Université de Montréal

# Méthodologie de synthèse d'acides aminés azacycloalcanes énantiopurs en solution et sur support solide

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présentée par:

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## Sommaire

Cette thèse relate le développement d'une méthodologie de synthèse d'acides aminés azacycloalcanes énantiopurs en solution et sur support solide. Nos travaux de synthèse en solution ont porté sur le développement d'une approche générale visant un contrôle de la dimension des systèmes hétérocycliques et des centres stéréogéniques, et qui offre la possibilité d'introduire des fonctions chimiques qui miment les chaînes latérales des acides aminés. Par ailleurs, nos travaux sur support solide ont porté sur l'élaboration d'une stratégie de protection et de manipulation de composés α-amino carbonyles ainsi que l'application d'une nouvelle technique d'analyse spectroscopique de la chimie organique sur support solide.

En utilisant une séquence d'oléfination de Horner-Wadsworth-Emmons / amination réductrice diastéréosélective / cyclisation de lactame, nous avons synthétisé l'acide aminé indolizidine-9-one à partir de l'acide aspartique. Subséquemment, l'élaboration des acides aminés quinolizidine-2-one et pyrroloazépine-2-one a démontré l'aspect général, versatile et pratique de notre approche. L'importance de la dimension des hétérocycles sur la géométrie des angles dièdres a été démontrée par l'analyse cristallographique des acides aminés. Nous avons développé une stratégie de synthèse des acides aminés azabicyclo[X.Y.0]alcanes qui rend maintenant possible l'étude systématique de la relation conformation-activité des peptides d'intérêt biologique.

L'utilisation de l'acide *N*-(BOC)aminé (2*S*, 6*R*, 8*S*)-indolizidine-9-one en mimétisme peptidique a indiqué son potentiel en tant que structure capable d'induire un repliement de type β dans les peptides biologiquement actifs. Nous avons incorporé cet acide aminé dans les structures peptidiques de la leucine-enképhaline et de l'hormone de libération de la gonadotropine (GnRH). Les résultats d'études conformationnelles par spectroscopie de résonance magnétique nucléaire (RMN) et dichroïsme circulaire (DC), ainsi que l'évaluation biologique semblent indiquer que l'acide aminé indolizidine-9-one stabilise la conformation bioactive de la leucine-enképhaline.

L'extension de la méthodologie mise sur pied pour la synthèse des systèmes azabicyclo[X.Y.0]alcanes, a fourni une synthèse pratique des acides aminés 6-alkylpipecoliques énantiopurs par une séquence de condensation aldolique / amination réductrice diastéréosélective à partir du β-aldéhyde de l'acide aspartique. L'étude de l'équilibre conformationnel d'analogues *N*-acétyles *N'*-méthylpipecolinamides comme peptides modèles a démontré l'effet produit par l'introduction d'un groupe stériquement encombrant en position 6 des pipecolinamides. Une augmentation de la population des isomères acétamides *cis* ainsi qu'une diminution de l'énergie d'activation du processus d'isomérisation ($\Delta G^{\ddagger}$) des acétamides ont été observés. Ces travaux ont indiqué l'impact de la dimension des hétérocycles sur l'équilibre conformationnel des acétamides.

Dans un deuxième temps, nous avons effectué les premiers pas d'une transition de la synthèse d'azacycloalcanes en solution vers une synthèse sur support solide. Nous avons voulu établir une stratégie flexible et applicable à la synthèse d'azacycloalcanes énantiopurs sur support solide.

Puisque l'approche envisagée impliquait la synthèse et la manipulation de composés α-amino carbonyles configurationnellement stables, nous avons développé un nouveau groupe protecteur, le 9-bromo-9-*p*-bromophénylfluorène, pour la fonction amine des acides aminés. Ce groupe protecteur permet la synthèse, l'ancrage au moment désiré, et la manipulation de composés α-amino carbonyles sur support solide. Nous avons évalué et validé le potentiel de cette approche par la synthèse et la manipulation d'un α-amino aldéhyde dans la préparation d'amino-alcools énantiopurs sur support solide.

Nous avons développé une nouvelle technique d'analyse non-destructive de la chimie organique sur support solide utilisant la spectroscopie infra-rouge photoacoustique à transformée de Fourier (FTIR-PA). Lors de ces travaux, nous avons développé une nouvelle synthèse des acides déshydroaminés en solution et sur support solide. L'application de la technique FTIR-PA lors de la synthèse d'un acide déshydroaminé a

permis de suivre chaque étape des manipulations chimiques sans destruction ni perte de matériel.

Les résultats obtenus lors de ces travaux auront un impact sur le design et le développement de nouveaux mimétismes peptidiques. En particulier, l'unification des divers aspects illustrés plus haut pourrait faciliter, accélérer et systématiser la conception et la production d'acides aminés énantiopurs et de structures hétérocycliques plus élaborées. Ces composés auront la capacité de mimer et de stabiliser les structures secondaires et la disposition spatiale des chaînes latérales des acides aminés à l'intérieur de peptides biologiquement actifs.

## Note

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

J'ai, bien sûr, entièrement rédigé le chapitre 1 de cette thèse.

Les articles du chapitre 2, qui décrivent la méthodologie de synthèse des acides aminés azabicyclo[X.Y.0]alcanes ont entièrement été rédigés par moi-même, sous la supervision du professeur William D. Lubell. J'ai conçu et effectué l'ensemble des travaux de recherche décrits dans ce chapitre.

L'article du chapitre 3, décrivant l'utilisation de l'acide aminé indolizidine-9-one en mimétisme peptidique a été rédigé par moi-même, sous la supervision des professeurs William D. Lubell et Dirk Tourwé. J'ai effectué l'ensemble des travaux de laboratoire décrits dans ce chapitre, sauf les expériences pour l'évaluation de l'activité biologique du peptide [I$^{9}$AA]$^{2,3}$-leucine-enképhaline.

Les articles du chapitre 4, qui décrivent la synthèse des acides 6-alkylpipecoliques ont été rédigés en collaboration avec Martin E. Swarbrick et le professeur William D. Lubell. J'ai conçu et développé la méthodologie de synthèse des 6-alkylpipecolates. J'ai préparé le 6-*tert*-butylpipecolate et j'ai étudié l'équilibre conformationnel des acétamides *N*-terminales de ce composé. J'ai étudié la stabilité du β-amino aldéhyde de l'aspartate et déterminé la structure de son produit de décomposition. Mon collègue a amélioré la synthèse du β-aldéhyde de l'aspartate en modifiant les conditions de réduction. En utilisant la méthodologie que j'ai développé, mon collègue a préparé les autres pipecolates, et déterminé, avec ma collaboration, leur pureté énantiomérique.

L'article du chapitre 5 décrit le développement d'une méthodologie d'ancrage et de synthèse sur support solide. L'article a été rédigé par moi-même, en collaboration avec mes collègues et le professeur William D. Lubell. J'ai conçu et effectué la synthèse du groupe protecteur, des composés α-amino carbonyles, l'ancrage aux divers supports solides, la synthèse de l'α-amino aldéhyde et l'ensemble des manipulations sur

support solide et déterminé la pureté énantiomérique du produit final. Mes collègues ont préparé le polystyrène non-réticulé, ils ont amélioré le rendement du couplage de Suzuki et les conditions de déprotection des produits liés au support solide.

L'article du chapitre 6 décrit l'utilisation d'une nouvelle technique d'analyse de la chimie organique sur support solide. L'article a été rédigé par l'ensemble des collaborateurs. J'ai conçu la synthèse de l'acide déshydroaminé sur support solide et en solution. J'ai effectué l'ensemble des manipulations chimiques. Mauro Di Renzo a effectué l'enregistrement des spectres.

Finalement, j'ai, bien sûr, entièrement rédigé la conclusion de cette thèse.

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## Liste des abréviations

| | |
|---|---|
| [α] | rotation spécifique [en (deg ml) / (g dm)] |
| Å | angstrom |
| AAI / IAA | acide aminé indolizidinone |
| Ac | acétyle |
| $Ac_2O$ | anhydride acétique |
| acac | acétylacétonate |
| AcOH | acide acétique |
| ACE | *angiotensine-converting-enzyme* |
| ANF | *atrial natriuretic factor* |
| Ar | aryle |
| atm | atmosphère(s) |
| Bn | benzyle |
| BOC | *tert*-butyloxycarbonyle |
| $(BOC)_2O$ | dicarbonate de di-*tert*-butyle |
| BOP | hexafluorophosphate de (1-azabenzotriazolyloxy) tris(diméthylamino)phosphonium |
| br | *broad* |
| BrPhF | 9-*p*-bromophénylfluorén-9-yle |
| BTD | *beta-turn dipeptide* |
| *n*-Bu | normal-butyle |
| *i*-Bu | iso-butyle |
| *t*-Bu | tert-butyle |
| *c* | concentration |
| °C | degré Celsius |
| calcd | *calculated* |
| Cbz | benzyloxycarbonyle |
| CD | *circular dichroism* |
| COSY | *correlated spectroscopy* |
| δ | déplacement chimique en parties par million |
| d | doublet |
| dd | doublet de doublet |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ène |
| DCC | *N,N*-dicyclohexylcarbodiimide |

| | |
|---|---|
| deg | degré |
| DHDP | dihydrodipicolinate |
| DIBAL-H | hydrure de diisobutylaluminium |
| DIC | *N,N*-diisopropylcarbodiimide |
| DIEA | *N,N*-diisopropyléthylamine |
| DMAP | 4-diméthylaminopyridine |
| DMF | *N,N*-diméthylformamide |
| DMS | diméthylsulfure |
| DMSO | diméthylsulfoxyde |
| DPPA | azidure de diphénylphosphoryle |
| ee | excès énantiomérique |
| EI | *electronic impact* |
| Et | éthyle |
| $Et_3N$ | triéthylamine |
| $Et_2O$ | éther diéthylique |
| EtOAc | acétate d'éthyle |
| EtOH | éthanol |
| FAB | *fast-atom-bombardment* |
| Fmoc | fluorénylméthyloxycarbonyle |
| Fmoc-OSu | hydroxysuccinimide de fluorénylméthyloxycarbonyle |
| FSH | *follicle stimulating hormone* |
| FTIR | *Fourier transform infra-red* |
| g | gramme(s) |
| GnRH | *gonadotropin releasing hormone* |
| GITC | isothiocyanate de 2,3,4,6-tétra-*O*-acétyl-β-D-glucopyranose |
| h | heure(s) |
| HATU | hexafluorophosphate d'hydroxy-azabenzotriazol-1-yle 1,1,3,3-tétraméthylaminium |
| HOAt | hydroxy-azabenzotriazole |
| HOBt | hydroxybenzotriazole |
| HPLC | *high performance liquid chromatography* |
| HRMS | *high resolution mass spectrometry* |

| | |
|---|---|
| IBTM | 2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate |
| *J* | constante de couplage (en RMN) |
| K | degré Kelvin |
| kcal | kilocalorie |
| λ | longueur d'onde |
| l, L | litre(s), *liter(s)* |
| LDA | diisopropylamidure de lithium |
| LH | *luteinizing hormone* |
| LHRH | *luteinizing hormone releasing hormone (GnRH)* |
| LRMS | *low resolution mass spectrometry* |
| µ | micro, micron |
| M | mole par litre |
| mM | millimole par litre |
| MAS-NMR | *magic angle spinning-NMR* |
| MBHA | méthylbenzyhydrylamine |
| *m*-CPBA | acide *meta*-chloroperoxybenzoique |
| Me | méthyle |
| MHz | mégahertz |
| min | minute(s) |
| mol | mole |
| mmol | millimole |
| m | multiplet (en RMN) |
| mp | *melting point* |
| Ms | méthanesulfonyle, mésyle |
| MS | *mass spectrometry* |
| MsCl | chlorure de méthanesulfonyle |
| NCPS | *non-cross-linked-polystyrene* |
| NCS | *N*-chlorosuccinimide |
| NEP | *neutral endopeptidase* |
| NMO | *N*-méthylmorpholine *N*-oxyde |
| NMR | *nuclear magnetic resonance* |
| nOe | *nuclear Overhauser effect* |
| NOESY | *nuclear Overhauser effect spectroscopy* |
| Nu | nucléophile |

| | |
|---|---|
| ORTEP | *Oak Ridge Thermal Ellipsoid Program* |
| PA | *photoacoustic* |
| PCC | chlorochromate de pyridinium |
| PEG | polyéthylène glycol |
| Ph | phényle |
| PhH | benzène |
| $PhCH_3$ | toluène |
| PhF | phénylfluorényle |
| PhT | phtalyle |
| ppm | parties par million (en RMN) |
| Pr | propyle |
| *i*-Pr | iso-propyle |
| q | quartet |
| $R_f$ | facteur de rétention (en chromatographie) |
| RMN | résonance magnétique nucléaire |
| ROESY | *rotating nuclear Overhauser effect spectroscopy* |
| rt | *room temperature* |
| s | singulet (en RMN) |
| $SN_1$ | substitution nucléophile unimoleculaire |
| $SN_2$ | substitution nucléophile bimoleculaire |
| t | triplet (en RMN) |
| TBTU | tétrafluoroborate de benzotriazol-1-yle 1,1,3,3-tétraméthylaminium |
| TEMPO | *2,2,6,6-tetramethyl-1-piperidinyloxy, free radical* |
| TFA | acide trifluoroacétique |
| THF | tétrahydrofuranne |
| TIPSOTf | trifluorométhanesulfonate de triisopropylsilyle |
| TLC | *thin layer chromatography* |
| TMS | tétramethylsilane, triméthylsilyle |
| TMSE | triméthylsilyléthyle |
| tosyl | *p*-toluènesulfonyle, tosyle |
| $t_R$ | temps de rétention (en chromatographie) |
| Tr | triphénylméthyle (trityle) |
| TRH | *thyrotropin releasing hormone* |
| *p*-TsOH | acide *para*-toluènesulfonique |

| | |
|---|---|
| U V | ultra-violet |

## Remerciements

Je tiens tout d'abord à remercier le professeur William D. Lubell, pour m'avoir donné l'opportunité d'effectuer ces travaux de recherche au sein de son groupe. Je considère sincèrement que mon séjour au sein du groupe Lubell a non seulement été une expérience scientifique extraordinaire, mais aussi une véritable initiation culturelle et une ouverture au monde.

Je voudrais remercier les membres du groupe Lubell, Eric Beausoleil, Laurent Bélec, Jérôme Cluzeau, Kenza Daïri, Zhe Feng, Patrice Gill, Liliane Halab, Mostafa Hatam, Henry-Georges Lombart, Trang Pham, Felix Polyak, Dany Rondeau, Simon Roy, Martin E. Swarbrick, Eryk Thouin, Jo Van Betsbrugge et Lan Wei pour avoir maintenu une atmosphère de travail exceptionnelle.

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A Michèle et Raymond,

# Chapitre 1

# Introduction

**1.1. Développement du peptidomimétisme**

Les peptides jouent un rôle fondamental en biologie. Ils agissent notamment comme hormones, neurotransmetteurs, neuromodulateurs, inhibiteurs d'enzymes, antibiotiques, toxines, et immunosuppressants.[1] Les peptides influencent une gamme de processus biologiques tels que le métabolisme, la sensibilité à la douleur, la reproduction, la digestion, la tension artérielle, la coagulation, et le comportement.[1] Bien qu'ils jouent ce rôle central dans la régulation des divers processus vitaux, certains facteurs associés aux peptides limitent leur usage thérapeutique. Ces facteurs incluent une dégradation rapide, un mauvais transport entre la voie gastro-intestinale et le sang ainsi qu'entre le sang et la barrière hématoencéphalique, une excrétion métabolique rapide et une spécificité faible due à leur flexibilité conformationnelle.[1,2]

En utilisant le peptidomimétisme comme approche pour étudier les peptides, on a développé une meilleure compréhension de la relation qui existe entre leur conformation et leur activité biologique.[2] Du point de vue chimique, un peptidomimétique peut être défini comme un composé qui maintient la topologie et l'activité biologique d'un peptide, mais qui ne possède pas nécessairement tous les liens amides caractéristiques des peptides. Par conséquent, les interactions entre les chaînes latérales du peptide avec les récepteurs biologiques sont maintenues et les problèmes relatifs aux peptides naturels sont potentiellement éliminés. Globalement, un peptidomimétisme est donc un composé qui peut mimer ou bloquer les effets biologiques d'un ligand peptidique au niveau du récepteur.[2d]

Durant les deux dernières décennies, les efforts combinés de la chimie organique, de la biochimie et de la pharmacologie ont initié et fait évoluer de façon significative le domaine du mimétisme peptidique.[2] Les retombées associées à une compréhension des effets physiologiques des peptides biologiquement actifs en relation avec leur structure tridimensionnelle seront une connaissance accrue de la biologie des peptides, ainsi que le design et le développement de nouveaux agents thérapeutiques.

Les efforts des chimistes ont porté sur le design et la synthèse de structures rigides capables d'induire ou de stabiliser les structures secondaires peptidiques.[2,3] Des mimétiques de structures secondaires tels que les hélices, les feuillets, les repliements de types β et γ ont été développés. En particulier, une gamme de mimétismes des repliements de type β ont été développés. Les repliements de type β sont caractérisés par un segment de quatre acides aminés qui renverse par 180° la direction de la chaîne peptidique (Figure 1). Ils se retrouvent régulièrement à la surface de protéines globulaires, exposant ainsi les chaînes latérales des acides aminés.[4]

Puisque ces chaînes latérales ont le potentiel d'agir en tant que groupement pharmacophore, on a émis l'hypothèse que cet élément de structure secondaire peptidique soit impliqué dans la reconnaissance moléculaire lors de processus biologiques.[4] On a pu démontrer que certains peptides plus courts possèdent aussi un repliement β dans leur conformation bioactive, encore une fois regroupant les chaînes latérales des acides aminés de façon à interagir avec des récepteurs biologiques.[4]

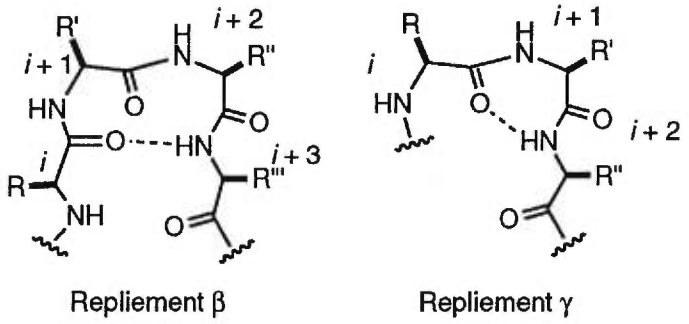


**Figure 1. Les repliements: structures secondaires peptidiques.**

Dans le but d'explorer l'hypothèse voulant que les repliements de type β soient impliqués dans la reconnaisance et la complexation entre un ligand peptidique et un récepteur biologique, on a introduit des contraintes qui permettent de rigidifier la structure flexible des peptides. L'introduction de contraintes conformationnelles réduit l'espace conformationnel et modifie l'équilibre dynamique entre les diverses conformations accessibles aux peptides. Dans le cas où la topologie du

mimétisme se rapproche de la conformation bioactive du ligand peptidique, la diminution de l'entropie lors de la complexation avec le récepteur sera moindre que celle due à la complexation du peptide naturel plus flexible. On peut donc s'attendre à ce qu'un tel peptidomimétisme possède une meilleure affinité avec un récepteur biologique. On a démontré que l'introduction de contraintes conformationnelles dans les ligands peptidiques produit des analogues qui possèdent une stabilité métabolique et une sélectivité accrues.[2]

Les travaux de Freidinger *et al.* ont indiqué qu'une lactame telle que **1** pourrait stabiliser la conformation d'un repliement de type β dans un peptide (Figure 2).[5] La préparation d'un analogue de la *gonadotropin releasing hormone* (GnRH) **2**, incorporant une γ-lactame possédant 8.9× la puissance du peptide naturel, a appuyé cette hypothèse de façon remarquable.[5] Ultérieurement, l'analyse conformationnelle par spectroscopie RMN du peptide GnRH contenant la γ-lactame a effectivement démontré que cette contrainte stabilisait la conformation d'un repliement de type β dans le peptide.[6] Subséquemment, des études sur des peptides capables d'altérer la fermentation à l'intérieur du rumen des ruminants[7] et sur les enképhalines[8] ont indiqué l'influence profonde de la dimension et la stéréochimie des lactames sur l'activité biologique de ces peptides.

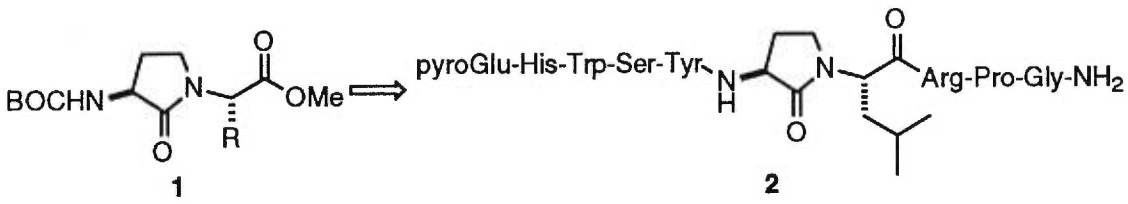


**Figure 2. Lactame de Freidinger et analogue de GnRH.**

Les lactames monocycliques ont fourni des résultats intéressants lors de leur incorporation dans les peptides. Toutefois, ces lactames ne permettent de rigidifier que les angles dièdres $\omega$ et $\psi$ (Figure 3). A la suite des travaux de Freidinger, on a poursuivi la quête de peptidomimétiques plus élaborés. On a donc recherché des peptidomimétiques qui rigidifient de façon accrue les différents angles dièdres du squelette peptidique et qui

**Note générale: Lorsque nécessaire, l'anglais est utilisé pour nommer les hormones, enzymes et autres entités biochimiques, et ce uniquement dans le but de simplifier la lecture.**

déploient les chaînes latérales pharmacophores de manière à interagir de façon optimale avec les récepteurs biologiques.

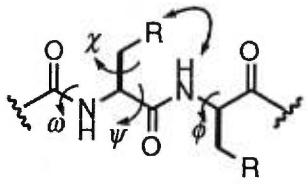


**Figure 3. Définition des angles dièdres au sein des peptides.**

Récemment, Hirschmann, Smith et Nicolaou ont développé des mimétismes non-peptidiques de la somatostatine et de RGD.[9,10] Ces mimétismes sont basés respectivement sur les squelettes d'un carbohydrate et d'un stéroïde (Figure 4). Ces composés miment l'arrangement tridimensionnel des chaînes latérales des peptides naturels. Cependant, ils ne miment pas la disposition spatiale du squelette peptidique. Cette approche est originale et élégante par sa simplicité puisque le squelette peptidique a été éliminé complètement et l'arrangement spatial des chaînes latérales a été maintenu en utilisant des charpentes moléculaires non-peptidiques (glucose et stéroïde). De plus, les composés **3** et **4** sont biologiquement actifs. Il est toutefois douteux que cette stratégie mimétique soit générale, particulièrement dans les cas où le squelette peptidique est impliqué dans une interaction, ou une complexation avec le récepteur biologique.

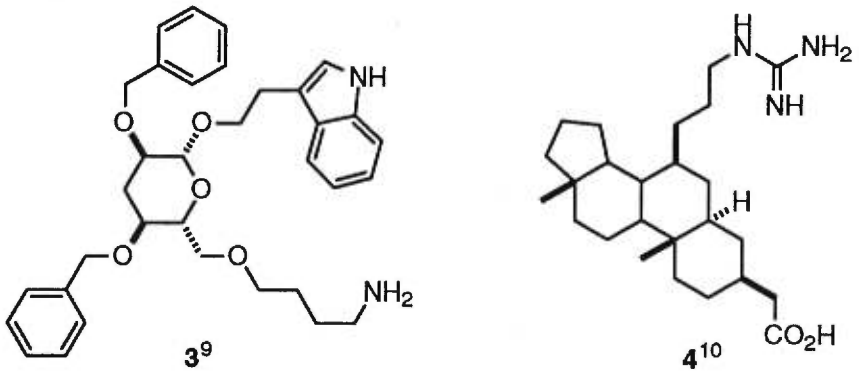


**Figure 4. Peptidomimétiques mimant la topologie des chaînes latérales.**

**1.2. Les acides aminés azabicycloalcanes comme peptidomimétiques**

Dans le but d'établir une approche systématique pour mimer et étudier les peptides, il est souhaitable et nécessaire d'appliquer des contraintes qui définissent plus clairement les divers angles dièdres de leur structure.

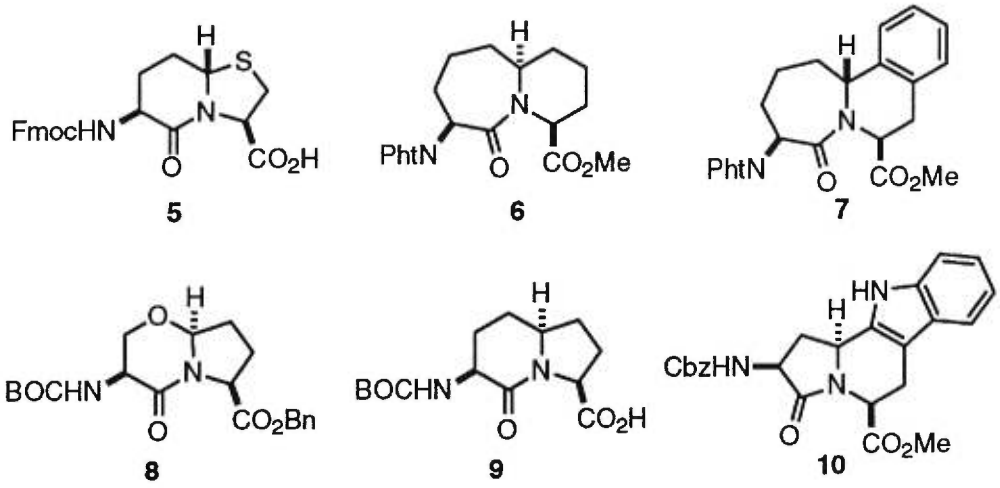


**Figure 5. Exemples représentatifs d'acides aminés azabicyclo[X.Y.0]alcanes.**

Dans cette optique, de nouveaux mimétismes peptidiques ont été développés pour rigidifier les angles dièdres $\psi$, $\phi$, $\omega$ et $\chi$ à l'intérieur des peptides. Parmi les mimétiques développés jusqu'à aujourd'hui, les acides aminés azabicycloalcanes, tels que présentés à la Figure 5, sont des structures bicycliques rigides qui ont été utilisées pour mimer les structures secondaires peptidiques telles que les repliements de type β.[3]

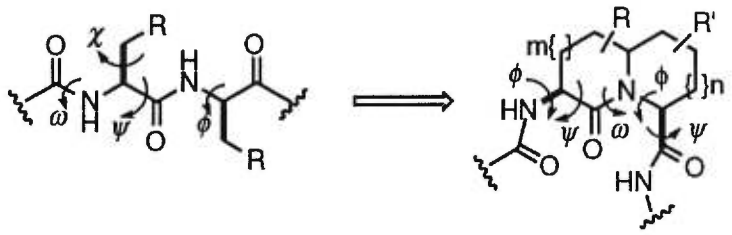

**Figure 6. Rigidification des angles dièdres dans un azabicycloalcane.**

Les acides aminés azabicycloalcanes possèdent la capacité de rigidifier trois angles dièdres internes $\psi$, $\phi$ et$\omega$. Ils imposent aussi une contrainte sur les angles dièdres externes $\psi$ et $\phi$, à travers les interactions stériques entre la

chaîne peptidique et l'hétérocycle (Figure 6). De plus, en déployant des groupes fonctionnels sur le squelette de l'hétérocycle, ces composés peuvent mimer la disposition des chaînes latérales des acides aminés d'une manière définie dans l'espace. Globalement, ces hétérocycles offrent donc la possibilité de mimer la géométrie retrouvée dans les peptides naturels biologiquement actifs, autant niveau du squelette peptidique que des chaînes latérales des acides aminés.[3]

**1.3. Utilisation des acides aminés azabicycloalcanes en peptidomimétisme**

Les acides aminés azabicycloalcanes ont été utilisés dans une gamme d'applications intéressantes tels que le design et la préparation de nouveaux mimétismes peptidiques et d'inhibiteurs d'enzymes biologiquement actifs.[3]

Par exemple, l'acide aminé thiaindolizidinone **11** (BTD) a été incorporé dans la structure du peptide cyclique gramicidine S (Figure 7).[11] Les résultats d'analyse conformationnelle par spectroscopie RMN et par dichroïsme circulaire ont démontré que le système bicyclique dans le peptide **12** était capable de stabiliser un repliement de type β.[11a-c] Ceci a été soutenu par les résultats d'évaluation biologique qui ont indiqué que l'analogue avait maintenu l'activité antibiotique du peptide naturel.

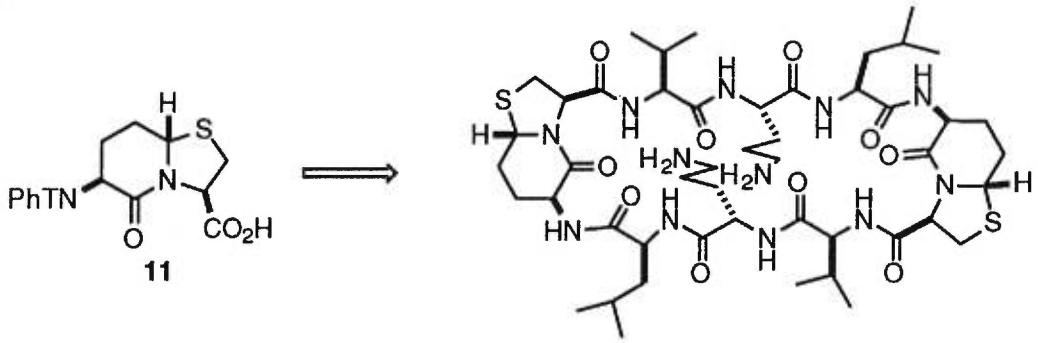


**Figure 7. Analogue BTD de la gramicidine S.**

Subséquemment, l'acide aminé IBTM **13** a été incorporé dans la gramicidine S (Figure 8). Les résultats de l'évaluation biologique du peptide **14** ont indiqué l'importance cruciale de la stéréochimie relative de l'IBTM **13** sur l'activité antibiotique. Alors que le peptide incorporant le

diastéréomère (11b*S*)-**13** était inactif, celui contenant l'isomère (11b*R*)-**13** possédait une activité comparable à celle du peptide naturel.[12]

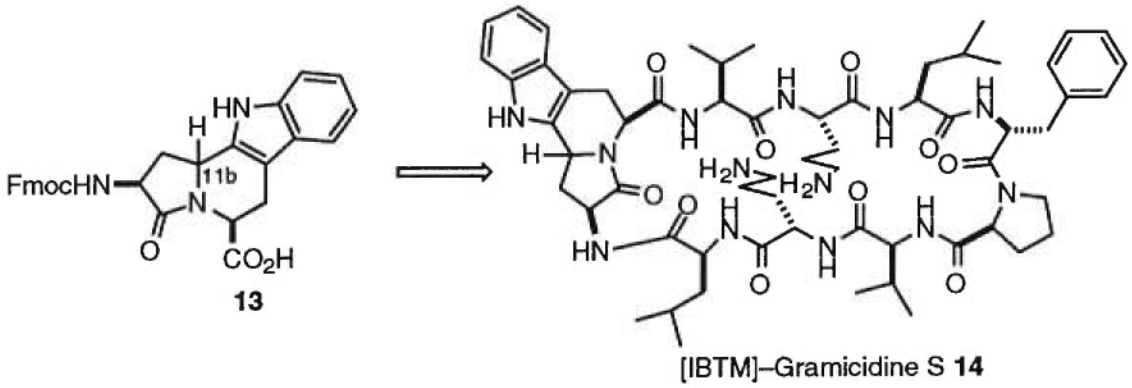


**Figure 8. Analogue IBTM de la gramicidine S.**

L'acide aminé thiaindolizidinone (BTD) **15** a aussi été incorporé dans la cyclosporine A (CsA), un agent immunosuppressant utilisé lors de transplantations d'organes (Figure 9). Le peptide tricyclique obtenu possédait une capacité d'inhiber la transduction de signal 3× plus puissante que celle du peptide naturel. Cet analogue **16** représente le premier composé qui est une variante structurale peptidique plus puissante que la CsA.[13]

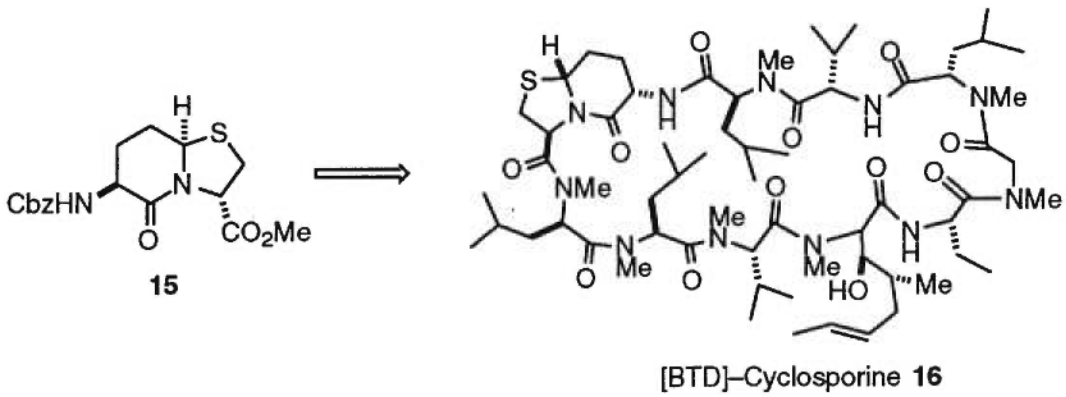


**Figure 9. Cyclosporine tricyclique incorporant le BTD.**

Par ailleurs, les acides aminés azabicycloalcanes ont été incorporés dans des structures d'inhibiteurs d'enzymes telles que la thrombine et *angiotensin-converting-enzyme / neutral endopeptidase* (ACE-NEP; Figure

10). Par exemple, des analogues **17** possédant une activité d'inhibition très puissante contre ACE-NEP ont été obtenus en attachant une chaîne mercaptoacyle à la fonction amine d'une gamme de systèmes bicycliques.[14] Ces travaux ont encore une fois indiqué l'importance de la dimension des hétérocycles sur l'activité biologique des analogues.[14] Les composés possédant une lactame de 6 membres étaient beaucoup moins actifs que les composés **17** avec une lactame à 7 membres.

Par ailleurs, des inhibiteurs de la thrombine, telle que la structure **18**, ont aussi été préparés en modifiant les chaînes latérales attachées aux fonctions amine et carboxylate.[15]

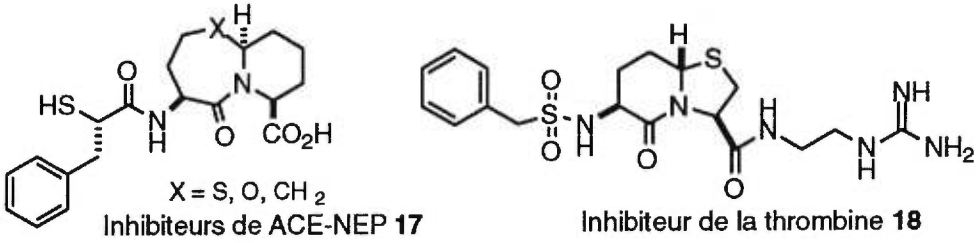


**Figure 10. Inhibiteurs de protéases.**

De plus, on a utilisé un azido acide benzyl-indolizidinone **19** dans la synthèse d'un agoniste sélectif **20** du récepteur tachykinine NK-2 (Figure 11).[16] Dans le composé **20**, le groupe benzyle sur le système indolizidinone mime la chaîne latérale d'une phénylalanine.

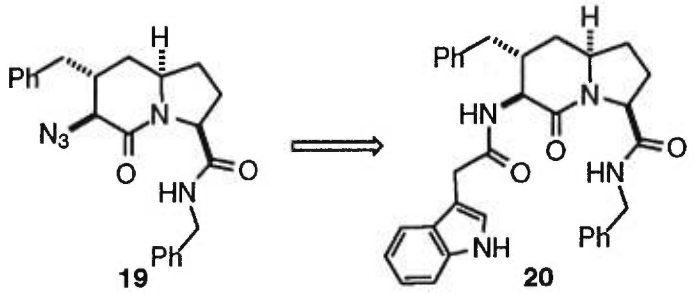


**Figure 11. Agoniste du récepteur NK-2.**

On a aussi incorporé un acide aminé indolizidinone dans un analogue de TRH (*thyrotropin releasing hormone*). Cet analogue bicyclique possède une activité qui varie selon la stéréochimie de

l'indolizidinone. L'analogue (6*R*)-**21** est 478× plus actif que l'isomère (6*S*)-**21**. De plus, le mimétisme (6*R*)-**21** est 4.8× plus puissant que l'hormone naturelle.[17] Il est à noter que la nature de la chaîne latérale est cruciale pour maintenir l'activité biologique puisque le remplacement du groupe cyclohexyle par un groupe phényle fourni un analogue inactif.

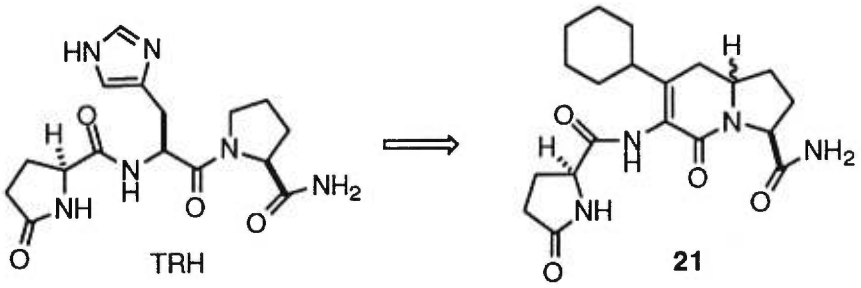


**Figure 13. Analogue de TRH.**

**1.4. Conclusion.**

Les exemples d'applications présentés ici démontrent la puissance de l'utilisation des contraintes conformationnelles pour étudier la conformation bioactive des peptides. Les composés peptidomimétiques ont permis le développement de modèles, ainsi que l'émission et la confirmation d'hypothèses préliminaires quant à la conformation bioactive et les éléments structuraux requis pour maintenir l'activité biologique de plusieurs peptides d'intérêt thérapeutique.

Dans cette optique, les acides aminés azabicycloalcanes ont été utilisés de façon remarquable comme outils pour stabiliser la structure de peptides. Les exemples décrits plus haut ont indiqué que la dimension du système hétérocyclique, la stéréochimie relative des substituants, de même que les groupes fonctionnels présents sur ces hétérocycles ont un impact profond sur l'activité biologique des peptides. Ces composés hétérocycliques ont donc un potentiel très intéressant pour mimer autant l'arrangement spatial du squelette peptidique que celui des chaînes latérales des acides aminés qui forment les groupes pharmacophores dans les peptides biologiquement actifs.

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# Chapitre 2

# Méthodologie de synthèse d'acides aminés azabicyclo[X.Y.0]alcanes énantiopurs

**2.1. Synthèse d'acides aminés azabicycloalcanes: problématique et objectifs**

Le champ d'application des acides aminés azabicycloalcanes en mimétisme peptidique et en chimie médicinale est relativement vaste. Les facteurs qui influencent la géométrie spatiale des peptides ont été explorés et influencés par l'utilisation d'acides aminés azabicycloalcanes. Toutefois, cette exploration ne semble pas systématique puisque généralement un seul analogue azabicycloalcane est incorporé dans la structure peptidique. Le développement d'une méthodologie générale de synthèse donnant accès à une variété d'acides aminés azabicycloalcanes pourrait systématiser l'étude de la relation structure-activité des peptides d'intérêt biologique.

L'usage d'un acide aminé azabicycloalcane peut effectivement fournir une information importante par rapport à la relation entre la conformation et l'activité biologique d'un peptide, mais ne permet toutefois pas d'établir des conclusions solides vis-à-vis cette relation. De plus, ce genre d'approche ne mène généralement pas à l'émission d'hypothèses plus avancées vis-à-vis l'élaboration d'analogues plus raffinés. Dans cette optique, l'utilisation de plusieurs acides aminés azabicycloalcanes différents devrait fournir une compréhension plus complète de la relation entre l'activité biologique et la conformation peptidique. Il est donc souhaitable et nécessaire de produire des acides aminés azabicycloalcanes de différentes dimensions pour mimer de façon systématique un large spectre de conformations peptidiques autant au niveau du squelette que des chaînes latérales. Ceci est crucial puisqu'un des objectifs ultimes est d'obtenir des peptidomimétiques possédant des qualités désirées comme candidats thérapeutiques, telles que l'augmentation de la stabilité métabolique, de meilleures propriétés de transport et une spécificité accrue avec le récepteur biologique.

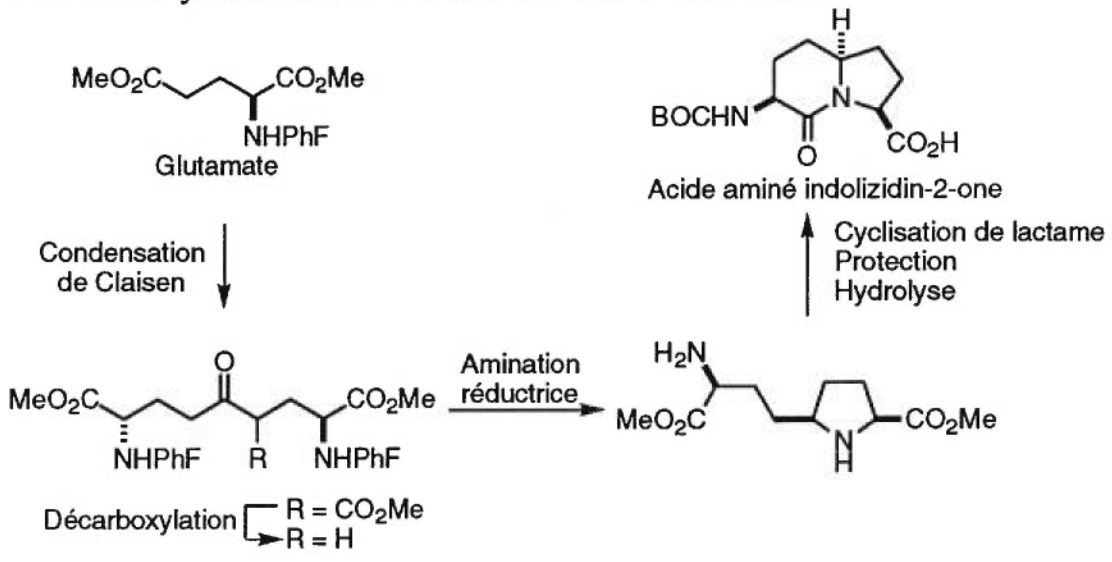
Une revue de la littérature sur les acides aminés azabicycloalcanes et autres acides aminés conformationnellement rigides a indiqué le grand nombre et la diversité des approches synthétiques qui permettent d'accéder à ces systèmes.[1a] En particulier, les stratégies basées sur la condensation des ω-aldéhydes d'acides aminés et les dérivés de la cystéine, ainsi que les approches utilisant la cyclisation des ions *N*-acyle-iminiums ont servi à

approfondir d'une façon significative les connaissances liées au domaine du peptidomimétisme.[1] Toutefois, les stratégies existantes ne tiennent généralement pas compte des problèmes relatifs au contrôle de la dimension des hétérocycles et des centres stéréogéniques ainsi que de la capacité d'introduire des fonctions chimiques sur le squelette hétérocyclique afin de mimer les chaînes latérales des acides aminés. De plus, ces approches utilisent des précurseurs qui fournissent généralement un seul système hétérocyclique après des synthèses relativement longues.

### 2.2. Stratégie de synthèse d'acides aminés azacycloalcanes énantiopurs

Dans le but de remédier à cette situation, nous avons voulu développer une méthodologie de synthèse efficace, simple et versatile qui puisse fournir une gamme d'acides aminés azabicycloalcanes sous forme énantiomériquement pure, avec contrôle sur la dimension des hétérocycles, sur la stéréochimie des substituants, et qui offre la possibilité d'introduire des chaînes latérales sur le squelette hétérocyclique.

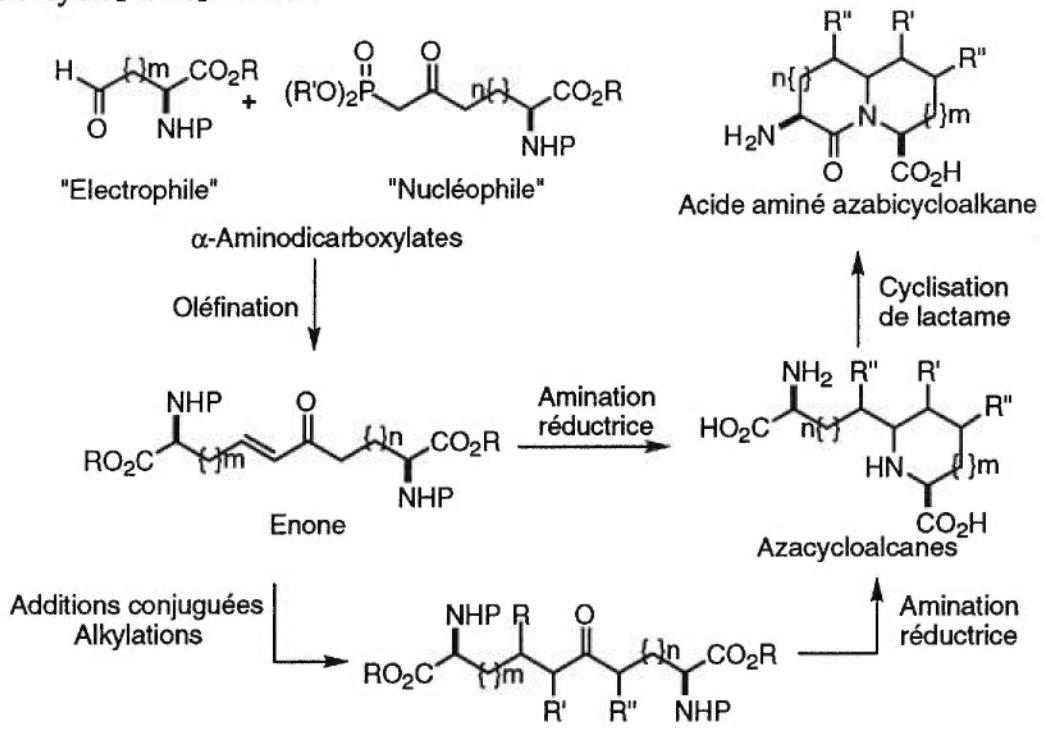
**Schéma 1. Synthèse de l'acide aminé indolizidine-2-one.**




Nous avons initialement exploré une modification de la synthèse stéréosélective de l'acide aminé indolizidine-2-one, développée par Lombart et Lubell (Schéma 1).[2] Cette synthèse efficace est basée sur une séquence d'autocondensation de Claisen entre deux glutamates /

amination réductive / cyclisation à la lactame bicyclique.[2] Cependant, lorsque nous avons tenté d'appliquer cette stratégie aux autres α-amino dicarboxylates tels que l'acide aspartique et l'acide α-amino adipique, tous nos efforts à tirer profit de la condensation de Claisen de ces analogues se sont révélés infructueux (voir Chapitre 2, Article 2).[3]

**Schéma 2. Stratégie d'oléfination pour la synthèse d'acides aminés azabicyclo[X.Y.0]alcanes.**




Nous avons donc envisagé une stratégie basée sur une réaction d'oléfination de Horner-Wadsworth-Emmons pour joindre les différents α-amino dicarboxylates. La stratégie est présentée au schéma 2. La réaction d'oléfination entre un amino aldéhyde et un β-céto phosphonate fournirait le précurseur diaminodicarboxylate. L'amination réductrice produirait un azacycloalcane et la formation de lactame fournirait finalement l'acide aminé azabicycloalcane. Cette approche offrirait les avantages de joindre deux α-amino dicarboxylates de différentes longueurs et stéréochimies et de contrôler la cyclisation des amines lors de l'amination réductrice. De plus, la cétone α,β-insaturée pourrait, en principe, être utilisée pour introduire des groupes fonctionnels mimant les chaînes latérales des acides aminés sur le système bicyclique via les alkylations et les additions conjuguées.

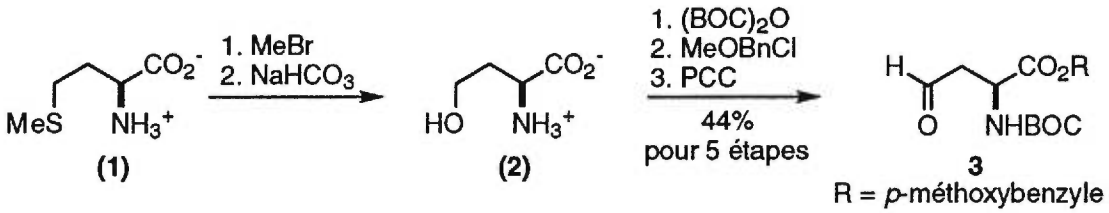
### 2.3. Synthèse et utilisation du β-aldéhyde de l'aspartate

Tel que décrit au schéma 2, la méthodologie envisagée implique la synthèse et l'utilisation d'un amino aldéhyde configurationnellement stable dans l'étape d'oléfination. Nous avons initialement porté nos efforts sur l'utilisation d'un β-amino aldéhyde dans cette séquence. Nous présentons donc un bref survol des méthodes de synthèse des β-amino aldéhydes de l'acide aspartique ainsi qu'une mention des applications de ces composés très utiles en synthèse organique. Les synthèses de β-aldéhydes de l'aspartate sont généralement basées sur l'oxydation de la méthionine, de l'allylglycine et de l'homosérine, ou sur la réduction régiosélective d'esters de l'acide aspartique.

#### 2.3.1. Méthodes basées sur l'oxydation de la méthionine

Le *N*-(BOC)aspartate β-aldéhyde **3** a été synthétisé à partir de la L-méthionine (**1**) (Schéma 3). La L-homosérine (**2**) a été formée par une alkylation du thioéther avec le bromure de méthyle suivie d'une substitution nucléophile par le bicarbonate aqueux sur le sulfonium formé. La protection de l'amine et du carboxylate suivie de l'oxydation de Jones de l'alcool instable a fourni l'aldéhyde **3** avec un bon rendement après 5 étapes. Cet aldéhyde a été utilisé dans la synthèse d'acides aminés non-naturels.[4]

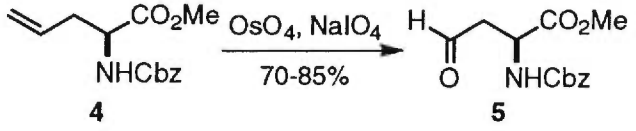
**Schéma 3.**




#### 2.3.2. Méthodes basées sur l'oxydation d'allylglycines

Le *N*-(Cbz)aspartate β-aldéhyde **5** a été préparé à partir de la L-allylglycine **4** en utilisant un clivage oxydatif par le periodate de sodium sur le diol formé par la dihydroxylation de l'oléfine avec le tétroxyde d'osmium (Schéma 4). Cette méthode a fourni un bon rendement de l'aldéhyde **5** qui a ensuite été utilisé dans la synthèse d'une gamme de produits naturels sidérophores (i.e. des agents naturels qui complexent le fer).[5]

**Schéma 4.**

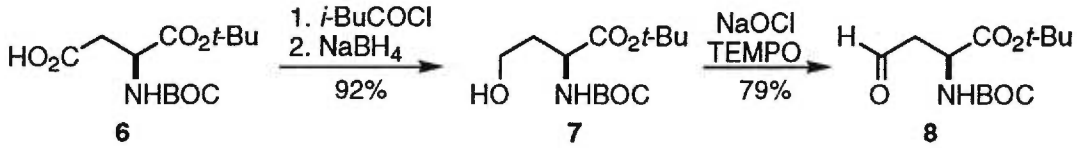



4 5

### 2.3.3. Méthodes basées sur la réduction d'aspartates

Le *N*-(BOC)aspartate β-aldéhyde **8** a été synthétisé à partir de l'aspartate **6** (Schéma 5). L'homosérine **7** a été produite par la réduction de l'anhydride mixte issu de la réaction entre l'acide **6** et le chloroformate d'isobutyle. On a mentionné que l'homosérine **7** est stable indéfiniment à 4°C. Cependant, les esters de méthyle, éthyle et benzyle de la *N*-(Cbz)homosérine se transforment en lactones correspondantes même lorsque gardés à –15°C. L'oxydation par l'hypochlorite de sodium a fourni l'aldéhyde **8** qui a été utilisé dans la synthèse d'analogues isostères de la phosphosérine.[6]

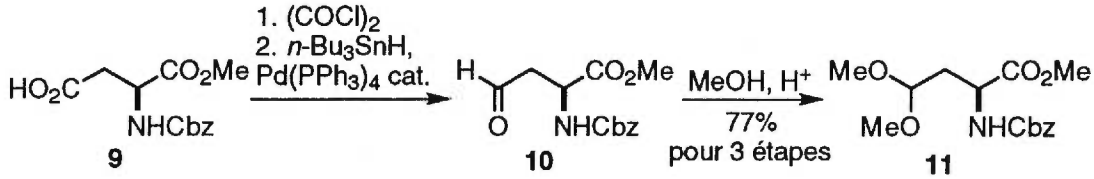
**Schéma 5.**




6 7 8

Le *N*-(Cbz)aspartate β-aldéhyde **10** a été obtenu par la réduction par l'hydrure de tributylétain du chlorure d'acide formé lors de la réaction entre le chlorure d'oxalyle et l'acide **9** (Schéma 6). L'aldéhyde a été isolé sous forme d'acétale de diméthyle **11** et a été utilisé dans la synthèse d'acides aminés neuroexcitatoires. [7]

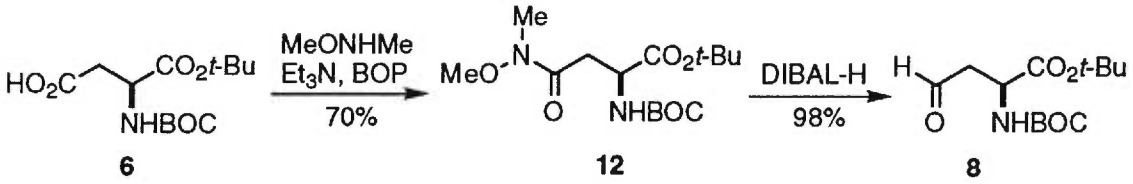
**Schéma 6.**




9 10 11

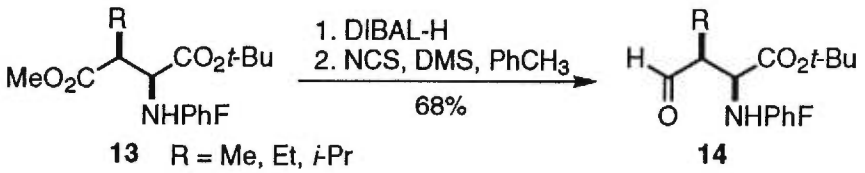
Le *N*-(BOC)aspartate β-aldéhyde **8** a été obtenu avec un excellent rendement par la réduction de l'hydroxamate de *N*-méthyle *O*-méthyle **12** par l'hydrure de diisobutylalumium (Schéma 7). L'aldéhyde a ensuite été utilisé dans la synthèse de l'acide L-α-aminosubérique, qui a été introduit comme un isostère du lien disulfure dans le peptide cyclique *atrial natriuretic factor* (ANF).[8]

**Schéma 7.**



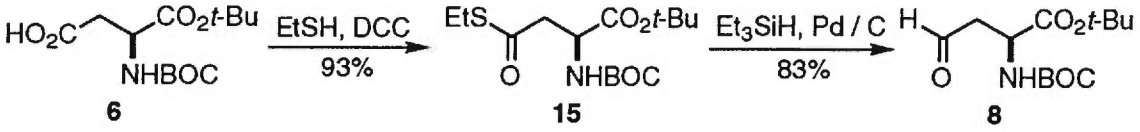

La réduction régiosélective du β-ester de l'aspartate **13** par l'hydrure de diisobutylaluminium a fourni une homosérine protégée qui a été transformée en β-aldéhyde **14** par une oxydation de Corey-Kim (Schéma 8). On a noté que le groupe phénylfluorényle (PhF) inhibait la réduction de l'ester en position α et la formation de la lactone de l'homosérine.[9] L'aldéhyde **14** a été utilisé dans la préparation de lactames énantiopures pour l'élaboration de peptidomimétismes.[9] En contraste, la γ-lactone de l'homosérine est le seul produit isolé lorsqu'on effectue la réduction avec DIBAL-H de l'aspartate de diméthyle ou de l'aspartate d'α-*tert*-butyle et de β-méthyle, quand l'amine est protégée par un groupe triphénylméthyle.[10] De plus, il est à noter que seul le produit de départ est récupéré, en faible rendement, après la réduction avec l'hydrure de diisobutylaluminium des aspartates de dibenzyles protégés à la fonction amine avec un groupe BOC, Cbz ou *p*-toluènesulfonyle.[10]

**Schéma 8.**



Le *N*-(BOC)aspartate β-aldéhyde **8** a été synthétisé avec un excellent rendement à partir de l'aspartate **6** (Schéma 9). La formation du thioester **15** est effectuée par un couplage de l'éthanethiol avec le *N,N*-dicyclohexylcarbodiimide. La réduction du thioester par le triéthylsilane a fourni l'aldéhyde **8**. Cet aldéhyde a été utilisé dans la synthèse de l'acide α-amino adipique et de précurseurs des nucléosides carbocycliques énantiopurs.[11]

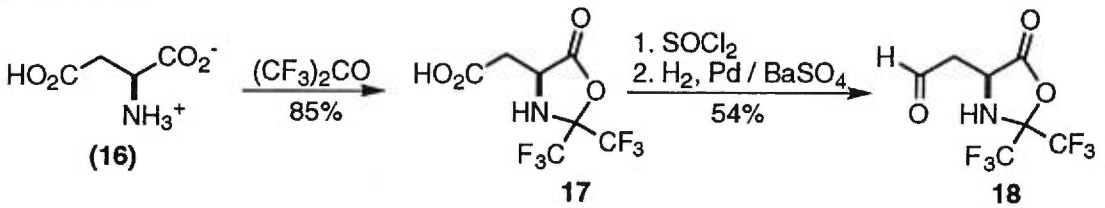
**Schéma 9.**




**6** **15** **8**

L'aldéhyde **18** a été synthétisé à partir de l'acide aspartique (**16**) (Schéma 10). La protection de l'amine et de l'acide carboxylique par l'hexafluoroacétone suivie de la réduction de Rosenmund du chlorure d'acide correspondant a fourni l'aldéhyde **18**. Cet aldéhyde a été utilisé dans la synthèse de la L-armentomycine.[12]

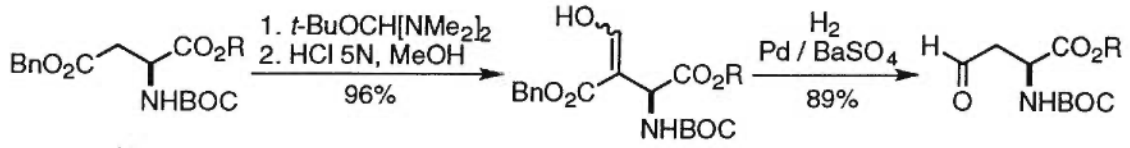
**Schéma 10.**




**(16)** **17** **18**

Le *N*-(BOC)aspartate β-aldéhyde **21** a été préparé en utilisant l'aspartate **19** comme produit de départ (Schéma 11). La condensation de l'ester **19** avec du (*tert*-butyloxy)bis(diméthylamino)méthane suivie de l'hydrolyse de l'intermédiaire énamine a fourni l'aspartate **20** comme un mélange d'aldéhyde et d'énol. L'aldéhyde **21** a été obtenu par l'hydrogénolyse et la décarboxylation de **20**. On a démontré que l'aldéhyde **21** était énantiopur par la formation des esters de Mosher avec l'homosérine correspondante.[13]

**Schéma 11.**




**19** **20** **21**

R = triméthylsilyléthyle (TMSE)

## 2.4. Conclusion

On peut noter que parmi toutes les méthodes présentées plus haut, la manipulation des aspartates avec un groupe protecteur 9-phénylfluorén-9-yle (PhF) sur la fonction amine est probablement la plus efficace, sécuritaire, pratique et versatile. Premièrement, le groupe PhF inhibe la formation de la γ-lactone de l'homosérine. Deuxièmement, le rendement de la réduction de l'aspartate est généralement excellent et nécessite un minimum d'étapes pour obtenir un aldéhyde protégé convenablement. Troisièmement, cette approche ne nécessite pas l'usage de composés organométalliques hautement toxiques. Quatrièmement, l'utilisation du groupe PhF assure l'intégrité stéréochimique au cours des diverses manipulations chimiques. Cinquièmement, les énolates du *N*-(PhF)aspartate sont générés régiosélectivement et peuvent subir les alkylations,[14a] hydroxylations[14b] ou aminations[14b] de manière diastéréosélective.

Ces facteurs ont donc orienté notre choix vers la synthèse et l'utilisation d'un *N*-(PhF)aspartate β-aldéhyde, en tenant compte de notre objectif de synthèse à long terme, c'est-à-dire la production d'une gamme d'analogues d'acides aminés azacycloalcanes énantiopurs et fonctionnalisés. Comme les prochains chapitres de cette thèse l'indiquent, ce β-amino aldéhyde a prouvé sa grande utilité dans la synthèse d'une gamme d'acides aminés azacycloalcanes énantiopurs.

Les trois articles qui suivent témoignent de l'efficacité, de la versatilité et de la simplicité de notre approche. Dans le premier et le deuxième article, nous avons démontré le potentiel de notre approche par la synthèse de l'acide aminé indolizidin-9-one. Dans le deuxième article,

nous avons étendu le spectre de diaminodicarboxylates accessibles par l'oléfination entre les β-céto-phosphonates et le *N*-(PhF)aspartate β-aldéhyde. Dans le troisième article, nous avons démontré la puissance de notre stratégie par la synthèse de deux acides aminés azabicycloalcanes à partir d'un même diaminodicarboxylate. Ces travaux ont indiqué l'importance de la dimension des hétérocycles sur la géométrie des angles dièdres internes.

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# Article 1

Gosselin, F.; Lubell, W. D. "Indolizidin-9-one Amino Acid: A New Rigid Dipeptide Turn Mimic." Publié dans *Peptides 1998*, Proceedings of the 25th European Peptide Symposium, Proceedings of the 25th European Peptide Symposium, S. Bajusz & F. Hudecz, Eds; Akadémia Kiadó: Budapest, Hungary, **1998**, 660-661.

## Indolizidin-9-one Amino Acid: A New Rigid Dipeptide Turn Mimic.

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### 2.6. Introduction

Engaged in the use of azabicycloalkane amino acids for studying biologically active peptides, we have strived to develop general approaches for synthesizing a variety of these rigid dipeptide mimics. Focusing on the stereocontrolled production of enantiopure heterocyclic amino acid, we reported first an efficient method for furnishing all isomers of indolizidin-2-one amino acid (IAA) **2** [1]. Extending our route towards introducing side-chain groups onto the heterocycle, we developed next a versatile method for synthesizing 5-, 7-, and 5,7-alkyl-branched IAAs **3-5** [2]. Exploring a new route to access a variety of heterocycle ring sizes, we introduce now an effective method for synthesizing enantiopure indolizidin-9-one amino acid **1** [3].

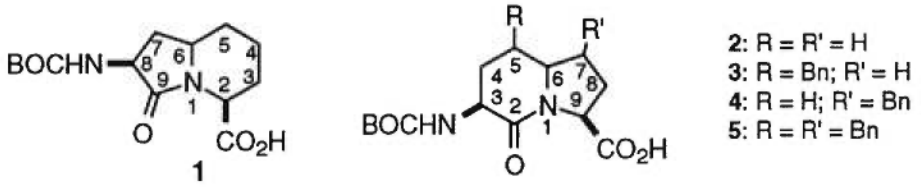


**Figure 1.**

### 2.7. Results and Discussion

Enantiopure 9-oxo-8-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-2-carboxylic acid (**1**) was synthesized in 9 steps and >25% yield from inexpensive aspartic acid via a Horner-Wadsworth-Emmons olefination / reductive amination / lactam cyclization approach. Initially, α-*tert*-butyl β-methyl *N*-(PhF)-aspartate **6** served as starting material for respectively synthesizing β-amino aldehyde **7** by DIBAL-H reduction and β-ketophosphonate **8** by nucleophilic addition of the lithium anion of dimethyl methylphosphonate in >95% and 71% yields (PhF = 9-phenylfluoren-9-yl). Subsequent Horner-Wadsworth-Emmons olefination

of β-amino aldehyde **7** with β-ketophosphonate **8** using $K_2CO_3$ in $CH_3CN$ provided enone **9** in 87% yield. Hydrogenation of α,ω-diaminoazelate **9** with Pd/C in 9:1 EtOH:AcOH under 6 atm of $H_2$ proceeded by reduction of the α,β-unsaturated ketone, cleavage of the PhF groups, imine formation, protonation and addition of hydrogen to the less hindered face of the iminium ion to provide 6-alkylpipecolate as a 9:1 mixture of *cis* and *trans* diastereomers. Treatment of the crude hydrogenation product with *p*-TsOH in toluene:MeOH followed by $Et_3N$ effected *tert*-butyl ester cleavage, esterification and lactam cyclization. Protection with $(BOC)_2O$ and $Et_3N$ in $CH_2Cl_2$, followed by hydrolysis of methyl ester **10** with $KOSiMe_3$ in $Et_2O$ gave *N*-(BOC)amino indolizidin-9-one acid **1** in 82% overall yield from ketone **9**.

The ring-fusion stereochemistry of the major isomer of **10** was assigned initially based on analogy to syntheses of indolizidin-2-ones **2-5** via reductive amination [1,2]. The assignment was later confirmed by X-ray analysis of crystals of **10** grown from EtOAc. Ester **10** was demonstrated to be >99% enantiomerically pure by conversion to (*R*)- and (*S*)-*N*-α-methylbenzylureas **11** followed by analysis of the diastereomeric methyl esters singlets using proton NMR spectroscopy.

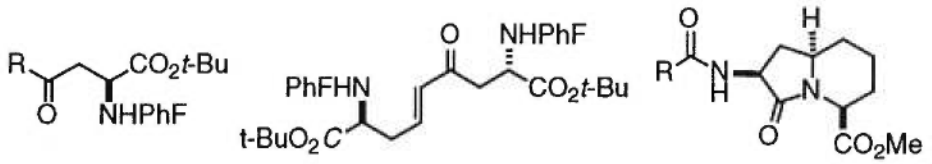


**Figure 2.**

We have developed an efficient synthesis of indolizidin-9-one *N*-(BOC)amino acid **1** for incorporation into peptides as a rigid dipeptide surrogate. Because alkyl substituents may be added to α,β-unsaturated ketone **9** by conjugate additions and alkylations, our route offers potential for introducing side-chain groups onto indolizidin-9-one **1**. Our method

may thus be used to synthesize analogs that mimic both the backbone and side-chain conformations of peptide secondary structures.

**Acknowledgements**
This research was supported by NSERC (Canada) and FCAR (Québec). F. G. is grateful for an award supporting travel expenses from the Ichikizaki Fund for Young Chemists.

**2.8. References**

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# Article 2

Gosselin, F.; Lubell, W. D. "An Olefination Entry for the Synthesis of Enantiopure α,ω–Diaminodicarboxylates and Azabicyclo[X.Y.0]alkane Amino Acids" Publié dans *The Journal of Organic Chemistry*, **1998**, *63*, 7463-7471.

## An Olefination Entry for the Synthesis of Enantiopure α,ω–Diaminodicarboxylates and Azabicyclo[X.Y.0]alkane Amino Acids

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### 2.9. Abstract

A new approach for synthesizing α,ω–diaminodicarboxylates of various chain lengths has opened the way for making a series of azabicyclo[X.Y.0]alkane amino acids of different ring sizes. β–Keto phosphonates **21-23** were synthesized in 71-90% yield by the addition of the lithium anion of dimethyl methyl phosphonate to the ω–methyl ester of α–*tert*-butyl *N*-(PhF)aspartate **3**, glutamate **9** and aminoadipate **12** (PhF = 9-phenylfluoren-9-yl). α,ω–Diaminodicarboxylates **24-26** of nine to eleven carbon chain lengths were prepared in 78-87% yield from the Horner-Wadsworth-Emmons olefination of α–*tert*-butyl *N*-(PhF)aspartate β–aldehyde (**5**) with aminodicarboxylate-derived β–keto phosphonates **21-23**. The power of this approach for making azabicyclo[X.Y.0]alkane amino acid was then illustrated by the first synthesis of enantiopure indolizidin-9-one amino acid **2** in 9 steps and >25% overall yield from inexpensive aspartic acid as chiral educt. Hydrogenation of (2*S*, 8*S*)-di-*tert*-butyl 4-oxo-2,8-bis[*N*-(PhF)amino]non-4-enedioate (**24**) in 9:1 EtOH:AcOH furnished a 9:1 diastereomeric mixture of 6-alkylpipecolate **28**, that was subsequently transformed into azabicyclo[4.3.0]alkane amino acid **2** via lactam cyclization and protecting group manipulations. Because α,ω–diaminodicarboxylates **25** and **26** may be similarly converted to heterocycles of larger ring sizes and because alkylation of similar ketones can be used to attach side-chains at different points on the heterocycle, this olefination strategy greatly expands our methodology for synthesizing azabicyclo[X.Y.0]alkane amino acids for the exploration of conformation-activity relationships of various biologically active peptides.

## 2.10. Introduction

The synthesis of azabicyclo[X.Y.0]alkanes has stimulated over a hundred years of innovative chemistry.[1,2] Organic chemists were first attracted to these structures because of their importance as components of biologically active natural products, such as the families of indolizidine, pyrrolizidine and quinolizidine alkaloids.[1] In recent years, peptide chemists have revived interest in their synthesis as constituents of azabicyclo[X.Y.0]alkane amino acids that restrain the back-bone and side-chain geometry of native proteins.[2] These bicyclic amino acids act as valuable building blocks for constructing conformationally rigid surrogates of peptide structures that have served to probe and mimic the spatial requirements for protein chemistry and biology.[2] Because azabicyclo[X.Y.0]alkane amino acids possess spatially defined amine and carboxylate handles suitable for functionalization by combinatorial technology, medicinal chemists have also become drawn towards their synthesis as inputs for generating libraries on which different pharmacophores are systematically displayed for studying recognition events.[2]

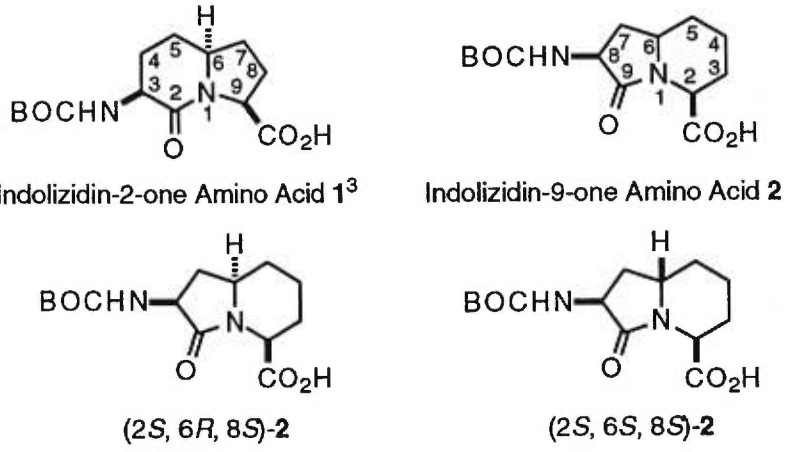



**Figure 1. Indolizidinone Amino Acids 1 and 2.**

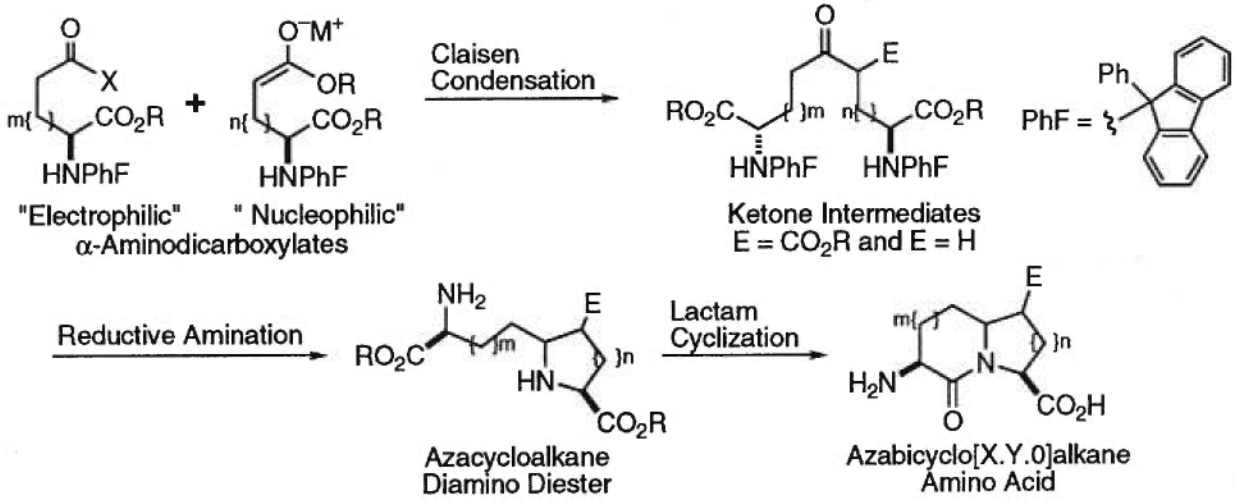
Engaged in the use of azabicyclo[X.Y.0]alkane amino acids in both peptide mimicry and combinatorial technology, we have strived to develop a general and practical synthesis to furnish a variety of these interesting ring systems.[3-5] Initially, we introduced a Claisen condensation /

reductive amination / lactam cyclization sequence for stereoselectively synthesizing azabicyclo[X.Y.0]alkane amino acid (Scheme 1, PhF = 9-(9-phenylfluorenyl)).[4] Our route gave access to all of the possible stereoisomers of enantiopure indolizidin-2-one amino acid **1** by employing inexpensive glutamic acid as chiral educt (Figure 1).[3] Lately, we have expanded our route to allow different amino acid side-chains to be appended onto the heterocyclic dipeptide surrogate via the alkylation of the ketone intermediate produced from decarboxylation of the Claisen condensation product.[5] In this report, we now present research to employ different aminodicarboxylates, such as aspartate and α–aminoadipate, in Scheme 1 in order to generate a variety of azabicyclo[X.Y.0]alkane heterocycle ring-sizes.

We have investigated the Claisen condensation between *N*-(PhF)aminodicarboxylates of three different chain lengths.[6-8] Using aspartate, glutamate and α–aminoadipate in Claisen condensations, we have demonstrated the unique reactivity of *N*-(PhF)glutamate and have illustrated aspects that interfere with the condensations of the other two aminodicarboxylates. Examination of an alternative strategy to link *N*-(PhF)aminodicarboxylates of different chain lengths opened a new route for synthesizing several of the key amino ketone intermediates involved in our original design. This new entry features olefination of *N*-(PhF)aspartate β–aldehyde and has furnished three different α,ω–diaminodicarboxylate intermediates for the synthesis of azabicyclo[X.Y.0]alkane amino acid.

We have illustrated the utility of these new α,ω–diaminodicarboxylate intermediates by the synthesis of a novel azabicyclo[4.3.0]alkane amino acid. Enantiopure indolizidin-9-one amino acid **2** was synthesized for the first time in 9 steps and >25% overall yield from aspartic acid via reductive amination and lactam cyclization of a γ–oxo-α,ω–diaminoazelate intermediate (Figure 1). Because the longer α,ω–diaminodicarboxylate intermediates may be similarly converted to heterocycles of larger ring sizes and because modification of these ketone intermediates may lead to the attachment of side-chains at different points

**Scheme 1.**
**Claisen Condensation Approach for Synthesizing Azabicyclo[X.Y.0]alkane Amino Acids**



on the azabicyclo[X.Y.0]alkane heterocycle, the olefination strategy greatly expands our methodology for constructing these important tools for studying the structure-activity relationships of biologically relevant peptides.

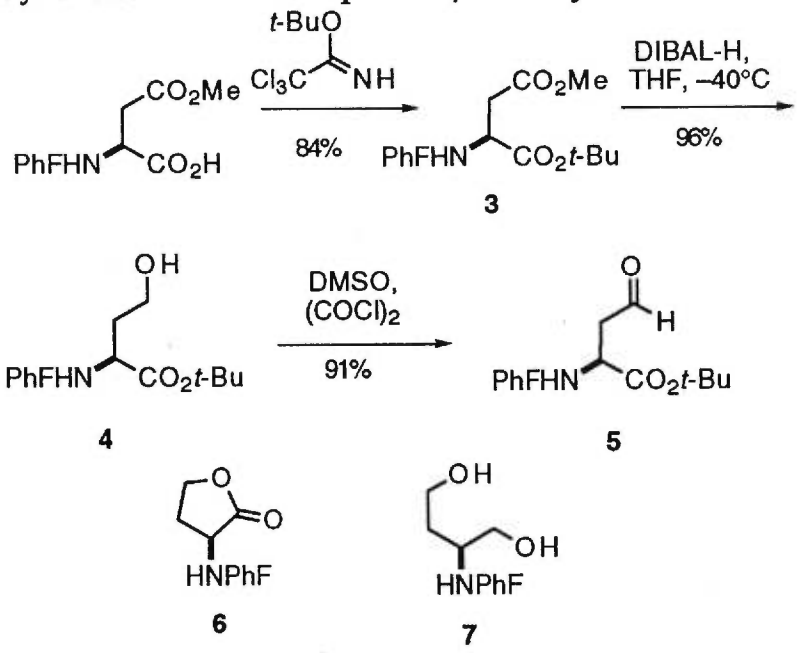
**2.11. Results and Discussion**

In principle, the Claisen condensations of the ω–carboxylates of aspartate, glutamate and α–aminoadipate followed by decarboxylation could provide six different α,ω–diaminodicarboxylates with chain lengths between seven and eleven carbons (Scheme 1). Subsequent reductive aminations and lactam cyclizations would then furnish nine different azabicyclo[X.Y.0]alkane amino acid heterocycles in which X varies from three to five and Y from two to four carbons. Symmetrical α,ω–diaminodicarboxylates provide a single heterocycle as demonstrated in the synthesis of indolizidin-2-one amino acid **1** from δ–oxo-α,ω–diaminoazelate. Unsymmetrical α,ω–diaminodicarboxylates may yield two possible heterocycles from reductive aminations between the ketone and one of two different amines. The three stereocenters at the ring-fusion and peptide back-bone carbons offer eight possible configurations per heterocycle. In sum, a library of 72 unique azabicyclo[X.Y.0]alkane amino acids may be generated by employing the L- and D-enantiomers of the three most common α–aminodicarboxylates, aspartate, glutamate and α–aminoadipate, in Scheme 1. Because each azabicycloalkane amino acid is expected to adopt a preferred set of dihedral angle geometries when incorporated into a peptide framework, this library of rigid dipeptide surrogates should mimic a comprehensive spectrum of peptide conformations.

Suitably protected α–aminodicarboxylates were initially required for employment in Claisen condensations. Diesters of *N*-(PhF)aspartate[6] and *N*-(PhF)glutamate[3,7] were obtained using literature methods. Enantiopure δ–methyl α–*tert*-butyl *N*-(PhF)-α–aminoadipate (**9**)[8,9] was synthesized by an improved method in 7 steps and 47% overall yield from aspartic acid (Schemes 2 and 3). β–Methyl *N*-(PhF)aspartate[6b] was esterified using *O*-*tert*-butyl trichloroacetimidate in dichloromethane which gave α–*tert*-butyl

ester **3** in 84% yield after chromatography.[10] Treatment of a solution of α–*tert*-butyl β–methyl *N*-(PhF)aspartate (**3**) in THF with DIBAL-H at –40 °C caused selective reduction of the β–methyl ester and provided homoserine **4** in excellent yield (96%, Scheme 2).[11]

**Scheme 2. Synthesis of *N*-(PhF)Aspartate β-Aldehyde 5**

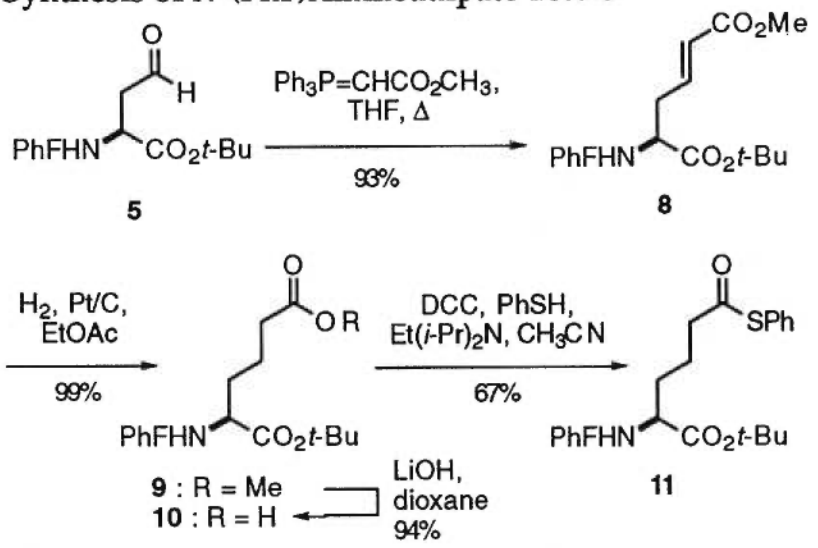



In agreement with a previous report demonstrating that *N*-(PhF)amine protection inhibits γ-lactone formation,[12] the DIBAL-H reduction was found to be sensitive to concentration. γ-Lactone **6** and 2-aminobutane-1,4-diol **7** were isolated in variable amounts when the reduction was conducted at higher concentrations. It is interesting to note that the corresponding reduction of *N*-trityl aspartate diesters has been reported to furnish *N*-(trityl)homoserine γ–lactone in good yield.[13] *N*-(PhF)Homoserine *tert*-butyl ester (**4**) showed no tendency to lactonize on standing nor during chromatography on silica gel. Subsequent oxidation of primary alcohol **4** using DMSO-oxalyl chloride in dichloromethane gave (2*S*)-*tert*-butyl 2-[*N*-(PhF)amino]-4-oxobutanoate (**5**) in 91% yield after chromatography on silica gel.[14] Aldehyde **5** was stable for several weeks under argon at –20°C, but samples of aldehyde dissolved in $CDCl_3$ decomposed within a few hours as judged by $^1H$ NMR spectroscopy.

Because decomposition of aldehyde **5** was presumed to be due to traces of acid in this solvent, exposure of **5** to chloroform was strictly avoided.

Wittig reaction of aldehyde **5** and methyl(triphenylphosphoranylidene)acetate in THF provided α,β–unsaturated ester **8** in 93% yield after chromatography (Scheme 3).[15,16] The formation of the *E*-isomer was confirmed by proton NMR which showed a vicinal coupling constant of 15.7 Hz between the vinyl protons. (2*S*)-δ–Methyl α–*tert*-butyl *N*-(PhF)aminoadipate (**9**) was then obtained in 99% yield from hydrogenation of olefin **8** using platinum-on-carbon as catalyst in EtOAc followed by removal of the catalyst by filtration through celite™.[17] Hydrogenolysis of the phenylfluorenylamine was not observed under these conditions. Selective hydrolysis of the methyl ester of **9** was readily accomplished with hydroxide ion and provided α–*tert*-butyl *N*-(PhF)-α–aminoadipate (**10**) in 94% yield. Acylation of thiophenol with **10** and DCC-DMAP in $CH_3CN$ gave (2*S*)-α–*tert*-butyl *N*-(PhF)amino adipate δ–thiophenyl ester **11** in 67 % yield.[18]

**Scheme 3. Synthesis of *N*-(PhF)Aminoadipate Esters**




Prior to the present investigation, we found that self condensations of *N*-(PhF)glutamate diesters **12** and **14** provided respectively β–ketoesters **15** and **16** in 82% and 75% yields accompanied by minor amounts of *N*-

(PhF)pyroglutamates **17** and **18** (Figure 2).[3,4] The self condensations of (2*S*)-α–*tert*-butyl β–methyl *N*-(PhF)aspartate (**3**), (2*S*)-dimethyl *N*-(PhF)aspartate (**19**) and (2*S*)-α–*tert*-butyl δ–methyl *N*-(PhF)aminoadipate (**9**) were thus examined using conditions previously optimized for the condensation of (2*S*)-α–*tert*-butyl γ–methyl *N*-(PhF)glutamate (**12**). Treatment of either aspartate diester **3** or **19** with $NaN(SiMe_3)_2$ in THF at –30°C returned only starting material. Exposure of α–aminoadipate **9** to the same conditions gave no Claisen condensation product; however, the Dieckmann cyclization product, cyclic β–keto ester **20** was isolated in less than 10% yield along with recovered starting material. Dieckmann cyclization of dimethyl *N*-(PhF)aminoadipate has previously been used in the synthesis of carbocyclic nucleosides.[8,17] Although α–*tert*-butyl ester **9** was employed, the Dieckmann reaction was still favored over the Claisen condensation.

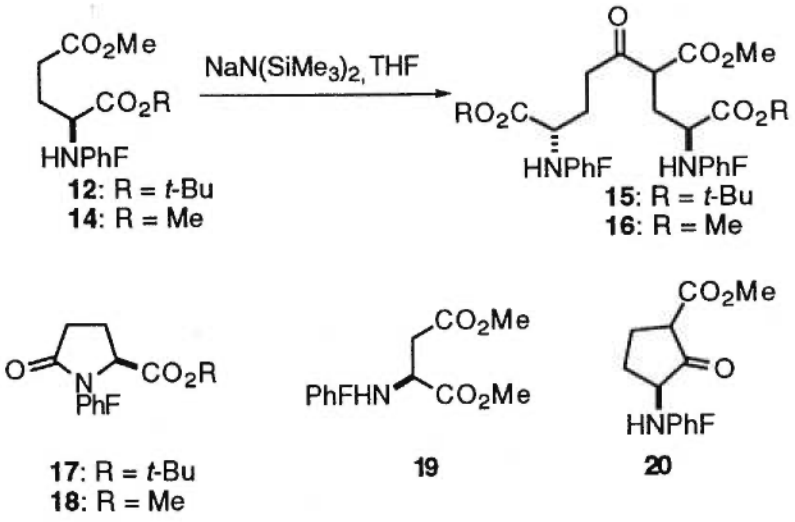



**Figure 2. Claisen Condensation Substrates and Products.**

Crossed Claisen condensations between glutamate and the two other aminodicarboxylates were also unsuccessful as discussed in the Supplementary Material. In summary, Claisen condensations failed to occur with aspartate diesters and returned starting material, glutamate diesters gave good yields of β–keto esters **15** and **16** in self-condensations, and α–aminoadipate diesters were not reactive in Claisen condensations and susceptible to Dieckmann condensation giving cyclic β–keto ester **20**. Attempts to use α–aminoadipate thioester **11** failed to provide the Claisen

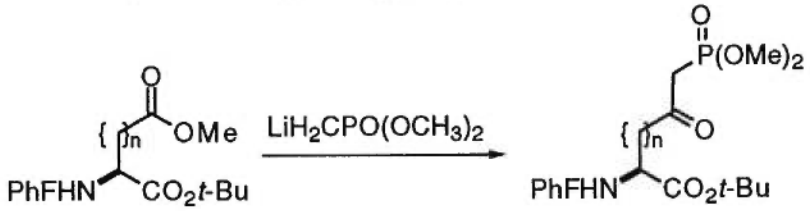
condensation products. Although continued investigation may eventually lead to conditions for Claisen condensations with aspartate and α–aminoadipate esters, we abandoned this approach in light of positive results from an alternative method for joining two amino dicarboxylates to make linear precursors for azabicycloalkane amino acid synthesis.

Successful olefination of aspartate β–aldehyde **5** in the synthesis of α–aminoadipate **9** inspired an investigation of the Horner-Wadsworth-Emmons olefination of **5** with aminodicarboxylate-derived β–keto phosphonates to synthesize α,ω–diaminodicarboxylates (Table 2). This olefination strategy overcomes the problems of regiocontrol inherent in the Claisen condensation approach, because different amino dicarboxylates are joined in a selective fashion.[19] All possible α,ω–diaminodicarboxylate configurations may thus be synthesized by combining D- and L-amino dicarboxylate starting materials. Furthermore, the geometry of the resulting olefin may later be used to direct the cyclization reactions of unsymmetrical α,ω–diaminodicarboxylate intermediates in order to form regioselectively azabicyclo[X.Y.0]alkane. Indeed, the resulting α,β–unsaturated ketone intermediates may also be used to introduce side-chains onto the heterocycle by both alkylations and conjugate additions.[5,20]

The prerequisite β–keto phosphonates were synthesized from the addition of the lithium anion of dimethyl methyl phosphonate to the ω–methyl ester of the *N*-(PhF)aminodicarboxylates **3**, **9** and **12** (Table 1).[21] Initial investigations were conducted with (2*S*)-α–*tert*-butyl γ–methyl *N*-(PhF)glutamate (**12**) to provide (2*S*)-α–*tert*-butyl 2-*N*-(PhF)amino-5-oxo-6-(dimethylphosphonyl)hexanoate (**22**). At first, **22** was obtained in low yield from reactions performed in THF and (2*S*)-*tert*-butyl *N*-(PhF)pyroglutamate (**17**) was isolated as a second product,[3] presumed to result from either direct deprotonation of the PhF amine and cyclization onto the methyl ester or by enolization of the glutamate ester, followed by decomposition to ketene and cyclization. Changes in the base favored the formation of pyroglutamate **17** (entries g and h). The yield of β–keto phosphonate **22** increased on lowering the concentration of dimethyl methyl phosphonate in THF. Switching solvents from THF to diethyl ether increased the

reaction yield and selectivity such that β–keto phosphonate **22** was isolated in 84% yield, along with 12% of pyroglutamate **17**. Treatment of (2*S*)-α–*tert*-butyl β–methyl *N*-(PhF)aspartate (**3**) under similar conditions in diethyl ether gave (2*S*)-α–*tert*-butyl 2-*N*-(PhF)amino-4-oxo-5-(dimethylphosphonyl)pentanoate (**21**) in 71% yield. Pentanoate **21** was obtained in the same yield when toluene was employed as solvent at slightly higher concentration. Exposure of (2*S*)-α–*tert*-butyl δ–methyl α–*N*-(PhF)aminoadipate (**9**) under similar conditions in diethyl ether gave (2*S*)-α–*tert*-butyl 2-*N*-(PhF)amino-6-oxo-7-(dimethylphosphonyl) heptanoate (**23**) in 40% yield contaminated with 6% of cyclic β–keto ester **20** as well as 54% of starting **9**. Alternatively, the use of toluene as solvent in the addition of dimethyl methyl phosphonate to aminoadipate **9** improved the yield of heptanoate **23** to 90% (Table 1).

**Table 1. Synthesis of β-Ketophosphonates 21-23**



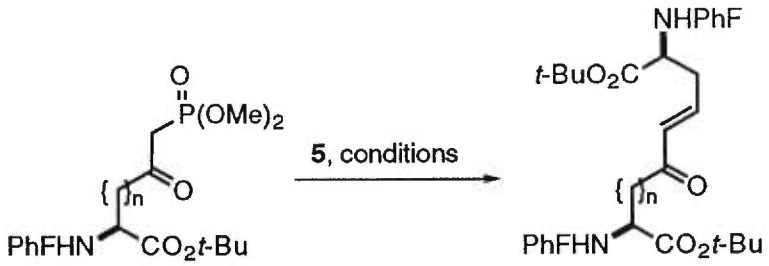

**3** : n = 1
**12** : n = 2
**9** : n = 3

**21** : n = 1
**22** : n = 2
**23** : n = 3

| entry | n | Base | Solvent | M[a] | % ketone | % rcvd diester | % **17** | % **20** |
|---|---|---|---|---|---|---|---|---|
| a | 1 | *n*-BuLi | $PhCH_3$ | 0.1 | 71 | - | - | - |
| b | 1 | *n*-BuLi | $Et_2O$ | 0.05 | 71 | 8 | - | - |
| c | 2 | *n*-BuLi | $Et_2O$ | 0.05 | 84 | 4 | 12 | - |
| d | 2 | *n*-BuLi | THF | 0.1 | 52 | 15 | 34 | - |
| e | 2 | *n*-BuLi | THF | 0.5 | 28 | 50 | 22 | - |
| f | 2 | *n*-BuLi | THF | 1.0 | 25 | 57 | 18 | - |
| g | 2 | $LiN(SiMe_3)_2$ | THF | 1.0 | - | <5 | >90 | - |
| h | 2 | *n*-BuLi•KO*t*-Bu | THF | 0.1 | - | - | >90 | - |
| i | 3 | *n*-BuLi | $PhCH_3$ | 0.1 | 90 | 5 | - | 5 |
| j | 3 | *n*-BuLi | $Et_2O$ | 0.05 | 40 | 54 | - | 6 |

[a]Concentration of $H_3CPO(OCH_3)_2$ (250 mol%).

**Table 2. Synthesis of Diaminodicarboxylates 24-26**




**21** : n = 1
**22** : n = 2
**23** : n = 3

**24** : n = 1
**25** : n = 2
**26** : n = 3

| entry | n | Base | Solvent | Temp. °C | Time | % olefin |
|---|---|---|---|---|---|---|
| a | 1 | $K_2CO_3$ | $CH_3CN$ | 25 | 72 h | 87 |
| b | 2 | $K_2CO_3$ | $CH_3CN$ | 10 | 72 h | 78 |
| c | 2 | $K_2CO_3$ | $CH_3CN$ | 25 | 48 h | 52 |
| d | 2 | $Cs_2CO_3$ | $CH_3CN$ | 10 | 36 h | 75 |
| e | 2 | $LiOH•H_2O$ | $Et_2O$ | 25 | 18 d | 73 |
| f | 2 | $Ba(OH)_2•8H_2O$ | 40:1 THF:$H_2O$ | 25 | 48 h | 45 |
| g | 2 | LiCl, DBU | $CH_3CN$ | 25 | 48 h | 33 |
| h | 3 | $K_2CO_3$ | $CH_3CN$ | 25 | 96 h | 78 |

With an efficient method for synthesizing β–keto phosphonates in hand, we explored next Horner-Wadsworth-Emmons olefinations with *N*-(PhF)aspartate β–aldehyde **5** (Table 2). (2*S*, 9*S*)-Di-*tert*-butyl 5-oxo-2,9-bis[*N*-(PhF)amino]dec-4-enedioate (**25**) was initially synthesized from the reaction of β–keto phosphonate **22** with aldehyde **5** at room temperature using a variety of bases and conditions.[22] We found that olefination proceeded slowly using anhydrous potassium carbonate in CH3CN at rt without any detectable decomposition of aldehyde **5** and gave unsaturated ketone **25** in up to 78% yield after chromatography. The reaction was accelerated by using cesium carbonate in CH3CN which gave olefin **25** in similar yield. (2*S*, 8*S*)-Di-*tert*-butyl 4-oxo-2,8-bis[*N*-(PhF)amino]non-4-enedioate (**24**) and (2*S*, 10*S*)-di-*tert*-butyl 6-oxo-2,10-bis[*N*-(PhF)amino]undec-4-enedioate (**26**) were then respectively obtained in 87% and 72% yields from the treatment of aspartate- and α–aminoadipate-derived β–keto phosphonates **22** and **23**

with *N*-(PhF)aspartate β–aldehyde **5** using potassium carbonate in $CH_3CN$ at rt. Olefins **24-26**, all were of the *E*-configuration as indicated by the large (15.9-16.0 Hz) vicinal coupling constant for the vinyl protons.

α,ω–Diaminodicarboxylates of nine to eleven carbon chain lengths were effectively synthesized using the olefination sequence. Their introduction into Scheme 1 offers potential to prepare five azabicyclo[X.Y.0]alkane amino acids having different ring sizes. Presently pursuing the production of this spectrum of rigid dipeptides, we have illustrated the utility of the olefination route by the first synthesis of indolizidin-9-one amino acid **2**.

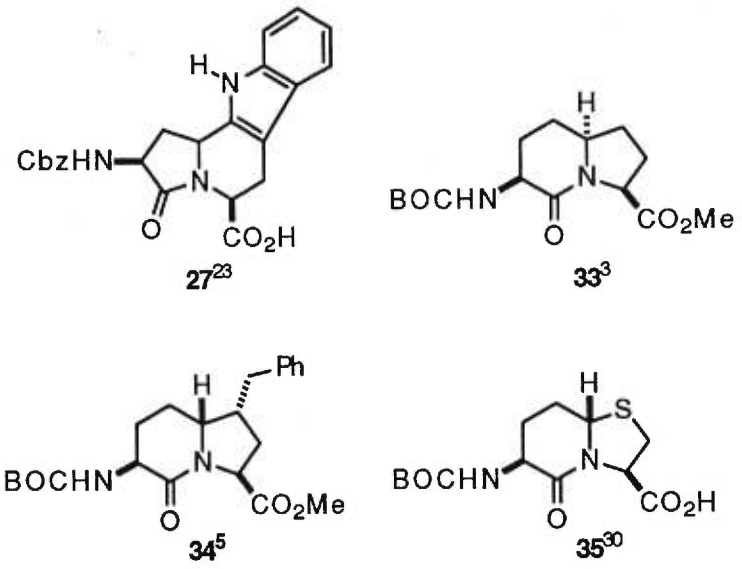
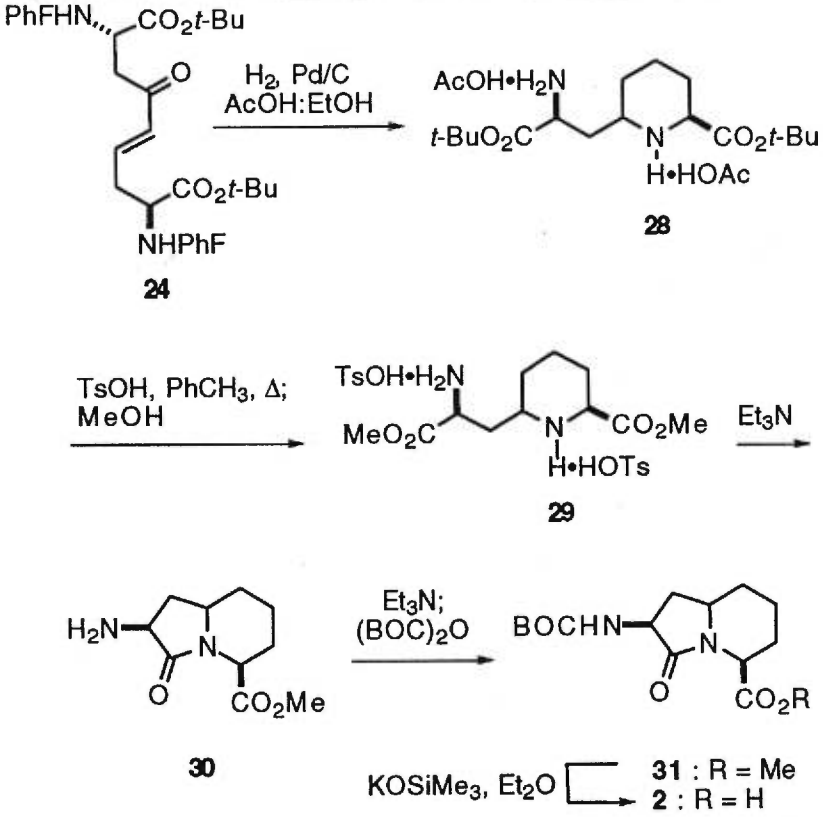



**Figure 3. Related Indolizidinone Amino Carboxylates.**

Interest in azabicyclo[X.Y.0]alkane amino acids like **2** has grown since the synthesis of the related indole-fused analogue **27** via a sequence featuring a Pictet-Spengler reaction between a protected aspartate β–aldehyde and tryptophan and subsequent lactam cyclization (Figure 3).[23] In analogs of gramicidin S, indole-fused indolizidinone **27** has recently been shown to serve as a type II' β–turn mimic contingent upon ring-fusion stereochemistry which influenced both the conformation and the bioactivity of the peptide antibiotic.[24] We selected to synthesize

indolizidin-9-one amino acid **2** as our first target because of its structural similarities to indolizidin-2-one amino acid **1** and because, to the best of our knowledge, **2** had never been synthesized.[2] (2*S*, 8*S*)-Di-*tert*-butyl 4-oxo-2,8-bis[*N*-(PhF)amino]non-4-enedioate (**24**) may be converted to both indolizidin-9-one amino acid **2** as well as an azabicyclo[5.2.0]alkane amino acid, subject to which amine reacts with the ketone at the azelate 4-position.

**Scheme 4. Synthesis of Indolizidin-9-one *N*-(BOC)Amino Esters**

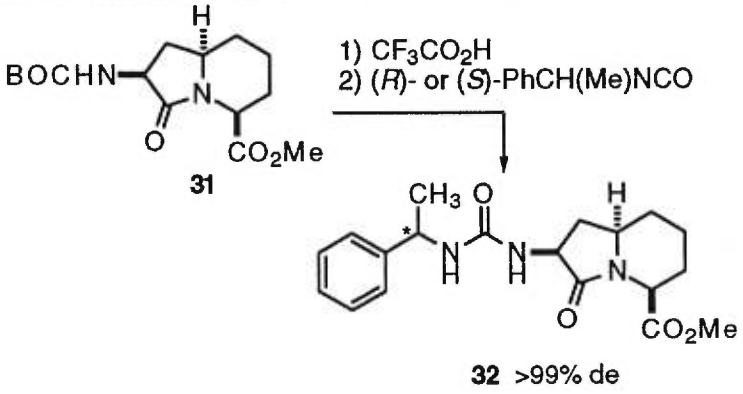



Presuming that reductive amination would favor formation of the piperidine instead of the azetidine ring system, we subjected γ–oxo-α,ω–diaminoazelate **24** to the same reductive amination conditions used in the synthesis of **1**.[3] Hydrogenation of azelate **24** with palladium-on-carbon as catalyst in 9:1 EtOH:AcOH proceeded by reduction of the α,β–unsaturated ketone, cleavage of the phenylfluorenyl groups, intramolecular imine formation, protonation, and hydrogen addition to the iminium ion intermediate (Scheme 4). 6-Alkylpipecolate **28** was formed as a 9:1 mixture of diastereomers, and was subsequently transformed into

azabicyclo[4.3.0]alkane amino ester **30** by a one-pot three-step reaction sequence featuring *tert*-butyl ester solvolysis with *p*-toluenesulfonic acid, esterification in methanol, and lactam cyclization on addition of triethylamine. Lactam formation was monitored by proton NMR spectroscopy by measuring the disappearance of the methyl ester singlets at 3.80 and 3.88 ppm for **29** and the appearance of a new methyl singlet at 3.72 ppm for **30** in $CD_3OD$. *N*-Protection of **30** with di-*tert*-butyldicarbonate and triethylamine in dichloromethane provided *N*-(BOC)amino indolizidin-9-one ester **31** in 85% overall yield from ketone **24**. Hydrolysis of methyl ester **31** using $KOSiMe_3$ in ether gave *N*-(BOC)amino indolizidin-9-one acid **2** in 96% yield.[25]

The enantiomeric purity of (2*S*,6*R*,8*S*)-**31**, produced as the major diastereomer from the reductive amination / lactam cyclization sequence on **24**, was determined after conversion to (1'*R*)- and (1'*S*)-*N*-α-methylbenzylureas **32** (Scheme 5). Trifluoroacetic acid in $CH_2Cl_2$ removed quantitatively the *N*-BOC protecting group and the TFA salt was acylated with either (*R*)- or (*S*)-α-methylbenzyl isocyanate in THF with triethylamine.[3] Measurement of the diastereomeric methyl ester singlets at 3.56 and 3.52 ppm in $C_6D_6$ by 600 MHz $^1H$ NMR spectroscopy demonstrated **32** to be of >99% diastereomeric excess. Hence diaminodicarboxylates **24-26**, *N*-(BOC)amino indolizidin-9-one ester **31** and acid **2**, all are presumed to be of >99% enantiomeric purity.

**Scheme 5. Enantiomeric Purity of Indolizidinone Ester 31**



The stereochemistry of the ring fusion carbon of *N*-(BOC)amino indolizidin-9-one acid (2*S*, 6*R*, 8*S*)-**2** was initially assigned based on analogy with *N*-(BOC)amino indolizidin-2-one acid (3*S*, 6*S*, 9*S*)-**1**.[3] Since hydrogenation of α–*tert*-butyl δ–oxo-α–*N*-(PhF)amino esters produces 5-alkylprolines with high selectivity in favor of the *cis*-diastereomer,[26] we presumed that reductive amination of amino ketone **24** proceeded to form *cis*-6-alkylpipecolate (2*S*, 6*R*, 2'*S*)-**28**. Crystallization of (2*S*, 6*R*, 8*S*)-methyl 9-oxo-8-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-2-carboxylate ((2*S*, 6*R*, 8*S*)-**31**) from EtOAc and X-ray crystallographic analysis confirmed our hypothesis (Figure 4).[27]

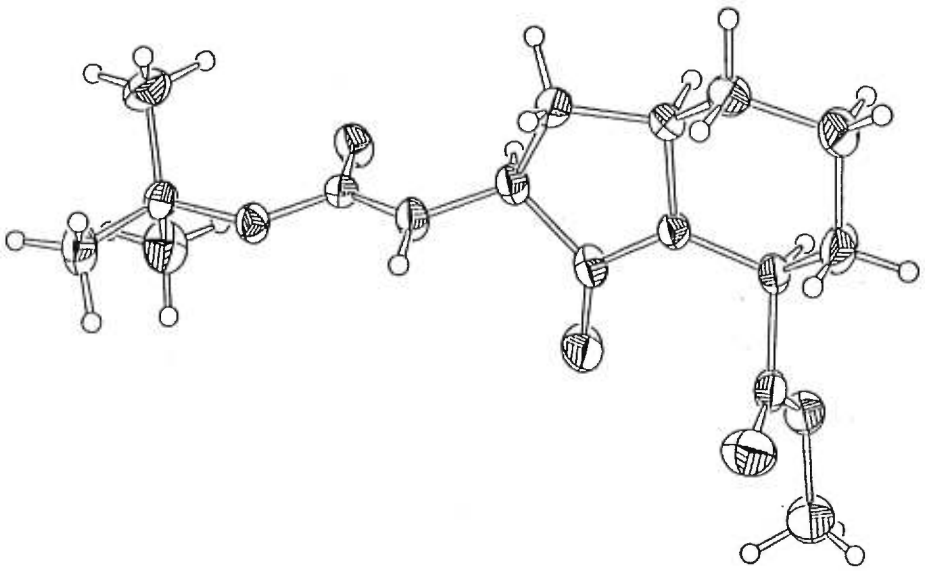


**Figure 4. ORTEP view of *N*-(BOC)Amino Indolizidin-9-one Methyl Ester (2*S*, 6*R*, 8*S*)-31. Ellipsoids drawn at 40% probability level. Hydrogens represented by spheres of arbitrary size.[27]**

In the crystal structure of *N*-(BOC)amino indolizidin-9-one ester **31**, the dihedral angles of the back-bone atoms constrained inside the heterocycle resemble the values of the central residues in an ideal type II' β–turn. For comparison, we have listed in Table 1 the values for an ideal type II' β–turn[28] and an ideal inverse γ–turn conformation[29] with the values for **31** and those observed in the crystal structures of the corresponding methyl esters of indolizidinone *N*-(BOC)amino acid, (3*S*, 6*S*, 9*S*)-**33**,[3] and (3*S*, 6*R*, 7*S*, 9*S*)-3-*N*-(BOC)amino 7-benzylindolizidin-2-one 9-

**Table 3. Comparison of the Dihedral Angles from Azabicycloalkane X-ray Data and Ideal Peptide Turns**

| entry | $\Psi$, deg | $\phi$, deg |
|---|---|---|
| (2*S*, 6*R*, 8*S*)-8-*N*-(BOC)Amino Indolizidin-9-one 2-Carboxylate **31**[27] | −141° | −34° |
| (3*S*, 6*R*, 7*S*, 9*S*)-3-*N*-(BOC)Amino 7-Benzylindolizidin-2-one 9-Carboxylate **34**[5] | −147° | −56° |
| (3*S*, 6*R*, 9*S*)-3-*N*-(BOC)Amino 7-Thiaindolizidin-2-one 9-Carboxylate **35**[30] | −161° | −69° |
| (3*S*, 6*S*, 9*S*)-3-*N*-(BOC)Amino Indolizidin-2-one 9-Carboxylate **33**[3] | −176° | −78° |
| Type II' β–turn $i + 1$ and $i + 2$ Residues[28] | −120° | −80° |
| Inverse γ–turn $i + 2$ Residue[29] | | −80° |

carboxylate **34**,[5] as well a (3*S*, 6*R*, 9*S*)-7-thiaindolizidin-2-one β–turn dipeptide analogue **35** (Figure 3).[30] Aside from crystal packing forces, this body of X-ray data begins to suggest that azabicycloalkane ring-size, stereochemistry and alkyl substituents, all may significantly influence peptide back-bone geometry.

**2.12. Conclusion**

We have developed a new approach for synthesizing α,ω–diaminodicarboxylates of various chain lengths for the construction of azabicyclo[X.Y.0]alkane amino acids of different ring sizes. α,ω–Diaminodicarboxylates of nine to eleven carbon chain lengths were effectively synthesized by the Horner-Wadsworth-Emmons olefination of α–*tert*-butyl *N*-(PhF)aspartate β–aldehyde (**5**) with β–keto phosphonates derived from aspartate, glutamate and α–aminoadipate. Our approach has been used in the first synthesis of azabicyclo[4.3.0]alkane amino acid **2**. Enantiopure indolizidin-9-one *N*-(BOC)amino acid **2** was synthesized in 9 steps and >25% overall yield from inexpensive aspartic acid as chiral educt via a route featuring reductive amination and lactam cyclization of γ–oxo-α,ω–diaminoazelate **24**. Because α,ω–diaminodicarboxylates **25** and **26** may be similarly converted to fused heterocycles having larger ring sizes and because alkylations and conjugate additions to **24-26** may be used to attach side-chains at different points on the heterocycle, this olefination strategy has greatly expanded our methodology for synthesizing azabicyclo[X.Y.0]alkane amino acids for the exploration of conformation-activity relationships of biologically active peptides.

**2.13. Experimental Section**

**General:** Unless otherwise noted all reactions were run under nitrogen atmosphere and distilled solvents were transferred by syringe. Tetrahydrofuran (THF) and ether were distilled from sodium / benzophenone immediately before use; toluene was distilled from sodium; $CH_2Cl_2$ and $CH_3CN$ were distilled from $CaH_2$; $CHCl_3$ from $P_2O_5$; triethylamine ($Et_3N$) was distilled from BaO; $CH_3CN$ was stored over 4Å sieves. Potassium carbonate was dried at 120°C for 18 h prior to use. Final reaction mixture solutions were dried over $Na_2SO_4$. Melting points are

uncorrected. Mass spectral data, HRMS and MS (EI and FAB), were obtained by the Université de Montréal Mass Spec. facility. Unless otherwise noted, $^{1}H$ NMR (300/400 MHz) and $^{13}C$ NMR (75/100 MHz) spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm (δ units) downfield of internal tetramethylsilane ($(CH_3)_4Si$), $CHCl_3$, and $C_6H_6$; coupling constants are reported in hertz. Chemical shifts of the vinyl carbons in **24-26** and of PhF aromatic carbons are not reported in the $^{13}C$ NMR spectra. Analytical thin-layer chromatography (TLC) was performed by using aluminum-backed silica plates coated with a 0.2 mm thickness of silica gel 60 $F_{254}$ (Merck). Chromatography was performed using Kieselgel 60 (230-400 mesh).

**(2*S*)-*tert*-Butyl β–Methyl *N*-(PhF)Aspartate (3)** To a stirred suspension of β–methyl *N*-(PhF)aspartate (22g, 56.4 mmol, prepared using the protocol in ref. 6b) in $CH_2Cl_2$ (120 mL) was added *O*-*tert*-butyl trichloroacetimidate (112 mL, 200 mol%, 1M in cyclohexane, prepared using the protocol in ref. 10), the mixture was stirred for 3 days, filtered, evaporated and the residue was resubmitted to the same conditions as above for 2 days. Filtration and evaporation, followed by chromatography (5-10% EtOAc in hexanes) gave diester **3** (21 g, 84%) as a clear crystalline solid: mp = 75-76°C, lit. 75-76°C;[6b] TLC $R_f$ = 0.40 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –233.7 (*c* 1.0, $CHCl_3$); $^{1}H$ NMR δ 7.71-7.67 (m, 2 H), 7.41-7.18 (m, 11 H), 3.67 (s, 3 H), 2.90 (t, 1 H, *J* = 5.7), 2.49 (dd, 1 H, *J* = 14.8, 5.8), 2.32 (dd, 1 H, *J* = 14.8, 5.6), 1.25 (s, 9 H); $^{13}C$ NMR δ 172.95, 171.17, 81.23, 72.93, 53.47, 51.49, 40.44, 27.71; HRMS calcd. for $C_{28}H_{30}NO_4$ [M+1]: 444.2175, found: 444.2157. Anal. Calcd for $C_{28}H_{29}NO_4$: C, 75.82; H, 6.59; N, 3.16. Found: C, 75.55; H, 6.79; N, 3.22.

**(2*S*)-*tert*-Butyl 2-[*N*-(PhF)amino]-4-hydroxybutanoate (4)** To a stirred solution of diester **3** (10.0 g, 22.6 mmol) in THF (760 mL) at –40 °C was added DIBAL-H (67.6 mL, 67.6 mmol, 300 mol%, 1.0 M in hexanes). The solution was stirred at –40 °C for 30 min, quenched with acetone (3.3 mL), diluted with MeOH (20 mL), let warm to rt and evaporated. The residue was dissolved in ether (200 mL), treated with $NaH_2PO_4$ (200 mL, 1M) and sodium-potassium tartrate (10 g), stirred vigorously for 30 min, and filtered. The biphasic filtrate was saturated with solid NaCl and the aqueous layer

was extracted with ether until TLC showed no UV active material. The combined organic layers were washed with brine, dried, filtered and evaporated. Chromatography (10-20% EtOAc in hexanes) afforded alcohol **4** as a white crystalline solid (9.0 g, 96% yield): mp = 121-122°C; TLC $R_f$ = 0.12 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –308.0 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 7.72-7.21 (m, 13 H), 3.71 (m 1 H), 3.59 (m, 1 H), 2.70 (dd, 1 H, *J* = 9.0, 4.3), 1.62 (m, 2 H), 1.17 (s, 9 H); $^{13}C$ NMR δ 174.25, 81.18, 73.16, 61.33, 56.07, 35.80, 27.81; HRMS calcd. for $C_{27}H_{30}NO_3$ [M+1]: 416.2226, found: 416.2240; Anal. Calcd for $C_{27}H_{29}NO_3$: C, 78.04; H, 7.03; N, 3.37. Found: C, 78.05; H, 7.22; N, 3.43.

Homoserine lactone **6** and diol **7** were encountered when the reduction was conducted at higher concentrations and with alternative reducing agents.

**(2*S*)-*N*-(PhF)Homoserine γ–lactone (6)** was obtained as a white solid: mp =195°C; TLC $R_f$ = 0.27 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –299.0 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 7.75-7.66 (m, 2 H), 7.43-7.19 (m, 11 H), 4.11 (t, 1 H, *J* = 9.0), 3.72 (m, 1 H), 2.94 (dd, 1 H, *J* = 11.5, 8.1), 1.80 (m, 1 H), 1.29 (m, 1 H); $^{13}C$ NMR δ 178.0, 72.93, 65.67, 52.2, 32.95; HRMS calcd. for $C_{23}H_{20}NO_2$ [M+1]: 342.1494, found: 342.1482; Anal. Calcd for $C_{23}H_{19}NO_2$: C, 80.92; H, 5.61; N, 4.10. Found: C, 80.59; H, 5.58; N, 3.87.

**(2*S*)-2-[*N*-(PhF)amino]-1,4-butanediol (7)** was obtained as an oil: TLC $R_f$ = 0.21 (4 : 1 EtOAc : hexanes); $[\alpha]_D^{20}$ –42.0 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 7.71-7.67 (m, 2 H), 7.40-7.18 (m, 11 H), 3.62 (dd, 1 H, *J* = 5.7, 5.1), 3.24 (br m, 2 H), 3.03 (dd, 1 H, *J* =11.0, 2.9), 2.83 (dd, 1 H, *J* = 11.0, 4.4), 2.36 (br m, 1 H), 1.59 (m, 1 H), 1.37 (m, 1 H); $^{13}C$ NMR δ 72.64, 64.14, 60.49, 53.20, 35.72; HRMS calcd. for $C_{23}H_{24}NO_2$ [M+1]: 346.1807, found: 346.1813.

**(2*S*)-*tert*-Butyl 2-[*N*-(PhF)amino]-4-oxobutanoate (5)** A solution of oxalyl chloride (3.95 mL, 45 mmol, 250 mol%) in $CH_2Cl_2$ (97 mL) at –60 °C was treated with DMSO (4.47 mL, 63 mmol, 350 mol%) in $CH_2Cl_2$ (13.3 mL), stirred for 30 min, and treated dropwise with a solution of alcohol **4** (7.48 g, 18 mmol) in $CH_2Cl_2$ (25 mL). The clear solution was stirred for 4 h, treated

with Et(*i*-Pr)$_2$N (18.8 mL, 108 mmol, 600 mol%), and warmed to rt over 60 min. The reaction mixture was added to aqueous $NaH_2PO_4$ (100 mL, 1M) and the layers were separated. The aqueous layer was saturated with solid NaCl and extracted repeatedly with EtOAc (4 × 50 mL). The combined organic layers were then washed with $H_2O$ (1 × 50 mL) and brine (60 mL). Aldehyde **5** was obtained as a colorless solid after chromatography using a gradient of 0-5% EtOAc in hexanes: 6.8 g, 91% yield; mp = 91-93°C; TLC $R_f$ = 0.41 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –263.9 (*c* 1.0, $CHCl_3$); $^1$H NMR δ 9.52 (dd, 1 H, *J* = 2.1, 2.8), 7.74-7.69 (m, 2 H), 7.41-7.16 (m, 11 H), 3.04 (dd, 1 H, *J* = 5.3, 7.6), 2.40 (m, 2 H), 1.23 (s, 9 H); $^{13}$C NMR δ 173.17, 158.10, 81.63, 72.94, 52.10, 48.34, 27.73; HRMS calcd. for $C_{30}H_{36}NO_5S$ [M+109 (thioglycerol)]: 522.2315, found: 522.2269. Anal. Calcd for $C_{27}H_{27}NO_3$: C, 78.42; H, 6.58; N, 3.39. Found: C, 78.67; H, 6.58; N, 3.42.

**(2*S*)-α–*tert*-Butyl δ–Methyl 2-[*N*-(PhF)Amino]-$\Delta^4$-dehydroadipate (8)** A solution of aldehyde **5** (2.0 g, 4.8 mmol) and methyl(triphenylphosphoranylidene) acetate[15] (2.4 g, 7.25 mmol, 150 mol%) in THF (48 mL) was stirred at a reflux for 24 h. Evaporation of the volatiles and chromatography (5-20% $Et_2O$ in hexanes) of the residue furnished diester **8** (2.11 g, 93% yield) as a thick clear oil: TLC $R_f$ = 0.43 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –116.1 (*c* 1.0, $CHCl_3$); $^1$H NMR δ 7.71-7.67 (m, 2 H), 7.44-7.19 (m, 11 H), 6.91 (m, 1 H), 5.79 (d, 1 H, *J* = 15.7), 3.76 (s, 3 H), 2.67 (dd, 1 H, *J* = 6.4, 6.1), 2.19 (m, 2 H), 1.20 (s, 9 H). $^{13}$C NMR δ 173.82, 166.58, 145.64, 122.90, 81.16, 72.98, 55.49, 51.39, 38.52, 27.82. HRMS calcd. for $C_{30}H_{32}NO_4$ [M+1]: 470.2331, found: 470.2314. Anal. Calcd for $C_{30}H_{31}NO_4$: C, 76.73; H, 6.65; N, 2.98. Found: C, 77.16; H, 6.80; N, 2.81.

**(2*S*)-α–*tert*-Butyl δ–Methyl 2-[*N*-(PhF)Amino]adipate (9)** A solution of **8** (1.96 g, 4.18 mmol) and Pt/C (196 mg, 5 wt %) in ethyl acetate (85 mL) was stirred under 2.75 atm of $H_2$ for 16 h. The mixture was filtered through a plug of celite™, which was washed thoroughly with EtOAc. Evaporation of the volatiles gave α–aminoadipate **9** (1.95 g, 99% yield) as a thick clear oil: TLC $R_f$ = 0.43 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –179.0 (*c* 4.4, $CHCl_3$); lit.[8] $[\alpha]_D^{25}$ –180 (*c* 4.4, $CHCl_3$); $^1$H NMR δ 7.67-7.63 (m, 2 H), 7.43-7.13 (m, 11 H),

3.62 (s, 3 H), 2.50 (m, 1 H), 2.15-2.11 (m, 2 H), 1.71-1.63 (m, 2 H), 1.44-1.41 (m, 1 H), 1.39-1.21 (m, 1 H), 1.17 (s, 9 H); $^{13}$C NMR δ 175.08, 173.73, 80.42, 72.90, 55.33, 51.29, 34.90, 33.58, 27.78, 20.77; HRMS calcd. for $C_{30}H_{34}NO_4$ [M+1] 472.2488, found: 472.2505; Anal. Calcd for $C_{30}H_{33}NO_4$ : C, 76.41; H, 7.05; N, 2.97. Found: C, 76.46; H, 7.15; N, 3.02.

**(2*S*)-α–*tert*-Butyl 2-[*N*-(PhF)Amino]adipate (10)** A stirred solution of diester **9** (504.8 mg, 1.07 mmol) in dioxane (10 mL) was treated with aqueous LiOH (2.70 mL, 500 mol%, 2 M), heated at a reflux for 2 h, cooled to rt, and acidified with concentrated $H_3PO_4$ to pH 2. The aqueous layer was saturated with NaCl, and extracted with EtOAc until TLC of the organic layer showed no UV active material. The combined organic layers were washed with brine (5 mL), dried, and evaporated to an oil which was chromatographed using a gradient of 0-10% *i*-PrOH in $CHCl_3$ as eluant to afford **10** (459 mg, 94% yield) as an oil: TLC $R_f$ = 0.42 (1:9 *i*-PrOH:$CHCl_3$); $[\alpha]_D^{20}$ –191.0 (*c* 1.0, $CHCl_3$); $^1$H NMR δ 7.72-7.65 (m, 2 H), 7.53-7.13 (m, 11 H), 2.55 (m, 1 H), 2.21 (m, 2 H), 1.73 (m, 2 H), 1.48 (m, 2 H), 1.29 (s, 0.5 H), 1.22 (s, 8 H), 1.16 (s, 0.5 H). $^{13}$C NMR δ 179.54, 175.03, 80.67, 73.01, 55.39, 34.65, 33.62, 27.83, 20.53. HRMS calcd. for $C_{29}H_{32}N04$[M+1]: 458.2331, found: 458.2342.

**(2*S*)-α–*tert*-Butyl δ–*S*-Phenyl 2-[*N*-(PhF)Amino]adipate (11)** To a solution of adipate **10** (100 mg, 0.22 mmol) and TBTU (105 mg, 0.33 mmol, 150 mol%) in $CH_3CN$ (2.2 mL) was added thiophenol (45 mL, 0.44 mmol, 200 mol%). The mixture was cooled to 0°C and $Et(iPr)_2N$ (76 mL, 200 mol%) was added. A slow reaction followed; however, addition of DMAP (10 mol%) and DCC (150 mol%) accelerated the reaction as witnessed by TLC. After 24 h, the reaction mixture was filtered and evaporated to a residue that was chromatographed (5% EtOAc in hexanes) to give thioester **11** (80.6 mg, 67%) as a thick clear oil: $^1$H NMR δ 7.71-7.68 (m, 2 H), 7.46-7.34 (m, 10 H), 7.27-7.20 (m, 6 H), 2.55-2.46 (m, 3 H), 1.85-1.74 (m, 2 H), 1.54-1.36 (m, 2 H), 1.21 (s, 9 H); $^{13}$C NMR δ 196.98, 175.05, 80.62, 72.98, 55.41, 43.30, 34.77, 27.87, 21.5; HRMS calcd. for $C_{35}H_{36}NO_3S$ [M+1]: 550.2416, found: 550.2398.

**(2S)-α-*tert*-Butyl 2-*N*-(PhF)Amino-4-oxo-5-(dimethylphosphonyl) pentanoate (21)** A –78°C solution of dimethyl methyl phosphonate (3.1 mL, 28,2 mmol, 250 mol%) in toluene (276 mL) was treated with *n*-butyllithium (11.4 mL, 28.2 mmol, 250 mol%, 2.5 M in hexanes), stirred for 20 min at –78°C and transferred by cannula dropwise over 30 min into a –78°C solution of α–*tert*-butyl β–methyl *N*-(PhF)aspartate (**3**, 5.0 g, 11.29 mmol) in toluene (112 mL). The solution was stirred for 1 h, warmed to rt, quenched with aqueous $NaH_2PO_4$ (40 mL, 1 M), and diluted with EtOAc (50 mL). The aqueous layer was saturated with solid NaCl and extracted with EtOAc until TLC of the organic layer showed no UV-active material. The combined organic layers were washed with brine, dried, and evaporated to a residue that was chromatographed using 10-100% EtOAc in hexanes as eluant to give **21** as a colorless solid (4.28 g, 71%): mp 113-114 °C; TLC $R_f$ = 0.17 (4 : 1 EtOAc : hexanes); $[\alpha]_D^{20}$ –136.6 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 7.69-7.66 (m, 2 H), 7.37-7.16 (m, 11 H), 3.78-3.74 (m, 6 H), 3.03 (s, 1 H), 2.97 (s, 1 H), 2.85 (dd, 1 H, *J* = 5.3, 5.5), 2.72 (dd, 1 H, *J* = 5.9, 16.1), 2.59 (dd, 1 H, *J* = 4.9, 16.0), 1.23 (s, 9 H) ; $^{13}C$ NMR (a mixture of keto-enol tautomers) δ 199.17, 199.11, 172.77, 81.39, 73.00, 53.09, 52.95, 52.89, 48.92, 42.25, 40.98, 27.67; HRMS calcd. for $C_{30}H_{35}NO_6P$ [M+1]: 536.2202, found: 536.2181. Anal. Calcd for $C_{30}H_{34}NO_6P$: C, 67.28; H, 6.40; N, 2.62. Found: C, 67.11; H, 6.70; N, 2.61.

**(2*S*)-α-*tert*-Butyl 2-*N*-(PhF)Amino-5-oxo-6-(dimethylphosphonyl) hexanoate (22).** A –78°C solution of dimethyl methyl phosphonate (2.97 mL, 27.2 mmol, 250 mol%) in $Et_2O$ (500 mL) was treated with *n*-butyllithium (11.0 mL, 27.2 mmol, 250 mol%, 2.5 M in hexanes) stirred for 20 min at –78°C, and transferred by cannula dropwise over 60 min into a –78°C solution of α–*tert*-butyl γ–methyl *N*-(PhF)glutamate **12** (4.98 g, 1.10 mmol, prepared according to ref. 3) in $Et_2O$ (50 mL). The solution was stirred for 2 h, quenched with aqueous $NaH_2PO_4$ (20 mL, 1 M), and diluted with EtOAc (100 mL). The aqueous layer was saturated with solid NaCl and extracted with EtOAc until TLC of the organic layer showed no UV-active material. The combined organic layers were washed with brine, dried, and evaporated to a residue that was chromatographed using a gradient of 5-100% EtOAc in hexanes as eluant. First to elute was glutamate **12** (188 mg, 4%), followed by α–*tert*-butyl *N*-(PhF)pyroglutamate (**17**,[3] 600 mg, 12-13%).

Last to elute was β–ketophosphonate **22** (5.04 g, 84%, oil): TLC $R_f$ = 0.19 (4 : 1 EtOAc : hexanes); $[\alpha]_D^{20}$ –168.7 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 7.68-7.65 (m, 2 H), 7.39-7.17 (m, 11 H), 3.79 (d, 3 H, *J* = 11.2), 3.78 (d, 3 H, *J* = 11.2), 3.08 (m, 3 H), 2.65 (m, 2 H), 2.50 (dd, 1 H, *J* = 5.3, 5.4), 1.66 (m, 2 H), 1.18 (s, 9 H); $^{13}C$ NMR (a mixture of keto-enol tautomers) δ 201.29, 201.23, 174.74, 80.81, 72.87, 54.88, 52.93, 52.87, 41.88, 40.60, 40.31, 40.30, 28.98, 27.77; HRMS calcd. for $C_{31}H_{37}NO_6P$ [M+1]: 550.2358, found: 550.2369; Anal. Calcd for $C_{31}H_{36}NO_6P$: C, 67.75; H, 6.60; N, 2.55. Found: C, 67.75; H, 6.74; N, 2.57.

**(2*S*)-α-*tert*-Butyl 2-*N*-(PhF)Amino-6-oxo-7-(dimethylphosphonyl) heptanoate (23)** A –78 °C solution of dimethyl methyl phosphonate (244 mL, 2.23 mmol, 250 mol%) in toluene (23 mL) was treated with *n*-butyllithium (898 mL, 2.23 mmol, 250 mol%, 2.5 M in hexanes) stirred for 20 min at –78°C, and transferred by cannula dropwise over 60 min into a –78 °C solution of α–*tert*-butyl δ–methyl *N*-(PhF)aminoadipate (**9**, 423 mg, 0.89 mmol) in toluene (8.9 mL). The solution was stirred for 1 h, let warm to rt (ca 1 h), quenched with aqueous $NaH_2PO_4$ (5 mL, 1 M), and diluted with EtOAc (20 mL). The aqueous layer was saturated with solid NaCl and extracted with EtOAc until TLC of the organic layer showed no UV-active material. The combined organic layers were washed with 10 mL of brine, dried, and evaporated to a residue that was chromatographed using a gradient of 10-100% EtOAc in hexanes as eluant. First to elute was aminoadipate **9** (21 mg, 5%), followed by β–keto ester **20** (20 mg, 5%). Last to elute was β–keto phosphonate **23** (427 mg, 90%), an oil: TLC $R_f$ = 0.16 (4 : 1 EtOAc : hexanes); $[\alpha]_D^{20}$ –144.1 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 7.69-7.65 (m, 2 H), 7.43-7.18 (m, 11 H), 3.77 (d, 3 H, *J* = 11.2), 3.75 (d, 3 H, *J* = 11.2), 3.05 (s, 1 H), 2.97 (s, 1 H), 2.49-2.34 (m, 3 H), 1.67-1.60 (m, 2 H), 1.44-1.26 (m, 2 H), 1.18 (s, 9 H); $^{13}C$ NMR (a mixture of keto-enol tautomers) δ 201.31, 201.25, 175.07, 80.54, 72.93, 55.37, 52.91, 52.85, 43.53, 41.74, 40.46, 34.52, 27.77, 19.19; HRMS calcd. for $C_{32}H_{39}NO_6P$ [M+1]: 564.2515, found: 564.2521. Anal. Calcd for $C_{32}H_{38}NO_6P$: C, 68.19; H, 6.80; N, 2.49. Found: C, 67.77; H, 7.17; N, 2.61.

**(2*S*, 8*S*)-Di-*tert*-butyl 4-Oxo-2,8-bis-[*N*-(PhF)amino]non-4-enedioate (24).** To a stirred solution of β–keto phosphonate **21** (1.20 g, 2.24 mmol) and aldehyde **5** (925 mg, 2.24 mmol, 100 mol%) in $CH_3CN$ (16 mL) was added

$K_2CO_3$ (326 mg, 2.35 mmol, 105 mol%). The mixture was stirred at rt for 72 h and evaporated to a residue that was suspended in toluene and added to a column of silica gel. Chromatography using a gradient of 0-10% EtOAc in hexanes and evaporation of the collected fractions gave **24** (1.61 g, 87%) as a white foam: TLC $R_f$ = 0.34 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –160.1 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 7.68-7.61 (m, 4 H), 7.41-7.13 (m, 22 H), 6.54-6.50 (m, 1 H), 5.90 (d, 1 H, *J* = 16), 3.29 (br s, 1 H), 3.19 (br s, 1 H), 2.93 (t, 1 H, *J* = 5.4), 2.64 (m, 2 H), 2.54 (m, 1 H), 2.25 (m, 2 H), 1.21 (s, 9 H), 1.17 (s, 9 H); $^{13}C$ NMR δ 197.05, 173.76, 173.31, 81.09, 80.95, 72.97, 72.93, 55.46, 53.36, 45.05, 38.95, 27.82, 27.69; HRMS calcd. for $C_{55}H_{55}N_2O_5$ [M+1]: 823.4111, found: 823.4140.

**(2*S*, 9*S*)-Di-*tert*-butyl 5-Oxo-2,9-bis[*N*-(PhF)amino]dec-4-enedioate (25).**
To a stirred solution of β–keto phosphonate **22** (488 mg, 0.88 mmol) in $CH_3CN$ (3.15 mL) was added $K_2CO_3$ (122 mg, 100 mol%). The suspension was stirred 30 min at rt, cooled to 0°C and treated with a solution of aldehyde **5** (367 mg, 0.88 mmol, 100 mol%) in $CH_3CN$ (3.15 mL). The mixture was stirred at 10°C for 72 h, diluted with EtOAc (15 mL) and quenched with $NaH_2PO_4$ (3 mL, 1 M). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with brine, dried and evaporated to give a yellowish oil that was chromatographed using a gradient of 5-20% $Et_2O$ in hexanes. Evaporation of the collected fractions gave **25** (574 mg, 78%) as a white foam: $^1H$ NMR δ 7.71-7.66 (m, 4 H), 7.43-7.15 (m, 22 H), 6.70 (m, 1 H), 6.00 (d, 1 H, *J* = 15.9), 2.71-2.63 (m, 2 H), 2.54-2.44 (m, 2 H), 2.36-2.28 (m, 2 H), 1.71-1.66 (m, 2 H), 1.20 (s, 9 H), 1.19 (s, 9 H). $^{13}C$ NMR δ 199.67, 174.99, 173.86, 81.20, 80.75, 73.02, 55.66, 55.37, 38.97, 36.27, 29.60, 27.87. HRMS calcd. for $C_{56}H_{57}N_2O_5$ [M+1]: 837.4268, found: 837.4281.

**(2*S*, 10*S*)-Di-*tert*-butyl 6-oxo-2,10-bis[*N*-(PhF)amino]undec-4-enedioate (26)**
To a stirred solution of β–keto-phosphonate **22** (1.19 g, 2.11 mmol) and aldehyde **5** (874 mg, 2.11 mmol) in $CH_3CN$ (20 mL) was added $K_2CO_3$ (293 mg, 2.22 mmol). The mixture was stirred at rt for 96 h and evaporated to a residue that was suspended in toluene and added to a column of silica gel. Chromatography using a gradient of 5-20% $Et_2O$ in hexanes and evaporation of the collected fractions gave **26** (1.39 g, 78%) as a white foam:

TLC $R_f$ = 0.36 (1:4 EtOAc:hexanes); $[\alpha]_D^{20}$ –151.6 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 7.74-7.66 (m, 4 H), 7.48-7.17 (m, 22 H), 6.67 (m, 1 H), 6.01 (d, 1 H, *J* = 15.9), 3.21 (bs, 1 H), 2.70 (dd, 1 H, *J* = 6.0, 6.1), 2.56 (dd, 1 H, *J* = 4.9, 7.3), 2.41-2.27 (m, 4 H), 1.78-1.67 (m, 2 H), 1.51-1.38 (m, 2 H), 1.22 (s, 18 H); $^{13}C$ NMR δ 199.72, 175.25, 173.89, 81.11, 80.42, 72.92, 55.53, 55.42, 39.17, 38.86, 35.13, 27.82, 19.73.; HRMS calcd for $C_{57}H_{59}N_2O_5$ [M+1]: 851.4423, found: 851.4444.

**(2*S*, 6*R*, 8*S*)-Methyl 9-Oxo-8-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-2-carboxylate ((2*S*, 6*R*, 8*S*)-31)** was obtained as a 9:1 diastereomeric mixture with (2*S*, 6*S*, 8*S*)-**31**. A solution of ketone **24** (375 mg, 0.456 mmol) in 15.4 mL of 9:1 EtOH : AcOH was transferred into a hydrogenation apparatus and treated with palladium-on-carbon (40 mg, 10 wt %). The pressure bottle was filled, vented and refilled four times with 6 atm of $H_2$. The reaction mixture was stirred over night, filtered onto a plug of Celite™ and washed thoroughly with EtOH. Evaporation of the volatiles gave a crude residue that was dissolved in toluene (10 mL) and MeOH (1 mL), and treated with *p*-toluenesulfonic acid mono-hydrate (428 mg, 2.25 mmol, 500 mol%). The solution was heated at a reflux for 1 h using a Dean-Stark apparatus. The Dean-Stark trap was then emptied and $Et_3N$ (200 mol%) was added to the solution, which was heated at a reflux for 48 h, cooled to rt, and evaporated to a residue, that was immediately dissolved in $CH_2Cl_2$ (12 mL). The solution was treated with $Et_3N$ (625 μL, 4.5 mmol, 1000 mol%) and di-*tert*-butyldicarbonate (119 mg, 0.54 mmol, 120 mol%), stirred at rt for 4 h, and evaporated to a residue, that was chromatographed using a gradient of 0-100% EtOAc in hexanes. Evaporation of the collected fractions gave ester **31** (119.4 mg, 85% from ketone **24**) as a 9 : 1 mixture of diastereomers: TLC $R_f$ = 0.43 (100% EtOAc); $^1H$ NMR (major isomer) δ 5.10 (bs, 1 H), 4.20 (bs, 1 H), 3.87 (dd, 1 H, *J* = 3.8, 9.4), 3.78 (s, 3 H), 3.32 (br m, 1 H), 2.81 (br m, 1 H), 1.97-1.12 (m, 7 H), 1.43 (s, 9 H); $^{13}C$ NMR (major isomer) δ 172.32, 171.45, 156.12, 80.04, 56.86, 55.52, 53.48, 52.62, 37.04, 30.62, 28.51, 27.32, 21.13. HRMS calcd. for $C_{15}H_{25}N_2O_5$ [M+1]: 313.1764, found: 313.1754. A sample of (2*S*, 6*R*, 8*S*)-**31** was obtained by HPLC purification using an isocratic solvent system of 45% $CH_3CN$ : 55% $H_2O$ on a reversed-phase $C_{18}$ column. Crystals of (2*S*, 6*R*, 8*S*)-**31** were obtained from a 9:1 mixture of (2*S*, 6*RS*, 8*S*)-**31** in EtOAc.

**(2*S*, 6*RS*, 8*S*)-9-Oxo-8-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-2-carboxylate ((2*S*, 6*RS*, 8*S*)-2).** A solution of methyl ester **31** (9:1 6*R*:6*S*, 35 mg, 0.112 mmol) in $Et_2O$ (4 mL) was treated with potassium trimethylsilanolate (21.6 mg, 0.168 mmol, 150 mol%), stirred for 30 min at rt, and treated with water (500 mL), followed by solid citric acid (32 mg, 0.168 mmol, 150 mol%). The layers were separated and the aqueous layer was extracted repeatedly with $Et_2O$ and then $CHCl_3$:*i*-PrOH (4:1) until TLC showed no ninhydrin active material. Evaporation of the combined organic layers, chromatography of the residue using 0-5% AcOH in ethyl acetate, and evaporation of the collected fractions gave **2** (32 mg, 96%) as a white solid: $R_f$ = 0.29 (5% AcOH in EtOAc); $^1H$ NMR ($CD_3OD$, major isomer) δ 4.66 (d, 1 H, *J* = 5.3), 4.24 (dd, 0.6 H, *J* = 10.2, 8.3), 3.65 (br m, 1 H), 2.57 (br m, 1 H), 2.28 (d, 1 H, *J* = 12.7), 1.93 (d, 1 H, *J* = 12.3), 1.87-1.52 (m, 4 H), 1.45 (s, 9 H), 1.24 (m, 2 H); $^{13}C$ NMR ($CD_3OD$, major isomer) δ 174.90, 174.40, 158.07, 81.79, 79.62, 53.29, 53.07, 35.35, 33.47, 28.87, 27.56, 21.72; HRMS calcd. for $C_{14}H_{23}N_2O_5$ [M+1]: 299.1607, found: 299.1616.

**Enantiomeric Purity of (2*S*, 6*R*, 8*S*)-methyl 9-oxo-8-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-2-carboxylate ((2*S*, 6*R*, 8*S*)-31).** A solution of (2*S*, 6*R*, 8*S*)-**31** (8.1 mg) in $CH_2Cl_2$ (1 mL) was treated with TFA (1 mL) and stirred for 2.5 h at rt when TLC (100% EtOAc) showed complete disappearance of starting **31**. The volatiles were removed under vacuum, and the residue was dissolved in THF (1 mL), treated with either (*R*)- or (*S*)-α–methylbenzylisocyanate (7.4 mL, 0.05 mmol, 200 mol%) and $Et_3N$ (7.4 mL, 0.05 mmol, 200 mol%) and heated at a reflux for 3 h. The mixture was cooled, the volatiles were removed under vacuum and the residue was directly examined by proton NMR. The limits of detection were determined by measuring the diastereomeric methyl ester singlets at 3.56 and 3.52 ppm in $C_6D_6$ in the 600 MHz $^1H$ NMR spectra. Less than 1% of the (1′*R*)-diastereomer was detected in the spectra for the (1′*S*)-urea **32**. Purification by chromatography using a gradient of pure hexanes to pure EtOAc as eluant gave ureas **32** having the following spectra.

**Urea (1′*R*)-32**: $^1H$ NMR δ 7.33-7.17 (m, 5 H), 5.45 (m, 1 H), 5.37 (d, 1 H, *J* = 5.3), 4.86 (m, 1 H), 4.22 (m, 1 H), 3.78 (m, 1 H), 3.76 (s, 3 H), 3.26 (m, 1 H), 2.77

(m, 1 H), 2.11 (m, 1 H), 1.98-1.44 (m, 4 H), 1.43 (d, 3 H, $J$ = 6.9), 1.38-1.20 (m, 2 H).

**Urea (1'*S*)-32**: $^1$H NMR δ 7.34-7.21 (m, 5H), 5.48 (d, 1 H, $J$ = 7.4), 5.38 (d, 1 H), 4.88 (m, 1 H), 4.43 (m, 1 H), 3.85 (m, 1 H), 3.72 (s, 3H), 3.30 (m, 1 H), 2.75 (m, 1 H), 1.96-1.47 (m, 4 H), 1.44 (d, 3 H, $J$ = 6.9), 1.21 (m, 3 H).

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**Supporting Information Available:** Experimental details for attempted Claisen condensations and β–methyl *N*-(PhF)aspartate;$^1$H and $^{13}$C NMR spectra of ketones **24-26**; and crystallographic data for **31** (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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# Article 3

Gosselin, F.; Lubell, W. D. "Rigid Dipeptide Surrogates: Syntheses of Enantiopure Quinolizidinone and Pyrroloazepinone Amino Acids from a Common Diaminodicarboxylate Precursor" Soumis à *The Journal of Organic Chemistry*, **1999**.

# Rigid Dipeptide Surrogates: Syntheses of Enantiopure Quinolizidinone and Pyrroloazepinone Amino Acids from a Common Diaminodicarboxylate Precursor

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## 2.15. Abstract

A versatile and practical approach for synthesizing azabicyclo[X.Y.0]alkane amino acids of different ring sizes from a common diaminodicarboxylate precursor has been developed as a means for mimicking different peptide conformations. (2*S*, 9*S*)-1-*tert*-Butyl 10-benzyl 5-oxo-2-[*N*-(PhF)amino] 9-[*N*-(BOC)amino]dec-4-enedioate (**18**) was first prepared in 83% yield by the Horner-Wadsworth-Emmons olefination of *N*-(PhF)aspartate β-aldehyde **8** with pyroglutamate-derived β-keto phosphonate **12** (PhF = 9-phenylfluoren-9-yl). The practicality of this approach for making azabicyclo[X.Y.0]alkane amino acids was then illustrated by the first synthesis of enantiopure quinolizidin-2-one amino acid **6** in 7 steps and 40% overall yield from pyroglutamic acid. Hydrogenation of δ-keto α,ω-diaminosebacate (**18**), followed by lactam cyclization and protection gave quinolizidin-2-one amino acid **6** as a single diastereomer. The versatility of this approach was next demonstrated by the synthesis of both ring-fusion isomers of pyrroloazepin-2-one amino acid **6** in 11 steps and 13% overall yield from pyroglutamic acid. Hydride reduction of **18**, followed by methanesulfonate displacement first gave 5-alkylproline intermediates **22**. Protective group manipulations, lactam cyclization and removal of the ester group afforded readily separable pyrroloazepinone amino acids (7*S*)- and (7*R*)-**7** in a 1:2 diastereomeric ratio. By introducing two new azabicycloalkane amino acids using our olefination approach, we have expanded the diversity of these important heterocycles for studying the conformational requirements for peptide biological activity.

## 2.16. Introduction

The biologically active conformation of a flexible peptide may be elucidated by using structural constraints to rigidify its back-bone and side-chain geometries. For this endeavor, azabicyclo[X.Y.0]alkane amino acids have been used as rigid dipeptide surrogates that constrain three back-bone dihedral angles within a fused bicyclic framework. Incorporation of these heterocyclic amino acids into a peptide can provide a better understanding of the spatial requirements for its biological activity.[1,2] Furthermore, these conformationally constrained dipeptide surrogates can serve as geometrically defined platforms onto which a variety of pharmacophores may be attached by modification of the amino and carboxylate handles using combinatorial techniques.[1,3] Their close structural relationship to the pyrrolizidine, indolizidine and quinolizidine alkaloids suggests the use of the related azabicyclo[X.Y.0]alkane amino acids as scaffolds for generating libraries of lead compounds that may possess similar biological activities as members of these large classes of alkaloid.[4]

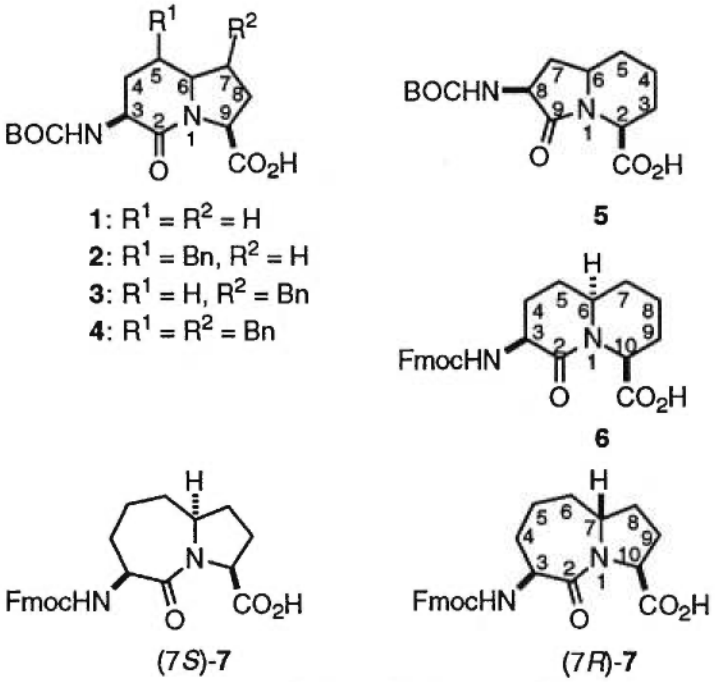


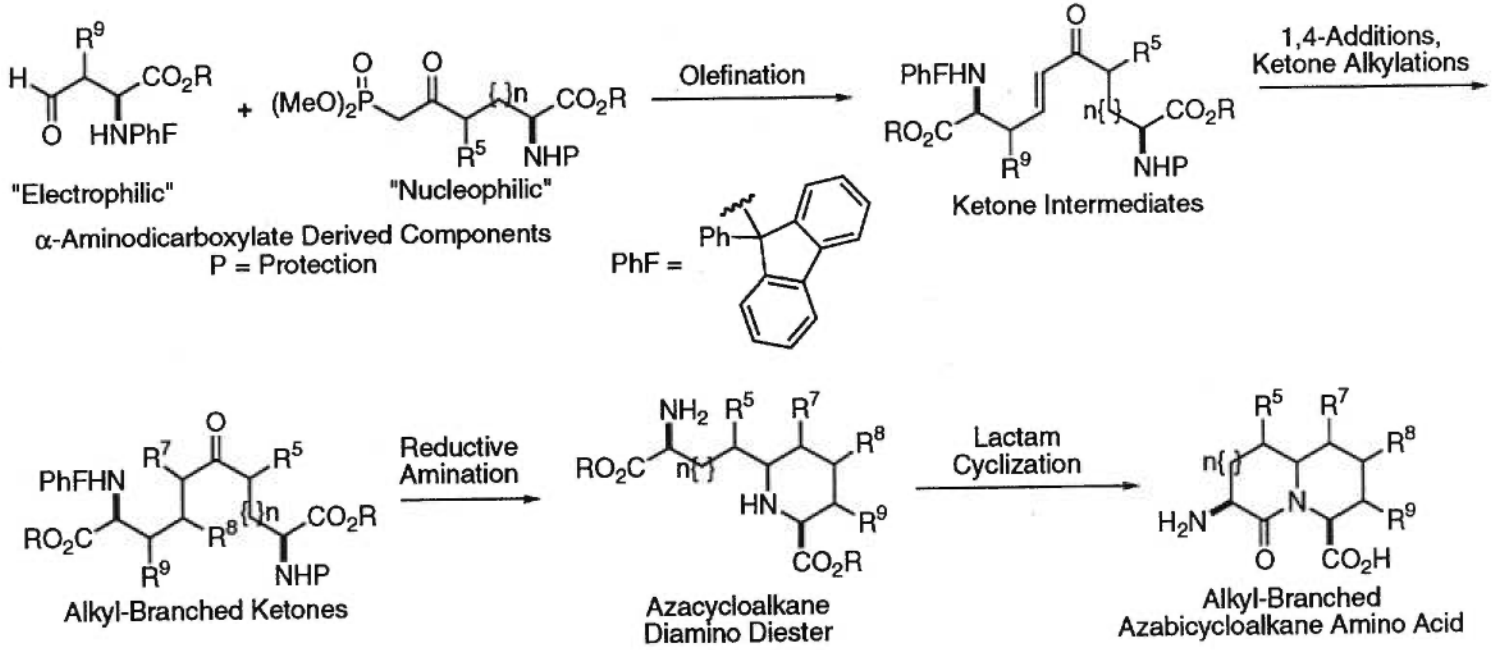

**Figure 1.** Indolizidinone, Quinolizidinone and Pyrroloazepinone Amino Acids **1-7** (ref. 1).

Ideally, the azabicycloalkane ring system should be formed unambiguously, with stereocontrol and the ability to append functional groups onto different positions of the heterocycle in order to mimic the side-chains of the common amino acids. Among the strategies for synthesizing azabicyclo[X.Y.0]alkane amino acid, methods based on *N*-acyl iminium ion cyclization as well as the condensation between a cysteine derivative and an amino acid ω-aldehyde have to date been the most successful for providing a variety of ring-systems.[5] Inability to control the stereochemistry of the ring-fusion center as well as low yields during the synthesis of alkyl substituted analogs have however been drawbacks encountered with these two strategies. For example, condensations between (*R*)-penicillamine and β-phenylcysteine with α-methyl-*N*-phthalyl-L-glutamate were plagued by low yields.[6] *N*-Acyl iminium ion cyclizations have given access to only one ring-fusion isomer in the synthesis of 5,7-fused pyrroloazepinone amino ester.[7] In the same light, although our Claisen condensation / alkylation / reductive amination / lactam cyclization strategy provided a stereoselective method for synthesizing indolizidin-2-one amino acid **1** and analogs **2**, **3** and **4** possessing amino acid side-chains at the 5- and 7-positions (Figure 1),[8,9] limitations in the Claisen condensation of aminodicarboxylates restricted this method's potential for generating a variety of ring-systems.[10a]

Striving to expand the variety of heterocycles that can be made by our approach, we introduced next an olefination entry for making a series of linear precursors for azabicyclo[X.Y.0]alkane synthesis (Scheme 1).[10] Employing aspartic acid as an inexpensive chiral educt in this olefination / reductive amination / lactam cyclization sequence, we synthesized enantiopure indolizidin-9-one amino acid **5**.[10] We envisioned that alkylation and conjugate addition to the α,β–unsaturated ketone intermediate in the synthesis of amino acid **5** may be used to introduce side-chains onto this heterocycle.

Having employed the olefination strategy to selectively join different amino dicarboxylates,[10] we investigated using the (*E*)-geometry of the resulting double bond to direct the cyclization of the unsymmetrical α,ω–

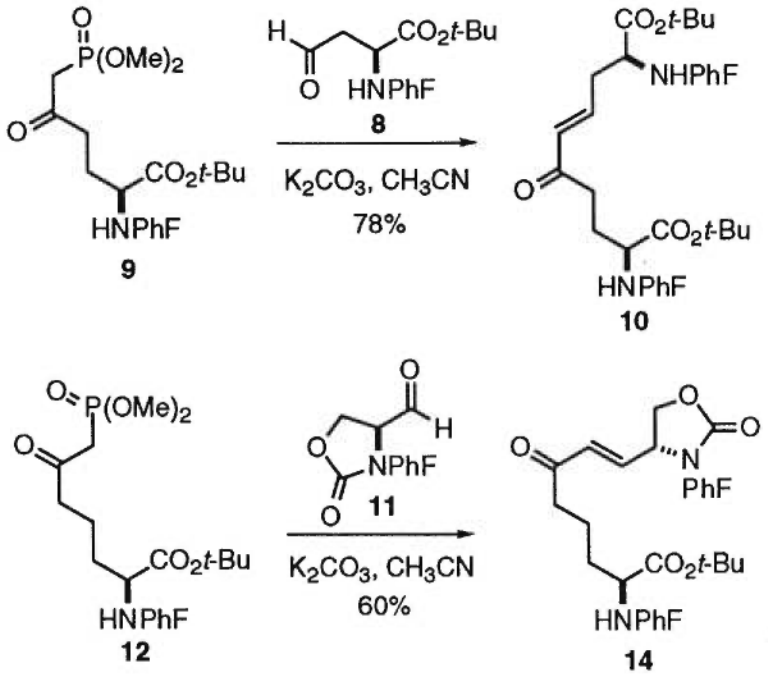
**Scheme 1. Olefination Approach for Synthesizing Alkyl-Branched Azabicyclo[X.Y.0]alkane Amino Acids**



diaminodicarboxylate intermediate in order to regioselectively produce different azabicyclo[X.Y.0]alkanes. Our investigation has now led to the synthesis of two azabicycloalkane ring-systems from a common linear olefin precursor. Quinolizidin-2-one amino acid **6** and pyrroloazepin-2-one amino acid **7**, both were prepared from δ-keto α,ω–diaminosebacate **18** (Schemes 3-5). The former, azabicyclo[4.4.0]alkane amino acid **6** had previously never been synthesized. Although the latter, azabicyclo[5.3.0]alkane amino acid **7** had been made, only the concave (7*S*)-isomer was produced because of conformational preferences during a route involving an *N*-acyl iminium ion cyclization,[7] and because of the precursor stereochemistry in a route featuring a radical cyclizations of an *N*-acyl acrylamide.[11] Besides yielding the concave isomer,[7] our route has now, for the first time, furnished the convex (7*R*)-isomer of pyrroloazepin-2-one amino acid **7**.

## 2.17. Results and Discussion

**Scheme 2. Synthesis of Diaminodicarboxylates by Olefination of Amino Aldehydes with Aminodicarboxylate-derived β-Ketophosphonates**



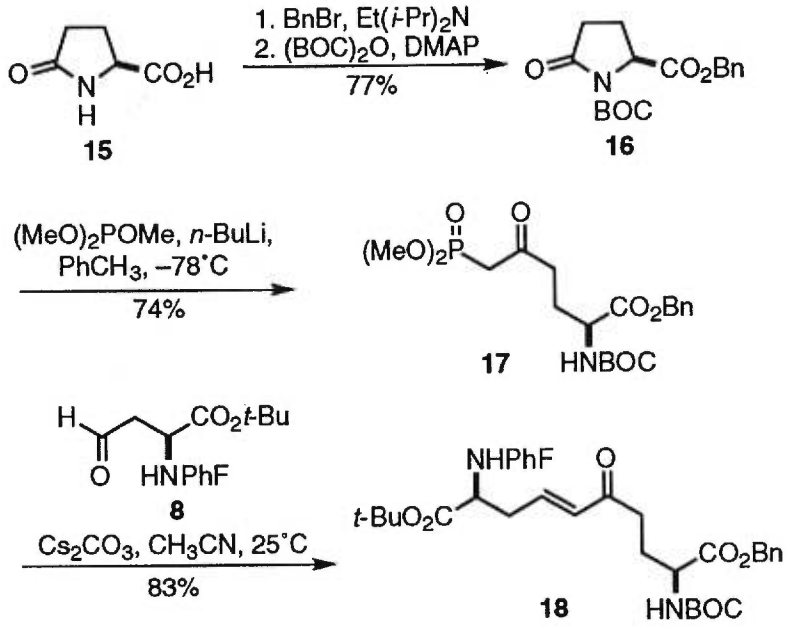
**Diaminodicarboxylate Precursor Synthesis** was initially investigated using different amino acid-derived aldehydes in the Horner-Wadsworth-Emmons olefination (Schemes 2 and 3). For example, olefination of *N*-(PhF)aspartate β-aldehyde **8** with *N*-(PhF)glutamate-derived β-ketophosphonate **9** in acetonitrile with potassium carbonate as base was initially found to provide α,β-unsaturated ketone **10** in 78% yield.[10a] Similarly, the reaction of configurationally stable *N*-(PhF)serinal **11**[12] with α-amino adipate-derived β-ketophosphonate **12**[10] in acetonitrile with $K_2CO_3$ as base gave α,β-unsaturated ketone **14** in 60% yield as described in the Supporting Information. In both cases, only the (*E*)-olefin was formed as confirmed by the large coupling constant ($J$ = 16.0 Hz) between the vinyl protons. Although 5-alkylproline and 6-alkylpipecolate intermediates could be respectively synthesized from α,β-unsaturated ketones **10** and **14**, we abandoned these approaches for preparing the 7,5- and 6,6-fused azabicycloalkanes because of difficulties encountered when trying to differentiate between the two *N*-(PhF)amines as well as between the two carboxylate functions.

We envisioned that a single linear olefin precursor could be used to synthesize both the 6,6- and 7,5-fused ring systems, if its amino and carboxylate functions were suitably protected. By employing a combination of *N*-BOC- and *N*-PhF-amines and *tert*-butyl and benzyl esters, we anticipated that acidic and hydrogenolytic conditions could be respectively used to unmask one member from each protected pair. Attention was thus turned towards the synthesis of 1-*tert*-butyl 10-benzyl 5-oxo-2-[*N*-(PhF)amino]-9-[*N*-(BOC)amino]dec-4-enedioate (**18**, Scheme 3).

Nucleophilic addition to *N*-carbamoyl pyroglutamate derivatives had previously been used to synthesize β-ketophosphonate.[13,14] Regioselective addition of diethyl methyl phosphonate to ethyl *N*-(BOC)pyroglutamate in THF afforded (2*S*)-α–ethyl 2-*N*-(BOC)amino-5-oxo-6-(diethylphosphonyl)hexanoate in 60% yield.[14b] In our route, benzyl *N*-(BOC)pyroglutamate **16** was first obtained in 77% overall yield by alkylative esterification of pyroglutamic acid with benzyl bromide and *N*,*N*-diisopropylethylamine in $CH_2Cl_2$, followed by *N*-protection using di-*tert*-

butyldicarbonate, DMAP and $Et_3N$ in $CH_3CN$.[15,16] By substituting benzyl bromide for the less reactive chloride, we found that the alkylation was complete in less than 24 h, instead of the previously required 7 days.[15] (2*S*)-α–Benzyl 2-*N*-(BOC)amino-5-oxo-6-(dimethylphosphonyl)hexanoate (**17**) was then prepared by the addition of the lithium anion of dimethyl methylphosphonate to benzyl *N*-(BOC)pyroglutamate **16** in toluene in 74% yield.[17] Earlier attempts to react this nucleophile on pyroglutamate **16** using THF as solvent gave lower yields (40-50%) of β-ketophosphonate **17**.

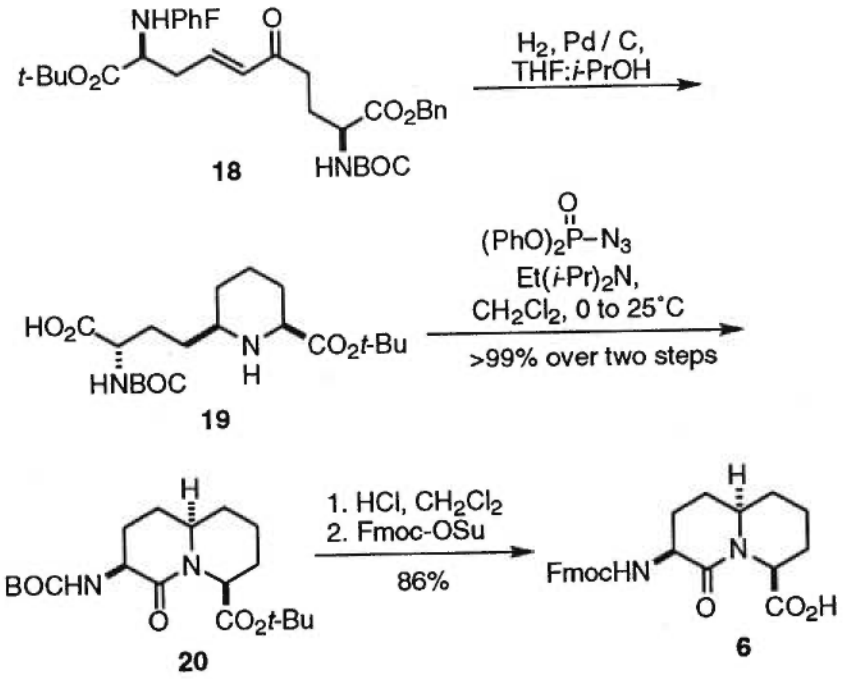
**Scheme 3. Synthesis of δ-Keto α,ω-Diaminosebacate 18**




The α,β-unsaturated ketone **18** was obtained by Horner-Wadsworth-Emmons olefination of *N*-(PhF)aspartate β-aldehyde **8** with β-ketophosphonate **17**.[10] Initially, longer times and high temperatures were required for the olefination reaction when $K_2CO_3$ was used as base alone as well as in the presence of 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane). Because of the limited stability of β-amino aldehyde **8** under these harsh conditions, α,β-unsaturated ketone **18** was isolated in less than 30% yield. Alternatively, the use of $Cs_2CO_3$ as base in $CH_3CN$ cleanly afforded **18** in 83% yield after 4-5 h at rt. No decomposition of the aldehyde to *tert*-butyl *N*-(PhF)-5-azapenta-2,4-dienoate[18a] was

observed under these conditions. The (*E*)-double bond geometry was confirmed by the $J$ = 16.0 Hz coupling constant between the vinyl protons in α,β-unsaturated ketone **18**.

**Scheme 4. Synthesis of Quinolizidin-2-one Amino Acid 6**




**Quinolizidin-2-one Amino Acid Synthesis** was studied using a reductive amination approach to selectively unmask the *N*-(PhF)amine and furnish the 6-alkylpipecolate. Previously, we have shown that δ- and ε-keto α-amino esters can be effectively converted to their respective 5-alkylprolines and 6-alkylpipecolates by catalytic hydrogenation with high diastereoselectivity.[8-10,18,19] In the case of (2*S*, 9*S*)-1-*tert*-butyl 10-benzyl 5-oxo-2-[*N*-(PhF)amino] 9-[*N*-(BOC)amino]dec-4-enedioate (**18**, Scheme 4), treatment with palladium-on-carbon in an *i*-PrOH:THF solution at 6 atm of hydrogen caused reduction of the double bond, hydrogenolysis of the benzyl ester and the PhF group, imine formation, and reduction from the less hindered face of the imine to afford pipecolate **19** as a single diastereomer. After filtration to remove the catalyst, the crude pipecolate mixture was then treated with diphenylphosphoryl azide (DPPA)[20] in $CH_2Cl_2$ in the presence of DIEA and provide crystalline azabicycloalkane

*N*-(BOC)amino ester **20** in >99% yield from ketone **18** after chromatography.

Because of the growing importance of Fmoc-based solid-phase peptide synthesis, 6,6-fused azabicycloalkane *N*-(BOC)amino ester **20** was converted into its Fmoc derivative.[21,22] First, the BOC group and the *tert*-butyl ester were simultaneously removed with HCl gas in $CH_2Cl_2$. The amino acid hydrochloride was acylated with 9-fluorenylmethyloxycarbonyl hydroxysuccinimide (Fmoc-OSu), in the presence of $NaHCO_3$ in a solution of acetone and water.[23] Quinolizidinone *N*-(Fmoc)amino acid **6** was finally obtained in 86% yield after column chromatography.

**Pyrroloazepin-2-one Amino Acid Synthesis** was next pursued using the same olefin precursor **18**. As mentioned, the concave (7*S*)-isomer of pyrroloazepin-2-one amino acid **7** had been previously synthesized by an *N*-acyl iminium ion cyclization from an allylglycine dipeptide precursor,[7] as well as by an intramolecular radical mediated cyclization of a 5-alkylproline derivative.[11] This 7,5-fused azabicycloalkane amino acid and its 4-thiapyrroloazepin-10-one counterpart have been used as D-Ala-Pro mimics in the preparation of potent angiotensin-converting-enzyme inhibitors.[24,25] Furthermore, this constrained dipeptide surrogate has been shown to stabilize preferentially γ-turn conformations in short peptides.[26]

Previously, we have shown that methanesulfonate displacements can be effectively used for synthesizing the proline ring in indolizidin-2-one amino acid and its 5- and 7-benzyl, and 5,7-dibenzyl derivatives.[8a,9] Intramolecular attack of *N*-(PhF)amine onto secondary methanesulfonates has provided excellent yields of 5-alkylprolines by what appears to be a selective $SN_2$ process in these cases. To examine the methanesulfonate displacement for making the proline in the pyrroloazepin-2-one amino acid, ketone **18** was first transformed into its corresponding allylic alcohol by hydride reduction with sodium borohydride in the presence of cerium trichloride in MeOH:THF to provide **21** as a 1:1 mixture of diastereomers in 86% yield (Scheme 5).[27] Treatment of the alcohol with methanesulfonyl

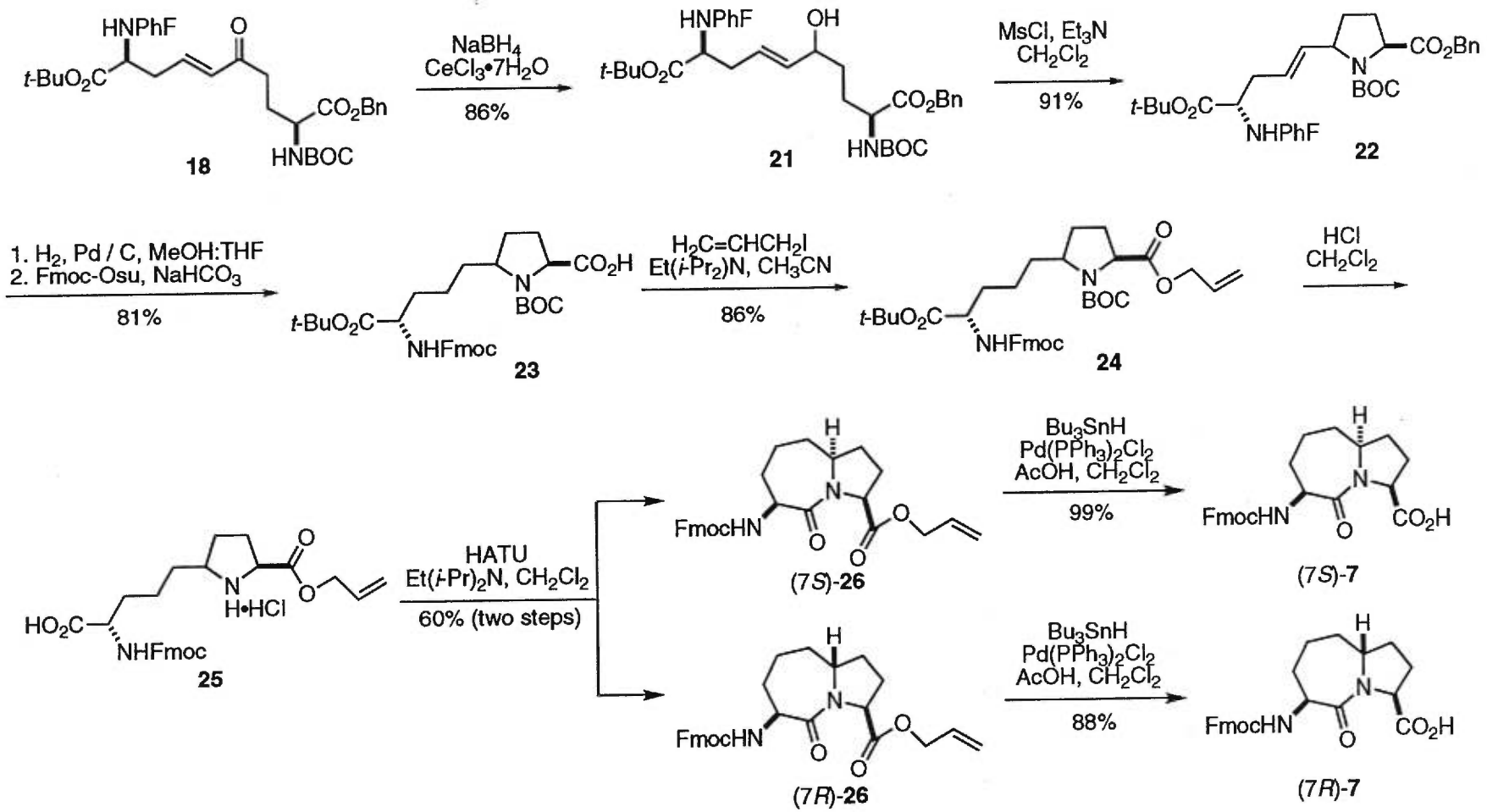
chloride and triethylamine in $CH_2Cl_2$, afforded cyclization to 5-alkylprolines **22** in 91% yield. The (*E*)-olefin geometry of alcohol **21** excluded the attack of the *N*-(PhF)amine onto the methanesulfonate such that exclusive cyclization of the *N*-(BOC)amine occurred.

5-Alkylprolines **22** were obtained as a 2:1 mixture of 5*R*:5*S* diastereomers as indicated by measurements of the *tert*-butyl ester singlets in the $^1H$ NMR spectra of analogs **23** and **24**. The enhanced stereochemical ratio relative to starting allylic alcohol **21** was ascribed to $SN_1$-type cyclization, presumably due to ionization of the methanesulfonates under the reactions conditions. As previously noted in a related synthesis of (–)-pyrrolidine-2,5-dicarboxylic acid,[28] the conformation of the allylic cation intermediate may have contributed to the enrichment of the 5*R* diastereomer of proline **22**.

Prior to the key lactam cyclization, the protecting groups were exchanged. First, the *N*-(PhF)amine and benzyl ester of **22** were cleaved with concurrent double bond reduction by catalytic hydrogenation in a THF / methanol solution. *N*-Acylation with Fmoc-OSu in an acetone / water solution gave prolines **23** in 81% overall yield from **22**. Alkylative esterification with allyl iodide in $CH_3CN$ heated at a reflux afforded ester **24** in 86% yield. Simultaneous removal of the BOC group and *tert*-butyl ester with HCl in $CH_2Cl_2$ then gave amino acid hydrochloride **25**.

Lactam cyclization of **25** using both azabenzotriazolyl-1,1,3,3-tetramethylaminium hexafluorophosphate (HATU)[29,30] and benzotriazolyl-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU)[30,31] with *N*,*N*-diisopropylethylamine in $CH_2Cl_2$ gave *N*-(Fmoc)-azabicycloalkane amino esters **26** in yields ranging between 50-60%.[32,33] Although TBTU gave essentially the same amount of conversion to lactam **26**, we found that the coupling reaction with HATU proceeded with an enhanced cyclization rate and yielded product of better purity.[29] Pyrroloazepin-2-one *N*-(Fmoc)amino esters **26** were isolated as a 2:1 mixture of diastereomers that were easily separated by chromatography on

**Scheme 5. Synthesis of Pyrroloazepin-2-one Amino Acid 7**



silica gel. Pyrroloazepin-2-one *N*-(Fmoc)amino acids (7*S*)-**7** and (7*R*)-**7** were finally synthesized by palladium catalyzed hydrostannolytic cleavage of their respective allyl esters (7*R*)-**26** and (7*S*)-**26** on treatment with $Pd(PPh_3)_2Cl_2$ and $nBu_3SnH$ in a $CH_2Cl_2$ / AcOH solution at room temperature in yields of 88% and 99% after chromatography.[34]

**Relative Stereochemistry** at the ring-fusion center of the new azabicycloalkane amino acids was confirmed by spectroscopic and crystallographic methods. Because quinolizidin-2-one *N*-(BOC)amino ester (3*S*, 6*R*, 10*S*)-**20** was prepared by a reductive amination sequence, we assigned the stereochemistry of its ring-fusion carbon initially based on analogy with *N*-(BOC)amino indolizidin-9-one acid (2*S*, 6*R*, 8*S*)-**5** and 6-alkylpipecolates.[10,18]

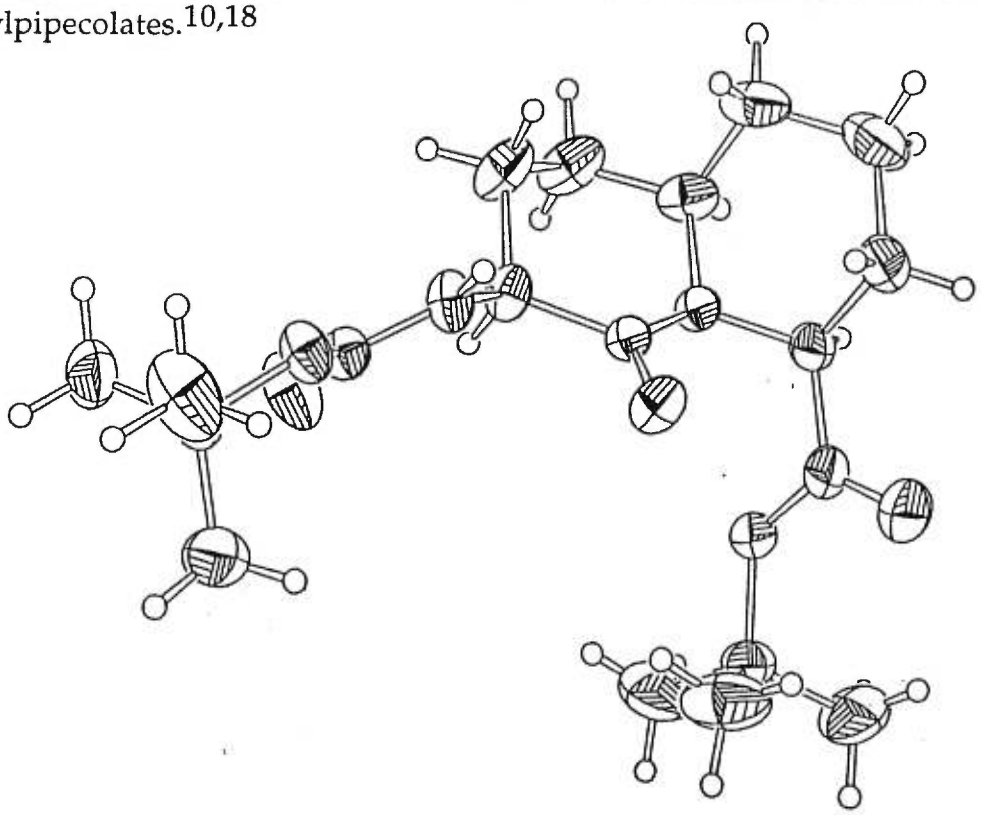


**Figure 2. ORTEP view of *N*-(BOC)amino quinolizidin-2-one *tert*-butyl ester (3*S*, 6*R*, 10*S*)-20. Ellipsoids drawn at 40% probability level. Hydrogens represented by spheres of arbitrary size.** [35]

Since hydrogenation of α–*tert*-butyl δ–oxo-α–*N*-(PhF)amino ester produced 6-alkylpipecolates with high selectivity in favor of the *cis*-diastereomer, we

presumed that the reductive amination with amino ketone **18** proceeded to form *cis*-6-alkylpipecolate (2*S*, 6*R*, 2'*S*)-**20**. Crystals of (3*S*, 6*R*, 10*S*)-*tert*-butyl 2-oxo-3-*N*-(BOC)amino-1-azabicyclo[4.4.0]decane-10-carboxylate **(20)** were later obtained from an EtOAc / hexanes solution. Subsequently, X-ray crystallographic analysis confirmed our hypothesis and indicated formation of the concave isomer (Figure 2).[35]

With both (7*S*)- and (7*R*)-pyrroloazepin-2-one *N*-(Fmoc)amino acids (7*S*)-**7** and (7*R*)-**7** in hand, a series of two dimensional NMR experiments were performed in order to assign the relative stereochemistry at the ring-fusion. First, the protons within the peptide back-bone and at the ring-fusion of each isomer were identified using their COSY spectra. The stereochemistry at the ring-fusion was next determined by NOESY experiments (Figure 3). Transfer of magnetization was observed between the ring-fusion *C*-7 proton (3.86 ppm) and the back-bone protons at *C*-3 (4.40 ppm) and *C*-10 (4.70 ppm) in the case of the (7*S*)-**7** minor diastereomer. By comparison, nuclear Overhauser effects were observed between the *C*-7 proton (3.70 ppm) and carbamate N-H (6.08 ppm) in the case of the (7*R*)-**7** major diastereomer.

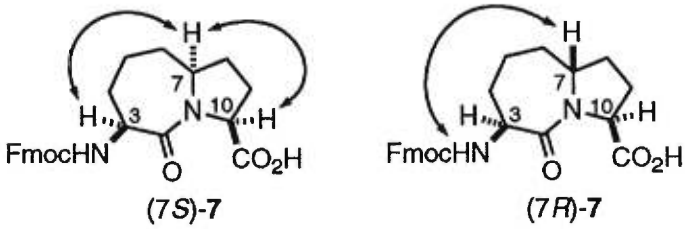
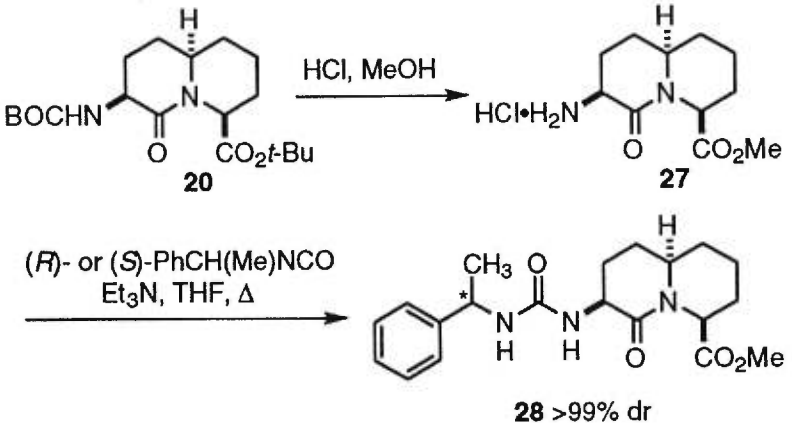



**Figure 3.** Observed diagnostic nOe's in NOESY experiments.

**Enantiomeric Purity** of quinolizidin-2-one amino ester (3*S*,6*R*,10*S*)-**20**, produced from the reductive amination / lactam cyclization sequence on ketone **18**, was determined after conversion to diastereomeric (1'*R*)- and (1'*S*)-*N*-α–methylbenzylureas **28** by analysis using NMR spectroscopy (Scheme 6). Hydrogen chloride in methanol removed both the *N*-BOC and *tert*-butyl ester protecting groups and esterified the acid intermediate to quantitatively furnish methyl ester hydrochloride **27**. Acylation of **27** with either (*R*)- or (*S*)-α–methylbenzyl isocyanate and triethylamine in THF

examined after evaporation of the volatiles. Measurement of the diastereomeric methyl ester singlets at 3.70 and 3.65 ppm in $CDCl_3$ by 400 MHz $^1H$ NMR spectroscopy demonstrated **28** to be of >99% in diastereomeric ratio. Hence diaminodicarboxylate **18**, *N*-(BOC)amino quinolizidin-2-one ester **20**, and its respective acid **6**, all are presumed to be of >99% enantiomeric purity. Because concurrent racemization of both stereocenters in ketone precursor **18** is very unlikely under the reaction conditions of the methanesulfonate displacement sequence, *N*-(Fmoc)amino pyrroloazepinone-2-one acids **7** are also assumed to be of >99% enantiomeric purity.

**Scheme 6. Enantiomeric Purity of Quinolizidin-2-one Amino Ester 20**




**Influences of Heterocycle Ring-Size on Peptide Back-Bone Conformation** were illustrated on comparison of the dihedral angles of indolizidin-2-one, indolizidin-9-one and quinolizidin-2-one *N*-(BOC)amino esters **1, 5** and **20** in the solid-state (Table 1). Because they possess the same relative stereochemistry, significant differences in the internal $\Psi$ and $\phi$ dihedral angles from the X-ray data of **1, 5** and **20** indicate that ring-size has a profound effect on conformation. Our data also indicates that a variety of azabicycloalkane amino acids of different ring-size may be used to mimic a comprehensive spectrum of peptide conformations. Because structure-activity relationship studies have already shown that peptides possessing $\gamma$, $\delta$, and $\varepsilon$-lactams can differ in

**Table 1. Comparison of the Dihedral Angles from Azabicycloalkane X-ray Data**

| entry | $\psi$, deg | $\phi$, deg |
|---|---|---|
| (3*S*, 6*R*, 10*S*)-3-*N*-(BOC)Amino Quinolizidin-2-one-10-carboxylate **20** | –163 | +48 |
| (2*S*, 6*R*, 8*S*)-8-*N*-(BOC)Amino Indolizidin-9-one-2-Carboxylate **5**[10] | –141 | –34 |
| (3*S*, 6*S*, 9*S*)-3-*N*-(BOC)Amino Indolizidin-2-one-9-Carboxylate **1**[8] | –176 | –78 |

bioactivity,[36] the application of a series of azabicycloalkane amino acids towards the study of biologically relevant peptides should provide detailed pictures of the conformational requirements for activity.

**2.18. Conclusion**

We have expanded the diversity of azabicyclo[X.Y.0]alkane amino acids by application of our olefination entry to synthesize their linear precursors. By taking advantage of the olefin geometry to direct the reductive amination and methanesulfonate displacement cyclizations on a common diaminodicarboxylate intermediate, two new ring systems of different sizes were prepared for the first time. Enantiopure (3*S*, 6*R*, 10*S*)-quinolizidin-2-one amino acid **6** was assembled in 7 steps and 40% overall yield from pyroglutamic acid. Convex (3*S*, 7*R*, 10*S*)-pyrroloazepin-2-one amino acid **7** was prepared as a 2:1 diastereomeric mixture with its previously synthesized concave counterpart in 11 steps and 13% overall yield from pyroglutamic acid.[38] Stereocontrol was achieved during the syntheses of 6 and 7 by selectively joining inexpensive aminodicarboxylate starting materials. Moreover, our approach is well poised for the introduction of side-chains onto the heterocycle framework through functionalization of the pyroglutamate and β-amino aldehyde precursors, as well as by conjugate additions and alkylations on the α,β-unsaturated ketone intermediate (Scheme 1). Our strategy now provides a series of azabicycloalkane amino acids for systematic mimicry of the back-bone and side-chain conformations of biologically relevant peptides.

**2.19. Experimental Section**

**General:** Unless otherwise noted all reactions were run under nitrogen atmosphere and distilled solvents were transferred by syringe. Tetrahydrofuran (THF) was distilled from sodium / benzophenone immediately before use; toluene was distilled from sodium; $CH_2Cl_2$ was distilled from $P_2O_5$; commercial anhydrous $CH_3CN$ was used without further purification; triethylamine ($Et_3N$) was distilled from BaO; *N,N*-diisopropylethylamine [Et(*i*-Pr)$_2$N] was distilled from $CaH_2$ and ninhydrin. Final reaction mixture solutions were dried over $Na_2SO_4$. Melting points are uncorrected. Mass spectral data, HRMS and MS (EI and FAB), were

obtained by the Université de Montréal Mass Spectrometry facility. Unless otherwise noted, IR spectra were recorded in mineral oil; $^{1}H$ NMR (300/400 MHz) and $^{13}C$ NMR (75/100 MHz) spectra were recorded in $CDCl_3$. IR bands are reported in reciprocal centimeters ($cm^{-1}$). Chemical shifts are reported in ppm (δ units) downfield of internal tetramethylsilane ($(CH_3)_4Si$), or residual $CHCl_3$; coupling constants are reported in hertz. Chemical shifts of the vinyl carbons in **18** and **21** and of PhF aromatic carbons are not reported in the $^{13}C$ NMR spectra. Analytical thin-layer chromatography (TLC) was performed by using aluminum-backed silica plates coated with a 0.2 mm thickness of silica gel 60 $F_{254}$ (Merck), visualized with UV light, ninhydrin solution and ceric ammonium molybdate solution. Chromatography was performed using Kieselgel 60 (230-400 mesh).

**(2*S*)-Benzyl *N*-(BOC)-pyroglutamate (16)**

A solution of pyroglutamic acid (5.0 g, 38.75 mmol) and *N*,*N*-diisopropylethylamine (13.5 mL, 77.5 mmol, 200 mol%) in $CH_2Cl_2$ (200 mL) was treated with benzyl bromide (18.5 mL, 155 mmol, 400 mol%), heated at a reflux for 12-24 h, cooled to rt and washed with aqueous $NaH_2PO_4$ (50 mL, 1 M). The aqueous layer was extracted with $CH_2Cl_2$ (2 × 25 mL) and the combined organic layers were washed with brine (50 mL), dried, filtered and evaporated. The crude product (HRMS calcd. for $C_{12}H_{14}NO_3$ [M+H]: 220.0974, found: 220.0967) was then dissolved in a solution of $CH_3CN$ (100 mL) and $Et_3N$ (5.4 mL, 100 mol%), treated with $(BOC)_2O$ (16.9 g, 200 mol%) and DMAP (473 mg, 10 mol%) and stirred at rt over night. The colored mixture was washed with $NaH_2PO_4$ (50 mL, 1 M) and brine (50 mL), dried, filtered and evaporated to a colored viscous solid that was filtered through a plug of silica gel using 1:1 EtOAc in hexanes as eluant. The collected fractions were evaporated to provide pyroglutamate **16** as a colorless crystalline solid: 9.56 g, 77%; mp 69-70°C (lit. 57-59°C[9f]; 72-74°C[10]); TLC $R_f$ = 0.19 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –37.8 (*c* 1.0, $CHCl_3$) (lit.[15] $[\alpha]_D^{25}$ –35.0 (*c* 0.98, $CHCl_3$ ); IR ($cm^{-1}$): 2973, 2922, 2882, 2841, 1782, 1739, 1702, 1457, 1366, 1156. $^{1}H$ NMR δ 1.42 (s, 9 H), 1.95-2.59 (m, 4 H), 4.64 (m, 1 H), 5.20 (s, 2 H), 7.36 (m, 5 H); $^{13}C$ NMR δ 21.7, 27.9, 31.3, 59.1, 67.5, 83.8, 128.7, 128.8, 128.9, 135.2, 149.4, 171.3, 173.4. HRMS calcd for $C_{17}H_{22}NO_5$ [M+H]: 320.1498,

found: 320.1508. Anal. calcd for $C_{17}H_{21}NO_5$: C, 63.94; H, 6.63; N, 4.39. Found: C, 63.96; H, 6.94; N, 4.43.

**(2*S*)-α–Benzyl 2-*N*-(BOC)Amino-5-oxo-6-(dimethylphosphonyl)hexanoate (17)** A –78°C solution of dimethyl methyl phosphonate (360 μL, 3.3 mmol, 105 mol%) in toluene (40 mL) was treated with *n*-butyllithium (2.53 mL, 3.3 mmol, 105 mol%, 1.3 M in hexanes), stirred for 20 min at –78°C and transferred by cannula dropwise over 60 min into a –78°C solution of (2*S*)-benzyl *N*-(BOC)pyroglutamate (**16**, 1.0 g, 3.1 mmol) in toluene (30 mL). The solution was stirred for 1 h, warmed to rt over 30 min, quenched with aqueous $NaH_2PO_4$ (20 mL, 1 M), and diluted with EtOAc (50 mL). The aqueous layer was separated, saturated with solid NaCl and extracted with EtOAc until TLC of the organic layer showed no material that stained with ceric ammonium molybdate. The combined organic layers were washed with brine, dried and evaporated to a residue that was chromatographed using 10-100% EtOAc in hexanes as eluant to afforded a colorless oil that crystallized (**17**, 1.02 g, 74%): mp 81-83°C; TLC $R_f$ = 0.26 (EtOAc); $[\alpha]_D^{20}$ –4.8 (*c* 1.0, $CHCl_3$); IR ($cm^{-1}$): 3542, 3270, 2983, 1738, 1736, 1701, 1621, 1529, 1394, 1363, 1265, 1213, 1166, 1042. $^1H$ NMR (keto-enol mixture) δ 1.37 (s, 2 H), 1.42 (s, 7 H), 1.88-1.98 (m, 1 H), 2.14-2.18 (m, 1 H), 2.62-2.78 (m, 2 H), 3.04 (s, 1 H), 3.09 (s, 1 H), 3.66 (m, 1 H), 3.75 (d, 5 H, *J* = 11.2), 4.28 (dd, 1 H, *J* = 4.7, 8.1), 5.13 (d, 1 H, *J* = 12.3), 5.18 (d, 1 H, *J* = 12.3), 5.48 (bd, 1 H, *J* = 8.1), 7.34 (s, 5 H); $^{13}C$ NMR (keto-enol mixture) δ 27.7, 28.2, 39.7, 40.4, 41.7, 52.7, 52.8, 52.9, 53.4, 62.1, 66.9, 79.7, 135.3, 155.5, 172.0, 200.6. HRMS calcd for $C_{20}H_{31}NO_8P$ [M+H]: 444.1787, found: 444.1802.

**(2*S*, 9*S*)-1-*tert*-Butyl 10-Benzyl 5-Oxo-2-[*N*-(PhF)amino] 9-[*N*-(BOC)amino]dec-4-enedioate (18)**. To a stirred solution of β–keto phosphonate **17** (1.02 g, 2.3 mmol) and *N*-(PhF)aspartate aldehyde **8** (950 mg, 2.3 mmol, 100 mol%, prepared according to references 10 and 18a) in $CH_3CN$ (20 mL) was added $Cs_2CO_3$ (899 mg, 2.76 mmol, 120 mol%). The mixture was stirred at rt for 4-5 h, quenched with aqueous $NaH_2PO_4$ (20 mL, 1 M) and diluted with EtOAc (25 mL). The aqueous layer was saturated with solid NaCl and extracted with EtOAc until TLC of the organic layer showed no UV-active material. The combined organic layers were washed

with 10 mL of brine, dried, and evaporated to a solid residue that was chromatographed using a gradient of 10-50% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave ketone **18** as a white crystalline solid: 1.39 g, 83%; mp 144-145°C; TLC $R_f$ = 0.40 (1 : 4 EtOAc : hexanes); $[\alpha]_D^{20}$ –81.8 (*c* 1.0, $CHCl_3$); IR ($cm^{-1}$): 3359, 2922, 2856, 1736, 1709, 1678, 1514, 1154. $^1H$ NMR δ 1.20 (s, 9 H), 1.44 (s, 9 H), 1.96-2.04 (m, 1 H), 2.14-2.20 (m, 1 H), 2.22-2.36 (m, 1 H), 2.52-2.72 (m, 3 H), 3.21 (bs, 1 H), 4.37 (dd, 1 H, *J* = 5.2, 7.6), 5.14 (d, 1 H, *J* =12.3), 5.20 (d, 1 H, *J* = 12.3), 5.28 (d, 1 H, *J* = 7.9), 5.99 (d, 1 H, *J* = 16.0), 6.69 (dt, 1 H, 7.4, 16.0), 7.19-7.43 (m, 16 H), 7.67-7.71 (m, 2 H); $^{13}C$ NMR δ 26.6, 28.0, 28.5, 35.8, 39.1, 53.4, 55.7, 67.3, 73.1, 80.1, 81.4, 155.6, 172.4, 174.1, 198.7. HRMS calcd for $C_{45}H_{51}N_2O_7$ [M+H]: 731.3696, found: 731.3676. Anal. calcd for $C_{45}H_{50}N_2O_7$: C, 73.95; H, 6.90; N, 3.83. Found: C, 73.90; H, 7.10; N, 3.84.

**(3*S*, 6*R*, 10*S*)-*tert*-Butyl 2-Oxo-3-*N*-(BOC)amino-1-azabicyclo[4.4.0]decane-10-carboxylate (20)** A solution of ketone **18** (1.46 g, 2.0 mmol) in THF (30 mL) and *i*-PrOH (20 mL) was transferred into a hydrogenation apparatus and treated with palladium-on-carbon (200 mg, 10 wt %). The pressure bottle was filled, vented and refilled four times with 6 atm of $H_2$. The reaction mixture was stirred for 18 h and filtered onto a plug of diatomaceous earth (Celite™) that was washed thoroughly with MeOH. Evaporation of the volatiles gave crude pipecolate **19** (HRMS calcd. for $C_{19}H_{35}N_2O_6$ [M+H]: 387.2495, found: 387.2507) that was dissolved in $CH_2Cl_2$ (50 mL), cooled to 0°C, treated with *N*,*N*-diisopropylethylamine (698 µL, 4 mmol, 200 mol%), diphenylphosphoryl azide (862 µL, 4 mmol, 200 mol%), stirred for 30 min, warmed to rt, and left to stir for 16 h. Evaporation of the volatiles and chromatography of the residue using 0-100% EtOAc in hexanes as eluant gave quinolizidin-2-one amino ester **20** as a clear oil that crystallized on standing: 736 mg, 99%, mp 115-117°C; TLC $R_f$ = 0.47 (1 : 1 EtOAc : hexanes); $[\alpha]_D^{20}$ –1.6 (*c* 1.0, $CHCl_3$); IR ($cm^{-1}$): 3346, 3005, 2987, 1721, 1708, 1657, 1526, 1363, 1324, 1302, 1246, 1163. $^1H$ NMR δ 1.33 (s, 9 H), 1.35 (s, 9 H), 1.26-1.75 (m, 7 H), 1.80-1.93 (m, 3 H), 2.24-2.30 (m, 1 H), 3.38-3.45 (m, 1 H), 3.99-4.05 (m, 1 H), 4.19-4.22 (dd, 1 H, *J* = 5.7, 5.8), 5.50 (bs, 1 H); $^{13}C$ NMR δ 18.8, 24.3, 25.3, 27.4, 27.9, 28.0, 28.3, 29.1, 50.7, 52.7, 55.3, 79.3, 81.1, 155.6, 170.6. HRMS calcd

for $C_{19}H_{33}N_2O_5$ [M+H]: 369.2390, found: 369.2403. Anal. calcd for $C_{19}H_{32}N_2O_5$: C, 61.93; H, 8.75; N, 7.60. Found: C, 61.85; H, 9.04; N, 7.51.

**(3*S*, 6*R* 10*S*)-2-Oxo-3-*N*-(Fmoc)amino-1-azabicyclo[4.4.0]decane-10-carboxylate (6)**

A solution of *N*-(BOC)amino-1-azabicyclo[4.4.0]decane-10-carboxylate **20** (350 mg, 0.95 mmol) in $CH_2Cl_2$ (15 mL) at 0°C was treated with HCl bubbles for 60 min, when TLC showed complete disappearance of the starting material. The volatiles were evaporated to give the crude hydrochloride as a white solid, that was dried under vacuum, dissolved in water (5 mL), treated with solid $NaHCO_3$ (160 mg, 1.9 mmol, 200 mol%), stirred for 10 min and treated with a solution of Fmoc-OSu (320 mg, 0.95 mmol, 100 mol%) in acetone (10 mL). The mixture was stirred for 3 h at rt, acidified with $H_3PO_4$ to pH 4 and extracted with EtOAc until TLC of the organic layer showed no UV-active material. The combined organic layers were evaporated to a residue that was chromatographed using 0-70% EtOAc in hexanes containing 2% AcOH as eluant. Evaporation of the collected fractions gave *N*-(Fmoc)amino acid **6** as a white foam: 356 mg, 86% TLC $R_f$ = 0.27 (EtOAc + 1% AcOH); $[\alpha]_D^{20}$ –5.2 (*c* 1.0, $CHCl_3$); IR ($cm^{-1}$): 3290, 2919, 2856, 1717, 1648, 1522, 1450, 1326, 1244. $^1H$ NMR δ 1.50-1.80 (bm, 6 H), 2.02-2.09 (bm, 2 H), 2.34-2.39 (bm, 1 H), 3.52 (bs, 1 H), 4.25 (m, 1 H), 4.31 (m, 1 H), 4.34 (m, 1 H), 6.35 (bd, 1 H, *J* = 6.0), 7.18-7.78 (m, 8 H), 10.04 (bs, 1 H); $^{13}C$ NMR δ 19.4, 24.4, 25.0, 27.0, 29.3, 47.1, 51.1, 54.0, 55.9, 67.1, 119.9, 125.3, 127.1, 127.7, 128.2, 129.0, 137.8, 141.2, 143.8, 144.0, 156.4, 171.2, 174.9, 176.2. HRMS calcd for $C_{25}H_{27}N_2O_5$ [M+H]: 435.1920, found: 435.1938.

**(2*S*, 6*RS*, 9*S*)-1-*tert*-Butyl 10-Benzyl 5-Hydroxy-2-[*N*-(PhF)amino]-9-[*N*-(BOC)amino]dec-4-enedioate (21)** A solution of ketone **18** (2.0 g, 2.72 mmol) in MeOH (20 mL) and THF (20 mL) was treated with $CeCl_3 \bullet 7H_2O$ (1.12 g, 110 mol%) and stirred for 10 min. Solid $NaBH_4$ (255 mg, 6.8 mmol, 250 mol%) was added and the solution was stirred for 10 min, quenched with aqueous $NaH_2PO_4$ (15 mL, 1 M) and diluted with EtOAc (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine, dried, and evaporated to a residue that was chromatographed using 0-50% EtOAc in

hexanes as eluant. Evaporation of the collected fractions gave allylic alcohol **21** as a 1:1 mixture of diastereomers: 1.71 g, 86%, white foam; TLC $R_f$ = 0.58 (1 : 1 EtOAc : hexanes); $^1$H NMR δ 1.17 (s, 9 H), 1.42 (s, 9 H), 1.46-1.55 (m, 2 H), 1.57-1.99 (m, 2 H), 2.07-2.11 (bm, 3 H), 2.57 (ddd, 1 H, *J* = 3.0, 6.0, 12.1), 3.09 (bs, 1 H), 3.98 (m, 1 H), 4.35 (bm, 1 H), 5.05-5.20 (m, 2 H), 5.23-5.44 (m, 2 H), 5.56-5.66 (m, 1 H), 7.14-7.19 (m, 7 H), 7.21-7.28 (m, 9 H), 7.30-7.42 (m, 1 H), 7.63-7.66 (m, 1 H); $^{13}$C NMR δ 28.0, 28.4, 32.7, 38.5, 38.6, 53.6, 56.2, 56.3, 65.1, 67.0, 72.0, 72.2, 73.1, 80.0, 80.8, 155.6, 172.7, 174.6. HRMS calcd for $C_{45}H_{53}N_2O_7$ [M+H]: 733.3853, found: 733.3869.

**(2*S*, 6*RS*, 9*S*)-Benzyl 5-[4'-[*N*-(PhF)amino]-4'-(*tert*-butyloxycarbonyl)but-1-enyl]-*N*-(BOC)prolinate (22)** A solution of allylic alcohol **21** (1.71 g, 2.3 mmol) in $CH_2Cl_2$ (30 mL) at 0°C was treated with $Et_3N$ (2.26 mL, 16.3 mmol, 700 mol%) and methanesulfonyl chloride (1.06 mL, 13.6 mmol, 580 mol%). The stirred mixture was left to warm to rt for 20 h, evaporated to dryness and the residue was chromatographed using a gradient of 0-20% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave a 2:1 diastereomeric mixture of prolines **22** (1.53 g, 91%, white foam): TLC $R_f$ = 0.57 (1 : 4 EtOAc : hexanes); $^1$H NMR δ 1.21 (s, 3 H), 1.23 (s, 4 H), 1.24 (s, 2 H), 1.32 (s, 1H), 1.41 (s, 2 H), 1.49 (s, 3 H), 1.50 (s, 3 H), 1.55-2.26 (m, 6 H), 2.66 (m, 1 H), 3.13 (bs, 1 H), 4.27-4.49 (m, 1 H), 5.15 (m, 2 H), 5.32 (m, 1 H), 5.45 (m, 1 H), 7.13-7.26 (m, 7 H), 7.31-7.49 (m, 9 H), 7.66-7.74 (m, 2 H); $^{13}$C NMR δ 27.4, 27.5, 27.9, 28.3, 28.4, 29.3, 29.5, 30.1, 31.7, 34.3, 34.4, 38.3, 38.5, 52.2, 53.1, 55.7, 56.0, 58.3, 58.7, 59.0, 59.2, 59.5, 59.6, 59.9, 62.0, 62.2, 66.5, 66.6, 66.8, 67.0, 72.96, 73.0, 76.3, 77.4, 79.7, 80.0, 80.4, 80.7, 153.3, 154.4, 155.3, 172.2, 172.5, 172.8, 174.2, 174.3. HRMS calcd for $C_{45}H_{51}N_2O_6$ [M+H]: 715.3747, found: 715.3735.

**(2*S*, 6*RS*, 9*S*)-5-[4'-[*N*-(Fmoc)amino]-4'-(*tert*-butyloxycarbonyl)butyl]-*N*-(BOC)proline (23)** A solution of prolines **22** (1.53 g, 2.14 mmol) in THF (15 mL) and MeOH (15 mL) was transferred into a hydrogenation apparatus and treated with palladium-on-carbon (153 mg, 10 wt %). The pressure bottle was filled, vented and refilled four times with 6 atm of $H_2$. The reaction mixture was stirred for 16 h and filtered onto a plug of diatomaceous earth (Celite™) that was washed thoroughly with MeOH. Evaporation of the volatiles gave a crude residue that was dissolved in

acetone (12 mL) and water (5 mL), treated with solid $NaHCO_3$ (180 mg, 2.14 mmol, 100 mol%) and Fmoc-OSu (721 mg, 2.14 mmol, 100 mol%), stirred for 2-3 h, acidified with solid citric acid to pH 4, saturated with solid NaCl and extracted with $CH_2Cl_2$ (3 × 25 mL). The combined organic layers were washed with brine, dried and evaporated to a residue that was chromatographed using a gradient of 20-100% EtOAc in hexanes containing 1% AcOH as eluant. Evaporation of the collected fractions gave acid **23** as a 2:1 diastereomeric mixture from **22**: 1.05 g, 81%, white foam, TLC $R_f$ = 0.30 (EtOAc + 1% AcOH); $^1$H NMR δ 1.25-1.55 (bm, 4 H), 1.44 (s, 3 H), 1.48 (s, 6 H), 1.50 (s, 9 H), 1.58-1.71 (bm, 2 H), 1.84-1.99 (bm, 2 H), 2.03-2.33 (bm, 2 H), 3.80-4.10 (m, 1 H), 4.04-4.58 (bm, 5 H), 5.14 (bd, 0.2 H, *J* = 7.4), 5.53 (bd, 0.4 H, *J* = 7.6), 5.68 (bd, 0.2 H, *J* = 8.0), 6.12 (bs, 0.2 H), 7.14-7.78 (m, 8 H); $^{13}$C NMR δ 22.3, 25.1, 25.3, 25.5, 28.1, 28.4, 28.5, 28.6, 29.1, 32.6, 32.8, 33.6, 33.9, 46.4, 47.3, 53.4, 54.3, 54.4, 58.0, 59.4, 59.6, 60.0, 67.5, 73.0, 80.1, 80.4, 80.8, 82.2, 151.7, 154.1, 155.7, 156.1, 156.3, 168.8, 172.1, 177.2. HRMS calcd for $C_{34}H_{44}N_2O_8Na$ [M+Na]: 631.2996, found: 631.3039.

**(2*S*, 6*RS*, 9*S*)-Allyl 5-[4'-[*N*-(Fmoc)Amino]-4'-(*tert*-butyloxycarbonyl)butyl]-*N*-(BOC)prolinate (24)** A solution of acid **23** (357 mg, 0.60 mmol) in $CH_3CN$ (12 mL) was treated with allyl iodide (108 μL, 2.4 mmol, 400 mol%) and *N*,*N*-diisopropylethylamine (210 μL, 1.2 mmol, 200 mol%), heated at a reflux for 3 h, cooled to rt and evaporated to dryness. The crude residue was chromatographed using a gradient of 0-30% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave **24** as a 2:1 diastereomeric mixture: 334 mg, 86%, white foam, TLC $R_f$ = 0.37 (1 : 4 EtOAc : hexanes); $^1$H NMR δ 1.17-1.50 (m, 4 H), 1.37 (s, 3 H), 1.41 (s, 6 H), 1.43 (s, 9 H), 1.57-1.59 (bm, 2 H), 1.66-1.97 (bm, 3 H), 2.15 (bm, 1 H), 3.75-4.05 (bm, 1 H), 4.15-4.49 (bm, 5 H), 4.49-4.62 (bm, 2 H), 5.23 (bm, 2 H), 5.61 (bm, 0.6 H), 5.73 (bm, 0.3 H), 5.86 (m, 1 H), 7.23-7.26 (m, 2 H), 7.31-7.33 (m, 2 H), 7.55-7.57 (m, 2 H), 7.68-7.70 (m, 2 H); $^{13}$C NMR δ 22.1, 24.9, 25.2, 27.3, 27.4, 27.9, 28.2, 28.3, 28.9, 32.3, 32.5, 33.4, 47.1, 53.4, 54.1, 54.2, 54.8, 57.5, 57.6, 57.8, 59.3, 59.6, 65.3, 65.5, 66.8, 79.5, 79.7, 79.8, 81.5, 81.7, 81.9, 153.7, 154.2, 155.3, 155.8, 156.0, 171.7, 171.8, 172.5, 172.7. HRMS calcd for $C_{37}H_{48}N_2O_8Na$ [M+Na]: 671.3308, found: 671.3333.

**(3*S*, 7*S*, 10*S*)- and (3*S*, 6*R*, 10*S*)-2-Oxo-3-*N*-(Fmoc)amino-1-azabicyclo[5.3.0]decane-10-carboxylate (22)** A solution of allyl ester **24** (793 mg, 1.25 mmol) in $CH_2Cl_2$ (40 mL) at 0°C was treated with HCl bubbles for 1.5 h. The solution was stirred for 1.5 h at 0°C, warmed to rt over 30 min and evaporated to dryness. Hydrochloride **25** was obtained as a white glassy solid: HRMS calcd for $C_{28}H_{33}N_2O_6$ [M+H]: 493.2339, found: 493.2330. A solution of hydrochloride **25** (439 mg, 0.83 mmol) in $CH_2Cl_2$ (200 mL) was treated with azabenzotriazolyl-1,1,3,3-tetramethylaminium hexafluorophosphate (HATU, 631 mg, 1.66 mmol, 200 mol%) and *N*,*N*-diisopropylethylamine (433 µL, 2.49 mmol, 300 mol%). The solution was stirred for 3 h at rt. The volatiles were evaporated and the residue was chromatographed using 0-50% EtOAc in hexanes as eluant. First to elute was concave (3*S*, 7*S*, 10*S*)-**26**: 65 mg, 17%, white foam, TLC $R_f$ = 0.32 (1 : 1 EtOAc : hexanes); TLC $R_f$ = 0.58 (4 : 1 EtOAc : hexanes); $[\alpha]_D^{20}$ –37.8 (*c* 0.5, $CHCl_3$); $^1$H NMR δ 1.61-1.90 (m, 5 H), 2.01-2.13 (m, 4 H), 2.23-2.31 (m, 1 H), 3.86 (m, 1 H), 4.22 (t, 1 H, *J* = 7.2), 4.31 (dd, 1 H, *J* = 5.0, 6.1), 4.34 (d, 2 H, *J* = 7.3), 4.66 (d, 2 H, *J* = 5.9), 4.67-4.70 (m, 1 H), 5.33 (m, 2 H), 5.97 (m, 1 H), 6.25 (d, 1 H, *J* = 5.9), 7.26-7.42 (m, 4 H), 7.60 (d, 2 H, *J* = 7.4), 7.76 (d, 2 H, *J* = 7.5); $^{13}$C NMR δ 27.8, 31.8, 33.1, 34.4, 47.3, 55.0, 59.4, 60.7, 66.0, 67.1, 118.9, 120.1, 125.4, 127.2, 127.8, 131.9, 141.4, 144.1, 144.2, 155.7, 171.3, 171.8. HRMS calcd for $C_{28}H_{31}N_2O_5$ [M+H]: 475.2233, found: 475.2245.

Next to elute was convex (3*S*, 7*R*, 10*S*)-**26**: 129 mg, 33%, white foam; TLC $R_f$ = 0.19 (1 : 1 EtOAc : hexanes); TLC $R_f$ = 0.45 (4 : 1 EtOAc : hexanes); $[\alpha]_D^{20}$ –54.6 (*c* 0.5, $CHCl_3$); $^1$H NMR δ 1.59-1.84 (m, 4 H), 1.97-2.11 (m, 5 H), 2.27 (m, 1 H), 3.78 (m, 1 H), 4.22 (t, 1 H, *J* = 7.0), 4.30 (bs, 1 H), 4.35 (d, 2 H, *J* = 6.5), 4.59-4.65 (bm, 3 H), 5.30 (m, 2 H), 5.93 (m, 1 H), 6.01 (bs, 1 H), 7.25-7.40 (m, 4 H), 7.61 (d, 2 H, *J* = 7.4), 7.75 (d, 2 H, *J* = 7.5); $^{13}$C NMR δ 23.2, 26.7, 28.5, 34.3, 47.3, 53.3, 59.8, 61.3, 65.8, 67.0, 118.6, 120.1, 125.3, 127.2, 127.8, 132.0, 141.4, 144.0, 144.1, 156.1, 170.4, 172.3. HRMS calcd for $C_{28}H_{31}N_2O_5$ [M+H]: 475.2233, found: 475.2245.

**(3*S*, 7*S*, 10*S*)-2-Oxo-3-*N*-(Fmoc)amino-1-azabicyclo[5.3.0]decane-10-carboxylic acid ((7*S*)-7)** A solution of (3*S*, 7*S*, 10*S*)-**26** (60 mg, 0.126 mmol) in $CH_2Cl_2$ (2 mL) and AcOH (18 µL, 0.315 mmol, 250 mol%) was degassed by bubbling nitrogen for 5-10 min, treated with $Pd(PPh_3)_2Cl_2$ (4 mol%) and *n*-$Bu_3SnH$

(68 μL, 0.252 mmol, 200 mol%). The mixture was stirred at rt for 1-2 min, when gas evolution was complete and the color of the solution changed from yellow to amber, and evaporated to dryness. Chromatography of the residue using a gradient of 50-100% EtOAc in hexanes containing 1% AcOH and evaporation of the collected fractions gave (7*S*)-**7** as a foam: 54 mg, 99%; TLC $R_f$ = 0.38 (1% AcOH in EtOAc); $[\alpha]_D^{20}$ –54.0 (*c* 0.3, $CHCl_3$); $^1H$ NMR δ 1.54-1.68 (m, 2 H), 1.83-1.85 (m, 3 H), 1.98-2.08 (m, 3 H), 2.11-2.35 (m, 2 H), 3.86 (m, 1 H), 4.21 (dd, 1 H, *J* = 7.1, 7.3), 4.35 (bd, 3 H, *J* = 7.5), 4.70 (d, 1 H, *J* = 7.7), 6.21 (d, 1 H, *J* = 6.2), 7.29-7.41 (m, 4 H), 7.60 (d, 2 H, *J* = 7.3), 7.76 (d, 2 H, *J* = 7.5); $^{13}C$ NMR δ 26.8, 27.4, 31.3, 32.9, 34.2, 47.0, 54.6, 59.5, 66.9, 119.9, 125.1, 126.9, 127.6, 141.1, 143.7, 143.8, 155.5, 172.4, 174.7. HRMS calcd for $C_{25}H_{27}N_2O_5$ [M+H]: 435.1920, found: 435.1929.

**(3*S*, 7*R* ,10*S*)-2-Oxo-3-*N*-(Fmoc)amino-1-azabicyclo[5.3.0]decane-10-carboxylic acid ((7*R* )-7)** was prepared using the same procedure as described for (3*S*, 7*S*, 10*S*)-**26** using (3*S*, 7*R* ,10*S*)-**26** (123 mg, 0.259 mmol). Evaporation of the collected fractions gave (7*R*)-**7** as a foam: 98 mg, 88%, solid; TLC $R_f$ = 0.19 (1% AcOH in EtOAc); $[\alpha]_D^{20}$ –40.0 (*c* 0.3, $CHCl_3$); $^1H$ NMR δ 1.57-2.29 (bm, 10 H), 3.69 (bm, 1 H), 4.21 (t, 1 H, *J* = 6.8), 4.31-4.35 (bm, 3 H), 4.58 (t, 1 H, *J* = 7.9), 6.08 (bs, 1 H), 7.25-7.46 (m, 4 H), 7.60 (d, 2 H, *J* = 7.3), 7.73 (d, 2 H, *J* = 7.4); $^{13}C$ NMR δ 23.0, 26.4, 28.4, 34.2, 47.3, 53.8, 59.7, 61.6, 67.1, 120.1, 125.4, 127.3, 127.8, 141.4, 144.0, 144.1, 156.3, 171.3, 176.0. HRMS calcd. for $C_{25}H_{27}N_2O_5$ [M+H]: 435.1920, found: 435.1929.

**Enantiomeric Purity of (3*S*, 6*R*, 10*S*)-*tert*-butyl 2-oxo-3-*N*-(BOC)amino-1-azabicyclo[4.4.0]decane-10-carboxylate ((3*S*, 6*R*, 10*S*)-20).** A solution of (3*S*, 6*R*, 10*S*)-**20** (8.1 mg) in MeOH (5 mL) at 0°C was treated with $SOCl_2$ (100 μL), stirred for 2.5 h at rt when TLC (100% EtOAc) showed complete disappearance of the starting material **20**. The volatiles were removed under vacuum, and the hydrochloride **27** was dissolved in THF (1 mL), treated with either (*R*)- or (*S*)-α–methylbenzylisocyanate (7.4 μL, 0.05 mmol, 200 mol%) and $Et_3N$ (7.4 μL, 0.05 mmol, 200 mol%), heated at a reflux for 3 h, cooled and evaporated to residue that was directly examined by proton NMR. The limits of detection were determined by measuring the

diastereomeric methyl ester singlets at 3.71 and 3.65 ppm in $CDCl_3$ in the 400 MHz $^1H$ NMR spectrum. Less than 1% of the (1'*R*)-diastereomer was detected in the spectrum for the (1'*S*)-urea **28**. Purification by chromatography using a gradient of pure hexanes to pure EtOAc as eluant gave ureas **28** having the following spectra.

**Urea (1'*R*)-28:** $^1H$ NMR δ 1.45 (d, 3 H, *J* = 6.8), 1.46-1.69 (m, 4 H), 1.70-1.80 (m, 1 H), 1.81-2.05 (m, 4 H), 2.35 (m, 1 H), 3.50 (m, 1 H), 3.71 (s, 3 H), 4.15 (dd, 1 H, *J* = 5.3, 7.2), 4.25 (m, 1 H), 4.83 (bm, 1 H), 5.27 (bs, 1 H), 5.59 (bs, 1 H), 7.19-7.34 (m, 5 H); HRMS calcd. for $C_{20}H_{28}N_3O_4$ [M+H]: 374.2080, found: 374.2077.

**Urea (1'*S*)-28:** $^1H$ NMR δ 1.45 (d, 3 H, *J* = 6.9), 1.51-1.59 (m, 2 H), 1.62-1.76 (m, 4 H), 1.81-2.06 (m, 3 H), 2.31 (m, 1 H), 3.51 (bd, 1 H, *J* = 4.9), 3.65 (s, 3 H), 4.14 (dd, 1 H, *J* = 5.3, 7.0), 4.34 (dd, 1 H, *J* = 6.0, 8.8), 4.88 (bs, 1 H), 5.37 (bs, 1 H), 5.69 (bs, 1 H), 7.20-7.40 (m, 5 H); HRMS calcd. for $C_{20}H_{28}N_3O_4$ [M+H]: 374.2080, found: 374.2077.

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**Supporting Information Available:** Experimental details for the preparation of **14**, $^1H$ and $^{13}C$ NMR spectra of **6, 7, 17, 21, 22, 24, 26, 28**; and crystallographic data for **20** (33 pages). This material, which is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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$^1$H NMR of its *O*-acetyl derivative, prepared by treating **21** with $Ac_2O$ in DMAP-pyridine at rt for 30 min; $^1$H NMR δ 1.18 (s, 4.5 H), 1.19 (s, 4.5 H), 1.45 (s, 9 H), 1.50-1.68 (m, 3 H), 1.86 (m, 1 H), 2.02 (s, 1.5 H), 2.03 (s, 1.5 H), 2.12 (m, 2 H), 2.60 (m, 1 H), 4.35 (bm, 1 H), 5.05-5.28 (m, 3 H), 5.30-5.38 (m, 1 H), 5.63-5.69 (m, 1 H), 7.16-7.43 (m, 16 H), 7.66-7.69 (m, 2 H).

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# Chapitre 3

# Utilisation de l'acide aminé indolizidine-9-one dans les mimétismes peptidiques

## 3.1 Introduction

Ce chapitre relate l'utilisation de l'acide aminé indolizidine-9-one comme composé mimétique de structures dipeptidiques dans deux peptides d'intérêt biologique. Nous avons incorporé cet acide aminé par synthèse peptidique sur support solide dans les structures de la GnRH et de la leucine-enképhaline.

Nous avons synthétisé un analogue [I$^9$AA]$^{6,7}$-GnRH de l'hormone hypothalamique GnRH dans le but d'évaluer la capacité de l'acide aminé indolizidine-9-one à stabiliser un repliement de type β et à mimer la séquence Gly$^6$-Leu$^7$ dans ce peptide. De plus, nous avons voulu explorer l'influence de la géométrie des angles dièdres et de la dimension des hétérocycles en comparaison avec les résultats présentés dans la littérature. Nous sommes présentement en attente des résultats d'activité biologique pour évaluer si le peptidomimétisme conserve la conformation requise pour l'interaction entre le ligand et le récepteur.

Nous avons aussi synthétisé un analogue de la leucine enképhaline où l'acide aminé indolizidine-9-one remplace la séquence Gly$^2$-Gly$^3$. La conformation de l'analogue [I$^9$AA]$^{2,3}$-leucine-enképhaline a été étudiée par spectroscopie RMN et par dichroïsme circulaire. L'activité biologique de ce peptide a été évaluée *in vitro* et *in vivo*. Le peptide exhibe une activité *in vitro* modeste au niveau des récepteurs opioïdes μ et δ. Les résultats indiquent aussi que le peptide possède une durée d'action analgésique *in vivo* plus longue que celle du peptide naturel.

Globalement, ces travaux démontrent que l'acide aminé indolizidine-9-one peut être incorporé dans les peptides d'intérêt biologique. De plus, nos résultats semblent indiquer que l'I$^9$AA possède la capacité de stabiliser les conformations bioactives de la leucine-enképhaline.

### 3.2. Importance des hormones peptidiques hypothalamiques et de GnRH

Les hormones peptidiques de l'hypothalamus jouent un rôle fondamental dans la régulation et le maintien des fonctions biologiques au sein des mammifères. La sécrétion de ces hormones dans le corps stimule notamment des processus tels que la réponse face au stress, la vasoconstriction, les contractions utérines et la croissance.[1]

Une substance appelée *Gonadotropin Releasing Hormone* (GnRH), qui est sécrétée par l'hypothalamus, est responsable de la cascade biochimique qui mène aux caractéristiques sexuelles et à la reproduction chez les mammifères.[2] Cette hormone peptidique est libérée de façon pulsatile par l'hypothalamus. Elle contrôle la synthèse et la libération des hormones gonadiques telles que LH et FSH à partir de l'hypophyse. Chez l'homme, la libération de LH (*luteinizing hormone*) régule la sécrétion de la testostérone, ainsi que la spermatogenèse. Chez la femme, la FSH (*follicle stimulating hormone*) stimule la formation de la progestérone dans les ovaires, et par conséquent mène à l'ovulation. En résumé, la GnRH contrôle donc le développement chez les enfants, alors que chez l'adulte elle module la fertilité.

Le décapeptide GnRH a été isolé, caractérisé et synthétisé par Schally et Guillemin, pour la première fois en 1971. La séquence des acides aminés a été déterminée à partir d'analyses chimiques et enzymatiques, ainsi que par spectrométrie de masse.[3]

$$(pyro)Glu^{1}\text{-}His^{2}\text{-}Trp^{3}\text{-}Ser^{4}\text{-}Tyr^{5}\text{-}Gly^{6}\text{-}Leu^{7}\text{-}Arg^{8}\text{-}Pro^{9}\text{-}Gly^{10}\text{-}NH_{2}$$

**Figure 1. Séquence des acides aminés de GnRH.**

Depuis sa découverte, plus de 5000 analogues de GnRH ont été synthétisés.[4a] Ceci illustre bien l'intérêt des scientifiques vis-à-vis le potentiel thérapeutique et médical de cette hormone. Les analogues peptidiques de GnRH sont utilisés principalement dans le traitement du cancer de la prostate, du sein, des ovaires, de l'endométriose, ainsi que dans le diagnostic de problèmes associés à l'hypophyse. Les agonistes de GnRH sont des agents capables d'induire l'ovulation chez les femmes qui ont des

problèmes de fertilité ou qui souffrent d'aménorrhée (absence de menstruation). Chez l'homme, ils servent à traiter la puberté tardive et l'infertilité. Puisque GnRH a un contrôle direct sur la fertilité, plusieurs chercheurs ont envisagé l'utilisation d'analogues comme agents de contraception et de castration biochimique réversible.[4] Cette hormone est aussi utilisée en pisciculture en tant qu'agent ovulatoire pour favoriser la reproduction chez les poissons.[5]

### 3.3. Relation structure-activité de GnRH

A partir d'un nombre important d'études de relation structure-activité et de modélisation moléculaire, on a pu tirer les conclusions suivantes (Figure 2).[4,6,7] Les acides aminés aux positions 1-3 sont requis pour maintenir l'activité de sécrétion hormonale, alors que les acides aminés aux positions 6 et 10 sont importants pour la complexation au récepteur. L'incorporation de la D-alanine en position 6 produit un analogue qui possède une activité 3.7 fois supérieure à celle du peptide naturel. Par contre, la substitution par la L-alanine en position 6 mène à un peptide qui possède seulement 4% de l'activité de la GnRH.[6b]

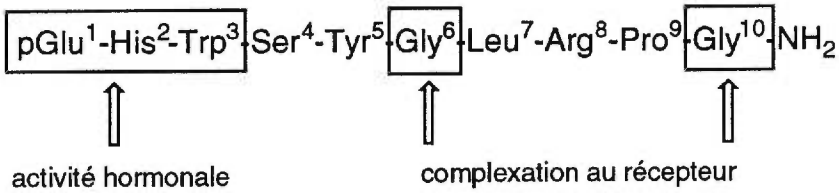



**Figure 2. Relation structure-activité de GnRH.**

Il est généralement reconnu que l'incorporation d'un acide aminé de configuration D suivi d'un acide aminé de configuration L induit une conformation de repliement β dans les peptides.[8] On a donc émis l'hypothèse de l'existence d'un repliement de type β autour des acides aminés 6-7. D'autre part, le remplacement de la leucine-7 par la *N*-méthylleucine maintient l'activité du peptide. La combinaison D-Ala$^6$, *N*-Me-L-Leu$^7$ a donné un analogue encore plus puissant (5.6 fois, Tableau 1). Ces résultats indiquent que le proton de l'amide de la leucine n'est pas impliqué dans un pont d'hydrogène avec les carbonyles de la sérine,

tyrosine, et arginine, et sont tous cohérents avec la présence d'une structure avec un repliement de type β.[6,7b]

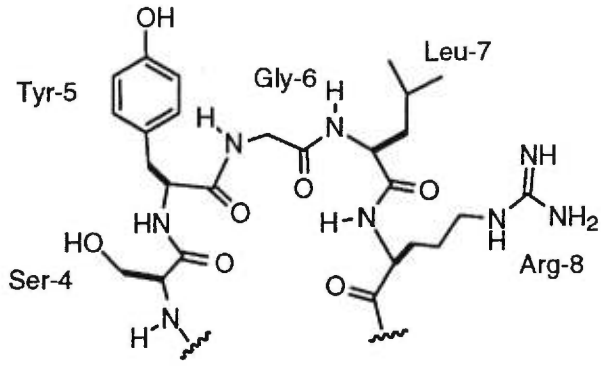



**Figure 3. Segment des résidus 4 à 8 de GnRH.**

**Tableau 1. Relation structure-activité de GnRH autour des résidus 6-7**

| Composé | Puissance relative |
|---|---|
| GnRH | 100 |
| (D-Ala$^6$)-GnRH | 370 |
| (L-Ala$^6$)-GnRH | 4 |
| (*N*-Me-L-Leu$^7$)-GnRH | 100 |
| (D-Leu$^7$)-GnRH | 1 |
| (D-Ala$^6$, *N*-Me-L-Leu$^7$)-GnRH | 560 |

Les résultats de modélisation moléculaire montrent que le peptide a une structure très flexible. On a cependant pu démontrer que les résidus 5 à 8 adoptent des conformations de basses énergies qui ressemblent encore une fois à une structure de repliement de type β.[7]

### 3.4. Rigidification du peptide GnRH avec une lactame de Freidinger

La naissance du peptidomimétisme a apporté une preuve supplémentaire sur la structure tridimensionnelle de GnRH. Les travaux pionniers de Freidinger ont montré l'efficacité des contraintes covalentes pour rigidifier une structure peptidique.[9] Ceci a été initialement démontré par l'utilisation d'une γ-lactame (Figure 3) pour remplacer la glycine-6 du peptide GnRH. En effet, lorsqu'incorporée dans le peptide GnRH, cette γ-

lactame impose une contrainte covalente sur l'angle dièdre $\psi$ de la glycine et force le lien peptidique à garder une géométrie *trans* (angle $\omega$).

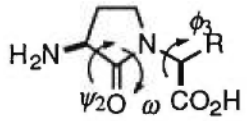



**Figure 4. Structure de la lactame de Freidinger et angles dièdres.**

L'introduction d'une contrainte covalente, sous forme d'une γ-lactame **1** en position 6, a mené à un analogue peptidique **2** qui possède une activité biologique 8.9× supérieure à celle de GnRH (Figure 4). Ces résultats, ainsi que ceux obtenus par substitution des acides aminés, semblent donc tous démontrer la présence d'un repliement de type β autour des résidus 5 à 8 au sein du peptide GnRH.[9]

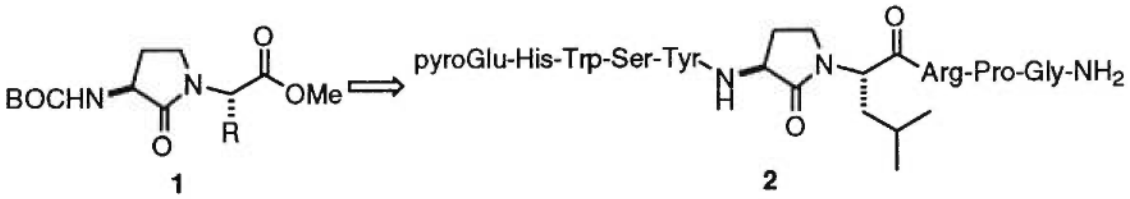



**Figure 5. Analogue de GnRH incorporant une lactame de Freidinger.**

Subséquemment, Nagai et Sato ont incorporé l'acide aminé BTD (**3**) pour remplacer le dipeptide Gly$^6$-Leu$^7$ dans la structure de GnRH. L'analogue peptidique **4** obtenu était 10× moins actif que le peptide naturel.[10]

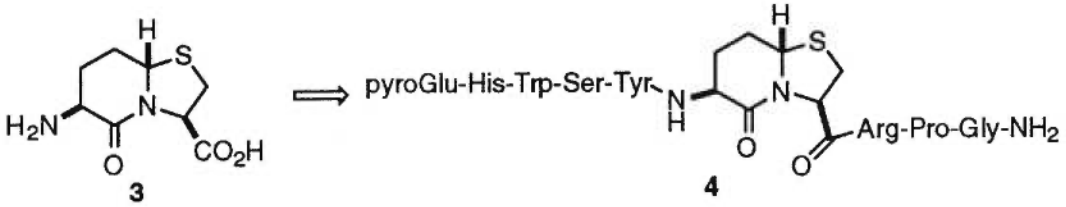



**Figure 6. Analogue de GnRH incorporant le BTD.**

Les auteurs ont émis l'hypothèse que l'analogue **4** possédait une affinité plus faible avec le récepteur en raison de l'absence de la chaîne latérale de la

leucine-7.[10] On peut aussi supposer que le BTD induit une conformation qui n'est pas appropriée pour une meilleure affinité entre le peptide et le récepteur.

### 3.5. Synthèse d'un analogue rigide de GnRH: [I$^9$AA]$^{6,7}$-GnRH

Nous avons synthétisé un acide aminé indolizidine-9-one **5** (I$^9$AA) qui possède des caractéristiques structurales semblables à la lactame de Freidinger.[11] A l'instar de la γ-lactame de Freidinger, le système azabicycloalcane **5** impose une contrainte covalente sur l'angle dièdre $\psi$ de la glycine et maintient le lien peptidique dans une géométrie *trans* (angle $\omega$). De plus, une deuxième contrainte covalente est introduite sur les angles $\phi$ et $\chi$ autour de la leucine-7.

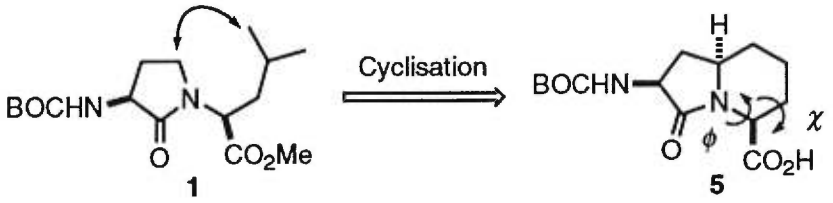



**Figure 7. Relation structurale entre la lactame de Freidinger et l'I$^9$AA.**

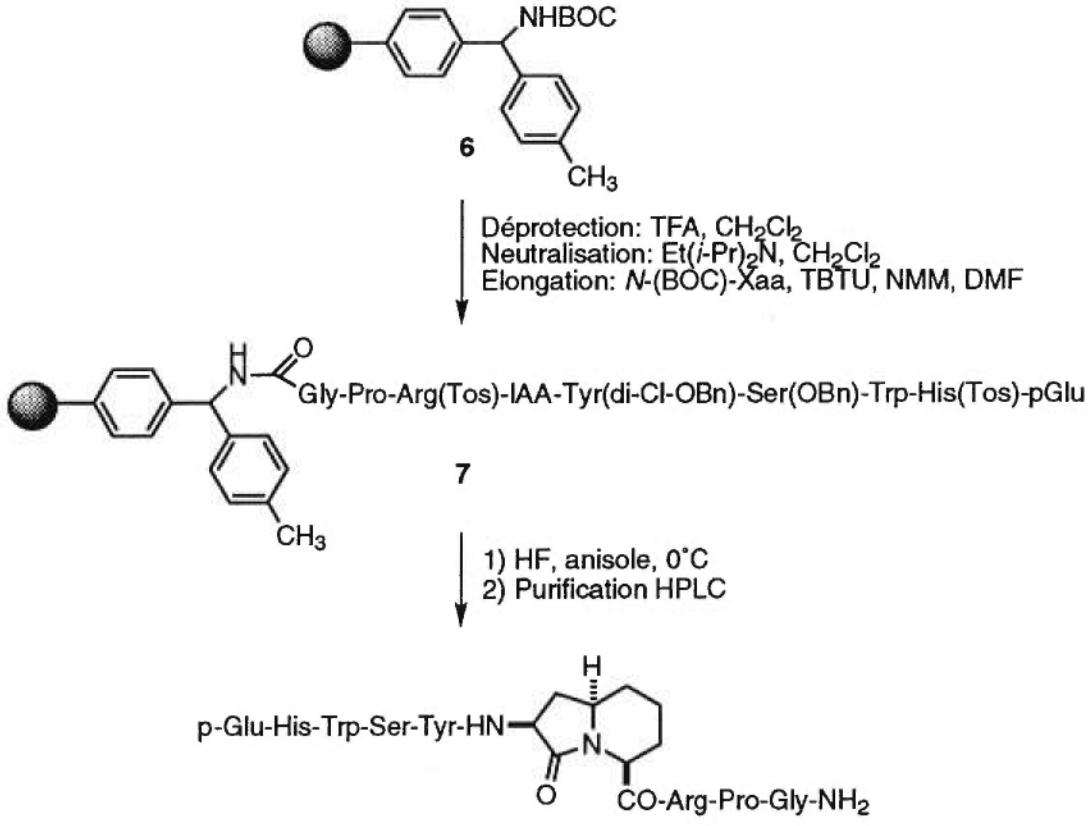
D'après les données cristallographiques des *N*-(BOC)amino esters de méthyle de l'acide aminé indolizidine-2-one et de l'I$^9$AA, on sait que la dimension a un impact important sur la géométrie des angles dièdres internes des hétérocycles.[11a] Dans le but de vérifier si l'I$^9$AA **5** pourrait mimer la séquence Gly$^6$-Leu$^7$ de GnRH et du même coup étudier l'effet de la grosseur de l'hétérocycle sur l'activité biologique, nous avons synthétisé [I$^9$AA]$^{6,7}$-GnRH en incorporant notre analogue bicyclique dans le peptide GnRH.

### 3.6. Résultats

L'incorporation de l'acide aminé indolizidine-9-one **5** dans la structure analogue de GnRH a été accomplie sur une résine de polystyrène méthylbenzhydrylamine **6**, en utilisant le protocole développé par Merrifield[12] pour le couplage peptidique (Schéma 1). Cette résine a été choisie dans le but d'obtenir une amide primaire en position *C*-terminale

du peptide.[13] Nous avons utilisé le tétrafluoroborate de benzotriazolyle 1,1,3,3-tétraméthylaminium (TBTU)[14] comme agent de couplage dans le *N*,*N*-diméthylformamide, en présence de *N*-méthylmorpholine comme base. La déprotection des groupes BOC a été effectuée avec un cocktail de TFA:$CH_2Cl_2$:anisole (49:49:2). L'assemblage du peptide a été fait par un processus de couplage / déprotection successifs. Le traitement de la résine avec l'acide fluorhydrique liquide (anhydre), en présence d'anisole comme capteur de carbocations, a fourni le peptide brut après lyophilisation. L'analyse et la purification de ce peptide par HPLC en phase inverse a donné le peptide final avec un rendement global de 3% à partir de l'acide aminé indolizidine-9-one. La pureté du peptide a été déterminée par analyse HPLC comme étant >99%. L'analyse du peptide par spectrométrie de masse a démontré que le peptide possédait une masse moléculaire conforme à la structure désirée.

**Schéma 1. Synthèse du peptide [I$^9$AA]$^{6,7}$-GnRH**




**[I$^9$AA]$^{6,7}$-GnRH 8**

Nous avons obtenu un rendement global de 3% (4 mg) du peptide [I$^9$AA]$^{6,7}$-GnRH **8** après une synthèse de 19 étapes à partir de la résine **6**. Le faible rendement obtenu provient probablement des couplages qui étaient plus difficiles, notamment entre Pro$^8$ et Arg$^9$, ainsi qu'entre His$^2$ et Trp$^3$. Les essais préliminaires de déprotection avec HF sur des périodes prolongées ont fourni essentiellement des fragments peptidiques de faibles masses. Ceci pourrait indiquer que le peptide est sensible aux conditions de déprotection. De plus, nous avons aussi isolé un peptide protégé par un groupe *p*-toluènesulfonyle.

L'activité biologique du peptide GnRH est évaluée *in vitro* en mesurant la capacité de l'analogue à stimuler la sécrétion des hormones LH et FSH à partir de l'hypophyse. Par ailleurs, l'évaluation *in vivo* est effectuée en déterminant le potentiel d'induction de l'ovulation chez la rate. Ces méthodes seront utilisées pour évaluer l'activité biologique du peptide [I$^9$AA]$^{6,7}$-GnRH **8**.

L'analyse conformationnelle du peptide [I$^9$AA]$^{6,7}$-GnRH **8** sera effectuée en utilisant la spectroscopie RMN et le dichroïsme circulaire. En raison des délais requis pour l'évaluation de l'activité biologique, nous sommes présentement en attente de la livraison d'un échantillon du peptide pour débuter l'analyse conformationnelle.

### 3.7. Conclusion

Les résultats obtenus montrent que l'acide aminé indolizidine-9-one peut être incorporé dans les peptides d'intérêt biologique. L'activité biologique du peptide [I$^9$AA]$^{6,7}$-GnRH est présentement sous évaluation en Belgique. Les résultats de ces tests permettront l'évaluation de la capacité de l'I$^9$AA à stabiliser une conformation de repliement de type β dans le peptide GnRH. De plus, l'évaluation biologique permettra une comparaison par rapport l'hormone naturelle, et les résultats obtenus avec l'analogue incorporant la γ-lactame de Freidinger et l'analogue incorporant le BTD de Nagai-Sato.

### 3.8. Partie expérimentale

**Général.** Le DMF a été distillé sur l'anhydride benzènetricarboxylique. Le dichlorométhane a été distillé sur $CaH_2$. Les solvants ont été entreposés à l'abri de la lumière, sur le tamis moléculaire 3Å. Les acides *N*-(BOC)aminés, l'acide trifluoroacétique, la *N*-méthylmorpholine ont été obtenus de sources commerciales et ont été utilisés sans purification. L'acide aminé *N*-(BOC)indolizidine-9-one a été synthétisé en 9 étapes et >25% de rendement à partir de l'acide aspartique par une séquence d'oléfination de Horner-Wadsworth-Emmons / amination réductrice / cyclisation de lactame.[11] La synthèse peptidique a été effectuée en utilisant un synthétiseur de peptide semi-automatique Labortec SP640. La chromatographie HPLC analytique et préparative ont respectivement été effectuées sur un instrument Spectra Physics 8100 et sur un appareil HPLC Gilson fonctionnant avec un contrôleur Gilson 712.

**Synthèse et analyse du peptide [I$^9$AA]$^{6,7}$-GnRH (8)**

La synthèse du peptide a été effectuée sur une résine de polystyrène méthylbenzhydrylamine **6** (MBHA, 1.12 mmol / g, 2% réticulée avec le divinylbenzène) comme support solide, en utilisant un synthétiseur de peptide semi-automatisé. Les acides *N*-(BOC)-aminés (200-400 mol%) ont été couplés en utilisant le TBTU (400 mol%) dans le DMF, en présence de *N*-méthyl morpholine (400 mol%) comme base. La déprotection des groupes BOC a été faite par un traitement avec une solution de TFA:$CH_2Cl_2$:anisole (49:49:2) pendant des périodes successives de 5 min et 15 min. La résine a ensuite été traitée avec un excès de *N*,*N*-diisopropyléthylamine (20% dans le $CH_2Cl_2$) pour neutraliser les amines. La synthèse a été poursuivie et la complétion de chaque étape de couplage a été évaluée avec le test de ninhydrine de Kaiser ainsi que le test chloranil.[15,16] Le peptide final lié au support solide a été séché dans un dessicateur sous vide pendant la nuit. Une portion de la résine **7** (0.2 mmol) a été traitée avec l'acide fluorhydrique liquide (HF, 5 mL) en présence d'anisole (1 mL) à 0°C pendant 90 minutes. *Attention: le HF est un gaz hautement toxique et corrosif qui doit être manipulé dans une hotte bien ventilée.* Le mélange réactionnel a été traité avec l'éther diéthylique anhydre (2x25 mL) pour précipiter le peptide. Les volatiles ont été évaporés

sous vide puisque le peptide ne précipitait pas et était soluble dans la plupart des solvants organiques. Le solide brut a été dissous dans l'acide acétique glacial et lyophilisé pendant la nuit. Le peptide brut [SMBR calc. pour $C_{56}H_{73}N_{17}O_{13}$ [$MH^+$] = 1192; trouvé: 1192] a été isolé sous forme d'une poudre colorée. Le peptide brut a été analysé par HPLC en phase inverse sur une colonne C18 (Vydac): $t_R$ = 13.8 min. (M); $t_R$ = 16.8 min.; (M+tosyl), en utilisant un gradient de 0-80% $CH_3CN$ dans $H_2O$ contenant 0.1% TFA, débit: 1.0 mL / min, $\lambda$ = 215 nm). La purification du peptide brut par HPLC préparatif en phase inverse sur une colonne C18 (Vydac, 15-20 $\mu$) en utilisant un gradient de 0-80% $CH_3CN$ dans $H_2O$ contenant 0.1% TFA (débit: 13.0 mL / min, $\lambda$ = 215 nm) a fourni, après lyophilisation des fractions récupérées, le peptide pur **8** (4 mg, 3% à partir de la résine **6**) sous forme d'une poudre blanche.

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# Article 4

Gosselin, F.; Tourwé, D.; Lubell, W. D. "Probing Opioid Receptor-Ligand Interactions by Employment of Indolizidin-9-one Amino Acid as a Constrained $Gly^2$-$Gly^3$ Surrogate in a Leucine-Enkephalin Mimic" Soumis à *The Journal of Peptide Research*, **1999**.

# Probing Opioid Receptor-Ligand Interactions by Employment of Indolizidin-9-one Amino Acid as a Constrained $Gly^2$-$Gly^3$ Surrogate in a Leucine-Enkephalin Mimic

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**3.10. Abstract**

The relationship between the conformation and biological activity of Leu-enkephalin was studied by the use of (2*S*, 6*R*, 8*S*)-9-oxo-8-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-2-carboxylic acid [(2*S*, 6*R*, 8*S*)-**1**, $I^9AA$] as a constrained $Gly^2$-$Gly^3$ dipeptide surrogate. [$I^9AA$]$^{2,3}$-Leu-enkephalin **12** was assembled using solid-phase peptide synthesis on Merrifield resin with TBTU as coupling reagent. In preliminary*in vitro* assays, [$I^9AA$]$^{2,3}$-Leu-enkephalin **12** exhibited agonist activity at the μ and δ receptors that was lower than that of Leu-enkephalin. In preliminary *in vivo* assays the indolizidinone analogue **12** showed significantly enhanced duration of action relative to the parent peptide. Conformational analysis was performed using NMR and CD spectroscopy. The amide temperature coefficients and $^3J$ NH-CαH coupling constants for **12** did not indicate a hydrogen-bonded β-turn structure; however, its CD spectrum supported a turn conformation. Incorporation of indolizidinone amino acid **1** into Leu-enkephalin, has thus provided additional support for the importance of a turn conformation for biological activity of the native peptide.

**Key Words:** Azabicyclo[X.Y.0]alkane amino acids, peptide mimics, conformational analysis, Leu-enkephalin, biological activity, indolizidinone amino acid.

### 3.11. Introduction

Replacement of a peptide secondary structure with a geometrically constrained surrogate can stabilize the biologically active conformation and increase the metabolic stability of the endogenous peptide (1). Because azabicyclo[X.Y.0]alkane amino acids can rigidify the three contiguous $\psi$, $\omega$ and $\phi$ dihedral angles within a peptide backbone (Figure 1), their insertion into a constrained peptide mimic can provide insight on the spatial requirements for peptide-receptor recognition (2). Among other examples, incorporation of azabicyclo[X.Y.0]alkane amino acids into biologically active peptides has led to active analogues of cyclosporin A (3), gramicidin S (2,4,5,6,7), and calcitonin gene related peptide8-37 (8).

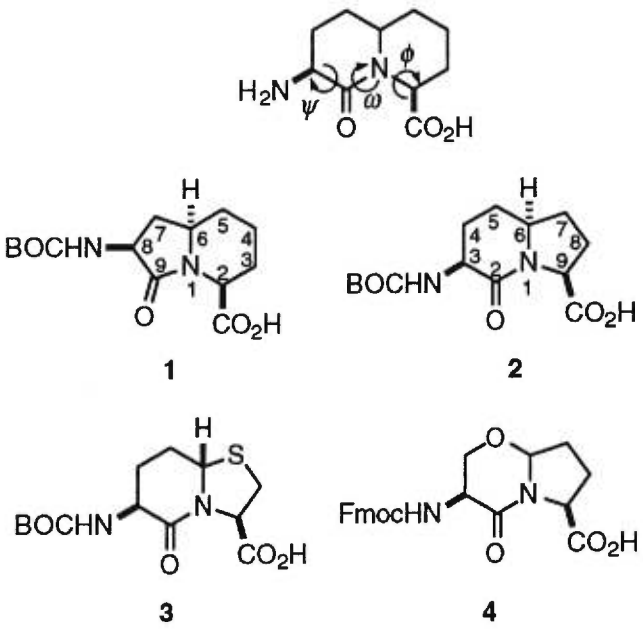



**Figure 1.** A Representative Azabicyclo[X.Y.0]alkane Amino Acid Analogue Exhibiting Constrained Dihedral Angles and Related Indolizidin-9-one, Indolizidin-2-one, 7-Thiaindolizidin-2-one and 5-Oxaindolizidin-2-one Amino Acids **1-4**.[2]

The enkephalins (Figure 2) are endogenous pentapeptides that display opioid-like biological activity (9). The use of enkephalins for the treatment of pain has been limited by their lack of metabolic stability and

low bioavailability. Furthermore, side-effects such as respiratory depression and physical dependence have been observed when opioid peptides were administered to rats. These side-effects may be a consequence of non-selective binding to more than one of the recognized μ, δ, and κ-receptors, and can be attributed to the conformational flexibility of the enkephalins (10).

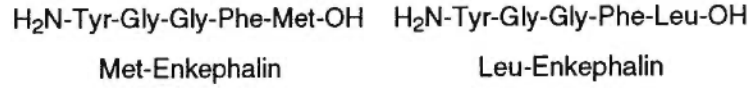



**Figure 2.** Amino Acid Sequence of the Enkephalins.

Leucine-enkephalin has been studied by X-ray crystallography, spectroscopic methods and molecular modeling (11-18). Although a number of reports have indicated that the enkephalins are flexible molecules that exist in several energetically similar conformational states, several lines of evidence have suggested the preference for a turn conformation centered around the $Gly^2$-$Gly^3$ as well as the $Gly^3$-$Phe^4$ residues. For example, in one X-ray crystallographic structure of Leu-enkephalin, hydrogen bonds could be inferred to exist between the $Phe^4$ NH and $Tyr^1$ C=O, and the $Leu^5$ NH and $Gly^2$ C=O (12). However, other studies of Leu-enkephalin by X-ray diffraction have shown extended structures (13). Conformational analysis of Leu-enkephalin by NMR spectroscopy has detected both extended and folded conformations depending on the environment (14-18). The folded structures of Leu-enkephalin have been found to exhibit either type I (16), I' (17), II' (14,17,18) and III (16) β-turn conformations. These results illustrate clearly the conformationally dynamic structure of Leu-enkephalin as well as the difficulties associated with determining its biologically active conformation.

In an intense effort to understand the relationship between their conformation and bioactivity and to improve their pharmacological profile, more than 1000 analogues of the enkephalins have been synthesized since their discovery in 1975.[9] Probing for the existence of turn

conformations, a number of conformationally constrained amino acids have been incorporated into analogues of enkephalins (Figure 3). For example, by constraining the $Gly^2$ residue of methionine-enkephalin in a lactam ring, activity was found to be dependent on ring size and stereochemistry (19). The (*R*)-δ-lactam **7** was most active, with 2-10% the activity of the parent peptide (19). Incorporation of the 7-thiaindolizidinone amino acid **3** (BTD) as a Gly-Gly replacement in Leu-enkephalin produced an analogue **9** which exhibited 1 /500 of the activity of the parent peptide (4). Similarly, the 5-oxaindolizidinone amino acid **4** has been used as a surrogate for the Gly-Phe residues in Leu-enkephalin analogue **10**; however, no biological data was reported (20). When an azepinone was used as a replacement of the Gly-Phe dipeptide, the doubly constrained Leu-enkephalin analogue **11** was also inactive (21).

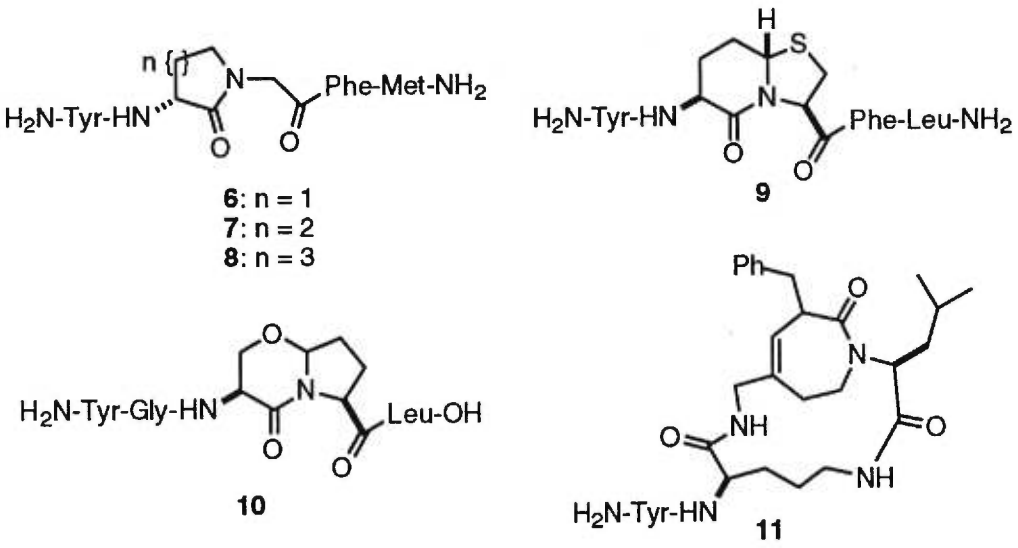



**Figure 3.** Structurally Constrained Enkephalins.

Comparison of X-ray crystallographic data of the methyl esters of indolizidinone amino acids **1** and **2** prepared in our laboratory (22-24), revealed that the ring size of the heterocycle had a profound impact on the geometry of the peptide backbone as illustrated in Table 1 and Figure 4 (22). Furthermore, the dihedral angles of the backbone atoms constrained inside (2*S*, 6*R*, 8*S*)-methyl 9-oxo-8-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-2-carboxylate ((2*S*, 6*R*, 8*S*)-**1**, $I^9AA$) resemble the values of the central residues in an ideal type II' β – turn (25,26). We became

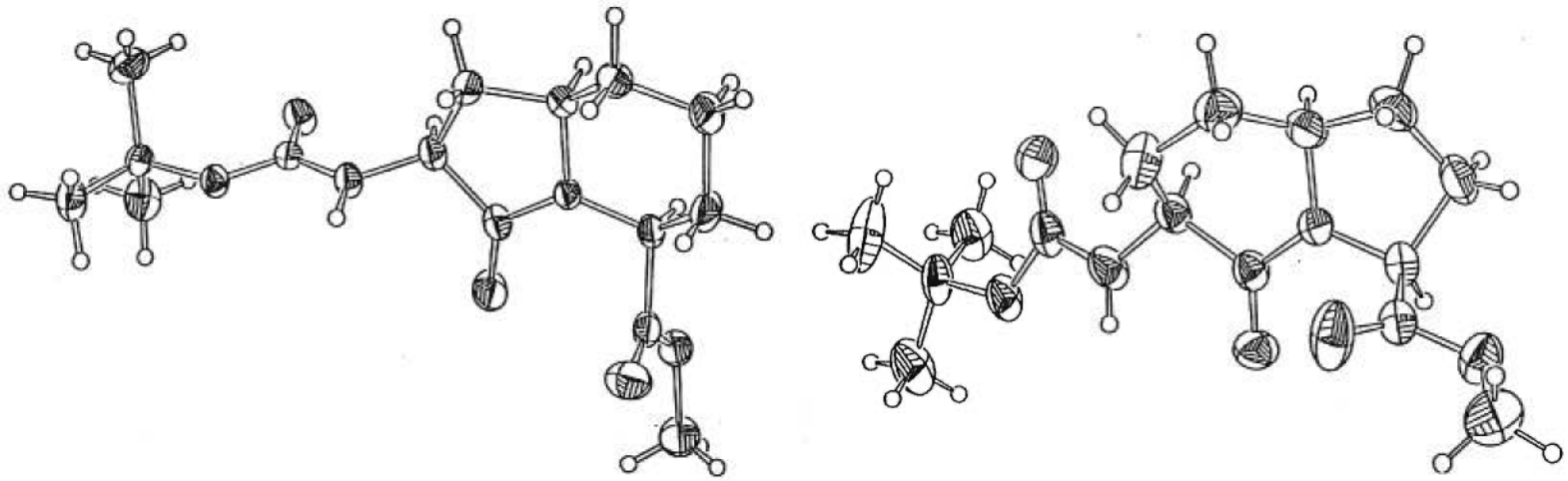


**Figure 4. X-ray Crystallographic Structures of N-(BOC)amino Indolizidin-9-one and Indolizidin-2-one Methyl Esters.**

**Table 1. Comparison of the Dihedral Angles from Azabicycloalkane X-ray Data and Ideal Peptide Turns**

| Entry | $\Psi$, deg | $\phi$, deg |
|---|---|---|
| (3*S*, 6*S*, 9*S*)–methyl-3-*N*-(BOC)amino indolizidin-2-one 9-carboxylate[22] | –176 | –78 |
| (2*S*, 6*R*, 8*S*)–methyl-8-*N*-(BOC)amino indolizidin-9-one 2-carboxylate[21] | –141 | –34 |
| Type II' β-turn *i*+1 and *i*+2 residues[24] | –120 | –80 |
| Inverse γ-turn *i*+2 residue[25] | | –80 |

thus interested in using the (2*S*, 6*R*, 8*S*)-indolizidin-9-one amino acid **1** as a turn mimic in Leu-enkephalin. Hypothesizing that this rigid scaffold would display the phenylalanine and tyrosine aromatic rings in positions required for interaction with the endogenous opioid receptors, $I^9AA$ **1** was used as a $Gly^2$-$Gly^3$ surrogate. Conformational analysis of the resulting constrained analogue of Leu-enkephalin was subsequently performed using NMR and CD spectroscopy. Although amide temperature coefficient measurements and $^3J_{NH}$ coupling constants in Leu-$I^9AA$-enkephalin **12** did not indicate a hydrogen-bonded β-turn structure, the CD spectrum supported a turn conformation. Furthermore, the biological activity of **12** demonstrated that azabicycloalkane amino acid **1** may stabilize a bioactive conformation and enhance the metabolic stability of Leu-enkephalin.

### 3.12. Experimental Section

**General.** DMF was distilled from benzenetricarboxylic acid anhydride, dichloromethane was distilled from $CaH_2$. Both solvents were stored protected from light over activated 3Å molecular sieves. *N*-(BOC)-L-Phenylalanine, *O*-(2,6-dichlorobenzyl) *N*-(BOC)-L-tyrosine, *N*-methylmorpholine and trifluoroacetic acid were used as purchased. Enantiopure indolizidin-9-one *N*-(BOC)amino acid **1** was synthesized in 9 steps and >25% overall yield from inexpensive aspartic acid as chiral educt via our Horner-Wadsworth-Emmons olefination / reductive amination / lactam cyclization sequence (22,24).

**Peptide Synthesis**

Peptide synthesis was performed on a Labortec SP640 semi-automated peptide synthesizer. Analytical HPLC was performed on a Spectra Physics 8100 instrument, and semi-preparative HPLC on a Gilson system run by the Gilson 712 HPLC system controller. *N*-(BOC)-L-Leucine was anchored by alkylation of its cesium carboxylate salt with Merrifield resin (chloromethylated cross-linked polystyrene, (27)) in *N*,*N*-dimethylformamide at 60-70°C to give a loading of 0.78 mmol / g as determined by a picric acid test (28). Cleavage of the BOC groups was effected by treatment with a cocktail of TFA:$CH_2Cl_2$:anisole (49:49:2) for successive periods of 5 min and 15 min. The resin was then neutralized

with 20% *N,N*-diisopropylethylamine in $CH_2Cl_2$ and peptide coupling was continued. *N*-(BOC)Amino acids (200-400 mol%) were coupled using benzotriazol-1-yl-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU, 400 mol%) in DMF, in the presence of *N*-methyl morpholine (400 mol%) as base (29). The completion of each coupling step was monitored using the Kaiser ninhydrin test and the chloranil test (30,31).

Cleavage of the final peptide from the solid support (256 mg, 0.2 mmol) was achieved by treatment with anhydrous liquid HF (5 mL) at 0°C for 90 minutes in the presence of anisole (1 mL). *Caution: HF is a highly toxic, corrosive gas that must be handled in a well ventilated hood.* The reaction mixture was treated with anhydrous diethyl ether (2 × 25 mL) to precipitate the peptide. Filtration, and washing of the resin with glacial acetic acid (2 × 50 mL) afforded a clear solution that was lyophilized overnight to give the crude peptide **12** (105 mg, 85% yield from the resin-bound *N*-(BOC)-L-leucine, LRMS calcd for $C_{33}H_{44}N_5O_7$ $[MH^+]$ = 622; Found: 622) as a white powder. A ninhydrin test was performed on the resin after cleavage and showed that no residual peptide remained attached to the polymer support. The crude peptide was shown to be of greater than 78% purity by analytical HPLC ($t_R$ = 17.7 min., Vydac 218TP54 C18 column, 0-80% $CH_3CN$ in $H_2O$ containing 0.1% TFA, flow rate: 1.0 mL / min, $\lambda$ = 215 nm). Purification of a portion of the crude peptide (80 mg) by reverse-phase preparative HPLC on a C18 column (15-20 μ) using a gradient of 0-80% $CH_3CN$ in $H_2O$ containing 0.1% TFA (flow rate: 13.0 mL / min, $\lambda$ = 215 nm) gave, after lyophilisation of the collected fractions, the pure $[I^9AA]^{2,3}$-Leu-enkephalin **12** (62 mg, 65% overall yield, >99% purity by analytical HPLC) as a white powder. HRMS calcd $C_{33}H_{44}N_5O_7$ $[MH^+]$ = 622.3241; Found: 622.3222.

**NMR Measurements**

$^1H$ and $^{13}C$ NMR experiments of 2.5 mM solutions of peptide were performed on a Bruker DMX 600 spectrometer. Chemical shifts are reported in ppm, with splitting patterns abbreviated as follows: s (singlet), d (doublet), t (triplet), m (complex multiplet). COSY, NOESY and ROESY spectra were obtained with 2048 by 512 data points. A mixing time of 500

ms was used for NOESY and ROESY spectra. The temperature coefficients of the amide proton chemical shifts in DMSO-$d_6$ were measured for 7 different temperatures in 5 degree intervals by varying the temperature between 298 and 328 K. The values of the temperature coefficients were obtained by a linear least-squares fit of the data (32).

**[I$^9$AA]$^{2,3}$-Leu-enkephalin 12**: $^1$H NMR ($H_2O$ containing 10% $D_2O$) δ 0.80 (d, 3 H, *J* = 6.2, Leu-$CH_3$), 0.84 (d, 3 H, *J* = 6.4, Leu-$CH_3$), 1.05 (m, 1 H, IAA-H$^4$), 1.44 (m, 1 H, IAA-H$^4$), 1.45 (m, 1 H, IAA-H$^7\alpha$), 1.57 (m, 2 H, Leu-β), 1.59 (m, 2 H, IAA-H$^3$), 1.71 (m, 1 H, IAA-H$^5$), 1.76 (m, 1 H, IAA-H$^5$), 2.36 (m, 1 H, IAA-H$^7\beta$), 2.86 (dd, 1 H, *J* = 10.4, 14.0, Phe-β), 3.01 (dd, 1 H, *J* = 8.0, 14.0, Tyr-β), 3.09 (dd, 1 H, *J* = 6.9, 13.9, Tyr-β), 3.19 (dd, 1 H, *J* = 5.2, 14.0, Phe-β), 3.47 (m, 1 H, IAA-H$^6$), 4.08 (dd, 1 H, *J* = 6.7, 7.9, Tyr-α), 4.15 (m, 1 H, IAA-H$^8\alpha$), 4.29 (m, 1 H, Leu-α), 4.52 (d, 1 H, IAA-H$^2$), 4.66 (m, 1 H, Phe-α), 6.82 (m, 2 H, Tyr-Ar$CH$), 7.10 (m, 2 H. Tyr-Ar$CH$), 7.19-7.31 (m, 5 H, Phe-Ar$CH$), 8.10 (d, 1 H. *J* = 8.3, Phe N-H), 8.19 (d, 1 H, *J* = 7.9, Leu N-H), 8.52 (d, 1 H, *J* = 7.4, IAA N-H).

**Circular Dichroism Measurements**

CD spectra of 0.1 mM solutions of peptide **12** in $H_2O$ were measured on a Jasco J-710 spectropolarimeter using a circular quartz cell with a path length of 1 mm at 23°C. Spectra were run with a bandwidth of 1 nm, a response time of 0.25 s, and a scan speed of 100 nm min$^{-1}$. Each measurement was the average result of 10 repeated scans in steps of 0.2 nm. Baseline spectra of the solvents were subtracted.

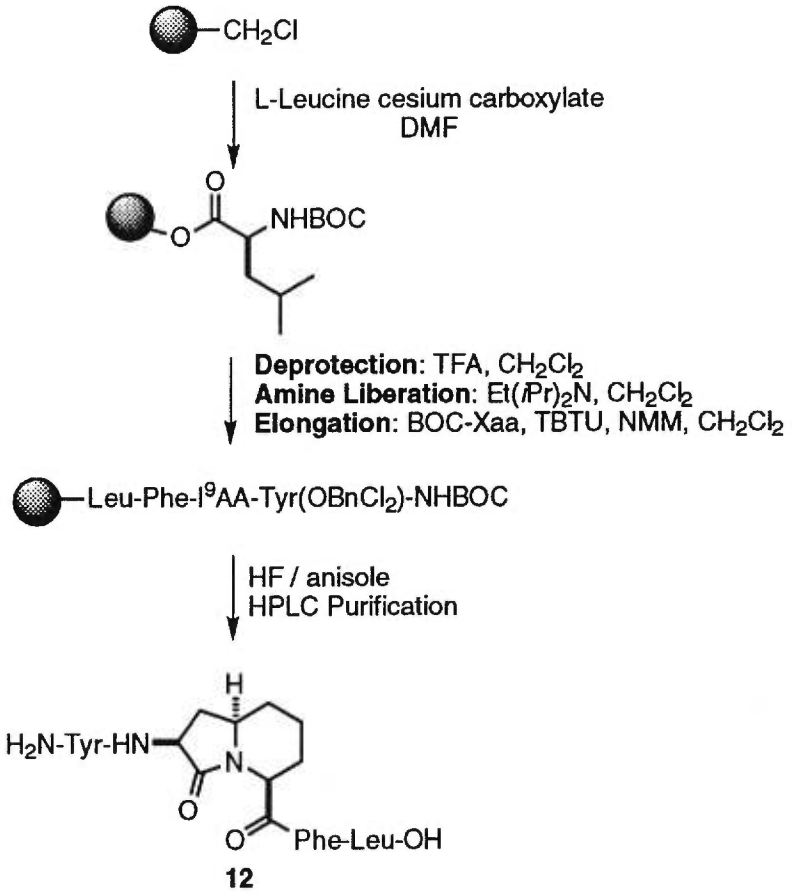
**Biological Evaluation**

The signal transduction properties of **12** (agonist / antagonist) were evaluated using receptor-activated [$^{35}$S] GTPγS binding measurement as a marker of the ability of an agonist to activate the receptor. Measurement of spinal analgesia in rats was performed on rats equipped four days prior to testing with a spinal catheter intrathecally. Animals (n = 3) were injected with compound **12** at doses of 5, 10, and 40 μg / rat. Tail withdrawal was measured at a water temperature of 55°C at different time intervals until 120 minutes after the treatment.

### 3.13. Results

[$I^9AA$]$^{2,3}$-Leu-enkephalin **12** was prepared using the solid-phase strategy introduced by Merrifield as illustrated in Scheme 1 (27). *N*-(BOC)Amino acids were sequentially coupled on chloromethylated polystyrene resin with benzotriazol-1-yl 1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) as coupling reagent in DMF (29).

**Scheme 1. Synthesis of [$I^9AA$]-Leu-Enkephalin**




Treatment of the resin with anhydrous liquid hydrogen fluoride in the presence of anisole as carbocation scavenger afforded the crude peptide that was lyophilized and purified by preparative reverse-phase HPLC. Freeze-drying of the collected fractions gave pure peptide in 65% overall yield from indolizidin-9-one amino acid **1**. The composition of peptide **12** was assessed using fast-atom bombardment mass spectrometry (FAB-MS), and

the purity of **12** was determined to be greater than 99% by analytical reverse-phase HPLC.

The conformation of the [$I^9$AA]$^{2,3}$-Leu-enkephalin **12** was first examined using NMR spectroscopy. The $^1$H NMR spectrum of **12** in 9:1 $D_2O$ / $H_2O$ showed well resolved signals that were assigned using COSY and NOESY experiments. First, the α-protons of all residues were assigned through connectivity with the amide NH protons and the methylene protons of the aromatic side-chains of Tyr and Phe. The relative stereochemistry between the bridgehead, *C*-terminal and *N*-terminal protons of the IAA-9-one residue was confirmed by the combined use of COSY, NOESY and ROESY methods. Transfer of magnetization was observed between the *C*-2, *C*-6 and *C*-8 protons of the indolizidinone residue. All other protons were assigned by analysis of the COSY spectrum.

Distinct $^3J_{NH\text{-}C\alpha H}$ coupling constants (~3 Hz ≥ $^3J_{NH\text{-}C\alpha H}$ ≥ ~10 Hz) can provide information on the peptide backbone conformation (32). Because coupling constants may be averaged due to conformational equilibrium on the NMR time scale, this parameter is less sensitive for analyzing flexible peptides (33, 34, 35). The $^3J_{NH\text{-}C\alpha H}$ coupling constants in **12** for all residues were not distinctive in the range of 7-9 Hz. As determined through the use of the Karplus-Bystrov equation, no clear conformational preference on the $\phi$ dihedral angles was indicated for peptide **12** (33). In addition, the NOESY and ROESY spectra did not show close through-space interactions between the Tyr and the Phe residues, as would be expected in a tight reverse-turn structure (36).

The ability of solvent to interact with the amide proton in a peptide is related to its temperature coefficient as measured by NMR spectroscopy. A value smaller than –2 ppb / K indicates usually a solvent shielded amide proton and a value greater than –4 ppb / K indicates a solvent-exposed amide proton in DMSO (34). Measurement of the chemical shift variation with temperature ($\Delta\delta$ / $\Delta$T) for the amide protons of **12** in DMSO-$d_6$, showed that all of the amide protons were solvent-exposed with temperature coefficients ranging from –4 to –7 ppb / K (32).

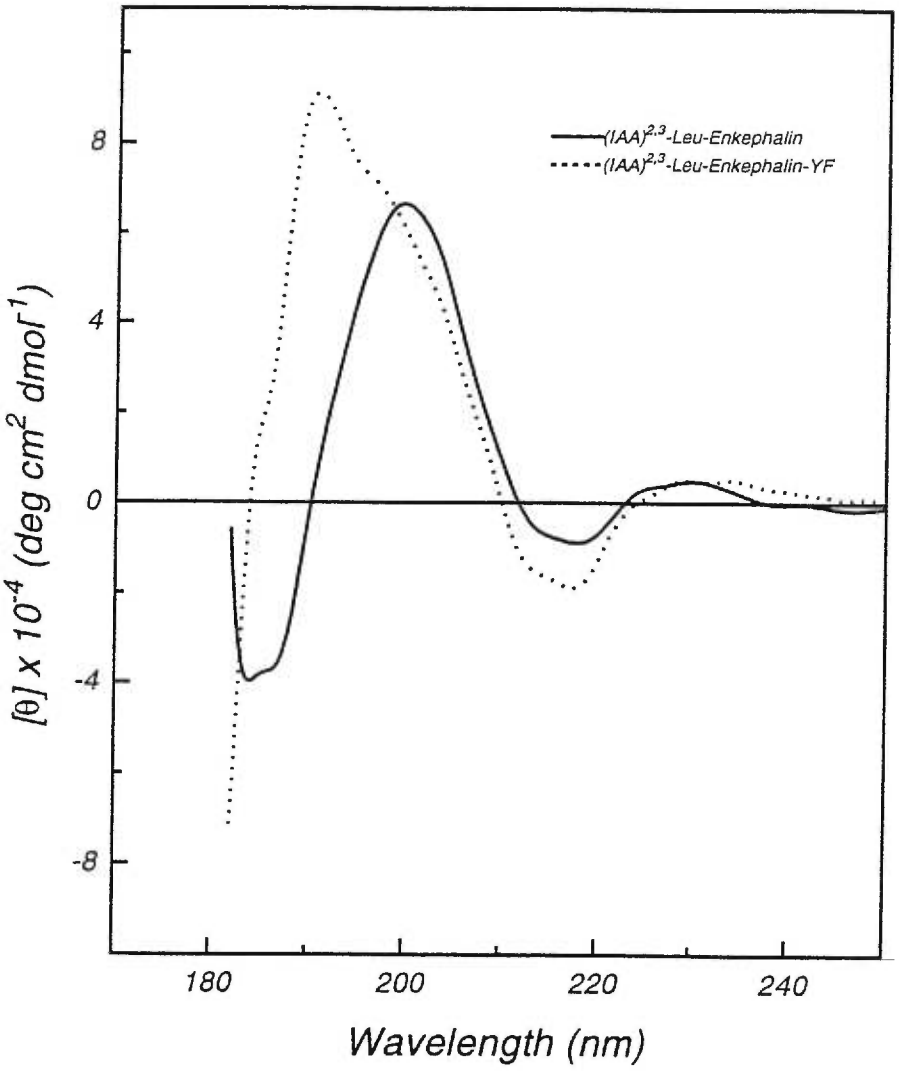



**Figure 5. Circular Dichroism Spectrum of [I$^9$AA]-Leu-Enkephalin 12 Recorded at a Concentration of 0.1 mM in $H_2O$ at 23°C, (Y = tyrosine, F = phenylalanine).**

Circular dichroism spectroscopy (CD) was next used to determine if the peptide was found to exist in a folded conformation in solution (Figure 5). The CD spectrum of **12** in water showed a minimum at 185 nm and a maximum at 200 nm. Previous studies of model peptides in water have shown that a CD curve shape of this type is consistent with β-turn conformations (34). Indeed, this data corresponds well with the theoretical and experimental values for β-turns which exhibit a CD spectrum with a

minimum at 190 nm and a maximum at 210 nm (35,37). In order to eliminate the dichroic effects of the aromatic side-chains of Tyr and Phe, we subtracted the spectrum of an equimolar mixture of L-tyrosine and L-phenylalanine, measured at the same sample concentration, from the CD spectrum of **12** (16). The general shape of the CD curve remained essentially unchanged, indicating that the main contribution was the conformation of the peptide.

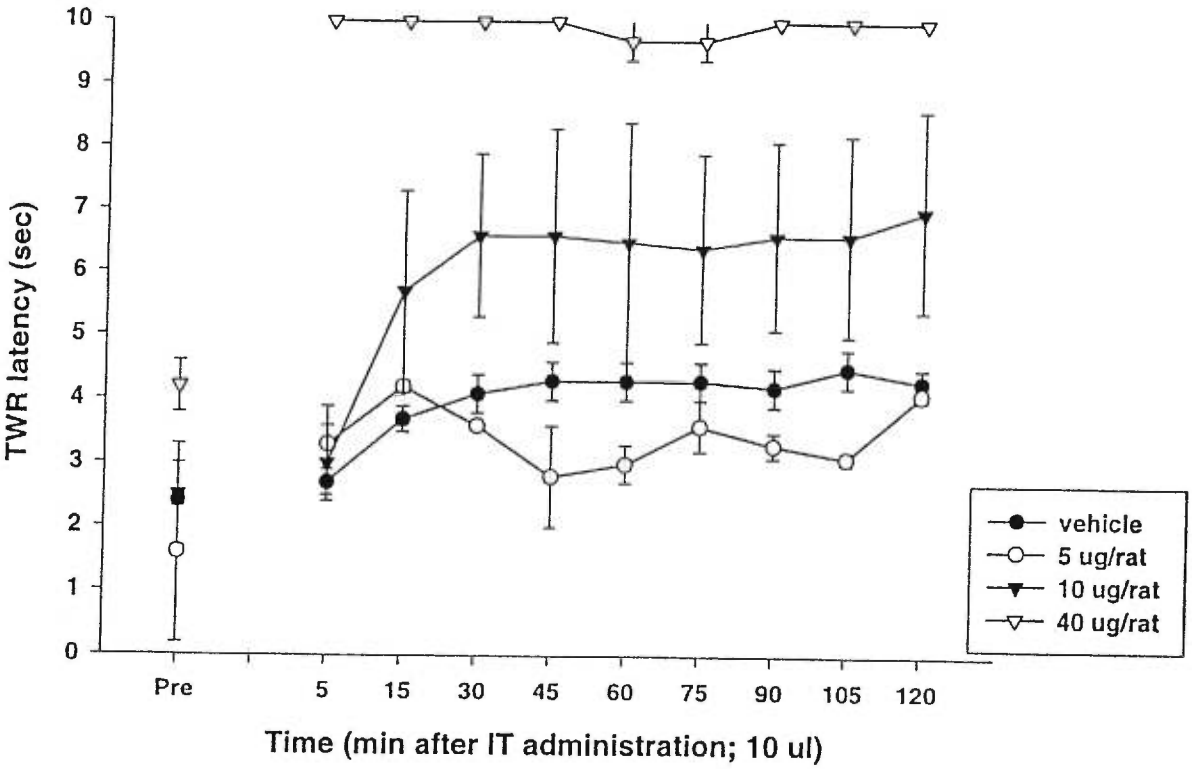



**Figure 6. Measurement of Spinal Analgesia Induced by [I$^9$AA]-Leu-Enkephalin 12.**

Leucine-enkephalin displays affinity for both the μ- and δ-receptors in rat brain, with $K_i$ values in the low nanomolar range. In the GPI and MVD assays, Leu-enkephalin behaves as a full agonist with $IC_{50}$ values around 250-300 nM and 11-13 nM respectively. When measuring the signal transduction of **12**, a $pEC_{50}$ value of 5.69 ($pEC_{50}$ = –log $EC_{50}$, μM units) was determined for its effect on GTPγS binding to μ-receptors. Similarly, a $pEC_{50}$ value of 6.05 was determined for its δ-receptor agonism. The data

indicated that analogue **1** is able to activate both the μ- and δ-receptors on binding. The affinity and potency were however lower than the native Leu-enkephalin. In the spinal analgesia test, the tail withdrawal latency after intrathecal injection of **12** showed a prolonged duration of action of more than a 120 minutes at a dose of 10 μg per rat (Figure 6). The maximum effect was obtained at a dose of 40 μg per rat.

These preliminary results were obtained in collaboration with Janssen Pharmaceutica, Belgium. As can be observed in Figure 6, the data points exhibit significant error margins. Similarly, no control experiments were performed in order to compare the activity of **12** with known opioid peptide standards such as Leu-enkephalin. The biology of peptide **12** is presently under further investigation.

**3.14. Discussion**

Structure-activity relationship studies have shown that modification at the glycine residues leads usually to reduced potency of the enkephalins. For example, replacement of $Gly^3$ with L- and D- amino acids caused a marked drop in activity. Similarly, replacement of $Gly^2$ with L-amino acids led to a decrease in activity. On the other hand, when D-amino acids such as D-alanine, D-methionine and D-serine were introduced at the $Gly^2$ position, increased potency was observed (38).

As mentioned, azabicycloalkane amino acids have been used to rigidify enkephalin with limited success. For example, replacement of the $Gly^2$-$Gly^3$ residues by the 7-thiaindolizidinone amino acid **3** produced an analogue **9** that displayed weak activity *in vitro* (4). Although 5-oxaindolizidinone **10** was also substituted for the $Gly^3$-$Phe^4$ residues of Leu-enkephalin, biological data has yet to be reported for this analogue. Amide temperature coefficients and nOe data were suggestive of a turn structure in this analogue (20).

Conformational analysis of $[I^9AA]^{2,3}$-Leu-enkephalin **12** using NMR spectroscopy did not provide any indication of a β-turn conformation possessing an intramolecular hydrogen bond. The peptide did not exhibit

$^3J_{NH\text{-}C\alpha H}$ coupling constants, amide temperature coefficients, nor nuclear Overhauser effects between the side-chains, that were characteristic of a folded conformation. On the other hand, the CD spectrum of **12** showed a curve indicative of a β-turn conformation. This dichotomy between the NMR and CD data seems to indicate that peptide **12** could exist in a open turn conformation that does not possess an intramolecular hydrogen bond.

The biological activity of peptide **12** suggested that the conformation retained the requirements for interaction with the biological receptors. In our study, the incorporation of the azabicycloalkane amino acid **1** as a replacement for the $Gly^2$-$Gly^3$ residues, introduced side-chains of L-configuration which have previously been shown to diminish potency. The lowered agonist activity of our analogue, relative to Leu-enkephalin, may be attributed to incorporation of these side-chains. Our analogue also exhibited similar receptor selectivity relative to Leu-enkephalin with greater affinity at the δ receptor than the μ receptor. Thus, peptide **12** may adopt a conformation preorganized for interaction with this receptor subtype. The biological activity *in vivo* showed that incorporation of **1** into Leu-enkephalin led to a significant increase in its duration of activity relative to the natural peptide. Leucine-enkephalin has been shown to be deactivated within 1-2 minutes at 37°C, through cleavage of the Tyr-Gly peptide bond by aminopeptidase (39). The increase in the duration of activity of $[I^9AA]^{2,3}$-Leu-enkephalin **12** relative to Leu-enkephalin may thus be attributed to increased metabolic stability towards proteases.

### 3.15. Conclusion

Our study has shown that indolizidin-9-one amino acid **1** can be used as a constrained surrogate of the $Gly^2$-$Gly^3$ dipeptide in the biologically relevant peptide Leu-enkephalin. The bioactivity of the $[I^9AA]^{2,3}$-Leu-enkephalin **12** indicated that the conformation required for interaction with the opioid receptors was maintained. We are now pursuing the incorporation of other azabicyclo[X.Y.0]alkane amino acids into biologically relevant peptides in order to further probe the effect of scaffold ring-size, stereochemistry and ring-substituents, on the biological activity of their peptide mimics.

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# Chapitre 4

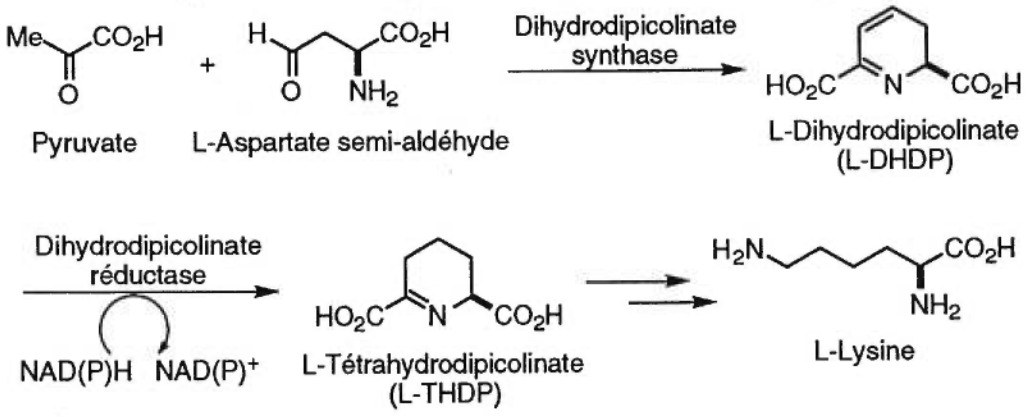
# Méthodologie de synthèse des acides 6-alkylpipecoliques énantiopurs. Analyse conformationnelle des dérivés *N*-acétyles *N'*-méthylamides comme peptides modèles

## 4.1. Introduction

Les acides pipecoliques se retrouvent dans la structure de plusieurs produits naturels[1] et ont été utilisés comme intermédiaires dans la synthèse d'acides aminés neuroexcitatoires,[2] de peptides[3] et d'alcaloïdes d'intérêt biologique.[4]

Toujours dans l'optique de développer une approche générale pour la synthèse d'acides aminés azacycloalcanes énantiopurs, nous avons porté nos efforts sur les acides pipecoliques. Nous avons relevé le défi de développer une approche de synthèse versatile, qui permet l'introduction stéréocontrôlée d'une gamme de substituants et de groupes fonctionnels sur chacune des positions de l'hétérocycle, tout en utilisant des produits de départ et des réactifs peu coûteux. Nous avons maintenu notre objectif de produire les acides pipecoliques sous forme énantiomériquement pure.

**Schéma 1. Biosynthèse de la lysine via le chemin diaminopimélate.**




En modifiant notre méthodologie de synthèse des acides aminés azabicyclo[X.Y.0]alcanes, nous avons élaboré une voie de synthèse biomimétique des acides pipecoliques. En effet, la biosynthèse de la L-lysine implique comme réaction clé une condensation aldolique catalysée par l'enzyme dihydrodipicolinate synthase (Schéma 1).[5] La L-lysine est un acide aminé nécessaire à la croissance et au développement normal chez les mammifères. Les végétaux synthétisent la L-lysine, où elle est utilisée pour la biosynthèse de protéines. Pour la plupart des bactéries, la L-lysine est essentielle à la réticulation de la paroi cellulaire pour résister à la forte

pression osmotique intracellulaire. Des inhibiteurs de la biosynthèse de la L-lysine pourraient vraisemblablement agir en tant que bactéricides ou herbicides non-toxiques pour les mammifères.[5]

**4.2. Equilibre conformationnel des prolinamides et des pipecolinamides**

Nos efforts initiaux ont porté en partie sur la synthèse d'acides 6-alkylpipecoliques parce que ces composés pourraient être des inhibiteurs potentiels de la biosynthèse de la L-lysine, mais surtout parce qu'ils nous permettaient étudier l'équilibre conformationnel des rotamères acétamides en position *N*-terminale des pipecolates.

La proline est le seul acide aminé protéinogénique qui possède un lien amide *N*-terminal pouvant exister sous deux formes isomériques *cis* et *trans* (Figure 1). Certains peptides adoptent une conformation de repliement β de type VI, dans laquelle on retrouve un isomère *cis* de l'amide *N*-terminale de proline.[6] Cette conformation a un rôle important dans la reconnaisance moléculaire entre les peptides et les immunophilines qui catalysent l'isomérisation du lien peptidyl-prolyle, telles que la FKBP et la cyclophiline.[7] En catalysant cette isomérisation, les immunophilines accélèrent le repliement de certaines protéines.[7]

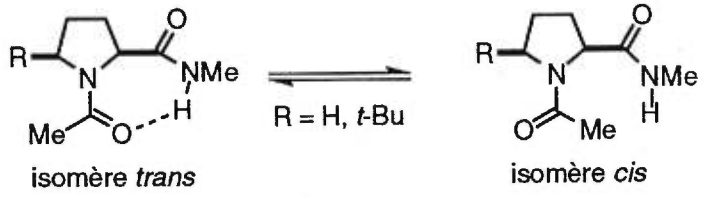



**Figure 1. Equilibre conformationnel des prolylacétamides.**

Précedemment, Beausoleil et Lubell ont démontré par spectroscopie RMN et infra-rouge, ainsi que par modélisation moléculaire que l'introduction d'un substituant stériquement encombré en position 5 des prolines a une influence marqué sur l'équilibre conformationnel des rotamères acétamides en position *N*-terminale de cet acide aminé (Figure 1).[8] Plus particulièrement, l'interaction stérique due à la présence du groupement *tert*-butyle en position 5 déstabilise le rotamère acétamide *trans* et, par conséquent, augmente la population du rotamère acétamide *cis* par rapport à celle observée chez l'acide aminé naturel. Nous avons donc

poursuivi cette étude dans le but de connaître l'influence de la dimension de l'hétérocycle sur cet équilibre conformationnel.

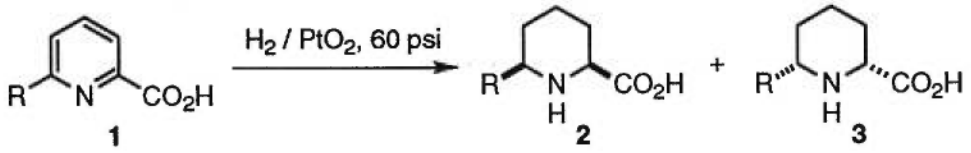
### 4.3. Méthodes de synthèse des acides 6-alkylpipécoliques

Une lecture attentive de la littérature montre que peu de méthodes pratiques et versatiles existent pour synthétiser les acides pipecoliques de façon stéréospécifique. Pour situer notre méthodologie en contexte, nous présentons donc ici un aperçu des méthodes existantes pour synthétiser les acides 6-alkylpipecoliques.

#### 4.3.1. Hydrogénation des pyridines

Les voies qui fournissent les pipecolates racémiques utilisent généralement la réduction catalytique des acides picoliniques **1** (Schéma 2).[9] La réduction sur $PtO_2$ produit les acides pipecoliques *cis*-racémiques **2** et **3** avec un excellent rendement (99%). Il est possible d'obtenir le diastéréoisomère *trans* par réduction catalytique avec un alliage de Ni-Al en présence de KOH.

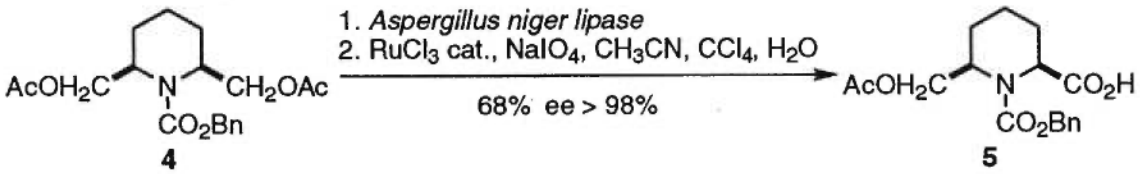
**Schéma 2. Hydrogénation des acides 6-alkylpicoliniques**




#### 4.3.2. Désymétrisation enzymatique

La désymétrisation enzymatique du diester **4** par une lipase a fourni un bon rendement du pipecolate **5** après oxydation de l'alcool primaire par le ruthénium en présence de periodate de sodium (Schéma 3).[10]

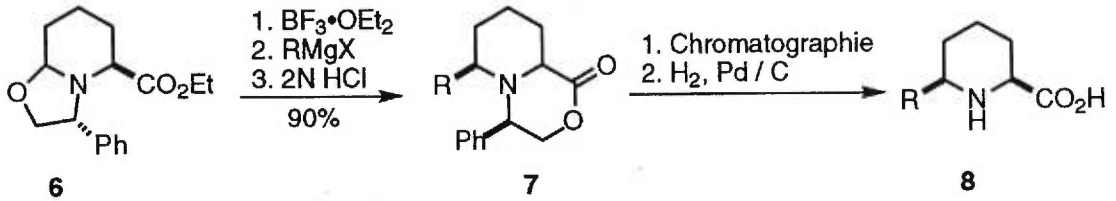
**Schéma 3. Désymétrisation enzymatique des piperidines-diacétates**



### 4.3.3. Additions organométalliques sur les ions iminiums chiraux

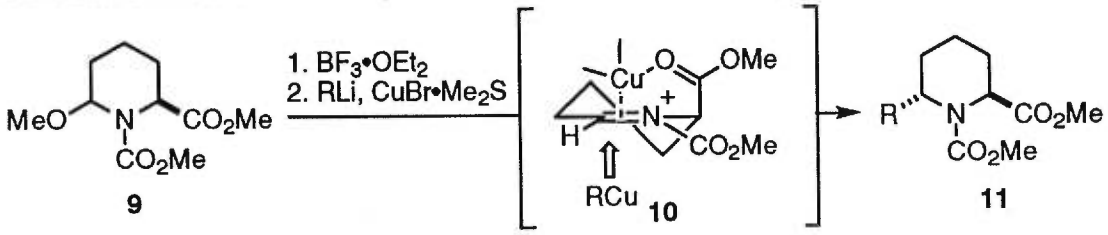
Les pipecolates 6-substitués ont été préparés par l'addition d'organomagnésiens sur une oxazolidine chirale dérivée du phénylglycinol (Schéma 4).[11] La diastéréosélectivité (85 : 15 à 99 : 1) de l'addition nucléophile provient d'une minimisation de la tension allylique 1,2 sur l'iminium formé après traitement de **6** avec $BF_3 \bullet Et_2O$. L'hydrogénolyse de la lactone **7** fournit les pipecolates *cis* énantiopurs **8**.

**Schéma 4. Addition d'organomagnésiens sur une oxazolidine chirale**




Les pipecolates *trans*-2,6-disubstitués **11** ont été obtenus avec de bons rendements par l'addition d'alkylcuivreux sur l'ion iminium généré par le traitement du méthoxypipecolate **9** avec un acide de Lewis (Schéma 5).[12] La très haute diastéréosélectivité observée semble être attribuable à une attaque nucléophile par la face la moins encombrée d'un complexe **10** entre l'iminium, l'ester et le cuivre (I). En effet, l'absence de la fonction ester mène à de faibles diastéréosélectivités.

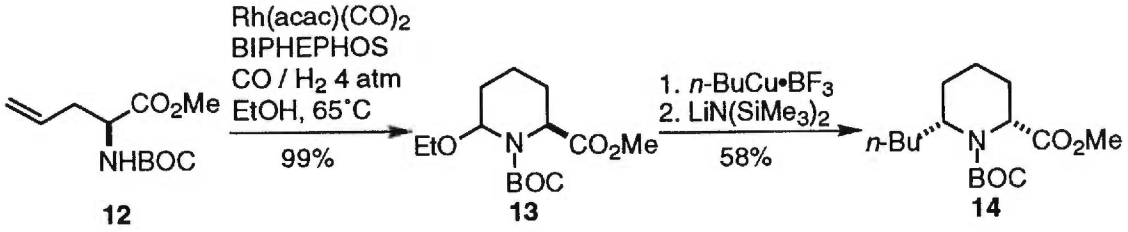
**Schéma 5. Addition d'alkylcuivreux sur un ion *N*-acyliminium**




Dans une approche similaire, on a préparé des pipecolates *cis*-2,6-disubstitués (Schéma 6).[13] La cyclohydrocarbonylation de l'allylglycine **12** catalysée par le rhodium a fourni un rendement quantitatif de l'éthoxypipecolate **13**. Le *cis*-pipecolate **14** est obtenu avec un excès diastéréomérique de 100% par l'addition d'un alkylcuivreux suivie d'une

épimérisation en conditions basiques avec le bis(triméthylsilyl)amidure de lithium.

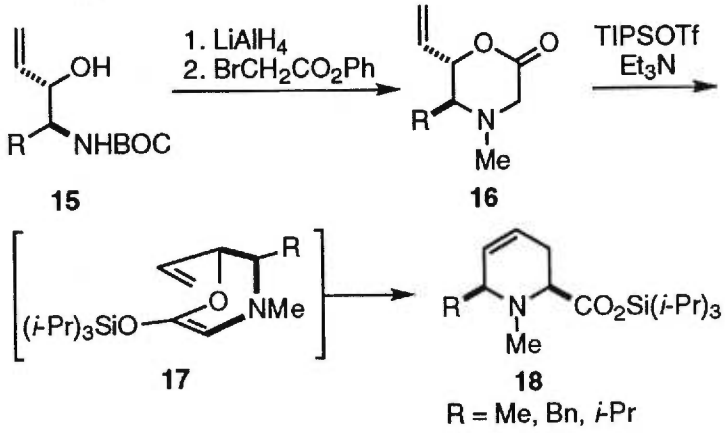
**Schéma 6. Addition d'un alkylcuivreux suivie d'une épimérisation**




### 4.3.4. Réarrangement sigmatropique des cétènes acétales silylés

Par ailleurs, on a synthétisé les pipecolates *cis*-2,6-disubstitués par un réarrangement de Claisen des cétènes acétales silylés (Schéma 7).[14] La lactone **16** est préparée par la réduction du *N*-(BOC)amino alcool **15**, obtenu par l'addition d'un vinylmétal (RMgX, $ZnCl_2$) sur l'α-amino aldéhyde correspondant, et le traitement avec le bromoacétate de phényle. La réaction subséquente avec le trifluorométhanesulfonate de triisopropylsilyle (TIPSOTf) donne le cétène acétale silylé **17** qui se réarrange spontanément en pipecolate **18**. Il est à noter que le diastéréoisomère *cis* du cétène acétale silylé ne se réarrange pas.

**Schéma 7. Réarrangement de Claisen des cétènes acétales silylés**




Tel que présenté plus haut, la plupart des méthodologies existantes ne permettent pas l'introduction des chaînes latérales sur chacun des

carbones du cycle avec sélectivité et stéréocontrôle. Bien que l'approche basée sur la désymétrisation enzymatique soit intéressante, la versatilité de cette méthode ainsi que la sensibilité de l'enzyme par rapport au substrat restent à déterminer. Les méthodes utilisant l'allyglycine et les dérivés de l'acide pipecolique sont efficaces. Toutefois, elles nécessitent des produits de départ coûteux. La méthodologie basée sur le réarrangement de Claisen est versatile, mais elle fournit un pipecolate qui doit être déalkylé pour fonctionnaliser l'amine.[14b]

Il serait donc avantageux de développer une stratégie de synthèse des acides pipecoliques qui remédierait à ces problèmes. Dans ce chapitre, nous présentons une méthodologie qui permet l'introduction d'une gamme de substituants en position 6 des acides pipecoliques. Notre synthèse est simple, pratique, et a le potentiel de résoudre les problèmes relatifs à la fonctionnalisation de chacun des carbones de l'hétérocycle de façon stéréocontrôlée. De plus, notre approche fournit les pipecolates sous forme énantiomériquement pure.

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# Article 5

Gosselin, F.; Swarbrick, M. E.; Lubell, W. D. "Synthesis of ε-Alkylpipecolates as Conformationally Rigid Proline Surrogates" Publié dans *Peptides 1998*, Proceedings of the 25th European Peptide Symposium, S. Bajusz & F. Hudecz, Eds; Akadémia Kiadó: Budapest, Hungary, **1998**, 678-679.

## Synthesis of ε-Alkylpipecolates as Conformationally Rigid Proline Surrogates

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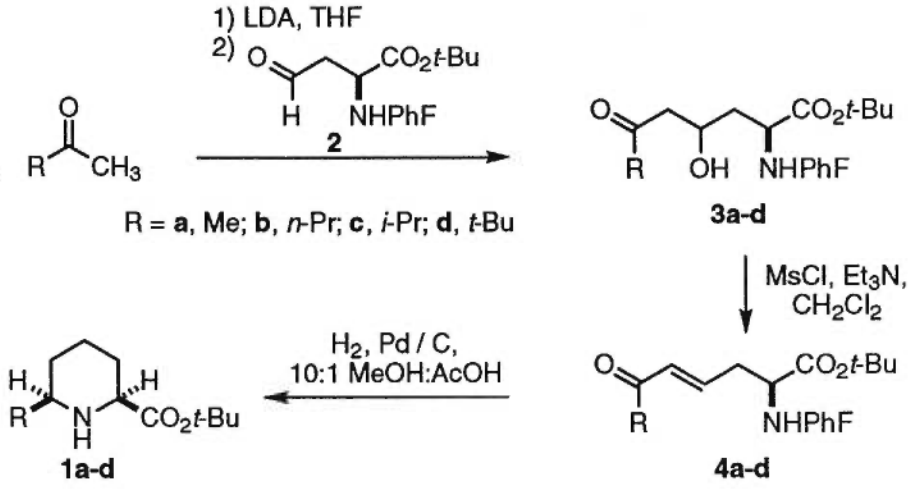
### 4.5. Introduction

Concurrent with research using alkylprolines in conformationally rigid mimics of peptide secondary structures [1,2], we initiated a program to synthesize and study alkylpipecolates in peptides in order to examine the influences of ring-size, alkyl-substituent and stereochemistry on peptide conformation. Since 5-alkylprolines exhibit steric effects that enhance the *cis*-isomer population *N*-terminal to prolyl residues [1], 6-alkylpipecolates became our first targets. Towards developing a general entry into this ring system, we report now effective methodology for synthesizing 6-alkylpipecolates and their examination in *N*-acetyl *N'*-methylamides.

### 4.6. Results and Discussion

**Scheme 1.**




*tert*-Butyl 6-alkylpipecolates **1a-d** were synthesized via an aldol condensation / reductive amination sequence (Scheme 1, PhF = 9-

phenylfluoren-9-yl). α-*tert*-Butyl *N*-(PhF)aspartate β-aldehyde (**2**) was quantitatively synthesized by hydride reduction of the corresponding β-methyl ester with DIBAL-H in toluene at –78°C. Aldol condensations with lithium enolates, generated from methyl ketones and LDA in THF provided β-hydroxy ketones **3** in 68-79% yields, which were dehydrated with $MeSO_2Cl$ and $Et_3N$ in $CH_2Cl_2$ to furnish ε-oxo-α-amino esters **4** in 64-79% yields. Hydrogenation of **4** using Pd/C as catalyst under 3 atm of $H_2$ in 10:1 MeOH:AcOH effected concurrent olefin reduction, PhF protecting group cleavage and reductive amination to *tert*-butyl 6-alkylpipecolates **1** in 60-83% yields, after removal of the phenylfluorene hydrocarbon by extraction with hexane.

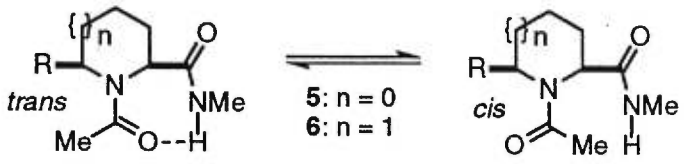



**Table 1. Amide Equilibrium *N*-Terminal to Proline and Pipecolate Amides**

| entry | n | R | % *cis*-isomer ±3% | Tc (°C) | $\Delta G^{\ddagger} \pm 0.3$ kcal/mol |
|---|---|---|---|---|---|
| **5a** | 0 | H | 27 | >85 | 20.4 |
| **5b** | 0 | *t*-Bu | 49 | 45 | 16.5 |
| **6a** | 1 | H | 28 | 80 | 17.8 |
| **6b** | 1 | *t*-Bu | 43 | 70 | 17.0 |

Conformational analysis of *N*-acetyl *N'*-methylpipecolinamide **6a** and its (2*S*, 6*R*)-6-*tert*-butyl counterpart **6b** by proton NMR spectroscopy showed that the bulky 6-position substituent increased the *N*-terminal amide *cis*-isomer population and lowered the barrier for amide isomerization as had been observed with proline amides **5a** and **5b** (Table 1) [1]. However, relative to the proline amides, the influence of the bulky *tert*-butyl substituent was less pronounced in the pipecolinamides. As observed when comparing acetamides of 2-methylpyrrolidine and 2-methylpiperidine, pipecolinamides possessed lower barriers for amide rotation than proline amides [3]. Greater conformational liberty and

reduced ring strain in 6- vs 5-member rings may account for the reduced steric effects in 6-alkylpipecolates relative to 5-alkylprolines. We are now synthesizing pipecolates possessing other alkyl substituents and studying the conformational effects of alkylpipecolates in biologically active peptides.

**Acknowledgements**
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# Article 6

Swarbrick, M. E.; Gosselin, F.; Lubell, W. D. "Alkyl Substituent Effects on Pipecolyl Amide Isomer Equilibrium: Efficient Methodology for Synthesizing Enantiopure 6-Alkylpipecolic Acids and Conformational Analysis of Their *N*-Acetyl *N*'-Methylamides" Publié dans *The Journal of Organic Chemistry*, **1999**, *64*, 1993-2002.

# Alkyl Substituent Effects on Pipecolyl Amide Isomer Equilibrium: Efficient Methodology for Synthesizing Enantiopure 6-Alkylpipecolic Acids and Conformational Analysis of Their *N*-Acetyl *N'*-Methylamides

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## 4.8. Abstract

Enantiopure 6-alkylpipecolic acid hydrochloride salts **1a-e** were synthesized in five steps and 15-59% overall yields from α-*tert*-butyl β-methyl *N*-(PhF)aspartate (**3**) via an approach featuring selective hydride reduction to the corresponding aspartate β-aldehyde **2**, aldol condensations with the enolates of various methyl alkyl ketones and diastereoselective intramolecular reductive aminations. The influence of the 6-position substituent on the equilibrium and the energy barrier for isomerization of the amide *N*-terminal to pipecolate was then explored via the synthesis of *N*-acetyl *N'*-methylpipecolinamide (**16**) and its (2*S*,6*R*)-6-*tert*-butylpipecolinamide counterpart **17**, and their conformational analysis by proton NMR spectroscopy and coalescence experiments. The presence of the *tert*-butyl substituent augmented the population of the amide *cis*-isomer and lowered the barrier for pipecolyl amide isomerization in water. Compared with the results from our previous examination of *N*-acetyl-5-*tert*-butylproline *N'*-methylamides (Beausoleil, E.; Lubell, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 12902), the consequences of the bulky 6-alkyl substituent on the acetamide geometry and isomerization barrier were less pronounced in the pipecolate series relative to the respective proline amides.

**4.9. Introduction**

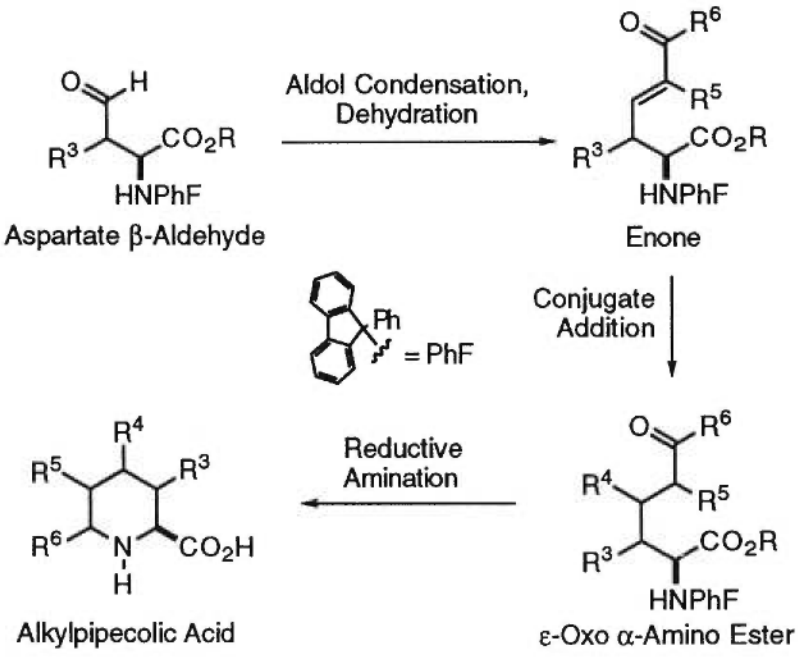
Pipecolic acid (piperidine-2-carboxylic acid) is a widespread, naturally-occurring non-proteinogenic amino acid found in many biologically interesting compounds. For example, pipecolate residues are components of the immunosuppressive drugs FK506[1] and rapamycin,[2] antibiotic peptides such as the virginiamycins[3] and the efrapeptins,[4] the antiprotozoal agent apicidin[5] and inhibitors of HIV protease.[6] Pipecolates have also served as proline substitutes in structure-activity studies of biologically relevant peptides.[7,8] Furthermore, alkyl substituted pipecolic acids have been employed as dihydropicolinic acid (DHDPA) synthase inhibitors[9] and *N*-methyl-D-aspartic acid (NMDA) receptor agonists[10] and antagonists.[11] In addition, alkylpipecolates have served as starting materials for synthesizing biologically active piperidine alkaloids.[12,13] For example, *N*-methoxycarbonyl-6-*n*-propylpipecolate was used in the synthesis of the *N*-methyl derivative of coniine,[13] the principal toxic constituent of hemlock.

Concurrent with our research on employing alkylprolines in conformationally rigid mimics of peptide secondary structures,[14,15] we became interested in the effects of azacycloalkane amino acids of larger ring-size. We initiated a program to synthesize and study alkylpipecolates in peptide structures in order to examine the influence of ring-size, alkyl-substituent and stereochemistry on peptide conformation.[16] Since 5-alkylprolines exhibited steric effects that greatly enhanced the *cis*-isomer of the amide *N*-terminal to prolyl residues in peptides,[14] 6-alkylpipecolates were chosen as our first targets in order to study the influence of bulky 6-position substituents on the equilibrium of pipecolyl amides.

The synthesis of alkylpipecolic acid derivatives[17] has received considerable attention;[18-22] however, few methods offer the potential for selective and stereocontrolled introduction of alkyl substituents at each of the ring carbons. We are exploring a biomimetic entry into this ring system in order to provide pipecolates with substituents at the 3-, 4-, 5- and 6-positions (Scheme 1, PhF = 9-phenylfluoren-9-yl). In analogy to the

diaminopimelate pathway for L-lysine biosynthesis, which features the enzyme catalyzed aldol condensation between pyruvate and aspartate β-aldehyde with subsequent cyclization to provide L-dihydropicolinic acid,[19] our route employs an aldol condensation / reductive amination sequence to transform *N*-(PhF)aspartate β-aldehyde **2** into alkylpipecolic acids. In principle, this route offers access to pipecolates with alkyl substituents at four of the five ring carbons by employment of β-alkyl branched aspartates[20] and alkyl substituted enolates, as well as by conjugate additions to the α,β-unsaturated ketone intermediate.[21] We report now the employment of this route for synthesizing enantiopure pipecolates with primary, secondary and tertiary alkyl as well as aryl 6-position substituents.[16]

**Scheme 1. General Strategy for Alkylpipecolate Synthesis**




The influence of the 6-position substituent on the pipecolyl amide isomer equilibrium was studied via the synthesis and conformational analysis of *N*-acetyl *N'*-methylpipecolinamide (**16**) and its (2*S*, 6*R*)-6-*tert*-butylpipecolinamide counterpart **17**. Using NMR spectroscopy and coalescence experiments, we observed that the consequences of the

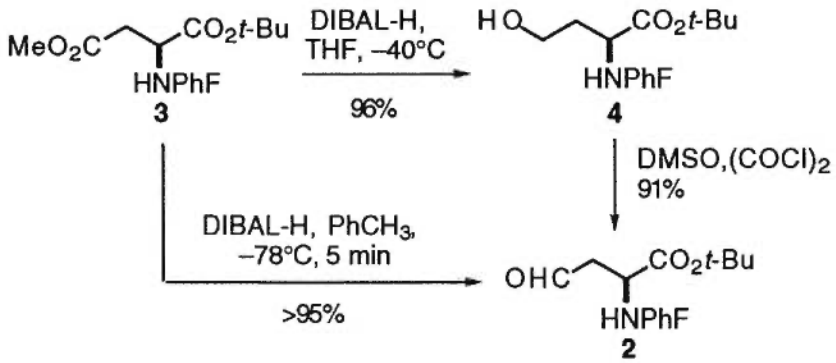
presence of the bulky (6*R*)-substituent on the pipecolyl amide isomer equilibrium in water were similar to those previously observed in our comparison of *N*-acetylproline *N'*-methylamide **18** with its (2*S*, 5*R*)-5-*tert*-butyl analogue **19**.[14] Steric interactions between the *N*-acetyl and 6-position substituents disfavored the amide *trans*-isomer and augmented the *cis*-amide population. In addition, the presence of the (6*R*)-*tert*-butyl substituent lowered the energy barrier for amide isomerization in **16** relative to **17**. On the other hand, the effects of the *tert*-butyl substituent were less pronounced in the pipecolate series relative to the respective proline amides. By elucidating their substituent effects on pipecolinamide conformation and by providing effective methodology for synthesizing 6-alkylpipecolates, our report is intended to further the employment of alkylpipecolates as tools for studying the structure-activity relationships of biologically relevant peptides.

### 4.10. Results and Discussion

**Synthesis and Rearrangement of Aspartate β-Aldehyde**

6-Alkylpipecolic acids **1** were synthesized from aspartic acid via a route featuring aldol condensation on α-*tert*-butyl *N*-(PhF)aspartate β-aldehyde **2**, dehydration and subsequent diastereoselective reductive amination of ε-oxo α-*N*-(PhF)amino esters **7** (Scheme 4).

**Scheme 2. Synthesis of *N*-(PhF)Aspartate β-Aldehyde 2**




We reported previously on the Wittig and Horner-Wadsworth-Emmons olefination chemistry of (2*S*)-*tert*-butyl 2-[*N*-(PhF)amino]-4-oxobutanoate

(**2**),[27] and its synthesis from α-*tert*-butyl β-methyl *N*-(PhF)aspartate (**3**)[24b] by selective β-ester reduction using DIBAL-H in THF at –40 °C to furnish *N*-(PhF)homoserine *tert*-butyl ester (**4**), followed by oxidation of the primary alcohol using DMSO and oxalyl chloride in dichloromethane. We report now that aldehyde **2** can be synthesized in one transformation and >95% yield from diester **3** by selective reduction with DIBAL-H in toluene at –78 °C for 5 min (Scheme 2).

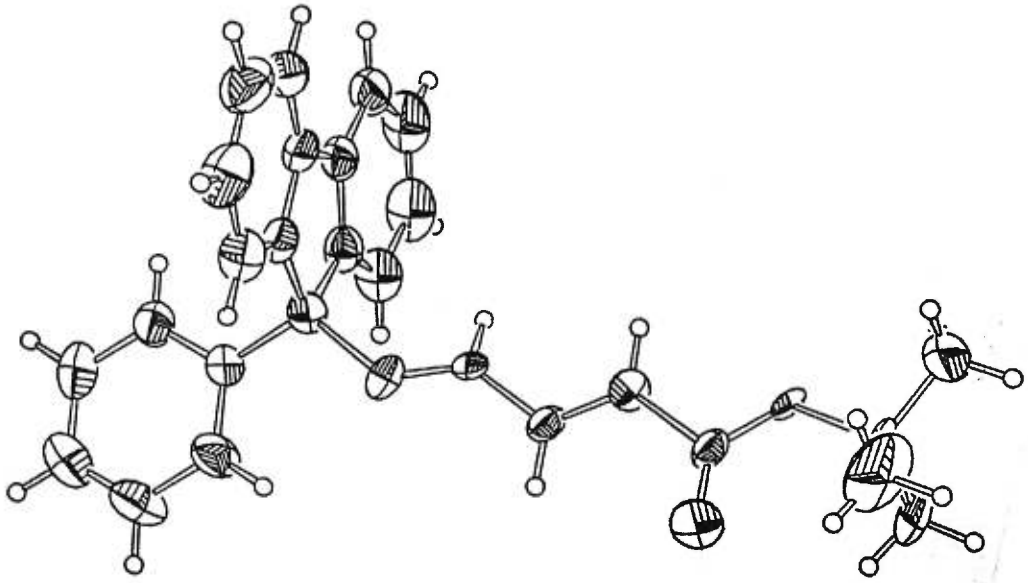
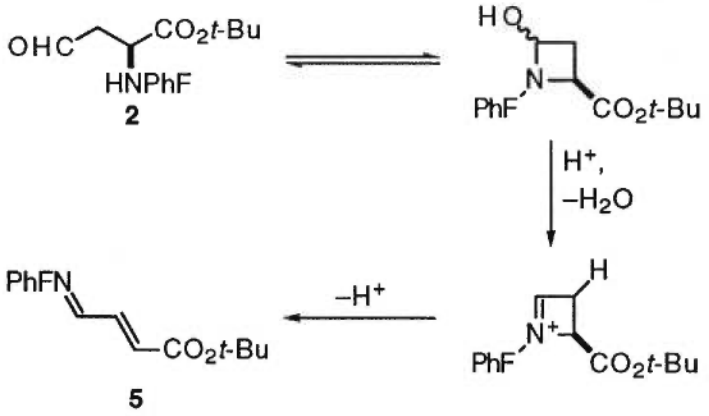


**Figure 1.** ORTEP view of *tert*-butyl *N*-(PhF)-5-azapenta-2,4-dienoate (**5**). Ellipsoids drawn at 40% probability level. Hydrogens represented by spheres of arbitrary size.[28]

As previously reported,[27] aldehyde **2** can be stored for several months at –20 °C without decomposition; however, when dissolved in $CDCl_3$, **2** was transformed into a new product **5** within a few hours as judged by the disappearance of the signals for the aldehyde, α- and β-protons respectively at 9.52, 3.04 and 2.40 ppm in the NMR spectrum. In the FT-IR spectrum of **5**, we observed bands at 1610 and 1714 $cm^{-1}$ corresponding respectively to the stretches for an α,β-unsaturated imine and ester. Mass spectrometric analysis of **5** by fast atom bombardment gave a peak with m/z = 396 indicating loss of water from aldehyde **2** when nitrobenzyl alcohol was used as the matrix, and with thioglycerol as the matrix, a peak was obtained with m/z = 504 which indicated loss of water

from **2** and formation of a thioglycerol adduct. Crystals of **5** were later grown from EtOAc-hexanes. Crystallographic analysis by X-ray diffraction demonstrated the product from decomposition of **2** to be *tert*-butyl *N*-(PhF)-5-azapenta-2,4-dienoate (**5**, Figure 1).[28]

**Scheme 3. Proposed Mechanism for the Rearrangement of Aldehyde 2 into Azadiene 5**




This conjugated imine is presumed to form by a process that commences with intramolecular attack of the *N*-(PhF)amine onto the β-aldehyde (Scheme 3). In the presence of trace amounts of acid in chloroform, the resulting aminol may lose water to provide a strained dehydroazetidine carboxylate that ring opens by elimination of the iminium ion nitrogen to furnish azadiene **5**. The thioglycerol adduct obtained in the mass spectral analysis of **5** may have thus resulted from 1,4- or 1,2-additions of this thiol to 5-azapenta-2,4-dienoate **5**.

**Synthesis of 6-Alkylpipecolates**

Aldol condensations with aldehyde **2** and the lithium enolates of a variety of methyl alkyl ketones furnished the respective ε-oxo γ-hydroxy α-*N*-(PhF)amino esters **6** in 61-93% yields (Scheme 4, Table 1). In the cases of 3-methylbutan-2-one and pentan-2-one, the enolate was generated under kinetically controlled conditions prior to the addition of aldehyde **2**.[29] Enones **7** were synthesized by dehydration of alcohols **6** using two different conditions. Initially, we subjected β-hydroxy ketones **6** to

**Scheme 4. Synthesis of 6-Alkylpipecolic Acid Hydrochloride Salts 1**

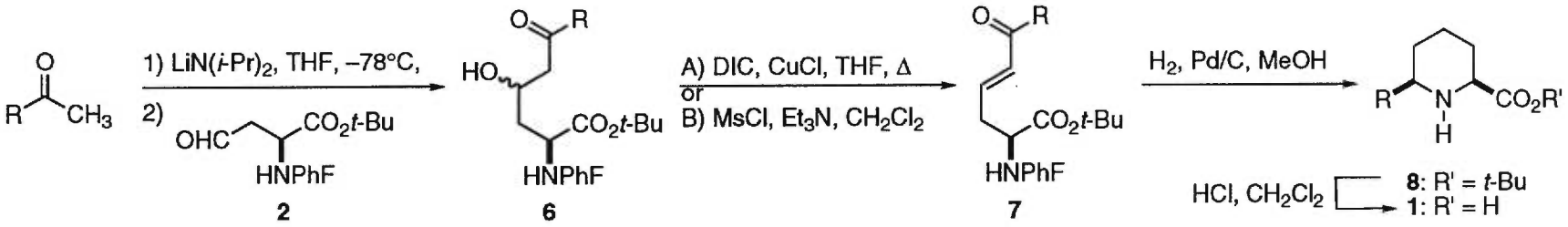



**Table 1. Isolated Yields in the Synthesis of 6-Alkylpipecolates**

| entry | Ketone | R | % Yield **6** (dr) | A) % Yield **7** | B) % Yield **7** | % Yield **8** | % Yield **1**•HCl |
|---|---|---|---|---|---|---|---|
| **a** | Pinacolone | *t*-Bu | 84 (1:1) | 87 | 64[a] | 86 | 98 |
| **b** | 3-Methyl-2-butanone | *i*-Pr | 77 (2:1) | 62 | 87 | 91 | 100 |
| **c** | 2-Pentanone | *n*-Pr | 72 (2:1) | 63 | 91 | 86 | 97 |
| **d** | Acetone | Me | 68 (2:1) | 62 | 86 | 90 | 98 |
| **e** | Acetophenone | Ph | 93 (2:1) | 81 | 92 | 20 | 96 |
| **f** | 2-Acetylpyridine | 2-Pyridyl | 61 (3:1) | – | 80 | – | – |

[a]Recoverred 32% methanesulfonate.

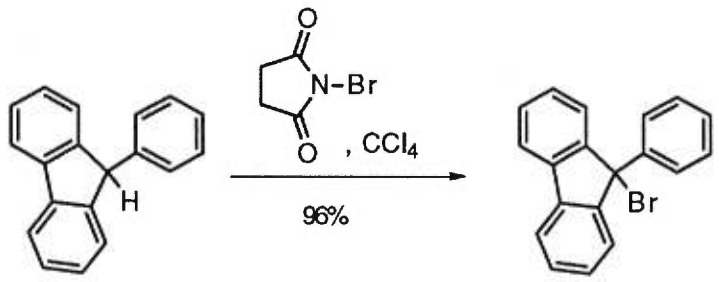
diisopropylcarbodiimide and catalytic copper(I)chloride in THF which furnished enones **7** in 62-87 % yields.[30] Improved yields of enones **7b-f** were obtained via the elimination of the corresponding methanesulfonates using methanesulfonyl chloride and triethylamine in dichloromethane. Treatment of **6a** under the latter conditions provided enone **7a** in 64% yield accompanied by 32% yield of a single diastereomeric methanesulfonate, which suggested that steric crowding prevented this diastereomer from attaining the conformation required for elimination. In all cases, formation of olefin having only the (*E*)-configuration was indicated by the large (15.2-16.0 Hz) vicinal coupling constants between the vinyl protons.

Hydrogenations of enones **7** were originally performed using 10% palladium-on-carbon as catalyst in a solution of 10:1 methanol : acetic acid under 3 atm of hydrogen.[31] Under these conditions, concurrent hydrogenation of the double bond and cleavage of the PhF protecting group were followed by iminium ion formation and subsequent reduction from the side opposite the *tert*-butyl ester to selectively furnish the *cis*-6-alkylpipecolate *tert*-butyl esters **8**. The relative stereochemistry of the pipecolate 6-position substituent was assigned initially based on analogy with our studies on the hydrogenation of α-*tert*-butyl δ-oxo-α-*N*-(PhF)amino esters which produced 5-alkylprolines with high selectivity in favor of the *cis*-diastereomer.[31] Using similar hydrogenation conditions to prepare related fused-pipecolate analogues, we later confirmed the assignment of the 6-position stereochemistry by crystallization of the (2*S*, 6*R*, 8*S*)-methyl 9-oxo-8-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-2-carboxylate and analysis by X-ray diffraction.[27]

6-*tert*-Butylpipecolate **8a** could be purified by chromatography after filtration and concentration; however, isolation of pure 6-alkylpipecolates **8b-d** was less straightforward, due largely to their volatility. Although chromatographic purification could be circumvented by partitioning the 9-phenylfluorene hydrocarbon into hexanes, subsequent concentration of the methanolic acetic acid solution under vacuum was accompanied by loss of material due to the volatility of the acetate salts of **8b-d**. Direct conversion of *tert*-butyl esters **8b-d** into amino acids **1b-d** was accomplished by

treatment of the crude hydrogenation product with 1:1 trifluoroacetic acid : dichloromethane, extraction of the pipecolate trifluoroacetates into water, concentration, and ion-exchange chromatography which furnished zwitterionic 6-alkylpipecolates **1b-d** in respective yields of 59%, 61% and 81%. Although amino acids **1b-d** were of adequate purity for subsequent use, their hygroscopic nature thwarted recrystallization. Hydrogenation of enones **7a-d** in the absence of acetic acid proved the most effective means for isolating 6-alkylpipecolates **8a-d**. Treatment of enones **7a-d** in methanol with palladium-on-carbon and 3 atm of hydrogen furnished *tert*-butyl 6-alkylpipecolates **8a-d** in 86-91% respective yields after chromatography with diethyl ether in dichloromethane as eluant. The 6-alkylpipecolate hydrochloride salts were then quantitatively obtained by exposure of *tert*-butyl esters **8** with HCl in dichloromethane.

**Scheme 5.**
**Recycle of 9-Phenylfluorene to 9-Bromo-9-phenylfluorene**




The 9-phenylfluorene hydrocarbon was quantitatively recovered from the hydrogenation of ε-oxo-α-*N*-(PhF)amino esters **7**. Endeavoring to recycle this hydrocarbon back to the protecting group precursor, 9-bromo-9-phenylfluorene, we found that simply heating 9-phenylfluorene with *N*-bromosuccinimide in carbon tetrachloride[32] provided the corresponding bromide in 96% yield after evaporation of the volatiles, digestion of the residue with hexanes and filtration of the insoluble succinimide (Scheme 5). This efficient recycle of 9-phenylfluorene to 9-bromo-9-phenylfluorene has thus provided an economic means for extending the duration of utility of the 9-phenylfluorenyl protecting group.[33]

**Synthesis of 6-Arylpipecolates**

Hydrogenation of (2*S*, 4*E*)-*tert*-butyl 6-oxo-6-phenyl-2-*N*-(PhF)-aminohex-4-enoate (**7e**) in the presence of acetic acid under the conditions described above for the synthesis of 6-alkylpipecolate did not furnish the desired 6-arylpipecolate **8e**; instead, (2*S*, 6*RS*)-*tert*-butyl 2-amino-6-hydroxy-6-phenylhexanoate (**9**) was isolated as a 1:1 mixture of diastereomers in 46% yield (Scheme 6). Furthermore, no cleavage of the PhF protecting group was observed on hydrogenation of (2*S*, 4*E*)-*tert*-butyl 6-oxo-6-(2'-pyridyl)-2-*N*-(PhF)aminohex-4-enoate (**7f**) under similar conditions; instead, (2*S*, 6*RS*)-*tert*-butyl 2-[*N*-(PhF)amino]-6-hydroxy-6-(2'-pyridyl)hexanoate (**10**) was obtained as a 1:1 mixture of diastereomers in 58% yield. These results prompted an investigation of the hydrogenation of **7e** in order to examine the competition between PhF group hydrogenolysis and aryl ketone reduction (Table 2).[34]

**Scheme 6. Hydrogenation of Enone 7e**

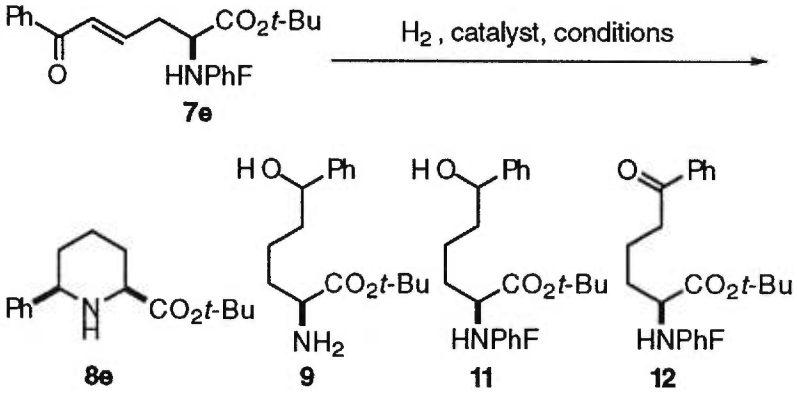
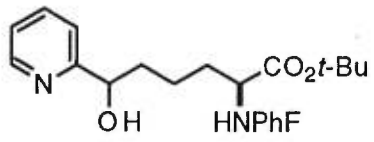



**Table 2. Catalytic Hydrogenation of Enone 7e**

| entry | catalyst | solvent | $H_2$ (atm) | isolated yields % **8e** | % **9** | % **11** | % **12** |
|---|---|---|---|---|---|---|---|
| a | Pd/C | MeOH/AcOH | 3 | - | 46 | - | - |
| b | Pd/C | MeOH/AcOH | 1 | 16 | 53 | - | - |
| c | Pd/C | MeOH | 1 | 20 | 13 | 31 | - |
| d | Pt/C | MeOH/AcOH | 3 | 9 | - | 9 | 64 |
| e | Pt/C | EtOAc | 2.75 | - | - | 14 | 84 |
| f | Ra Ni | EtOH | 3 | - | - | 95 | - |




**10**

The rates of ketone hydrogenation and N–PhF bond hydrogenolysis were competitive using palladium and platinum catalysts such that *tert*-butyl 6-phenylpipecolate **8e** was isolated in low yield. The best yield (20%) of **8e** was obtained on hydrogenation of **7e** over palladium-on-carbon in methanol under 1 atmosphere of hydrogen. Selective reduction of the double bond and aryl ketone without loss of the phenylfluorenyl group was achieved on hydrogenation of **7e** with Raney nickel as catalyst to give *N*-(PhF)amino alcohol **11**. As previously noted,[35] selective olefin hydrogenation without PhF removal was accomplished with a platinum catalyst and gave (2*S*)-*tert*-butyl 6-oxo-6-phenyl-2-*N*-(PhF)aminohexanoate (**12**) from **7e**. Attempts to convert **12** to 6-arylpipecolate **8e** gave similar product distributions as those obtained from hydrogenations of **7e** under identical conditions. In summary, the reductive amination route proved more effective for preparing 6-alkyl rather than 6-arylpipecolates due to competitive aryl ketone reductions.

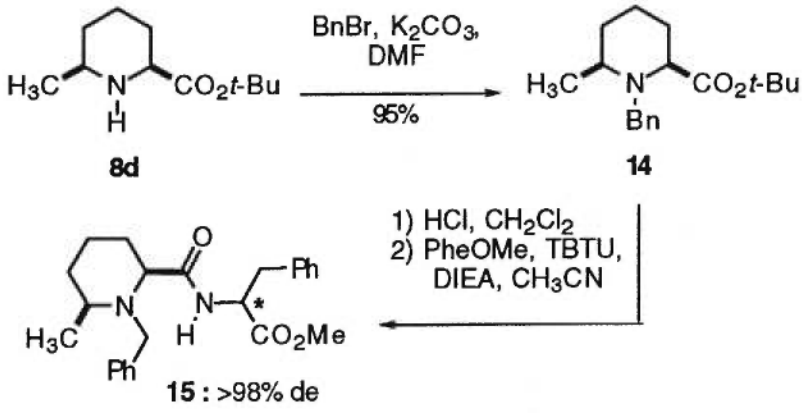
**Enantiomeric Purity**

Determination of the enantiomeric purity of 6-alkylpipecolate **1** by the preparation and analysis of diastereomeric derivatives proved more difficult than purity studies we had previously conducted on related 5-alkylprolines.[31] As noted in a failed attempt to determine the enantiomeric purity of an alkylpiperidine,[36] although amides and ureas could be synthesized by acylation of the piperidine nitrogen, analysis by proton NMR spectroscopy did not indicate sufficiently distinct diastereomeric signals for ascertaining the limits of the minor isomer. Furthermore, these diastereomeric derivatives eluted with similar retention times in cursory examinations by reverse-phase HPLC.

The enantiomeric purity of 6-alkylpipecolate **1** was successfully determined after conversion to the respective *N*-benzylpipecolate and subsequent coupling to a chiral amine (Scheme 7). (2*S*, 6*S*)-6-

Methylpipecolate *tert*-butyl ester (**1d**) was treated with benzyl bromide and potassium carbonate in DMF to provide *N*-(benzyl)pipecolate **14** in 95% yield. Dipeptides **15** were synthesized by *tert*-butyl ester solvolysis on exposure of **14** to HCl gas in dichloromethane, followed by coupling to D- and L-phenylalanine methyl ester hydrochloride using benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[37] and DIEA in acetonitrile. Measurement of the methyl ester singlets at 3.68 and 3.52 ppm in $CDCl_3$ by 400 MHz $^1H$ NMR spectroscopy and incremental additions of diastereomeric (2*S*, 6*R*, 2'*S*)-**15** demonstrated (2*S*, 6*R*, 2'*R*)-dipeptide **15** to be of >99% diastereomeric purity. Hence 6-alkylpipecolic acids **1** are presumed to be of >99% enantiomeric purity.

**Scheme 7.**
**Enantiomeric Purity of (2*S*, 6*S*)-6-Methylpipecolate 8d**




**Conformational Analysis of *N*-Acetyl *N'*-Methyl Pipecolinamides**

*N*-Acetyl *N'*-methyl pipecolinamide **16** and its (2*S*, 6*R*)-6-*tert*-butyl counterpart **17** were synthesized in order to examine the influence of the bulky 6-position substituent on the amide isomer equilibrium and the rate of amide isomerization *N*-terminal to the pipecolyl residue. Pipecolic acid was *N*-protected with di-*tert*-butyl dicarbonate in an aqueous dioxane solution containing sodium hydroxide and the resulting *N*-(BOC)-pipecolate was coupled to methylamine using TBTU in acetonitrile. Pipecolinamide **16** was then obtained after solvolysis of the BOC group with gaseous HCl in dichloromethane, followed by acetylation on heating in the presence of acetic anhydride and triethylamine in dichloromethane.

(2*S*, 6*R*)-*N*-Acetyl *N'*-methyl 6-*tert*-butylpipecolinamide was synthesized without *N*-protection by coupling to methylamine using TBTU in acetonitrile, followed by *N*-acetylation on heating with acetic anhydride, sodium acetate and triethylamine in toluene at reflux.

**Table 3. Proline[14] and Pipecolate *N*-Acetyl-*N'*-Methyl Amide Equilibrium**


*trans*-isomer ⇌ *cis*-isomer

| | n | R | %*cis*-isomer ±3% | $T_c$ (°C) | $\Delta G^{\ddagger}$ ±0.3 kcal/mol |
|---|---|---|---|---|---|
| **16** | 1 | H | 28 | 80[a] | 17.8 |
| **17** | 1 | *t*-Bu | 43 | 70[a] | 17.0 |
| **18** | 0 | H | 27 | >85[b] | 20.4 |
| **19** | 0 | *t*-Bu | 49 | 45[b] | 16.5 |

[a]Measured at 400 MHz. [b]Measured at 300 MHz in ref. 14.

The pipecolinamide isomer equilibrium and the energy barrier for amide isomerization were determined in water ($D_2O$) because of its physiological importance and for comparison with literature examples.[14] In comparison to reaction rates in non-protic and non-polar solvents, amide isomerization *N*-terminal to proline has been shown to proceed slower in water, which stabilizes the polar amide ground states relative to the less polar transition state.[38] The *cis*-isomer geometry was assigned based on the cross-peak arising from the nuclear Overhauser effect between the *N*-acetyl and prolyl α-hydrogens in the NOESY and ROESY spectra of **16** and **17** in water. The populations of the amide isomers were measured by integration of their isomeric acetyl methyl singlets in the $^1H$ NMR spectra. The energy barriers ($\Delta G^{\ddagger}$) for amide isomerization in **16** and **17** were determined by recording a series of $^1H$ NMR spectra at 400 MHz with increasing temperatures until the resonances for the two isomer populations were observed to coalesce. The energy barriers for amide

isomerization in **16** and **17** were then calculated to respectively be 17.8 and 17.0 kcal/mol in $D_2O$.[39] For comparison, the isomer populations and the energy barriers for isomerization of **16** and **17** are listed next to the values for *N*-(acetyl)proline *N*'-methylamide (**18**) and its (2*S*, 5*R*)-5-*tert*-butyl counterpart **19** in Table 3.[14]

As observed in our comparison of *N*-acetyl *N*'-methylamides of proline and (2*S*, 5*R*)-5-*tert*-butylproline, the bulky substituent caused steric interactions that disfavored the *trans*-isomer and increased the *cis*-amide isomer population. Furthermore, a lower barrier for amide isomerization was observed for the (6*R*)-*tert*-butylpipecolinamide **17** relative to the simple pipecolyl amide **16** (Table 3). The influences of the *tert*-butyl substituent were less pronounced in the pipecolinamides relative to their proline counterparts.

The 2.6 kcal/mol lower barrier for isomerization of pipecolinamide **16** relative to *N*-(acetyl)proline *N*'-methylamide (**18**) compared well with the 2.8 kcal/mol lower barrier for isomerization of *N*-acetyl 2-methylpyrrolidine (15.3 kcal/mol) relative to *N*-acetyl 2-methylpiperidine (18.1 kcal/mol).[40] The barrier for amide isomerization was also observed to be lower for pipecolyl than prolyl residues in peptides.[8] A tendency for greater amide *cis*-isomer populations *N*-terminal to pipecolyl versus prolyl residues has been recorded in peptides and attributed to increased steric interaction between the *N*-terminal amino acid and the bulkier piperidine ring.[8] Since the amount of *cis*-isomer exhibited by *N*-acetyl *N*'-methylpipecolinamide (**16**) is similar to that of *N*-(acetyl)proline *N*'-methylamide (**18**) in water, the *N*-acetyl residue does not appear to have sufficient bulk to discriminate between the piperidine and pyrrolidine ring-sizes. The greater conformational liberty of the 6- versus 5-member ring may also account for the lower acetamide *cis*-isomer population for 6-*tert*-butylpipecolinamide **17** relative to 5-*tert*-butylproline amide **19**.

### 4.11. Conclusion

Our investigation has provided effective means for preparing novel alkylpipecolates and better understanding of their conformational effects in peptide structures. Enantiopure 6-alkylpipecolates were synthesized by employment of α-*tert*-butyl *N*-(PhF)aspartate β-aldehyde **2** in an aldol condensation, dehydration, reductive amination sequence. Primary, secondary and tertiary alkyl as well as aromatic groups, all were stereoselectively introduced at the 6-position of pipecolic acid. Furthermore, extension of this methodology offers potential to provide pipecolates with other substitution patterns. Comparison of *N*-acetyl *N'*-methyl pipecolinamide **16** and its (2*S*, 6*R*)-6-*tert*-butyl counterpart **17** demonstrated that the 6-position substituent augmented the pipecolyl amide *cis*-isomer population and lowered the barrier for amide isomerization in a manner similar albeit less pronounced than a 5-*tert*-butyl substituent influenced the amide of the corresponding proline series.[14] This approach has thus expanded our capacity for synthesizing and employing azacycloalkane carboxylates as tools for the exploration of conformation-activity relationships of biologically active peptides.

### 4.12. Experimental Section

**General.** Unless stated otherwise, solvents and reagents were used as supplied, and all reactions were carried out under nitrogen. Toluene was distilled from sodium, tetrahydrofuran from sodium/benzophenone, dichloromethane from $P_2O_5$ and the methyl ketones from $CaSO_4$ immediately prior to use. Diisopropylamine, DIEA and triethylamine were distilled from $CaH_2$. Diisopropylcarbodiimide was distilled from $KMnO_4$. Copper(I)chloride was purified by precipitation from hydrochloric acid.[41] Methanesulfonyl chloride was passed through a pad of basic alumina immediately prior to use. *n*-Butyllithium was titrated using menthol in THF containing fluorene as indicator. Final reaction solutions were dried over $Na_2SO_4$. Flash-column chromatography[42] was performed on 230-400 mesh silica gel; TLC was performed on aluminum-backed silica plates with visualization by UV-light, iodine or ninhydrin. Mass spectral data, HRMS (FAB and ES), were obtained by the Université de Montréal Mass Spec. facility. $^1$H NMR and $^{13}$C NMR spectra were respectively recorded at 400

MHz and 100 MHz in $CDCl_3$. Chemical shifts are in ppm (δ units) relative to internal $(CH_3)_4Si$ or residual solvent for $^1H$ NMR spectra, and relative to solvant signals for $^{13}C$ NMR spectra. Chemical shifts for aromatic and vinyl carbons are not reported for PhF containing compounds. Catalytic hydrogenation was conducted at 1 atm in a round-bottomed flask equipped with a balloon and at 3 atm in a high-pressure hydrogenation apparatus.

**(2*S*)-*tert*-Butyl 2-[*N*-(PhF)amino]-4-oxobutanoate (2).** Diisobutylaluminum hydride (46.6 mL of a 1.0 M solution in toluene, 0.047 mol) was added dropwise over 10 min to a stirred solution of (2*S*)-α-*tert*-butyl β-methyl-*N*-(PhF)aspartate (**3**, 18.8 g, 0.042 mol, prepared according to reference 26) in toluene (420 mL) at –78 °C. Upon complete addition, the reaction was stirred for an additional 5 min and quenched by the addition of acetone (10 mL) followed by water (10 mL). Sodium bicarbonate (approx. 45 g) was added to the mixture which was allowed to warm to rt with vigorous stirring and filtered through a pad of sodium bicarbonate on Celite™. The filter cake was washed thoroughly with ethyl acetate, and the filtrate was concentrated *in vacuo* to furnish the title compound[27] in >95% yield as a viscous colorless oil which was subsequently used without further purification: TLC $R_f$ = 0.44 (20% ethyl acetate in hexanes); $^1H$ NMR (300 MHz) δ 1.21 (s, 9 H), 2.40 (m, 2 H), 3.03 (dd, 1 H, $J$ = 7.4, 5.5), 3.34 (br s, 1 H), 7.15-7.72 (m, 13 H), 9.48 (dd, 1 H, $J$ = 2.8, 2.2).

***tert*-Butyl *N*-(PhF)-5-azapenta-2,4-dienoate (5)** was isolated, after stirring **3** in $CDCl_3$, by chromatography using a gradient of 0-5% EtOAc in hexanes as eluant. Evaporation of the collected fractions and recrystallization from EtOAc and hexanes gave colorless needles: mp = 148-149 °C; $R_f$ = 0.57 (1:4 EtOAc:hexanes); $^1H$ NMR δ 7.83-7.74 (m, 3 H), 7.39-7.22 (m, 13 H), 6.04 (d, 1 H, $J$ = 15.8), 1.49 (s, 9 H); $^{13}C$ NMR δ 28.0, 80.2, 81.1, 158.1, 164.9; FT-IR (nujol, $cm^{-1}$) 1714, 1610; m/z (nitrobenzyl alcohol): 396 ($MH^+$); m/z (thioglycerol): 504 (M+109).

**General Procedure for Aldol Condensation of Aspartate β-Aldehyde 2 with 3-Methylbutan-2-one and Pentan-2-one.** A solution of *n*-butyllithium (1.2 mmol) in hexanes was added dropwise to a stirred solution of diisopropylamine (164 μL, 1.2 mmol) in THF (1 mL) at –10 °C to 0 °C. Upon complete addition, the solution was cooled to –78 °C, stirred for 15 min, and treated with the ketone (1.1 mmol, 110 mol %). After stirring an additional 15 min, the reaction mixture was treated dropwise via cannula with a solution of aldehyde **2** (414 mg, 1 mmol) in THF (1 mL) and stirred at –78 °C until analysis by TLC indicated the complete consumption of aldehyde **2** (1-2 h). The reaction was quenched by the addition of a solution of saturated aqueous sodium bicarbonate (0.5 mL), allowed to warm to rt, and treated with additional saturated aqueous sodium bicarbonate (10 mL). The mixture was separated and the aqueous phase was extracted with diethyl ether (3 × 10 mL). The combined organic layers were dried and concentrated *in vacuo* to a crude product which was purified by flash-column chromatography, eluting with an ethyl acetate:hexanes system of appropriate polarity.

**General Procedure for Aldol Condensation of Aspartate β-Aldehyde 2 with Acetone, Pinacolone, Acetophenone and 2-Acetylpyridine.** A solution of *n*-butyllithium (1.4 mmol) in hexanes was added dropwise to a stirred solution of diisopropylamine (273 μL, 2.0 mmol) in THF (1 mL) maintaining the temperature between –10 °C and 0 °C. Upon complete addition, the solution was cooled to –78 °C, stirred for 15 min, treated with the ketone (1.4 mmol, 140 mol %), stirred 30 min, and treated dropwise via cannula with a solution of aldehyde **2** (414 mg, 1 mmol) in THF (1 mL). Stirring was continued at –78 °C until TLC analysis indicated complete consumption of aldehyde (1-2 h). Quench, work-up and purification, all were performed in the same manner as described above.

**(2*S*, 4*RS*)-*tert*-Butyl 4-Hydroxy-6-oxo-7,7-dimethyl-2-[*N*-(PhF)amino]octanoate (6a)** was isolated as a white foam containing an inseparable 2:1 mixture of diastereomers (84 % from **2**): TLC $R_f$ = 0.24 (20% ethyl acetate in hexanes); $^1$H NMR (major isomer) δ 1.11 (s, 9 H), 1.15 (s, 9 H), 1.50-1.56 (m, 2 H), 2.36 (dd, 1 H, *J* = 17.2, 5.4), 2.70 (dd, 1 H, *J* = 17.2, 6.8),

2.71-2.75 (m, 1 H), 4.04-4.15 (m, 1 H), 7.18-7.38 (m, 10 H), 7.46-7.52 (m, 1 H), 7.67-7.72 (m, 2 H); $^{1}$H NMR (minor isomer) δ 1.09 (s, 9 H), 1.23 (s, 9 H), 1.50-1.56 (m, 2 H), 2.44 (dd, 1 H, *J* = 17.4, 5.5), 2.63 (dd, 1 H, *J* = 17.4, 7.2), 2.71-2.75 (m, 1 H), 4.31-4.38 (m, 1 H), 7.18-7.38 (m, 10 H), 7.46-7.52 (m, 1 H), 7.67-7.72 (m, 2 H); $^{13}$C NMR (major isomer) δ 26.1, 27.7, 40.0, 43.6, 55.8, 67.0, 72.9, 81.0, 174.1, 214.4; $^{13}$C NMR (minor isomer) δ 26.1, 27.7, 39.7, 44.1, 53.8, 65.3, 72.9, 81.1, 174.5, 214.7; HRMS calcd for $C_{33}H_{40}NO_4$ ($MH^+$) 514.2957, found 514.2973.

**(2*S*, 4*RS*)-*tert*-Butyl 4-Hydroxy-7-methyl-6-oxo-2-[*N*-(PhF)amino]octanoate (6b)** was isolated as a pale-yellow, glassy solid containing an inseparable 2:1 mixture of diastereomers (77 % from **2**): TLC $R_f$ = 0.48 (30% ethyl acetate in hexanes); $^{1}$H NMR (major isomer) δ 1.07 (d, 3H, *J* = 6.9), 1.08 (d, 3H, *J* = 6.9), 1.15 (s, 9H), 1.50-1.55 (m, 2H), 2.33 (dd, 1H, *J* = 16.3, 5.0), 2.51-2.64 (m, 2H), 2.70-2.74 (m, 1H), 4.02-4.08 (m, 1H), 7.20-7.75 (m, 13H); $^{1}$H NMR (minor isomer) δ 1.06 (d, 3H, *J* = 6.9), 1.07 (d, 3H, *J* = 6.9), 1.24 (s, 9H), 1.50-1.55 (m, 2H), 2.39 (dd, 1H, *J* = 16.1, 4.9), 2.51-2.64 (m, 2H), 2.70-2.74 (m, 1H), 4.26-4.34 (m, 1H), 7.20-7.75 (m, 13H); $^{13}$C NMR (major isomer) δ 17.8, 27.7, 39.9, 41.1, 47.5, 55.9, 67.2, 72.9, 81.0, 174.0, 213.2; $^{13}$C NMR (minor isomer) δ 17.9, 27.7, 39.7, 41.3, 47.3, 53.9, 65.4, 72.9, 81.3, 174.3, 213.4; HRMS calcd for $C_{32}H_{38}NO_4$ ($MH^+$) 500.2801, found 500.2774. Anal. Calcd for $C_{32}H_{37}NO_4$: C, 76.92; H, 7.46; N, 2.80. Found C, 76.84; H, 7.55; N, 2.80.

**(2*S*, 4*RS*)-*tert*-Butyl 4-Hydroxy-6-oxo-2-[*N*-(PhF)amino]nonanoate (6c)** was isolated as an off-white solid containing an inseparable 2:1 mixture of diastereomers (72 % from **2**): TLC $R_f$ = 0.30 (20% ethyl acetate in hexanes); $^{1}$H NMR (major isomer) δ 0.91 (t, 3H, *J* = 7.4), 1.14 (s, 9H), 1.47-1.63 (m, 4H), 2.23-2.73 (m, 5H), 3.99-4.05 (m, 1H), 7.15-7.80 (m, 13H); $^{1}$H NMR (minor isomer) δ 0.89 (t, 3H, *J* = 7.4), 1.23 (s, 9H), 1.47-1.63 (m, 4H), 2.23-2.73 (m, 5H), 4.25-4.30 (m, 1H), 7.15-7.80 (m, 13H); $^{13}$C NMR (major isomer) δ 13.6, 16.9, 27.7, 39.8, 45.4, 49.9, 55.9, 67.4, 72.9, 81.1, 173.9, 209.7; $^{13}$C NMR (minor isomer) 13.6, 16.9, 27.7, 39.6, 45.5, 49.7, 54.0, 65.5, 72.9, 81.4, 174.2, 209.8; HRMS calcd for $C_{32}H_{38}NO_4$ ($MH^+$) 500.2801, found 500.2815.

**(2*S*, 4*RS*)-*tert*-Butyl 4-Hydroxy-6-oxo-2-[*N*-(PhF)amino]heptanoate (6d)** was isolated as a white solid containing an inseparable 2:1 mixture of diastereomers (68 % from **2**): TLC $R_f$ = 0.26 (30% ethyl acetate in hexanes); $^1$H NMR (major isomer) δ 1.14 (s, 9 H), 1.47-1.52 (m, 2 H), 2.15 (s, 3 H), 2.29 (dd, 1 H, *J* = 16.0, 4.7), 2.51 (dd, 1 H, *J* = 16.0, 8.0), 2.69-2.73 (m, 1 H), 3.98-4.05 (m, 1 H), 7.14-7.73 (m, 13 H); $^1$H NMR (minor isomer) δ 1.24 (s, 9 H), 1.47-1.52 (m, 2 H), 2.13 (s, 3 H), 2.34 (dd, 1 H, *J* = 16.5, 4.8), 2.54 (dd, 1 H, *J* = 16.0, 8.0), 2.69-2.73 (m, 1 H), 4.20-4.30 (1 H, m), 7.14-7.73 (m, 13 H); $^{13}$C NMR (major isomer) δ 27.7, 30.5, 39.7, 50.8, 55.9, 67.4, 72.9, 81.2, 173.9, 207.6; $^{13}$C NMR (minor isomer) δ 27.7, 30.7, 39.5, 50.7, 54.1, 65.4, 72.9, 81.4, 174.1, 207.5; HRMS calcd for $C_{30}H_{34}NO_4$ ($MH^+$) 472.2488, found 472.2505. Anal. Calcd for $C_{30}H_{34}NO_4$: C, 76.41; H, 7.05; N, 2.97. Found C, 76.28; H, 7.15; N, 3.01.

**(2*S*, 4*RS*)-*tert*-Butyl 4-Hydroxy-6-oxo-6-phenyl-2-[*N*-(PhF)amino]hexanoate (6e)** was isolated as a white solid containing an inseparable 2:1 mixture of diastereomers (93 % from **2**): TLC $R_f$ = 0.28 (20% ethyl acetate in hexanes); $^1$H NMR (major isomer) δ 1.16 (s, 9 H), 1.60-1.70 (m, 2 H), 2.77 (dd, 1 H, *J* = 9.4, 5.4), 2.84 (dd, 1 H, *J* = 16.4, 5.4), 3.32 (t, 1 H, *J* = 7.1), 4.24-4.31 (m, 1 H), 7.12-7.96 (m, 18 H); $^1$H NMR (minor isomer) δ 1.23 (s, 9 H), 1.60-1.70 (m, 2 H), 2.78 (m, 1 H), 2.92 (dd, 1 H, *J* = 16.6, 5.5), 3.36 (t, 1 H, *J* = 7.1), 4.46-4.54 (m, 1 H), 7.12-7.96 (m, 18 H); $^{13}$C NMR (major isomer) δ 27.8, 40.1, 45.7, 55.8, 67.4, 73.0, 81.1, 174.1, 198.8; $^{13}$C NMR (minor isomer) δ 27.7, 39.7, 45.8, 54.1, 65.8, 73.0, 81.4, 174.2, 198.7; HRMS calcd for $C_{35}H_{36}NO_4$ ($MH^+$) 534.2644, found 534.2662.

**(2*S*, 4*RS*)-*tert*-Butyl 4-Hydroxy-6-oxo-6-(2-pyridyl)-2-[*N*-(PhF)amino]hexanoate (6f)** was isolated as a white solid containing an inseparable 3:1 mixture of diastereomers (61 % from **2**): TLC $R_f$ = 0.33 (30% ethyl acetate in hexanes); $^1$H NMR (major isomer) δ 1.15 (s, 9H), 1.57-1.68 (m, 2H), 2.75 (dd, 1H, *J* = 9.8, 4.8), 3.09 (dd, 1H, *J* = 16.1, 4.4), 3.30 (dd, 1H, *J* = 16.1, 8.1), 4.26-4.33 (m, 1H), 7.10-8.70 (m, 17H); $^1$H NMR (minor isomer) δ 1.22 (s, 9H), 1.57-1.68 (m, 2H), 2.78-2.82 (m, 1H), 3.21 (dd, 1H, *J* = 16.5, 4.6), 3.30-3.35 (m, 1H), 4.48-4.56 (m, 1H), 7.10-8.70 (m, 17H); $^{13}$C NMR (major isomer) δ 27.7, 40.2, 45.1, 55.8, 67.4, 73.0, 81.0, 174.1, 200.2; $^{13}$C NMR (minor

isomer) δ 27.7, 40.0, 45.5, 54.1, 65.7, 73.0, 81.2, 174.4, 200.2; HRMS calcd for $C_{34}H_{35}N_2O_4$ ($MH^+$) 535.2597, found 535.2612.

**General Procedure A for the Dehydration of β-Hydroxy Ketones 6a-e.** A solution of β-hydroxy ketone **6** (1 mmol, 100 mol%) in THF (8 mL) was treated with copper(I)chloride (40 mg, 0.4 mmol) and diisopropylcarbodiimide (470 μL, 3 mmol), and the suspension was heated at a reflux for 14 h. After cooling to rt, the solvent was removed *in vacuo* and the residue was purified by flash-column chromatography, eluting with an ethyl acetate:hexanes system of appropriate polarity.

**General Procedure B for the Dehydration of β-Hydroxy Ketones 6a-f.** Methanesulfonyl chloride (85 μL, 1.1 mmol) was added dropwise to a stirred solution of β-hydroxy ketone **6** (1 mmol) and triethylamine (418 μL, 3 mmol) in dichloromethane (2.5 mL) at 0 °C. Upon complete addition, the reaction was stirred for 30 min at 0 °C, allowed to warm to rt, and stirred until TLC analysis indicated the consumption of the starting material (1-2 h). The reaction was diluted with dichloromethane (10 mL) and washed with water (10 mL). The aqueous phase was extracted with dichloromethane (3 × 5 mL), and the combined organic layer was dried and concentrated *in vacuo* to a crude oil that was purified by flash-column chromatography eluting with an ethyl acetate:hexanes system of appropriate polarity to furnish enones **7**.

**(2*S*, 4*E*)-*tert*-Butyl 6-Oxo-7,7-dimethyl-2-[*N*-(PhF)amino]oct-4-enoate (7a)** was isolated as an oil from **6a** in 87% yield with method A and 62% with method B: TLC $R_f$ = 0.57 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ –144.5 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 1.19 (s, 9 H), 1.23 (s, 9 H), 2.28-2.41 (m, 2 H), 2.73 (m, 1 H), 3.19 (d, 1 H, *J* = 8.1), 6.50 (d, 1 H, *J* = 15.2), 6.97 (dt, 1 H, *J* = 15.1, 7.6), 7.16-7.40 (m, 10 H), 7.45-7.48 (m, 2 H), 7.67-7.72 (m, 2 H); $^{13}C$ NMR δ 26.0, 27.7, 38.7, 42.6, 55.5, 72.8, 80.8, 173.8, 203.24; HRMS calcd. for $C_{33}H_{38}NO_3$ ($MH^+$) 496.2852, found 496.2836. Anal. Calcd for $C_{33}H_{37}NO_3$: C, 79.97; H, 7.52; N, 2.83. Found C, 80.21; H, 7.90; N, 2.96.

**(2*S*, 4*E*)-*tert*-Butyl 7-Methyl-6-oxo-2-[*N*-(PhF)amino]oct-4-enoate (7b)** was isolated as a white solid from **6b** in 62 % yield with method A and 87 % yield with method B: TLC $R_f$ = 0.69 (30% ethyl acetate in hexanes); mp 94-96°C; $[\alpha]_D^{20}$ –145.4 (*c* 1.5, $CHCl_3$); $^1H$ NMR δ 1.11 (d, 3 H, *J* = 7.0), 1.12 (d, 3 H, *J* = 6.9), 1.18 (s, 9 H), 2.22-2.36 (m, 2 H), 2.67 (t, 1 H, *J* = 6.2), 2.84 (sept., 1 H, *J* = 6.9), 3.16 (br s, 1 H), 6.09 (d, 1 H, *J* = 15.7), 6.80 (dt, 1 H, *J* = 15.7, 7.4), 7.15-7.73 (m, 13 H); $^{13}C$ NMR δ 18.4, 27.8, 38.0, 38.8, 55.5, 72.9, 81.1, 173.8, 203.5; HRMS calcd for $C_{32}H_{36}NO_3$ ($MH^+$) 482.2695, found 482.2686.

**(2*S*, 4*E*)-*tert*-Butyl 6-Oxo-2-[*N*-(PhF)amino]non-4-enoate (7c)** was isolated as a white solid from **6c** in 63 % yield with method A and 91 % yield with method B: TLC $R_f$ = 0.50 (20% ethyl acetate in hexanes); mp 88-89 °C; $[\alpha]_D^{20}$ –135.1 (*c* 1.5, $CHCl_3$); $^1H$ NMR δ 0.97 (t, 3 H, *J* = 7.4), 1.18 (s, 9 H), 1.67 (sex., 2 H, *J* = 7.4), 2.22-2.36 (m, 2 H), 2.52 (td, 2 H, *J* = 7.4, 1.2), 2.68 (t, 1 H, *J* = 6.5), 3.19 (br. s, 1 H), 6.03 (dt, 1 H, *J* = 16.0, 1.4), 6.74 (dt, 1 H, *J* = 16.0, 7.4), 7.15-7.70 (m, 13 H); $^{13}C$ NMR δ 13.8, 17.6, 27.8, 38.8, 41.6, 55.5, 72.9, 81.1, 173.8, 200.2; HRMS calcd for $C_{32}H_{36}NO_3$ ($MH^+$) 482.2695, found 482.2681.

**(2*S*, 4*E*)-*tert*-Butyl 6-Oxo-2-[*N*-(PhF)amino]hept-4-enoate (7d)** was isolated as a white solid from **6d** in 62 % yield with method A and 86 % yield with method B: TLC $R_f$ = 0.37 (20% ethyl acetate in hexanes); mp 81-82°C; $[\alpha]_D^{20}$ –148.0 (*c* 1.3, $CHCl_3$); $^1H$ NMR δ 1.19 (s, 9 H), 2.25 (s, 3 H), 2.31 (m, 2 H), 2.66 (t, 1 H, *J* = 6.1), 3.19 (br. s, 1 H), 6.00 (d, 1 H, *J* = 15.9), 6.70 (dt, 1 H, *J* = 15.9, 7.4), 7.15-7.73 (m, 13 H); $^{13}C$ NMR δ 26.5, 27.8, 38.8, 55.4, 72.9, 81.1, 173.8, 198.3; HRMS calcd for $C_{30}H_{32}NO_3$ ($MH^+$) 454.2382, found 454.2391.

**(2*S*, 4*E*)-*tert*-Butyl 6-Oxo-7-phenyl-2-[*N*-(PhF)amino]hept-4-enoate (7e)** was isolated as a white solid from **6e** in 81 % yield with method A and 92 % yield with method B: TLC $R_f$ = 0.44 (20% ethyl acetate in hexanes); mp 43-45 °C; $[\alpha]_D^{20}$ –108.5 (*c* 1.1, $CHCl_3$); $^1H$ NMR δ 1.18 (s, 9H), 2.32-2.46 (m, 2H), 2.71 (t, 1H, *J* = 6.4), 3.20 (br.s, 1H), 6.79 (dt, 1H, *J* = 15.4, 1.2), 6.95 (dt, 1H, *J* = 15.4, 7.5), 7.15-7.95 (m, 18H); $^{13}C$ NMR δ 27.8, 39.1, 55.7, 73.0, 81.1, 173.9, 190.6; HRMS calcd for $C_{35}H_{33}NO_3$ ($MH^+$) 454.2382, found 454.2391.

**(2*S*, 4*E*)-*tert*-Butyl 6-Oxo-7-(2'-pyridyl)-2-[*N*-(PhF)amino]hept-4-enoate (7f)** was isolated as an off-white solid from **6f** in 65-80 % yield with method B: TLC $R_f$ = 0.53 (30% ethyl acetate in hexanes); mp 118 °C (dec.); $[\alpha]_D^{20}$ –91.7 (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 1.18 (s, 9 H), 2.36-2.51 (m, 2 H), 2.72 (dd, 1 H, *J* = 6.9, 5.9), 3.21 (br s, 1 H), 7.14-7.70 (m, 14 H), 7.86 (m, 1 H), 8.14 (m, 1 H), 8.70 (m, 1 H); $^{13}C$ NMR δ 27.7, 39.1, 55.7, 72.9, 81.0, 173.9, 189.0; HRMS calcd for $C_{34}H_{33}N_2O_3$ ($MH^+$) 517.2492, found 517.2500.

**General Procedure for the Hydrogenation of ε-Oxo-α-*N*-(PhF)amino Acid Analogs 7a-f Over Palladium and Platinum Catalysts.** A solution of enone **7** (1 mmol) in either ethyl acetate (20 mL), methanol (20 mL) or 10:1 methanol/acetic acid (22 mL) was treated with either palladium-on-carbon (10 % by weight, 100 mg) or platinum-on-carbon (5 % by weight, 200 mg). The reaction vessel (see general experimental details) was filled, vented and refilled with hydrogen three times, and the suspension was stirred under hydrogen at rt for 14 h. The catalyst was filtered onto Celite™ and washed thoroughly with methanol (**7e** and **f**) or dichloromethane (**7a-d**). The filtrate was concentrated *in vacuo* and the residue was purified by flash-column chromatography with 9-phenylfluorene as the first product to elute. In cases where the filter cake had been washed with methanol, subsequent washing with dichloromethane provided an additional crop of 9-phenylfluorene hydrocarbon.

**(2*S*, 6*R*)-*tert*-Butyl 6-(1,1-Dimethylethyl)-2-piperidinecarboxylate (8a)** was isolated as a colorless oil in 86% yield from **7a**: TLC $R_f$ = 0.24 (5% $Et_2O$ in $CH_2Cl_2$); $[\alpha]_D^{20}$ –19.9 (c 0.6, $CHCl_3$); $^1H$ NMR ($CD_3OD$) δ 0.93 (s, 9 H), 1.13-1.02 (m, 1 H), 1.22-1.44 (m, 2 H), 1.47 (s, 9 H), 1.65 (m, 1 H), 1.92 (m, 2 H), 2.23 (dd, 1 H, *J* = 2.2, 11.3), 3.17 (dd, 1 H, *J* = 2.6, 11.5); $^{13}C$ NMR ($CD_3OD$) δ 25.9, 27.0, 27.1, 28.4, 29.8, 34.3, 61.5, 67.2, 82.1, 173.8; HRMS calcd for $C_{14}H_{28}NO_2$ ($MH^+$) 242.21201, found 242.21130.

**(2*S*, 6*R*)-*tert*-Butyl 6-(1-Methylethyl)-2-piperidinecarboxylate (8b)** was isolated as a colorless oil in 91% yield from **7b**: TLC $R_f$ = 0.22 (10% diethyl ether in dichloromethane); $[\alpha]_D^{20}$ –19.5 (c 1.0, $CHCl_3$); $^1H$ NMR δ 0.88 (d,

3H, J = 6.7), 0.91 (d, 3H, J = 6.7), 1.00 (dtd, 1H, J = 11.1, 8.8, 4.0), 1.21-1.39 (m, 2H), 1.42 (s, 9H), 1.58 (sept., 1H, J = 6.7), 1.59-1.65 (m, 1H), 1.82-1.99 (m, 3H), 2.19 (ddd, 1H, J = 11.1, 6.5, 2.5), 3.15 (dd, 1H, J = 11.3, 2.8); $^{13}$C NMR δ 18.7, 19.1, 24.7, 28.0, 28.4, 29.3, 33.1, 60.0, 62.3, 80.7, 172.7; HRMS calcd for $C_{13}H_{26}NO_2$ ($MH^+$): 228.1964, found 228.1957.

**(2*S*, 6*S*)-*tert*-Butyl 6-Propyl-2-piperidinecarboxylate (8c)** was isolated as a colorless oil in 90% yield from **7c**: TLC $R_f$ = 0.20 (20% diethyl ether in dichloromethane); $[\alpha]_D^{20}$ –13.7 (*c* 1.0, $CHCl_3$); $^1$H NMR 0.89 (t, 3H, *J* = 7.0), 0.98-1.09 (m, 1H), 1.23-1.44 (m, 6H), 1.45 (s, 9H), 1.61-1.67 (m, 1H), 1.84-1.90 (m, 1H), 1.95-2.00 (m, 1H), 2.51 (dtd, 1H, *J* = 11.0, 5.9, 2.6), 3.22 (dd, 1H, *J* = 11.3, 2.9); $^{13}$C NMR δ 14.2, 19.0, 24.6, 28.0, 29.3, 31.9, 39.3, 56.1, 59.7, 80.8, 172.6; HRMS calcd for $C_{13}H_{26}NO_2$ ($MH^+$) 228.1957, found 228.1964.

**(2*S*, 6*S*)-*tert*-Butyl 6-Methyl-2-piperidinecarboxylate (8d)** was isolated as a colorless oil in 86% yield from **7d**: TLC $R_f$ = 0.28 (10% diethyl ether in dichloromethane); $[\alpha]_D^{20}$ –7.4 (*c* 1.04, $CHCl_3$); $^1$H NMR δ 0.94-1.06 (m, 1H), 1.08 (t, 3H, *J* = 6.2), 1.21-1.45 (m, 2H), 1.43 (s, 9H), 1.53-1.59 (m, 1H), 1.78-1.85 (m, 2H), 1.90-1.96 (1H, m), 2.61 (dqd, 1H, *J* = 10.9, 5.7, 2.6), 3.21 (dd, 1H, *J* = 11.3, 2.8); $^{13}$C NMR δ 22.7, 24.6, 28.0, 28.9, 33.7, 51.7, 59.7, 80.7, 172.5; HRMS calcd for $C_{11}H_{22}NO_2$ ($MH^+$) 200.1651, found 200.1644.

**(2*S*, 6*R*)-*tert*-Butyl 6-Phenyl-2-piperidinecarboxylate (8e)** was isolated as a white solid: mp = 74-75°C; TLC $R_f$ = 0.57 (20% ethyl acetate in hexanes); $[\alpha]_D^{20}$ –28.0 (*c* 1.3, $CHCl_3$); $^1$H NMR δ 1.40 (s, 9H), 1.38-1.52 (m, 3H), 1.68-1.74 (m, 1H), 1.89-2.00 (m, 2H), 2.18 (br. s, 1H), 3.31 (dd, 1H, *J* = 11.1, 2.7), 3.57 (dd, 1H, *J* = 10.8, 2.3), 7.19-7.45 (m, 5H); $^{13}$C NMR δ 25.1, 28.0, 28.5, 34.2, 60.3, 61.7, 76.7, 126.7, 127.1, 128.3, 144.6, 172.2; HRMS calcd for $C_{16}H_{24}NO_2$ ($MH^+$) 262.1807, found 262.1813.

**(2*S*, 6*RS*)-*tert*-Butyl 2-Amino-6-hydroxy-6-phenylhexanoate (9)** was isolated as a viscous colorless oil containing an inseparable 1:1 mixture of diastereomers (46% from **7e**): TLC $R_f$ = 0.33 (10% $Et_3N$ in $Et_2O$); $^1$H NMR δ 1.34-1.58 (m, 3H), 1.41 (s, 9H), 1.63-1.81 (m, 3H), 2.15 (br.s, 3H), 3.24-3.29 (m,

1H), 4.62-4.68 (m, 1H), 7.21-7.36 (m, 5H); $^{13}C$ NMR (second diastereomer in parentheses where resolved) δ 21.7, (21.8), 27.9, 29.6, 34.3, (34.5), 38.7, 54.6, (54.7), 73.9, 81.0, 125.8, 127.3, 128.3, 144.8, (144.9), 175.2.

**(2*S*, 6*RS*)-*tert*-Butyl 6-hydroxy-2-[*N*-(PhF)amino]-6-(2'-pyridyl)hexanoate (10)** was isolated as a viscous colorless oil containing an inseparable 1:1 mixture of diastereomers (67% from **7f**): TLC $R_f$ = 0.37 (40% EtOAc in hexanes); $^1H$ NMR (second diastereomer in brackets where resolved) δ 1.10 (s, 9H), [1.11 (s, 9H)], 1.25-1.70 (m, 6H), 2.44-2.49 (m, 1H), 4.64-4.70 (m, 1H), 7.15-7.80 (m, 16H), 8.51-8.56 (m, 1H); $^{13}C$ NMR (second diastereomer in parentheses where resolved) δ 21.0, (21.1), 27.8, 35.6, 38.1, 55.8, 72.4, (72.5), 73.0, 80.3, 175.4; HRMS calcd for $C_{34}H_{37}N_2O_3$ ($MH^+$) 521.2804, found 521.2795.

**(2*S*)-*tert*-Butyl 6-Oxo-6-phenyl-2-[*N*-(PhF)amino]hexanoate (12)** was isolated as a white solid in 84% yield from **7e**: mp = 91-92°C; TLC $R_f$ = 0.44 (20 % EtOAc in hexane); $[\alpha]_D^{20}$ –159.4 (*c* 1.2, $CHCl_3$); $^1H$ NMR δ 1.19 (s, 9H), 1.40-1.57 (m, 2H), 1.76-1.88 (m, 2H), 2.54 (br. s, 1H), 2.71 (ddd, 1H, *J* = 17.0, 7.0, 6.6), 2.79 (ddd, 1H, *J* = 17.0, 8.3, 6.7); $^{13}C$ NMR δ 19.9, 27.6, 34.9, 38.0, 55.3, 72.9, 80.4, 175.2, 199.9; HRMS calcd for $C_{35}H_{36}NO_3$ ($MH^+$) 518.2695, found 518.2686.

**(2*S*, 6*RS*)-*tert*-Butyl 6-Hydroxy-6-phenyl-2-[*N*-(PhF)amino]hexanoate (11).** To a stirred suspension of Raney nickel (*ca.* 10 mg of a 50% slurry in water, pore size 50μ, surface area 80-100 $m^2/g$, washed 3 times with ethanol) in ethanol (4 mL) was added a solution of (2*S*, 4*E*)-*tert*-butyl 6-oxo-7-phenyl-2-[*N*-(PhF)amino]hept-4-enoate (**7e**, 445 mg, 0.86 mmol) in ethanol (5 mL). The resulting suspension was stirred under 3 atm of hydrogen at rt for 14 h. Careful filtration followed by concentration furnished **11** (425 mg, 95%) as a white foam containing an inseparable 1:1 mixture of diastereomers: TLC $R_f$ = 0.18 (10% EtOAc in hexanes): $^1H$ NMR δ 1.15 (s, 9 H), 1.32-2.07 (m, 6 H), 2.46-2.50 (m, 1 H), 3.05 (br. s, 1 H), 4.59-4.65 (m, 1 H), 7.15-7.71 (m, 18 H); $^{13}C$ NMR (second diastereomer in parentheses where resolved) δ 21.6, (21.7),

27.8, 35.5, 38.7, 55.9, 73.0, 74.3, 80.4, 175.4; HRMS calcd for $C_{35}H_{38}NO_3$ ($MH^+$) 520.2852, found 520.2834.

**General Method for the Deprotection of *tert*-Butyl 6-Alkylpipecolates 8.** Dry HCl gas was bubbled through a solution of 6-alkylpipecolate *tert*-butyl ester **8** (0.5 mmol) in dichloromethane (2-3 mL) at 0°C until complete disappearance of the *tert*-butyl ester singlet was observed by $^1H$ NMR analysis (2-4 h). Removal of the solvent *in vacuo* furnished pipecolate hydrochlorides **1•HCl**.

**(2*S*, 6*R*)-6-(1,1-Dimethylethyl)-2-piperidinecarboxylic Acid Hydrochloride (1a•HCl)** was isolated as a white powder in 98% yield from **8a**: mp >220 °C dec.; $[\alpha]_D^{20}$ –25.4 (*c* 0.7, 0.1N HCl); $^1H$ NMR ($CD_3OD$) δ 1.08 (s, 9H), 1.44-1.57 (m, 1H), 1.63-1.82 (m, 2H), 1.99-2.06 (m, 2H), 2.28-2.34 (m, 1H), 3.02 (dd, 1H, *J*=12.5, 2.5), 3.97 (dd, 1H, *J* = 12.7, 3.3); $^{13}C$ NMR ($CD_3OD$) δ 23.9, 24.7, 26.9, 27.0, 34.5, 60.7, 68.0, 171.2.

**(2*S*, 6*R*)-6-(1-Methylethyl)-2-piperidinecarboxylic Acid Hydrochloride (1b•HCl)** was isolated as a white powder in 100% yield from **8b**: mp >215 °C dec.; $[\alpha]_D^{20}$ –18.8 (*c* 0.6, 0.1N HCl); $^1H$ NMR ($CD_3OD$) δ 0.88 (d, 3H, *J* = 6.7), 0.91 (d, 3H, *J* = 6.7), 1.00 (dtd, 1H, *J* = 11.1, 8.8, 4.0), 1.21-1.39 (m, 2H), 1.42 (s, 9H), 1.58 (sept., 1H, *J* = 6.7), 1.59-1.65 (m, 1H), 1.82-1.99 (m, 3H), 2.19 (ddd, 1H, *J* = 11.1, 6.5, 2.5), 3.15 (dd, 1H, *J* = 11.3, 2.8); $^{13}C$ NMR ($CD_3OD$) δ 18.0, 19.7, 23.6, 25.5, 27.2, 32.3, 59.6, 64.1, 171.3; m/z 172 ($MH^+$).

**(2*S*, 6*S*)-6-Propyl-2-piperidinecarboxylic Acid Hydrochloride (1c•HCl)** was isolated as a white powder in 97% yield from **8c**: mp >209 °C dec.; $[\alpha]_D^{20}$ –13.0 (*c* 1.0, 0.1N HCl); $^1H$ NMR ($CD_3OD$) δ 0.99 (t, 3H, *J* = 7.3), 1.34-1.78 (m, 7H), 1.95-2.07 (m, 2H), 2.30-2.39 (m, 1H), 3.11-3.19 (m, 1H), 3.94 (dd, 1H, *J* = 12.4, 3.2); $^{13}C$ NMR ($CD_3OD$) δ 14.2, 19.6, 23.5, 27.4, 28.8, 36.7, 58.2, 58.9, 171.3; m/z 172 ($MH^+$).

**(2*S*, 6*S*)-6-Methyl-2-piperidinecarboxylic Acid Hydrochloride (1d•HCl)** was isolated as a white powder in 98% yield from **8d**: mp >212 °C dec.; $[\alpha]_D^{20}$

–11.7 (*c* 1.1, 0.1N HCl); $^{1}$H NMR ($CD_3OD$) δ 1.37 (d, 3H, *J* = 6.6), 1.41-1.50 (m, 1H), 1.58-1.74 (m, 2H), 1.89-1.99 (m, 2H), 2.29-2.36 (m, 1H), 3.22-3.29 (m, 1H), 3.94 (dd, 1H, *J* = 12.4, 3.3); $^{13}$C NMR ($CD_3OD$) δ 19.4, 23.6, 27.1, 31.1, 54.4, 58.7, 171.3; m/z 144 ($MH^+$).

**(2*S*, 6*R*)-6-Phenyl-2-piperidinecarboxylic Acid Hydrochloride (1e•HCl)** was isolated as a white powder in 96% yield from **8e**: mp >230 °C dec.; $[\alpha]_D^{20}$ –17.0 (*c* 0.4, 0.1N HCl); $^{1}$H NMR ($CD_3OD$) δ 1.81-2.14 (m, 5H), 2.38-2.45 (m, 1H), 4.18 (dd, 1H, *J* = 12.4, 3.4), 4.35 (dd, 1H, *J* = 11.6, 3.0), 7.41-7.57 (m, 5H); $^{13}$C NMR ($CD_3OD$) δ 24.1, 26.9, 30.9, 59.7, 62.4, 128.6, 130.5, 130.7, 137.9. 171.0; m/z 206 ($MH^+$).

**(2*S*, 6*S*)-*tert*-Butyl *N*-Benzyl-6-methylpiperidine-2-carboxylate (14).** Benzyl bromide (34 μL, 0.29 mmol, 120 mol%) and potassium carbonate (66 mg, 0.48 mmol, 200 mol%) were added to a solution of (2*S*, 6*S*)-*tert*-butyl 6-methylpiperidine-2-carboxylate (**8d**, 48 mg, 0.24 mmol) in DMF (0.2 mL). After stirring for 36 h, the suspension was diluted with ethyl acetate (5 mL), washed successively with water (3 × 5 mL) and brine (5 mL), dried and concentrated *in vacuo* to give a pale yellow oil (79 mg). Purification by flash-column chromatography (dichloromethane followed by 5% diethyl ether in dichloromethane) furnished (2*S*, 6*S*)-*tert*-butyl *N*-benzyl-6-methylpiperidine-2-carboxylate (**14**, 66 mg, 95%) as a colorless oil: TLC $R_f$ = 0.64 (10% diethyl ether in dichloromethane); $[\alpha]_D^{20}$ –24.8 (*c* 0.5, $CHCl_3$); $^{1}$H NMR δ 1.00 (d, 3H, *J* = 6.3), 1.21-1.36 (m, 1H), 1.38 (s, 9H), 1.49-1.55 (m, 1H), 1.60-1.69 (m, 2H), 1.74-1.81 (m, 1H), 2.36 (dqd, 1H, *J* = 9.6, 6.2, 2.9), 3.01 (dd, 1H, *J* = 10.5, 3.3), 3.65 (d, 1H, *J* = 15.9), 3.84 (d, 1H, *J* = 15.9), 7.15-7.38 (m, 5H); $^{13}$C NMR δ 21.2, 23.0, 27.8, 30.2, 34.1, 56.2, 56.4, 66.5, 80.5, 126.3, 127.7, 128.7, 139.5, 173.5.

**Enantiomeric Purity of (2*S*, 6*S*)-*tert*-Butyl *N*-Benzyl-6-methylpiperidine-2-carboxylate (14).** Dry HCl gas was bubbled through a stirred solution of (2*S*, 6*S*)-*tert*-butyl *N*-benzyl-6-methylpiperidine-2-carboxylate (**14**, 36 mg) in dichloromethane at 0 °C for 2 h. Evaporation of the volatiles *in vacuo* gave a white solid, from which portions (10 mg, 0.04 mmol) were dissolved in

acetonitrile (0.5 mL) and treated with D- or L-phenylalanine methyl ester hydrochloride (11 mg, 0.05 mmol, 120 mol%), TBTU (16 mg, 0.05 mmol, 120 mol%) and diisopropylethylamine (35 μL, 0.20 mmol, 500 mol%) at rt. After stirring for 1 h, the yellow solution was diluted with ethyl acetate (5 mL), washed sequentially with water (2 × 5 mL) and brine (5 mL), dried and concentrated *in vacuo* to a crude viscous yellow oil which was directly examined by $^1$H NMR spectroscopy. The limits of detection were determined by measuring the diastereomeric methyl ester singlets at 3.52 and 3.68 ppm in $CDCl_3$ in the 400 MHz $^1$H NMR spectra. Less than 1% of (2'*S*)-**15** was detected in the spectrum of (2'*R*)-**15**. Purification by chromatography using a gradient of 0-5% diethyl ether in dichloromethane as eluant gave dipeptides having the following spectra.

**(2*S*, 6*S*)-*N*-Benzyl-6-methylpipecolyl-D-phenylalanine Methyl Ester ((2'*R*)-15)** $^1$H NMR δ 1.11 (d, 3 H, *J* = 6.1), 1.15-1.36 (m, 2 H), 1.53-1.67 (m, 4 H), 1.83-1.87 (m, 1 H), 2.36-2.45 (m, 1 H), 2.81 (dd, 1 H, *J* = 13.9, 7.6), 2.96 (dd, 1 H, *J* = 13.9, 6.2), 3.01 (dd, 1 H, *J* = 9.9, 3.7), 3.47 (d, 1 H, *J* = 15.4), 3.67 (d, 1 H, *J* = 15.4), 3.68 (s, 3H), 4.78 (td, 1 H, *J* = 8.0, 6.2), 7.00-7.31 (m, 10 H).

**(2*S*, 6*S*)-*N*-Benzyl-6-methylpipecolyl-L-phenylalanine Methyl Ester ((2'*S*)-15)** $^1$H NMR δ 1.05 (d, 3 H, *J* = 6.1), 1.15-1.39 (m, 3 H), 1.55-1.68 (m, 3 H), 1.83-1.90 (m, 1 H), 2.32-2.41 (m, 1H), 3.01 (dd, 1 H, *J* = 10.6, 3.4), 3.06 (dd, 1 H, *J* = 13.9, 6.7), 3.14 (dd, 1 H, *J* = 13.9, 6.7), 3.52 (s, 3 H), 3.67 (d, 1H, *J* = 15.5), 3.85 (d, 1 H, *J* = 15.5), 4.79 (dt, 1 H, *J* = 8.1, 6.2), 7.11-7.53 (m, 10 H).

**Recycle of 9-Phenylfluorene to 9-Bromo-9-phenylfluorene** was accomplished by heating a mixture of 9-phenylfluorene (969 mg, 4.00 mmol) and *N*-bromosuccinimide (711 mg, 4.00 mmol) in $CCl_4$ (4 mL) at a reflux for 1 h. After cooling, the reaction was concentrated *in vacuo*, and the residue was successively digested with volumes of hexanes until TLC analysis showed that no UV-active material was contained in the hexanes. Concentration of the combined hexane volumes gave 9-bromo-9-phenylfluorene as a yellow solid (1.23 g, 96%): mp 98-100°C (lit[43] mp 99°C).

***N*-Acetyl *N'*-Methylpipecolinamide (16).** A solution of pipecolic acid (1.0 g, 7.7 mmol) in dioxane (15 mL) and water (8 mL) was treated with NaOH (12 mL, 1 M) followed by di-*tert*-butyl dicarbonate (4.06 g, 18.6 mmol, 240 mol%), stirred for 48 h at rt, and evaporated to a 5 mL volume that was diluted with EtOAc (20 mL) and acidified with 5% HCl to pH 2-3. The aqueous layer was saturated with solid NaCl and extracted with EtOAc until TLC of the organic layer showed no ninhydrin-active material. The combined organic layers were washed with brine (5 mL), dried, and evaporated to a solid residue that was dried for 48 h in a drying pistol. *N*-(BOC)-Pipecolic acid (1.62 g, 91%) was obtained as a white powder: mp 125°C, (lit. mp 124°C); HRMS calcd for $C_{11}H_{20}NO_4$ ($MH^+$) 230.1392, found 230.1388.

A solution of *N*-(BOC)-pipecolic acid (500 mg, 2.18 mmol), TBTU (1.05 g, 3.3 mmol, 150 mol%) and methylamine hydrochloride (295 mg, 4.36 mmol, 200 mol%) in acetonitrile (20 mL) was then treated with DIEA (1.33 mL, 7.6 mmol, 350 mol%), stirred for 24 h at rt, and evaporated to a residue, that was dissolved in EtOAc (40 mL), washed with 5% HCl (2 × 5 mL), $H_2O$ (5 mL), $NaHCO_3$ (2 × 5 mL, saturated solution), $H_2O$ (2 × 5 mL) and brine (5 mL), dried, and evaporated to give *N*-(BOC)-pipecolate *N'*-methylamide (393 mg, 74%) as a clear crystalline solid: mp = 86-88°C; $^1H$ NMR ($CD_3OD$) δ 4.61 (bs, 1 H), 3.94 (m, 1 H), 2.99 (t, 1 H, *J* = 13), 2.72 (s, 3H), 2.12 (d, 1 H, *J* = 13), 1.60 (m, 3 H), 1.43 (s, 9 H), 1.48-1.31 (m, 3 H); HRMS calcd for $C_{12}H_{23}N_2O_3$ ($MH^+$) 243.1709, found 243.1701.

*N*-(BOC)-Pipecolate *N'*-methylamide (390 mg, 1.6 mmol) was dissolved in $CH_2Cl_2$ (10 mL), cooled to 0°C, treated with gaseous HCl bubbles for 5 min, stirred for 1 h at rt, and evaporated to a residue that was dissolved in $CH_2Cl_2$ (8 mL), and treated with $Et_3N$ (444 μL, 3.2 mmol, 200 mol%) and acetic anhydride (302 μL, 3.2 mmol, 200 mol%). The mixture was heated at a reflux overnight and evaporated to dryness. Chromatography of the residue using a gradient of 0-5% MeOH in $CHCl_3$ as eluant gave **16** (128 mg, 65%) as a white crystalline solid: mp = 99-100 °C; $^1H$ NMR ($D_2O$, *trans*-isomer) δ 1.38-1.46 (m, 1H), 1.51-1.66 (m, 1H), 1.68-1.71 (m, 4H), 2.22 (s, 3H), 2.78 (s, 3H), 3.21 (m, 1H), 3.87 (bd, 1H, *J* = 14.7), 5.09 (bd, 1H, *J* = 2.2); (*cis*-isomer) δ 1.38-1.46 (m, 1H), 1.51-1.66 (m, 1H), 1.68-1.71 (m, 4H), 2.13 (s, 3H), 2.18 (bm, 1H), 2.28 (bm, 1H), 2.71 (bm, 1H), 2.81 (s, 3H), 4.30 (bd, 1H, *J* = 13.7),

4.65 (bd, 1H); $^{13}C$ NMR ($D_2O$, *trans*-isomer) δ 20.0, 21.2, 24.6, 26.2, 26.8, 45.1, 53.8, 173.8, 174.6; (*cis*-isomer) δ 20.2, 21.0, 24.3, 26.3, 26.4, 40.4, 58.5, 173.2, 174.4; HRMS calcd for $C_9H_{17}N_2O_2$ ($MH^+$) 185.1290, found 185.1285.

**(2*S*, 6*R*)-*N*-Acetyl *N'*-Methyl 6-*tert*-Butylpipecolinamide (17).** To a solution of acid **1a** (100 mg, 0.33 mmol), TBTU (159 mg, 0.495 mmol, 150 mol%) and methylamine hydrochloride (44.6 mg, 0.66 mmol, 200 mol%) in acetonitrile (2.3 mL) was added $Et_3N$ (229 μL, 1.65 mmol, 500 mol%). The clear solution was stirred for 24 h at rt and then evaporated to a residue that was treated with an ion-exchange resin (3 g, Dowex® SBR) in $H_2O$ (20 mL) to remove excess methylamine hydrochloride. The resin was filtered and the solution was evaporated to dryness. The crude amide product was heated for 1 week in the presence of acetic anhydride (5 mL) and sodium acetate (82 mg, 1 mmol) in toluene (5 mL). Evaporation of the volatiles and chromatography of the residue using a gradient of 0-5% MeOH in $CHCl_3$ as eluant gave **17** (36 mg, 28%) as a viscous oil: $^1H$ NMR ($D_2O$, *trans*-isomer) δ 0.96 (s, 9 H), 1.23-1.40 (m, 1 H), 1.69-1.84 (m, 2 H), 1.86-2.09 (m, 3 H), 2.33 (s, 3 H), 2.76 (s, 3 H), 4.01 (m, 1H), 5.04 (dd, 1 H, $J$ = 8.4, 8.2); (*cis*-isomer) δ 0.91 (s, 9 H), 1.23-1.40 (m, 1 H), 1.69-1.84 (m, 2 H), 1.86-2.09 (m, 3 H), 2.22 (s, 3 H), 2.79 (s, 3 H), 4.57 (m, 1 H), 4.71 (dd, 1 H, $J$ = 7.6, 7.3); $^{13}C$ NMR ($D_2O$, *trans*-isomer, ) δ 17.7, 23.8, 24.0, 25.0, 26.2, 27.2, 36.7, 54.9, 61.9, 175.2, 177.9; (*cis*-isomer) δ 17.9, 22.6, 23.5, 25.2, 26.4, 27.1, 36.3, 56.7, 58.1, 174.2, 177.5; HRMS calcd for $C_{13}H_{25}N_2O_2$ ($MH^+$) 241.1916, found 241.1907.

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**Supporting Information Available:** $^{1}H$ and $^{13}C$ NMR spectra of x; and crystallographic data for **5** (x pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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# Chapitre 5

# Développement d'une stratégie d'ancrage pour la chimie organique sur support solide

## 5.1. Introduction

La chimie sur support solide et la technologie combinatoire[1] ont eu un impact extraordinaire sur la recherche et le développement de nouveaux catalyseurs,[2] polymères,[3] matériaux[4] et agents thérapeutiques.[5]

Le découverte d'agents thérapeutiques potentiels par la chimie combinatoire a reçu une attention particulière. Puisque les azacycloalcanes jouent un rôle fondamental dans une multitude de processus biologiques, ils ont été traditionnellement utilisés dans la préparation d'une gamme d'agents thérapeutiques. Bien que plusieurs approches existent pour synthétiser des hétérocycles aromatiques[6] sur support solide, les composés azacycloalcanes chiraux ont reçu une attention limitée. En raison de leurs propriétés biologiques, il est remarquable que peu de méthodologies pratiques et flexibles aient été développées jusqu'à aujourd'hui pour synthétiser ces composés sur support solide.[6,7,8] Le développement d'une méthode de synthèse flexible pour accéder à ces hétérocycles est donc un défi intéressant.

Dans le but d'étudier de façon systématique les divers facteurs qui influencent la reconnaissance moléculaire entre un récepteur biologique et les ligands azacycloalcanes, il serait souhaitable de développer une approche permettant de produire une gamme de ces composés sous forme énantiomériquement pure. Une méthodologie de synthèse d'azacycloalcanes énantiopurs sur support solide serait potentiellement avantageuse pour effectuer cette exploration.

### 5.2. Potentiel et intérêt des bibliothèques moléculaires d'azacycloalcanes

Au chapitre 1, nous avons présenté des exemples représentatifs d'acides aminés azabicycloalcanes en tant que mimétismes peptidiques. On peut voir que ces composés possèdent des structures intimement reliées aux alcaloïdes de la famille des pyrrolizidines, indolizidines et quinolizidines (Figure 1). Les composés azacycloalcanes sont ubiquistes dans la nature. En effet, plus de 30% de tous les alcaloïdes connus aujourd'hui incorporent dans leur structure un noyau dérivé des composés de la classe des pyrrolizidines, des indolizidines, et des

quinolizidines.[9] L'intérêt des chimistes a été stimulé par le défi de synthèse imposé par la complexité structurale et stéréochimique des produits naturels azacycloalcanes. De plus, ces composés présentent un large spectre d'effets physiologiques. Par exemple, l'hélifoline (**1**) est un agent hépatotoxique qui a causé certains problèmes économiques au niveau de l'alimentation des bovins. La (–)-slaframine (**2**) et la (+)-pumiliotoxine B (**3**) possèdent une activité de stimulation cholinergique et cardiaque. Les indolizidines polyhydroxylées telle que la castanospermine (**4**), sont de puissants inhibiteurs de glycosidases. La géphyrotoxine (**5**) possède une activité au niveau des récepteurs muscariniques. L'indolizomycine (**6**) est un composé antibiotique. L'épilupinine *N*-oxyde (**7**) et la multiflorine (**8**) possèdent respectivement une activité hypoglycémique et antidiabétique. Par ailleurs, la spartéine (**9**) inhibe la croissance des cellules T.[9]

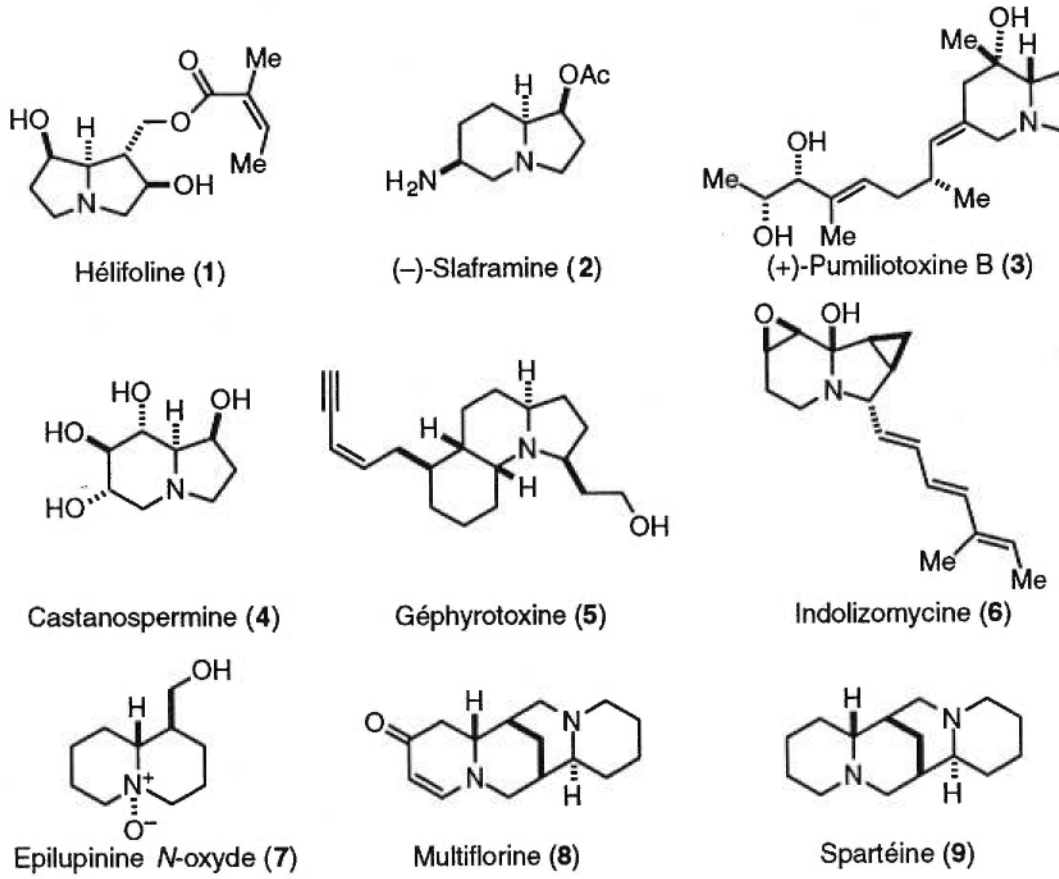



**Figure 1. Exemples de produits naturels azacycloalcanes.**

Puisque les acides aminés azabicycloalcanes possèdent des structures rigides, on peut les utiliser comme plate-formes moléculaires pour projeter dans l'espace des groupements pharmacophores. Ces composés peuvent être perçus comme des charpentes pour générer des bibliothèques moléculaires en modifiant les fonctions amine et carboxylate par les techniques combinatoires.[1] En contrôlant les divers centres stéréogéniques, la dimension de l'hétérocycle et les fonctions chimiques, ou chaînes latérales, sur le système hétérocyclique, on pourrait explorer de façon systématique des cibles biologiques.

L'élaboration de bibliothèques moléculaires basées sur les charpentes d'acides aminés azacycloalcanes pourrait vraisemblablement mener à des analogues possédant des propriétés biologiques similaires à ces familles d'alcaloïdes. La facilité avec laquelle on peut diversifier les fonctions chimiques sur le squelette azabicycloalcane et la grande variété d'applications, autant en chimie peptidique qu'en chimie médicinale, rend donc ces composés particulièrement intéressants comme cibles de synthèse.

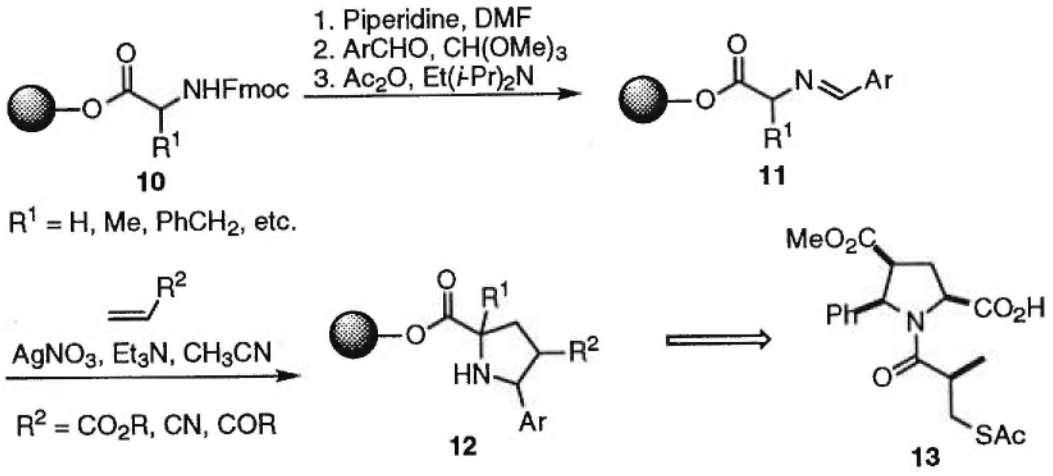
Nous avons effectué ici la transition d'une méthodologie de synthèse d'azacycloalcanes en solution, tel que présenté dans les chapitres précédents, vers une synthèse sur support solide. Dans le but d'établir une approche flexible et applicable à la synthèse d'azacycloalcanes énantiopurs sur support solide, nous avons envisagé une stratégie impliquant la synthèse et la manipulation de composés α-amino carbonyles configurationnellement stables.

### 5.3. Exemples de synthèse d'azacycloalcanes chiraux sur support solide

Tel que mentionné plus haut, la littérature présente très peu d'exemples de synthèse d'azacycloalcanes chiraux sur support solide. Une approche intéressante utilise la cycloaddition 1,3-dipolaire des ylures d'azométhines avec les oléfines (Schéma 1).[7] Les pyrrolidines **12** formées sont cependant racémiques en raison de la perte de stéréochimie lors de la formation de l'intermédiaire métallodipolaire. Après fonctionnalisation de la fonction amine par acylation avec une chaîne mercaptoacétyle, cette approche a permis la découverte d'un inhibiteur puissant **13** ($K_i$ ~ 160 pM)

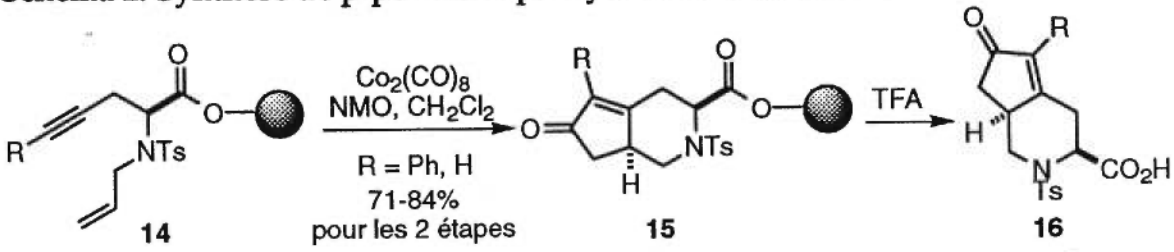
de l'enzyme *angiotensin converting enzyme*. Les résultats d'évaluation biologique ont indiqué que l'inversion des centres stéréogéniques sur la pyrrolidine de **13** produit un analogue essentiellement inactif.[7]

**Schéma 1. Synthèse de pyrrolidines par cycloaddition 1,3-dipolaire.**




Par ailleurs, la cycloaddition [2+2+1] de Pauson-Khand des énynes **14** en présence de cobalt octacarbonyle et de *N*-méthylmorpholine *N*-oxyde a été utilisée pour préparer les composés bicycliques **15** avec des rendements variant de 71 à 84% à partir de **14** (Schéma 2). Les acides aminés **16** étaient cependant racémiques.[8]

**Schéma 2. Synthèse de piperidines par cycloaddition [2+2+1].**



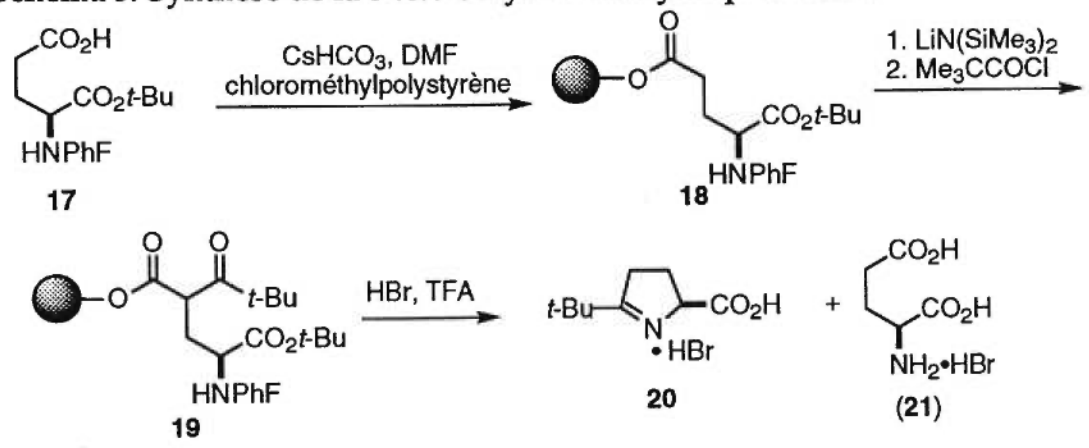

### 5.4. Résultats préliminaires

Dans le but de développer une méthodologie de synthèse d'azacycloalcanes énantiopurs sur support solide, nous avons initialement utilisé une stratégie basée sur la fonctionnalisation du glutamate **18** lié à la résine de Merrifield[10] à travers le carboxylate en position γ (Schéma 2).

Nous avons choisi la résine de Merrifield comme support polymérique puisque la littérature montrait qu'il est possible d'effectuer avec succès des réactions d'acylation[11] et d'alkylation[12] d'énolates d'esters en utilisant ce support solide.

Le (2*S*)-*N*-(PhF)glutamate d'α-*tert*-butyle a été ancré par un lien ester, formé par la réaction du sel de césium du glutamate **17** avec le polystyrène chlorométhylé. Nous avons au départ exploré l'acylation du glutamate **18** avec le chlorure de pivaloyle de façon à développer une synthèse de la 5-*tert*-butylproline sur support solide. En solution, l'acylation du *N*-(PhF)glutamate d'α-*tert*-butyle et de γ-méthyle avec le chlorure de pivaloyle donne un rendement de 58% du β-céto ester correspondant en utilisant le bis(triméthylsilyl)amidure de lithium comme base.[13]

**Schéma 3. Synthèse de la 5-*tert*-butyl-Δ$^5$-déshydroproline 20.**




Nous avons choisi des conditions similaires avec le glutamate **18**. La déprotonation régiosélective suivie de l'acylation de l'énolate avec le chlorure de pivaloyle, a fourni le β-céto ester **19**. Dans le but d'évaluer la conversion en β-céto ester **19**, nous avons retiré le produit de réaction de la résine par traitement avec une solution d'acide hydrobromique dans l'acide trifluoroacétique. Ceci a entraîné la solvolyse du groupe PhF, l'hydrolyse et la décarboxylation de l'ester et du même coup la cyclisation fournissant la 5-*tert*-butyl-Δ$^5$-déshydroproline **20** et l'acide glutamique (**21**).[14] Une comparaison de l'intensité des signaux pour le singulet du *tert*-

butyle avec le multiplet du proton α de l'acide glutamique dans le spectre $^1$H RMN a démontré que l'acylation de **18** donnait une conversion de 14% au β-céto ester **19**. Puisque cette conversion était médiocre, nous avons répété trois fois, de façon séquentielle, l'acylation du glutamate **18** selon les conditions décrites plus haut. Après l'acidolyse, nous avons obtenu une conversion maximale de 38% à la proline **20**.

Il est clair que les résultats préliminaires obtenus en utilisant cette approche étaient peu favorables. Cette stratégie rendait la récupération du produit de réaction difficile et ne permettait pas de fonctionnaliser ou de modifier la fonction ester liée au support solide. Nous avons donc choisi de modifier notre approche de façon à en améliorer la versatilité. Puisque la plupart des manipulations sur des composés α-amino carbonyles envisagées impliquaient les fonctions carbonyles, nous avons envisagé d'utiliser la fonction amine pour lier les substrats aux supports solides.

### 5.5. Protection d'amines pour la chimie sur support solide

Plusieurs stratégies ont été développées pour ancrer des fonctions amines sur support solide. Les groupes *tert*-butyloxycarbonyle (BOC),[15] fluorénylméthyloxycarbonyle (Fmoc),[16] benzyloxycarbonyle (Cbz),[17] triphénylméthyle (Tr, "Trityl"),[18] possèdent tous leurs équivalents sur des polymères (Figure 2).

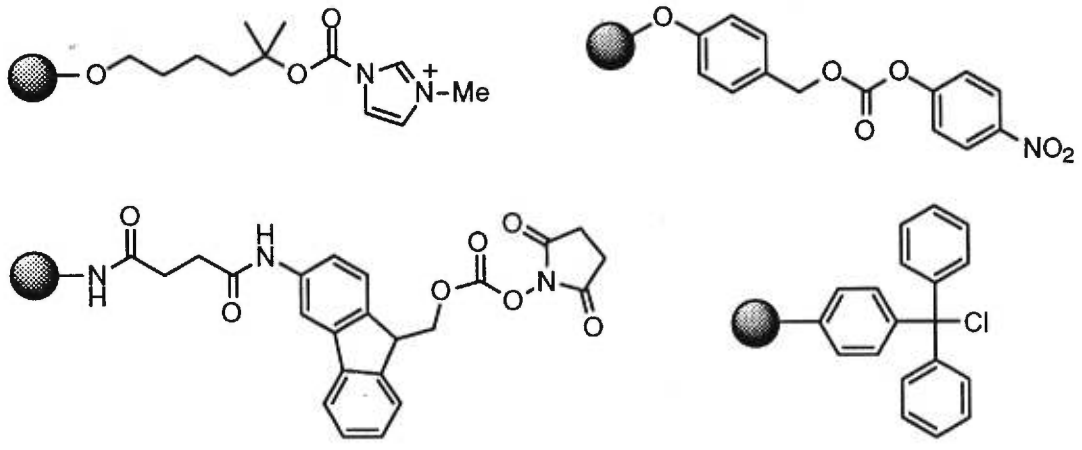



**Figure 2. Exemples de groupes d'ancrage d'amines sur support solide.**

Toutefois, la plupart de ces groupes protecteurs ne sont pas stables en conditions basiques, acides, ou en présence de réactifs organométalliques. Ils ont généralement été utilisés pour effectuer la synthèse de peptides sur support solide. De plus, ces groupes protecteurs nécessitent l'ancrage des intermédiaires à un stade souvent prématuré de la synthèse sur support solide. Il est clair que ces attributs compliquent l'utilisation de ces groupes protecteurs en synthèse. Dans le but de fournir une solution à cette situation, nous avons donc développé un nouveau groupe protecteur, le 9-bromo-9-*p*-bromophénylfluorène, pour protéger la fonction amine des acides aminés. Ce groupe protecteur permet la synthèse, l'ancrage au moment désiré, et la manipulation de composés α-amino carbonyles sur support solide.

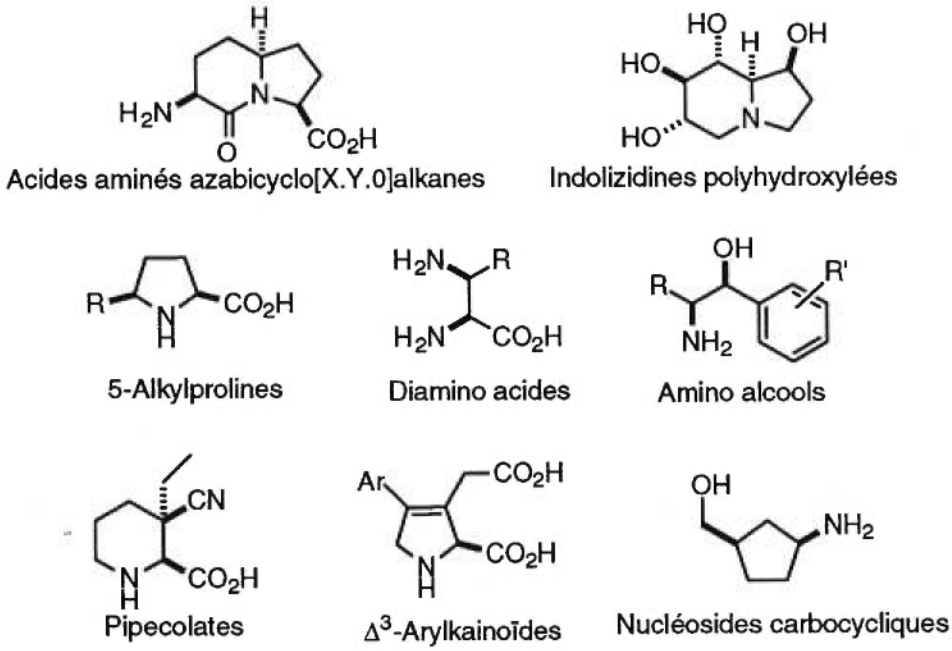



**Figure 3. Exemples de molécules énantiopures synthétisées à partir de composés *N*-(PhF)amino carbonyles.**

Précedemment, les travaux de Rapoport et de ses disciples ont établi l'utilité et la puissance du groupe protecteur phénylfluorényle (PhF) dans la synthèse de composés énantiopurs.[19-28] Par exemple, les composés *N*-(PhF)amino carbonyles ont été utilisés dans la synthèse d'alcaloïdes, d'hétérocycles et d'acides aminés énantiopurs (Figure 3).[19-28]

A travers la combinaison d'un effet stérique et d'un effet stéréoélectronique[27] qui placent le groupement carbonyle dans une géométrie planaire défavorable à la déprotonation, le groupe PhF assure l'intégrité stéréochimique des composés α-amino carbonyles.[19-28] On peut observer cette relation géométrique coplanaire entre le proton α et la fonction carbonyle dans les structures cristallographiques de composés *N*-(PhF)amino carbonyles.[27,28] La géométrie la plus favorable à la déprotonation est celle qui place le lien proton-carbone en position α dans un plan orthogonal au plan du système carbonyle. De cette façon, le recouvrement des orbitales est maximal lorsque l'hybridation du carbone α passe de $sp^3$ à $sp^2$.[27] Par ailleurs, le groupe PhF est 6000 fois plus stable que le groupe triphénylméthyle (Trityle, Tr) à la solvolyse. En raison de ses attributs exceptionnels, nous avons choisi ce groupe protecteur pour effectuer la chimie sur support solide.

Dans le chapitre 5, nous présentons le développement d'une nouvelle stratégie d'ancrage pour la synthèse et la manipulation d'α-amino aldéhydes configurationnellement stables sur des supports polymériques. Nous avons illustré et validé ce concept par la synthèse d'amino-alcools énantiopurs. Nous avons donc développé un procédé qui a converti le groupe protecteur 9-phénylfluorén-9-yle en un groupe d'ancrage pour la synthèse et la manipulation de composés α-amino carbonyles.

**5.6. Références.**

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# Article 7

Gosselin, F.; Van Betsbrugge, J.; Hatam, M.; Lubell, W. D. "A Novel Linking-Protecting Group Strategy for Solid-Phase Organic Chemistry with Configurationally Stable α-[*N*-(Phenylfluorenyl)]Amino Carbonyl Compounds: Synthesis of Enantiopure Norephedrines on Solid Support" Publié dans *The Journal of Organic Chemistry*, **1999**, *64*, 2486-2493.

# A Novel Linking-Protecting Group Strategy for Solid-Phase Organic Chemistry with Configurationally Stable α-[*N*-(Phenylfluorenyl)]Amino Carbonyl Compounds: Synthesis of Enantiopure Norephedrines on Solid Support

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## 5.7. Abstract

A novel linking strategy has been developed for synthesizing configurationally stable α-amino aldehyde on polymeric supports. Alkylation of L-alanine methyl ester with 9-bromo-9-*p*-bromophenylfluorene, followed by ester hydrolysis and coupling to isoxazolidine provided *N*-(9-*p*-bromophenylfluoren-9-yl)alanine isoxazolidide (**5**) which was transformed into its corresponding boronate **2** by a palladium catalyzed cross-coupling reaction with diboron pinacol ester. Boronate **2** was anchored to four different polymeric aryl halides **6-9** in 70-99% yields. Polymer-bound alaninal **1b** was then synthesized on non-cross-linked polystyrene by hydride reduction of isoxazolidide **10b**. Treatment of alaninal **1b** with phenylmagnesium bromide, cleavage of the resulting amino alcohol in a 1:2:2 TFA:$CH_2Cl_2$:anisole cocktail, and acylation with di-*tert*-butyldicarbonate furnished *N*-(BOC)norephedrines **14** that were demonstrated to be enantiopure by conversion to diastereomeric thioureas **15** and analysis by HPLC. In summary, we have developed a process by which the 9-phenylfluoren-9-yl protecting group has been converted into a new linker for the solid-phase synthesis and manipulation of α-amino carbonyl compounds.

**5.8. Introduction**

The development of novel strategies for effectively linking organic compounds to solid supports has become essential as solid-phase chemistry and combinatorial technology have evolved into fundamental tools for drug discovery.[1] In principle, the ideal linker should provide effective loading onto the support, stability under a diverse variety of reaction conditions, and easy product removal without contamination from the linker.[2] Although several strategies have emerged for attaching amines to solid supports,[3-5] many of these linkers are not stable under strong base, strong acid nor organometallic reaction conditions. Furthermore, because such linkers often require amine attachment to the support early in the synthesis, multiple reactions may be necessary prior to steps for generating molecular diversity. Solution-phase amine protection that converts into a solid-phase amine linker anytime during synthesis may offer advantages over conventional support-bound protecting groups, because penultimate intermediates could be initially synthesized in solution on large scale and then attached to supports for transformation via molecular diversification chemistry.

The 9-phenylfluoren-9-yl (PhF) amine protecting group has insured configurational stability in the preparation and manipulation of α-amino carbonyl compounds during solution-phase syntheses of enantiopure alkaloids,[6,7] heterocycles,[8] and amino acids.[9,10] The PhF group prevents the racemization of α-amino carbonyl compounds by sterically shielding the α-proton and by positioning the α-proton and carbonyl in a planar geometry in which deprotonation is stereoelectronically disfavored.[10,11] Relative to the trityl group,[5,12] the PhF group is also significantly more stable to solvolysis in acid because of the anti-aromatic character of the fluorenyl cation.[13] Because of these attributes, we began to study the conversion of the PhF protecting group into a new linker for the solid-phase synthesis and manipulation of α-amino carbonyl compounds.

Initially, we synthesized 9-bromo-9-phenylfluorenyl cross-linked polystyrene by lithiation of cross-linked polystyrene,[14,15] followed by addition of fluorenone and treatment of the tertiary alcohol with acetyl

bromide in benzene.[15,16] L-Alanine methyl ester hydrochloride was anchored to the polymer using $Et_3N$ in 1:1 $CH_2Cl_2$:$CH_3CN$, as indicated by a strong band for the ester carbonyl at 1736 $cm^{-1}$ in the PA-FTIR spectrum.[16,17] During the course of our investigation, communications appeared describing the preparation of 9-chloro-9-phenylfluorenyl cross-linked polystyrene resin and a phenylfluorenyl acetic acid linker, as well as their employment in peptide chemistry and manipulations of achiral amines, which demonstrated the greater acid stability of the PhF supports relative to trityl resins.[18] Focusing our attention on the synthesis and configurational stability of resino α-amino carbonyl compounds such as α-amino aldehyde **1** (Figure 1), we realized that a limitation of the phenylfluorenyl cross-linked polystyrene resins was the requirement to employ several steps on solid-phase in order to convert the starting resino ester into the desired α-amino aldehyde. Since such reactions could be more effectively accomplished on large scale in solution,[11] we discontinued our studies on the PhF-resin; instead, we selected to develop a PhF linker for synthesizing the penultimate intermediate prior to resin attachment.

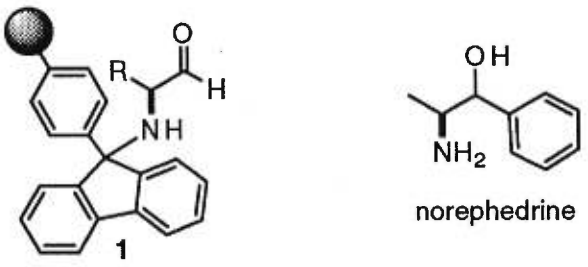



**Figure 1. Resino *N*-(PhF)-α-Amino Aldehyde 1 and Norephedrine.**

In developing a PhF linker, we pursued a protecting-linking group that was synthesized in few steps from inexpensive starting materials, compatible with solution and solid-phase chemistry, and attachable to an assortment of different solid supports. The employment of esters for joining the PhF group to the resin was judged inadequate due to their reactivity with organometallic reagents. Similarly, the phenylfluorenyl acetic acid linker[18] was deemed unsuitable because the amide used to link the PhF group to the resin was also expected to react competitively with

organometallic reagents during transformations of the resino α-amino carbonyl compounds. Furthermore, initial attempts to attach the PhF group to the support by an ether bond proved cumbersome due to difficulties in synthesizing PhF groups possessing protected ethers.

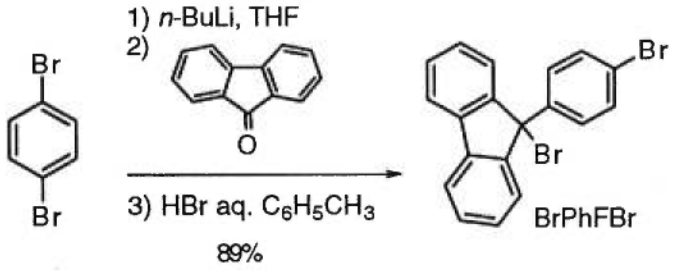
Transition metal catalyzed cross-coupling chemistry was examined for linking the PhF group and support, because these reactions are usually high yielding both in solution and on solid phase.[19,20] Mild conditions for converting aryl bromides into their respective aryl boronates had recently been reported and appeared compatible with a broad spectrum of functional groups.[21] We perceived that amino acids could be protected with 9-bromo-9-*p*-bromophenylfluorene in solution and that palladium catalyzed cross-coupling reactions could then be used to link the *N*-(BrPhF)amino acid derivative to the solid support (BrPhF = 9-(9-*p*-bromophenylfluorenyl)).

This concept has now been validated by the solution-phase synthesis of *N*-(BrPhF)alanine isoxazolidide and its conversion into polymer-bound α-amino aldehyde **1** (Figure 1, R = $CH_3$). Alaninal **1** was examined because α-amino aldehydes have been previously shown to be susceptible to racemization from various reactions in solution.[11,22] By employing polymer-bound α-amino aldehyde **1** in the synthesis of enantiopure norephedrines, we have demonstrated that the PhF protecting-linking group approach can provide configurational stability during the synthesis and modification of support-bound α-amino carbonyl compounds.

**5.9. Results**

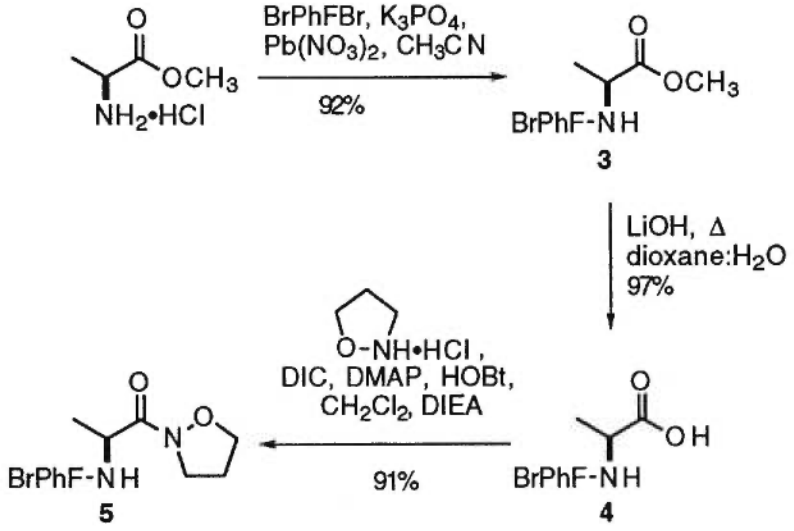
9-Bromo-9-*p*-bromophenylfluorene (BrPhFBr) was first synthesized in two steps and 89% overall yield from fluorenone by a procedure that was patterned closely after the preparation of 9-bromo-9-phenylfluorene published originally by Christie and Rapoport (Scheme 1).[6,23] Metal-halogen exchange on 1,4-dibromobenzene with *n*-butyllithium in THF at –78°C gave *p*-bromophenyllithium,[24] which was treated with fluorenone to furnish 9-*p*-bromophenylfluoren-9-ol. The crude alcohol was subsequently agitated vigorously with aqueous HBr in toluene to afford the desired bromide after crystallization from isooctane.

**Scheme 1.**
**Synthesis of 9-Bromo-9-*p*-bromophenylfluorene (BrPhFBr)**




L-*N*-[*p*-(Pinacolboronato)phenylfluorenyl]alanine isoxazolidide (**2**), the polymer-bound aldehyde precursor was synthesized in four steps and 74% overall yield using solution-phase chemistry (Schemes 2 and 3). Initially, L-*N*-(BrPhF)alanine methyl ester **3** was synthesized in 92% yield from L-alanine methyl ester hydrochloride and 9-bromo-9-*p*-bromophenylfluorene using the literature procedure for phenylfluorenation of α-amino esters.[25]

**Scheme 2.**
**Synthesis of *N*-(BrPhF)Alanine Isoxazolidide 5**



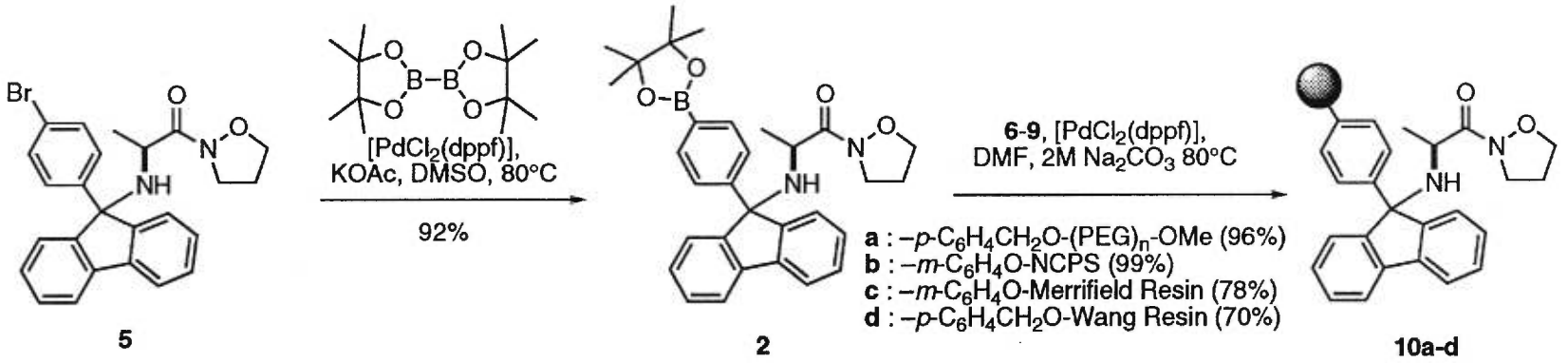

Hydrolysis of the methyl ester with aqueous LiOH in dioxane furnished *N*-(BrPhF)alanine **4** in 97% yield. *N*-(BrPhF)Alanine isoxazolidide **5** was

isolated as a white crystalline solid in 91% yield from coupling alanine **4** and isoxazolidine hydrochloride with *N,N*-diisopropylcarbodiimide and Et(*i*-Pr)$_2$N in dichloromethane.[11] Conversion of *p*-bromophenylfluorenyl protected amide **5** into its corresponding boronate **2** was accomplished in 92% yield after chromatography by treatment of isoxazolidide **5** with diboron pinacol ester, potassium acetate, and [PdCl$_2$(dppf)][26] in DMSO at 80°C for 20 h (Scheme 3).[21]

Different supports were examined in the palladium catalyzed cross-coupling reaction with boronate **2**. For example, boronate **2** was reacted with two soluble polymer supports.[27] Polyethylene glycol monomethyl ether (MeO-PEG-5000)[28] and non-cross-linked-polystyrene (NCPS)[29] were used to anchor *N*-(phenylfluorenyl)amino acid analogs, because reaction conversions could be monitored by $^1$H NMR spectroscopy of these supports in solution. Boronate **2** was also cross-coupled with two non-soluble supports. Chloromethyl cross-linked polystyrene (Merrifield Resin)[30] and *p*-benzyloxybenzyl alcohol resin (Wang Resin)[31] were linked to *N*-(phenylfluorenyl)amino acid analogs because of the importance of these supports in solid-phase organic synthesis.

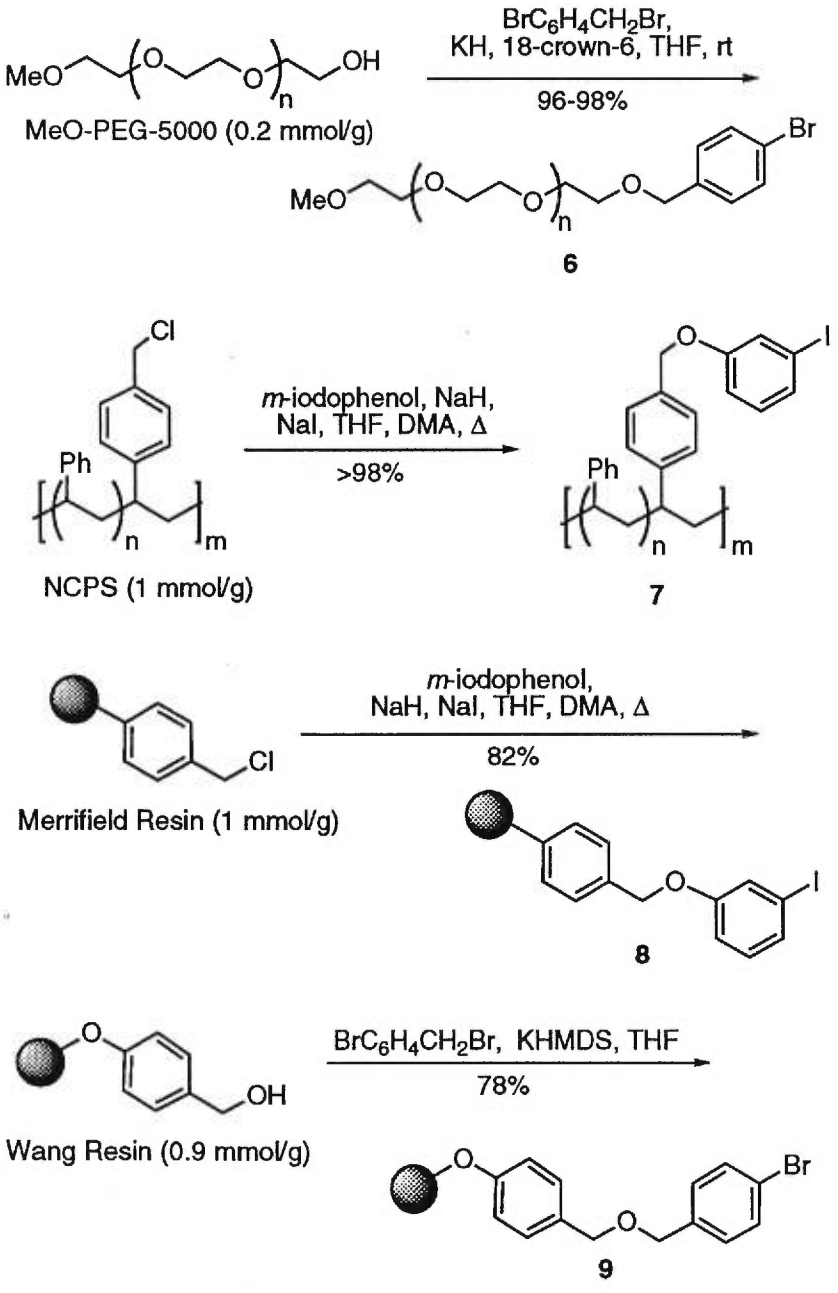
Aryl halide terminals were attached to the four different supports (Scheme 4). For example, MeO-PEG-5000-*p*-bromobenzyl ether **6** was synthesized by alkylation of MeO-PEG-5000 using *p*-bromobenzyl bromide in THF in the presence of NaI and 18-crown-6.[28] Non-cross-linked polystyrene (NCPS) *m*-iodophenyl ether **7** was prepared by anionic polymerization of styrene using *n*-butyllithium as initiator in toluene,[32] chloromethylation using chloromethyl methyl ether in the presence of ZnCl$_2$,[33] and ether formation with *m*-iodophenol, NaI and NaH in a solution of 1:1 DMA:THF. Proton NMR spectroscopy was used to determine the yield of the ether forming reactions to synthesize **6** and **7**. Measurement of the methylene proton signals of the benzylic ethers showed that MeO-PEG-5000-*p*-bromobenzyl ether **6** and NCPS-*m*-iodophenyl ether **7**, both were prepared in >96% yield. Treatment of Merrifield resin under similar conditions as described for the synthesis of NCPS-*m*-iodophenyl ether **7** gave Merrifield resin *m*-iodophenyl ether **8** in

**Scheme 3. Attachment of *N*-(BrPhF)Alanine Isoxazolidide 5 to Polymeric Supports via Arylbroronate 2**




**5** **2** **10a-d**

**Scheme 4. Preparation of Resino Aryl Halides 6-9**



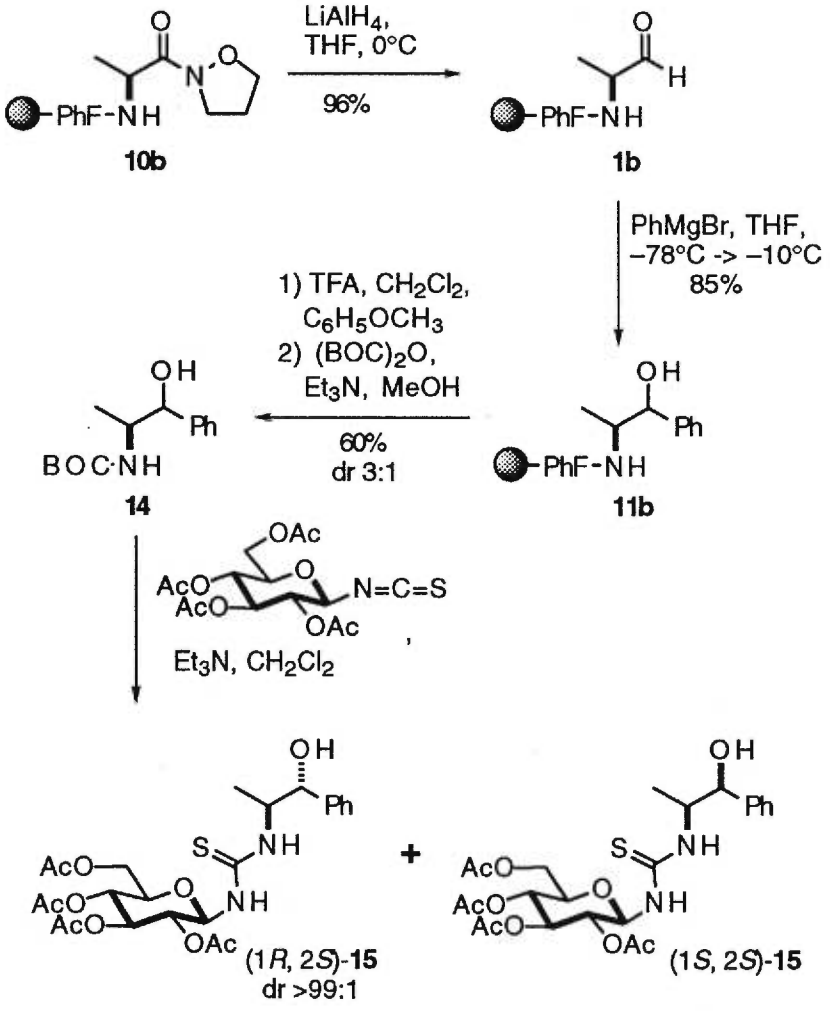
82% yield as ascertained by the increase in polymer weight. Alkylation of Wang resin using $KN(SiMe_3)_2$ and *p*-bromobenzyl bromide in THF at rt furnished Wang resin *p*-bromobenzyl ether **9** in 78% yield as determined by the increase in polymer weight.

Boronate **2** was coupled to polymer supports **6-9** using $[PdCl_2(dppf)]$ as catalyst and $Na_2CO_3$ as base in DMF at 80°C (Scheme 3).[21] In the case of soluble polymer supports **6** and **7**, conversions were estimated by comparing the integrations of the resino benzylic protons and the alanine methyl doublet in the $^1H$ NMR spectra. The cross-coupling of boronate **2** and MeO-PEG-5000-*p*-bromobenzyl ether **6** gave a 50-60% conversion to resino isoxazolidide **10a**. Cross-coupling of NCPS-iodophenyl ether **7** with boronate **2** gave NCPS-supported isoxazolidide **10b** in >98% conversion. Merrifield resin **8** reacted with **2** to provide resino isoxazolidide **10c** in 78% conversion based on proton MAS-NMR spectroscopic analysis and comparison of the integrations for the alanine methyl doublet and benzyl methylene protons.[34] Finally, cross-coupling of **2** to Wang resin **9** gave a 70% conversion to resino isoxazolidide **10d** as determined by weighing the cleavage product, (*p*-hydroxymethylphenyl-PhF)alanine isoxazolidide,[35] obtained from exposing the polymer to a 1:1 TFA:$CH_2Cl_2$ solution.

At present, we have focused our attention on the use of the soluble polymer-supported isoxazolidides **10a** and **10b**, because reactions on these materials were effectively monitored using solution-phase NMR spectroscopy. Alaninals **1a** and **1b** were respectively synthesized by reduction of polymer-bound isoxazolidides **10a** and **10b** with $LiAlH_4$ in THF and identified by $^1H$ NMR spectroscopy which showed an aldehyde peak at 9.1-9.2 ppm (Scheme 5).[11] In the case of MeO-PEG-5000-supported alaninal **1a**, the reaction was conducted at rt because the polymer had poor solubility in THF at lower temperatures. The polymer was isolated by precipitation in diethyl ether and filtration. Although $^1H$ NMR spectroscopy indicated that MeO-PEG-5000-supported alaninal **1a** was obtained in 66% yield, aldehyde **1a** was not stable and decomposed within 24 h at –20°C. An attempt to immediately react aldehyde **1a** with excess phenylmagnesium bromide in THF at rt failed to provide polymer

displaying amino alcohol signals in the $^1H$ NMR spectrum. These experiments indicated that MeO-PEG-5000-polymer was not suitable for α-amino aldehyde chemistry. In our hands, the breadth of chemical transformations performed with the MeO-PEG-5000-polymer was greatly limited due to low solubility in $CH_2Cl_2$, THF, and toluene below room temperature.

**Scheme 5.**
**Synthesis and Conversion of Polymer-bound Alaninal 1 into Enantiopure Norephedrines 14**




The NCPS-supported α-amino aldehyde, alaninal **1b** was prepared by $LiAlH_4$ reduction in THF at 0°C and stored overnight at rt without

noticeable decomposition. Polymer-bound norephedrines **11b** were synthesized by addition of a THF solution of phenylmagnesium bromide to a –78°C solution of NCPS-supported α-amino aldehyde **1b** in THF. Parallel experiments were conducted in solution to transform *N*-[BrPhF]alanine isoxazolidide **5** into a 1:1 mixture of diastereomeric *N*-(BrPhF)norephedrines **12** in 92% overall yield as described in the experimental section. Comparison of the proton NMR spectrum of NCPS-supported norephedrines **11b** with that of *N*-(BrPhF)norephedrines **12** verified the formation of the desired product from addition of phenylmagnesium bromide to polymer-bound aldehyde **1b**.

Cleavage of the amino alcohol product from the NCPS-support was initially studied using solutions containing TFA in $CH_2Cl_2$ at rt; however, the polymer became insoluble under these conditions, presumably due to cross-linking of polystyrene in acid,[29b] and norephedrine could not be isolated. In examinations of the cleavage conditions using both NCPS-supported norephedrines **11b** and *N*-(BrPhF)norephedrines **12**, we found that the polymer remained soluble in solutions of TFA in $CH_2Cl_2$ containing anisole as cosolvent. Anisole was introduced to trap carbocations formed during the acid induced cleavage.[36] The reaction of the NCPS-polymer and anisole was indicated by the presence of a new peak at 3.74 ppm for the anisole methoxy group in the $^1H$ NMR spectrum of the polymer after the cleavage reaction. Treatment of polymer **11b** in a 1:2:2 TFA:$CH_2Cl_2$:anisole solution for 48 h at rt provided norephedrines that were conveniently isolated as their *N*-BOC-derivatives. After an aqueous extraction and evaporation of the aqueous phase, the purity of the crude norephedrine was estimated to be >75% by comparison of the integration of the methyl doublets for the product with that of other doublets in the same spectral region. Treatment of the crude product with di-*tert*-butyldicarbonate and $Et_3N$ in MeOH, followed by column chromatography on silica gel to provide a 3:1 mixture of diastereomeric *N*-(BOC)-norephedrines **14** in 60% yield from NCPS-supported ephedrines **11b**. For comparison, we note that a 1:1 mixture of diastereomeric *N*-(BOC)-norephedrines **14** were obtained in 66% yield from the solution-phase solvolysis of *N*-(BrPhF)norephedrines **12** and protection with di-*tert*-

butyldicarbonate. In summary, *N*-(BOC)-norephedrines **14** were produced in 46% overall yield from L-*N*-[*p*-(pinacolboronato)phenylfluorenyl] alanine isoxazolidide **2**.

The enantiomeric purity of norephedrines **14**, that were synthesized from support-bound alaninal **1b**, was determined by HPLC analysis of their 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl thioureas **15**. Diastereomeric *N*-(BOC)amino alcohols **14** were treated with trifluoroacetic acid in $CH_2Cl_2$ to remove the BOC group and the crude trifluoroacetates were acylated using 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) and triethylamine in $CH_2Cl_2$.[37] Thioureas **15**, prepared from material cleaved from the NCPS-polymer, were then analyzed by reverse-phase HPLC using conditions that resolved all four diastereomers.[37] Comparison of the magnitudes of the major peak for the (1*R*, 2*S*)-thiourea **15** and the peak that co-eluted with a sample of (1*S*, 2*R*)-thiourea **15** synthesized from authentic (1*S*, 2*R*)-norephedrine indicated a >99:1 diastereomeric ratio of (1*R*, 2*S*)- and (1*S*, 2*R*)-thioureas **15**. Hence amino alcohols **14** and polymer-bound α-amino aldehyde **1b**, all are presumed to be of a >99:1 enantiomeric ratio.

**5.10. Discussion**

The solid-phase synthesis of enantiopure product has traditionally been addressed in peptide chemistry where a wide milieu of reagents, protecting groups and linkers have been used to circumvent racemization.[38] The current renaissance of interest in solid-phase methods for synthesizing different organic structures in enantiopure form has created the necessity for new tools to manipulate intermediates that may be configurationally labile on polymeric supports.[1] Towards the development of a new linker for anchoring α-amino carbonyl compounds onto polymer, we selected to adapt the PhF protecting group to solid-phase chemistry because of its proven effectiveness in providing enantiomerically pure amine-bearing products in solution.[6-11] Our studies have provided the first example of a configurationally stable polymer-bound α-amino aldehyde which has been used in the synthesis of enantiopure norephedrines **14**.

Towards the development of an ideal linker, we have demonstrated that the phenylfluorenyl linking/protecting group strategy is effective for loading advanced intermediates onto both soluble and insoluble supports. Alanine isoxazolidide **2** was successfully anchored to polymer-bound aryl halides by palladium-catalyzed cross-couplings in 70-99% yields. Linker stability was demonstrated both in the lithium aluminum hydride reduction of support-bound isoxazolidide **10b** and in the addition of phenylmagnesium bromide to α-amino aldehyde **1b**; both organometallic reactions proceeded without linker degradation. Final product removal was effected with TFA in $CH_2Cl_2$ containing anisole as cosolvent which gave crude material of >75% purity after a simple aqueous extraction and evaporation.

Because this method may be extended to other amino acids and organometallic reagents, the potential exists to apply polymer-bound α-amino aldehydes in syntheses of amino alcohol libraries.[39,40] However, the present design has its limitations, and we are currently exploring modifications to make this approach more amenable to combinatorial chemistry. For example, to link the PhF group to the resin, we are now examining alternative transition metal-catalyzed cross-coupling procedures that do not involve the employment of the relatively expensive diboron pinacol ester.[21] We are also investigating similar chemistry with resino carbonyl compounds such as isoxazolidides **10c** and **10d**, because we believe that insoluble resins may be easier to manage in library syntheses relative to their soluble polymer counterparts. Considering the numerous biologically active compounds that possess amino alcohol components, this PhF protecting-linking group strategy may find significant use in combinatorial science for drug discovery.

In summary, we have developed a novel strategy for linking protected amino carbonyl compounds to solid supports that employs transition metal catalyzed cross-coupling chemistry. By employing this strategy to anchor PhF-protected amines to soluble polymers, we found that non-cross-linked polystyrene (NCPS) was better suited for the synthesis of configurationally-stable polymer-bound alaninal **1** than the MeO-PEG-5000-

polymer. The addition of an organometallic reagent to α-amino aldehyde **1**, polymer cleavage and isolation via *N*-acylation provided enantiopure *N*-(BOC)norephedrines **14**. The application of this strategy to the synthesis of different configurationally stable polymer-bound α-amino carbonyl compounds is presently under investigation to furnish a variety of enantiomerically pure amine-bearing products.

### 5.11. Experimental Section

**General**: Unless otherwise noted all reactions were run under argon atmosphere and distilled solvents were transferred by syringe. THF was distilled from sodium / benzophenone immediately before use; $CH_2Cl_2$ and $CH_3CN$ were distilled from $CaH_2$; $CHCl_3$ was distilled from $P_2O_5$; MeOH was distilled from Mg. DMSO and DMF were distilled and stored over 4Å molecular sieves. Potassium acetate was recrystallized from 1:1 EtOH:$H_2O$ and dried at >100°C under vacuum before use. For reactions involving palladium catalysis, the solutions were degassed for 10-15 minutes by bubbling argon through the solution before addition of the catalyst. Final reaction mixture solutions were dried over $Na_2SO_4$. Chromatography was performed on 230-400 mesh silica gel; TLC on aluminum-backed silica plates. Melting points are uncorrected. Mass spectral data, HRMS, were obtained by the Université de Montréal Mass Spec. facility. $^1H$ NMR (400 MHz) and $^{13}C$ NMR (75 MHz) spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm (δ units) downfield of internal TMS and coupling constants are reported in Hz. Aromatic carbons of compounds having PhF groups are not reported.

**9-*p*-Bromophenylfluoren-9-yl bromide (BrPhFBr)** A solution of 1,4-dibromobenzene (5.0 g, 21 mmol) in THF (120 mL) at –78°C was treated dropwise with *n*-butyllithium (7.98 mL, 19.95 mmol, 95 mol%, 2.5 M in hexanes), stirred for 20-25 min at –78°C, and treated dropwise with a solution of fluorenone (3.60 g, 20 mmol, 95 mol%) in THF (30 mL). The mixture was stirred for 5 min at –78°C, warmed to rt over 60 min, and quenched with $H_2O$ (20 mL). The layers were separated and the aqueous layer was extracted with $Et_2O$ (2 × 100 mL). The combined organic layers were washed with brine (20 mL), dried and evaporated. The crude residue

was immediately dissolved in toluene (70 mL), treated with HBr (60 mL, 48% aqueous solution) and stirred vigorously, protected from light, for 24 h at rt. The layers were separated and the aqueous layer was extracted with toluene (2 × 20 mL). The combined organic layers were washed with brine (25 mL), dried and evaporated to yield **BrPhFBr** as an oil. Crystallization from isooctane gave 7.14 g of colorless needles (89 %): mp 112-113°C; $^{1}$H NMR δ 7.78-7.76 (m, 2 H), 7.62-7.60 (m, 2 H), 7.57-7.54 (m, 2 H), 7.50-7.43 (m, 4 H), 7.40-7.36 (m, 2 H); $^{13}$C NMR δ 149.0, 140.3, 137.9, 131.3, 129.3, 129.13, 129.11, 129.0, 128.8, 128.7, 128.6, 125.8, 122.2, 120.4, 66.4. Anal Calcd for $C_{19}H_{12}Br_2$: C, 57.04; H, 3.02. Found: C, 57.48; H, 3.00.

**(2*S*)-*N*-(BrPhF)Alanine Methyl Ester (3)** A suspension of L-alanine methyl ester hydrochloride (3.66 g, 26.25 mmol, 150 mol%) in $CH_3CN$ (70 mL) was treated with $K_3PO_4$ (11.70 g, 55.13 mmol, 210 mol%), $Pb(NO_3)_2$ (7.40 g, 22.31 mmol, 85 mol%) and BrPhFBr (7.0 g, 17.5 mmol), stirred for 36 h at rt, and treated with MeOH (7.10 mL, 175 mmol). After stirring for 30 min, the mixture was filtered through a plug of Celite™. The filter cake was thoroughly washed with $CHCl_3$ until the filtrate contained no UV active material. Evaporation of the volatiles and chromatography of the residue using 5% EtOAc in hexanes as eluant gave ester **3** (7.31 g, 92%) as an oil that solidified on standing: mp 71–73°C; $R_f$ = 0.25 (1:9 EtOAc in hexanes); $[\alpha]_D^{20}$ –173.0 (*c* = 1, $CHCl_3$); $^{1}$H NMR δ 7.69-7.67 (m, 2 H), 7.37-7.16 (m, 10 H), 3.29 (s, 3 H), 2.93 (bs, 1 H), 2.76 (q, 1 H, *J* = 7.0), 1.11 (d, 3 H, *J* = 7.0); HRMS calcd for $C_{23}H_{21}NO_2{}^{79}Br$ [$MH^+$]: 422.0756; found: 422.0747.

**(2*S*)-*N*-(BrPhF)Alanine (4)** A solution of (2*S*)-*N*-(BrPhF)alanine methyl ester (**3**, 3 g, 7.10 mmol) in dioxane (30 mL) was treated with a 2M aqueous solution of LiOH (17.75 mL, 500 mol%), heated at a reflux for 3 h, cooled to rt, and acidified with concentrated $H_3PO_4$ to pH 5-6. The mixture was then saturated with solid NaCl and extracted with EtOAc until TLC of the organic layer contained no UV active material. The combined organic layers were washed with brine (15 mL), dried and evaporated to give **4** (2.80 g, 97%) as a white foam: $^{1}$H NMR δ 7.76-7.69 (m, 2H), 7.23 (m, 10 H), 2.70 (q, 1H, *J* = 7.2), 1.16 (d, 3H, *J* = 7.2). $^{13}$C NMR ($CDCl_3$) δ 176.1, 72.9, 52.8, 19.3; HRMS calcd for $C_{22}H_{19}NO_2{}^{79}Br$ [$MH^+$]: 408.0599, found: 408.0617.

**(2*S*)-*N*-(BrPhF)Alanine Isoxazolidide (5)** A stirred mixture of *N*-(BrPhF)alanine (**4**, 1.50 g, 3.68 mmol), hydroxybenzotriazole (596 mg, 4.41 mmol, 120 mol%), isoxazolidine hydrochloride (806 mg, 7.36 mmol, 200 mol%) in $CH_2Cl_2$ (36 mL) was treated with Et(*i*-Pr)$_2$N (1.92 mL, 300 mol%) and DMAP (45 mg, 10 mol%), cooled to 0°C for 15 min, and treated with *N,N*-diisopropylcarbodiimide (1.15 mL, 200 mol%). The mixture was stirred for 3 h at 0°C, warmed to rt, and evaporated to dryness. The residue was purified by chromatography using 20-30% EtOAc in hexanes as eluant to yield isoxazolidide **5** as a white solid. Recrystallization from $Et_2O$ in hexanes gave white needles (1.54 g, 91%): mp 143-144°C; $[\alpha]_D^{20}$ –140.5 (*c* = 1, MeOH); $R_f$ = 0.41 (1:1 EtOAc:hexanes); $^1$H NMR δ 7.66-7.17 (m, 12 H), 3.56 (bm, 3 H), 3.13-3.01 (bm, 2 H), 2.64 (bs, 1 H), 1.97 (bm, 1 H), 1.80 (bm, 1 H), 1.12 (d, 3 H, *J* = 7.0); $^{13}$C NMR δ 176.7, 73.1, 68.4, 48.6, 42.9, 27.0, 22.5; HRMS calcd for $C_{25}H_{24}N_2O_2{}^{79}Br$ [$MH^+$] 463.1021; found : 463.1010. Anal calcd for $C_{25}H_{23}N_2O_2{}^{79}Br$: C, 64.80; H, 5.00; N, 6.05. Found: C, 64.92; H, 5.12; N, 6.12.

**(2*S*)-*N*-[*p*-(Pinacolboronato)phenylfluorenyl]Alanine Isoxazolidide (2)** A solution of isoxazolidide **5** (247 mg, 0.53 mmol), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi-1,3,2-dioxaborolane (diboron pinacol ester, 137 mg, 0.53 mmol, 100 mol%), and KOAc (157 mg, 1.59 mmol, 300 mol%) in DMSO (5 mL) was degassed for 10-15 min and treated with [$PdCl_2$(dppf)] (10 mg, 3 mol%). The mixture was heated at 80 °C overnight. Isoxazolidides **2** and **5** had the same $R_f$ values in a variety of eluants; however staining with a ceric ammonium molybdate solution showed a pink color for bromide **5**, and a deep-blue color for boronate **2**. After complete disappearance of the pink staining bromide was observed by TLC, the reaction was cooled to rt and diluted with $Et_2O$ (10 mL) and $H_2O$ (10 mL). The layers were separated and the aqueous layer was extracted with $Et_2O$ until TLC of the organic layer showed no UV active material. The combined organic layers were washed with brine (10 mL), dried and evaporated to a residue that was chromatographed using 20-30% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave boronate **2** (251 mg, 92%) as a colorless solid: mp >230°C; $[\alpha]_D^{20}$ –164.4 (*c* = 0.23, $CHCl_3$); $R_f$ = 0.41 (1:1 EtOAc:hexanes); $^1$H NMR δ 7.65-7.64 (m, 4 H), 7.41-7.15 (m, 8 H), 3.64-3.56 (bm, 3 H), 3.03-2.98

(bm, 2 H), 2.63 (bs, 1 H), 1.95-1.94 (bm, 1 H), 1.84-1.79 (bm, 1 H), 1.28 (s, 12 H), 1.80 (d, 3 H, $J$ = 7.0); $^{13}$C NMR δ 176.9, 83.7, 73.5, 68.3, 48.5, 42.9, 27.0, 24.9, 22.6; HRMS calcd for $C_{31}H_{36}N_2O_4B$ [$MH^+$]: 511.2768; found: 511.2753. Anal calcd for $C_{31}H_{35}N_2O_4B$: C, 72.95; H, 6.91, N, 5.49. Found: C, 72.88; H, 7.06; N, 5.47.

**MeO-PEG-5000 *p*-Bromobenzyl Ether 6** To a suspension of KH (574 mg, 5 mmol, 500 mol%, 35% wt in mineral oil) prewashed with hexanes and 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane, 10 mg) in THF (50 mL) at rt was added MeO-PEG-5000 (5 g, 1 mmol, 0.2 mmol/g, dried at rt overnight under vacuum in the presence of $P_2O_5$). After stirring for 10 min, *p*-bromobenzyl bromide (1.25 g, 5 mmol, 500 mol%) was added to the mixture, which was stirred for 24 h at rt, and filtered on Celite™. The filter cake was washed with $CH_2Cl_2$ (15 mL) and evaporated to a volume of 15-20 mL of solvent. Diethyl ether was added to precipitate the polymer, which was then filtered and washed with ether. The polymer was recrystallized from EtOH in $Et_2O$ in the freezer, filtered and dried under vacuum to give **6** (4.4 g, 85%) as a white powder: $^1$H NMR δ 7.45 (d, 2 H, $J$ = 8.4), 7.22 (d, 2 H, $J$ = 8.6), 4.51 (s, 2 H).

**Non-Cross-Linked Polystyrene *m*-Iodophenyl Ether (NCPS-*m*-Iodophenyl Ether) 7** A solution of *m*-iodophenol (1.32 g, 6 mmol) in THF (50 mL) was treated with NaH (720 mg, 18 mmol, 60% in mineral oil), stirred for 30 min and treated with a solution of chloromethylated polystyrene (2 g, 2 mmol, 1 mmol/g, molecular weight = 38634, molecular weight distribution = 1.56) and NaI (300 mg, 2 mmol) in *N*,*N*-dimethylacetamide (25 mL). The mixture was heated at 60°C for 24 h and poured slowly into MeOH (200 mL) with vigorous agitation. The precipitated polymer was collected, dissolved in a minimal amount of THF and reprecipitated on addition of MeOH. The polymer was dried under vacuum to give NCPS-*m*-iodophenyl ether **7** (2.1 g, 97%) as a powder: $^1$H NMR δ 7.35-6.25 (m, 40 H), 4.89 (bs, 2 H), 2.25-1.15 (m, 22 H).

**Merrifield Resin *m*-Iodophenyl Ether 8** A suspension of *m*-iodophenol (6.05 g, 27.5 mmol, 250 mol%) in THF (50 mL) and *N*,*N*-dimethylacetamide

(50 mL) was treated with NaH (1.32 g, 33 mmol, 300 mol%, 60% in mineral oil), stirred for 15 min and treated with chloromethylated cross-linked polystyrene (Merrifield resin, 2 % cross-linked, 10 g, 11 mmol, 1.1 mmol/g) and NaI (300 mg, 2 mmol). The mixture was heated at 70°C for 48 h, cooled to rt, filtered, and washed sequentially with 15 mL/g resin of the following solvents, dioxane, THF, water, acetone, MeOH and $Et_2O$. The polymer was then dried under vacuum to give 11.7 g of ether **8** (82% yield by weight increase).

**Wang Resin *p*-Bromobenzyl Ether 9** A suspension of Wang resin (1.0 g, 0.92 mmol, 0.92 mmol/g) in THF (15 mL) at rt was treated with $KN(SiMe_3)_2$ (3.68 mL, 1.84 mmol, 200 mol%, 0.5 M in toluene), stirred for 30 min and treated with *p*-bromobenzyl bromide (460 mg, 1.84 mmol, 200 mol%). After stirring overnight, the reaction mixture was quenched with excess water, filtered, and washed sequentially with 15 mL/g resin of the following solvents: dioxane, THF, water, acetone, MeOH and $Et_2O$. The resin was dried under vacuum to give 1.12 g of ether **9** (78%).

**MeO-PEG-5000-Supported Isoxazolidide 10a** was prepared using the reaction conditions described below for **10b** in the general protocol, precipitated from cold diethyl ether, filtered and washed with cold diethyl ether. The polymer was then dried under vacuum. Proton NMR spectroscopic analysis of the polymer **10a** showed a conversion of 50-60% by integration of the methyl doublet of alanine isoxazolidide relative to the benzylic methylene protons of the polymer: $^1$H NMR δ 7.50-7.21 (m, 16 H), 4.58 (bs, 2 H), 3.15 (bs, 1 H), 3.05 (bs, 1 H), 2.60 (bs, 1 H), 1.95 (bm, 1 H), 1.80 (bm, 1 H), 1.15 (bd, 3 H).

**General Procedure for Cross-Coupling to Polymeric Aryl Halides; Synthesis of NCPS-Supported Isoxazolidide 10b.** A solution of polymer **7** (350 mg, 0.35 mmol) and boronate **2** (241 mg, 0.47 mmol, 134 mol%) in DMF (10 mL) containing aqueous 2M $Na_2CO_3$ (588 µL, 1.18 mmol, 250 mol%) was degassed for 10-15 min, treated with [$PdCl_2$(dppf)] (14 mg, 5 mol%), heated at 80°C overnight and then cooled to rt. The polymer was precipitated by addition of excess MeOH and collected by decanting the solvent away from

the solid after placing the mixture in a centrifuge for 3-5 min. The collected polymer was dissolved in a minimal amount of $CHCl_3$, precipitated with MeOH and recollected using a centrifuge as described. This process was repeated three times and the recovered polymer was dried under vacuum which provided 411 mg, (93%) of isoxazolidide **10b**: $^1$H NMR δ 4.94 (bs, 2 H), 3.64 (bs, 1 H), 3.59 (bs, 2 H), 3.15 (bs, 1 H), 3.07 (bs, 1 H), 2.62 (bs, 1 H), 1.16 (bd, 3 H, $J$ = 6.2).

**Merrifield Resino Isoxazolidide 10c** was prepared using the reaction conditions described for **10b**, filtered and washed sequentially with 15 mL/g resin of the following solvents: DMF, water, acetone, THF, MeOH and $Et_2O$. The resin was dried under vacuum to give **10c**, that was shown by $^1$H MAS-NMR spectroscopic analysis to be of 78% conversion by integration of the methyl doublet of alanine isoxazolidide relative to the benzylic methylene protons of the polymer: $^1$H MAS-NMR (300 MHz with nanoprobe) δ 4.90 (bs, 2 H), 3.60 (bm, 3 H), 3.15-3.00 (bm, 2 H), 2.60 (bs, 1 H), 1.15 (bs, 3 H).

**Wang Resino Isoxazolidide 10d** was prepared using the reaction conditions described for **10b**, isolated by filtration and washed sequentially with 15 mL/g resin of the following solvents: DMF, water, acetone, THF, MeOH and $Et_2O$. The resin was dried under vacuum to give **10d**, that was estimated to be of 70% conversion by cleavage of a 60 mg sample (0.04 mmol, 0.72 mmol/g) using TFA (2 mL) in $CH_2Cl_2$ (2 mL), which gave 15 mg of *N*-(*p*-hydroxymethylphenyl-PhF)alanine isoxazolidide.[35]

**NCPS-Supported (2*S*)-*N*-(PhF)Alaninal 1b** A solution of polymer-supported isoxazolidide **10b** (60 mg, 0.05 mmol) in THF (6 mL) was cooled to 0°C and treated with a 1.5 M solution of $LiAlH_4$ (50 μL, 0.075 mmol, 150 mol%) in THF. The clear solution was stirred for 25 min at 0°C, quenched by the addition of EtOAc (2 mL) followed by water (1 mL), and filtered through a plug of sodium bicarbonate on Celite™. The filter cake was washed thoroughly with EtOAc, and the filtrate was washed with brine, dried and evaporated to furnish polymer-bound aldehyde **1b** (50 mg, 96%): $^1$H NMR δ 9.25 (bs, 1 H), 4.93 (bs, 2 H), 2.65 (bs, 1 H), 0.98 (bs, 3 H).

**NCPS-Supported (1*RS*, 2*S*)-1-Phenyl-2-*N*-(PhF)amino-1-propanol 11b** A solution of alaninal **1b** (106 mg, 0.11 mmol) in THF (6 mL) was cooled to –78°C and treated with a 1 M solution of PhMgBr (450 μL, 400 mol%) in THF. The stirred clear solution was allowed to warm to –10°C over 3 h, and quenched with MeOH (5 mL). The polymer was precipitated by addition of excess MeOH and collected by decanting the solvent away from the solid after placing the mixture in a centrifuge for 3-5 min. The collected polymer was dissolved in a minimal amount of $CHCl_3$, precipitated with MeOH and recollected using a centrifuge as described. This process was repeated three times and the recovered polymer was dried under vacuum which provided amino alcohol **11b** (111 mg, 85% yield): $^1$H NMR δ 4.93 (bs, 2 H), 4.11 (bs, 1 H), 2.41 (bs, 0.5 H), 2.28 (bs, 0.5 H), 0.57 (1.5 H), 0.44 (bs, 1.5 H).

**(1*RS*, 2*S*)-*N*-(BOC)norephedrines 14** A solution of polymer **11b** (100 mg, 0.08 mmol) in dichloromethane (2 mL) and anisole (2 mL) was treated dropwise with TFA (950 μL), stirred for 48 h at rt, and treated with $H_2O$ (5 mL). The layers were separated, and the aqueous layer was evaporated to a crude amino alcohol that was treated with $(BOC)_2O$ (120 mol%) and $Et_3N$ (200 mol%) in dichloromethane (1 mL). After stirring for 20 h at rt, the solution was evaporated to a residue that was chromatographed using a gradient of 0-50% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave 12 mg of *N*-protected norephedrine **14** (60%) as a 3:1 mixture of diastereomers: $^1$H NMR δ 7.35-7.26 (m, 5 H), 4.86 (d, 0.3 H, $J$ = 2.9), 4.64 (bm, 0.5 H), 4.56 (d, 0.7 H, $J$ = 6.1), 4.03 (bm, 0.4 H), 3.88 (bm, 0.1 H), 1.47 (s, 6 H), 1.41 (s, 3 H), 1.08 (d, 0.8 H, $J$ = 6.9), 1.00 (d, 2.2 H, $J$ = 6.9); HRMS calcd for $C_{14}H_{22}NO_3$ [$MH^+$]: 252.1600; found: 252.1607.

**Enantiomeric Purity of Amino Alcohols 14** A solution of **14** (2 mg) in dichloromethane (500 μL) was treated with TFA (500 μL) for 1 h at rt. The volatiles were removed under vacuum and the residue was treated with GITC (3.7 mg, 120 mol%) and $Et_3N$ (1.3 μL, 120 mol%) in dichloromethane (500 μL) at rt for 20 min. The volatiles were removed under vacuum and the ureas **15** were dissolved in HPLC grade methanol (1 mg/mL) and examined by reversed-phase HPLC using $H_2O$:MeOH:AcOH (51:48:1) as

eluant on a $C_{18}$ column with a flow rate of 1.0 mL/min.[37] The limits of detection were determined by measuring the relative integrations of the peaks for diastereomeric thioureas **15** from the (1*R*, 2*S*)-and (1*S*, 2*R*)-norephedrines. Less than 1% of the (1*S*, 2*R*)-isomer was detected in the HPLC trace of (1*R*, 2*S*)-thiourea **15**.

**9-Bromo-9-Phenylfluorenyl Polystyrene Resin** A suspension of polystyrene (2.8 g, 27 meq, washed according to the procedure described in ref 14) in cyclohexane (20 mL) was treated with *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (4 mL) and stirred for 20 min at rt. A 2.5 M solution of *n*-butyllithium (13.5 mL, 33.8 mmol) in hexanes was added and the mixture was heated at 65°C for 4.5 h. After cooling to rt, removal of the solvents by cannula, and washing of the resin with cyclohexane (2 × 10 mL), the resin was swollen in THF (15 mL) at 0°C and treated with a solution of fluorenone (2.02 g, 11.2 mmol, 200 mol%) in THF (10 mL). After 10 min, the cooling bath was removed and the suspension was left to stir at rt overnight, quenched with water (10 mL) and stirred for 5 min. The resin was filtered, washed with 15 mL/g of the following solutions: MeOH, THF, acetone, 2:1 THF:$H_2O$, $Et_2O$, $H_2O$, THF, MeOH and $Et_2O$. The resin was dried overnight under vacuum at 55°C to provide 3.54 g of a yellow colored resin (PA–FTIR: 3548 $cm^{-1}$) containing 1.15 mmol of alcohol per gram of resin as determined by mass increase. A suspension of resin (1.0 g, 1.15 mmol) in benzene (15 mL) was treated with acetyl bromide (444 µL, 6 mmol, 522 mol%) and heated at a reflux overnight. The solution was cooled and the resin was filtered, washed thoroughly with $Et_2O$ and dried overnight under vacuum at 55°C to furnish 1.08 g of a yellow colored resin with a loading of 1 mmol/g resin as ascertained by mass increase. Spectroscopic analysis by PA–FTIR indicated complete disappearance of the alcohol band at 3548 $cm^{-1}$.

**Solution-Phase Synthesis of *N*-(BOC)-norephedrines** A solution of (2*S*)-*N*-(BrPhF)alanine isoxazolidide (**5**, 463 mg, 1 mmol) in THF (10 mL) at 0°C was treated with a solution of $LiAlH_4$ (730 µL, 1.1 mmol, 110 mol%, 1.5 M in THF), stirred for 20 min at 0°C, quenched with EtOAc (2 mL) and water (2 mL) and filtered through a plug of sodium bicarbonate on Celite™. The

filtrate was diluted with EtOAc (10 mL), washed with brine (5 mL), dried, and evaporated to a solid, that was dissolved in THF (10 mL), cooled to –78°C, and treated with a 1 M solution of phenylmagnesium bromide (5 mL, 500 mol%) in THF, stirred for 1.5 h at –78°C, warmed to rt over 30 min, quenched with water (2 mL) and diluted with EtOAc (15 mL). The organic layer was washed with brine (5 mL), dried, and evaporated to a residue that was chromatographed using a gradient of 0-30% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave a 1:1 diastereomeric mixture of *N*-(BrPhF)-norephedrines **12** (448 mg, 95% from isoxazolidide **5**) as an oil: $^1$H NMR δ 7.75-6.86 (m, 17 H), 4.21 (d, 0.5 H, *J* = 3.3), 4.13 (d, 0.5 H, *J* = 8.4), 3.37 (bs, 1 H), 2.40 (m, 0.5 H, *J* = 3.3, 6.7), 2.30 (dq, 0.5 H, *J* = 1.9, 6.4), 0.56 (d, 1.5 H, *J* = 6.7), 0.44 (d, 1.5 H, *J* = 6.4); HRMS calcd for $C_{28}H_{25}NO^{79}Br$: 470.1119; found: 470.1132.

A solution of *N*-(BrPhF)norephedrines **12** (42.3 mg, 0.1 mmol) in $CH_2Cl_2$ (1 mL) and anisole (1 mL) was treated with TFA (900 μL), stirred at rt overnight, and diluted with water (5 mL). The layers were separated and the aqueous layer was evaporated to a residue. A solution of the residue in MeOH (1.2 mL) was treated with $Et_3N$ (13 μL) and $(BOC)_2O$ (12 mg, 120 mol%), stirred overnight at rt, and evaporated to a residue that was chromatographed using a 0-50% gradient of EtOAc in hexanes. Evaporation of the collected fractions gave **14** (15 mg, 66%) as a 1:1 diastereomeric mixture that exhibited the same characteristics as material prepared on the NCPS-polymer.

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**Supporting Information Available:** $^1$H NMR spectra of **3, 4, 6, 7, 10a-c, 1b, 11b**, and **14**, and HPLC trace of thioureas **15**. This material is contained in libraries on microfiche, immediately follows this article in the microfilm

version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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# Chapitre 6

# Développement d'une technique d'analyse spectroscopique non-destructrice pour la chimie organique sur support solide

## 6.1. Introduction

Dans le développement d'une synthèse sur support solide, une étape d'optimisation des conditions réactionnelles est nécessaire avant de passer à la synthèse parallèle ou combinatoire. Lorsqu'on effectue la synthèse organique en solution, la chromatographie sur couche mince est utilisée comme technique rapide pour évaluer qualitativement la progression des réactions et la pureté des produits. Puisqu'il n'est pas possible d'utiliser cette technique en chimie sur support solide, on doit trouver une méthode alternative pour effectuer cette évaluation.

Une des méthodes d'évaluation existantes consiste à effectuer la réaction sur le support solide et ensuite à récupérer le produit de réaction par clivage du lien entre le substrat et le support polymérique. L'analyse du produit est alors possible en utilisant les mêmes techniques que pour la chimie en solution. Toutefois, cette méthode nécessite plusieurs manipulations physiques et chimiques, et mène ultimement à la destruction de l'échantillon sur support solide.

Les méthodes spectroscopiques offrent une alternative moins pénible. La spectroscopie infra-rouge est sensible aux groupements fonctionnels, elle est donc une technique de choix pour analyser les transformations organiques de ces groupes sur support solide. Traditionnellement, la méthode utilisée consiste à enregistrer le spectre infra-rouge d'un échantillon de résine polymérique que l'on moule sous forme de pastille avec du bromure de potassium. Cette technique est destructrice et nécessite plusieurs manipulations sur l'échantillon. De plus, la qualité des spectres est dépendante de l'homogénéité de l'échantillon.[1]

Plusieurs méthodes basées sur la spectroscopie infra-rouge ont été développées pour analyser la chimie organique sur support solide.[2] Ces méthodes permettent la caractérisation de composés organiques sur support solide en utilisant 5-10 mg de résine. Toutefois, certaines de ces techniques non-destructrices nécessitent soit l'écrasement d'une bille

polymérique,[3] un appareillage complexe[2] ou l'usage d'un microscope[2,3] pour manipuler les résines.

Nous avons voulu développer une technique spectroscopique commode et routinière, peu coûteuse, non-destructrice et sensible pour l'analyse de la chimie organique sur support solide. Nous avons démontré que la spectroscopie infra-rouge photoacoustique à transformée de Fourier (FTIR-PA) est supérieure à la spectroscopie FTIR traditionnelle pour analyser les réactions chimiques sur support solide. En détectant seulement la composante d'absorption du rayon infra-rouge, la FTIR-PA soustrait les effets de dispersion et de réflexion de la lumière. Ces effets compliquent les spectres obtenus par les méthodes de spectroscopie FTIR traditionnelles pour les molécules liées à des supports solides.

Puisqu'aucune préparation d'échantillon n'est nécessaire, la spectroscopie FTIR-PA a été utilisée pour examiner une séquence de réactions chimiques sur un même échantillon de résine polymérique, sans perte de matériel. En particulier, des spectres FTIR-PA utiles ont été enregistrés avant et après chacune des quatre étapes utilisées pour transformer un échantillon de 10 mg d'un amino ester polymérique en déshydroalanine.

Nous avons choisi d'élaborer une synthèse d'un acide déshydroaminé sur support solide pour démontrer le potentiel de la spectroscopie infra-rouge photoacoustique. Lorsque ces acides aminés sont incorporés dans les peptides, ils peuvent stabiliser des structures secondaires peptidiques (Figure 1).[4] Par exemple, la déshydroalanine (Figure 1, R = H) favorise les conformations peptidiques étendues.[4] Par comparaison, la déshydrophénylalanine (Figure 1, R = Ph) stabilise les repliements β de type II dans les peptides courts.[4] Dans les peptides plus longs, cet acide aminé permet la nucléation d'hélices α.[4] On retrouve les acides déshydroaminés dans une gamme de peptides antibiotiques.[5] De plus, ces composés peuvent être utiles comme intermédiaires réactifs en synthèse sur support solide, par exemple dans les additions de Michael anioniques ou radicalaires sur le système conjugué.[6]

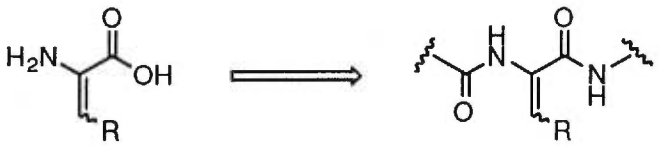

**Figure 1. Structures d'un acide déshydroaminé et d'un déshydropeptide.**

Il existe plusieurs méthodes de synthèse des acides déshydroaminés en solution. Ces méthodes impliquent généralement des réactions de β-élimination sur les dérivés chloro,[7] acétyle,[8] toluènesulfonyle[9] et isourée[10] de la sérine, ou les dérivés sulfonium,[11] et sulfoxyde[12] de la cystéine. Par ailleurs, on a aussi utilisé l'oxydation des azalactones,[13] l'oléfination des aldéhydes par les phosphonates[14] et la dégradation de Hoffmann des acides diaminopropioniques.[15] Ces méthodes nécessitent souvent plusieurs étapes, des conditions drastiques et fournissent parfois des rendements variables de l'acide déshydroaminé désiré ainsi que des produits secondaires.

Nous avons développé une méthode qui permet d'obtenir les acides déshydroaminés en conditions douces et compatibles avec les structures peptidiques. Dans ce chapitre, nous présentons l'application de la spectroscopie infra-rouge photoacoustique comme technique non-destructrice pour analyser la synthèse d'un acide *N*-acyldéshydroaminé dans une séquence de déprotection / acylation / oxydation / β-élimination sur support solide.

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# Article 8

Gosselin, F.; Di Renzo, M.; Ellis, T. H.; Lubell, W. D. "Photoacoustic FTIR Spectroscopy, A Non-Destructive Method for Sensitive Analysis of Solid-Phase Organic Chemistry" Publié dans *The Journal of Organic Chemistry*, **1996**, *61*, 7980-7981.

## Photoacoustic FTIR Spectroscopy, A Non-Destructive Method for Sensitive Analysis of Solid-Phase Organic Chemistry

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### 6.3. Abstract

Photoacoustic Fourier-transform infrared spectroscopy (PA-FTIR) is superior to conventional FTIR spectroscopy for monitoring chemical reactions on solid-phase. By detecting only the absorption component of the IR beam, PA-FTIR spectroscopy eludes the effects of light scattering and reflection that complicate conventional FTIR methods. Because no sample preparation is required, PA-FTIR spectroscopy was used to examine a sequence of reactions on the same resin sample without product loss. In particular, useful PA-FTIR spectra were recorded before and after each of the four steps to convert resino-(2*R*)-*S*-benzyl-*N*-(BOC)cysteinate (**1**) into resino-*N*-(*p*-cyanobenzoyl)dehydroalanine **5** using the same 10 mg sample of resin. Photoacoustic FTIR spectroscopy should thus find general use as a convenient, non-destructive method for sensitive analysis of solid-phase organic chemistry.

### 6.4. Summary

In comparison with conventional Fourier-transform infrared spectroscopy, photoacoustic Fourier-transform infrared spectroscopy was shown to be a superior, non-destructive method for monitoring chemical reactions on solid phase.

The growing use of combinatorial chemistry for drug discovery has accelerated the development of analytical techniques for studying organic chemistry on solid phase.[1-6] Although Fourier-transform infrared (FTIR) spectroscopy is most commonly used for detecting functional group changes of resin-bound compound,[1-3] the acquisition of quality spectra from resino compound by FTIR spectroscopy is complicated by light scattering and reflection.[7] Furthermore, methods for FTIR spectroscopy of derivatized resin are usually destructive and require inconvenient sample preparation.[1] Since spectral quality may be influenced by matrix homogeneity, the resin samples are typically examined in KBr pellets after thorough mixing.[1a] Single-bead FTIR microspectroscopy[2] and internal reflection FTIR spectroscopy[3] have recently been shown to offer advantages compared to conventional FTIR spectroscopy; however, these techniques require some sample preparation as well as mechanical contact with the sample.

We have explored the use of photoacoustic FTIR spectroscopy in order to develop a simple, rapid, non-destructive technique to monitor chemical reactions on solid support.[8] Photoacoustic FTIR (PA-FTIR) spectroscopy involves the measurement of oscillating pressure variations of a confined inert gas situated above the sample. This variation is caused by a thermal transfer from the sample to the surrounding gas upon absorption of modulated infrared radiation. The absorbed radiation diffuses as heat through the sample towards the surface where it heats a boundary layer of gas and induces a pressure change resulting in an acoustic wave. The acoustic wave is then detected by a sensitive microphone. Therefore, the photoacoustic signal is a direct measurement of the amount of absorbed radiation.

Photoacoustic FTIR spectroscopy offers a number of important advantages over conventional and the above-mentioned surface-sensitive IR techniques. First, because only the absorbed radiation is measured, PA-FTIR spectroscopy eludes the effects of light scattering and reflection. Moreover, PA-FTIR spectroscopy does not require sample preparation. The sample is simply placed into a sample cup, introduced into the

photoacoustic cell and purged with an inert gas (helium in the present experiments). Photoacoustic FTIR spectroscopy has been used to characterize many surfaces ranging from asbestos fibers to calcified tissues.[9-16] The technique is sensitive and non-destructive. Spectra can be recorded on a few milligrams of resin which can be recovered, treated in chemical reactions and reexamined repeatedly. To the best of our knowledge, we are the first to apply PA-FTIR spectroscopy towards the analytical characterization of solid-phase organic chemistry.

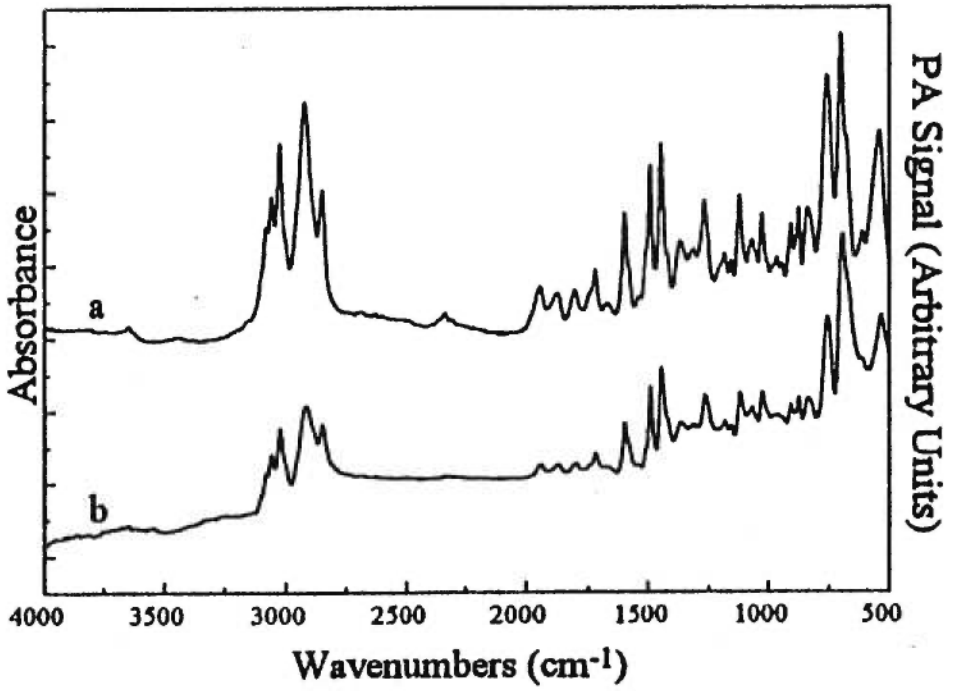
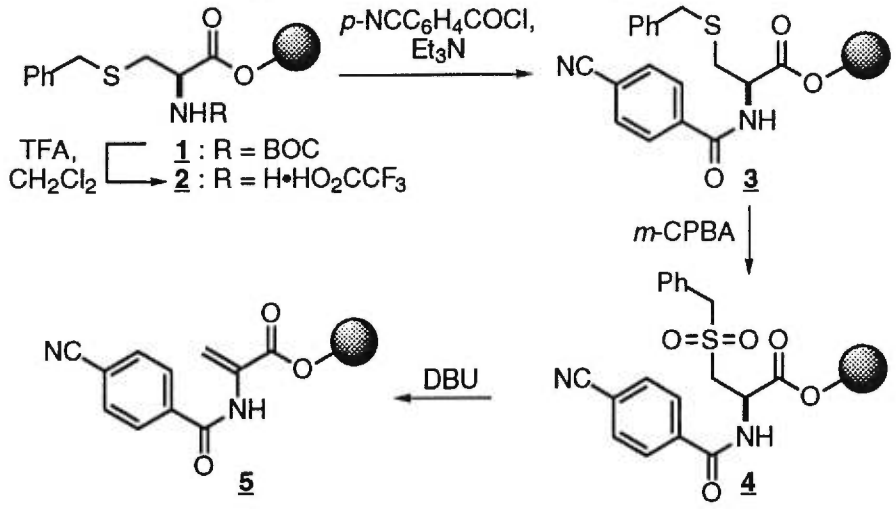



**Figure 1. FTIR spectra of Merrifield's resin (a) PA-FTIR; (b) FTIR of KBr pellet.**

In comparison with conventional FTIR spectroscopy, we have found that PA-FTIR spectroscopy produces spectra of superior quality as is clearly evidenced in the spectra of Merrifield's peptide resin (chloromethylated polystyrene[17]) that were obtained by using PA-FTIR spectroscopy[18] on neat resin (Figure 1a) and by employing standard FTIR spectroscopy[19] on the resin in a KBr pellet[20] (Figure 1b). The PA-FTIR spectrum is devoid of the light scattering and artifacts due to non-homogeneity of the KBr pellet. Such effects generally require a baseline correction in order to obtain better quality spectra.

Three different resino amino esters were next examined by PA-FTIR spectroscopy.[21]

**Scheme 1. Synthesis of Resino Dehydroalanine 5**




We next monitored a sequence of reactions using the same sample of resin.[22] Resino-*N*-(*p*-cyanobenzoyl)dehydroalanine **5** was synthesized in four steps from resino-(2*R*)-*S*-benzyl-*N*-(BOC)cysteinate (**1**, Scheme 1).[23] The spectra were recorded on the same 10 mg sample. Resin **1** was first treated with TFA in dichloromethane and anisole to yield resino-(2*R*)-*S*-(benzyl)cysteinate trifluoroacetate **2**. The formation of **2** was indicated by the disappearance of the carbamate carbonyl and N-H bands at 1718 $cm^{-1}$ and 3420 $cm^{-1}$, respectively, and the appearance of the amine-trifluoroacetate salt band at 1681 $cm^{-1}$ (Figures 2a and 2b). Treatment of **2** with 4-cyanobenzoyl chloride in the presence of triethylamine in THF-$CH_2Cl_2$ afforded resino-(2*R*)-*S*-benzyl-*N*-(*p*-cyanobenzoyl)cysteinate **3** as determined by the appearance of the amide carbonyl band at 1674 $cm^{-1}$, the nitrile band at 2231 $cm^{-1}$ and the amide N-H at 3413 $cm^{-1}$ (Figure 2c). Oxidation of the thioether using excess *m*-CPBA in dichloromethane then yielded the resino sulfone **4** as observed by the appearance of the strong O=S=O stretch at 1140 $cm^{-1}$ (Figure 2d).[24] The formation of resino-*N*-(*p*-cyanobenzoyl)dehydroalanine **5** on treatment of resino sulfone **4** with DBU was finally indicated by the disappearance of the sulfone stretch at 1140 $cm^{-1}$ (Figures 2d and 2e).[25] As one can see from the results presented in Figure

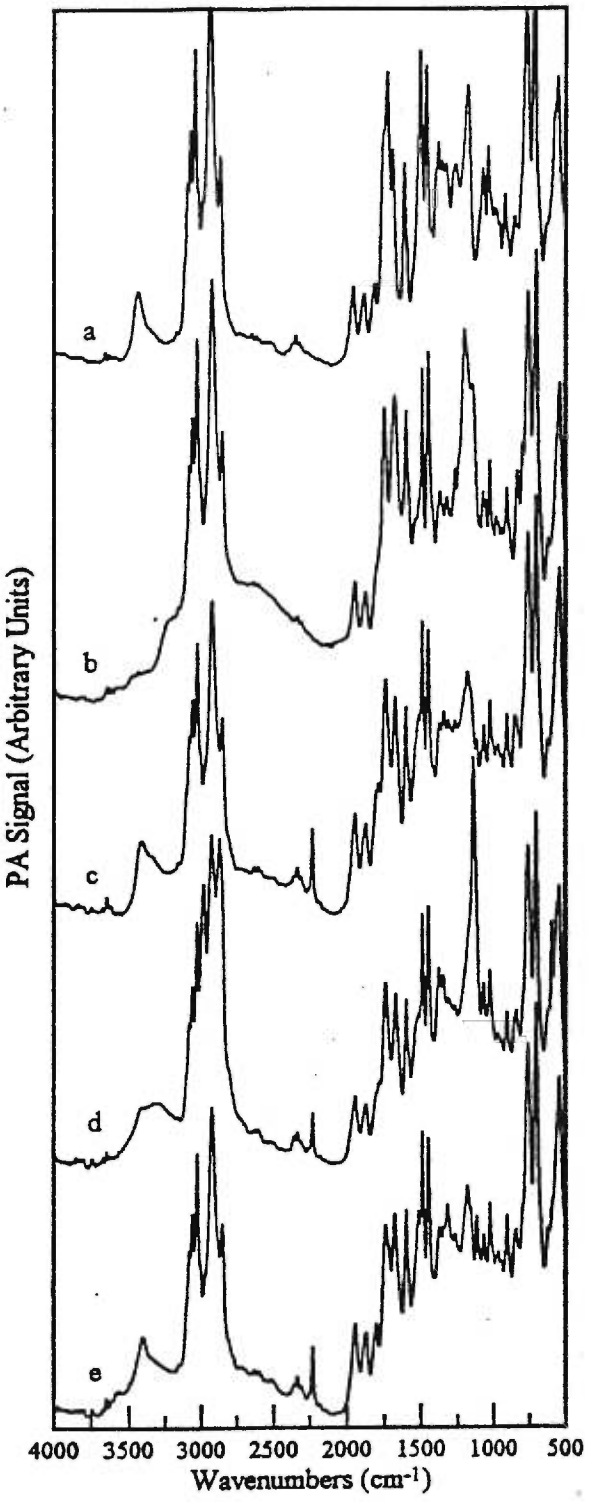



**Figure 2.** (a-e) PA-FTIR spectra of resino compounds **1-5**.

2, useful PA-FTIR spectra were recorded on the same 10 mg sample of resino amino ester before and after each transformation. Moreover, no resin was lost.

Effective ways to monitor chemical transformations on solid-phase are needed in order to ascertain reaction conversion and product purity during combinatorial chemistry. We have shown that PA-FTIR spectroscopy is superior to conventional FTIR spectroscopy for monitoring chemical reactions on solid-phase. By detecting only the absorption component of the IR beam, PA-FTIR spectroscopy eludes the effects of light scattering and reflection that complicate conventional FTIR methods. Because no sample preparation is required, PA-FTIR spectroscopy can be used to examine a sequence of reactions on the same resin sample without product loss. Photoacoustic FTIR spectroscopy should thus find general use as a convenient, non-destructive method for sensitive analysis of solid-phase organic chemistry.

### 6.5. Supplementary Materiel

**General:** Unless otherwise noted all reactions were run under nitrogen atmosphere and distilled solvents were transferred by syringe. THF was distilled from sodium / benzophenone immediately before use; $CH_2Cl_2$ was distilled from $CaH_2$. DMF (HPLC grade) was stored over 4 Å molecular sieves. Final reaction mixture solutions were dried over $Na_2SO_4$. Chromatography was on 230-400 mesh silica gel; TLC on aluminum-backed silica plates. Melting points are uncorrected. Mass spectral data, HRMS, were obtained by the Université de Montréal Mass Spec. facility. $^1H$ NMR (300 MHz) and $^{13}C$ NMR (75 MHz) spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm (δ units) downfield of internal TMS and coupling constants are reported in Hz.

**General Procedure for the anchoring of amino acids to Merrifield's peptide resin.**

**A) Preparation of the cesium salt of the amino acid**

The *N*-(BOC)amino acid (0.32 mmol) was dissolved in EtOH (1.5 mL) and $H_2O$ (0.3 mL). Cesium bicarbonate (93 mg, 150 mol%) was added and the

solution was stirred until evolution of gas had ceased (120 minutes). The solution was evaporated and the residue was dried under vacuum.

**B) Anchoring to the resin**. A suspension of the cesium salt (0.32 mmol), DMF (3 mL) and Merrifield's resin (640 mg, 1 meq Cl/g, 1% cross-linked) was stirred at 55-60 °C for 3 days. The resin was then filtered, washed thoroughly with DMF (2 × 5 mL), DMF/$H_2O$ (2 × 5 mL, 9:1) and EtOH (2 × 5 mL, anhydrous), dried under vacuum and kept in the freezer.

The samples were prepared by anchoring the cesium salts of (2*S*)-γ-benzyl-*N*-(BOC)glutamate, (2*R*)-*S*-benzyl-*N*-(BOC)cysteine and (2*S*)-$N^{\varepsilon}$-nitro-*N*-(BOC)arginine to Merrifield's resin which was washed with solvent and dried under vacuum.[21] The PA-FTIR spectra were recorded using 10 mg of the resin-bound compound. Bands for the ester, carbamate carbonyl and carbamate N-H, all were clearly observed (Table 1).[22] In addition, the formation of the resino ester was indicated by the negative chloromethylene band at 679 $cm^{-1}$ and the positive carbonyl bands at 1736 $cm^{-1}$ and 1721 $cm^{-1}$ in the spectrum obtained upon subtraction of the spectrum of Merrifield's resin from that of resino (2*S*)-γ-benzyl-*N*-(BOC)glutamate (Figure 2).

**Resino-(2*S*)-γ-benzyl-*N*-(BOC)glutamate**: PA-FTIR ($cm^{-1}$) 3421 (N-H, carbamate), 1736 (C=O, ester), 1721 (C=O, carbamate).

**Resino-(2*S*)-$N^{\varepsilon}$-nitro-*N*-(BOC)argininate**: PA-FTIR ($cm^{-1}$) 3327 (N-H carbamate), 1739 (C=O, ester), 1716 (C=O, carbamate).

**Resino-(2*R*)-*S*-benzyl-*N*-(BOC)cysteinate**: PA-FTIR ($cm^{-1}$) 3420 (N-H, carbamate), 1740 (C=O, ester), 1718 (C=O, carbamate).

**Resino-(2*R*)-*S*-(benzyl)cysteinate Trifluoroacetate (2).** A suspension of (2*R*)-*S*-(benzyl)-resino-*N*-(BOC)cysteinate (10 mg, 5 μmol, 0.5 mmol/g) in $CH_2Cl_2$ (200 μL) and anisole (2 μL) was treated with $CF_3CO_2H$ (250 μL), stirred at rt for 25 min, filtered, washed thoroughly with EtOH and $Et_2O$, and dried under vacuum. PA-FTIR ($cm^{-1}$) 1748 (C=O, ester), 1681 (amine-trifluoroacetate salt).

**Resino-(2*R*)-*S*-benzyl-*N*-(*p*-cyanobenzoyl)cysteinate (3).** Resin **2** (10 mg, 5 μmol) was treated with $Et_3N$ (95 μL, 250 mol%, 0.018M in THF) and a solution of 4-cyanobenzoyl chloride (100 μL, 600 mol%, 0.3M) in $CH_2Cl_2$. The suspension was stirred for 4 h at rt, then the resin was washed thoroughly with EtOH and $Et_2O$, and dried under vacuum. PA-FTIR ($cm^{-1}$) 3413 (N-H, amide), 2231 (nitrile), 1739 (C=O, ester), 1674 (C=O, amide).

**Benzyl Resino-(2*R*)-*S*-*N*-(*p*-cyanobenzoyl)cysteinyl Sulfone (4).**
A suspension of resin **3** (10 mg, 5 μmol) in $CH_2Cl_2$ (300 μL) at 0°C was treated with a solution of *m*-CPBA (12.5 μL, 12.5 μmol, 250 mol%, 1M) in $CH_2Cl_2$. The mixture was stirred for 3 h, warmed to rt, and the resin was washed thoroughly with EtOH and $Et_2O$, then dried under vacuum. PA-FTIR ($cm^{-1}$) 3412 (N-H, amide), 2231 (nitrile), 1742 (C=O, ester), 1672 (C=O, amide), 1140 (O=S=O, sulfone).

**Resino-*N*-(*p*-cyanobenzoyl)dehydroalanine (5).**
A suspension of resin **4** (10 mg, 5 μmol) in $CH_2Cl_2$ (200 μL) at 0°C was treated with DBU (50 μL, 120 mol%, 6 μmol, 0.12 M) in $CH_2Cl_2$ and stirred for 5 min. The resin was washed with EtOH and $Et_2O$, then dried under vacuum. PA-FTIR ($cm^{-1}$) 3404 (N-H, amide), 2231 (nitrile), 1741 (C=O, ester), 1679 (C=O, amide).

**(2*R*)-Methyl-*S*-benzyl-*N*-(*p*-cyanobenzoyl)cysteinate (6)**
To a solution of (2*R*)-*S*-(benzyl)cysteine methyl ester hydrochloride (131 mg, 0.5 mmol) in $CH_2Cl_2$ (5 mL) was added 4-cyanobenzoyl chloride (124 mg, 150 mol%). The mixture was cooled to 0°C, treated with $Et_3N$ (174 mL, 250 mol%) and stirred for 30 min when the ice bath was removed. After stirring for 3 h at rt, the mixture was diluted with $CHCl_3$ (15 mL), washed with HCl (3 × 5 mL, 5%), $H_2O$ (2 × 3 mL) and $NaHCO_3$ (3 × 3 mL), dried, filtered and evaporated. Chromatography (5-25% EtOAc in hexanes) of a sample of the crude product afforded the desired amide as a white solid: $R_f$ = 0.65 (EtOAc/hexanes 1:1); mp = 109-110 °C; PA-FTIR ($cm^{-1}$) 3311 (N-H, amide), 2231 (nitrile), 1739 (C=O, ester), 1646 (C=O, amide); $^1H$ RMN ($CDCl_3$) δ 7.87 (m, 2H), 7.75 (m, 2H), 7.26 (s, 5H), 6.95 (d, 1H, $J$ = 7.4), 4.98 (m, 1H), 3.79 (s, 3H), 3.71 (d, 2H, $J$ = 1.8), 3.03 (m, 2H); $^{13}C$ RMN ($CDCl_3$) δ 171.0,

165.2, 137.4, 132.4, 128.8, 128.6, 127.9, 127.3, 117.9, 115.4, 52.9, 52.0, 36.7, 33.3; HRMS calcd. for $C_{19}H_{19}O_3N_2S$ [M+1]: 355.1116, found: 355.1122.

**(2*R*)-*S*-Benzyl-*N*-(*p*-cyanobenzoyl)cysteine Sulfone Methyl Ester (7).**
To a solution of **6** (7.6 mg, 0.021 mmol) in $CH_2Cl_2$ (320 µL) at 0°C was added *m*-CPBA (5.2 mg, 0.025 mmol, 120 mol%, 85% technical grade). The mixture was stirred for 60 min. Another portion of *m*-CPBA (5.2 mg) was added. Stirring was maintained for 3 h, when the mixture was brought to rt and diluted with $CH_2Cl_2$ (1 mL). The organic layer was washed with $NaHSO_3$ (1 mL, 10% solution), $NaHCO_3$ (0.5 mL, saturated solution) and brine (0.5 mL), dried, filtered, evaporated and chromatographed (20–50% EtOAc in hexanes) to afford sulfone **7** as a white solid (7.5 mg, 92%): $R_f$ = 0.29 (EtOAc/hexanes 1:1); mp = 190 °C; PA-FTIR ($cm^{-1}$) 3323 (N-H, amide), 2232 (nitrile), 1734 (C=O, ester), 1647 (C=O, amide), 1119 (O=S=O, sulfone); $^1H$ RMN ($CDCl_3$) δ 7.90 (d, 2H, *J*=8.4), 7.76 (d, 2H, *J* = 8.4), 7.40 (s, 5H), 7.36 (d, 1H, *J* = 7.7), 5.15, dd, 1H, *J* = 2.8, 4.8), 4.29 (d, 2H, *J* = 3.5), 3.81 (s, 3H), 3.64 (m, 2H); $^{13}C$ RMN ($CDCl_3$) δ 169, 165.3, 136.8, 132.5, 130.6, 129.3, 129.1, 127.9, 126.8, 117.8, 115.7, 61.8, 53.4, 51.3, 48.5.

***N*-(*p*-cyanobenzoyl)Dehydroalanine Methyl Ester (8)**
To a stirred solution of **7** (4 mg, 0.01 mmol) in $CH_2Cl_2$ (400 µL) at 0°C was added DBU (2.3 µL, 150 mol%). The mixture was stirred for 7 min, quenched with $NaH_2PO_4$ (150 µL, 1M), and extracted with $CH_2Cl_2$ (2 × 1 mL). The combined organic layers were washed with brine (150 µL). After filtration through a plug of silica gel eluting with EtOAc-hexanes (1:1), the pure dehydroamino acid was obtained as a white solid (2.2 mg, 93%): $R_f$ = 0.67 (EtOAc/hexanes 1:1); mp = 135 °C dec.; PA-FTIR ($cm^{-1}$) 3397 (N-H, amide), 2231 (nitrile), 1725 (C=O, ester), 1680 (C=O, amide); $^1H$ RMN ($CDCl_3$) δ 8.55 (bs, 1H), 7.95 (m, 2H), 7.80 (m, 2H), 6.81 (s, 1H), 6.06 (d, 1H, *J* = 1.3), 3.92 (s, 3H); $^{13}C$ RMN ($CDCl_3$) δ 164.6, 163.9, 138.1, 132.7, 130.7, 127.7, 117.8, 115.7, 109.9, 53.3.

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**Supplementary Material Available:** Experimental details[23] as well as PA-FTIR and FTIR spectra (18 pages).

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18. Spectra were taken on a Mattson Research Series FTIR spectrometer using an MTEC model 300 photoacoustic cell. Less than 10 mg of the sample was placed in the sample cup. Samples were evaluated at a mirror speed of 0.098 cm/s (1.6 kHz) and a resolution of 8 $cm^{-1}$. Five hundred scans were co-added between 500 and 4000 $cm^{-1}$. Helium was used as the carrier gas. PA spectra were ratioed against carbon black spectra obtained from the reference from MTEC photoacoustics.
19. Absorption spectra were taken on the spectrometer described in ref. 18 using a DTGS detector with the samples pressed in KBr pellets. One hundred spectra were co-added at a mirror speed of 0.6 cm/s (10 kHz) and ratioed against the source emission spectrum. Beyond ratioing and in one case spectral subtraction, the spectra were subjected to no

other mathematical treatment such as smoothing or baseline correction.

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# Chapitre 7

# Conclusion

Les acides aminés azacycloalcanes sont utilisés dans une grande variété d'applications autant en mimétisme peptidique qu'en chimie médicinale. L'arsenal de mimétismes peptidiques basés sur les acides aminés azacycloalcanes est relativement vaste. Cependant, peu de méthodes générales, pratiques et versatiles existent pour préparer ces hétérocycles. Une gamme de mimétismes est requise pour étudier les relations qui existent entre la structure tridimensionnelle et l'activité biologique des peptides. Nous avons donc développé un protocole général pour synthétiser les acides aminés azacycloalcanes comme composés peptidomimétiques et comme plate-formes moléculaires pour étudier de façon systématique les peptides biologiquement actifs. Notre approche permet de contrôler la dimension des hétérocycles et la stéréochimie des centres stéréogéniques. De plus elle offre la possibilité d'introduire des chaînes latérales sur le squelette hétérocyclique.

Dans un premier temps, nous avons porté nos efforts sur la synthèse des acides aminés azabicyclo[X.Y.0]alcanes. Nous avons démontré le potentiel de la séquence d'oléfination de Horner-Wadsworth-Emmons / amination réductrice / cyclisation de lactame pour synthétiser ces hétérocycles. En utilisant les acides aminés dicarboxyliques tels que l'acide aspartique, l'acide glutamique et l'acide α-amino adipique comme produits de départ, nous avons préparé une gamme de diaminodicarboxylates. Ces diaminodicarboxylates ont été utilisés efficacement dans la synthèse d'acides aminés azabicycloalcanes. Notre méthodologie a fourni, pour la première fois, trois nouveaux acides aminés azabicyclo[X.Y.0]alcanes. L'acide aminé indolizidine-9-one a été synthétisé en 9 étapes à partir de l'acide aspartique, avec un rendement global supérieur à 25%. Par ailleurs, deux acides aminés azabicycloalcanes ont été synthétisés à partir d'un même précurseur diaminodicarboxylate. L'acide aminé quinolizidine-2-one a été préparé en 7 étapes à partir de l'acide pyroglutamique, avec un rendement global de 40%. L'acide aminé pyrroloazépine-2-one a été obtenu en 11 étapes à partir de l'acide pyroglutamique, avec un rendement global de 13%.

Nous avons démontré l'importance de la dimension des hétérocycles sur la géométrie des angles dièdres internes $\psi$ et $\phi$, par l'analyse

cristallographique de l'acide aminé indolizidine-9-one et de l'acide aminé quinolizidine-2-one. Lors de leur incorporation dans les peptides, ces divers acides aminés azabicycloalcanes pourraient donc mimer un large spectre de conformations peptidiques.

La pleine puissance de notre méthodologie sera mise à profit lorsque nous introduirons sur le squelette hétérocyclique des fonctions chimiques qui miment les chaînes latérales des acides aminés dans les peptides biologiquement actifs. La disposition du squelette peptidique et l'arrangement spatial des chaînes latérales pourront alors être mimés de façon combinée.

Dans un deuxième temps, en préparant des analogues de l'hormone GnRH et de la leucine-enképhaline, nous avons montré que l'acide aminé indolizidine-9-one peut être incorporé dans les peptides d'intérêt biologique. La synthèse de l'analogue de GnRH a été effectuée dans le but de connaître la capacité de l'acide aminé indolizidine-9-one à stabiliser un repliement de type β. L'analyse conformationnelle et les tests biologiques du peptide devraient permettre d'évaluer ce potentiel. Par ailleurs, la synthèse, l'analyse conformationnelle et l'évaluation biologique de l'analogue de la leucine-enképhaline ont indiqué que l'acide aminé indolizidine-9-one peut stabiliser la conformation bioactive du peptide naturel.

Dans un troisième temps, en modifiant la méthodologie développée pour la synthèse des acides aminés azabicycloalcanes, nous avons fourni une méthode pratique pour préparer les acides 6-alkylpipecoliques énantiopurs. Les acides pipecoliques ont été synthétisés en 5 étapes à partir de l'acide aspartique, avec un rendement global de 15-59%. Nous avons étudié l'équilibre conformationnel des acétamides en position *N*-terminale du *N*-acétyl-6-*tert*-butylpipecolate *N'*-méthylamide. En comparant nos observations avec les résultats obtenus lors l'analyse conformationnelle des prolylacétamides, cette étude a encore une fois indiqué l'effet de la dimension de l'hétérocycle sur la conformation peptidique. Nous avons remarqué que l'introduction d'un groupement *tert*-butyle en position 6 des

pipecolates a un effet moins prononcé sur l'équilibre conformationnel des prolylacétamides que celui observé lorsque l'on introduit un groupement *tert*-butyle en position 5 de l'acide aminé proline.

Finalement, nous avons initié une transition vers le développement d'une méthodologie de synthèse d'azacycloalcanes sur support solide. Dans cette optique, nous avons développé une stratégie de protection et d'ancrage qui permet la synthèse et la manipulation de composés α-amino carbonyles sur support solide. Bien que nous n'ayons pas préparé d'hétérocycles en utilisant cette approche, nous avons validé le concept par la synthèse d'amino alcools énantiopurs à partir d'un α-amino aldéhyde. De plus, nous avons appliqué la spectroscopie infra-rouge photoacoustique comme technique non-destructrice et pratique pour analyser la chimie organique sur support solide.

L'unification de la méthodologie de synthèse d'acides aminés azacycloalcanes en solution avec la stratégie développée pour manipuler les composés α-amino carbonyles sur support solide devrait permettre la préparation d'une gamme de composés hétérocycliques comme outils pour étudier de façon systématique des événements de reconnaissance moléculaire d'importance biologique.

L'ensemble des travaux présentés devrait contribuer à l'avancement des connaissances dans le domaine du mimétisme peptidique et pourrait mener au développement de nouveaux agents thérapeutiques. De façon plus importante, nous espérons que notre contribution puisse fournir une compréhension accrue des phénomènes biologiques qui impliquent les peptides.