**) et Qaa (peptide **4**) sont actifs sur ORL1 (K*i* = 44 et 35 nM respectivement) mais que seul le peptide **4** est sélectif pour ce récepteur (Figure 1).

La présente étude, menée en collaboration avec les laboratoires des professeurs Tourwé (VUB, Bruxelles, Belgique) et Simonin (CNRS-INSERM, Strasbourg, France) avait pour but d'étudier différents pharmacophores du peptide **4**. Ainsi, l'importance des fonctions guanidines des arginines 1 et 5 a été étudiée. Chacune des deux fonctions a successivement été remplacées par des fonctions urées des citrulines (peptides **5** et **6**). Cette modification permet d'étudier l'importance des charges positives sur les arginines.[11] Les autres éléments étudiés, par troncation du peptide, sont l'importance des différents acides aminés (peptides **7**, **8** et **9**) et de la fonction acétamide *N*-terminale (peptide **10**, Figure 1).

7.2. Synthèse.

Les étapes de synthèse et de synthèse peptidique ont été effectuées en commun avec Mlle Van Cauwenberghe (voir note, page III). Le Boc-Qaa-O*t*-Bu a été préparé à partir d'acide aspartique et d'acide pyroglutamique tel que décrit dans la littérature (voir aussi Schéma 39, chapitre 2).[12] Il a ensuite été transformé en Boc-Qaa-OH par déprotection des deux groupes protecteurs par HCl gaz et re-protection de la fonction amine par Boc_2O en présence de

$NaHCO_3$ dans un mélange H_2O / dioxane. Les peptides ont ensuite été synthétisés sur support solide en utilisant la résine MBHA en stratégie Boc. Les couplages ont été effectués en utilisant DIC / HOBt dans une solution DCM / DMF. Les déprotections des fonctions amines ont été effectuées dans un mélange TFA / anisole. L'amine *N*-terminale a été acétylée avant la libération du support solide par traitement avec de l'anhydride acétique et de la pyridine. La libération du support solide ainsi que la déprotection des groupes protecteurs toluènesulfonyle des arginines ont été effectuées simultanément par traitement avec du HF liquide anhydre. Le peptide **10** a été préparé par couplage finale de l'acide *N*-(Boc)-ω-amino valérique qui après déprotection du groupe Boc a été guanylé par du *N*,*N*'-bis(Boc)-1-guanylpyrazole en présence de DIEA dans le DMF.[13,14] Les peptides ont ensuite été purifiés et caractérisés par Mlle Van Cauwenberghe à Bruxelles et les testes biologiques ont été effectués à Strasbourg dans les laboratoires du professeur Simonin.

Le peptide **4** a également été re-synthétisé et re-testé en même temps que les peptides **5** à **10**. Il semble intéressant en premier lieu de noter que les valeurs de K*i* pour le peptide **4** sont différentes entre les précédentes mesures ($Ki_{(ORL1/\mu/\kappa/\delta)}$ = 35 / 496 / 441 / 7050) et celles remesurées ($Ki_{(ORL1/\mu/\kappa/\delta)}$ = 27 / 90 / 105 / >5000). L'activité reste semblable mais la sélectivité est moindre avec un ratio de $Ki_{(ORL1/\mu/\kappa/\delta)}$ de 1/3/4/>185 comparé à 1/14/13/201 calculé précédemment. Seules les nouvelles données seront donc comparées car elles ont toutes été effectuées en même temps et dans les mêmes conditions.

7.3. Discussion.

L'ablation d'un des acides aminés du peptide (peptides **7**, **8** et **9**) conduit à une perte presque totale d'activité pour tous les récepteurs, confirmant l'importance de la séquence. L'ablation de la partie acétamide *N*-terminale (peptide **10**), a conduit à un maintien d'activité et de sélectivité ($Ki_{(ORL1/\mu/\kappa/\delta)}$: 45 / 85 / 153 / >5000), indiquant la possibilité de supprimer cette fonction sans perte significative d'affinité pour le récepteur, ni de sélectivité. La charge de la fonction guanidine de la D-arginine 5 joue un rôle crucial dans l'activité du peptide. En effet, son remplacement par une D-citruline (peptide **6**) conduit à

une perte totale d'activité. La charge de l'arginine 1 joue, en revanche, un rôle déterminant dans la sélectivité du peptide. En effet, le remplacement par une citruline conduit à un gain notable de sélectivité avec un ratio de $Ki_{(ORL1/\mu/\kappa/\delta)}$ de 1/35/59/>69. Ce gain de sélectivité indique l'importance de la fonction guanidine à la position 1 pour maintenir une interaction avec les récepteurs μ et κ, comparé à ORL1.

Des testes complémentaires, essais d'affinité avec [^{35}S]GTPγS, ont également montrés une absence totale d'activité agoniste pour **5** et **10** ainsi que l'augmentation de l'activité antagoniste de **5** avec un K*e* = 136 nM comparé à **4**, K*e* = 300 nM.

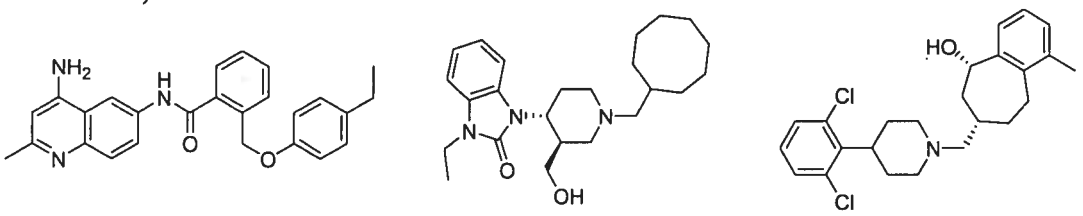
7.4. Conclusion.

Tableau 1. Affinités des antagonistes de ORL1 pour les récepteurs hORL, hMOR, hKOR et hDOR.

	Ki (nM)				Ratios
Compound	hORL	hMOR	hKOR	hDOR	
Nociceptin	0.079 ± 0.021	128.5 ± 7.5	49.4 ± 9.3	>2500	1:1626:620:31600
SB-612111	0.33 ± 0.03	57.6 ± 8	160.5 ± 22.4	2109 ± 570	1:174:485:6390
5	72 ± 21	2555 ± 635	4270 ± 100	>5000	1:35:59:69
(+)-J-113397	2 ± 0.7	30.7 ± 7.6	58.3 ± 6.9	>2500	1:15:29:1250
JTC-801	8.2 ± 0.3	102.9 ± 5.9	1057 ± 128	8647 ± 557	1:13:132:1080

Une meilleure compréhension des éléments structuraux et électroniques requis pour l'affinité et la sélectivité à ORL1 a été obtenue par la synthèse et l'analyse d'analogues du peptide **4** antagonistes de ORL1. L'ablation d'un de l'acide aminé de la séquence, ainsi que le remplacement de la fonction guanidine de la D-arginine 5 par une fonction urée d'une D-citruline conduisent à une perte totale d'activité vis-à-vis de tous les récepteurs opioïdes. Le remplacement de l'autre arginine par une citruline conduit en revanche à une nette augmentation de la sélectivité pour ORL1. L'ablation de l'acétamide *N*-

terminale peut être effectuée sans perte significative d'affinité ou de sélectivité. Le peptide **5**, Ac-Cit-D-Cha-Qaa-D-Arg-D-*p*ClPhe-NH_2, représente une avancée dans le développement d'antagoniste puissant et sélectif de ORL1. En effet, le peptide **5**, bien que moins actif que les antagonistes non peptidiques connus de ORL1 tels que JTC-801,[15] (+)-J-113397[16] et SB-612111,[17] ce situe au niveau de ces composés pour la sélectivité vis-à-vis des récepteurs opioïdes (Figure 2, Tableau 2).



JTC-801[15] J-113397[16] SB-612111[17]

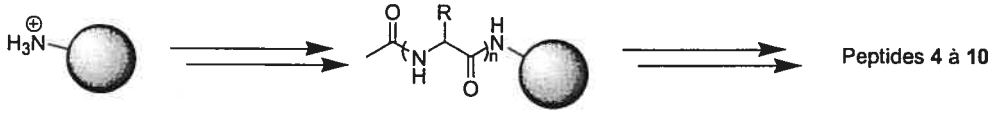
FIGURE 2. Antagonistes non peptidiques sélectifs de ORL1

7.5. Partie expérimentale.

Acide (3*S*,6*R*,10*S*)-2-oxo-3-*N*-(Boc)amino-1-azabicyclo[4.4.0]decane-10-carboxylique :

Le Boc-Qaa-O*t*-Bu (320 mg, 0.87 mmol) est dissout dans le DCM et mis à 0°C. Du HCl gazeux est bullé durant 2 h puis le solvant est évaporé. Le solide est repris dans H_2O / dioxane (20 ml), traité avec $NaHCO_3$ (165 mg, 230 mol%) puis avec Boc_2O (211 mg, 110 mol%) et la solution est agitée pour la nuit. Les solvants sont évaporés et l'huile est reprise dans Et_2O / $NaHCO_3$ saturé. La phase organique est ré-extraite 2 fois avec $NaHCO_3$ saturé puis les phases aqueuses sont réunies, acidifiées avec acide citrique solide jusqu'à pH=4 puis extraite 5 fois avec un mélange $CHCl_3$ / *i*-PrOH (80 / 20). Les phases organiques sont réunies, séchées avec $MgSO_4$ puis évaporées pour donner un solide blanc (131 mg, 41%) pf 126-128 °C ; $[\alpha]^{25}_D$ 18.8° (*c* 0.8, $CHCl_3$); RMN ^1H δ 5.65 (sl, 1H), 4.34 (dd, 1H, *J* = 5.79, 5.87), 4.20 (m, 1H), 3.56-3.50 (m, 1H,), 2.36-2.30 (m, 1H), 2.07-2.01 (m, 3H), 1.85-1.57 (ml, 6H), 1.44 (s, 9H); RMN ^{13}C δ 175.2, 171.3, 155.7, 79.4, 55.1, 53.4, 50.6, 29.0, 28.1, 27.0, 25.1, 24.0, 18.8; MS (ESI, *m/z*) 312.9 $[M+H]^+$.

Synthèse sur support solide : élongation du peptide.



La synthèse a été réalisée sur résine MBHA avec une stratégie Boc. La fonction guanidine des arginines est protégée par un groupement *para*-toluènesulfonyle (Tos) durant toute la synthèse. Avant le début des couplages, la résine sous forme $NH3^+$ est lavée avec DIEA (2x) puis DCM (2x). Pour chacun des couplages, la séquence suivante est utilisée :

1. Acide aminé Boc (300 mol%), DIC (300 mol%) et HOBt (300 mol%) dans un mélange 1 :1 de DMF/DCM agité pour 3 h.
2. La complétion de la réaction est vérifié en utilisant le test de Kaiser à la ninhydrine.[18] Si la réaction n'est pas complète, la résine est re-soumise aux mêmes conditions de couplage.
3. La résine est lavée avec du DMF (3x)
4. La résine est lavée avec du *i*-PrOH (3x)
5. La résine est lavée avec du DCM (3x)
6. Le groupe protecteur Boc est déprotégé avec un mélange TFA/DCM/anisole (49/49/2) pendant 5 min (2x) et 15 min (2x).
7. La résine est lavée avec du DCM (2x)
8. La résine est lavée avec de la DIEA (2x)
9. La résine est lavée avec du DCM (2x)

L'amine *N*-terminale est finalement acétylée (sauf peptide **10**) en utilisant de l'anhydride acétique (150 mol%) et de la pyridine (150 mol%) dans une solution 1 :1 de DMF/DCM pour 1 h. Les peptides et les groupements Tos sont simultanément libérés en traitant la résine par du HF liquide anhydre à 0°C. Après évaporation du HF, chaque peptide est précipité par ajout d'éther (2x). Le peptide est ensuite dissout avec de l'acide acétique (2x) et ainsi séparé de la résine. L'acide acétique est lyophilisé puis les peptides ont été purifié par HPLC en phase inverse.

7.6. Références.

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Chapitre 8.

Conclusion.

Le mimétisme peptidique est de plus en plus répandu dans l'étude des structures peptidiques comme dans le développement d'agents thérapeutiques, notamment afin de résoudre les problèmes de stabilité des chaînes peptidiques *in vivo*. Une autre utilisation est la rigidification des structures peptidiques dans le but de faciliter l'identification des conformations actives. Ainsi, de nombreux analogues peptidiques plus stables et/ou plus rigides ont été développés. Nos recherches sur le développement de mimes de repliements β et plus particulièrement sur les acides aminés azabicycloalcanes, nous ont porté à développer une méthode d'introduction de chaîne latérale sur l'acide aminé indolizidin-9-one.

Dans un premier temps, nous avons montré les possibilités d'introduction d'une chaîne latérale sur l'I^9aa par addition conjuguée sur l'intermédiaire de synthèse linéaire diamino azélate. Nous avons étudié les différents paramètres impliqués dans l'obtention de bonnes régio et stéréosélectivité pour l'addition de réactifs de Grignard. Nous avons ainsi remarqué l'importance des deux groupements acides aminés protégés pour le maintient de ces sélectivités.

Dans un deuxième temps, après avoir développé une méthode permettant de séparer les deux diastéréoisomères de l'intermédiaire linéaire comportant une chaîne latérale phényle, nous avons effectué la synthèse des acides aminés 4-phényl-indolizidin-9-ones. La cyclisation du diastéréoisomère 6*S* a conduit à la formation d'un seul isomère du 4-Ph-I^9aa. La cyclisation du diastéréoisomère 6*R* c'est révélé beaucoup plus complexe. Ainsi, l'amination réductrice de cet isomère dans les conditions standard (H_2, Pd/C, EtOH, AcOH) a conduit majoritairement à la formation d'un produit ayant perdu une fonction amine. Cette élimination issue d'une β-élimination à partir d'une énamine a pu être contournée par saturation de la réaction par de l'acétate d'ammonium. L'utilisation de ces nouvelles conditions a conduit à la formation de trois diastéréoisomères du 4-Ph-I^9aa. Deux de ces isomères ont subi des épimérisations durant le processus de cyclisation. Les différents diastéréoisomères ont ensuite été identifié par RMN 1D et 2D du proton.

Par la suite, dans le cadre d'une étude structure / activité des analogues antagonistes du peptide CGRP, nous avons utilisé le 4-Ph-I^9aa ainsi que deux autres azabicycloalcanes, l'I^2aa et l'I^9aa. Treize nouveaux peptides de 9 ou 11 acides aminés, antagonistes potentiels de CGRP, ont été synthétisé. Les résultats du test biologique pour ces peptides ne sont malheureusement pas encore disponibles.

Finalement, dans le cadre d'une coopération entre notre groupe et le groupe du professeur Tourwé (VUB, Bruxelles), une étude structure / activité sur un hexapeptide (Ac-Arg-D-Cha-Qaa-D-Arg-D-*p*ClPhe-NH_2) antagoniste de ORL1 a été menée. Cette étude a porté sur l'importance des fonctions guanidine des arginines, de l'acétamide *N*-terminal et de la séquence peptidique. Elle a montré que la fonction guanidine sur l'arginine 5 et la séquence peptidique sont nécessaires pour l'activité du peptide. Elle a également montré la possibilité d'ablation de l'acétamide *N*-terminal sans perte d'activité. Enfin, cette étude a montré l'importance de la fonction guanidine sur l'arginine 1 pour la sélectivité et a ainsi permis d'obtenir un nouvel antagoniste (Ac-Cit-D-Cha-Qaa-D-Arg-D-*p*ClPhe-NH_2) sélectif pour ORL1 vis-à-vis des autres récepteurs opioïdes.

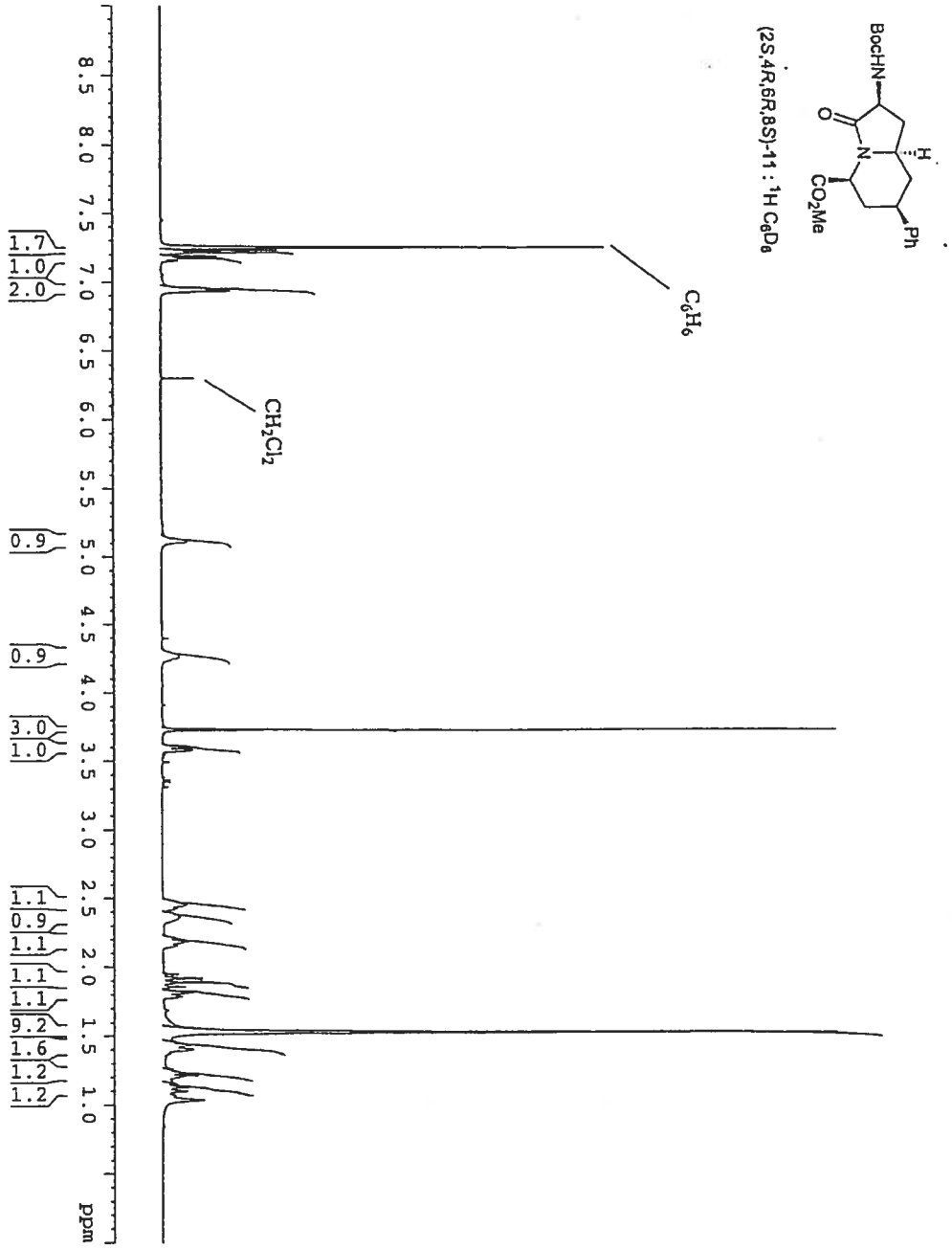
Le développement d'analogues substitués des acides aminés quinolizidinone et pyrroloazépinone par la méthode utilisée ici semble être un des éléments intéressant à développer dans le futur. En effet, leur intermédiaire de synthèse commun possède également une fonction énone (cf. chapitre 2 schémas 39 et 41). Ceci permettra de comparer les angles dièdres d'un repliement tout en conservant la chaîne latérale. Il semble également très intéressant de pouvoir comparer l'effet des différents diastéréoisomères de 4-Ph-I^9aa lors d'une étude structure / activité afin de déterminer l'orientation d'une chaîne latérale.

L'ensemble des travaux présentés dans le cadre de cette thèse devrait contribuer à la compréhension de la réactivité des acides aminés et à l'avancement des connaissances dans le domaine du mimétisme peptidique. Enfin, nous espérons que les connaissances apportées par les études structure /

activité sur les récepteurs de nociceptine et de CGRP aident à la compréhension du fonctionnement et de l'utilité de ces récepteurs et permettent l'étude et le développement de nouveaux agents thérapeutiques.

Annexe 1.

Donnés RMN de l'article 4 au chapitre 5.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N
H
CO2Me
Ph
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C6H6
CH2Cl2
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ppm
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1.0
2.0
0.9
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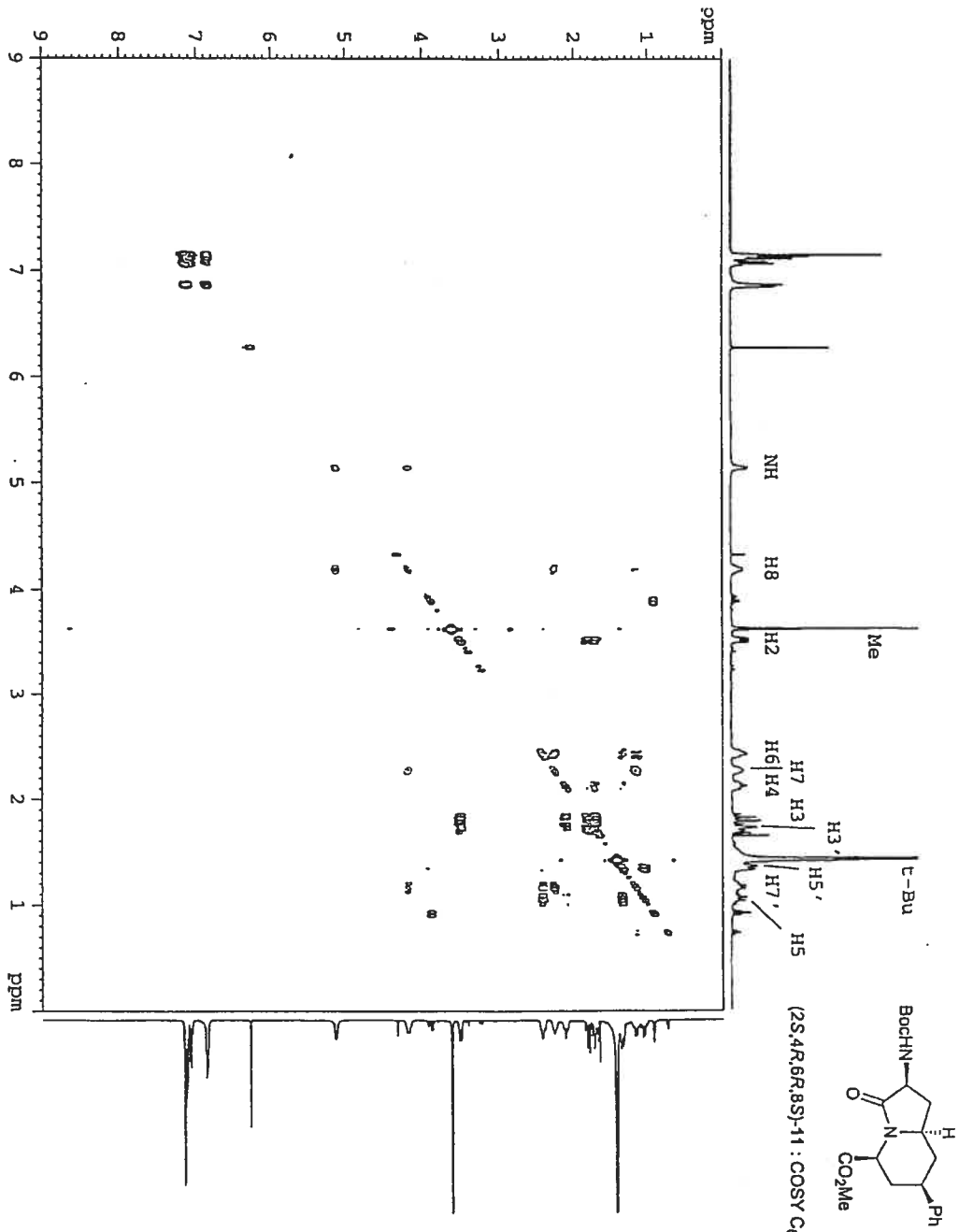
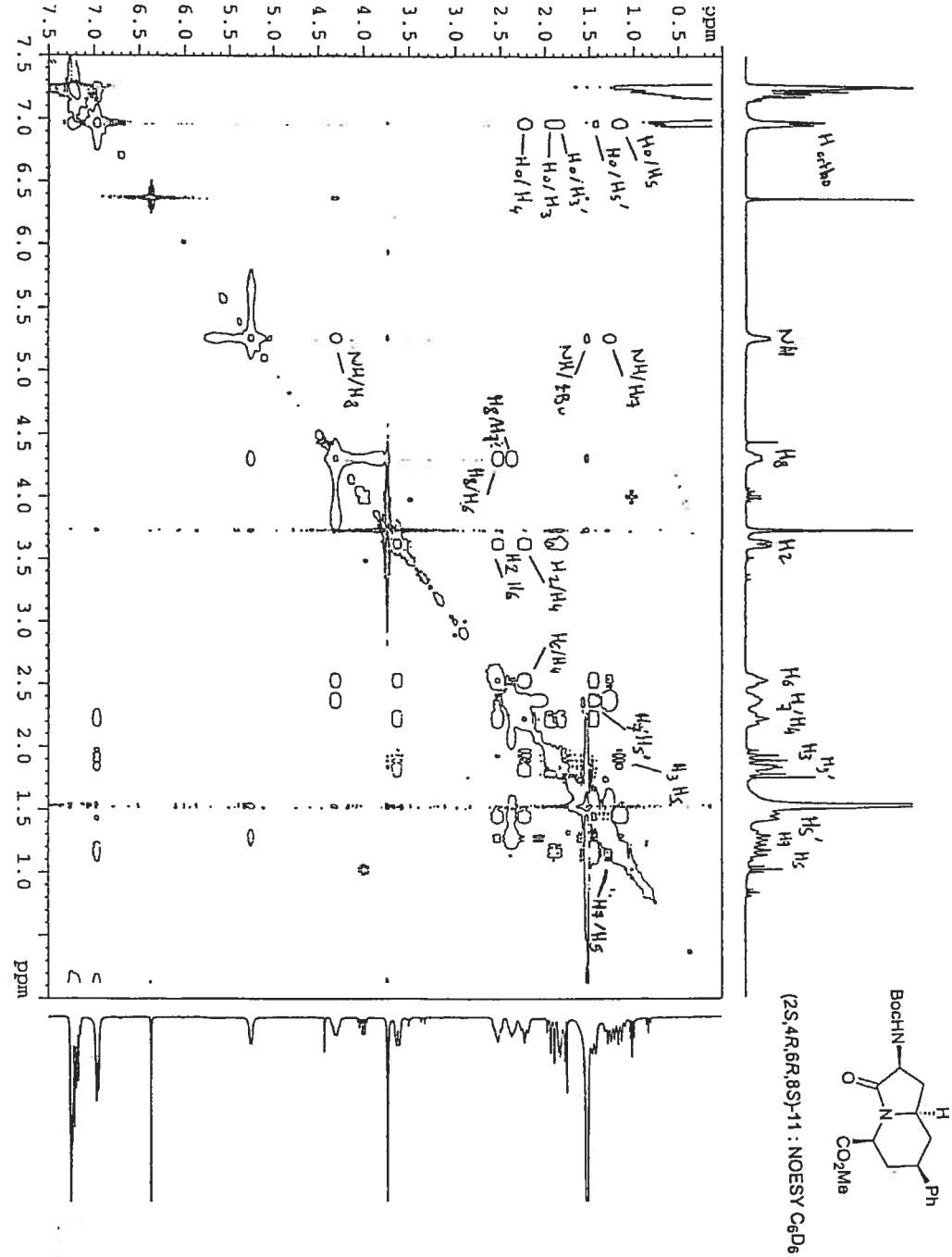
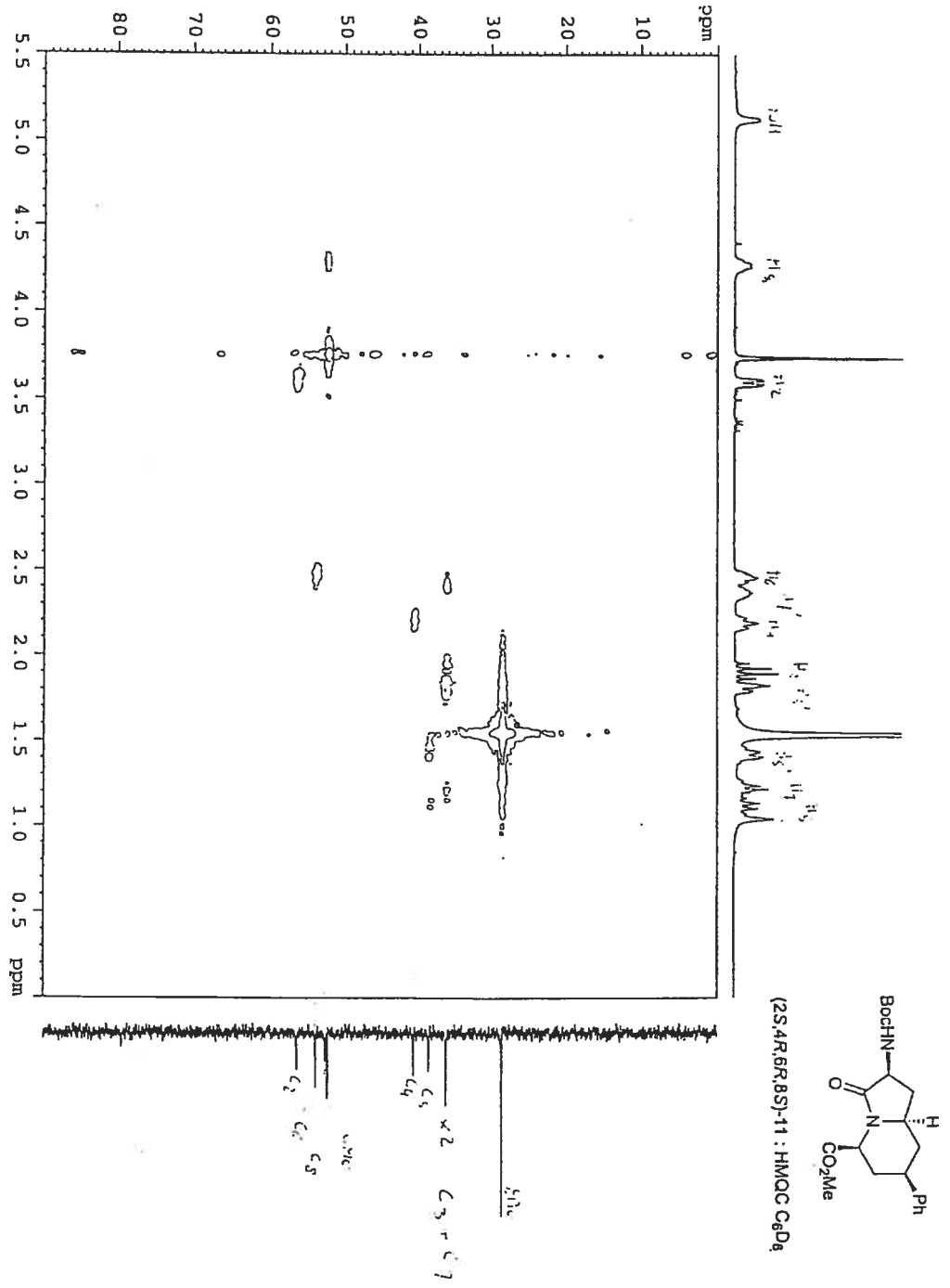
1.1
0.9
1.1
1.1
1.1
9.2
1.6
1.2
1.2



(2S,4R,6R,8S)-11 : COSY C_6D_6


BocHN
H
O
N
CO2Me
Ph
(2S,4R,6R,8S)-11 : NOESY C6D6
NH
H8
H2
H6 H4
H3'
H3
H5'
H5
H1
ppm
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5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H
N
O
CO2Me
Ph
(2S,4R,6R,8S)-11 ; HMQC C6D6
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4.5
4.0
3.5
3.0
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1.5
1.0
0.5
ppm
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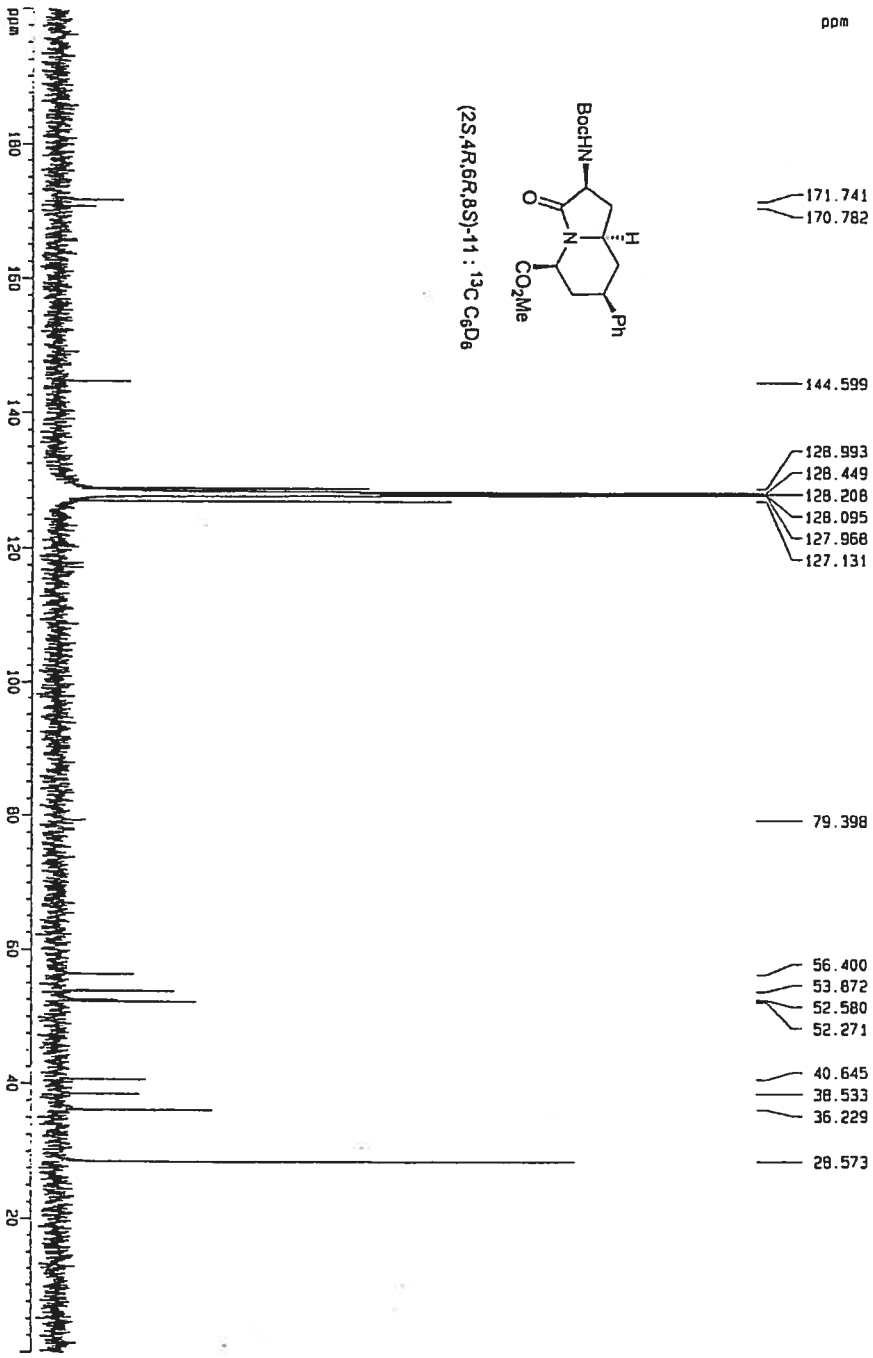
60
50
40
30
20
10
0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120
100
80
60
40
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O
N
H
CO_2Me
Ph
ppm
171.741
170.782
144.599
128.993
128.449
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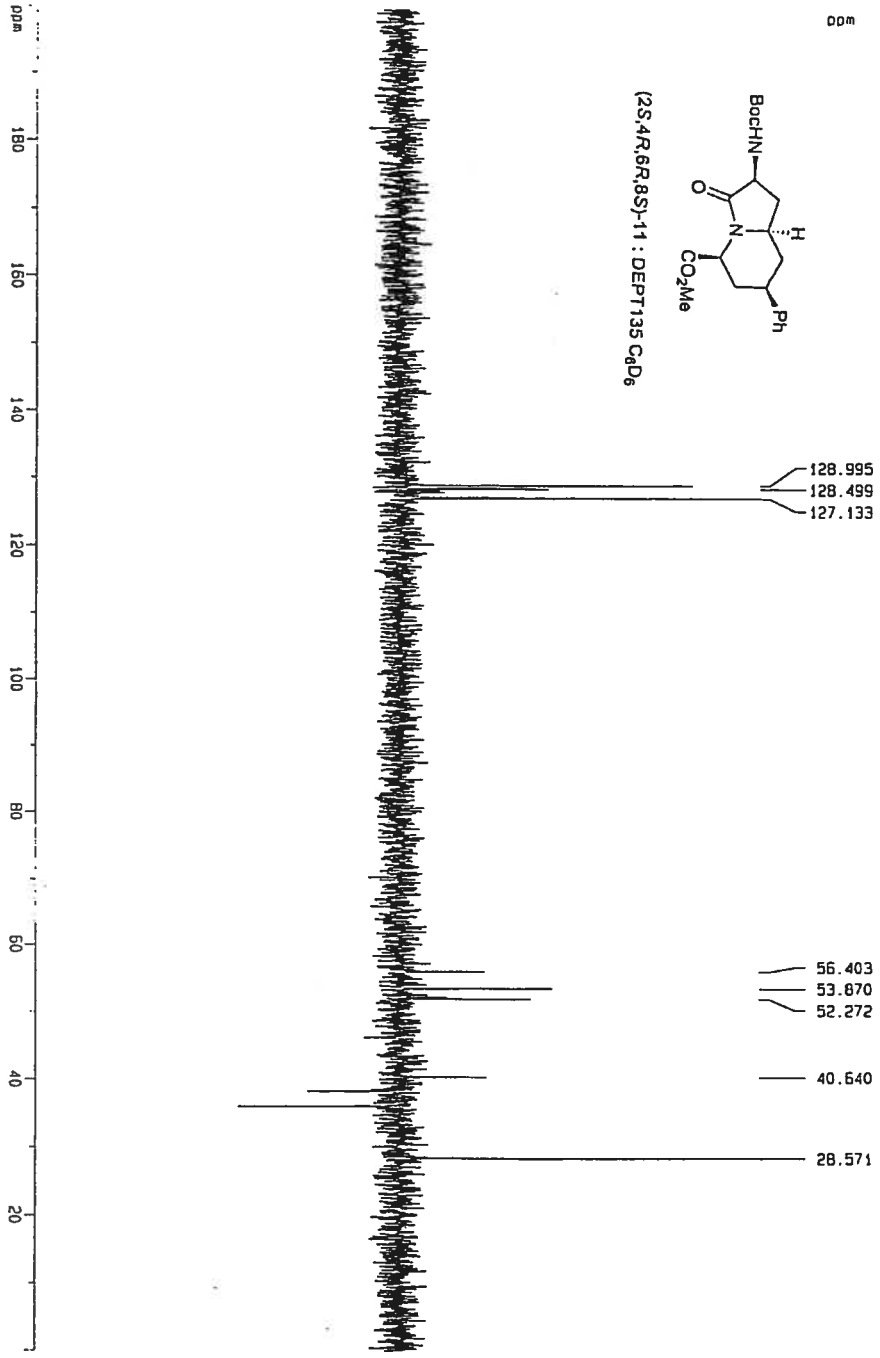
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120
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80
60
40
20
ppm
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CO_2Me
Ph
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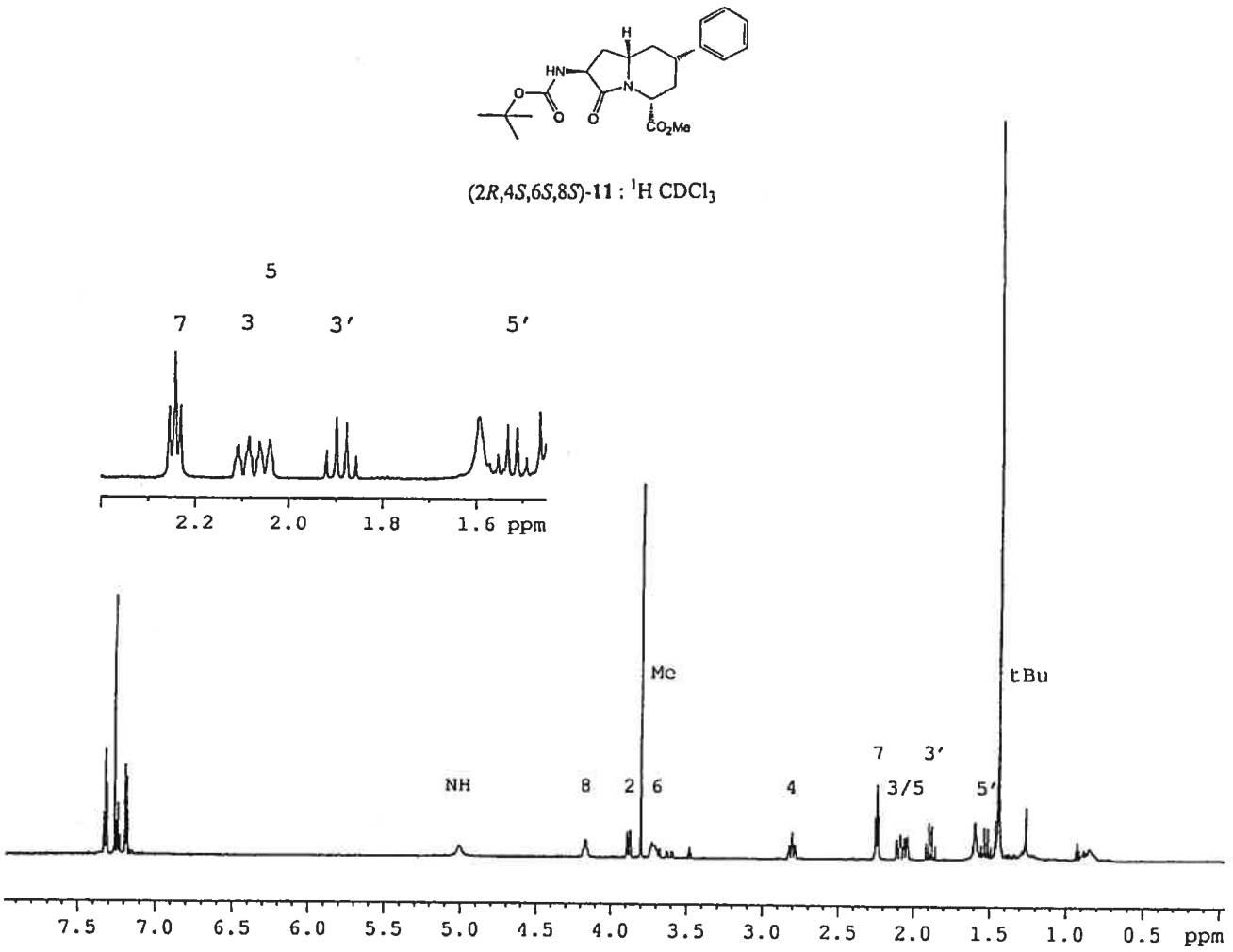
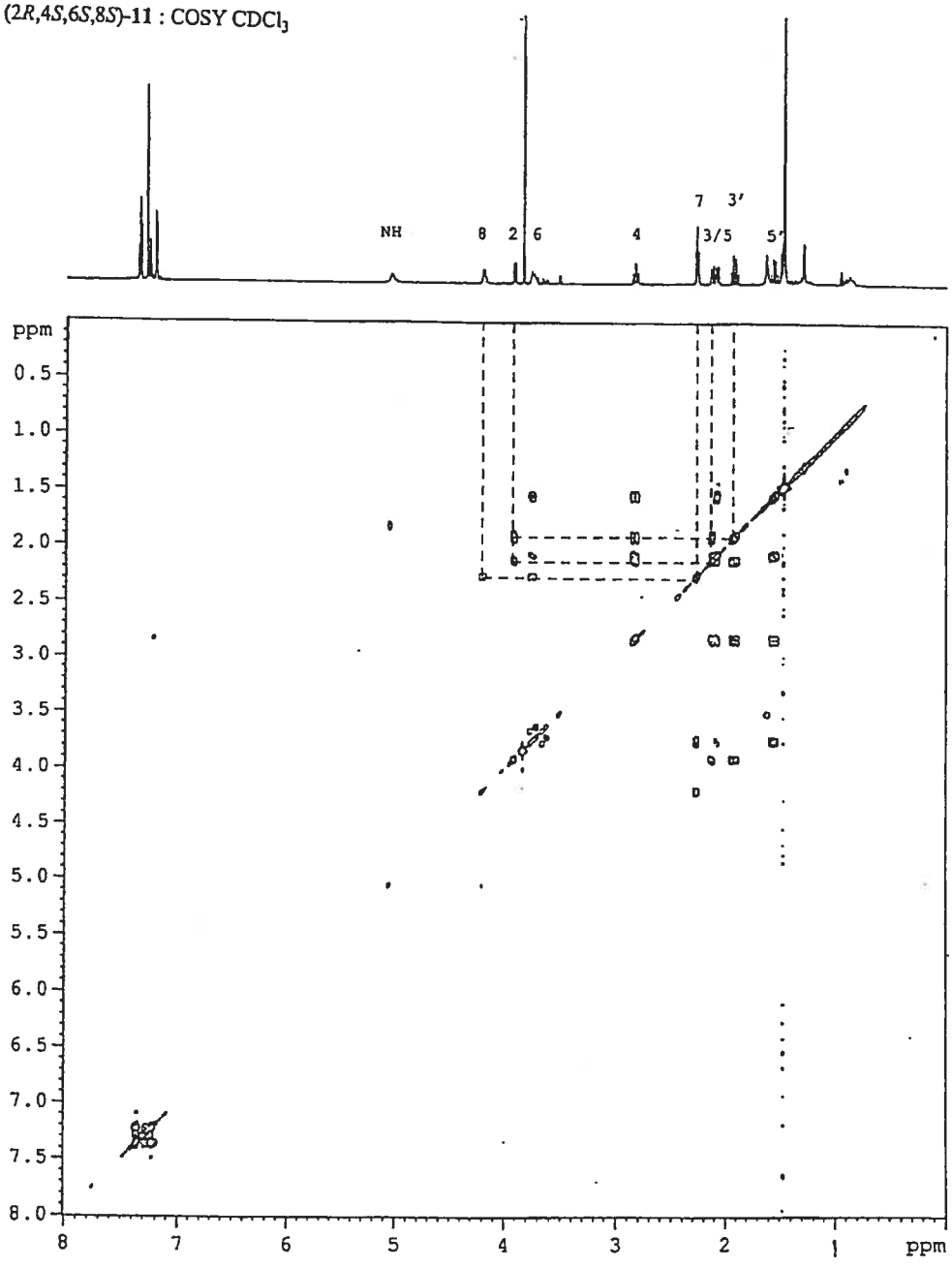
40.640
28.571

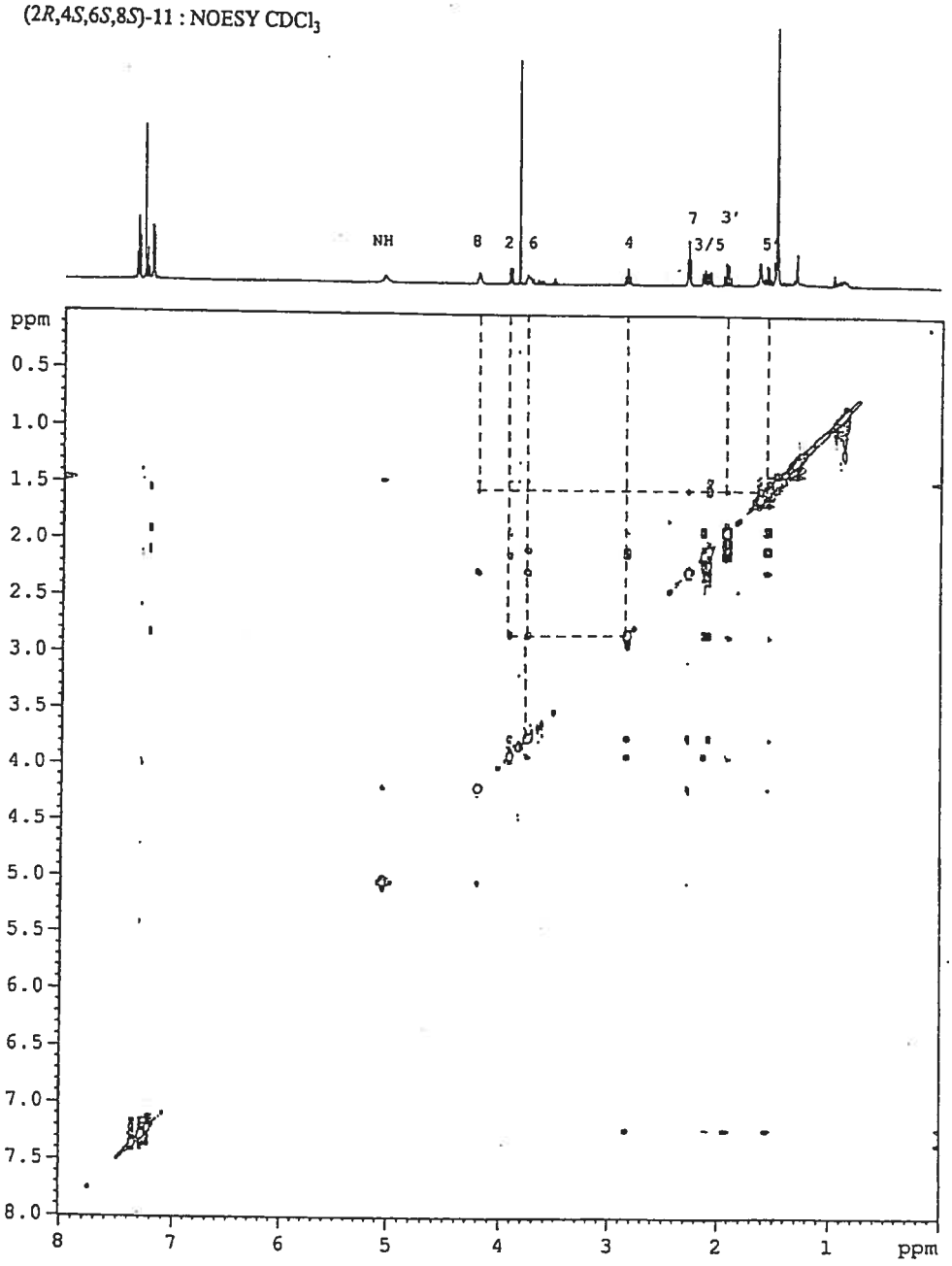


(2*R*,4*S*,6*S*,8*S*)-**11** : ^{1}H $CDCl_3$

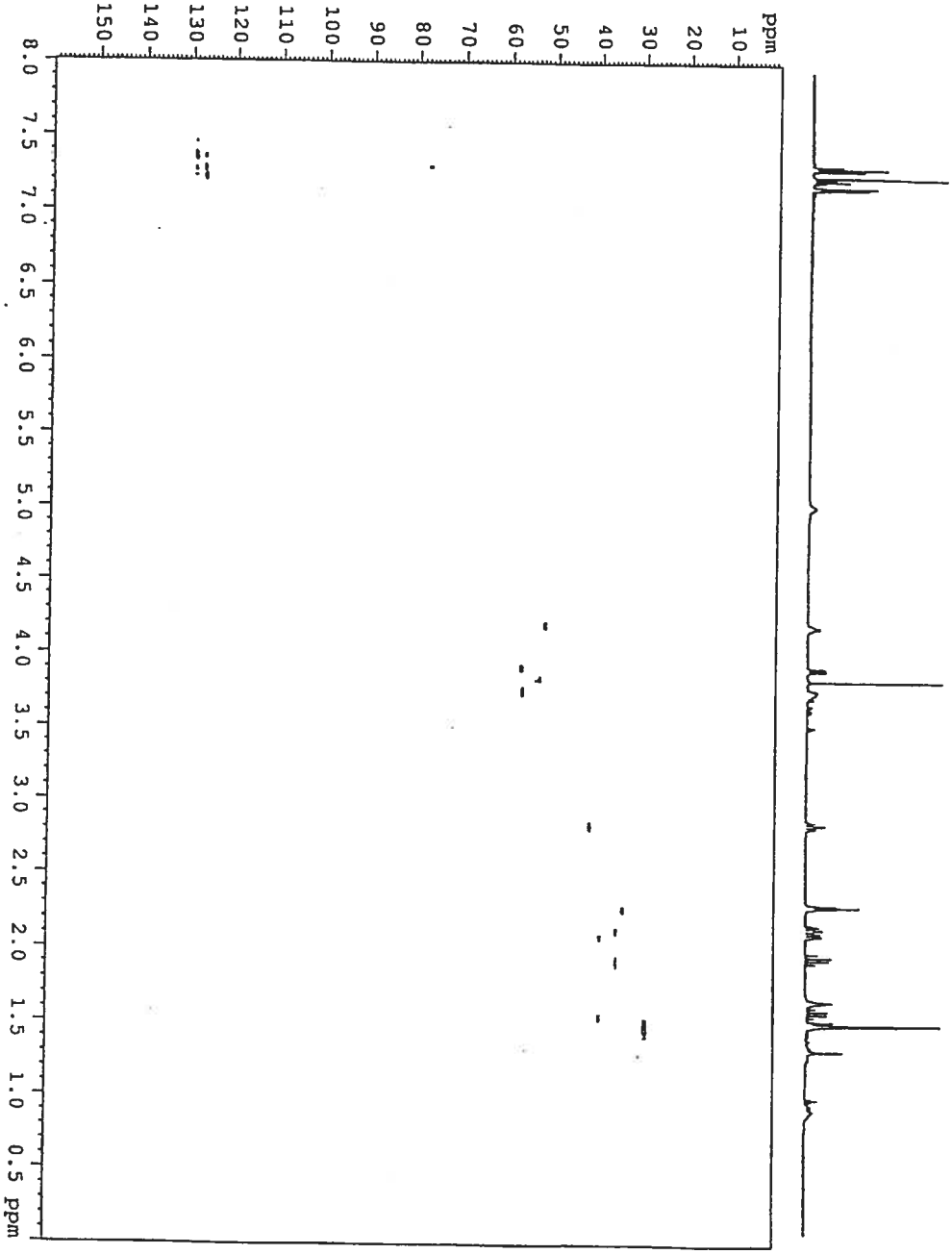
(2*R*,4*S*,6*S*,8*S*)-**11** : COSY $CDCl_3$

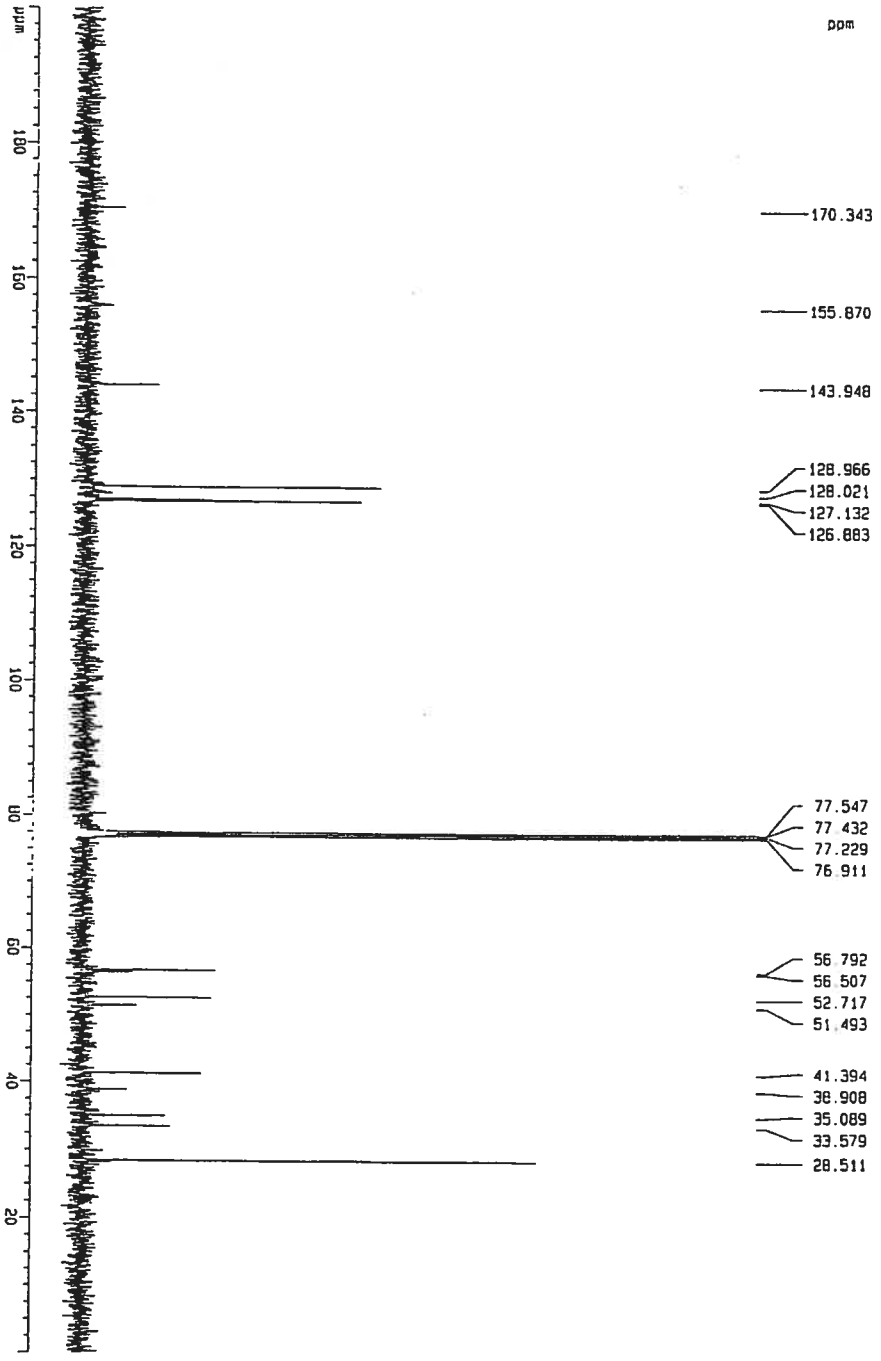


(2*R*,4*S*,6*S*,8*S*)-11 : NOESY $CDCl_3$

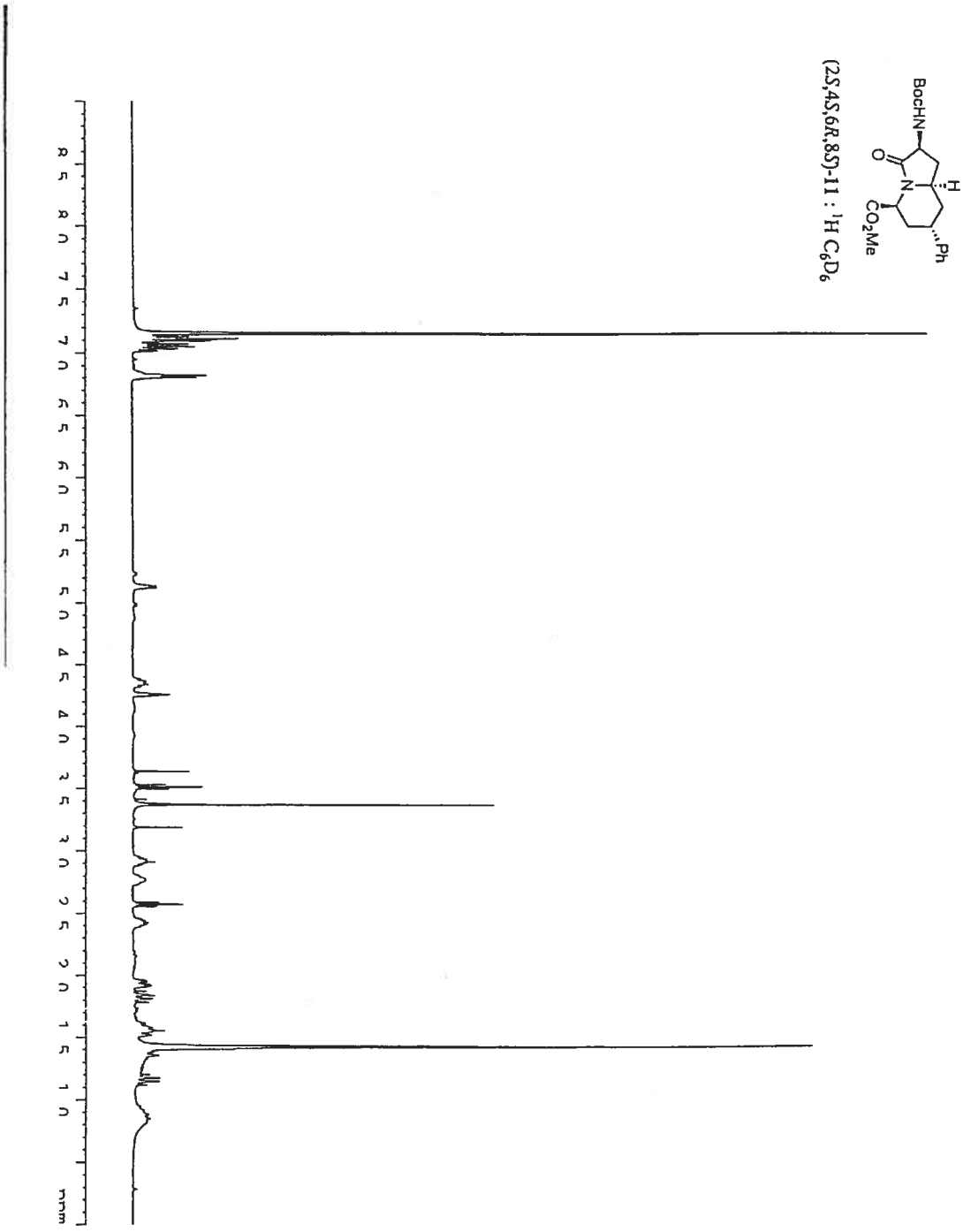


(2*R*,4*S*,6*S*,8*S*)-11 : HMQC $CDCl_3$





(2*R*,4*S*,6*S*,8*S*)-11 : ^{13}C $CDCl_3$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N
H
CO2Me
Ph
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7.5
7.0
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4.0
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3.0
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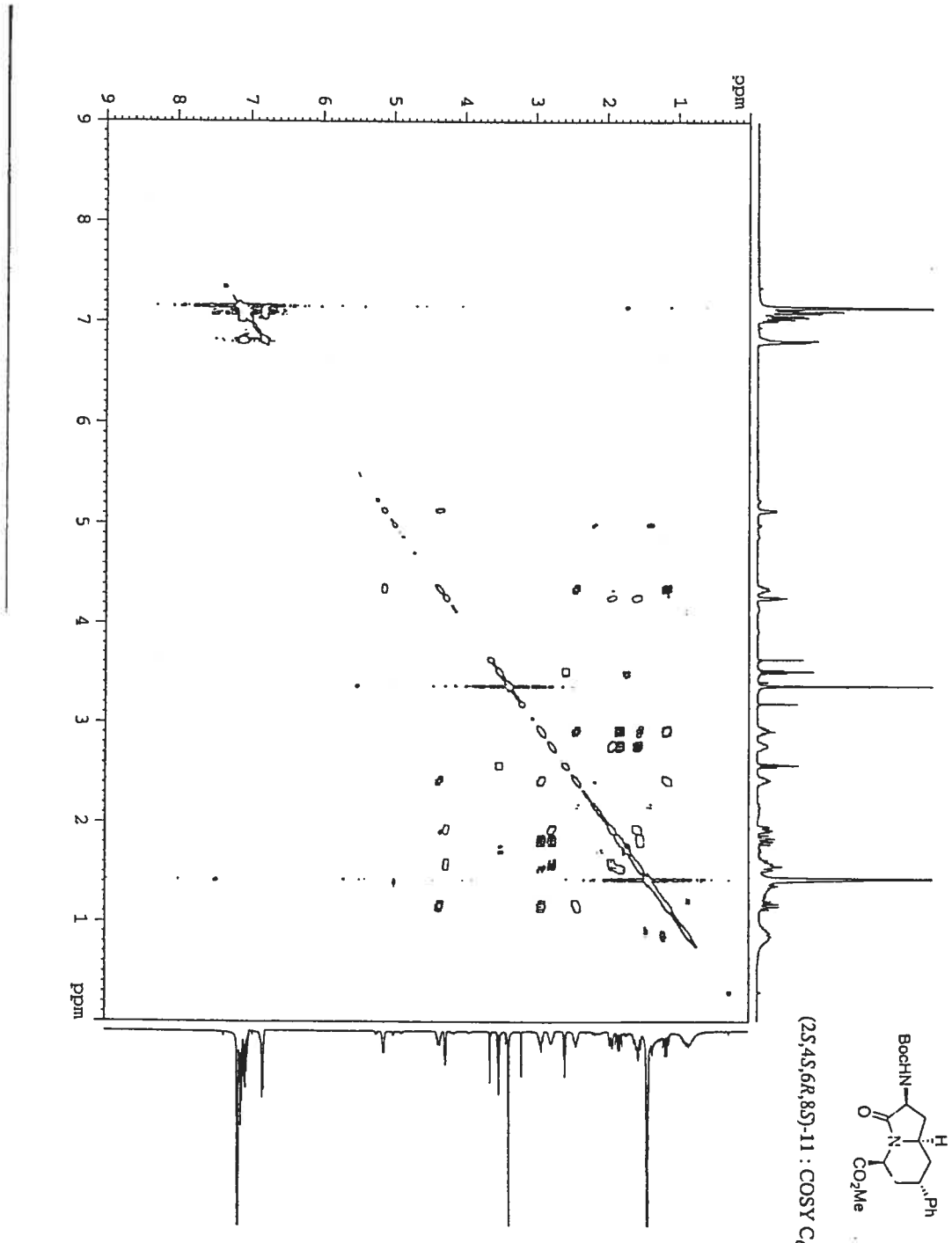
ppm



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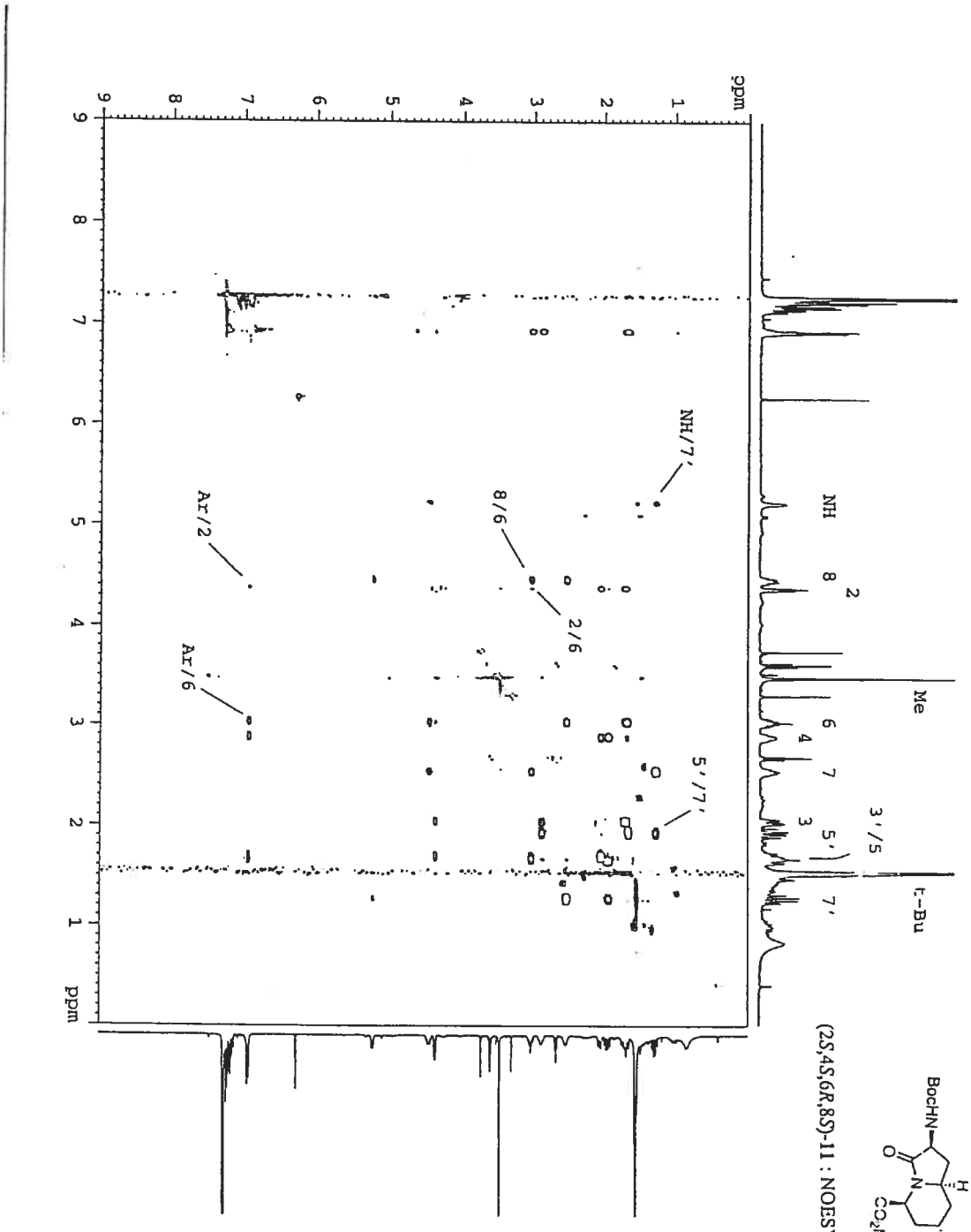
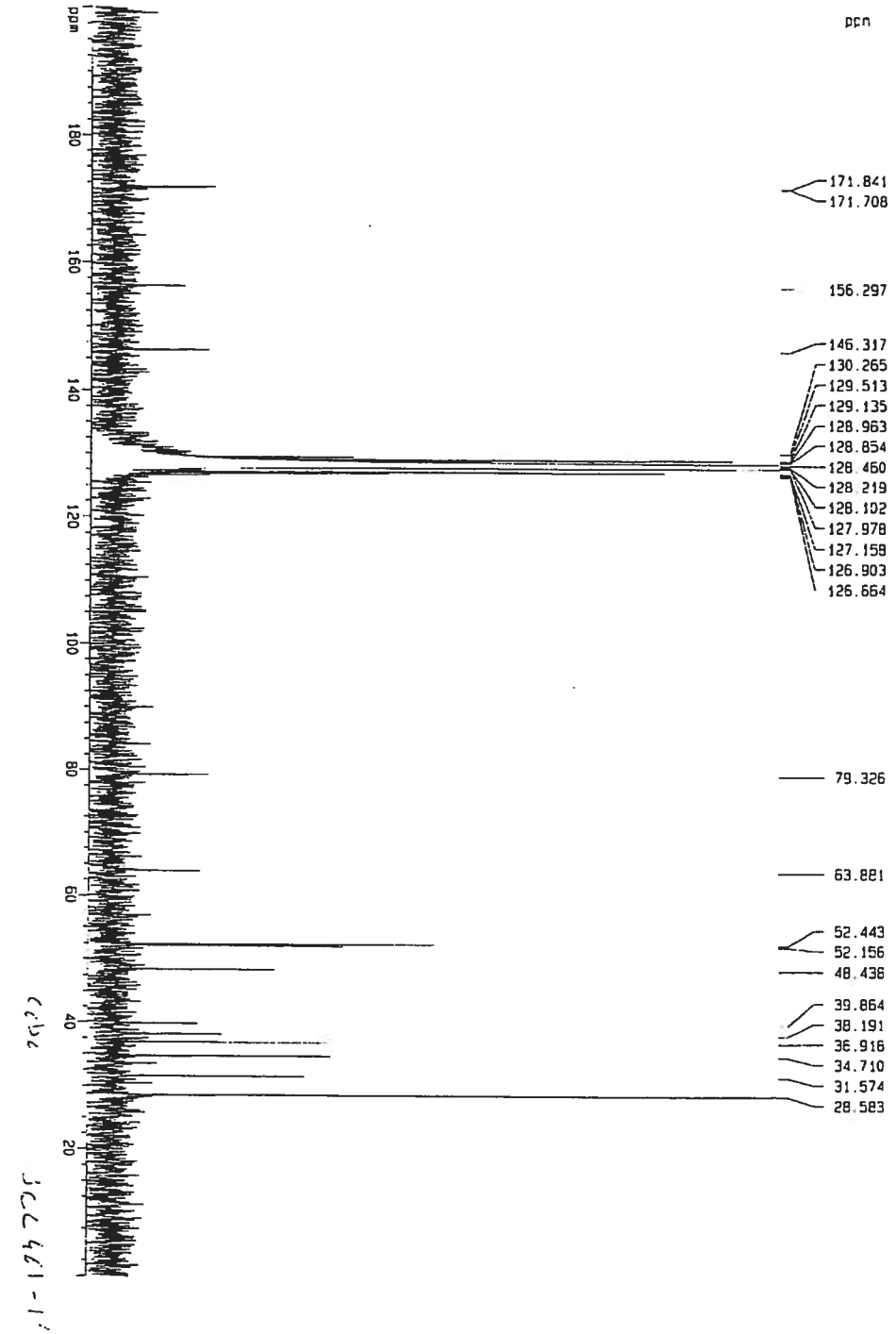
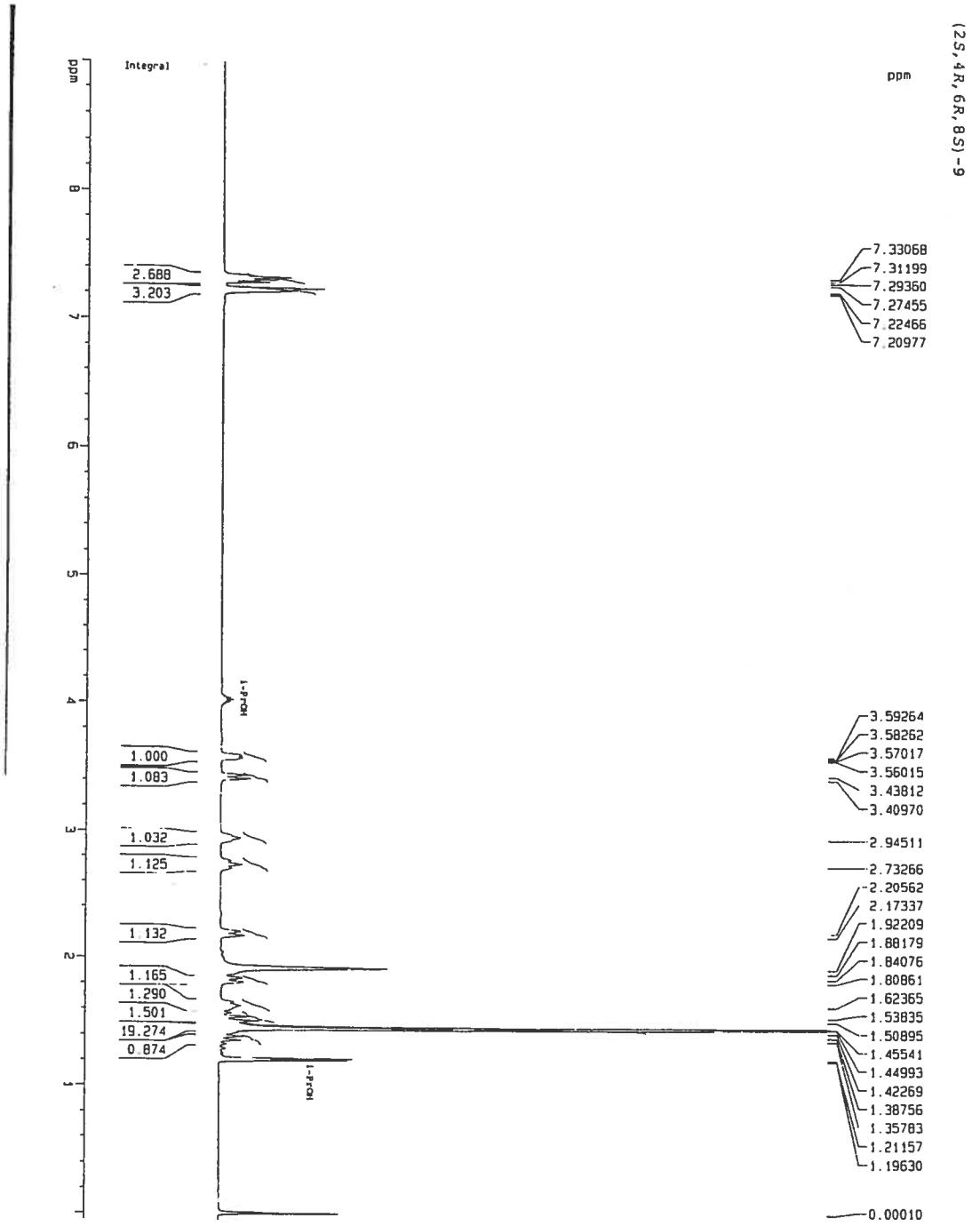


(2S,4S,6R,8S)-11 : NOESY C_6D_6

(2*S*,4*S*,6*R*,8*S*)-**11** : ^{13}C C_6D_6

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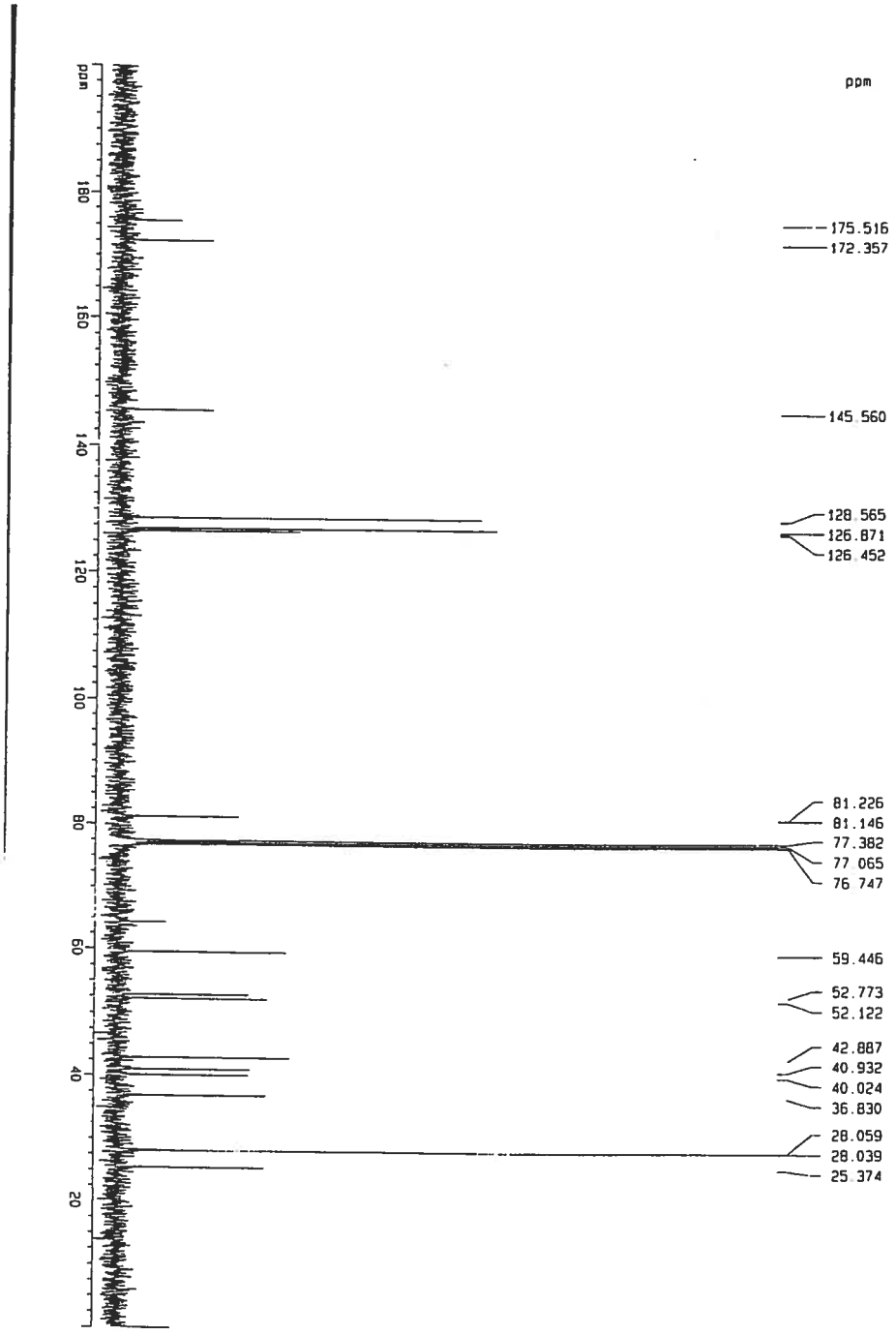
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81.146
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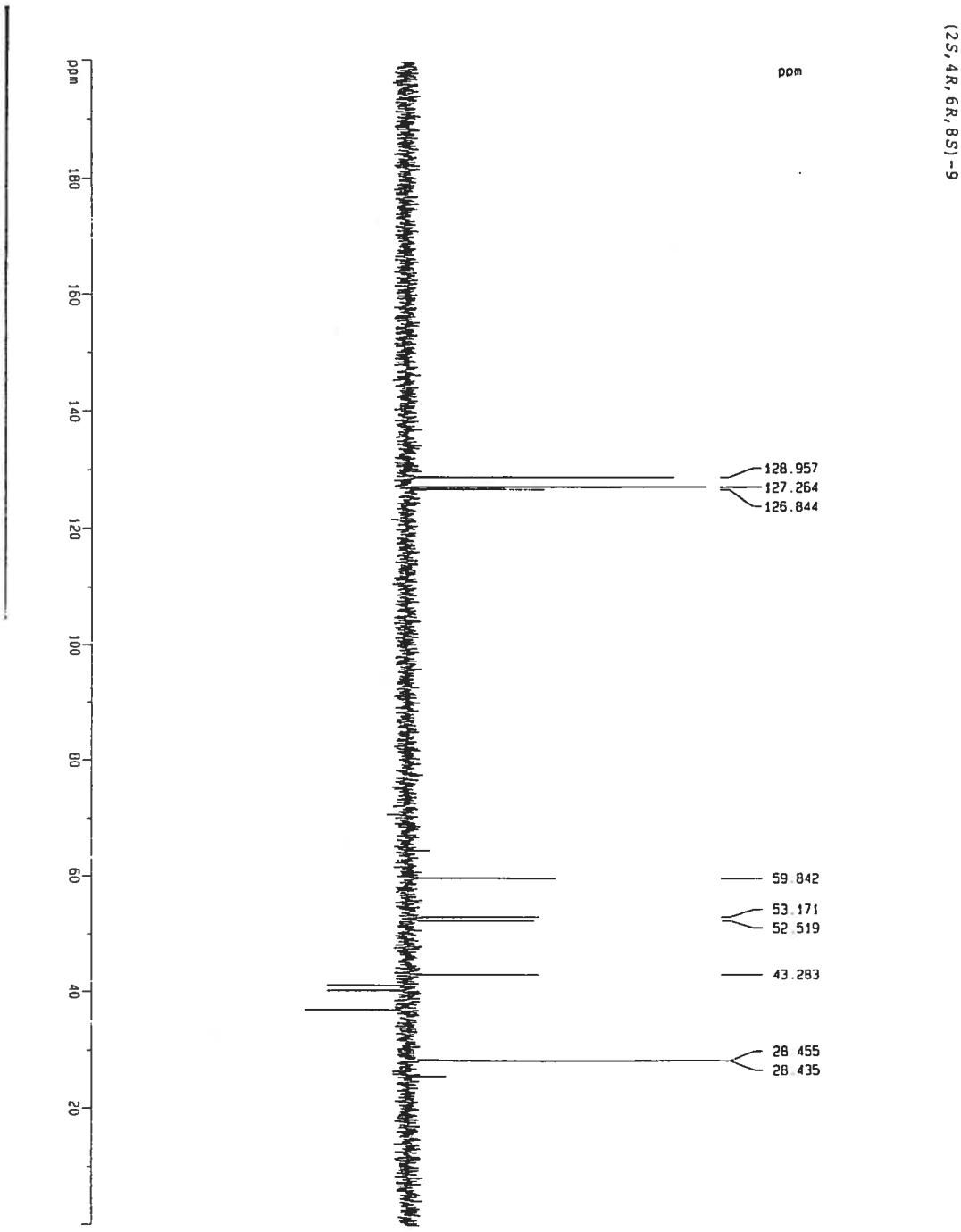
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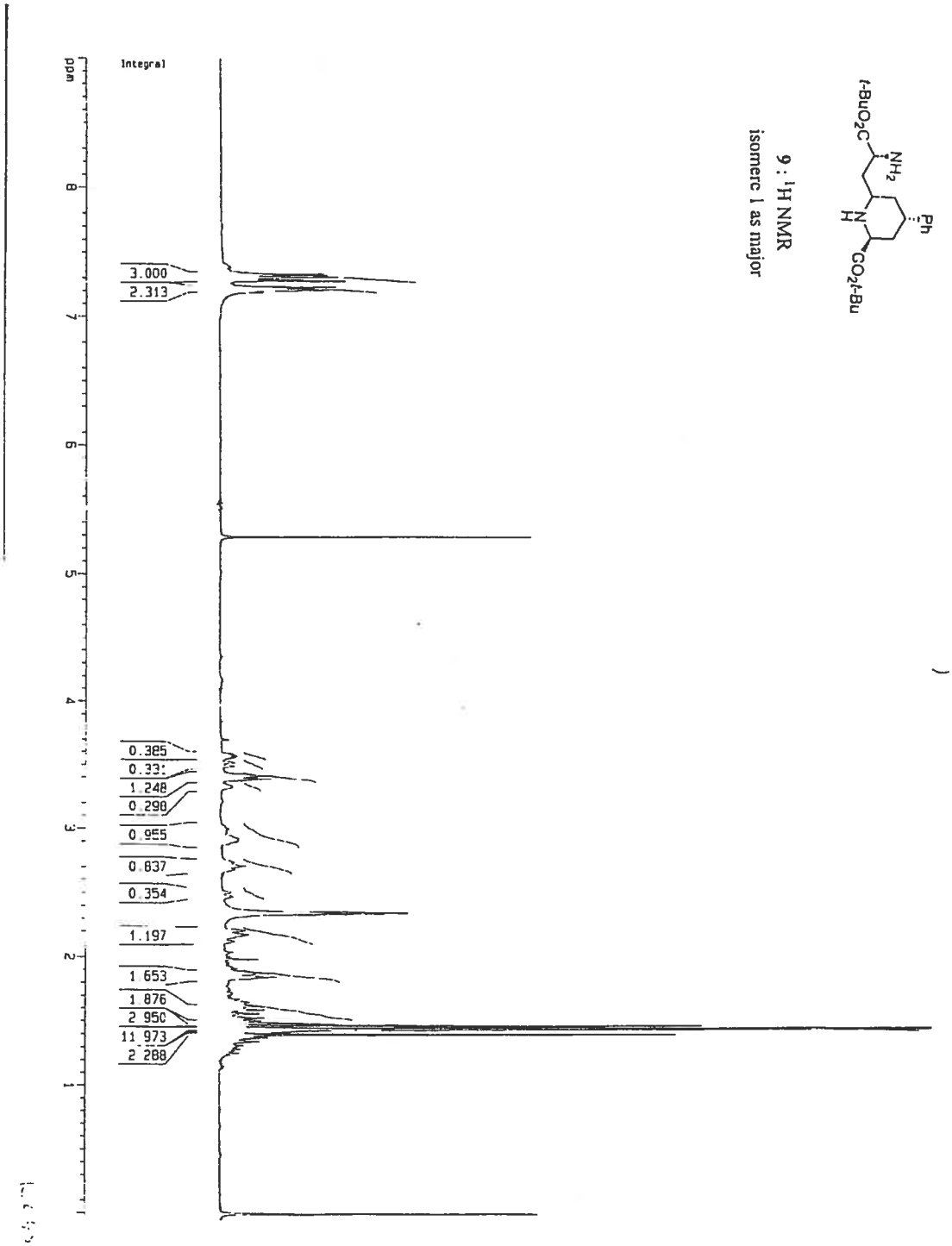

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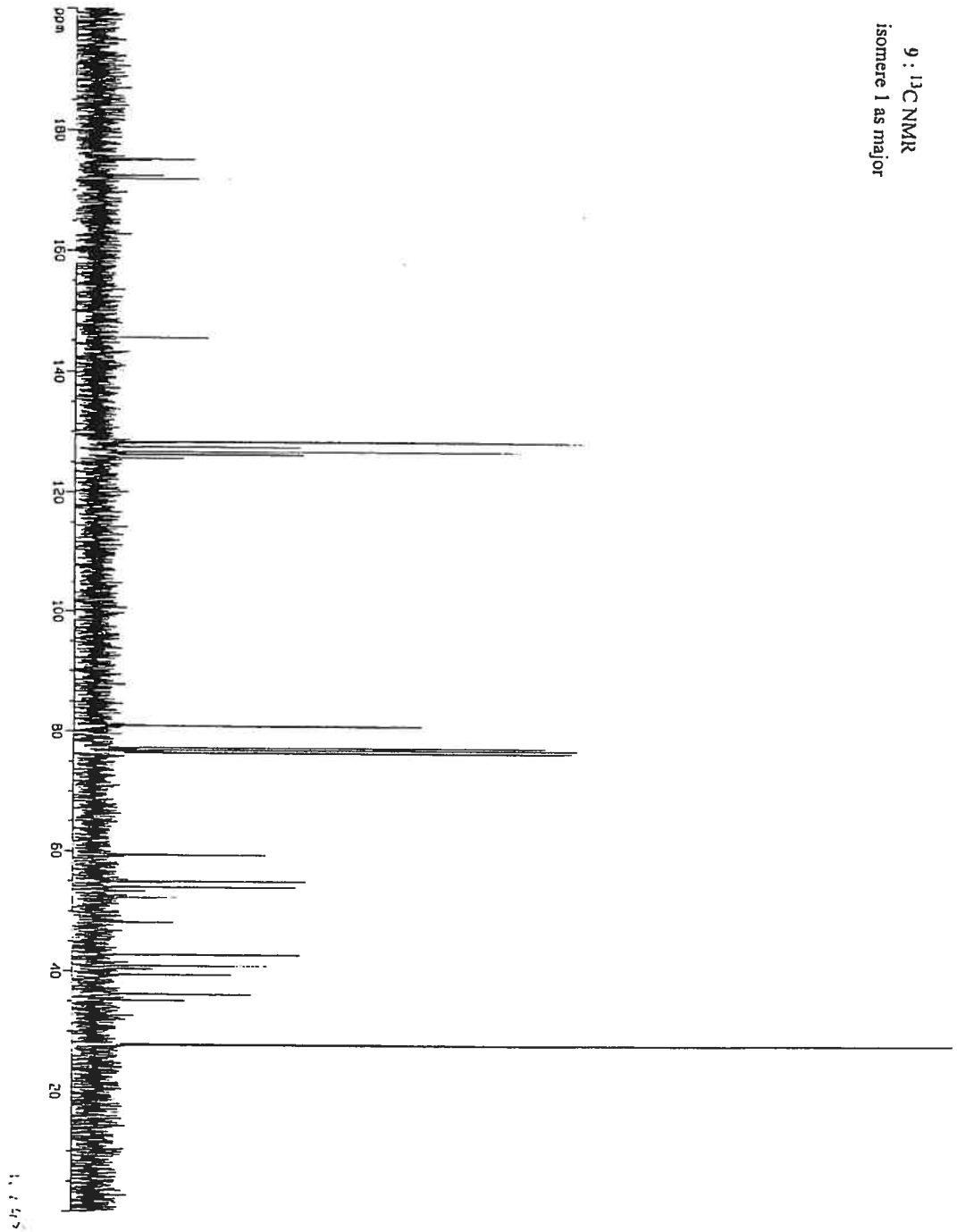

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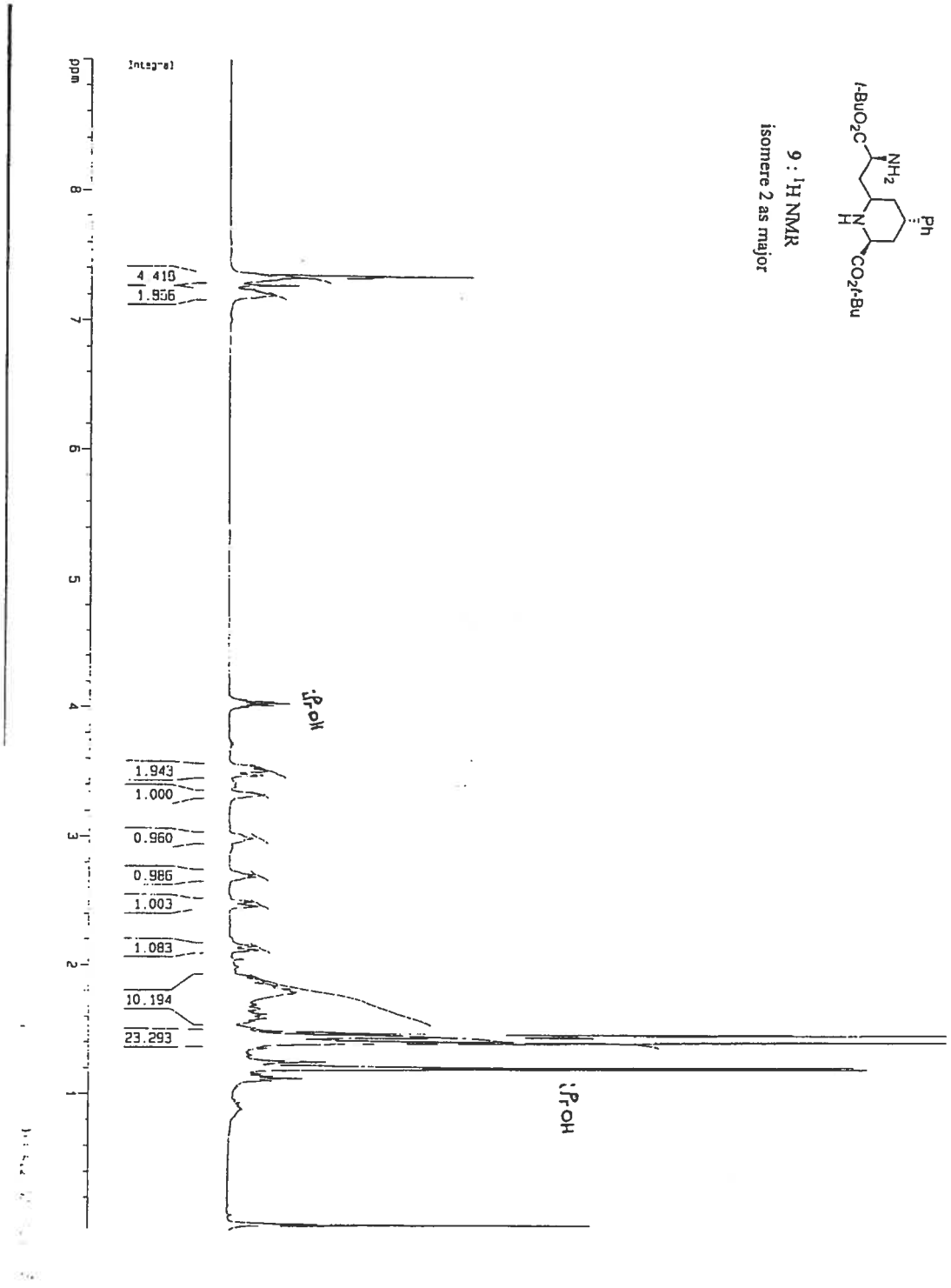
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N
H
Ph
CO2t-Bu
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isomere 2 as major
ppm
Integral
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1.000
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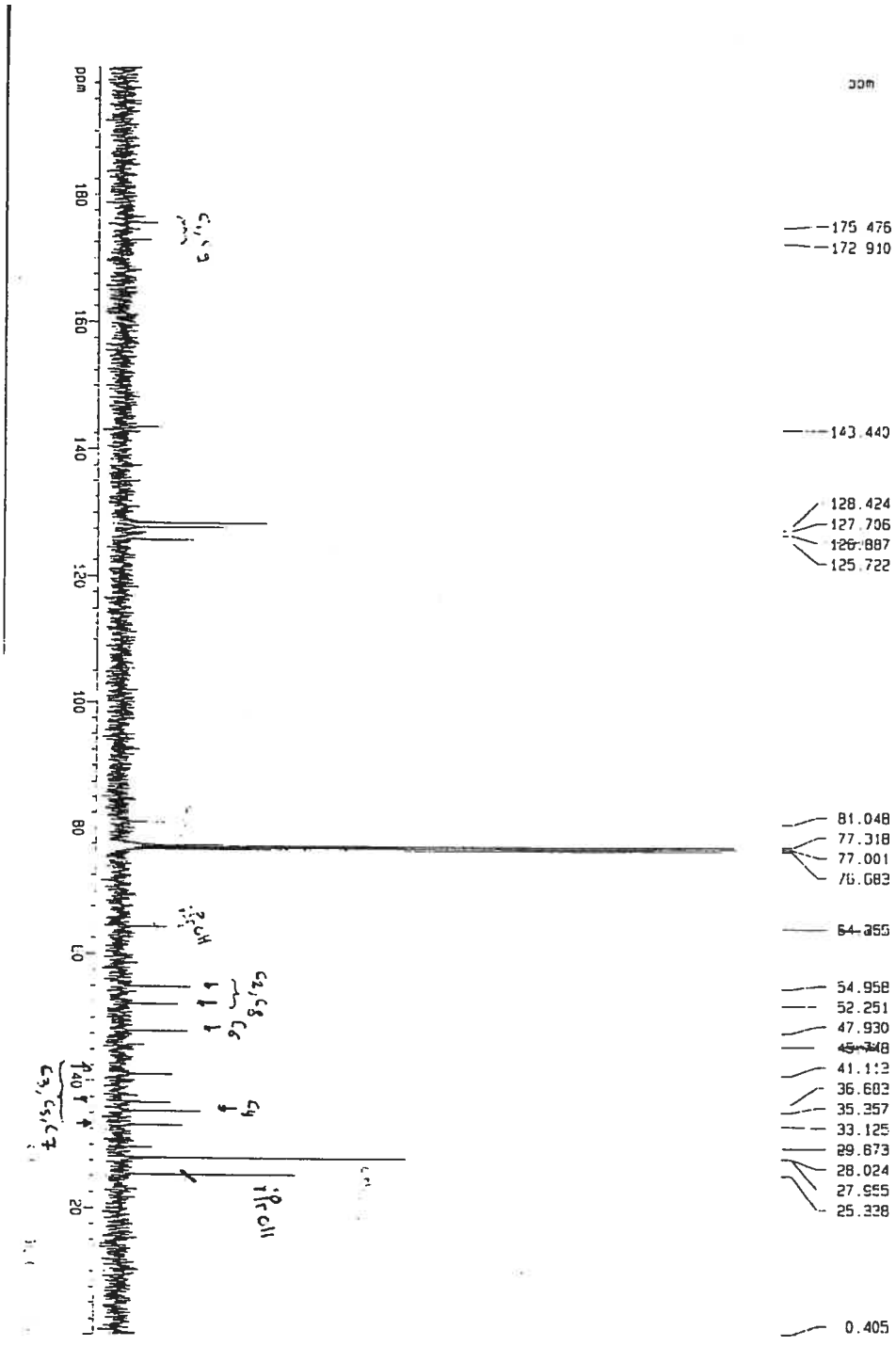
10.194
23.293
iPrOH
iPrOH

9 : ^{13}C NMR
isomere 2 as major



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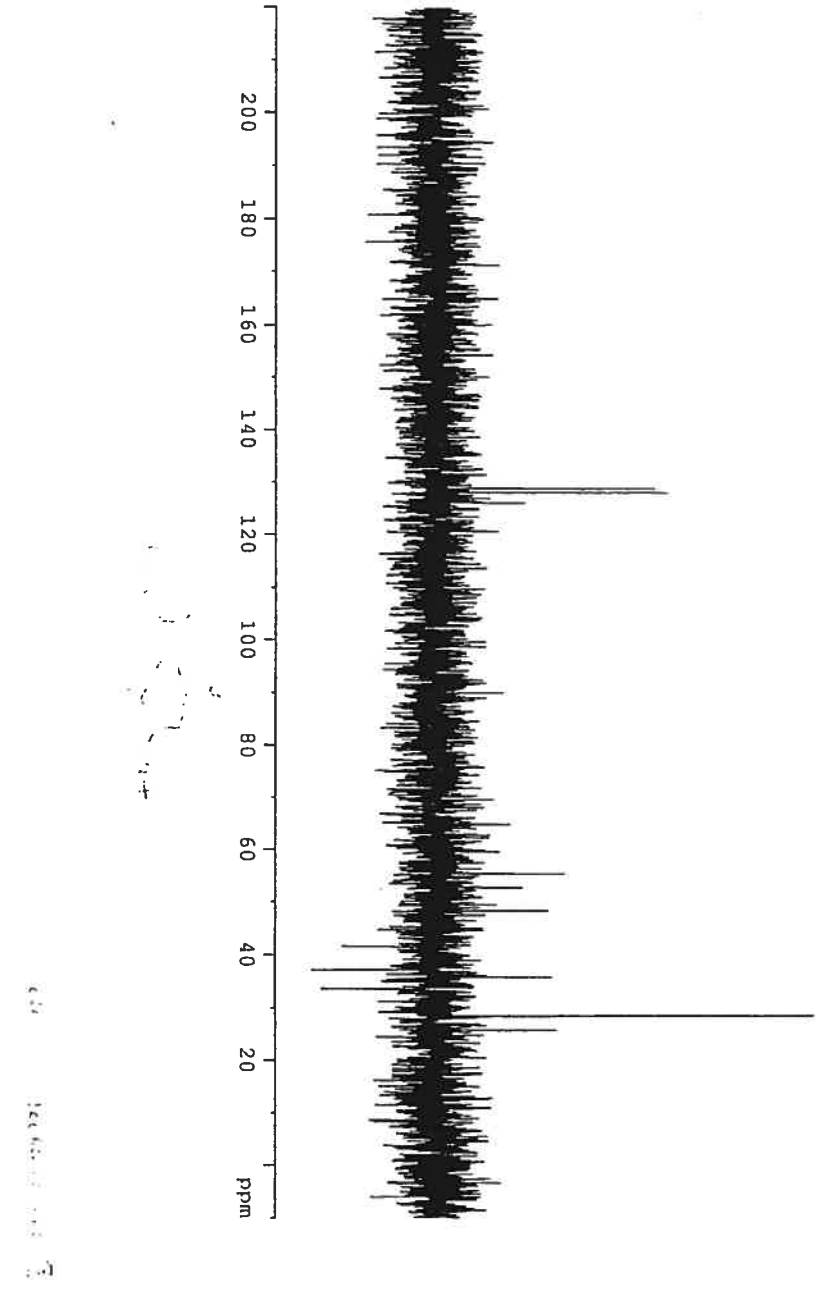

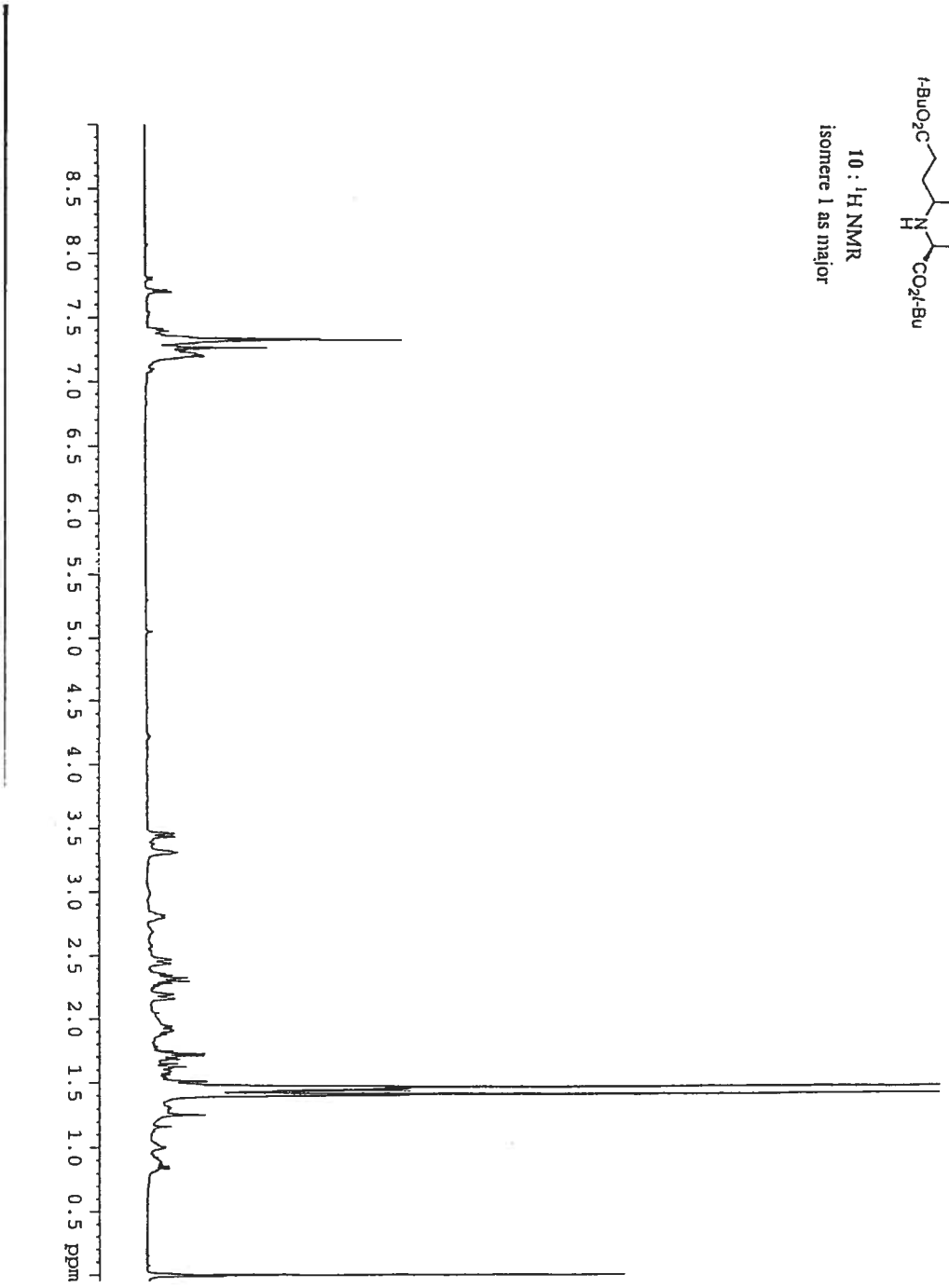

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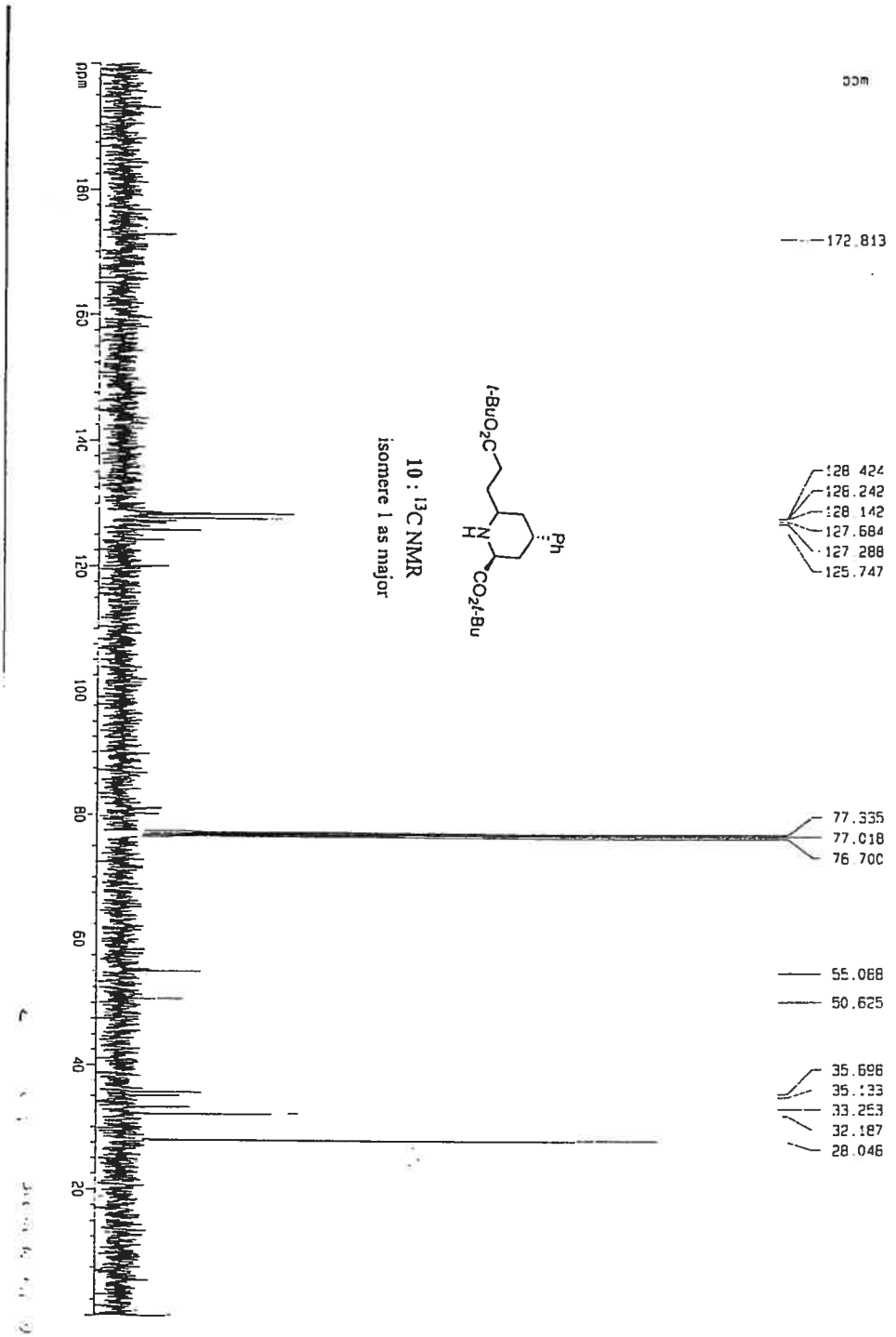

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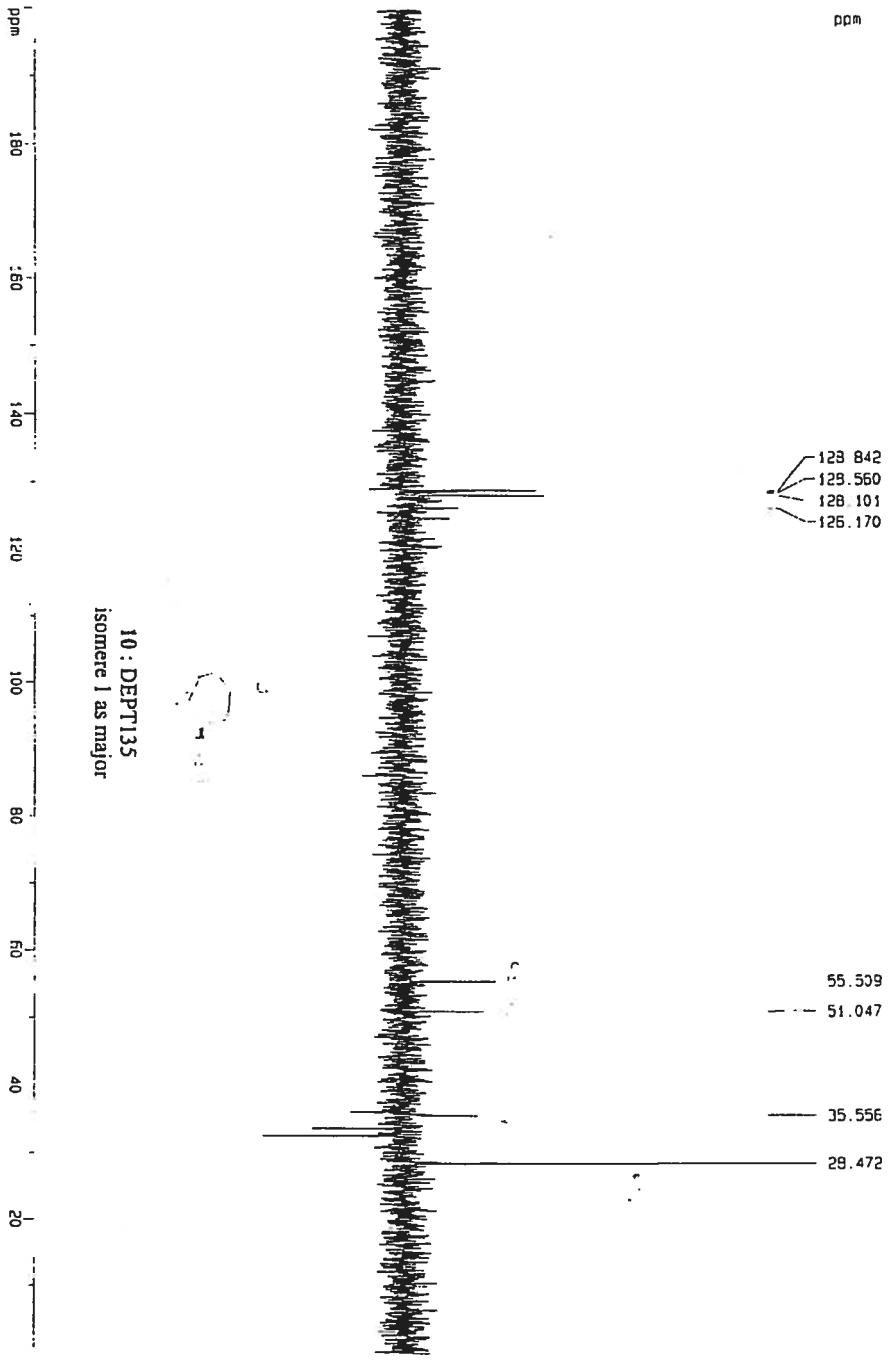
isomere 1 as major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128.560
128.101
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51.047
35.556
29.472
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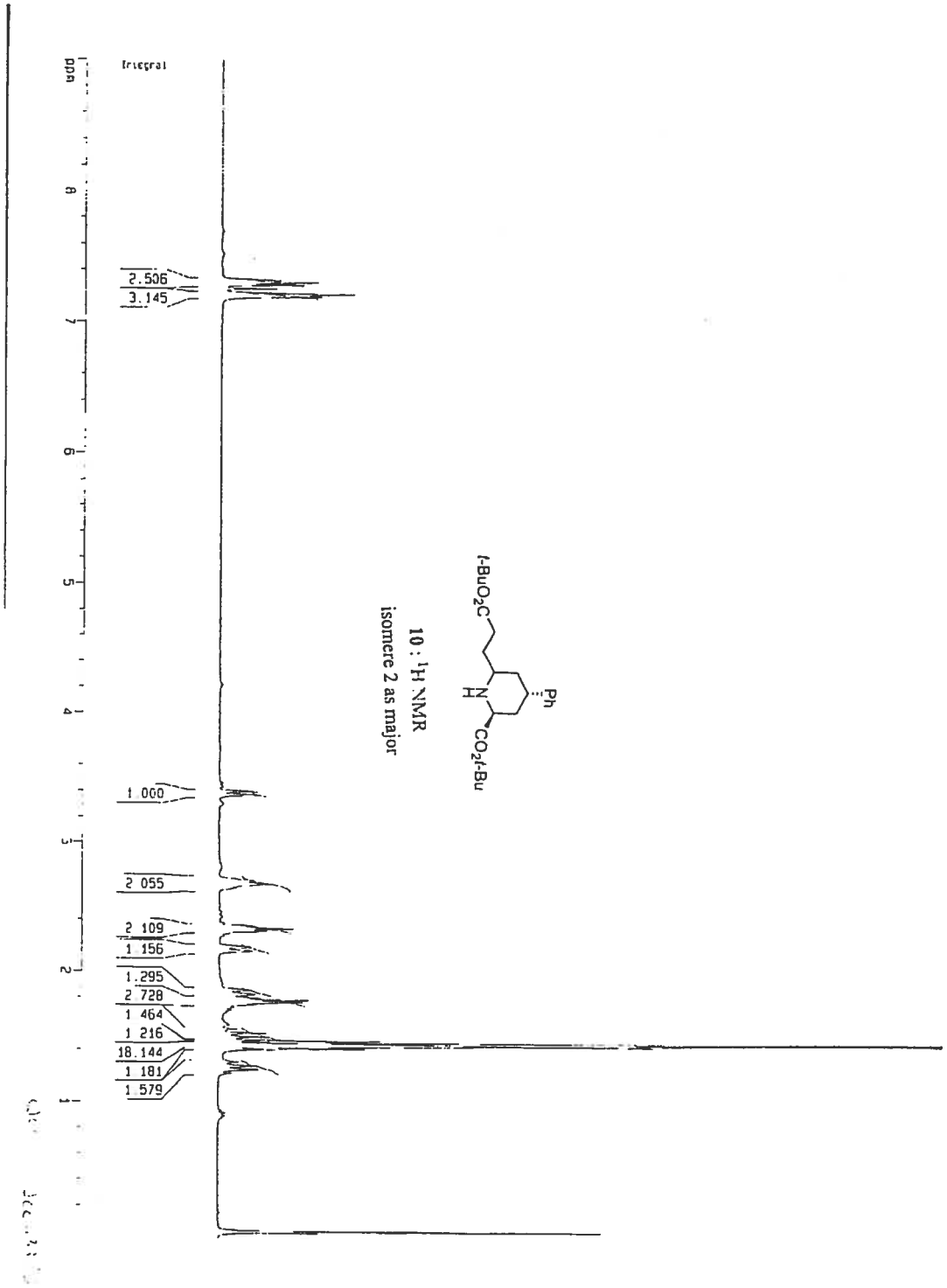

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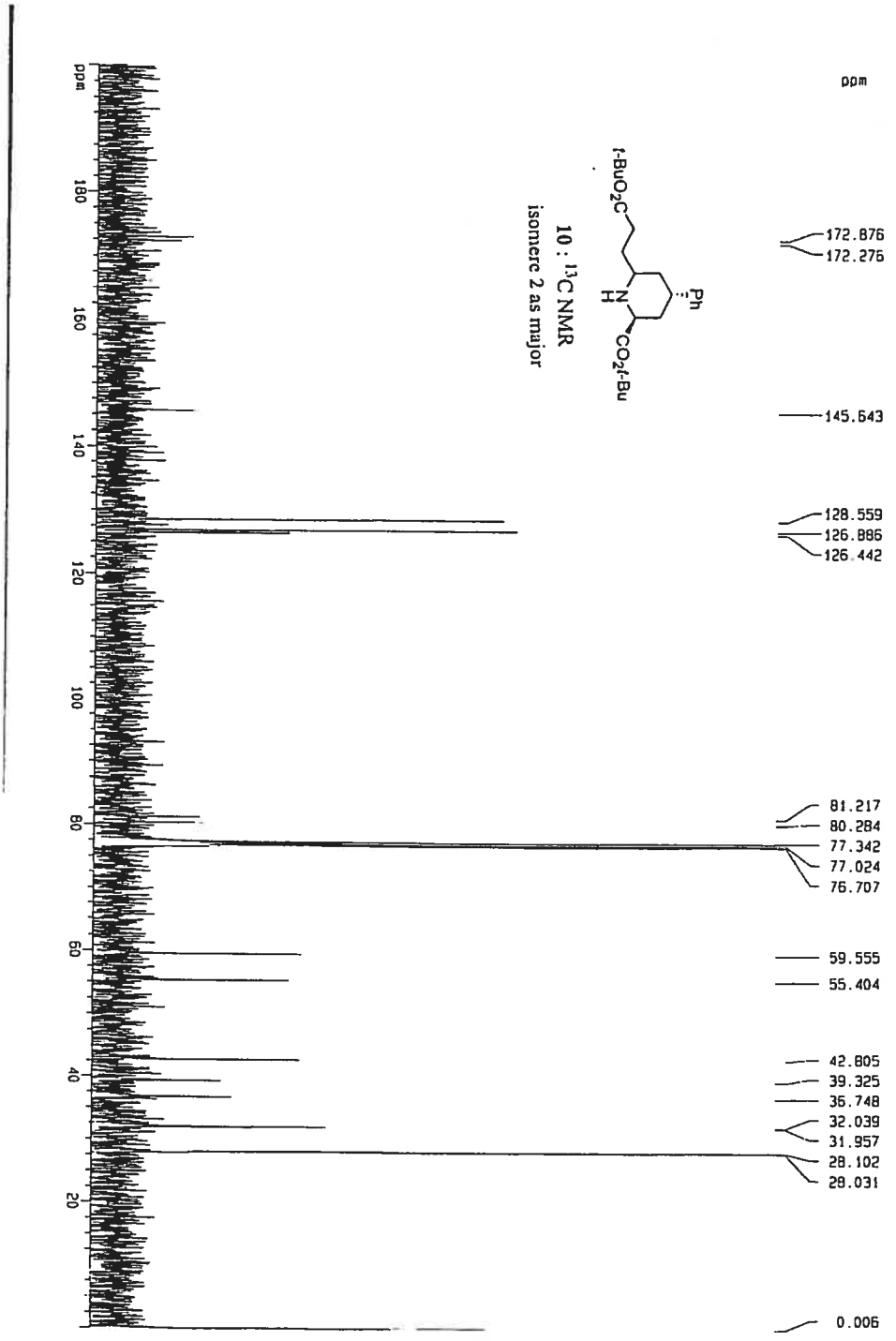
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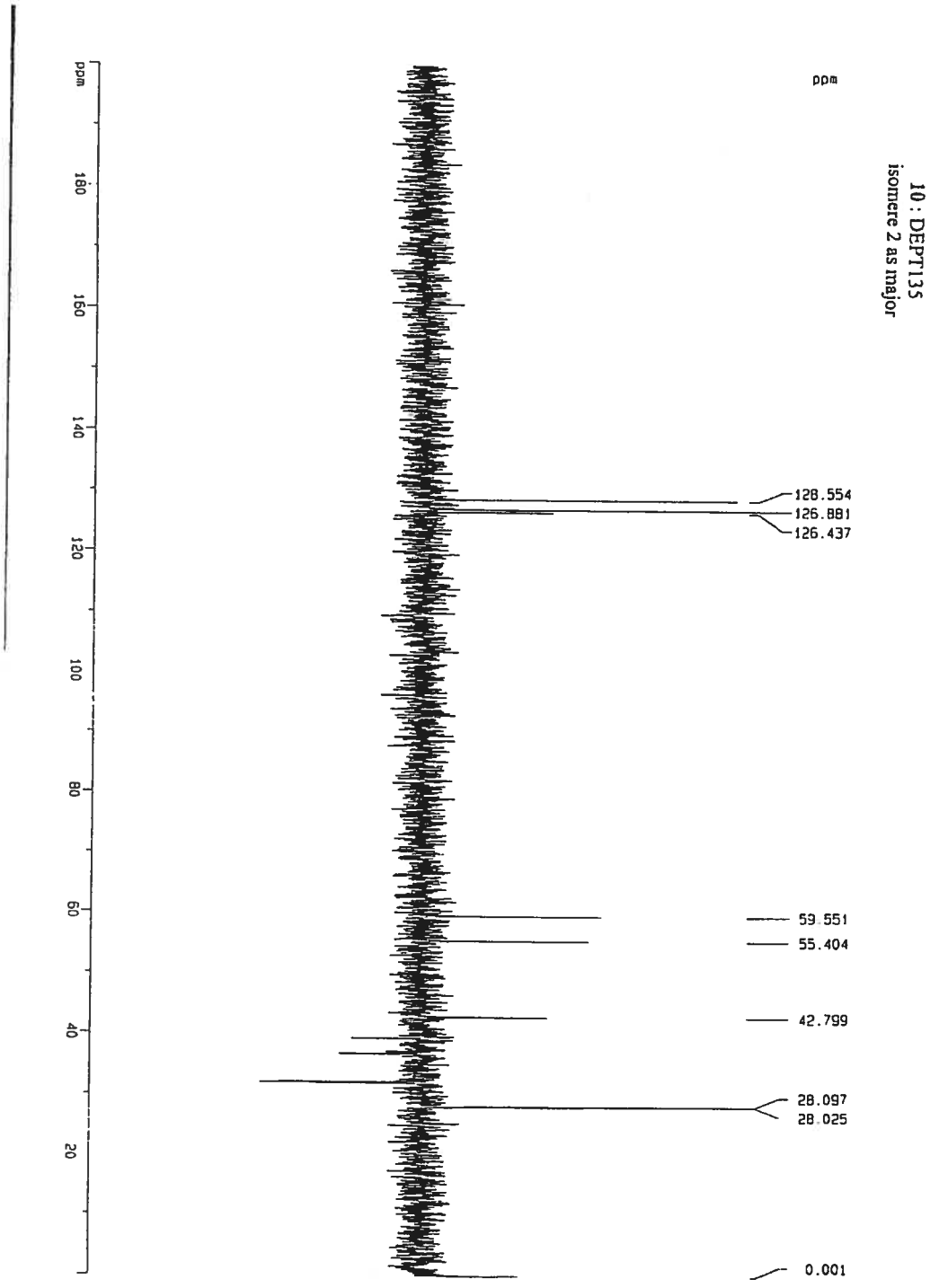
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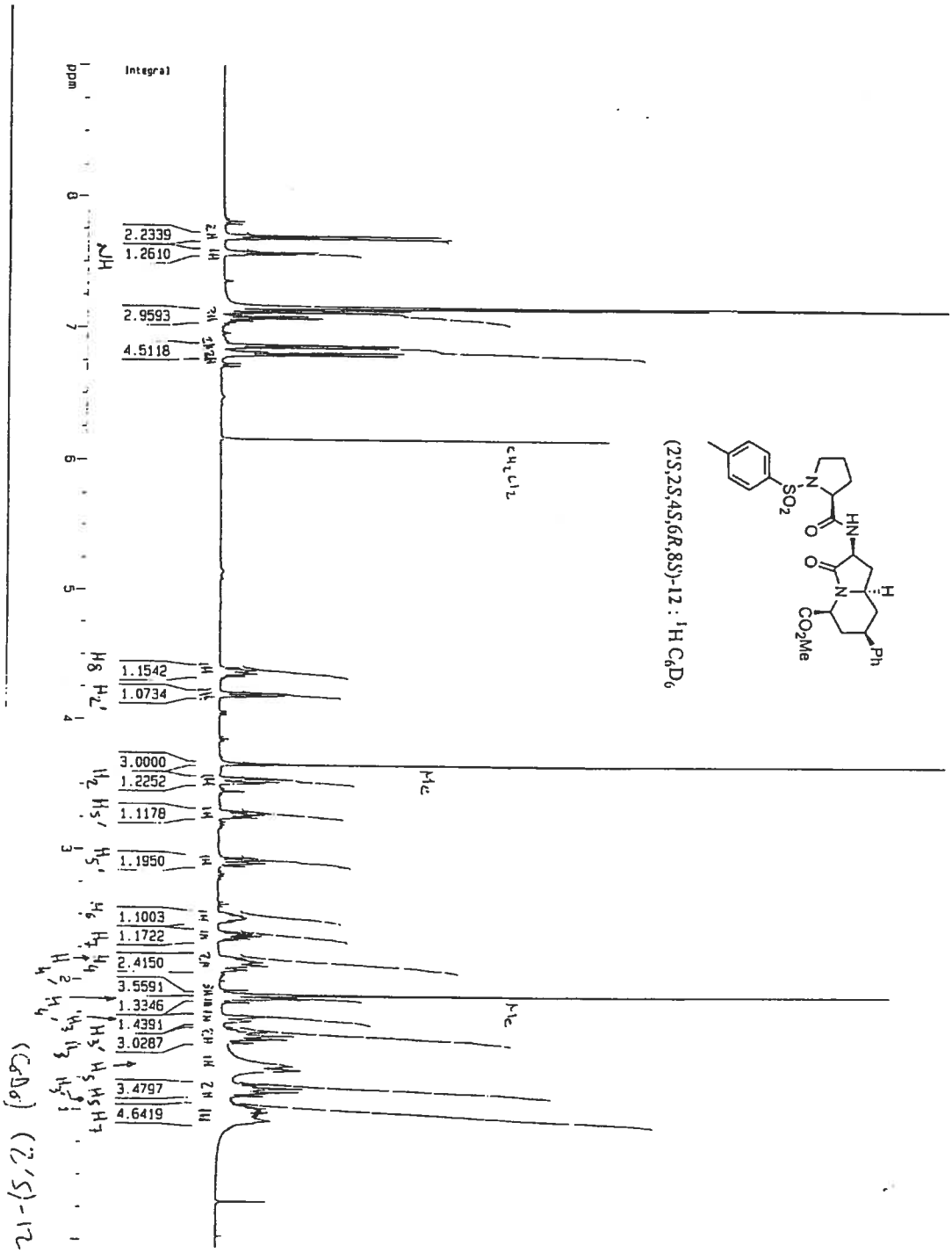
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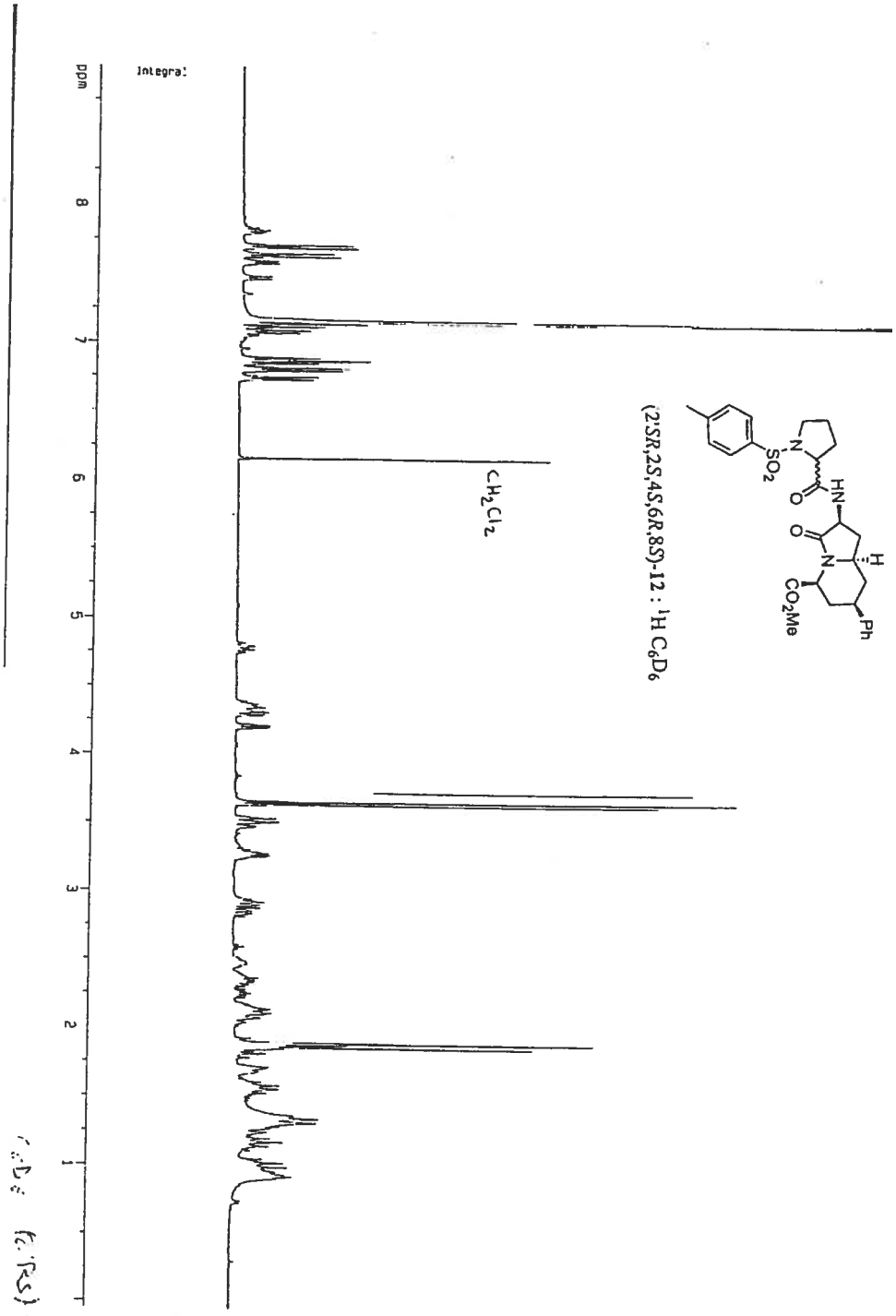
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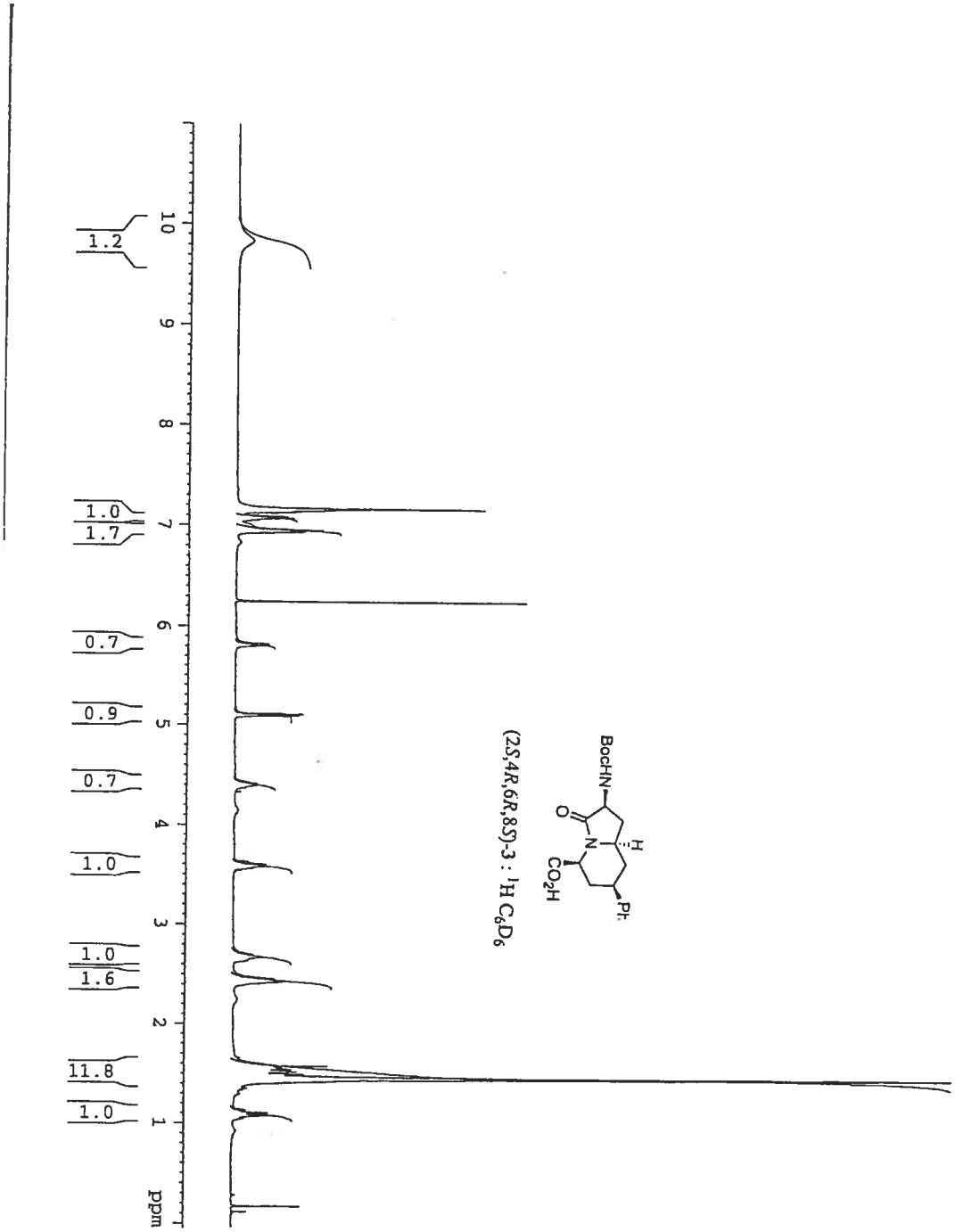
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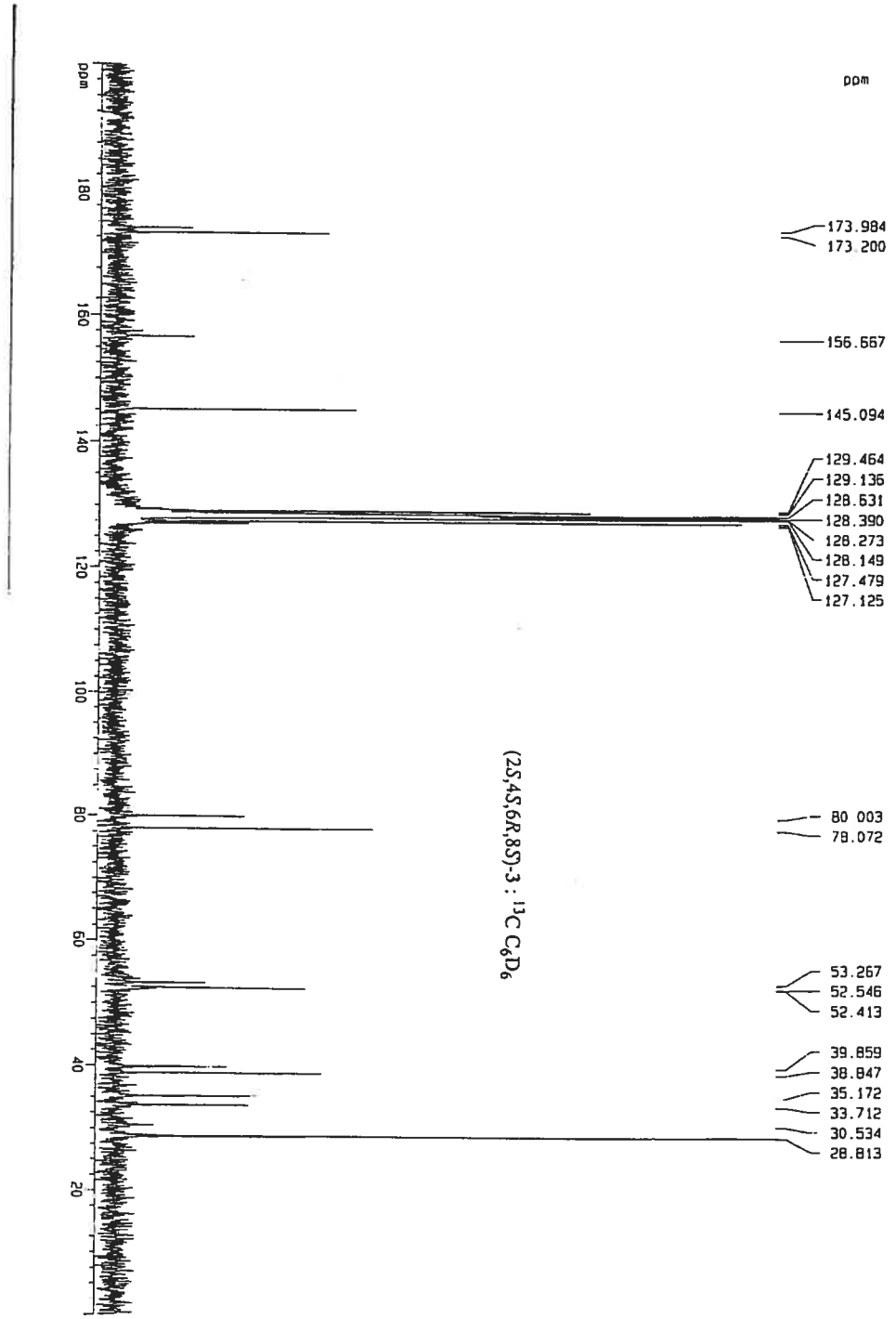
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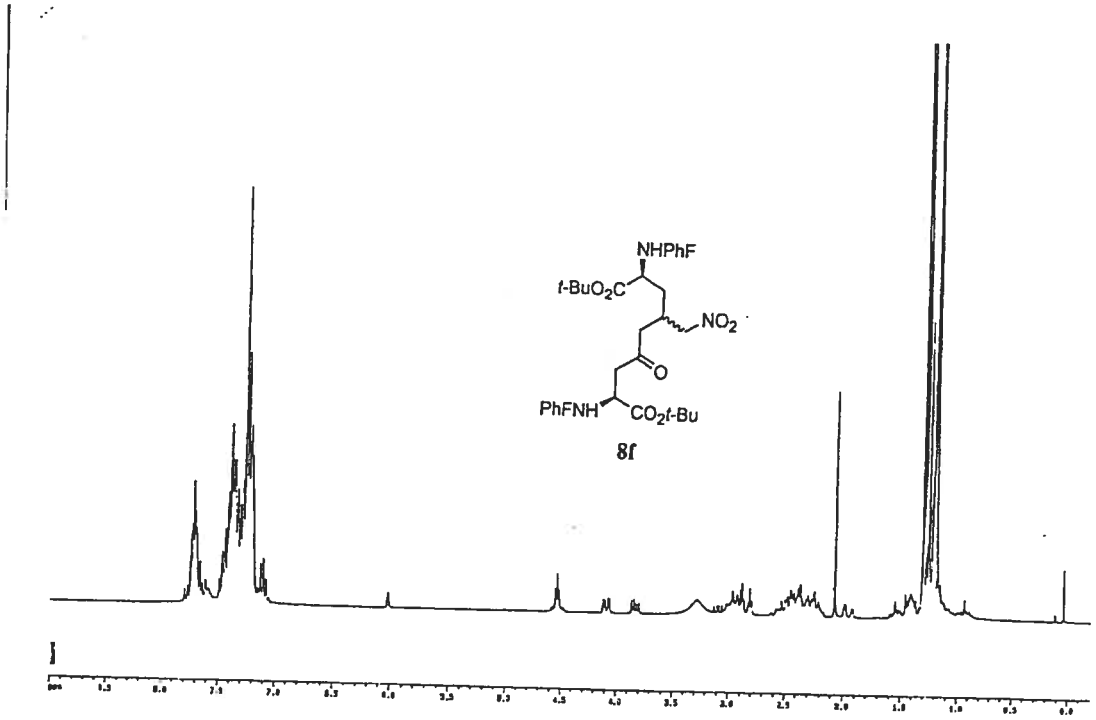
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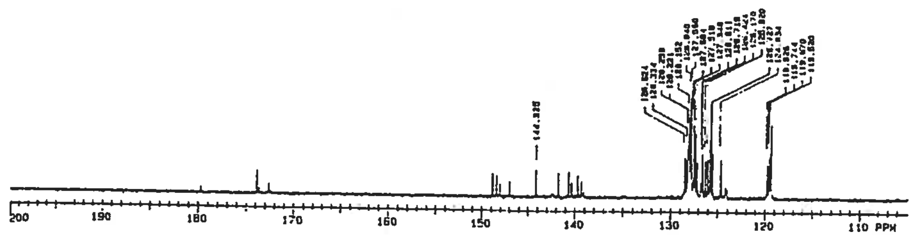

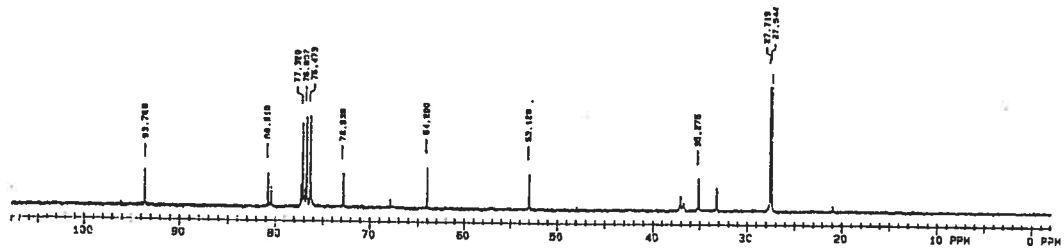

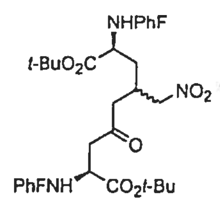

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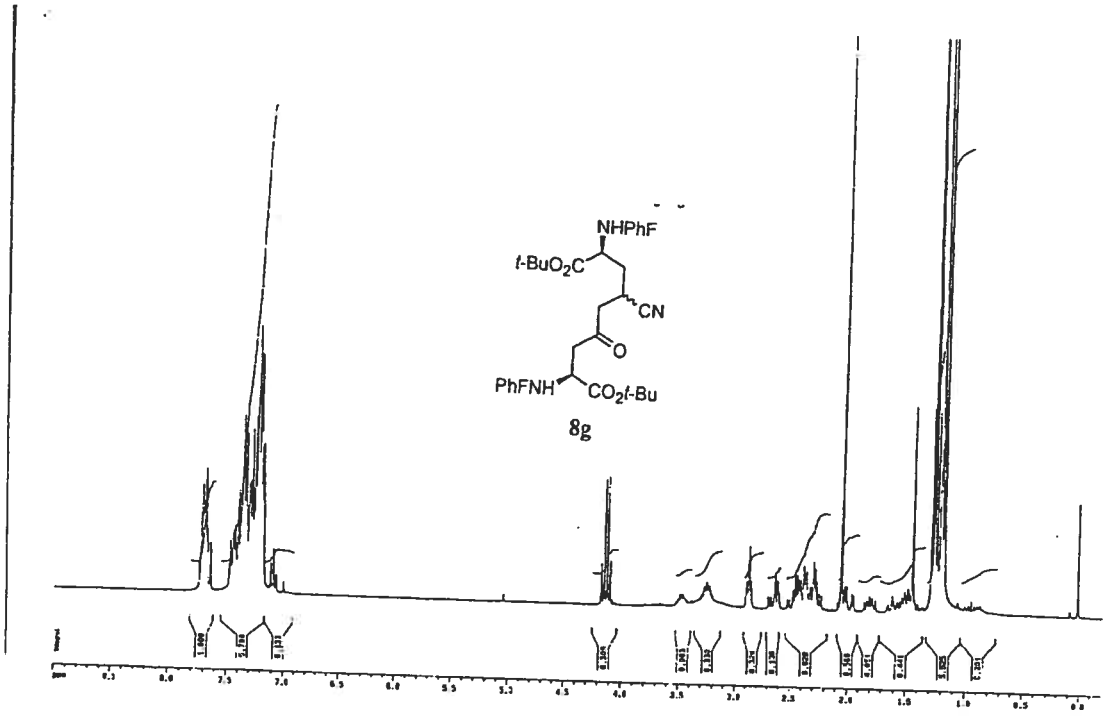

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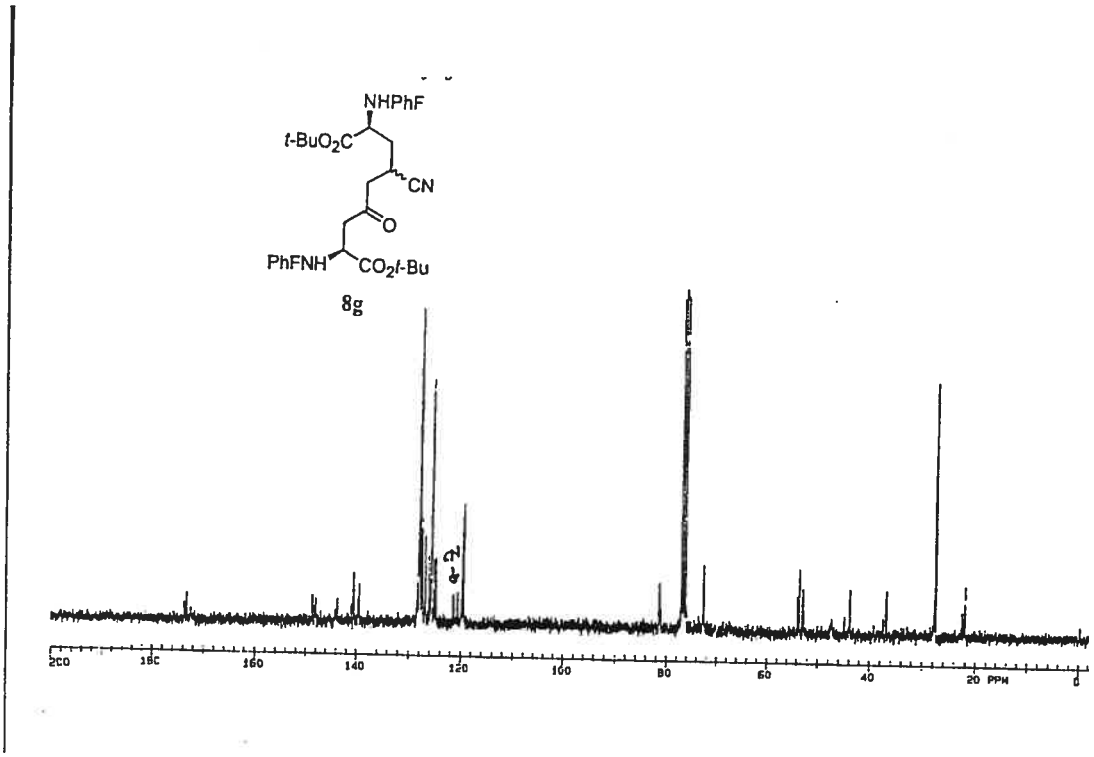
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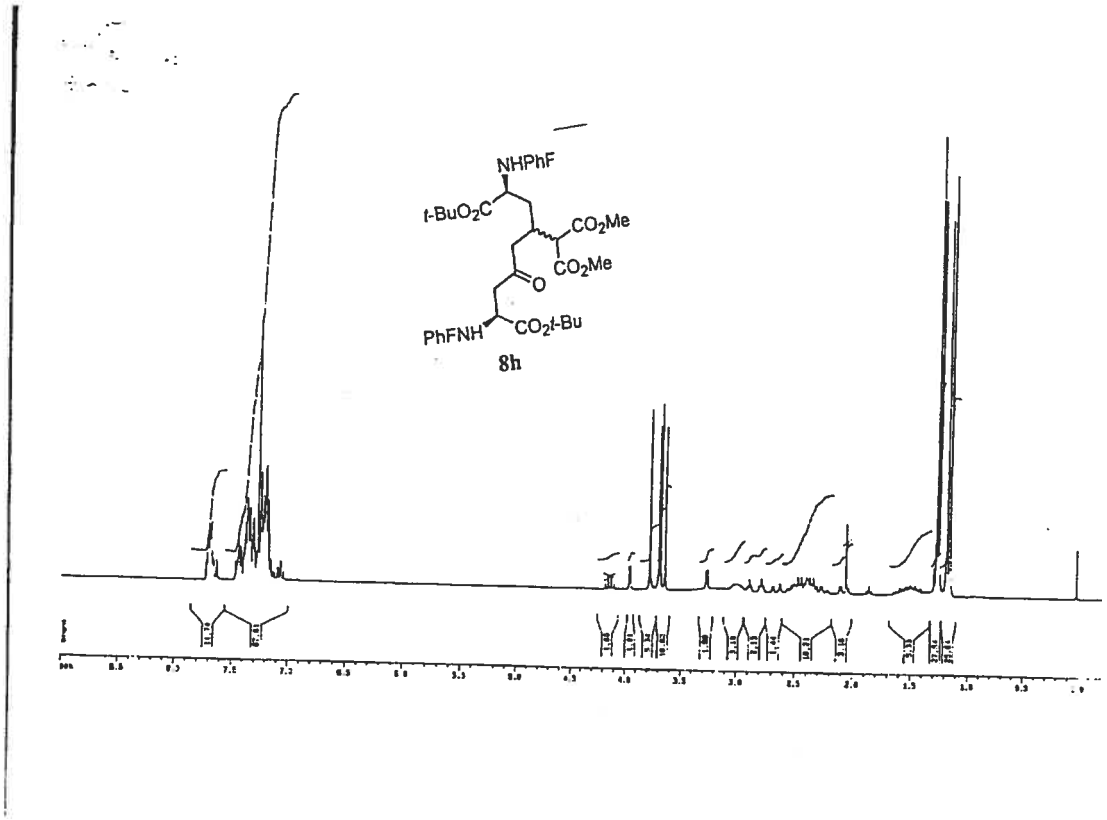
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Annexe 2.

Article relié aux travaux du chapitre 7.

J. Med. Chem. 2004, *47*, 1864−1867

Structure−Activity Study of the ORL1 Antagonist Ac-Arg-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2

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Received September 15, 2003

The structure−activity requirements of the ORL1 antagonist Ac-Arg-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2 **4** were investigated by varying the position, structure, and charge of the Arg residues. Attempts to abridge the peptide by removal of the Arg, D-Cha, and D-*p*-ClPhe residues abolished affinity for the ORL1 receptor, whereas deletion of the acetamido N-terminus maintained receptor affinity and selectivity. This series of analogues has provided an improved potent and selective ORL1 receptor antagonist, Ac-Cit-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2.

Introduction

The opioid receptor like 1 (ORL1) is a G-protein-coupled receptor structurally related to μ-, δ-, and κ-opioid receptors. However, it has been shown to display poor affinity for opioid receptor ligands.[1−4] The endogenous ligand of ORL1 was shown to be a heptadecapeptide known as nociceptin/orphanin FQ.[5,6] Although the physiological role for the ORL1 receptor is still poorly defined, nociceptin/orphanin FQ has exhibited a broad spectrum of pharmacological actions, including pain modulation, anxiolytic-like effects, stimulation of food intake, and modulation of spontaneous locomotor activity.[7] The development of new ORL1 ligands with high selectivity and bioavailability remains an important challenge for the elucidation and control of the physiological role of this receptor.

From screening of a synthetic combinatorial constrained peptide library on the human μ-, δ-, and κ-opioid receptors, and the ORL1 receptor, a hexapeptide analogue (**1**) was identified as a ligand exhibiting good affinity and modest selectivity for the ORL1 receptor (Figure 1).[8] Hexapeptide analogue **1** contains a thiaindolizidinone β-turn mimic and acts as a competitive ORL1 receptor antagonist. At the opioid receptors, analogue **1** also exhibited agonist activity at higher concentrations. Replacement of the thiaindolizidinone β-turn mimic in **1** with indolizidinone and quinolizidinone amino acids produced a series of related peptides **2**−**4**. Peptide **4**, which contained the quinolizidinone dipeptide mimic, Qaa, displayed similar affinity and antagonist potency as **1** at the ORL1 receptor; moreover, it exhibited enhanced receptor selectivity (Table 2).[9]

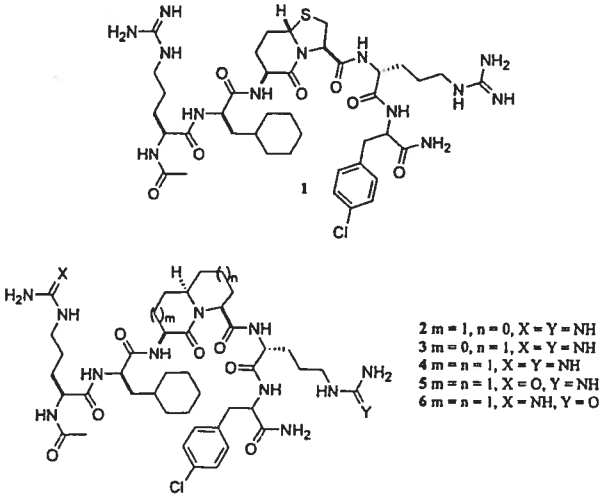

Figure 1. Structure of peptide III−BTD **1** and peptides containing azabicyclo[*X.Y.*0]alkane amino acids **2**−**6**.

Probing the structural and electronic requirements for antagonist activity and selectivity for the ORL1 receptor, we have now synthesized and evaluated a new series of analogues of Ac-Arg-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2 (**4**). In particular, we have varied the position, structure, and charge of the arginine residues in **4** in order to better understand their importance for activity and selectivity. The neutral amino acid citrulline (Cit) has been used to replace each of the Arg residues in **4** to study the importance of their positive charge for interaction with the ORL1 receptor (analogues **5** and **6**).[10] Furthermore, the size of the hexapeptide analogue was reduced by systematic deletions of the Arg, D-Cha, and D-*p*-ClPhe residues as well as the acetamido N-terminus in analogues **7**−**10** in order to assess their importance for biological activity (Tables 1 and 2).

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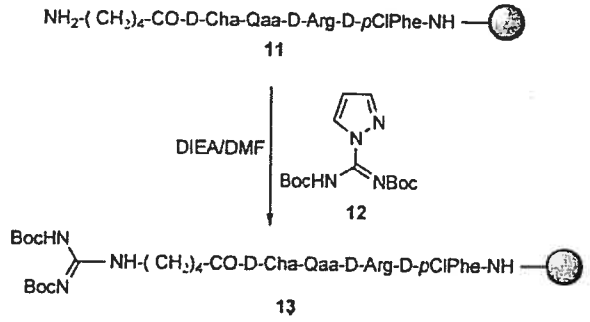
Table 1. Qaa-Containing Peptides to Investigate the Structure–Activity Relationship of Hexapeptide **4** at the ORL1 Receptor

	peptide	formula	calcd: (M + H⁺)	MS: (M + H⁺)	HPLC,[a] min	HPLC,[b] min	TLC, R_f
4	Ac–Arg-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2	$C_{42}H_{66}ClN_{13}O_7$	900.5	900.5	15.5	20.67	0.64
5	Ac–Cit-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2	$C_{42}H_{65}ClN_{12}O_8$	901.5	901.5	14.7	20.56	0.70
6	Ac–Arg-D-Cha-Qaa-D-Cit-D-*p*-ClPhe-NH_2	$C_{42}H_{65}ClN_{12}O_8$	901.5	901.5	14.6	20.11	0.63
7	Ac–Arg-Qaa-D-Arg-D-*p*-ClPhe-NH_2	$C_{33}H_{51}ClN_{12}O_6$	747.4	747.6	11.8	17.05	0.50
8	Ac–Arg-D-Cha-Qaa-D-Arg-NH_2	$C_{33}H_{58}N_{12}O_6$	719.5	719.6	13.9	17.55	0.48
9	Ac-D-Cha-Qaa-D-Arg-NH_2	$C_{27}H_{46}N_8O_5$	563.4	563.6	15.3	19.29	0.56
10	$H_2NC(=NH)NH(CH_2)_4CO$-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2	$C_{40}H_{63}ClN_{12}O_6$	843.5	843.7	15.7	21.06	0.69

[a] HPLC conditions 1. [b] HPLC conditions 2. See Supporting Information.

Scheme 1. Synthesis of the Guanidine End of Peptide **10** by Guanylation



Results and Discussion

Chemistry. Enantiopure *N*-(Boc)aminoquinolizidinone acid was synthesized from aspartic and pyroglutamic acids as inexpensive chiral educts according to the literature procedure.[9,11] Peptides **5**–**9** were synthesized using the solid-phase method of Merrifield[12] on MBHA resin in a semiautomatic apparatus as described in the Experimental Section. Elongation of the peptide involved coupling of *N*-Boc protected amino acids in the presence of HOBt and DIC as coupling reagent in a CH_2Cl_2/DMF solution. Amine deprotection was performed with TFA and anisole in CH_2Cl_2. Before the peptide was cleaved from the resin, the N-terminal end was acetylated by treatment with acetic anhydride and pyridine in a CH_2Cl_2/DMF solution. Cleavage of the peptide and deprotection of the tosyl groups of the arginyl residues was accomplished simultaneously by treatment of the resin with anhydrous liquid HF in the presence of anisole. Crude peptide was purified by reverse-phase HPLC. Peptide purity and composition were respectively ascertained by analytical HPLC and mass spectrometry (Table 1). Peptide **10** was synthesized by a similar protocol involving an additional guanylation of resin-bound 5-aminovaleryl peptide **11** using *N,N*-bis(*tert*-butyloxycarbonyl)-1-guanylpyrazole **12**[13] and excess of DIEA in DMF (Scheme 1).[14]

Receptor Binding Activity. The receptor binding affinities of peptides **5**–**10** were compared with the parent peptide, Ac-Arg-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2 (**4**), in assays on membrane homogenates of COS-1 or CHO cells expressing recombinant human μ-, δ-, and κ-opioid receptors (hMOR, hDOR, hKOR) and the human opioid receptor-like (hORL1, Table 2). Like peptide **4**, peptides **5**–**10**, all displayed affinities higher than 5000 nM at the DOR. Peptides **6**–**9** exhibited drastically reduced binding affinities for the ORL1 receptor, indicating the importance of a positively charged D-Arg residue as well as the difficulty in abridging peptide **4** by removal of the Arg, D-Cha, and D-*p*-ClPhe residues. On the other hand, peptide **10** exhibited similar affinity as peptide **4** (45 and 27 nM, respectively) for hORL1 as well as similar modest selectivity for the hORL1 versus the hMOR and hKOR, indicating that the N-terminus acetamido group could be removed without influencing binding and receptor specificity. Furthermore, peptide **5**, in which the N-terminal Arg residue was replaced by Cit, showed similarly good affinity for ORL1 as **4** (72 and 27 nM, respectively). In addition, peptide **5** displayed a greatly enhanced selectivity for hORL1 versus hMOR and hKOR (1:36:59 K_i ratio of hORL1/hMOR/hKOR) relative to that observed for peptide **4** (1:3:4 K_i ratio of hORL1/hMOR/hKOR). The maintained activity and improved selectivity of peptide **5** relative to the parent peptide **4** demonstrated that the positively charged guanidine at the N-terminus of **4** was more important for maintaining interactions with the μ- and κ-opioid receptors relative to the ORL1 receptor.

Table 2. Binding Affinities for hMOR, hKOR, and hORL1 of Peptides

	K_i (nM)[a]			
peptide	hORL	hMOR	hKOR	hDOR
1	34 ± 8[b]	53 ± 20[b]	78 ± 14[b]	222 ± 44[b]
4	27 ± 7	90 ± 12	105 ± 18	>5000
5	72 ± 21	2555 ± 635	4270 ± 100	>5000
6	>5000	>5000	>5000	>5000
7	>5000	833 ± 187	779 ± 176	>5000
8	>5000	>5000	>5000	>5000
9	>5000	>5000	>5000	>5000
10	45 ± 18	85 ± 25	153 ± 55	>5000

[a] K_i values were determined using [^{3}H]diprenorphine for hKOR, hMOR, hDOR, and [leucyl-^{3}H]nociceptin. Experiments were conducted on hMOR and hDOR transiently transfected into COS-1 cells, and hORL1 and hKOR stably expressed into CHO cells. Values are means ± SEM from two or more separated experiments, performed in duplicate. [b] From Becker et al., 1999 (ref 8).

[^{35}S]GTPγS Binding Assay. Because they both exhibited submicromolar affinities for hORL1, peptides **5** and **10** were further characterized in a functional assay consisting of agonist promoted stimulation of [^{35}S]-GTPγS binding to hORL1, hMOR, and hKOR cell membranes. At high concentrations (up to 50 μM), neither peptide **5** nor **10** increased nor decreased the [^{35}S]GTPγS binding to cell membranes expressing these receptors (data not shown). Peptide **5** (7 μM) shifted the concentration–effect curve of orphanin FQ/nociceptin (100 K_i concentration) to the right by about 55-fold, confirming its antagonist activity toward hORL1 (Figure 2). Moreover, peptide **5** displayed higher antagonist potency (K_e = 136 ± 32 nM) than was previously observed for peptide **4** (K_e = 300 ± 60 nM).

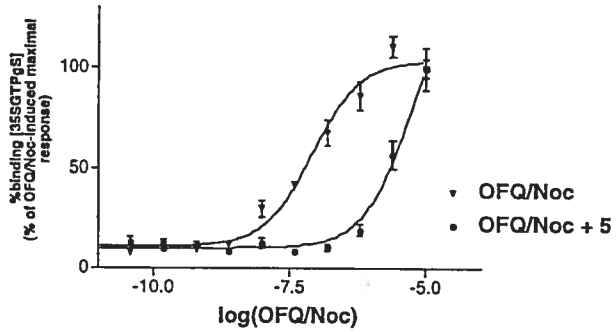


Figure 2. Stimulation of [^{35}S]GTPγS binding by orphanin FQ/nociceptin on hORL1 in the presence of putative antagonist peptide **5**. CHO-hORL1 membranes (5 μg of protein) were incubated 1 h at 37 °C with [^{35}S]GTPγS (0.2 nM) and GDP (40 μM), with increasing concentrations of ligands: nociceptin/orphanin FQ (▼) and 7 μM of peptide **5** (●). Peptide **5** shifted the concentration-effect curve of orphanin FQ/nociceptin to the right by about 55-fold. Data are expressed as percentage Nociceptin/Orphanin FQ-induced maximal [^{35}S]GTPγS binding and represent mean ± SEM from at least two separated experiments.

Conclusions

A better insight into the structural and electronic requirements for affinity and selectivity at the ORL1 receptor has been obtained by the synthesis and analysis of a series of analogues of the ORL1 antagonist **4**. Contraction of the peptide caused typically a dramatic loss of biological activity; however, the N-terminal acetamido group could be removed with little influence on affinity and selectivity. Replacement of each of the guanidine moieties with a urea, by substitution of Arg with Cit, demonstrated the importance of positive charge for affinity and selectivity. In particular, replacement of the C-terminal D-Arg residue with D-Cit in peptide **6** abolished affinity at the ORL1 receptor and indicated an essential electrostatic interaction. On the other hand, replacement of the charged moiety at the N-terminus by a neutral hydrogen-bonding surrogate had little effect on affinity, yet improved antagonist potency and selectivity for the ORL1 receptor. Peptide **5** (Ac-Cit-D-Cha-Qaa-D-Arg-D-*p*-ClPhe-NH_2) represents a new improved potent and selective ORL1 receptor antagonist.

Experimental Section

Solid-phase synthesis was conducted using a semiautomatic peptide synthesizer by a DIC/HOBt-mediated BOC-protection strategy on a 4-methylbenzhydrylamine resin (MBHA, 1.10 mmol/g). Before the coupling was started, the resin was washed with DIEA (2 ×) and CH_2Cl_2 (2 ×). The following schedule was employed: (1) Boc-protected amino acid (3 equiv)/DIC (3 equiv)/HOBt (3 equiv) in DMF/CH_2Cl_2 (1:1) for 3 h; (2) the coupling reaction was monitored by Kaiser ninhydrin test[15] (in cases of incomplete couplings, the resin was resubmitted to the same coupling conditions); (3) DMF wash (3 ×); (4) ^{i}PrOH wash (3 ×); (5) CH_2Cl_2 wash (3 ×); (6) BOC-deprotection with TFA/CH_2Cl_2/anisole (49/49/2) (2 ×, 5 and 15 min); (7) CH_2Cl_2 wash (2 ×); (8) DIEA wash (2 ×); (9) CH_2Cl_2 wash (2 ×). N-Terminal acetylation of the peptides was accomplished by treating the resin with Ac_2O (1.5 eq) and pyridine (1.5 equiv) in DMF/ CH_2Cl_2 (1/1) for 1 h. The side chain protection group used for Arg was Tos (toluene-4-sulfonyl). The peptides were cleaved from the resin and side chain groups were deprotected by treating the peptide on solid support with liquid HF and anisole at 0 °C for 90 min. After HF evaporation, the residue was treated with diethyl ether (2 ×), to precipitate the peptide, and filtered. Separation of the peptide from the resin was performed by adding acetic acid to obtain a solution that was lyophilized. The crude material was purified by semipreparative RP-HPLC, with UV-detection at 215 nm, on a Supelco, Discovery BIO Wide Pore column (C18, 25 cm × 21.2 mm, 10 μm particle size) using a flow rate of 20 mL/min and a gradient from 97:3 to 80:20 water/CH_3CN containing 0.1% TFA over 30 min, followed by a 10 min isocratic run with the final eluant.

Abreviations

BTD ((3*S*,6*S*,9*R*)-2-oxo-3-amino-7-thia-1-azabicyclo-[4.3.0]nonane-9-carboxylic acid); CI-977, [5*R*-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzo[*b*]furan-4-acetamide; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin; hDOR, human δ-opioid receptor; hKOR, human κ-opioid receptor; hMOR, human μ-opioid receptor; hORL1, human opioid receptor-like; MBHA, 4-methylbenzhydrylamine; Qaa, (3*S*,6*R*,10*S*)-2-oxo-3-amino-1-azabicyclo[4.4.0]decane-10-carboxylate.

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Supporting Information Available: Experimental details as well as protocols for the biological testing of **4**−**10** in cell culture, cell transfections, and cell membrane preparations, receptor binding assay, and [^{35}S]GTPγS binding assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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