Université de Montréal

Interactions stériques à l'intérieur de peptides contenant des prolines non-naturelles.

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Cette thèse intitulée

Interactions stériques à l'intérieur de peptides
contenant des prolines non-naturelles.

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Sommaire

Les études décrites à travers les différents chapitres de cette thèse sont le fruit de travaux dont l'objectif est de démontrer qu'en combinant les outils de l'analyse conformationnelle des peptides et de la synthèse organique, il est possible d'utiliser et de mieux comprendre les effets stériques à l'intérieur des peptides. Dans ces études, la méthodologie utilisée consiste à synthétiser de nouveaux acides aminés possédant des groupes produisant des effets d'encombrement stérique. Par la suite, ces acides aminés ont été insérés à l'intérieur de peptides modèles et l'influence des effets stériques a été mesurée. En étudiant la structure des peptides de cette manière, il est possible de développer nouvelles stratégies permettant de contrôler la structure tridimensionelle des peptides.

Une introduction méthodologique ainsi qu'une revue de la littérature constituent le premier chapitre de cette thèse. Elles ont pour objectif de présenter l'intérêt de l'étude des interactions stériques à l'intérieur de peptides contenant des prolines non-naturelles. Nous procéderons en établissant l'état des connaissances sur quatre thèmes importants. Ceci permettra au lecteur de garder une vision globale de l'importance des résultats obtenus durant cette thèse. Ainsi nous débuterons par établir ce que sont: les peptides, le peptidomimétisme, la relation entre l'acide aminé proline et la conformation des peptides, et le 5-*tert*-butylprolines les (3,3)-diméthylprolines, des nouveaux outils pour le peptidomimétisme.

Par la suite les travaux effectués seront présentés en trois parties et regroupés sous les thèmes suivants: La synthèse des 5-alkylprolines, l'utilisation des effets stériques afin de contrôler la conformation de peptides modèles et l'utilisation des 5-*tert*-butylprolines afin de contrôler la nucléation de l'hélice PPI en milieu aqueux.

Ainsi, la première partie de cette thèse décrit la synthèse des 5-*tert*-butylprolines. Elle est constituée de deux articles qui sont suivis d'une description de la contribution relative de chacun des auteurs.

La deuxième partie de cette thèse décrit l'influence des effets stériques de la 5-*tert*-butylprolines et de la 3,3-dimetylproline sur la conformation de peptides modèles. Les trois articles présentés sont suivis d'une description de la contribution relative de chacun des auteurs.

La troisième partie de cette thèse est constituée de deux articles et elle décrit l'utilisation des 5-*tert*-butylprolines afin de contrôler la nucléation de l'hélice PPI en milieu aqueux.

Chacune des parties 1, 2 et 3 est accompagnée d'une brève introduction qui met en évidence les objectifs de la recherche effectuée ainsi que les principaux résultats obtenus.

Pour terminer, un chapitre de conclusion permet de présenter une perspective globale des résultats obtenus et l'importance de la contribution de ce travail dans le domaine du peptidomimétisme.

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Liste des Abbréviations

[α]	rotation spécifique [en (deg ml) / (g dm)]
Å	ångström
Ac	acétyle
Ac_2O	anhydride acétique
AcOH	acide acétique
atm	atmosphère
BOC	*tert*-butyloxycarbonyle
$(BOC)_2O$	dicarbonate de di-*tert*-butyle
BOP-Cl	*N,N'*-bis(2-oxo-3-oxazolidinyl)phosphonic chloride
br	*broad*
n-Bu	normal-butyle
t-Bu	*tert*-butyle
c	concentration
°C	degré Celsius
calcd	*calculated*
CD	*circular dichroism*
COSY	*correlated spectroscopy*
δ	déplacement chimique en parties par million
d	doublet
DCM	*dichloromethane*
dd	doublet de doublet
ΔG	Energie libre de Gibbs
$\Delta G^{\ddagger}$	Energie d'activation
DIEA	di-isopropyléthylamine
DMF	diméthylformamide
DMSO	diméthylsulfoxide
Dtc	5,5-dimethylthiazolidine-4-carboxylate
Et	éthyle
Et_2O	diéthyl ether
EtOAC	ethyl acetate
FAB	*fast atom bombardment*
FMOC	fluorenyloxycarbonyl

FT-IR	*Fourier transform infrared spectroscopy*
Pro	proline
i-Pr	*iso*-propyle
Rink resin	4-(2',4'-di-methoxyphenyl-Fmoc-aminomethyl-polystyrene resin
PPI	polyproline type I
PPII	polyproline type II
RMN	résonance magnétique nucléaire
TBTU	tetrafluoroborate de benzotriazol-1-yl-1,1,3,3-tetraméthyluronium
TFA	*trifluoroacetic acid*
TFE	trifluoroéthanol
THF	tetrahydrofurane
TLC	*thin layer chromatography*
p-TsOH	*para*-toluene sulfonic acid

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À mon père...

CHAPITRE 1
Interactions stériques à l'intérieur de peptides contenant des prolines non-naturelles.

1. Introduction

Dans la nature, l'assemblage linéaire des acides aminés conduit à la formation de peptides qui se distinguent principalement par le nombre et la séquence d' acides aminés qui les composent. Cette séquence définit la structure primaire du peptide. Cependant les différentes propriétés pharmacologiques qui résultent de l'interaction des peptides avec d'autres constituants cellulaires, telles que les enzymes, les récepteurs cellulaires et les oligonucléotides, ne peuvent être expliquées uniquement que par la structure primaire des peptides.

Afin de mieux comprendre l'origine de ces interactions, une description plus précise de la structure d'une chaîne peptidique s'impose. Celle-ci doit tenir compte de l'organisation spatiale des atomes de chaque acide aminé. Cette organisation est appelée conformation. Cependant, la coexistence de plusieurs conformations énergétiquement similaires pour un seul peptide rend complexe la compréhension de la relation entre sa structure et son activité biologique.

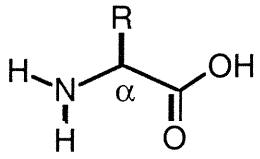
Les travaux décrits dans cette thèse discuteront de la synthèse ainsi que de l'utilisation de nouveaux acides aminés ayant pour fonction de favoriser la formation de certaines conformations peptidiques pour explorer le repliement des peptides.

1.1 Les peptides

Dans cette première partie nous décrirons les peptides, les différentes structures qu'ils adoptent, les facteurs qui influencent leur structure, quelques exemples représentatifs du type d'activité biologique qu'ils peuvent produire, et enfin, différents moyens qui sont à notre disposition pour comprendre la relation entre la structure d'un peptide et son activité biologique.

Les peptides sont des polymères dont l'unité de répétition est l'acide α-aminé. Chaque acide α-aminé se distingue par le type de substituant se trouvant sur le carbone α (figure 1).

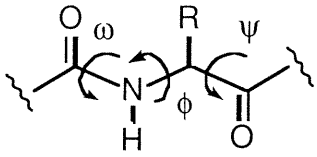
Figure 1. Structure de l'acide α-aminé



En 1959, Linderström-Lang et Shellman[1] ont décrit la structure des peptides en terme de niveau d'organisation. Cette classification hiérarchique des structures peptidiques définit la structure primaire par la séquence et le nombre d'acides aminés, la structure secondaire par les angles dièdres ω, ϕ et ψ du squelette peptidique (figure 2), la structure tertiaire par la séquence des structures secondaires d'une seule chaîne peptidique et la structure quaternaire par le mode d'assemblage de plusieurs chaînes peptidiques. Par exemple, la séquence primaire du peptide opiacé morphiceptine[2a], fait référence à la séquence des acides aminés Phe-Pro-Phe-Pro-NH_2. La structure secondaire d'un peptide fait référence aux angles dièdres adoptés par les acides

aminés composant le peptide. Par exemple, pour une séquence d'acides aminés dont les angles dièdres prennent les valeurs de $\omega = 180°$, $\phi = -55°$ et $\psi = -45°$ il y a une structure secondaire appelée hélice α.

Figure 2. Les angles dièdres du squelette peptidique



La formation d'une structure peptidique secondaire, tertiaire et quaternaire est favorisée à la fois par la nature et la séquence des acides aminés qui composent un peptide et par le milieu dans lequel le peptide se trouve. Ces paramètres détermineront la présence de ponts d'hydrogène, de liaisons ioniques, d'interactions hydrophobes, de forces de Van der Waals, de liaisons disulfures, de coordinances métalliques et de répulsions stériques.

L'intérêt que nous portons à la structure des peptides réside dans le fait que l'importance d'un peptide dans un milieu biologique dépendra principalement de sa structure. En effet, au niveau cellulaire, un peptide peut intervenir dans plusieurs processus biochimiques et produire différents effets biologiques. Par exemple, l'activité biologique de plusieurs peptides a été identifiée et selon le type de récepteur biologique avec lequel ils interagissent, certains peptides se sont révélés avoir des propriétés antibiotiques[2], antifongiques[3], neurotransmettrices[4], chimiotactiques[5] et régulatrices de l'expression de gènes[6]. Cependant l'interaction conduisant à l'activité biologique attendue ne se produira que lorsque le peptide possédera la conformation requise par le récepteur biologique.

Ainsi, puisque les peptides sont des entités moléculaires flexibles, il est par conséquent très utile de pouvoir disposer de différentes stratégies permettant d'identifier la conformation prérequise à l'activité biologique d'un peptide. Cela permet de mieux comprendre la relation entre la structure et l'activité de peptides biologiquement actifs. Dans cet ordre d'idée, l'utilisation de plusieurs techniques d'analyse moléculaire telles que la résonance magnétique nucléaire (RMN), la spectroscopie infrarouge à transformée de Fourier (FT-IR), la diffraction des rayons X, le dichroïsme circulaire ainsi que l'analyse conformationnelle assistée par ordinateur utilisant la mécanique moléculaire se sont révélés être des outils puissants. En utilisant la synthèse organique, le peptidomimétisme s'est développé comme une approche complémentaire permettant de répondre aux nombreuses questions qui concernent la relation entre le repliement et la biologie des peptides.

Par ailleurs, différentes approches ont été envisagées pour déterminer quels acides aminés peuvent induire préférentiellement certaines conformations.

L'observation d'un nombre élevé de certains acides aminés tel que la sérine, thréonine et la proline à l'intérieur de séquences ne formant pas de conformation hélicale, a conduit au développement d'une approche statistique de la détermination de la structure secondaire des protéines[7]. Ainsi, à partir de la détermination de la structure de certaines protéines telles que myoglobine et l'hémoglobine par l'analyse de la diffraction des rayons X, Guzzo[7a] et Prothero[7b] ont débuté le classement des acides aminés naturels selon leurs potentiels à favoriser et à empêcher la formation d'hélices. Ils ont déterminé ce potentiel en calculant la fréquence d'apparition de chaque acide aminé à l'intérieur d'une hélice. Chou et Fasman[7c] ont poursuivi ce travail en incluant le potentiel de chaque acide aminé à former des feuillets β. En

utilisant ce classement, il est possible de prédire pour des protéines à l'état solide, la formation d'hélice α, de feuillets β ou l'absence de ces conformations avec une précision allant de 70 à 80%[7a].

Cependant, il est difficile à ce point de prédire avec certitude la structure secondaire d'un peptide avec la séquence d'acide aminé qui le compose, puisque la structure secondaire d'un peptide est souvent dépendante du milieu dans lequel il se trouve[8a]. Par exemple l'étude par spectroscopie infrarouge à transformée de Fourier du peptide antibiotique nisine suggère que sa conformation dans l'eau est différente de celle près du milieu membranaire. Dans ce dernier cas, l'analyse de la bande amide I a montré que l'association de ce peptide à une membrane phospholipidique augmente la proportion de repliement β du peptide[8b]. Parallèlement, l'interaction de certains peptides amphiphiles avec un milieu hydrophobe similaire à celui de membranes conduit préférentiellement à la formation d'hélices α, tandis que les mêmes peptides en milieu aqueux ne possèdent pas de structures secondaires définies[8b]. Ces résultats montrent que l'approche statistique de Chou et Fasman procure une grande incertitude dans la prédiction de la structure secondaire des peptides en solution puisque celle-ci ne tient pas compte du milieu.

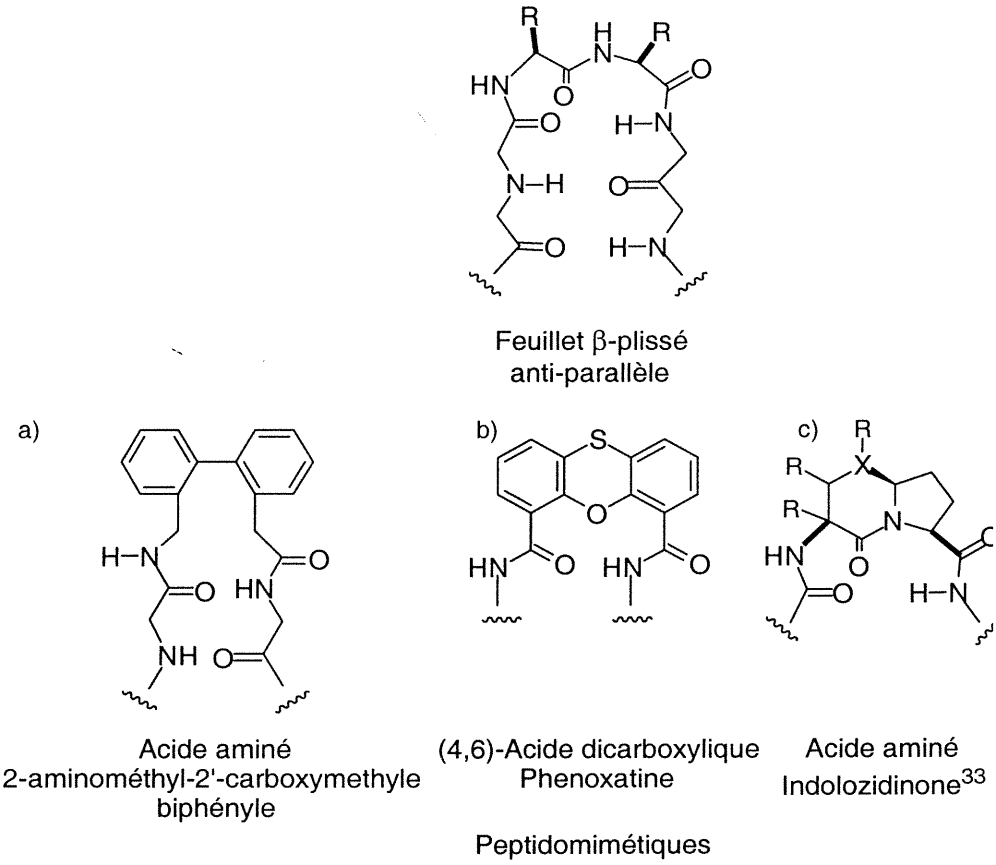
Afin de connaître la conformation des peptides biologiquement actifs dans l'environnement dans lequel ils fonctionnent, il serait utile de disposer d'acides aminés conformationnellement rigides.

Dans ce contexte, nous verrons dans la prochaine section, différentes approches qui ont été utilisées pour mieux déterminer l'importance de la structure secondaire des peptides dans la compréhension de leurs activités biologiques.

1.2 Le peptidomimétisme

Il est difficile de donner une définition simple du peptidomimétisme puisqu'il en existe de nombreuses. Cependant, de façon générale, le peptidomimétisme consiste à

Figure 3. Peptidomimétiques avec contraintes structurales

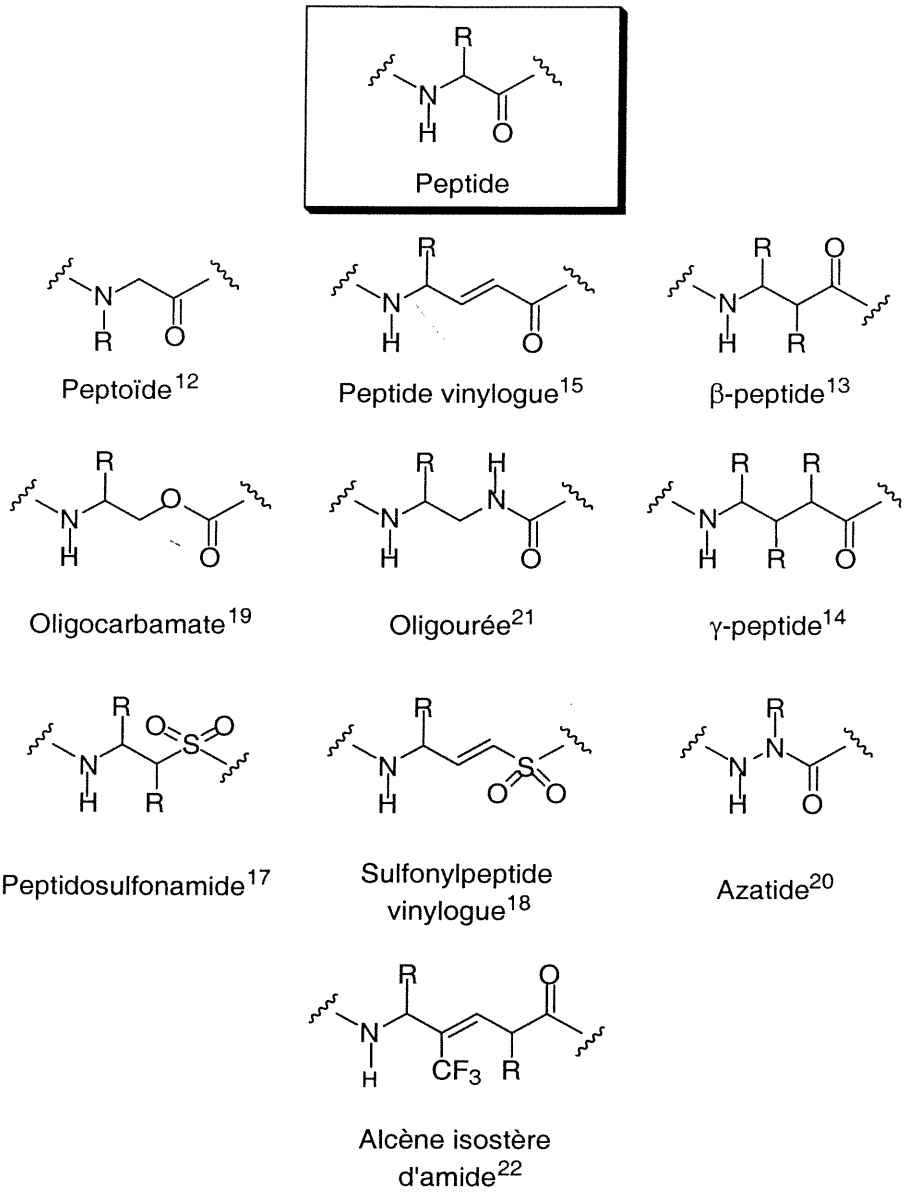


synthétiser des structures moléculaires qui peuvent reproduire, imiter, améliorer, ou amplifier certaines caractéristiques des peptides telles que leur propension à former des structures secondaires précises et à jouer le rôle des peptides dans leur milieu biologique. À ce titre, de nouvelles structures peptidomimétiques peuvent servir de substrat d'enzymes ou servir de ligands de récepteur cellulaire. Selon Hirshmann[9b]: "La force motrice de la recherche de peptidomimétiques se trouve dans le désir de

découvrir des structures non-peptidiques qui se lient aux récepteurs de peptides avec une meilleure bio-disponibilité, une meilleure stabilité métabolique et avec une plus grande sélectivité que le ligand peptidique endogène ou synthétique". Par exemple, il est possible d'utiliser les acides aminés α–disubstitués[9b] afin de stabiliser les hélices α. D'autre part de nouvelles structures synthétiques possédant des contraintes structurales ont été utilisées pour stabiliser des feuillets β plissés[11] (Figure 3a et 3b) ainsi que des repliements β[10] (Figure 3c) à l'intérieur de peptides.

Une autre approche au peptidomimétisme consiste à modifier le squelette polyamidique (figure 4). Par exemple, afin d'explorer de nouvelles alternatives à ce squelette, des acides β-[13a] et γ-[14] aminés ont été utilisés. Dans certains hétéropolyamides constitués d'acides β-aminés, on a observé la formation d'hélices et de feuillets[13b] tandis que l'utilisation d'acides γ–aminés a conduit à la formation d'hélices. Dans cette approche on retrouve la formation de poly-*N*-alkyl-glycines (peptoïde)[12], peptidosulfonamides[17], sulfonylpeptide vinylogues[18], d'oligocarbamates[19], d'azatides[20], d'oligourées[21], d'alcènes isostères d'amide[22] de peptides vinylogues[15] ainsi que la formation de squelettes mixtes peptides-peptoïdes (peptomères)[16](figure 4). Dans le cadre du peptidomimétisme, ces hétéropolymères procurent des alternatives au squelette polyamide classique tout en conservant la diversité de chaînes latérales communes aux peptides constitués d'acides α-aminés. Dans l'optique d'utiliser ces nouvelles structures comme agent thérapeutique, certaines d'entres elles ont démontré posséder une plus grande résistance à l'action des protéases[12].

Figure 4. Modification du squelette polyamidique



De façon à pouvoir concevoir de nouveaux peptidomimétiques, il est essentiel de connaître la structure primaire du peptide ciblé, mais il est aussi important de considérer quel type de structure secondaire peut adopter le peptide naturel. Puisque

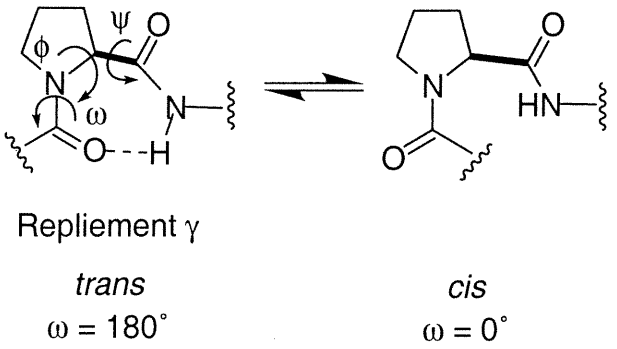
de nombreux peptides biologiquement actifs contiennent l'acide aminé proline, nous avons dirigé notre travail vers la synthèse et l'étude de peptidomimétiques de la proline. Ainsi dans la prochaine section nous discuterons des structures secondaires de peptides qui contiennent l'acide aminé proline.

1.3 La conformation des peptides et l'acide aminé proline.

La structure secondaire des peptides qui contiennent l'acide aminé proline est fortement influencée par les différentes caractéristiques structurales de la proline. Premièrement, la présence d'un cycle pyrrolidine à cinq membres impose une contrainte structurale qui réduit considérablement l'espace conformationnel de l'angle dièdre ϕ (figure 5). Ensuite, le carbone δ du cycle de la proline occasionne une répulsion stérique avec le carbone α d'un acide aminé en position *N-terminale* de la proline. Ceci conduit à une déstabilisation de l'amide en conformation *trans* (ω = 180°) et promouvoit la conformation *cis* (ω = 0°). Enfin dans une séquence peptidique le type d'acide aminé précédant la proline fait varier la population d'amide *cis*.[23].

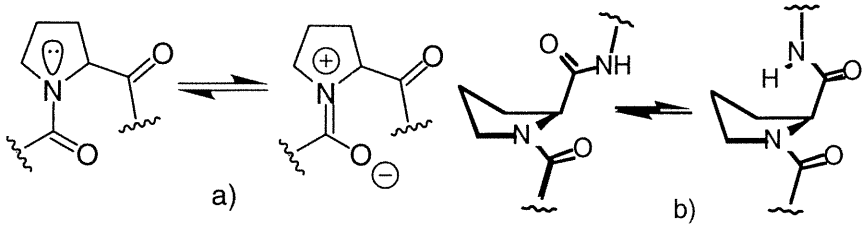
La présence d'un amide secondaire en position *C*-terminale permet à la proline d'être engagé dans une conformation du type repliement γ. Dans cette conformation un pont d'hydrogène intramoléculaire forme un cycle à sept membres qui stabilise l'amide en position *N*-terminale dans une conformation *trans*. La population de l'amide *cis* est plus importante en présence de solvants polaires et protiques. Dans ces conditions la formation du pont d'hydrogène stabilisant l'amide en conformation *trans* est inhibée par le solvant qui compétitionne avec cette interaction (figure 5).

Figure 5. Équilibre *cis-trans* de l'amide en position *N*-terminale



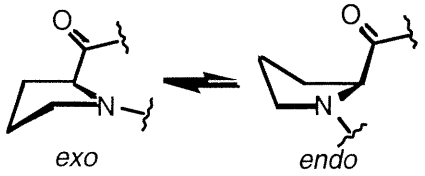
En plus de la stabilité relative de chaque isomère *cis* et *trans*, il est important de considérer la flexibilité du peptide. Cette flexibilité peut être évaluée en mesurant l'énergie d'activation du processus d'isomérisation. Cette énergie qui varie selon le type de solvant est proportionnelle à la vitesse d'isomérisation $K^{\ddagger} = (kT/h)\, e^{-\Delta G\ddagger/RT}$. Il a été suggéré que la pyramidalisation de l'amide (figure 6a) ainsi que la présence d'une interaction avec l'hydrogène de l'amide en position *C*-terminale(figure 6b) d'un résidu proline peuvent diminuer l'énergie d'activation et augmenter la vitesse d'isomérisation[24a].

Figure 6. Facteurs influençant l'isomérisation de l'amide *N*-terminale de la proline



Par ailleurs, les enveloppes *endo-exo* (figure 7) du cycle pyrolidine sont d'énergies similaires lorsque l'amine en position *N*-terminale est libre. Cependant, l'acylation de l'amine conduit préférentiellement à la formation de l'enveloppe *endo* qui est plus stable que l'enveloppe *exo* par 1.1 kcal/mole[24b].

Figure 7 Équilibre entre les formes *endo* et *exo* des pyrolidines



En conséquence, ces éléments structuraux ont des effets importants sur la stabilité de plusieurs structures secondaires. Par exemple, alors que les hélices α sont déstabilisées par la présence d'un acide aminé proline[25], les hélices polyprolines type II (PPII) se retrouvent fréquemment dans les peptides riches[26]en cet acide aminé. Par ailleurs les repliements β type I' et II' se trouvent favorisés dans les séquences D-Pro-Xaa[27] et l'aptitude de la proline à former des amides *cis* en position *N*-terminale le prédispose à la formation de repliements β de type VIa et VIb[28]. De plus des calculs de mécanique moléculaire ont montré qu'un résidu L-prolyl favorise la formation d'un repliement β type II et qu'un résidu D-prolyl favorise la formation d'un repliement β type I'.[29] Dans la section suivante, nous verrons comment nous avons abordé le peptidomimétisme de proline.

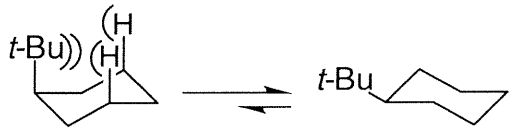
1.4. La 5-*tert*-butylproline et la 3,3-diméthylproline, des nouveaux outils pour le peptidomimétisme

Compte tenu de la présence du résidu proline au sein de plusieurs structures secondaires tels que les repliements β type VIa et VIb[30], les hélices de type polyproline I et II[30,31], les spirales de ruban β plissés[32], nous avons voulu disposer d'acides aminés pouvant stabiliser préférentiellement l'une ou l'autre de ces structures secondaires. Nous avons donc choisi de construire des peptidomimétiques qui conserveront la rigidité des prolines, ce qui permettra de contrôler la valeur de l'angle dièdre ϕ. Puisque les valeurs des angles ω et ψ sont dépendantes du solvant ainsi que

de la séquence d'acides aminés dans laquelle chaque proline se trouve, nous avons exploré les effets de différents substituants sur le cycle de proline afin de déterminer la possibilité de concevoir des analogues de l'acide aminé proline qui peuvent exercer un contrôle conformationnel sur ces angles dièdres.

Le groupe *tert*-butyle crée des effets de répulsion stérique conduisant à l'adoption de la conformation de plus basse énergie[34]. Lorsqu'un substituant encombrant tel que le groupe *tert*-butyle se trouve en position axiale du cycle cyclohexane, des intéractions répulsives se produisent avec les autres hydrogènes axiaux. La différence d'énergie libre entre la conformation axiale et équatoriale est une mesure quantitative du volume de ce substituant, aussi appelée la valeur A de ce substituant[34]. Par exemple la valeur A d'un groupe *tert*-butyle (5 Kcal/mole) est significativement plus élevée que celle d'un groupe méthyle (1.7 Kcal/mole).

Figure 8. Effets Stériques du groupe *tert*-butyle en position axiale



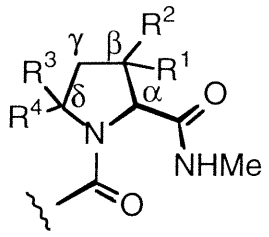
$$A = \Delta G^{\circ}_{\text{eq-> ax}} = -RT \ln([\text{ax}]/[\text{eq}]) = 5 \text{ Kcal/mole}^{33}$$

Nous avons utilisé la mécanique moléculaire pour estimer la valeur de l'effet d'encombrement stérique dans les peptides (chapitres 3 et 4) et nous avons dirigé la synthèse d'analogues exhibant des répulsions stériques ayant le plus grand potentiel de générer des conformations uniques.

La synthèse de ces acides aminés représente un défi qui se trouve dans la conception d'une synthèse réalisable en peu d'étapes en contrôlant la création de

nouveaux centres chiraux tout en maintenant l'intégrité stéréochimique déjà existante (chapitre 2).

Figure 9. Prolines non-naturelles substituées



En position δ du cycle pyrrolidine (figure 9), nous avons introduit le groupe *tert*-butyle (chapitre 2) afin de contrôler l'angle dièdre ω. En position β nous avons étudié l'effet d'un groupe *gem*-diméthyle afin de contrôler l'angle dièdre ψ (chapitre 4). De plus, puisque la vitesse de l'isomérisation de l'amide en position *N*-terminale de la proline est relativement lente sur l'échelle de temps RMN nous avons choisi d'évaluer les effets de ces substituants sur la vitesse d'isomérisation.

Enfin, après avoir établi quels étaient les effets de ces acides aminés non-naturels sur la conformation de peptides modèles, nous avons choisi d'utiliser un de ces acides aminés afin de favoriser une conformation dont le rôle est peu connu, l'hélice polyproline de type I (PPI). La conformation helicoïdale PPI se caractérise principalement par la présence de liaison d'amide *cis* en position *N*-terminale de proline ainsi que par le fait que sa formation n'a pas été observée en milieu aqueux. Puisque la *tert*-butylproline favorise la formation d'isomères d'amide *cis* par la destabilisation d'isomères d'amide *trans*, nous avons choisi d'utiliser cet acide aminé pour induire l'hélice PPI en milieu aqueux. (chapitre 5).

En résumé nous avons développé de nouveaux outils permettant de contrôler la conformation des peptides, défini leurs propriétés à l'intérieur de peptides modèles

et examiné leurs effets à l'intérieur d'une classe importante de polypeptides.

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Première partie:

La synthèse des 5-alkylprolines

Chapitre 2

Ce chapitre est constitué d'une brève introduction des articles 1 et 2 qui est suivie de la version intégrale des articles 1 et 2.

Introduction

Le projet de construire des acides aminés possédant des substituants en position 5 de la proline a été initié par Houda Haj Ibrahim. Elle a réalisé, en collaboration de Mohammed Atfani, toutes les réactions décrites dans le premier article de la première partie. Ce travail m'a conduit à démarrer des études théoriques préliminaires dont l'objectif a été d'envisager l'utilisation des intéractions stériques à l'intérieur de peptides modèles. Ces calculs effectués par modélisation utilisant la mécanique moléculaire sur les *N*-acétyl-5-alkylprolines ont indiqué que les effets stériques du groupe *tert*-butyle peuvent produire des changements importants de la valeur des angles ω et ψ. Ces études préliminaires nous ont poussés à nous intéresser plus particulièrement à l'importance des effets stériques du groupe *tert*-butyle en position 5 de la proline sur la conformation des prolylpeptides.

De par la stratégie de protection utilisée lors de la synthèse des alkylprolines, cette approche synthétique est demeurée très coûteuse et n'a pas conduit à l'obtention d'une quantité permettant l'étude conformationelle d'analogues peptidiques possédant les acides aminés non-naturels décrits dans l'article 1. De plus, cette approche synthétique n'a permis la préparation que d'un seul des diastéréoisomères des 5-*tert*-butylprolines. J'ai donc entrepris le développement d'une méthode de synthèse permettant l'obtention des quatre diastéréoisomères de la 5-*tert*-butylproline.

Dans le développement d'une méthode de synthèse d'analogue de l'ATPA, Mohamed Atfani a montré qu'il est possible de réaliser l'acylation du γ-méthyl-*N*-(PhF)-glutamate en position γ avec l'acide pivaloïque sans utiliser de protection de la fonction acide en position α. Ceci est devenu la méthode d'acylation avec l'acide pivaloïque la plus simple.

Par ailleurs, parallèlement à l'idée de déterminer les effets conformationnels des *tert*-butylprolines, Benoît L'Archevêque a débuté des travaux dont l'objectif était d'insérer des acides aminés non-naturels tels que la 5-*tert*-butylproline à l'intérieur du peptide oxytocin et ainsi explorer la possibilité de produire des antagonistes d'oxytocin. Il a contribué donc au deuxième article en réalisant les études de puretés énantiomériques du diastéréoisomère *cis*. Par ailleurs, il a entrepris les études préliminaires d'épimérisation sous contrôle thermodynamique et cinétique qui m'ont conduit à l'obtention du diastéréoisomère *trans* procédant par l'épimérisation du diastéréoisomère *cis*.

Laurent Bélec, qui a travaillé comme étudiant sous-gradué sous la supervision du professeur Lubell, a produit des quantités importantes de 5-*tert*-butylproline afin de permettre l'obtention de diastéréoisomère *trans* par l'isolation de l'imminium. Sans réel succès, ces efforts m'ont permis cependant d'obtenir des quantités suffisantes de l'imminium et ainsi étudier différentes réductions diastéréosélectives. Enfin, je me dois de mentionner que Laurent procura une aide considérable et très appréciable dans les révisions ainsi que dans la rédaction de la version finale du manuscrit qui a conduit à l'article 2.

Puisque l'amination réductrice telle que décrite dans le deuxième article de la première partie m'a permis d'obtenir des quantités importantes du diastéréoisomère *cis*, j'ai conduit l'évaluation de l'influence de la protection de l'acide en position α. J'ai été par la suite attiré vers la possibilité de produire le diastéréoisomère *trans* via la réduction diastéréosélective de l'imminium. Après avoir produit des quantités importantes du diastéréoisomère *trans* et déterminé qu'il était possible de réduire de façon diastéréosélective l'iminino acide **11**, j'ai pu démontrer que la formation de l'iminium produisait un mélange racémique. En utilisant l'iminoamide **15** la stabilité du centre stéréochimique en position α a pu être préservée mais la formation majoritaire du diastéréoisomère *trans* n'a pas été observée. Par la suite, j'ai conduit une série d'épimérisations de l'isomère *cis* qui ont montré qu'il est possible d'obtenir le diastéréoisomère *trans* de cette manière. En résumé, il est possible de voir que la synthèse des alkylprolines a été au centre de nombreux autres projets de recherche. Ceci provient du fait que l'approche de synthèse utilisée a permis l'exploration de nombreux chemins synthétiques. Cependant en intégrant l'ensemble des résultats préliminaires obtenus, j'ai pu développer et terminer la synthèse des quatres diastéréoisomères des 5-*tert*-butylprolines, des nouveaux outils pour le peptidomimétisme.

En effet, l'influence des effets stériques sur l'équilibre des isomères d'amide en position *N*-terminale de proline peut être explorée avec des 5-alkylprolines possédant des substituants encombrés en position 5. Les 5-*tert*-butylprolines énantiomériquement pures ont donc été préparées à partir de l'acide glutamique via une séquence d'acylation et d'amination réductive diastéréoselective. Une double déprotonation du γ-méthyl-*N*-(PhF)glutamate (**2**) avec $LiN(SiMe_3)_2$ et une *C*-acylation avec le chlorure d'acide pivaloïque ont procuré le β-cétoester **3**, qui, sous hydrolyse du γ-méthylester et d'une décarboxylation a donné l'acide δ-oxo-α-[*N*-(PhF)amino]heptanoïque (**4**). Les

synthèses des (2*S*, 5*R*)- et (2*R*, 5*S*)-*N*-(BOC)-5-*tert*-butylprolines ((2*S*, 5*R*)-1 et (2*R*, 5*S*)-1) ont été accomplies par hydrogénation catalytique de leurs (2*S*)- et (2*R*)-δ-oxo-α-[*N*-(PhF)amino]heptanoates de méthyle ((2*S*)-**5a** et (2*R*)-**5a**) dans le méthanol avec le di-*tert*-butyle dicarbonate suivi d'une chromatographie sur gel de silice et d'une hydrolyse de l'ester avec le potassium triméthylsilanolate. La pureté énantiomérique observée est > 99% après conversion aux diastéréoisomériques α-méthylbenzylamides **10**. Une bonne diastéréosélectivité en faveur du diastéréoisomère *trans* a été observée quand la (2*S*, 5*R*)-5-*tert*-butylproline a été synthétisée à partir du (2*S*)-δ-oxo-α-[*N*-(PhF)amino]heptanoate ((2S)-**4**) par solvolyse du groupe PhF dans l'acide trifluoroacétique suivi d'une réduction du 5-*tert*-butyl-Δ^5-dehydroproline (**11**) avec le triacétoxyborohydrure de tetraméthylammonium. Cependant, l'imino-acide **11** s'est montré configurationnellement labile et a racémisé sous conditions acides. Le 5-*tert*-butyl-Δ^5-dehydroproline *N*'méthylamide **15** a été configurationnellement stable en milieu acide, cependant des études préliminaires pour réduire **15** montrent que le diastéréoisomère *cis* **16** est favorisé. Alternativement, le *trans*-diastéréoisomère énantiomériquement pure, (2*R*, 5*R*)-*N*-(BOC)-2-méthyl-5-*tert*-butyleprolinate (**9**) a été préparé par épimérisation du (2*S*, 5*R*)-**9**. En résumé cette méthodologie synthétique donne accès à tous les quatre isomères énantiomériquement pures des 5-*tert*-butylproline à partir du L- et du D-glutamate comme précurseur chiral.

Publication 1:

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Synthesis of 5-alkylprolines and their use in the design of X-proline *cis*-amide surrogates

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Introduction

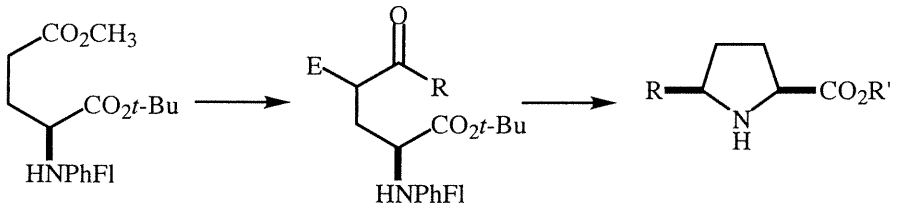
Secondary amides exist primarily as the energetically favored *trans*-rotamer which avoids steric interactions between adjacent α-carbon substituents. In contrast, the *cis*- and *trans*-rotamers of tertiary amides *N*-terminal to proline have more similar energies [1,2]. Significant populations of X-Pro *cis*-rotamer have been observed by NMR in many biologically active peptides under physiological conditions [3-6]. Furthermore, increasing evidence suggests that X-Pro rotamer geometry plays an important role in the recognition and reactivity of bioactive peptides [1]. Hydrolysis of X-L-Pro peptide bonds by proline-specific peptidases requires the *trans*-rotamer [7], and isomerization of X-Pro amide bonds has been proposed as a rate-limiting step in protein folding [8]. The X-Pro *cis-trans* isomerization energy barrier of 13 kcal/mol can be overcome by peptidyl prolyl isomerase enzymes, which can facilitate refolding of denatured proteins [1,8]. The rotamer equilibrium of acylprolyl residues can also be influenced by local peptide sequence patterns [6,9], protonation of *N*-terminal residue side-chains [5], solvent conditions [10], and artificial hydrogen bonding receptors [11].

Interest in understanding the relation of X-Pro *cis*-amide rotamers to peptide bioactivity has led to the synthesis of prolines with ring alkyl substituents designed to constrain rotamer and torsion angle geometries [12-14]. Methyl substituents on the 5-position of proline can exert a steric effect on the *N*-acyl residue that alters the rotamer isomerization barrier and augments the population of *cis*-amide in *N*-acetylproline *N'*-methylamide [13]. Similarly, the use of 5,5-dimethyl proline was recently demonstrated to produce 90% of the *cis*-peptide bond isomer in a dipeptide [14]. Addition of bulkier alkyl substituents to the 5-position of proline should thus be expected to produce even more pronounced effects on rotamer and torsion angle geometry.

Results and Discussion

We have developed an efficient synthesis of 5-alkylprolines based on the reductive amination of δ-oxo α-amino esters derived from glutamic acid [15]. This method provides unnatural prolines substituted at the 5-position with primary, secondary and tertiary alkyl groups, as well as with aromatic substituents (Scheme 1).

Scheme 1



1

2 : E = CO_2CH_3
3 : E = H

4 : R' = *t*-Bu
5 : R' = H

R = Et, *n*-Bu, $PhCH_2$, *i*-Pr, *t*-Bu

Synthesis of 5-substituted prolines begins with α-*tert*-butyl γ-methyl *N*-(9-(9-phenylfluorenyl))glutamate (**1**) [16]. The 9-(9-phenylfluorenyl), (PhFl) group for nitrogen protection allowed selective enolization of the γ-ester without racemization of the chiral α-amino center; in addition, it shielded the amine from acylation [15-17].

Treatment of **1** with lithium bis(trimethylsilyl)amide provided the γ_lithium enolate, which reacted with acid chlorides to give good yields of β-keto esters **2**. Selective hydrolysis of the γ-methyl ester and decarboxylation was achieved on exposure of β-keto esters **2** to lithium hydroxide in a THF : water solution to provide δ-oxo α-amino esters **3**. Hydrogenation of δ-oxo α-amino esters **3** with palladium-on-carbon in a 9:1 methanol : acetic acid solution proceeds by cleavage of the phenylfluorenyl group, intramolecular imine formation, protonation, and hydrogen addition to the less hindered face of the iminium ion intermediate to furnish *cis*-5-alkylprolines **4** with the 5-substituent and carboxylate on the same side of the proline ring. Although imine products from incomplete hydrogenation were sometimes observed by proton NMR analysis of the crude reaction mixtures, *trans*-diastereomer **4** was not detected. The *tert*-butyl ester was cleaved by treatment with trifluoroacetic acid to give prolines **5** as their TFA salts after evaporation of the volatiles. 5-*n*-Butylproline **4** was determined to be of >99% enantiomeric purity as ascertained by preparation of diastereomeric ureas on reaction with α-methylbenzylisocyanate and observation of the *tert*-butyl ester singlets in the proton NMR spectrum during incremental additions of the diastereomer prepared from D-glutamate. Initial modeling studies of *N*-acetyl-5-*tert*-butylproline *N'*-methylamides suggest that the bulkier 5-*tert*-butyl substituent exerts effects on both the *N*-acyl and α-carboxylate groups to favor the *cis*-rotamer as well as to constrain the proline Ψ torsion angle.

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5-*tert*-Butylproline

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Abstract

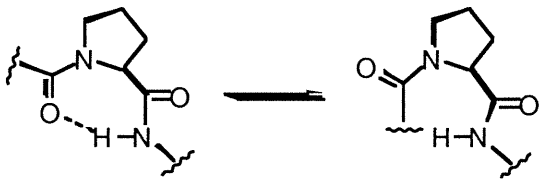
Steric effects on the isomer equilibrium of amides *N*-terminal to proline can be explored with 5-alkylprolines having bulky 5-position substituents. Enantiopure 5-*tert*-butylprolines were thus synthesized from glutamic acid via an acylation / diastereoselective reductive amination sequence. Double deprotonation of γ-methyl *N*-(PhF)glutamate (**2**) with $LiN(SiMe_3)_2$ and *C*-acylation with pivaloyl chloride provided β-ketoester **3**, which upon γ-ester hydrolysis and decarboxylation gave δ-oxo-α-[*N*-(PhF)amino]heptanoate (**4**). Syntheses of (2*S*, 5*R*)- and (2*R*, 5*S*)-*N*-BOC-5-*tert*-butylprolines ((2*S*, 5*R*)-**1** and (2*R*, 5*S*)-**1**) were accomplished by catalytic hydrogenation of their respective (2*S*)- and (2*R*)-methyl δ-oxo-α-[*N*-(PhF)amino]heptanoates ((2*S*)-**5a** and (2*R*)-**5a**) in methanol with di-*tert*-butyldicarbonate followed by chromatography and ester hydrolysis with potassium trimethylsilanolate. The 5-*tert*-butylproline *cis*-diastereomers were proven to be of >99% enantiomeric purity after their conversion to diastereomeric α-methylbenzylamides **10**. Good diastereoselectivity in favor of the *trans*-diastereomer

was observed when (2*S*, 5*S*)-5-*tert*-butylproline was synthesized from (2*S*)-δ-oxo-α-[*N*-(PhF)amino]heptanoate ((2*S*)-**4**) by solvolysis of the PhF group in trifluoroacetic acid and subsequent reduction of 5-*tert*-butyl-Δ^5-dehydroproline **11** with tetramethylammonium triacetoxyborohydride; however, imino acid **11** was shown to be configurationally labile and racemized under acidic conditions. 5-*tert*-butyl-Δ^5-dehydroproline *N*'-methylamide **15** was configurationally stable in acid, yet preliminary attempts to reduce **15** favored *cis*-diastereomer **16**. Alternatively, enantiopure *trans*-diastereomer, (2*R*, 5*R*)-methyl *N*-BOC-5-*tert*-butylprolinate **9** was prepared by epimerization of (2*S*, 5*R*)-**9**. In sum, this synthetic methodology now provides access to all four enantiopure 5-*tert*-butylproline isomers from inexpensive L and D-glutamate as chiral educts.

Introduction

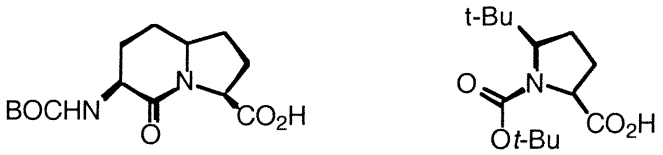
Amides *N*-terminal to proline possess energetically similar *cis*- and *trans*-isomers that are separated by a significant barrier for isomerization (Figure 1).[1] Consequently, isomer geometry plays an important role in the recognition, reactivity and stability of bioactive peptides and proteins which possess prolyl residues.[2-5] For example, proline-specific peptidases require the *trans*-isomer to hydrolyze X-Pro peptide bonds.[3] In addition, the acceleration of the folding of particular proteins by peptidyl prolyl *cis/trans* isomerase catalyzed isomerization of X-Pro amide bonds may implicate substrate binding as X-Pro amide *cis*-isomers in type VI β-turn conformations.[4,6,7]

The conformational heterogeneity of peptides possessing X-Pro residues has often confounded efforts to elucidate bioactive structures of native peptides using X-ray diffraction and NMR spectroscopy.[2] The identification of a bioactive structure is made more complicated due to influences on isomer geometry from environmental conditions such as solvent polarity and pH.[6,8] Because knowledge of the bioactive conformation is principal to the rational design of therapeutics based on peptide leads, conformationally rigid surrogates of the *cis*- and *trans*-amide isomers of X-Pro residues have emerged as important tools for elucidating structure-activity relationships of peptides that possess prolyl residues.[9]



***Trans*-Rotamer** **Cis-Rotamer**

Figure 1. Amide Isomer Equilibrium *N*-Terminal to Proline Derivatives.



A B

Figure 2. δ-Alkylprolines as Conformationally Rigid Amide Isomer Surrogates.

We are exploring the relationship between X-Pro amide conformation and peptide bioactivity through the use of 5-alkylprolines that function as fixed *trans*- and *cis*-isomer surrogates (Figure 2). We recently reported the synthesis of enantiopure 2-oxo-3-*N*-(BOC)amino-1-azabicyclo[4.3.0]nonane-9-carboxylic acid in which the amide is locked in the *trans*-isomer by linking the 5-position of proline to its *N*-terminal amino acid in a six membered lactam (Figure 2A).[10] We are currently investigating incorporation of this indolizidinone amino acid into peptides in order to examine its potential to serve as a conformationally fixed surrogate of β- and γ-turn conformations.[11]

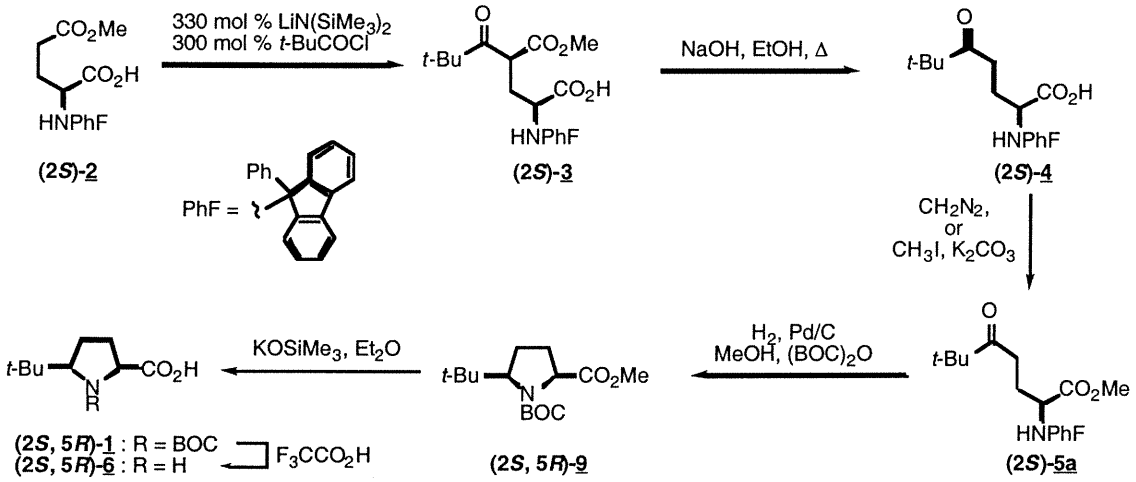
In this manuscript, we report methodology for synthesizing 5-alkylprolines possessing bulky 5-position substituents.[12] All four stereoisomers of 5-*tert*-butylproline can now be synthesized in >99% enantiomeric purity from inexpensive glutamic acid as chiral educt. By allowing for the selective introduction of sterically demanding tertiary alkyl substituents at the proline 5-position, our process offers a unique advantage when compared to previous methods to synthesize 5-alkylprolines.[15,16] Furthermore, our method provides *N*-BOC-5-*tert*-butylprolines (**1**) that are suitable for incorporation into peptides using standard coupling techniques.[12a]

Since the steric interactions between the 5-position substituent and the *N*-terminal residue disfavor the X-Pro amide *trans*-isomer, they increase the *cis*-isomer population.[12,13,14] We synthesized specifically 5-*tert*-butylprolines in order to study

the importance of X-Pro amide *cis*-isomer populations to the activity of prolyl peptides (Figure 2B). We have also synthesized and examined the conformational preferences of *N*-acetyl-5-*tert*-butylproline *N*'-methylamides.[14] Besides increasing the X-Pro *cis*-amide isomer population, incorporation of 5-*tert*-butylprolines into peptides influences the proline ψ dihedral angle and alters the energy barrier for X-Pro isomerization.[12,13,14] *N*-BOC-5-*tert*-Butylproline should thus be useful for examining X-Pro amide *cis*-isomers in bioactive peptides as well as for synthesizing type VI β-turn mimetics.[17]

Results and Discussion

Cis-*N*-BOC-5-*tert*-Butylprolines: (2*S*, 5*R*)-1 and (2*R*, 5*S*)-1

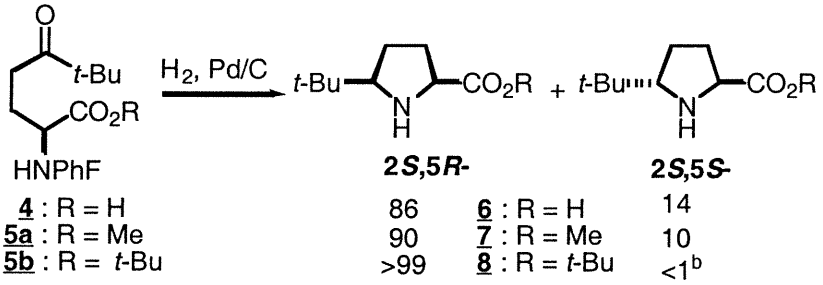


Scheme 1. Synthesis of (2*S*, 5*R*)-5-*tert*-Butylproline 1

We demonstrated previously that acylation of the lithium γ-enolate of α-*tert*-butyl γ-methyl *N*-(9-(9-phenylfluorenyl))glutamate with different acid chlorides provides β-keto esters.[18] Hydrolysis and decarboxylation of the γ-ester then furnishes enantiomerically pure δ–oxo α-amino esters possessing alkyl and aromatic δ-substituents.[18] In the acylation of the γ-enolate to prepare β-keto ester, we have found that α-carboxylate protection and pyroglutamate formation can both be avoided and

high yields can be achieved by double deprotonating γ-methyl *N*-(PhF)glutamate (**2**, Scheme 1, PhF = 9-(9-phenylfluorenyl)).[19] Treatment of **2** with 200 mol % of lithium bis(trimethylsilyl)amide deprotonates the α-carboxylate and generates the γ-enolate which reacts with pivaloyl chloride to provide β-keto ester **3** after aqueous work-up and chromatography.[20] We have optimized acylation of **2** to furnish β-keto ester **3** in 75% yield by using ~300 mol % of both $LiN(SiMe_3)_2$ and pivaloyl chloride. β-Keto ester **3** was observed by proton NMR as a mixture of diastereomers each exhibiting a γ-proton appearing as a doublet of doublets in the region between 4 and 4.7 ppm.

Hydrolysis and decarboxylation of β-keto ester **3** with sodium hydroxide provided the δ-oxo α-*N*-(PhF)amino acid **4** in 78% yield after chromatography. Esterification of acid **4** was accomplished quantitatively with diazomethane in ether as well as with iodomethane and potassium carbonate in acetonitrile providing methyl δ-oxo-α-[*N*-(PhF)amino]heptanoate **5a** after chromatography. On large scale, chromatographic isolation of acids **3** and **4** was shown to be unnecessary. Multi-gram quantities of δ-oxo α-amino ester **5a** can now be obtained in ~50% overall yield from glutamate **2** by the acylation, hydrolysis, decarboxylation and esterification sequence presented in Scheme 1.



	2*S*,5*R*-		2*S*,5*S*-
4 : R = H	86	**6** : R = H	14
5a : R = Me	90	**7** : R = Me	10
5b : R = *t*-Bu	>99	**8** : R = *t*-Bu	<1[b]

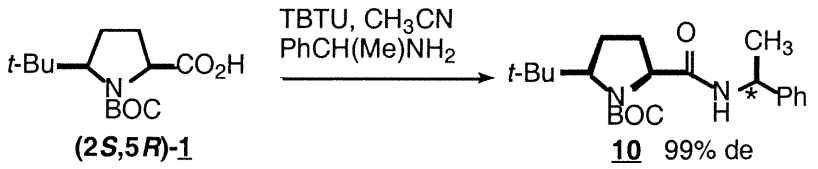
[a]Performed in a 0.6 M 9:1 MeOH:AcOH solution with 5 mol % Pd/C at 4 atm H_2. [b]2*S*, 5*S*-**8** was not detected.

Table 1. Influence of α-Carboxylate Protection in the Diastereoselective Hydrogenation of 4, 5a and 5b.[a]

Hydrogenations of δ-oxo-α-[*N*-(PhF)amino]heptanoate (**4**), methyl ester **5a** and *tert*-butyl ester **5b**[18] were examined in order to study the influence of the α-carboxylate on the diastereoselectivity of the reductive amination. We performed our study in 0.6 M 9:1 methanol : acetic acid solutions with palladium-on-carbon as catalyst under 4 atm of hydrogen for 24 h (Table 1). Under these conditions, hydrogenation of δ-oxo-heptanoates **4**, **5a** and **5b** proceeds by cleavage of the phenylfluorenyl group, intramolecular imine formation, protonation, and hydrogen addition to the iminium ion intermediate.[15s] The diastereomeric ratios of (2*S*, 5*R*)- and (2*S*, 5*S*)-5-*tert*-butylprolines **6**, **7** and **8** were ascertained by proton NMR of the crude product after removal of the catalyst by filtration and evaporation of the volatiles under vacuum. The stereochemistry of esters **7** and **8** was assigned based on analogy with our previous work.[18] Deprotection of (2*S*, 5*R*)-*tert*-butyl ester **8** with trifluoroacetic acid gave an authentic sample of (2*S*, 5*R*)-*tert*-butylproline ((2*S*, 5*R*)-**6**). Our examination demonstrates clearly that steric bulk at the α-carboxylate favors hydrogen addition to the less hindered face of the iminium ion providing the *cis*-diastereomers (Table 1). Hydrogenation of (2*S*)-*tert*-butyl ester **5b** proceeds diastereospecifically furnishing (2*S*, 5*R*)-*tert*-butylproline **8**.[12b]

We next employed a one-pot reductive amination / nitrogen protection process in order to prepare (2*S*, 5*R*)-*N*-BOC-5-*tert*-butylproline ((2*S*, 5*R*)-**1**). Hydrogenation of (2*S*)-δ-oxo-α-[*N*-(PhF)amino]heptanoate (2*S*)-**5a** with palladium-on-carbon and di-*tert*-butyldicarbonate in methanol without acetic acid proceeds by cleavage of the PhF protection, cyclization, *N*-acylation and hydrogen addition furnishing selectively (2*S*, 5*R*)-*N*-BOC-5-*tert*-butylproline methyl ester ((2*S*, 5*R*)-**9**) in 77 % yield after chromatography. Since hydrogenation of Δ^1-pyrrolidine in alcoholic solvents without acid is normally a poor reaction,[15s] in this one-pot process, an *N*-acyl iminium salt is presumably reacting with hydrogen in the presence of the palladium catalyst.

Examination of **9** by proton NMR prior to chromatography showed a 97:3 ratio of *cis:trans* diastereomers and demonstrated that hydrogenation of **5a** via the *N*-acyl iminium proceeded with higher diastereoselectivity than the hydrogenation of **5a** via the protonated iminium ion (90:10, Table 1). Hydrolysis of methyl ester (2*S*, 5*R*)-**9** with potassium trimethylsilanolate (150 mol%) in Et_2O furnished (2*S*, 5*R*)-*N*-BOC-5-*tert*-butylproline ((2*S*, 5*R*)-**1**) as a crystalline solid after aqueous extraction.[21] Removal of the BOC protecting group with trifluoroacetic acid in dichloromethane and evaporation of the volatiles under vacuum provided quantitatively (2*S*, 5*R*)-5-*tert*-butylproline ((2*S*, 5*R*)-**6**) as a TFA salt which was free based by ion exchange chromatography on a Dowex 1-X8 resin (Scheme 1). When D-glutamic acid was employed in the same sequence of reactions, (2*R*, 5*S*)-**1** was obtained in comparable overall yield.

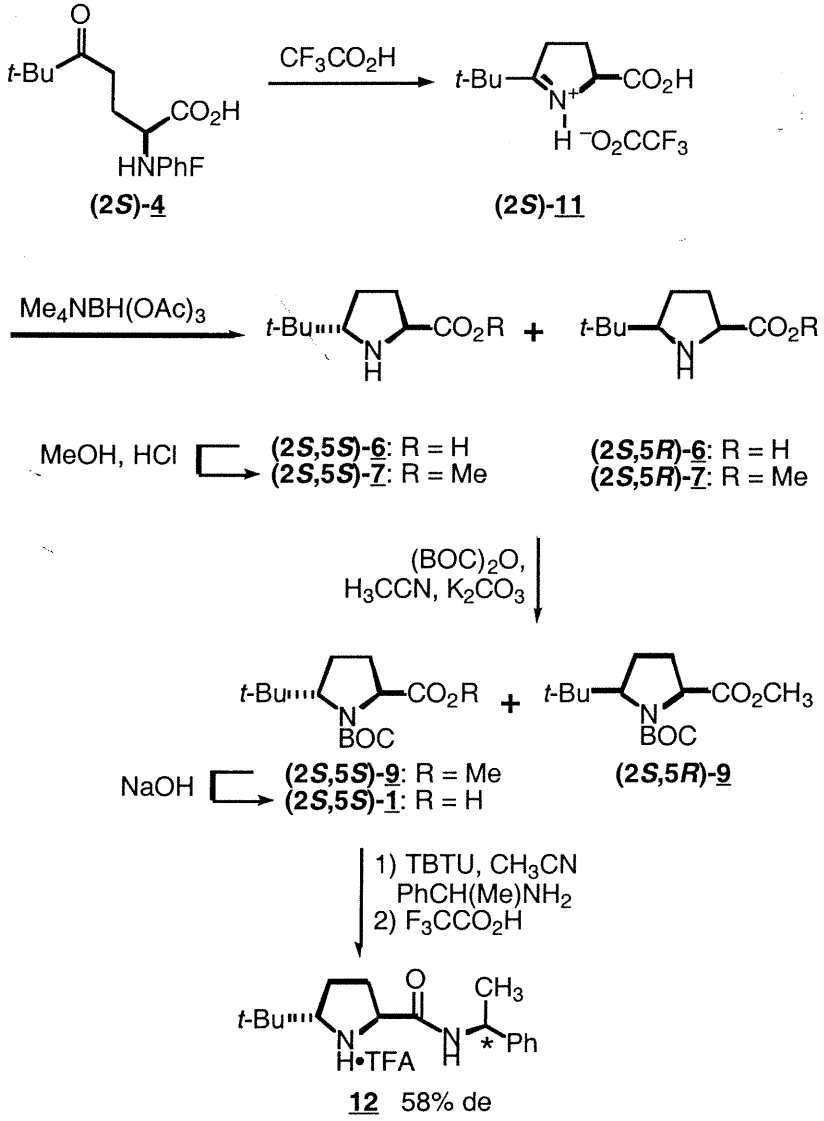


Scheme 2. Enantiomeric Purity of (2*S*, 5*R*)-5-*tert*-Butylproline 1

In order to ascertain if any racemization had occurred during the syntheses of the 5-*tert*-butylproline *cis*-diastereomers from glutamic acid, the enantiomeric purity of proline (2*S*, 5*R*)-**1** was investigated after conversion to diastereomeric α-methylbenzylamides **10** (Scheme 2). Both (*R*)- and (*S*)-α-methylbenzylamine were coupled to proline (2*S*, 5*R*)-**1** in high yield using benzotriazol-1-yl-1,1,3,3-tetramethyl uronium tetrafluoroborate (TBTU) in acetonitrile.[22] By integrating the diastereomeric 5-*tert*-butyl singlets (δ = 0.74, 0.93), we established amide (1'*S*)-**10** to be of 99% diastereomeric purity. Since (*S*)-α-methylbenzylamine of 99% ee was used in the

coupling step, proline (2*S*, 5*R*)-**1** is presumed to be of >99% enantiomeric purity. Hence, no racemization was observed in the synthesis of *cis*-diastereomer **1**.

***Trans*-*N*-BOC-5-*tert*-Butylprolines: (2*S*, 5*S*)-1 and (2*R*, 5*R*)-1**

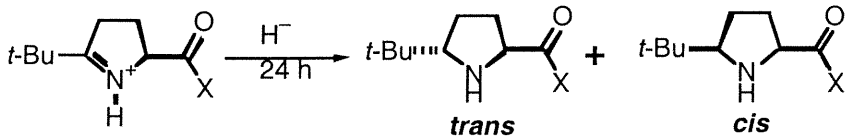


Scheme 3. Synthesis of (2*S*, 5*S*)-*N*-BOC-5-*tert*-Butylproline 1 via Imminium Ion 11.

With an efficient route in hand to synthesize enantiopure *cis*-diastereomers of 5-*tert*-butylproline, (2*S*, 5*R*)- and (2*R*, 5*S*)-**1**, we began the more formidable task to prepare *trans*-isomers (2*S*, 5*S*)-**1** and (2*R*, 5*R*)-**1**. In order to synthesize *trans*-

diastereomer, we investigated a novel approach involving carboxylate directed hydride reduction of an iminium ion intermediate. (2*S*)-5-*tert*-Butyl-Δ^5-dehydroproline trifluoroacetate ((2*S*)-**11**) was synthesized in quantitative yield from δ-keto α-amino acid (2*S*)-**4** (Scheme 3). Treatment of (2*S*)-δ-oxo-α-[*N*-(PhF)amino]heptanoate ((2*S*)-**4**) with trifluoroacetic acid in dichloromethane effected solvolysis of the phenylfluorenyl group and intramolecular cyclization to imino acid (2*S*)-**11** which was isolated as the trifluoroacetate after removal of the hydrocarbon impurities by trituration with hexanes. The zwitterionic form of imino acid **11** was also prepared by ion exchange chromatography.

We hypothesized that coordination of a borohydride salt by the α-carboxylate of (2*S*)-**11** could direct hydride addition to the iminium ion and give *trans*-diastereomer (2*S*, 5*S*)-**6**. Carboxylate complexation of metal ion has been suggested to bias the direction of organocuprate additions to *N*-acyl-Δ^5-dehydroprolinates.[15e] Since hydroxyl-directed reductions with tetramethylammonium triacetoxyborohydride proceed with good diastereoselectivity,[23] and because triacetoxyborohydrides effectively reduce imines,[24] we examined $Me_4NHB(OAc)_3$ in the reduction of **11** (Table 2).



11 : X = OH
15 : X = NHMe

6 : X = OH
16 : X = NHMe

Entry	Substrate	Hydride	Solvent	Temp.	*Trans* : *Cis*
a	**11**•TFA	$NaCNBH_3$	THF	66 °C	43 : 57
b	**11**•TFA	$Me_4NBH(OAc)_3$	THF	66 °C	83 : 17[b]
c	**11**•TFA	$Me_4NBH(OAc)_3$	THF	0 °C	66 : 33
d	**11**	$Me_4NBH(OAc)_3$	CH_3CN	–70 °C	33 : 66
e	**11**	$NaCNBH_3$	CH_3CN	–40 °C	50 : 50
f	**11**	$Me_4NBH(OAc)_3$	CH_3CN	0 °C	58 : 42
g	**11**	$Me_4NBH(OAc)_3$	CH_3CN	–40 °C	54 : 46
h	**15**•TFA	$Me_4NBH(OAc)_3$	THF	66 °C	37 : 74
i	**15**•TFA	$Me_4NBH(OAc)_3$	CH_3CN	0 °C	50 : 50[c]

[a]Unless noted 100% conversion. [b]100% conversion after 6 h.
[c]66% conversion after 96 h.

Table 2. Hydride Additions to Imino Acid 11 and Imino Amide 15.[a]

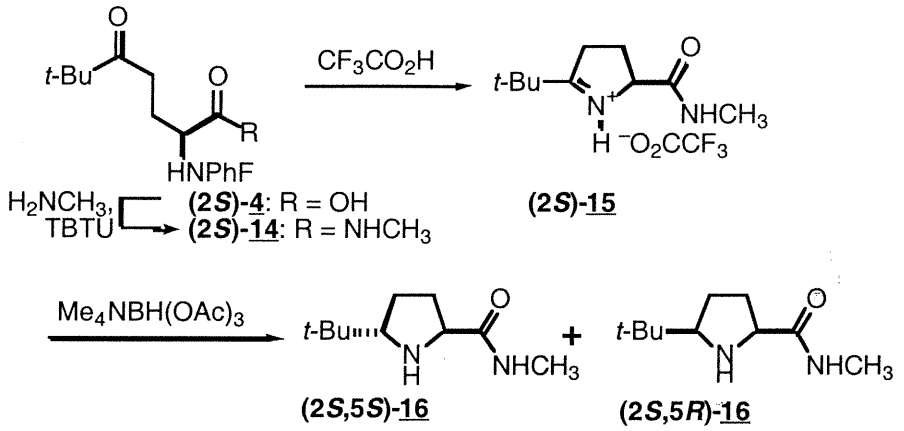
tert-Butylprolines **6** were purified by ion exchange chromatography; however, the diastereoselectivity of imine reductions was ascertained by proton NMR of the crude (2*S*, 5*S*)- and (2*S*, 5*R*)-*tert*-butylprolines (**6**) after addition of 1M HCl and evaporation. The signals of the diastereomeric α-, δ- and *tert*-butyl protons of **6** were all well resolved in CD3OD acidified with TFA. In general, we have noted that the coupling pattern of the α-proton signal of 5-*tert*-butylproline analogues (**6**,**7** and **16**) is different for the *cis*- and *trans*-diastereomers. In the ^1H NMR, the α-proton signal of the *trans*-diastereomer is observed as a triplet and that of the *cis*-diastereomer appears as a doublet of doublets.

In our best conditions, solid Me4NHB(OAc)3 was added to a solution of 5-*tert*-butyl-Δ^5-dehydroproline trifluoroacetate (**11**) in THF at reflux (entry b in Table 2). We received an 86:14 ratio of *trans*:*cis* diastereomers (2*S*, 5*S*)- and (2*S*, 5*R*)-**6** in 96%

yield after ion exchange chromatography. The predominant formation of *trans*-diastereomer (2*S*, 5*S*)-**6** is presumed to be due to a carboxyl-directed addition of hydride to the iminium. Employment of $NaCNBH_3$ in lieu of $Me_4NBH(OAc)_3$ under the same conditions gave a 43:57 ratio of (2*S*, 5*S*)-**6** : (2*S*, 5*R*)-**6**. Reduced diastereoselectivity also resulted at lower temperature. Similarly, the diastereoselectivity decreased in acetonitrile and when the zwitterionic form of **11** was employed.

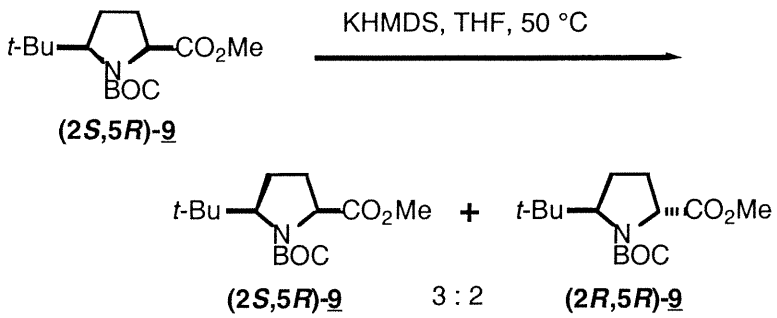
Diastereomers (2*S*, 5*S*)- and (2*S*, 5*R*)-**6** were converted to *N*-BOC-5-*tert*-butylproline methyl esters (**9**) on treatment with methanolic HCl followed by acylation with di-*tert*-butyldicarbonate in acetonitrile with potassium carbonate.[25] Separation of the diastereomers was then achieved by chromatography on silica gel with 0-2% EtOAc in hexanes as eluant. (2*S*, 5*S*)-*N*-BOC-5-*tert*-butylproline (**1**) was obtained on hydrolysis of methyl ester **9** using potassium hydroxide in dioxane (Scheme 3).

The enantiomeric purity of (2*S*, 5*S*)-*N*-BOC-5-*tert*-butylproline (**1**) was next ascertained by the preparation of diastereomeric amides **12**. Acid **1** was coupled to both (*R*)- and (*S*)-α-methylbenzylamine using TBTU in acetonitrile, and the BOC group was subsequently removed using TFA. Examination of the *tert*-butyl singlets (1.02 and 1.03 ppm) as well as the α-proton signals in the 400 MHz ^1H NMR in CD_3OD indicated that amides **12** were of only 58% diastereomeric excess. Racemization had obviously occurred after the removal of the PhF protection either during the deprotection or the reduction steps to produce (2*S*, 5*S*)-**1**. By monitoring the decrease in the value of the specific rotation of **11** upon exposure to the deprotection conditions (TFA in CH_2Cl_2 at reflux),[26] we confirmed that imino acid **11** was configurationally labile under the acidic conditions.



Scheme 4. Synthesis of (2*S*, 5*S*)-*N*-BOC-5-*tert*-Butylproline 1 via Imminium Ion 15.

In light of these results, we decided to explore amide directed hydride addition to 5-*tert*-butyl-Δ^5-dehydroproline *N*'-methylamide trifluoroacetate (**15**). Since racemization of imino acid **11** presumably arose from enolization toward the α-carbon on protonation of the carboxylate, we expected **15** to be more configurationally stable than **11** because the amide would be less prone to enolize under acidic conditions.[27] (2*S*)-δ-Oxo-α-[*N*-(PhF)amino]heptanoate *N*'-methylamide (2*S*)-**14** was synthesized in 78% yield from acid (2*S*)-**4** and methylamine using TBTU in acetonitrile. Solvolysis of the PhF group and imine formation proceeded slowly when amide (2*S*)-**14** was treated with TFA in CH_2Cl_2 at reflux, yet furnished imino amide **15** in 92% yield. An examination of **15** after exposure to TFA in refluxing CH_2Cl_2 for 24 and 48 h showed no decrease in its specific rotation. Although amide **15** was shown to be of greater configurational stability than acid **11**, in preliminary studies, hydride reduction of imino amide **15** proceeds slowly and tends to favored *cis*-diastereomer **16** (entry h, Table 2).[28]



Scheme 5. Epimerization of (2*S*, 5*R*)-5-*tert*-Butylprolinates 9.

In order to synthesize enantiomerically pure *trans*-diastereomer, we examined epimerization of *N*-protected *cis*-5-*tert*-butylproline methyl esters (Scheme 5). Overall, the epimerization route to *trans*-isomers provided at best a 1:1 ratio of (2*S*, 5*R*)- and (2*R*, 5*R*)-isomers. For example, treatment of (2*S*, 5*R*)-**9** with potassium bis(trimethylsilyl)amide (125 mol %) in THF for 16 h at 50 °C followed by a methanol quench and aqueous work-up gave a separable 3:2 mixture of (2*S*, 5*R*)- and (2*R*, 5*R*)-esters **9** in 97% yield. Epimerization of (2*S*, 5*R*)-methyl *N*-benzyl-5-*tert*-butylprolinate using potassium *tert*-butoxide in *tert*-butanol at 50°C for 16 h yielded a 1:1 *cis* : *trans* diastereomeric mixture.[29,30] Treatment of (2*S*, 5*R*)-5-*tert*-butylproline ((2*S*, 5*R*)-**6**) with acetic anhydride in acetic acid at 50°C, conditions previously used to racemize L-proline,[31] provided a 3:1 *cis*:*trans* ratio of diastereomers **6**. Among these epimerization methods, the most practical in our hands was epimerization of *N*-(BOC)amino ester (2*S*, 5*R*)-**9** with $KN(Si(CH_3)_3)_3$ as described below in the experimental section.

Conclusion

We have developed efficient methodology for synthesizing enantiopure 5-alkylprolines possessing tertiary 5-position substituents. All four stereoisomers of 5-*tert*-butylproline can be synthesized from glutamic acid via our acylation / diastereoselective reductive amination sequence. Because the 5-*tert*-butyl substituent can effect the X-Pro amide geometry to favor the *cis*-isomer and because *N*-BOC-5-

tert-butylproline may be introduced into a variety of peptides via standard coupling techniques, these 5-alkylprolines should find general use in the conformational analysis of bioactive peptides possessing energetically similar *cis*- and *trans*-isomers *N*-terminal to prolyl residues.

Experimental

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; 1,1,1,3,3,3-hexamethyldisilazane (HMDS), CH_3CN and CH_2Cl_2 were distilled from CaH_2; Et_3N was distilled from BaO. 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 (EI and FAB), were obtained by the Université de Montréal Mass Spectroscopy facility. ^1H 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 ($(CH_3)_4Si$). Chemical shifts for aromatic PhF carbons are not reported. The chemical shifts for the carbons and protons of minor isomers are respectively reported in parentheses and in brackets.

(2*S*)-Methyl 6,6-Dimethyl-5-oxo-2-[*N*-(PhF)amino]heptanoate (5a). A –10°C solution of HMDS (20 mL, 95.9 mmol) in 20 mL of THF was treated with n-butyllithium (34 mL of a 2.5 M solution in hexane, 85 mmol), stirred for 30 min, cooled to –78°C, and treated with a solution of γ-methyl *N*-(9-(9-phenylfluorenyl))-L-glutamate (**2**, 10.3 g , 25.7 mmol)[15o] in THF (30 mL). The reaction mixture was stirred at –78°C for 1.5 h, treated with a –78°C solution of trimethylacetyl chloride (9 mL, 73.2 mmol) in THF (6 mL), stirred an additional 45 min, and poured into 1 M NaH_2PO_4 (50 mL). The mixture was extracted with EtOAc

(4 × 50 mL), and the combined organic phases were washed with cold water (3 × 20 mL) and brine (2 × 30 mL), dried, filtered, and evaporated. The residue was normally used without purification in the next reaction. Purification of the residue by chromatography on silica gel with an eluant of 17-60% EtOAc in hexane provided a 1.5:1 mixture of diastereomers, **(2*S*, 4*RS*)-6,6-dimethyl-5-oxo-4-methyloxycarbonyl-2-[(*N*-(PhF)amino] heptanoate** (**3**, 75%): ^{1}H NMR δ 1.07 (s, 9 H), 1.22 (s, 9 H), 1.55 (m, 2 H), 2.03 (m, 1 H), 2.25 (m, 1 H), 2.5 (m, 2 H), 3.47 (s, 3 H), 3.7 (s, 3 H), 3.87 (dd, 1 H, *J* = 2.8, 8.8), 4.37 (dd, 1 H, *J* = 2.6, 10), 7.1-7.6 (m, 26 H); ^{13}C NMR δ 25.9, 26, 33, 33.6, 45.3, 45.6, 48, 49.7, 52.1, 52.3, 53.9, 54.6, 72.6, 72.7, 170.1, 170.2, 178.2, 178.3, 210, 210.2; HRMS calcd for $C_{30}H_{32}NO_5$ (MH^+) 486.2280, found 486.2250.

Crude β-keto ester **3** was dissolved in EtOH (125 mL), treated with 2 N NaOH (125 mL), and stirred at a reflux for 48 h. The mixture was cooled to room temperature and brought to pH 5 using 10% HCl. The solution was extracted with EtOAc (4 × 75 mL), and the combined organic phases were washed with brine, dried, filtered, and evaporated to a residue that was normally used without purification in the next reaction. When pure β-keto ester **3** was used, purification by chromatography on silica gel using 1:1 EtOAc:hexane gave a 78 % yield of **(2*S*)-6,6-dimethyl-5-oxo-2-[*N*-(PhF)amino]heptanoate** (**4**): mp 170°C; $[\alpha]^{20}_D$ -61.1° (*c* 1.1, MeOH); ^{1}H NMR δ 1.12 (s, 9 H), 1.7 (m, 2 H), 2.5 (m, 2 H), 2.6 (dd, 1 H, *J* = 4.7, 6.6), 7.2-7.8 (m, 13 H); ^{13}C NMR δ 26.5, 27.37, 33.4, 44.2, 56, 72.8, 175.4, 212.2; FT-IR ($CHCl_3$) 2971, 1708, 1704, 1447, 1368, 1284; HRMS calcd for $C_{28}H_{30}NO_3$ (MH^+) 428.2226, found 428.2205.

Crude acid **4** was dissolved in acetonitrile (190 mL), treated with K_2CO_3 (6.5 g, 47.2 mmol) and MeI (5 mL, 80.1 mmol), and stirred at room temperature for 19 h. Brine (200 mL) was added to the reaction mixture which was extracted with EtOAc (3

× 100 mL). The organic phases were combined, washed with 0.65 M sodium thiosulfate (200 mL) and brine, dried, filtered, and evaporated to an oil that was purified by chromatography on silica gel using a gradient of 0-25% EtOAc in hexane. Evaporation of the collected fractions gave 5.5 g (12.5 mmol, 49% overall from **2**) of **(2*S*)-methyl 6,6-dimethyl-5-oxo-2-[*N*-(PhF)amino]heptanoate (5a)** as a thick oil. $[\alpha]^{20}_D$ -137.7° (*c* 1, MeOH); ^{1}H NMR δ 1.11 (s, 9 H), 1.63 (m, 2 H), 2.3 (ddd, 1 H, *J* = 6, 9, 15), 2.54 (dd, 1 H, *J* = 5, 8), 2.7 (ddd, 1 H, *J* = 5.6, 9, 15), 3 (br s, 1 H), 3.27 (s, 3 H), 7.14-7.44 (m, 11 H), 7.17 (m, 2 H); ^{13}C NMR δ 26.5, 29.1, 33.1, 44, 51.5, 55, 72.9, 176.5, 215.2; FT-IR ($CHCl_3$) 2965, 1732, 1704, 1477, 1448, 1197, 1168; HRMS calcd for $C_{29}H_{32}NO_3$ (MH+) 442.2382, found 442.2365; Anal. Calcd for $C_{29}H_{31}NO_3$: C, 78.9; H, 7.1; N, 3.2. Found : C, 78.7; H, 7.2; N, 3. **(2*R*)-Methyl 6,6-dimethyl-5-oxo-2-[*N*-(PhF)amino] heptanoate** (*R*-**5a**) was prepared by the same procedure in similar yield from γ-methyl *N*-(9-(9-phenylfluorenyl))-D-glutamate: $[\alpha]^{20}_D$ 159.5° (*c* 1, MeOH).

(2*S*, 5*R*)-*N*-(BOC)-5-*tert*-Butylproline Methyl Ester ((2*S*, 5*R*)-9). A solution of (2*S*)-methyl 6,6-dimethyl-5-oxo-2-[*N*-(PhF)amino]heptanoate (**5a**, 0.73 g, 1.66 mmol) and di-*tert*-butyldicarbonate (1g, 4.6 mmol) in MeOH (50 mL) was treated with palladium-on-carbon (10 wt %, 90 mg), and stirred under 4 atm of hydrogen for 48 h. The mixture was filtered on celite, the catalyst was washed with MeOH (2 × 30 mL), and the combined organic phase was evaporated to a residue that was purified by chromatography on silica gel using a gradient of 0-25% EtOAc in hexane. Evaporation of the collected fractions gave 400 mg (85%) of (2*S*, 5*R*)-**9** as an oil. On larger scale, hydrogenation of **5a** (4.5 g ,10.3 mmol) under similar conditions [500 mg of 10 wt % Pd/C, 4 atm H_2 and 5.8 g (26.6 mmol) of $(BOC)_2O$ in 70 mL of MeOH] and purification gave 2.1 g (71%) of (2*S*, 5*R*)-**9**: $[\alpha]^{20}_D$ -32.2° (*c* 1, MeOH); ^{1}H NMR δ 0.88 (s, 9 H), 1.35 (s, 9 H), 1.84 (m, 3 H), 2.13 (m, 1 H), 3.65 (s, 3 H),

3.73 (br d, 1 H, J = 6.4), 4.22 (m, 1 H); ^{13}C NMR δ 26.6, 27.3, 28.1, 29.4, 36.2, 51.6, 61.4, 66.5, 79.8, 155.9, 173.7; HRMS calcd for $C_{15}H_{28}NO_4(MH^+)$ 286.2018, found 286.2025; Anal. Calcd for $C_{15}H_{27}NO_4$: C, 63.1; H, 9.5; N, 4.9. Found : C, 63.4; H, 9.3; N, 4.9. **(2*R*, 5*S*)-*N*-(BOC)-5-*tert*-Butylproline Methyl Ester ((2*R*, 5*S*)-9)** was prepared with the same procedure from heptanoate (2*R*)-**5a** in 84% yield: $[\alpha]^{20}_D$ 30.3° (*c* 1, MeOH).

(2*S*, 5*R*)-*N*-(BOC)-5-*tert*-Butylproline ((2*S*, 5*R*)-1). Methyl ester *cis*-**9** (0.4 g, 1.4 mmol) was dissolved in 10 mL of Et_2O, treated with KOSi(Me)$_3$ (200 mg, 1.56 mmol), and stirred for 22 h at room temperature. Another 200 mg of KOSi(Me)$_3$ was added and the reaction was stirred an additional 2 h. The reaction mixture was extracted with water (5 × 20 mL), the aqueous phases were combined, acidified with citric acid to pH 2, saturated with NaCl, and extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried, filtered and evaporated to give 0.36 g (1.33 mmol, 94%) of (2*S*, 5*R*)-**7**: mp 119-121°C; $[\alpha]^{20}_D$ -22.5° (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 0.95 (s, 9 H), 1.43 (s, 9 H), 1.95 (m, 3 H), 2.27 (m, 1 H), 3.77 (br d, 1 H, J = 7.6), 4.27 (m, 1 H); ^{13}C NMR (CD_3OD) δ 27.7, 28.1, 28.7, 30.6, 37.3, 62.8, 68.3, 81.4, 158, 176.6; HRMS calcd for $C_{14}H_{26}NO_4$ (MH^+) 272.1862, found 272.1848; Anal. Calcd for $C_{14}H_{25}NO_4$: C, 62; H, 9.3; N, 5.2. Found : C, 61.7; H, 9.1; N, 5.1. The same conditions provided **(2*R*, 5*S*)-*N*-(BOC)-5-*tert*-butylproline ((2*R*, 5*S*)-1)** from methyl ester ((2*R*, 5*S*)-**9**) in similar yield: mp 120°C; $[\alpha]^{20}_D$ 22.2° (*c* 0.5, MeOH).

(2*S*)-6,6-Dimethyl-5-oxo-2-[*N*-(PhF)amino]heptanoate (4) via Hydrolysis of 5a. A solution of methyl ester **5a** (1.9 g, 4.3 mmol) in EtOH (80 mL) was treated with 2 N NaOH (65 mL), and stirred at a reflux for 48 h. The solution was cooled to room temperature, acidified to pH 5 with 10% HCl, saturated with NaCl, and extracted with EtOAc (2 × 250 mL). The organic phases were combined, washed

with brine, dried, filtered and evaporated to a minimum volume of EtOAc from which **4** was allowed to crystallize giving 1.37 g (75%) of a white solid; mp 170°C.

(2*S*, 5*R*)-5-*tert*-Butylproline ((2*S*, 5*R*)- 6). A solution of (2*S*, 5*R*)-*N*-(BOC)-5-*tert*-butylproline ((2*S*, 5*R*)-**1**, 90 mg, 0.33 mmol) in CH_2Cl_2 (10 mL) was treated with trifluoroacetic acid (0.5 mL), stirred at room temperature for 12 h, and evaporated to a clear oil: (2*S*, 5*R*)- 5-*tert*-butylproline trifluoroacetate ; ^{1}H NMR δ 1.08 (s, 9 H), 1.68 (m, 1 H), 2.06 (m, 1 H), 2.44 (m, 2 H), 3.55 (m, 1 H), 4.44 (m, 1 H), 6.51 (br m, 1 H), 10.07 (br m, 1 H), 11.77 (br s, 1 H); ^{13}C NMR δ 25, 25.9, 29.1, 32, 58.9, 71,9, 172.7. The oil was dissolved in water and passed through an ion exchange column of Dowex 1-X8 (hydroxide form) eluting with 0.01 M AcOH and provided **6** as a white solid: mp 265°C dec.; $[\alpha]^{20}_D$ –29.7° (*c* 0.26, 1 N HCl).

Hydrogenation of δ-Oxo α-[*N*-(PhF)Amino]heptanoates 4, 5a, and 5b. A 0.6 M solution of (2*S*)-6,6-dimethyl-5-oxo-2-[*N*-(PhF)amino]heptanoate (**4**, **5a**, and **5b**, 100 mol %) in 9:1 MeOH:AcOH was treated with palladium-on-carbon (10 wt %, 5 mol % of Pd), and stirred under 4 atm of hydrogen for 24 h. The mixture was filtered on celite, the catalyst was washed with MeOH, and the combined organic phase was evaporated to a residue that was analyzed directly by ^{1}H NMR in order to determine the *cis*:*trans* ratios reported in Table 1.

(2*S*, 5*R*)-5-*tert*-Butylproline Methyl Ester Trifluoroacetate ((2*S*, 5*R*)-7): a sample for comparison was obtained as a low melting solid from stirring a 0.1 M solution of (2*S*, 5*R*)-*N*-(BOC)-5-*tert*-butylproline methyl ester ((2*S*, 5*R*)-**9**) in 4:1 CH_2Cl_2 : CF_3CO_2H for 18 h at room temperature followed by evaporation of the volatiles under vacuum; $[\alpha]^{20}_D$ –18.7° (*c* 0.4, MeOH); ^{1}H NMR δ 1.09 (s, 9 H), 1.67 (m, 1 H), 2.06 (m, 1 H), 2.3 (m, 1 H), 2.46 (m, 1 H), 3.64 (m, 1 H), 3.9 (s, 3 H),

4.58 (br d, 1 H, J = 9), 6.12 (br s, 1 H), 12 (br s, 1 H); ^{13}C NMR δ 25.2, 26.1, 29, 32.1, 54.2, 58.7, 71.3, 170.9.

(2*S*, 5*R*)-5-*tert*-Butylproline *tert*-Butyl Ester ((2*S*, 5*R*)-8): ^{1}H NMR δ 0.95 (s, 9 H), 1.46 (s, 9 H), 1.47 (m, 1 H), 1.75 (m, 2 H), 2.04 (m, 1 H) 2.84 (dd, 1 H, J = 6.2, 9.8), 3.65 (dd, 1 H, J = 5.5, 9); ^{13}C NMR δ 26.5, 26.6, 27.2, 27.9, 30.8, 60.4, 69.7, 81, 174.1.

(2*S*)-5-*tert*-Butyl-Δ^{5}-dehydroproline Trifluoroacetate (11). A solution of acid **4** (320 mg, 0.74 mmol), in CH_2Cl_2 (15 mL) was treated with TFA (0.76 mL) and anisole (0.38 mL), heated at reflux for 48 h, cooled to room temperature and evaporated to a solid that was dissolved in a minimum volume of $CHCl_3$ and precipitated with excess hexane. The precipitate was recovered by the aid of a centrifuge, redissolved and retreated in the same way two additional times to provide pure **11** (201 mg, 96%). $[\alpha]^{20}_{D}$ 81° (*c* 0.1, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.39 (s, 9 H); 2.37 (m, 2 H); 2.65 (m, 2 H); 5.05 (m, 1 H); ^{13}C NMR (CD_3OD) δ 25.6, 25.7, 27.6, 35.9, 38.7, 171.5, 208.1; HRMS calcd for $C_9H_{16}NO_2$ (M^+) 170.1181, found 170.1178.

Hydride Reduction of (2*S*)-10. A solution of (2*S*)-5-*tert*-butyl-Δ^{5}-dehydroproline (**10**, 141 mg, 0.5 mmol) in THF (30 mL) was heated to a reflux, treated with solid $(H_3C)_4NHB(OAc)_3$ (197 mg, 0.75 mmol, 150 mol %) and stirred for 6 h. The solution was cooled to room temperature, treated with 1 N HCl (10 mL) and evaporated to a residue. The proton NMR spectrum of the residue exhibited a 85:15 *trans* : *cis* ratio of diastereomers. The residue was dissolved in water and purified on 11 g of Dowex 1-X8 ion exchange resin (hydroxide form, 20-50 mesh) using a gradient of 0-10 % acetic acid in water as eluant. Evaporation of the collected ninhydrin positive fractions gave 76 mg (96 %) of solid (2*S*, 5*RS*)-5-*tert*-butylprolines (**6**) possessing an 87:13 ratio of diastereomers.

(2*S*, 5*S*)-*N*-BOC-5-*tert*-Butylproline Methyl Ester ((2*S*, 5*S*)-9). A 0 °C solution of gaseous HCl (1g, 27 mmol) in 161 mL of MeOH was treated with a solution of 5-*tert*-butylproline (490 mg, 3.1 mmol, 5*R*:5*S* = 8.6:1) in MeOH (25 mL). The mixture was allowed to warm to rt, stirred for 16 h, and evaporated to a yellow solid containing an 8.6:1 mixture of (2*S*, 5*R*)- and (2*S*, 5*S*)-**7** (522 mg, 76%). The spectral characteristics of (2*S*, 5*S*)-5-*tert*-butylproline methyl ester hydrochloride ((2*S*, 5*S*)-**7**) are as follows: ^{1}H NMR ($CHCl_3$ acidified with TFA) δ 1.07 (s, 9 H), 1.9 - 2.2 (m, 3 H), 2.5 (m, 1 H), 3.65 (m, 1 H), 3.79 (s, 3 H), 4.51 (t, 1 H, *J* = 7.5), 9.0 (br s, 2 H).; ^{13}C NMR (CD_3OD) δ 26.5, 27, 29.9, 33.7, 54.1, 61.4, 71.9, 170.2; HRMS calcd for $C_{10}H_{20}NO_2$ $[MH]^+$, 186.1494 found 186.1501.

A solution of **7** (100 mg, 0.45 mmol) in CH_3CN (3 mL) was then treated with solid K_2CO_3 (124 mg, 0.90 mmol, 200 % mol), stirred for 30 min, and treated with a solution of di-*tert*-butyldicarbonate (147 mg, 68 mmol, 150 % mol) in CH_3CN (1 mL). After stirring for 24 h at rt, a second portion of di-*tert*-butyldicarbonate (147 mg, 68 mmol, 150 % mol) in CH_3CN (1 mL) was added and the solution was stirred 24 h, evaporated to a yellow solid, and chromatographed on silica gel using a gradient of 0-7% Et_2O in $CHCl_3$ as eluant. (2*S*, 5*S*)-*N*-BOC-5-*tert*-butylproline methyl ester ((2*S*, 5*S*)-**9**, 99 mg, 77 %) was first to elute: $[\alpha]^{20}_D$ –43.6° (*c* 0.94, CH_3OH); ^{1}H NMR δ 0.9 (s, 9 H), 1.51 (s, 9 H), 1.8 - 2.4 (m, 4 H), 3.74 (s, 3 H), 3.97 (d, 0.3 H, *J* = 8.4), 4.06 (d, 0.7 H, *J* = 8.5), 4.4 (d, 1 H, *J* = 9.5); ^{13}C NMR δ 25.2 (26.1), (27.3) 27.4, 27.5, (29) 30, (36.3) 37, (52.2) 52.4, (61.3) 61.7, (67.5) 67.6, (84.6) 84.7, (146.8) 149.9, (172.5) 172.8; HRMS calcd for $C_{18}H_{11}NO_3$ $[M - O\textit{t}\text{-Bu}]^+$, 212.1287 found 212.1275. Next to elute was (2*S*, 5*R*)-*N*-BOC-5-*tert*-butylproline methyl ester (2*S*, 5*R*)-**9** (11 mg, 9 %).

Epimerization of (2*S*, 5*R*)-*N*-(BOC)-5-*tert*-Butylproline Methyl Ester ((2*S*, 5*R*)-9). A solution of methyl ester (2*S*, 5*R*)-**9** (143 mg, 0.5 mmol) in

10 mL of THF at –78 °C was treated with 1.2 mL of $KN(SiMe_3)_2$ (120 mol %, 0.5 M in toluene), heated to 50 °C, and stirred for 16 h. Methanol (6 mL) was added, and the mixture was partitioned between 1 M NaH_2PO_4 (15 mL) and EtOAc (15 mL). The aqueous phase was extracted with EtOAc (3 × 15 mL), and the combined organic phase was washed with brine, dried, evaporated and purified by chromatography using 0-25% EtOAc in hexanes. First to elute was (2*S*, 5*R*)-**9** (84 mg, 59%) followed by (2*R*, 5*R*)-**9** (54 mg, 38%): $[\alpha]^{20}_D$ 21.6° (*c* 0.25, CH_3OH). Last to elute was a mix of acids **1** (4 mg, 3 %).

(2*S*, 5*S*)-5-*tert*-Butyl-*N*-(BOC)proline ((2*S*, 5*S*)-1). A solution of methyl ester (2*S*, 5*S*)-**9** (50 mg, 0.18 mmol) in 1 mL of dioxane was treated with 1 mL of 1 N NaOH, and stirred for 48 h at room temperature. The reaction mixture was extracted with water (5 × 1 mL), the aqueous phases were combined, acidified with citric acid to pH 2, saturated with NaCl and extracted with EtOAc (3 × 2 mL). The organic phases were combined, dried, filtered and evaporated to give 39 mg (82 %) of (2*S*, 5*S*)-**1**: $[\alpha]^{20}_D$ –16.3° (*c* 0.7, MeOH); ^{1}H NMR δ 0.9 (s, 9 H), 1.41 (s, 5.4 H), [1.46 (s, 3.6 H)], 1.90 (m, 2 H), 2.05 (m, 1 H), 2.30 (m, 1 H), [3.85 (d, 0.4 H, *J* = 8.5)] 3.98 (d, 0.6 H, *J* = 8.5), 4.28 (d, 0.6 H, *J* = 9.3) [4.34 (d, 0.4 H, *J* = 9.4)]; ^{13}C NMR δ 25.0 (26.1), 27.5, 28.1 (28.3), 29.1, 36.5 (36.8), 61.0, 66.2, 80.2 (80.6), 155.3, 179.9.

Enantiomeric Purity of (2*S*, 5*R*)-*N*-(BOC)-5-*tert*-Butylproline ((2*S*, 5*R*)-1). A room temperature solution of (2*S*, 5*R*)-*N*-(BOC)-5-*tert*-butylproline ((2*S*, 5*R*)-**1**, 20 mg, 0.07 mmol) and either (*R*)- or (*S*)-α-methylbenzylamine (24 μL, 0.19 mmol) in 1 mL of acetonitrile was treated with benzotriazol-1-yl-1,1,3,3-tetramethyl uronium tetrafluoroborate (26 mg, 0.08 mmol) and stirred 2 h when TLC showed complete disappearance of the starting acid. Brine (2 mL) was added to the reaction mixture that was then extracted with EtOAc (2 × 3 mL). The combined organic

phase was extracted with 2 N HCl (2 × 2 mL) and $NaHCO_3$ (2 × 2 mL), washed with H_2O (2 × 2 mL) and brine, dried, filtered and evaporated to a residue that was directly examined by ^1H NMR. When (*S*)-α-methylbenzylamine of 99% diastereomeric purity was used, examination of the *tert*-butyl singlets in the ^1H NMR in $CDCl_3$ demonstrated (*S*)-**10** to be of 99% diastereomeric purity. Hence, *cis*-**1** is presumed to be of >99% enantiomeric purity.

(1'*S*, 2*S*, 5*R*)-*N*-(BOC)-5-*tert*-Butylproline *N*'-α-Methylbenzylamide (*S*)-10: ^1H NMR δ 0.93 (s, 9 H), 1.34 (s, 9 H), 1.47 (d, 3 H, *J* = 6.9), 1.78 (m, 2 H), 2.2 (m, 2 H), 3.9 (dd, 1 H, *J* = 3, 7.7), 4.3 (t, 1 H, *J* = 8.8), 5.1 (quintet, 1 H, *J* = 6.9), 7.3 (m, 5 H).

(1'*R*, 2*S*, 5*R*)-*N*-(BOC)-5-*tert*-Butylproline *N*'-α-Methylbenzylamide (*R*)-10: ^1H NMR δ 0.74 (s, 9 H), 1.47 (s, 9 H), 1.51 (d, 3 H, *J* = 7), 1.84 (m, 2 H), 2.1 (m, 1 H), 2.25 (m, 1 H), 3.83 (m, 1 H), 4.34 (t, 1 H, *J* = 8.7), 5.1 (quintet, 1 H, *J* = 7), 7.3 (m, 5 H).

Enantiomeric purity of (2*S*, 5*S*)-*N*-(BOC)-5-*tert*-butylproline ((2*S*, 5*S*)-1). (2*S*, 5*S*)-*N*-(BOC)-5-*tert*-Butylproline ((2*S*, 5*S*)-**7**, 22 mg, 0.06 mmol) was transformed into its respective (1'*R*)- and (1'*S*)-α-benzylamides **10** via the route described above for (2*S*, 5*R*)-**1**. Amides **10** were then dissolved in CH_2Cl_2 (0.6 mL), treated with 45 μL (0.6 mmol, 1000 mol%) of trifluoroacetic acid and stirred for 24 h. Evaporation of the volatiles gave amides **12** that were analyzed, without further purification, by ^1H NMR in CD_3OD via integration of the *tert*-butyl singlets.

(1'*S*, 2*S*, 5*S*)-5-*tert*-Butylproline *N*'-α-Methylbenzylamide Trifluoroacetate (*S*)-12: ^1H NMR (CD_3OD) δ 1,02 (s, 9 H), 1.47 (d, 3 H, *J* = 7,0), 1.88 (m, 2 H), 2.14 (m, 2 H), 2,49 (m, 1 H), 3,61 (m, 1 H), 4,07 (m, 1 H) 5,03 (m, 1 H).

(1'*R*, 2*S*, 5*S*)-5-*tert*-Butylproline *N*'-α-Methylbenzylamide Trifluoroacetate (*R*)-12: ^{1}H NMR (CD_3OD) δ 1,03 (s, 9 H), 1.49 (d, 3 H, *J* = 7,0), 1.9 (m, 2 H), 2.15 (m, 2 H), 2,42 (m, 1 H), 3,58 (m, 1 H), 4,14 (m, 1 H) 5,03 (m, 1 H).

(2*S*)-6,6-Dimethyl-5-oxo-2-[*N*-(PhF)amino]heptanoate *N*'-Methylamide ((2*S*)-14). A suspension of (2*S*)-6,6-dimethyl-5-oxo-2-[*N*-(PhF)amino]heptanoate ((2*S*)-**4**, 1.37 g, 3.2 mmol) and methylamine hydrochloride (238 mg, 3.52 mmol, 110 mol %) in acetonitrile (100 mL) at room temperature was treated with triethylamine (1.42 mL, 10.24 mmol, 320 % mol) followed by TBTU (1.13 g, 3.52 mmol, 110% mol). The mixture was stirred for 48 h. The volatiles were removed under vacuum leaving a residue that was purified by chromatography on silica gel using an eluant of 20-50% EtOAc in hexane. Evaporation of the collected fractions gave 1.094 g (78%) of (2*S*)-**14** as a white solid: $[\alpha]^{20}_D$ –28.7° (*c* 2.0, MeOH);[26] mp 156-158°C; ^{1}H NMR δ 1.05 (s, 9 H), 1.64 (q, 2 H, *J* = 6.7), 2.33 (t, 1H, *J* = 6.2), 2.43 (t, 2 H, *J* = 6.4), 2.49 (d, 3 H, *J* = 5), 3.2 (s, 1 H), 6.2 (d, 1 H, *J* = 4.7); ^{13}C NMR δ 25.6, 26.4, 29, 33.1, 43.9, 56.6, 72.9, 175.5, 216.4; HRMS Calcd for $C_{29}H_{33}N_2O_2$ (MH^+) 441.2542, found 441.2552.

(2*S*)-5-*tert*-Butyl-Δ^5-dehydroproline *N*'-Methylamide Trifluoroacetate ((2*S*)-15). A solution of amide (2*S*)-**14** (100 mg, 0.23 mmol), in CH_2Cl_2 (11 mL) was treated with TFA (0.23 mL) and anisole (0.1 mL, 0.92 mmol), heated at reflux for 72 h, cooled to room temperature and extracted with water (3 × 10 mL). The aqueous extractions were then evaporated to furnish (2*S*)-**15** as an oil (62 mg, 92%): $[\alpha]^{20}_D$ 86.6° (*c* 0.6, CH_2Cl_2); ^{1}H NMR δ 1.38 (s, 9 H); 2.33 (m, 1 H); 2.68 (m, 1 H); 2.81 (d, 3 H, *J* = 4.7), 3.04 (m, 1 H), 3.27 (m, 1 H), 5.13 (m, 1 H), 8.19 (m, 1 H); ^{13}C NMR (CD_3OD) δ 24.1, 26.5, 27.6, 35.4, 37.5, 69.1, 167.5, 202.3; HRMS calcd for $C_{10}H_{19}N_2O$ (MH^+) 183.1497, found 183.1503.

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Supporting Information Available: The ^{1}H and ^{13}C NMR spectra of **1-16** (37 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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Deuxième partie:
L'utilisation des effets stériques afin de contrôler la conformation de peptides modèles.

Chapitre 3

Ce chapitre est constitué d'une brève introduction de l'article 3 qui est suivie de la version intégrale de l'article 3.

Introduction

L'obtention de la méthodologie de synthèse des quatres diastéréoisomères de la 5-*tert*-butylproline m'a permis de synthésiser les peptides modèles décrits dans l'article 3. Par la suite j'ai analysé les conséquences conformationnelles de l'ajout d'interactions stériques en position 5 du cycle pyrrolidine de proline en utilisant la résonance magnétique nucléaire (RMN), la spectroscopie infra-rouge (IR) ainsi que la mécanique moléculaire tel que discuté dans l'article 3.

Afin d'étendre l'étude de l'influence des effets stériques des substituants à la position 2 du cycle de proline, j'ai ensuite utilisé la spectroscopie infrarouge pour étudier les 3,3-diméthylprolines. Les composés **2**, **3**, **5** et **6** ont été obtenus par la méthodologie de synthèse développée par Raman Sharma tel que mentionné à la page 91. Cependant, j'ai dû compléter la synthèse des composés modèles **1** et **4** de l'article 4. Ces résultats préliminaires sont décrits dans le deuxième article de la deuxième partie. Ils m'ont conduit à effectuer une analyse conformationnelle complète utilisant la spectroscopie RMN, IR ainsi que la mécanique moléculaire (article 5). La préparation de cristaux qui a permis l'obtention de la structure tridimensionnelle du (2*S*,4*S*)-*N*-acétyl-3,3-diméthyl-4-hydroxyproline *N*'-méthylamide par la diffraction des rayons X a été réalisée par Raman Sharma. Par ailleurs, j'ai affectué l'évaluation de l'énergie d'activation de l'isomérisation composés en utilisant un programme de minimisation écrit en langage fortran par le professeur Stephen W. Michnick. Enfin ces études m'ont

permises d'étendre notre compréhension sur la possibilité d'utiliser les effets stériques en position 2 de la proline afin de contrôler la géométrie de la liaison amide *N*-teminale de la proline dans l'état de transition de l'isomérisation.

L'influence d'un substituant encombré en position 5 de la proline sur l'équilibre des isomères d'amide de prolylpeptides a été explorée via la synthèse et l'analyse de *N*-(acétyl)-proline *N'*-méthylamide (**1**) ainsi que de ses diastéréomères *cis*- et *trans-tert*-butylproline **2** et **3.** Les populations relatives des isomères d'amide *cis* et *trans* ainsi que l'énergie d'activation de l'isomérisation des amides **1-3** dans le D_2O ont été déterminées par RMN en utilisant des expériences de transfert de magnétisation et de coalescence. Les populations relatives des amides en position *C* terminale libre de pont hydrogène ainsi que celles formant un pont hydrogène dans un repliement γ ont aussi été estimées par l'intégration de la bande d'élongation N-H dans le spectre infrarouge des composés **1-3** dans le $CHCl_3$ et le CCl_4. Dans les prolylpeptides, le substituant *tert*-butyle en position 5 a démontré avoir des effets profonds sur l'équilibre des isomères d'amide, sur l'énergie d'activation de l'isomérisation de l'amide et sur la stabilité du repliement γ. Des interactions stériques entre les substituants en position 5 et le groupe *N*-acétyle défavorisent l'amide *trans* et augmentent la population d'isomère *cis*: 25% pour **1**, 48% pour **2** et 66% pour **3**. Dans le cas de l'isomérisation de l'amide du *cis*-5-*tert*-butylproline **2**, l'énergie d'activation observée est de 3.9 kcal/mol plus basse que celle de **1**. D'autre part l'isomérisation de l'amide du *cis*-5-*tert*-butylproline **3** est similaire à celle de **1**. Dans le spectre infrarouge de **3** dans le $CHCl_3$, une seule bande d'élongation de l'amide est observée à 3454 cm^{-1} indiquant ainsi que l'amide est libre de tout pont hydrogène intramoléculaire. Ainsi l'amide du *trans*-5-*tert*-butylproline **3** n'adopte pas de conformation en repliement γ, qui est une des conformations favorisées pour **1** et **2** dans le $CHCl_3$. Des cartes dans lesquelles sont représentées

l'énergie conformationnelle à des intervalles 30° sur les angles dièdres ψ et ω prédisent qualitativement et montrent clairement tous les effets observés des substituents 5-*tert*-butyle sur l'isomérie des *N*-(acétyl)-proline *N*'-méthylamides. Les résultats de cette étude suggèrent l'utilisation des 5-*tert*-butylprolines afin de préparer des succédanés d'isomère d'amide *cis* et d'amides pyramidalisés pour étudier la conformation du résidu prolyle à l'intérieur de peptides biologiquement actifs.

Publication 3:

Eric Beausoleil and William D. Lubell

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Steric Effects on the Amide Isomer Equilibrium of Prolyl Peptides. Synthesis and Conformational Analysis of *N*-Acetyl-5-*tert*-Butylproline *N*'-Methylamides.

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Abstract

The influence of a bulky 5-position substituent on the amide isomer equilibrium *N*-terminal to proline has been explored via the synthesis and analysis of *N*-(acetyl)proline *N*'-methylamide (**1**) and its respective *cis*- and *trans*-5-*tert*-butylproline amide diastereomers **2** and **3**. The relative populations of the amide *cis*- and *trans*-isomers as well as the energy barriers for amide isomerization of **1**-**3** in D_2O were ascertained using NMR with coalescence and magnetization transfer experiments. The

relative populations of free *C*-terminal amide and hydrogen-bonded amide in the γ-turn conformation were also estimated by integrating the N–H stretch absorbances in the FT-IR spectra of **1**-**3** in $CHCl_3$ and CCl_4. In the prolyl peptides, the 5-*tert*-butyl substituent was found to exhibit profound effects on the amide isomer equilibrium, on the energy barrier for amide isomerization, and on the stability of the γ-turn conformation. Steric interactions between the 5-position substituent and the *N*-acetyl group disfavor the amide *trans*- and augment the *cis*-isomer population: 25% in **1**, 48% in **2**, and 66% in **3**. In the case of *cis*-5-*tert*-butylproline **2**, the energy barrier for amide isomerization is observed to be 3.9 kcal/mole lower than that of **1**. On the other hand, the amide isomerization barrier for *trans*-5-*tert*-butylproline **3** is similar to that for **1**. Only a single amide N–H stretch band is observed at 3454 cm^{-1} in the FT-IR spectrum of **3** in $CHCl_3$ and indicates that the NH group is free of intramolecular hydrogen bonding. Hence, *trans*-5-*tert*-butylproline amide **3** does not adopt a seven member γ-turn conformation, which is a favored conformer for **1** and **2** in $CHCl_3$. Maps, in which the ψ- and ω-dihedral angles are plotted at 30° intervals against the calculated energy of the local minimum conformation, predict qualitatively and display clearly all of the observed effects of the 5-*tert*-butyl substituent on the amide isomer in the *N*-(acetyl)proline *N'*-methylamides. The results of this study suggest the use of 5-*tert*-butylprolines to prepare both X-Pro *cis*-amide isomers and twisted amide surrogates for examining prolyl residue conformations in bioactive peptides.

Introduction

The rational design of therapeutics based on peptide lead structures requires detailed knowledge of their conformational requirements for biological activity. Since energetically similar amide *cis*- and *trans*-isomers *N*-terminal to proline may create multiple low energy conformers (Figure 1), prolyl residues can complicate the characterization of peptide structures using techniques such as X-ray diffraction and

NMR spectroscopy.[1] Conformationally rigid surrogates of the *cis*- and the *trans*-isomers of X-Pro amide bonds have thus emerged as important tools for studying the relationship between isomer geometry and peptide bioactivity.[2-10] Since conformational changes about proline can influence the stability, folding, denaturation, and recognition of prolyl peptides,[11] analogues that restrict prolyl geometry can also serve as sensitive probes for examining protein biology at the molecular level.

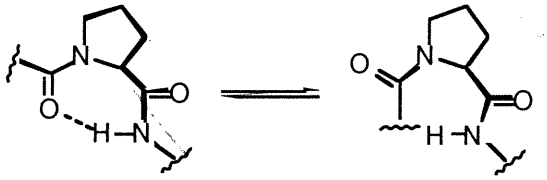


Figure 1. Amide Isomer Equilibrium *N*-Terminal to Proline Derivatives
Figure 2. Representative Examples of Conformationally Rigid Amide Surrogates[2-9]

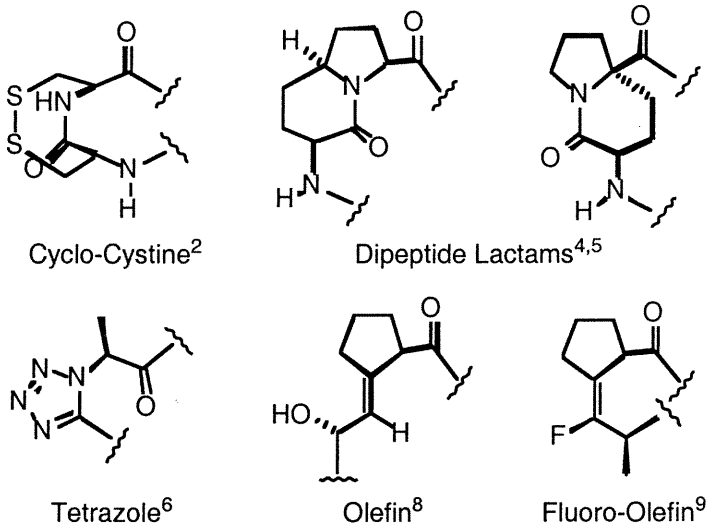


Figure 2. Representative Examples of Conformationally Rigid Amide Surrogates[2-9]

Attempts to understand the relationship between X-Pro amide isomer geometry and protein bioactivity have led to many strategies for preparing conformationally rigid isosteres of X-Pro amide bonds (Figure 2).[2-10] Efforts to mimic X-Pro dipeptide

residues in peptides have focused on the geometry of the back-bone, the hydrogen acceptor properties of the amide carbonyl, as well as the shape, function and geometry of the amino acid side-chains. For example, cyclo-cystine[2] and cyclo-lanthione[3] derivatives have been examined as amide *cis*-isomer mimics that replicate the back-bone geometry of X-Pro residues.[2a] Dipeptide lactams in which either the 2- or the 5-position carbons of the pyrrolidine ring is tethered to the α-carbon of the *N*-terminal amino acid residue have been used to mimic the back-bone geometry of type VI and II' β-turn conformations.[4,5] In addition, rigid sp^2 hybridized amide isomer surrogates, that forfeit the potential hydrogen acceptor properties of the carbonyl group, have been generated using heterocycles such as tetrazoles[6] and pyrroles,[7] as well as olefin[8] and fluoro-olefin[9] amide bond replacements.

Rigid amide isomer analogues with biological activities similar to the parent peptide can support the importance of a particular conformation for bioactivity. For example, the replacement of the Phe-Pro residues in a somatostatin analogue[12] with *cyclo*-cystine,[2a] dipeptide lactams[4d] and tetrazole derivatives,[6c] all have given potent peptide mimetics that implicate the significance of a type VI β-turn conformation for bioactivity. Inhibitors of proline specific enzymes, such as peptidyl prolyl isomerase (PPIase)[8] and proline-specific protease,[9b] have also been designed using olefins and fluoro-olefins as non-isomerizable, non-hydrolizable amide isosteres. Since analogues without biological activity may arise both from mimicking an inactive conformation and from using a surrogate having structural features that interfere with receptor recognition, various innovative approaches to generate constrained X-Pro amide isomers are needed in order to precisely probe prolyl peptide geometry.

The employment of alkylprolines and their steric interactions to control amide isomer geometry is a particularly attractive method for preparing conformationally rigid amide isomers because libraries of X-Pro derivatives may be prepared by coupling

different amino acid residues to the *N*-terminal of the alkylproline. Pioneering studies on the influence of methyl substituents on the amide isomer equilibrium of *N*-(acetyl)proline *N'*-methylamides have provided fundamental understanding of the steric effects of alkylprolines as well as an effective means for preparing rigid X-Pro amide *trans*-isomers.[13-17] For example, only the *trans*-isomer (>98%) was observed in the ^{13}C NMR spectrum of *N*-acetyl-2-methylproline *N'*-methylamide.[13] This result motivated synthesis of 2,4-methanoproline,[14] a natural achiral proline analogue,[15] that was also shown to furnish X-Pro amide *trans*-isomer (>95% by ^{13}C NMR) in model peptides.

The steric interactions of methylprolines have also been employed to augment the population of the X-Pro amide *cis*-isomer.[16,17] A single methyl substituent at the proline 5-position was shown to have a subtle influence on the X-Pro amide isomer equilibrium of *N*-(acetyl)proline *N'*-methylamide.[16] This effect was contingent on the relative stereochemistry of the 5-methylproline. In the case of the *trans*-diastereomer, the steric interactions of the 5-methyl group destabilized the amide *trans*-isomer in *N*-acetyl-5-methylproline *N'*-methylamide without affecting the isomerization energy barrier. A 5% increase in the amide *cis*-isomer population was observed in water for the 5-methyl *trans*-diastereomer. In the case of the *cis*-diastereomer, the presence of the 5-methyl group had no effect on the ratio of amide isomers. However, the amide isomerization energy barrier was observed to be 1.2 kcal/mol lower for *N*-acetyl-5-methylproline *N'*-methylamide *cis*-diastereomer in water.[16] The combined effect of two methyl substituents at the proline 5-position was recently studied in *N*-BOC-phenylalanyl-5,5-dimethylproline methyl ester which was reported to exist as a 9:1 mix of amide *cis*:*trans* isomers.[17] The results so far obtained with methyl substituents,[16,17] all point to the use of alkylprolines with bulkier 5-position substituents in order to prepare X-Pro amide analogues with greater *cis*-isomer

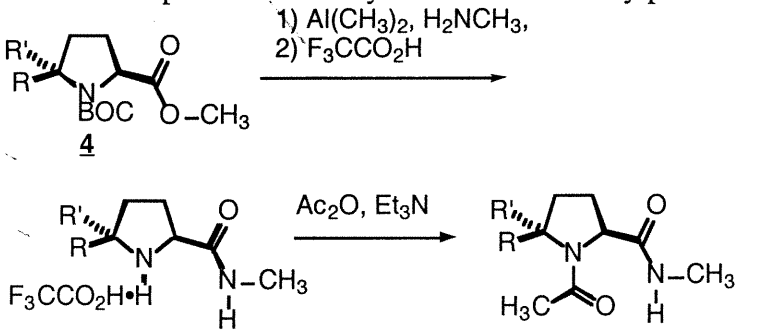
populations. In addition, since the lower energy barrier for amide isomerization of the 5-methylproline *cis*-diastereomer arises via destabilization of both the *cis*- and *trans*-isomer conformations, larger 5-position substituents may give "twisted" amide mimetics by perturbing the planar amide sp^2 hybridized geometry.[18]

We have recently developed efficient methodology for synthesizing all four stereoisomers of 5-*tert*-butylprolines from glutamic acid as an inexpensive chiral educt.[19] We are presently using these 5-alkylprolines to alter the *cis-trans* isomer equilibrium of the amide bond *N*-terminal to prolyl residues in order to study the importance of X-Pro *cis*-isomer populations in the recognition and reactivity of bioactive peptides.[20] In this manuscript, we describe the synthesis and conformational analysis of *N*-acetyl-5-*tert*-butylproline *N*'-methylamides. These simple prolyl peptides are ideal model systems for illustrating the influence of the bulky 5-*tert*-butyl substituent on the amide isomer equilibrium *N*-terminal to proline. In the model peptides, the 5-*tert*-butyl substituent exhibits profound effects on both the amide isomer equilibrium and the energy barrier for amide isomerization. Moreover, we observe that the 5-*tert*-butyl group can alter the stability of the γ-turn conformation. In addition, the effects of the 5-*tert*-butyl substituent on the amide isomer in *N*-(acetyl)proline *N*'-methylamides were predicted and displayed qualitatively by maps portraying energy vs conformation as the ψ- and ω-dihedral angles are rotated about 30° intervals. In sum, our results indicate that 5-*tert*-butylprolines may be used to generate prolyl peptide libraries possessing X-Pro amide *cis*-isomers that mimic type VI β-turn as well as twisted amide conformations.

Results and Discussion

Synthesis of *N*-(Acetyl)proline *N*'-Methylamides

5-*tert*-Butylprolines were synthesized from glutamic acid as described.[19] Acylation of the dianion of *N*-(9-(9-phenylfluorenyl))glutamate γ-methyl ester with pivaloyl chloride, hydrolysis of the methyl ester and decarboxylation gave 6,6-dimethyl-5-oxo-2-[*N*-(9-(9-phenylfluorenyl))amino]heptanoates that were diastereoselectively reduced to the *cis*- and *trans*-5-*tert*-butylprolines. The *cis*- and *trans*-diastereomers were purified as methyl *N*-BOC-5-*tert*-butylprolinates **4**.

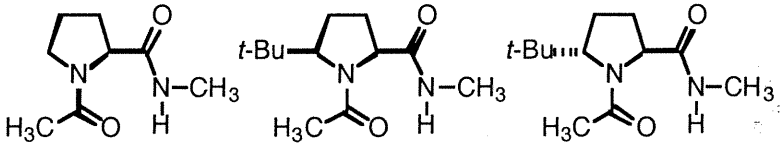


Scheme 1. Synthesis of *N*-(Acetyl)proline *N*'-Methylamides 2 and 3

N-Acetyl-*cis*-5-*tert*-butylproline *N*'-methylamide (**2**) and *N*-acetyl-*trans*-5-*tert*-butylproline *N*'-methylamide (**3**), both were synthesized from their respective *N*-BOC-5-*tert*-butylproline methyl esters **4** (Scheme 1). 5-*tert*-Butylproline *N*'-methylamides **5** were initially synthesized in 70% and 81% respective yield by treatment of methyl ester **4** in CH_2Cl_2 with a solution of methylamine hydrochloride and trimethyl aluminum in benzene,[21] followed by solvolysis of the BOC group with trifluoroacetic acid. Subsequent *N*-acetylation of *N*'-methylamides **5** with acetic anhydride and triethyl amine provided the respective *N*-(acetyl)proline *N*'-methylamides **2** and **3** in 88% and 96% yield after purification by chromatography. Amide **1** was obtained from proline methyl ester hydrochloride via treatment with a premixed solution of $Al(CH_3)_3$ and

H_2NCH_3•HCl in benzene as described for **4** followed by *N*-acetylation as described for **5** in the experimental section.[22]

NMR Analysis of *N*-(Acetyl)proline *N'*-Methylamides 1-3



1 2 3

Figure 3. *N*-(Acetyl)Proline *N'*-Methylamides 1-3 (amide *trans*-isomers shown)

Table 1.
Selected Carbon Chemical Shift Values of Prolyl Amides 1-3

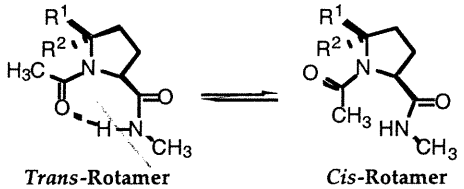
Prolyl Amide	C^{α}		C^{β}		C^{γ}		C^{δ}	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
1	62.3	60.8	31.9	30.2	22.8	24.5	47.5	49.1
2	69.9	67.6	36	35.9	28.3	30.7	62.6	63.6
3	69.4	66.7	31.6	29.5	25.4	26.6	62.4	63.1

Table 1. Selected Carbon Chemical Shift Values of Prolyl Amides 1-3

Water (H_2O and D_2O) was chosen as the solvent for measuring the populations of the amide *cis*- and *trans*-isomers as well as the energy barrier for amide isomerization. The rate of amide isomerization *N*-terminal to proline is known to be slower in water than in polar non-protic and in nonpolar solvents.[23] Since the conformational distribution of *N*-(acetyl)proline *N'*-methylamides has been shown to be solvent-dependent,[24] the effects of solvent on the isomer population in **1-3** are presently being examined and will be reported in due time.

The assignments of the isomer geometry were made based on the chemical shift values for the signals of the α- and δ-carbons in D_2O (Table 1).[25] In the ^{13}C NMR spectra, the α-carbon signal of the *trans*-isomer appears upfield to that of the *cis*-isomer. The δ-carbon signal of the *trans*-isomer appears downfield from that of the *cis*-

isomer. Furthermore, the γ-carbon of the *cis*-isomer appears upfield to that of the *trans*-isomer and the β-carbon of the *cis*-isomer appears downfield from that of the *trans*-isomer. The populations of the amide isomers were measured in the ^{1}H NMR spectra by integration of the isomeric α- and *N*-acetyl proton signals in **1** as well as the δ- and *tert*-butyl proton signals in **2** and **3**. The ratios of amide isomers in **1**-**3** are listed as the percent of *cis*-isomer in Table 2.



Trans-Rotamer ⟷ *Cis*-Rotamer

R^1	R^2	ΔH (Calcd) (kcal/mole)[a,e]	*cis*-rotamer % Calcd[a]	*cis*-rotamer % ± 3%[b]	T_c (°C)[c]	$\Delta G^{\ddagger}$ (kcal/mole)[c]	ΔG (*cis* – *trans*) (kcal/mole)[b,e]
H	H	1.02	15	27	>85	20.4	0.57
Me	H	-	-	25	82	19.0	0.65
t-Bu	H	0.23	46	49	45	16.5	0.03
H	Me	-	-	30	>85	-	0.50
H	*t*-Bu	–0.72	77	66	>85	20.2	–0.38

[a]Calculated with Macromodel 3.5x and the AMBER force field. [b]Determined by 300 MHz NM D_2O at 25°C. [c]Determined by 300 MHz NMR in D_2O. [d]Values for 5-Me examples are from ref. [e]Negative values indicate a predominant population of *cis*-rotamer.

Table 2. Amide Isomer Equilibrium of Prolyl *N*-Acetyl *N*'-Methyl Amides in D_2O[d]

The energy barriers (?G‡) for amide isomerization in **1**-**3** were determined by two different NMR techniques. In the case of *cis*-diastereomer **2**, a series of ^{1}H NMR spectra were recorded at increasing temperatures until the resonances for the two isomer populations were observed to coalesce at 45°C. The energy barrier for amide isomerization in **2** was calculated to be 16.5 kcal/mol in D_2O.[26] Insufficient exchange broadening below the boiling point of water prevented us from obtaining the coalescence temperatures for *N*-(acetyl)proline *N*'-methyl amide (**1**) and 5-*tert*-

butylproline *trans*-diastereomer **3**. Instead, a series of magnetization (saturation) transfer experiments were performed in order to ascertain the energy barriers for amide isomerization in **1** and **3**. In these experiments, the signal of the α-carbon of the major amide isomer was irradiated in H_2O and the rate for magnetization transfer to the minor isomer α-carbon signal was measured over a temperature range of 60-85°C. The barriers for amide isomerization in **1** and **3** were respectively calculated to be 20.4 and 20.2 ±0.2 kcal/mole.[27] The results of these studies are presented in Table 2 in which data for *N*-acetyl-5-methylproline *N*'-methyl amides in water has also been listed for comparison.[16]

FT-IR Analysis of *N*-(Acetyl)proline *N*'-Methylamides 1-3

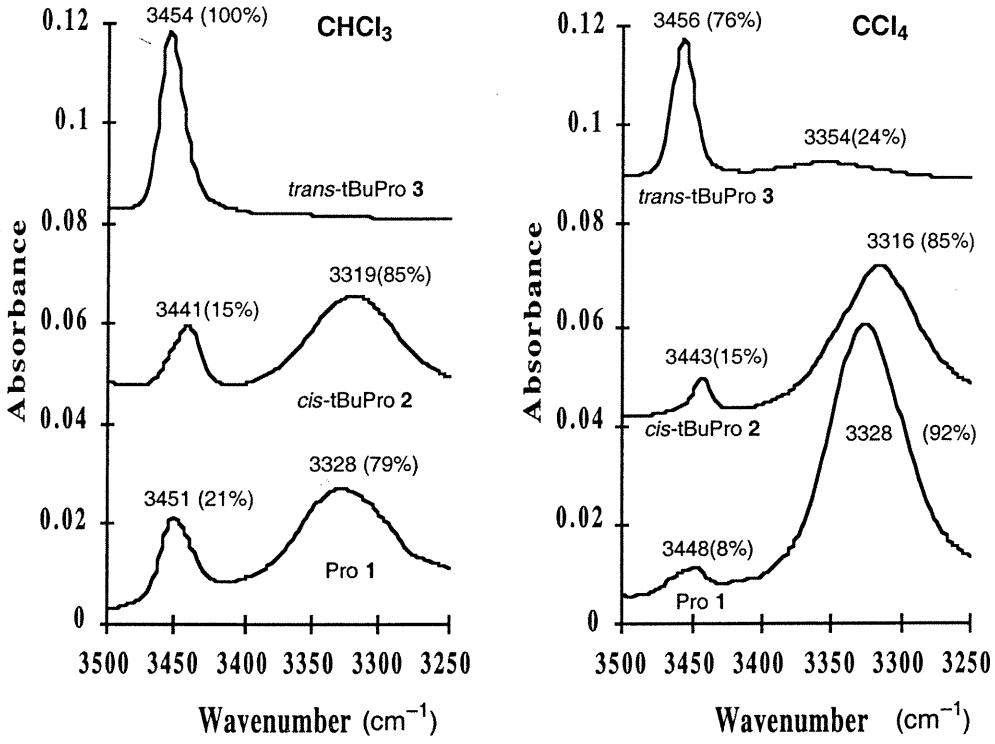


Figure 4. FT-IR spectra of the N–H Stretch Region of Prolyl *N*-Acetyl *N*'-Methylamides 1-3 in $CHCl_3$ and CCl_4

The FT-IR spectra of prolyl amides **1**-**3** were measured at 25°C at 1 ∞ 10^{-4} M concentrations in $CDCl_3$ and in CCl_4 in order to examine the presence of a γ-turn conformation in which a seven-membered-ring intramolecular hydrogen bond exists between the *C*-terminal amide NH and the carbonyl of the *N*-terminal amide *trans*-isomer.[28,29] The solvents $CDCl_3$ and CCl_4 were chosen in order to directly compare spectra for **2** and **3** with the reported spectra of *N*-(acetyl)proline *N'*-methylamide (**1**).[13] The presence of the free N–H stretch band was observed at 3441-3454 cm^{-1} for the non-hydrogen bonded amide in all cases (Figure 4). In prolyl amide **1** and *cis*-diastereomer **2** in $CHCl_3$, an additional N–H stretch band was respectively observed at 3328 and 3319 cm^{-1} as the result of hydrogen bonding in the γ-turn conformation.[28] A predominance of hydrogen-bonded amide in **1** and **2** is indicated by the ratios of the relative areas of the lower vs higher energy absorbance for the N–H stretches which are

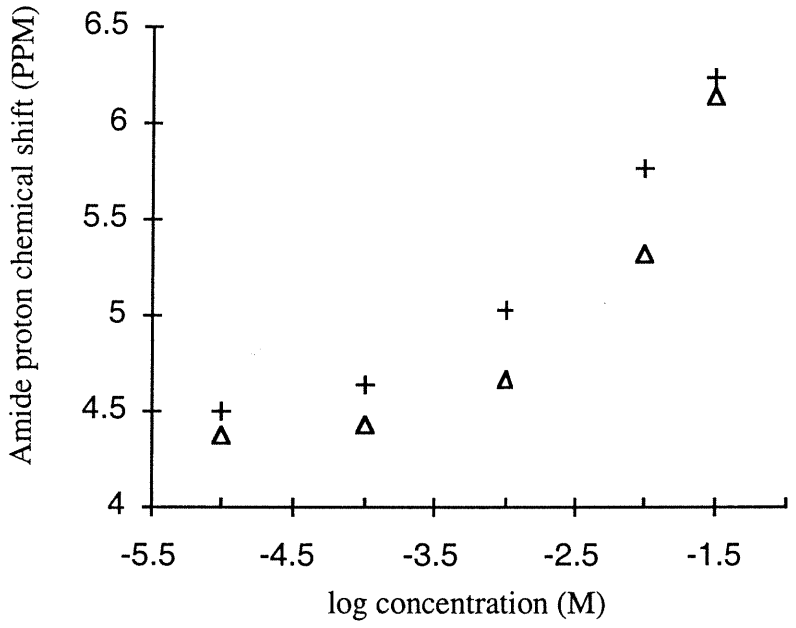


Figure 5. Amide proton NMR chemical shifts in CCl_4 at room temperature, as a function of the logarithm of the concentration of 3: (Δ), NH amide *cis*-isomer; (+), NH amide *trans*-isomer.

79:21 in **1** and 85:15 in **2**. On the other hand, no second lower energy N–H stretch band was observed for *trans*-diastereomer **3**. This result indicates the absence of a γ-turn conformation for **3** in $CHCl_3$.

In CCl_4, the area of the lower energy N–H stretch band increased in **1** and remained the same in **2**. When the FT-IR spectrum of prolyl amide **3** was measured at $1 \infty 10^{-4}$ M concentration in CC14 a second broader N–H stretch band was observed at 3354 cm^{-1}. The relative areas of the lower energy absorbance and that of higher energy for **3** in CCl_4 was 1:3. Since the N–H stretch for **3** at 3354 cm^{-1} in CCl_4 could be due to intermolecular instead of intramolecular hydrogen bonding, the effect of concentration on the chemical shift of the NH signal of **3** was measured by ^1H NMR (Figure 5). The ?δ/?concentration for the NH signal was still noticeable at $1 \infty 10^{-4}$ M and indicated intermolecular hydrogen bonding. Attempts to record the FT-IR spectrum of **3** at lower concentration were, however, unsuccessful at $1 \infty 10^{-5}$ M. The predominance of the N–H stretch at 3456 cm^{-1} demonstrates clearly that the γ-turn conformation is not energetically favored for *trans*-diastereomer **3**.

Molecular Mechanics Calculations and Maps of Energy vs Conformation

In recent years, considerable effort has been made to model the conformational preferences of the *cis*- and *trans*-isomers of *N*-acetylproline *N'*-methylamide (**1**) and the transition states for X-Pro amide isomerization.[30,31] These investigations have focused on calculating the energy difference between the *cis*- and *trans*-isomers and the energy barrier for amide isomerization.[30,31] We initially used the MacroModel 3.5x program and the AMBER force field with the GB/SA solvent model for water in order to calculate the energy differences between the amide *cis*- and *trans*-isomers in **1–3** by comparing the minima for each isomer (Table 2).[32]

We sought next to develop a visual model by which the effects of the 5-alkyl substituent could be compared with proline **1**. A systematic analysis was performed in which the ψ- and ω-dihedral angles were rotated at 30° intervals and the energy of the local minimum was calculated for each conformation. Maps 1-3 were then constructed for *N*-acetylproline *N'*-methylamides **1–3** by plotting the minimum energy value at each interval against the values for the ψ- and ω-dihedral angles.

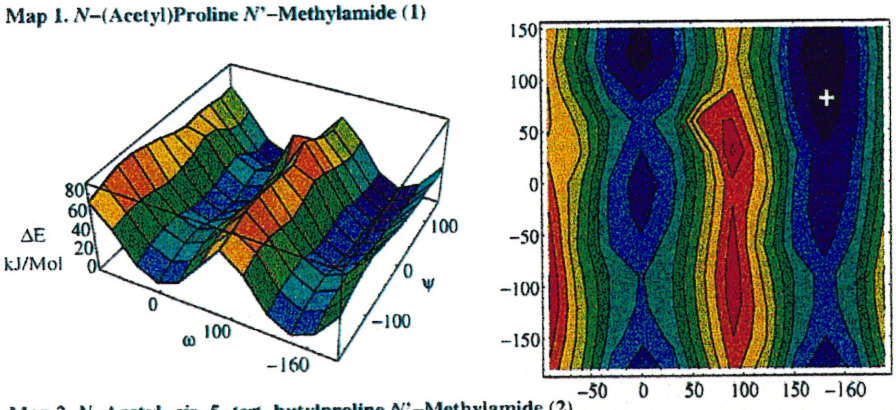
Maps 1-3 provide a useful graphical representation of the effects of the 5-*tert*-butyl substituent on the amide isomer in *N*-(acetyl)proline *N'*-methylamides **1–3**. For example, the relative sizes of the regions of minimum energy (blue lakes) at the *cis*- and *trans*-isomers ($\omega \sim 0°$ and 180°) compare well with the populations observed by proton NMR. The magnitude of the saddles between the minima reflect the energy barriers ascertained by coalescence and magnetization transfer experiments. Furthermore, the white cross at $\omega = 180°$ and $\psi = 80°$ marks an ideal γ-turn conformation,[24] which is located at energy minima in the maps for **1** and **2**, yet appears at higher energy in the map for **3**. Maps 1-3 provide a visual aid that shows the conformational liberty about the ψ dihedral angle. Relative to proline **1**, the conformational freedom about ψ appears to be greater in *cis*-5-*tert*-butylproline **2** and more restricted in *trans*-5-*tert*-butylproline **3**.

Conclusion

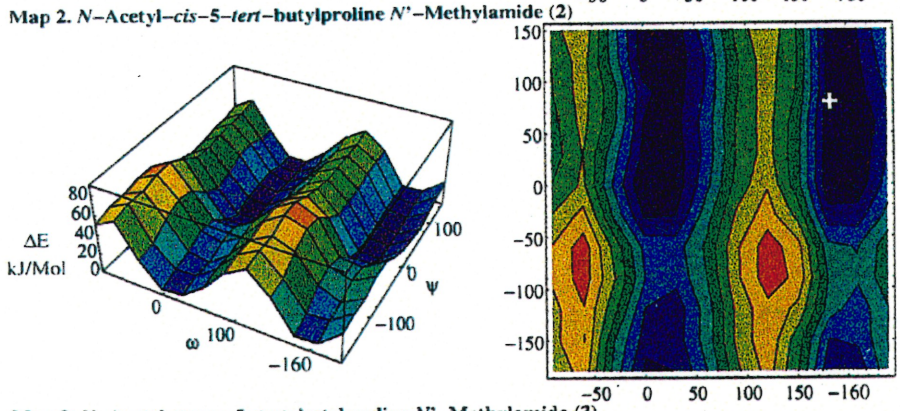
Amide *cis*-isomers *N*-terminal to prolyl residues in peptides are presently being identified as important recognition elements in protein biology. For example, the type VI β-turn conformation, which features a *cis*-isomer, has been suggested to be a key intermediate in the mechanism for PPIase catalyzed isomerization of X-Pro amide from *cis*- to *trans*-isomer.[33] In addition, a type VI conformation has been suggested as a requirement for thrombin catalyzed cleavage of the V_3 loop of HIV gp120, a

Chart 1. Maps 1−3: Conformation vs Energy of Prolyl Amides **1**−**3**

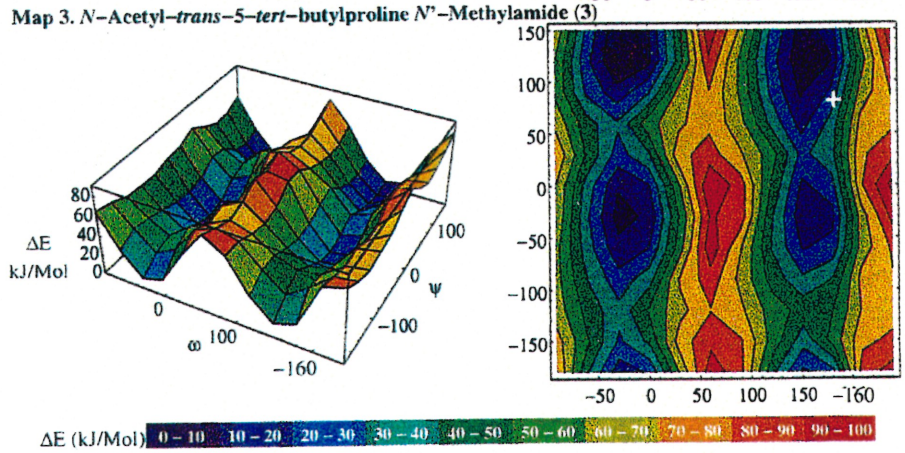
Map 1. *N*–(Acetyl)Proline *N'*–Methylamide (1)



Map 2. *N*–Acetyl–*cis*–5–*tert*–butylproline *N'*–Methylamide (2)



Map 3. *N*–Acetyl–*trans*–5–*tert*–butylproline *N'*–Methylamide (3)



prerequisite to viral infection.[34] Further examination of X-Pro amides in type VI β-turns as well as in alternative structural motifs, such as type I poly-proline helices,[35] is likely to provide additional examples of *cis*-isomer significance in other protein recognition events. In order to facilitate their study, methodology is thus desired for generating libraries of different peptide structures possessing conformationally restrained X-Pro amide *cis*-isomers.

Steric effects have been used to control the X-Pro amide conformation and favor the *cis*-isomer population. 5-*tert*-Butylprolines were synthesized with stereocontrol and examined in model peptides **2** and **3**. In *N*-(acetyl)proline *N'*-methylamides **1**-**3**, the interactions of the *tert*-butyl group on the amide geometry augment significantly the *cis*-isomer population in water (Table 2). The barrier for amide isomerization is 3.9 kcal/mole lower in *cis*-diastereomer **2** relative to proline **1**. Maps 2 and 3 illustrate that the bulky 5-position substituent skews the amide bond away from planarity such that twisted amide conformations appear with energy minima at $\omega > 0°$ and $> 180°$ for *cis*-diastereomer **2** and $\omega < 0°$ and $< 180°$ for *trans*-diastereomer **3**. Moreover, the *tert*-butyl group influences the ψ dihedral angle such that minima are found at $\psi \sim 125°$ for **1** and **3**, and at $\psi \sim 0°$ for **2**.[32] The lower barrier for isomerization in *cis*-diastereomer **2** may be due in part to this effect on the ψ dihedral angle, because the *C*-terminal NH group at $\psi \sim 0°$ may stabilize the pyramidalized amide transition states by interacting with either the nitrogen lone pair or the carbonyl oxygen.[30] In *trans*-diastereomer **3**, a combination of effects on the ω and ψ dihedral angles leads to a destabilization of the γ-turn conformation and no N–H stretching band corresponding to a hydrogen-bonded amide is observed by FT-IR spectroscopy in $CHCl_3$ (Figure 4).

In conclusion, by studying model peptides **1**-**3**, we have been able to itemize both the effects of steric bulk and stereochemistry of 5-position substituents on prolyl

conformation. Since the bulky 5-position substituent influences amide geometry by disfavoring the *trans*-isomer, the population of *cis*-isomer will augment as the size of the *N*-terminal group increases. Furthermore, because the *tert*-butyl group distorts the amide away from planarity, potential exists to use 5-*tert*-butylprolines to generate twisted amide mimetics. Our preliminary results demonstrate already that 5-*tert*-butylproline can be introduced into different amide and peptide structures.[19,20] In future work, incorporation of 5-*tert*-butyl proline into a library of X-Pro analogues will be explored as a promising new means for studying the importance of *cis*-isomer geometry in protein chemistry and biology.

Experimental Section

Experiments involving chemical reactions were performed under the general guidelines described in reference 19. The chemical shifts for the carbons and protons of minor isomers are respectively reported in parentheses and in brackets.

General Procedure for the Synthesis of *N*-Acetyl-5-*tert*-butylproline *N'*-Methylamides 2 and 3. Methylamine hydrochloride (1.35 g, 20 mmol) in benzene (20 mL) at 5°C was treated with a solution of trimethylaluminum in hexane (2 M, 10 mL, 20 mmol), brought to room temperature and stirred until the loss of CH_4 gas was no longer detectable.[21] From the resulting solution (0.067 M), 2.7 mL (1.74 mmol, 300 mol %) was added dropwise to a room temperature solution of *cis*-*N*-BOC-5-*tert*-butylproline methyl ester (*cis*-**4**, 166 mg, 0.58 mmol, 100 mol %) in CH_2Cl_2 (6 mL). The mixture was stirred for 72 h, treated with another 1.73 mL of the benzene solution and stirred 48 h when TLC showed complete disappearance of starting ester. The reaction mixture was treated with 6 mL of saturated K_2CO_3, and extracted with CH_2Cl_2 (3 ∞ 15 mL). The organic layers were combined, washed with brine, dried with Na_2SO_4, filtered, and evaporated to 122 mg of an oil (74%) that was used in the next step without further purification. *cis*-*N*-BOC-5-*tert*-Butylproline *N'*-methylamide:

^{1}H NMR δ ($CDCl_3$) 0.88 (s, 9 H), 1.47 (s, 9 H), 1.9 (m, 2 H), 2.15 (m, 1H), 2.25 (m, 1 H), 2.83 (d, 3 H, *J* = 4.9), 3.87 (dd, 1 H, *J* = 3.1, 7.8), 4.32 (t, 1 H, *J* = 8.7), 6.8 (s, 1 H); ^{13}C NMR δ ($CDCl_3$) 26.15, 26.18, 26.5, 27.4, 28.3, 35.6, 62.9, 67.6, 80.8, 157.8, 173.2.

cis-N-BOC-5-*tert*-Butylproline *N'*-methylamide (100 mg, 0.35 mmol) was dissolved in 4 mL of dichloromethane, treated with 260 μL of trifluoroacetic acid (3.5 mmol), and stirred at room temperature for 5 h. Evaporation of the volatiles on a rotary evaporator gave 99 mg (95%) of *cis*-5-*tert*-butylproline *N'*-methylamide trifluoroacetate (**5a**): ^{1}H NMR δ ($CDCl_3$) 1.0 (s, 9 H), 1.6 (m, 1 H), 1.9 (m, 1H), 2.1 (m, 1 H), 2.3 (m, 1 H), 2.76 (d, 3 H, *J* = 4.7), 3.36 (m, 1 H), 4.49 (m, 1 H), 6.86 (br s, 1 H), 7.57 (br s, 1 H), 10.43 (br s, 1 H).

trans-5-*tert*-Butylproline *N'*-methylamide trifluoroacetate (**5b**) was generated in a similar way from *trans*-**4** in 81% overall yield: ^{1}H NMR δ ($CDCl_3$) 0.9 (s, 9 H), 1.38 (m, 1 H), 1.77 (m, 2 H), 2.25 (m, 1 H), 2.8 (d, 3 H, *J* = 5), 2.81 (m, 1 H), 3.73 (t, 1 H, *J* = 7.5), 7.5 (br s, 1 H).

cis-5-*tert*-Butylproline *N'*-methylamide trifluoroacetate (**5a**, 50 mg, 0.31 mmol) and Et_3N (60 μL, 0.5 mmol) in CH_2Cl_2 (3 mL) were treated with acetic anhydride (44 μL, 0.47 mmol), stirred for 48 h at room temperature and evaporated to a residue that was purified by chromatography using an eluant of 5% methanol in $CHCl_3$. Evaporation of the collected fractions gave 61 mg (88%) of *N*-acetyl-*cis*-5-*tert*-butylproline *N'*-methylamide (**2**): ^{1}H NMR δ (D_2O) 0.92 (s, 4.5 H) [0.97 (s, 4.5 H)], 1.8-2.5 (m, 4 H), 2.02 (s, 1.5 H) [2.24 (s, 1.5 H)], 2.75 (s, 1.5 H) [2.8 (s, 1.5 H)], 3.97 (d, 0.5 H, *J* = 7.6) [4.1 (d, 0.5 H, *J* = 8.2)], 4.55 (m, 1 H); ^{13}C NMR δ (D_2O) 22.3 (23), 26 (26.2), 26.9, 27.4, (28.3) 30.7, 35.9 (36), (62.6) 63.6, 67.6 (69.9), 175.3, 176.6; FAB MS *m/e* 227.2 $[MH]^+$ (100%), 185.2, 168.2, 126.2. HRMS calcd for $C_{12}H_{23}N_2O_2$ (MH^+) 227.1760, found 227.1768.

N-Acetyl-*trans*-5-*tert*-butylproline *N'*-methylamide (**3**) was prepared from **5b** in a similar way in 96% yield; ^{1}H NMR δ (D_2O) 0.85 (s, 3 H) [0.9 (s, 6 H)], 1.7-2.2 (m, 3 H), 1.92 (s, 1 H) [2.16 (s, 2 H)], 2.4 (m, 1 H), 2.69 (s, 2 H) [2.73 (s, 1 H)], 4.02 (m, 0.66 H) [4.19 (d, 0.33 H, J = 6.2)], 4.32 (d, 0.66 H, J = 8.5) [4.57 (d, 0.33 H, J = 10)]; ^{13}C NMR δ (D_2O) (22.9) 23.6, (25.4) 26.1, (26.3) 26.6, 27.3 (27.6), 29.5 (31.6), (37.3) 37.9, 62.4 (63.1), (66.7) 69.4, 175.3 (175.5), 176 (176.1); FAB MS *m/e* 227.2 $[MH]^+$ (100%), 196.2, 168.2, 126.2. HRMS calcd for $C_{12}H_{23}N_2O_2$ (MH^+) 227.1760, found 227.1766.

Magnetization Transfer NMR experiments were performed on Bruker AMX600 and DMX600 MHz spectrometers, both equipped with selective excitation units, and ^{1}H and broadband heteronuclear probes. Experiments were performed over several temperatures between 313°K and 348°K for each compound. Selective inversion of C^α carbon *trans*- and *cis*-isomer signals were done with gaussian pulses centered on resonance (+/-) 25 Hz. Relaxation delays of 20 seconds and inversion-recovery delays of between 1 msec and 20 seconds were used. Data for each inversion recovery point were averaged over 32 to 128 points, depending on the concentration of the compound used. Amides **1** and **3** were dissolved in distilled and deionized H_2O or D_2O at concentrations between $1 \infty 10^{-2}$ M and $2 \infty 10^{-2}$ M and extensively exchanged with nitrogen gas in NMR tubes. For each compound at an individual temperature, inversion-recovery results were collected with selective inversion of the C^α *trans*-isomer peak. Data were fitted according to equations 1 and 2.[27]

1. $M_Z(A)/M_8(A) = 1 - (2k_A/\beta)\, I_A\{\exp[-1/2(\alpha - \beta)\tau] - \exp[-1/2(\alpha + \beta)\tau]\}$

2. $M_Z(X)/M_8(X) = 1 - (1/\beta)\, I_X\{(\beta + R_X - R_A)\exp[-1/2(\alpha + \beta)\tau] + (\beta + R_A - R_X)\exp[-1/2(\alpha - \beta)\tau]\}$

Where $\alpha = R_A + R_X$, $\beta = [(R_A - R_X)^2 + 4k_Ak_X]^{1/2}$ and $R_A = k_A + (1/T_{1A})$, $R_X = k_X + (1/T_{1X})$. Where X is the inverted C^α signal; A is the passive signal; T_{1A} and T_{1X} are longitudinal relaxation times; k_A and k_X are rate constants for isomerization; $M_Z(A)$, $M_8(A)$, $M_Z(X)$ and $M_8(X)$ are longitudinal magnetizations; I_A and I_X are proportionality constants used to correct for incomplete return of magnetization to equilibrium. We used conjugate-gradient optimization (Marquart-Levenberg method) to simultaneously fit all eight parameters in 1 and 2 starting with estimates of values for the individual parameters.[36] Analytical derivatives of 1 and 2 were calculated with the program Mathematica™ (version 2.0).

^1H NMR experiments to determine the effect of concentration on the chemical shift of **3** were performed in CCl_4 (freshly distilled from P_2O_5). The solutions were transferred to an NMR tube and into the sample, a second tube was inserted containing acetone d_6 that was used for the Lock signal and as the internal reference.

FT-IR spectra were measured at 25°C on a Perkin-Elmer 1600 FT-IR spectrometer using a DTGS detector at 4 cm^{-1} resolution. Chloroform and CCl_4 were distilled from P_2O_5. Peptide solutions of $1 \infty 10^{-4}$ M were examined in 1 cm quartz glass Infrasil cell and the spectra were recorded with 1024 scans.

Molecular Mechanics Calculations were performed on a Silicon Graphics Personal Iris Workstation using the AMBER force field and the GB/SA solvent model for water within the Macromodel program 3.5x.[37] The modeling protocol on amides **1-3** was conducted as follows. For each *N*-(acetyl)proline *N'*-methylamide, a *trans*-isomer amide bond was drawn and minimized in water using the Steepest Descent (SD) procedure followed by a Full Matrix Newton Raphson (FMNR) method until a 0.001 kJ/Å•mol gradient was reached. The resulting structure was then used as the starting point for the generation of the conformational energy maps. The ω and ψ dihedral angles were rotated about 30° increments in order to generate 144 conformers. For

each conformer, two pyrrolidine ring puckerings were applied in order to generate 288 starting conformers that were then minimized in water using a Polack-Ribiere Conjugate Gradient (PRCG) and a force of 1000 kJ/mol in order to restrain the ω and ψ dihedral angles. The resulting minima were then plotted against the ω and ψ dihedral angles using the Mathematica™ program in order to furnish Maps 1-3. The global minima for the *cis*- and *trans*-isomers of **1-3** were obtained using a similar protocol in which no constraints were placed on the ω and ψ dihedral angle geometries.[32]

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Supplementary Material Available: The ^{1}H and ^{13}C NMR spectra of **1-3** and ^{1}H NMR of **5**; plots of intensity vs mixing time for the magnetization transfer experiments on **1** and **3** (9 pages). This material is contained in many 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 4

Ce chapitre est constitué d'une brève introduction des articles 4 et 5 qui est suivie de la version intégrale des articles 4 et 5.

Introduction

L'influence des substituants alkyles en position 3 sur la vitesse d'isomérisation des amides en position *N*-terminal des prolines et des hydroxyprolines a été explorée en synthétisant et analysant des (2*S*)-*N*-(acétyl)-proline *N*'-méthylamides (**1**), (2*S*,4*R*)- et (2*S*,4*S*)-*N*-acétyl-4-hydroxyproline *N*'-méthylamides **2** et **3**, et respectivement leurs analogues 3,3-diméthyles **4**-**6**. Les populations relatives des isomères *cis* et *trans* des amides ainsi que les vitesses d'isomérisation de *cis* à *trans* et de *trans* à *cis* de **1**-**6** dans l'eau ont été déterminées par des expériences de spectroscopie RMN et de transfert de magnétisation. Les populations relatives des amides en position *C*-terminale, libre de pont d'hydrogène ainsi que celles ayant un pont d'hydrogène impliqué dans un repliement γ ont été estimées en intégrant la bande d'élongation N-H dans le spectre infrarouge de **1** et **4** dans le $CHCl_3$. De plus, la structure de l'isomère *trans* de l'amide (2*S*,4*S*)-*N*-acétyl-3,3-diméthyl-4-hydroxyproline *N*'-méthylamide (**6**) a été déterminée à l'état solide par l'analyse cristallographique des rayons X. Dans les peptides **1**-**6**, les substituants 3,3-diméthyles et hydroxyles ont très peu d'effet sur l'équilibre des isomères de l'amide. Une baisse importante de la vitesse d'isomérisation de l'isomère *cis* à *trans* de l'amide a été observée pour le *N*-acétyl-3,3-diméthylproline *N*'-méthylamide (**4**) qui a montré une constante k_{ct} près de 7 fois plus faible que celle observée pour **1**. Des effets similaires ont été observés pour les substituants 3,3-diméthyle, cependant à un moindre degré, dans le cas des peptides d'hydroxyproline.

diméthyle, cependant à un moindre degré, dans le cas des peptides d'hydroxyproline. Les données par FT-IR pour **4** et les données obtenues par rayons X pour **6** ont démontré que les substituants 3,3-diméthyle restreignent l'angle dièdre ψ de proline et prévient la formation d'un repliement γ possédant un pont hydrogène entre le NH de l'amide en position C-terminale et le carbonyle en position N-terminale. Par ailleurs, la restriction de l'angle dièdre ψ par les groupes méthyles a été observée par une analyse conformationelle de **1-6** assistée par ordinateur, dans laquelle l'énergie des angles ψet ω a été déterminée à des intervalles de 30° afin de localiser les minimum locaux. La diminution de la vitesse d'isomérisation de *cis* vers *trans* de l'amide *N*-terminale des analogues diméthylés peut être attribuée aux interactions stériques favorisant un angle dièdre ψ où le carbonyle de l'amide *C*-terminale déstabilise l'état de transition par une répulsion de Coulomb provenant soit de la paire d'électron libre se développant ou de l'oxygène du carbonyle de l'amide pyramidalisé en position *N*-terminale. Les conséquences de substituants alkyles en position 3 ou de substituants hydroxyles en position 4 sur la vitesse d'isomérisation de l'amide de proline dans l'eau, dont la diminution est dans l'ordre de $\mathbf{1} \approx \mathbf{3} > \mathbf{2} > \mathbf{6} > \mathbf{5} > \mathbf{4}$, peuvent provenir de l'influence de la géométrie de l'angle dièdre ψ, des effets inductifs et de ponts d'hydrogène intramoléculaires.

Publication 4:

Eric Beausoleil, Raman Sharma, Stephen Michnick
and William D. Lubell 82

In *Peptides 1996* (*Proceedings of the 24th European Peptides Symposium*); Ramage, R., Epton, R., Ed.; ESCOM: Leiden, The Netherlands, 1997; pp 241-242.

Steric Effects of Alkylprolines on the Conformation of *N*-(Acetyl)Proline *N'*-Methylamides

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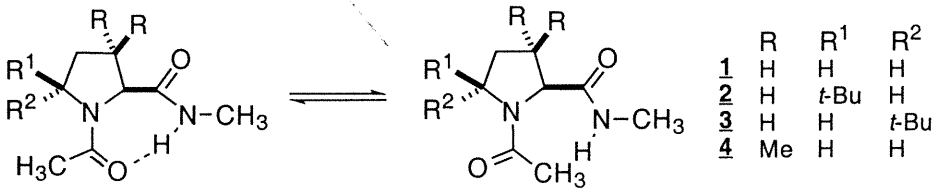
Introduction

Alkylprolines that exhibit steric interactions on peptide geometry can be used to explore relationships between conformation and ioactivity. Model peptides that possess prolyl residues are useful for ascertaining the specific influences of alkyl substituents on prolyl conformation. Herein, we try to elucidate factors effecting prolyl amide rotamer equilibrium and prolyl peptide geometry via the examination of *N*-(acetyl)proline *N'*-methylamides **1-4** by NMR and FT-IR in aqueous and non-polar solvents.

Results and Discussion

We have reported the synthesis of *cis*-5-*tert*-butylproline [1] and 3,3-dimethylproline [2]. Epimerization of *N*-Boc-*cis*-5-*tert*-butylproline methyl ester furnished the *trans*-diastereomer. Prolines were converted to amides **1**-**4** using standard methods. Rotamers were assigned using ^{13}C NMR and their populations were measured by integration of the relative intensities of the α-, N-Me, and acetyl methyl proton resonances (Table 1). Ratios were measured at lower temperature when signal coalescence was noted at 25°C.



trans-Rotamer *cis*-Rotamer

Figure 1. *N*-(Acetyl)Proline *N'*-Methylamide Rotamer Equilibrium

Amide **1** exists predominantly as the *trans*-rotamer and adopts a γ-turn conformation in non-polar solvents and α-helix or polyproline II-like conformations in polar solvents [3-5]. Steric effects of alkyl substituents can alter these preferences [6]. For example, interactions between the bulky 5-*tert*-butyl substituent and the *N*-terminal residue disfavor the *trans* and increase the *cis*-rotamer population in amides **2** and **3**. On the other hand, 3,3-dimethylproline amide **4** exhibits similar rotamer populations as **1**. In all cases, the amide *cis*-rotamer population diminishes in non-polar solvents [3-5].

Free and hydrogen-bound *N*-methylamide populations were measured by FT-IR in $CHCl_3$ (Table 2) [6]. In contrast to **1** and **2**, which exist predominantly in 7-member hydrogen-bonds, we observe no γ-turn conformation for amides **3** and **4** in $CHCl_3$.

Table 1. Solvent Effects on the Rotamer Equilibrium of Amides **1-4**.[a]

Amide	R	R^1	R^2	D_2O (% *cis*)	$CDCl_3$ (% *cis*)	CCl_4 (% *cis*)
1	H	H	H	27[b]	22[c]	5[d,e]
2	H	*t*-Bu	H	49	18[f]	13[e]
3	H	H	*t*-Bu	66	47	54
4	Me	H	H	27	22	-

[a]Unless noted, determined by 300 MHz ^1H NMR at 25°C, error ± 4%. [b]Lit. 27 [4], 30 [5]. [c]Lit. 16 [3], 13 [4], 20 [5]. [d]Lit. 18 [3], 4 [4]. [e]At –15°C. [f]At 5°C.

Table 2. FT-IR Bands for NH Stretch of *N*-(Acetyl)Proline *N'*-Methylamides **1-4**.[a]

Amide	Solvent	ν(free), cm^{-1}	ν(bound), cm^{-1}	% free	% bound
1	$CHCl_3$	3451	3328	21	79
2	$CHCl_3$	3441	3319	15	85
3	$CHCl_3$	3454	-	>99	<1
4	$CHCl_3$	3455	-	>99	<1

[a]Determined at 25°C on samples of 10^{-4}M concentration.

The factors that favor the amide *trans*-rotamer *N*-terminal to proline appear to be more numerous than stabilization from hydrogen bonding in a γ-turn conformation and from the relief of steric repulsion between the α-carboxylate and *N*-terminal residue. For example, a γ-turn is not adopted by **4** because of steric interactions that alter the ψ dihedral angle, yet the rotamer populations for **4** are identical to **1**. Although a *tert*-butyl group is much more sterically bulky than a carboxylate group [7], the rotamer populations for **2** and **3** do not reflect such a competition of steric force. Clearly, other

factors, maybe solvation, dipole and ring puckering effects, are working to stabilize the prolyl amide *trans*-rotamers in **2-4**.

Acknowledgments

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et William D. Lubell

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Alkyl 3-Position Substituents Retard Isomerization of Prolyl and Hydroxyprolyl Amides in Water

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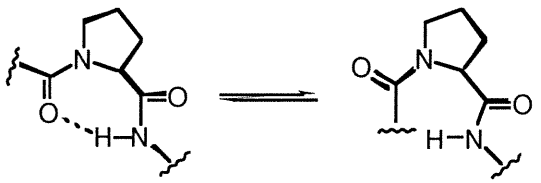
Abstract

The influence of alkyl 3-position substituents on the rate of amide isomerization *N*-terminal to proline and hydroxyproline has been explored via the synthesis and analysis of (2*S*)-*N*-(acetyl)proline *N*'-methylamide (**1**), (2*S*, 4*R*)- and (2*S*, 4*S*)-*N*-acetyl-4-hydroxyproline *N*'-methylamides **2** and **3** and their respective 3,3-dimethyl analogs **4-6**. The relative populations of the amide *cis*- and *trans*-isomers as well as the rates for *cis* to *trans* and *trans* to *cis* isomerization of **1-6** in water were ascertained by NMR spectroscopy and magnetization transfer experiments. The relative populations of free *C*-terminal and hydrogen-bonded amides in the γ-turn conformation were also estimated by integrating the N–H stretch absorbances in the FT-IR spectra of **1** and **4** in $CHCl_3$. In addition, the structure of the amide *trans*-isomer of (2*S*, 4*S*)-*N*-

acetyl-3,3-dimethyl-4-hydroxyproline *N*'-methylamide (**6**) was determined in the solid state by X-ray crystallographic analysis. In prolyl peptides **1-6**, the 3,3-dimethyl and hydroxyl substituents had little effect on the amide isomer equilibrium. A dramatic decrease in the rate of *cis*- to *trans*-amide isomerization was observed for *N*-acetyl-3,3-dimethylproline *N*'-methylamide (**4**) which exhibited k_{ct} nearly 7-fold slower than **1**. Similar effects of the 3,3-dimethyl substituents were observed, albeit to a lesser degree, in the cases of the hydroxyprolyl peptides. The FT-IR data for **4** and X-ray data for **6**, both demonstrated that the 3,3-dimethyl substituents restricted the proline ψ dihedral angle and prevented the formation of a γ-turn conformation, having a 7-membered hydrogen-bond between the *C*-terminal amide NH and *N*-terminal amide carbonyl. Furthermore, restriction of the ψ dihedral angle by the methyl groups was observed in systematic computational conformational analyses of **1-6**, in which the ψ- and ω-dihedral angles were rotated at 30° intervals and the energies of the local minima were determined. Retardation of the rate of *cis*- to *trans*-amide isomerization in the dimethyl analogs may be attributed to steric interactions favoring a ψ dihedral angle at which the *C*-terminal amide carbonyl destabilized the transition state through Coulomb repulsion of either the developing nitrogen lone pair or carbonyl oxygen of the pyramidalized *N*-terminal amide. The consequences of 3-alkyl and 4-hydroxyl substituents on the rate of proline amide isomerization in water, which was observed to decrease in the order **1** $\approx$ **3** > **2** > **6** > **5** > **4**, may result from influences on the ψ dihedral angle geometry, inductive effects and intramolecular hydrogen-bonding.

Introduction

Prolyl residues can profoundly influence the conformation and reactivity of peptide structures.[1,2] Since amides *N*-terminal to proline possess energetically similar *cis*- and *trans*-isomers,[1] prolyl residues can act as junctions that alter peptide chain direction creating multiple low energy conformers. Consequently, prolyl amide isomerization can be a rate limiting step in protein folding[3] and may induce functional changes in peptides and proteins.[4] Prolyl peptide *cis-trans* isomerases (PPIases),[5] as well as catalytic[6,7] and autocatalytic[8-10] mechanisms for prolyl amide isomerization have thus been recognized as accelerators of protein folding that may serve important roles in various biological systems.



***trans*-Isomer** ***cis*-Isomer**

Figure 1. Amide Isomer Equilibrium *N*-Terminal to Proline Derivatives

Alkylprolines that exhibit steric interactions on peptide geometry can serve as probes for exploring relationships between conformation and activity.[11-15] Alkylprolines can also be used to dissect conformational effects on prolyl isomerization.[11,12] For example, we have reported that 5-*tert*-butylprolines augment the amide *cis*-isomer population in prolyl peptides and may be used to construct type VI β-turn mimics.[12] In the case of the *cis*-diastereomer of *N*-acetyl-5-*tert*-butylproline *N'*-methylamide, we observed that the 5-*tert*-butyl substituent reduced the barrier for amide isomerization by 3.7 kcal/mol compared to the barrier for *N*-(acetyl)proline *N'*-methylamide (**1**).[12] Acceleration of amide isomerization was attributed in part to ground-state destabilization resulting from the bulky 5-position substituent skewing the

amide bond away from planarity.[16,17] In addition, the *tert*-butyl derivative adopted an energy minimum conformation with the ψ dihedral angle at $\psi \approx 0°$,[12] which has been suggested to stabilize the pyramidalized amide transition states,[12] because the *C*-terminal NH group is able to interact with the nitrogen lone pair or the carbonyl oxygen of the rotating *N*-terminal amide.[8-10] Having demonstrated that steric interactions can lower the barrier for amide isomerization of 5-alkylprolyl peptides, we now report that 3-alkylprolines can create steric interactions that raise the barrier for amide isomerization. The results of the present study again draw attention to the importance of *C*-terminal amide interactions on prolyl peptide isomerization.

We have recently introduced methodology for efficiently synthesizing 3-alkylproline and 3-alkyl-4-hydroxyprolines.[15] Attachment of the side-chain functions of natural amino acids to the 3-position of the pyrrolidine ring provides proline-amino acid chimeras that can be used to explore the geometric relation of side-chain groups to the peptide back-bone. Replacement of natural amino acids with such proline-amino acid chimeras has been used to study relationships between biological activity and conformation,[15] because the 3-alkyl substituents can impose steric interactions that restrict the ψ dihedral angle to prevent formation of a γ-turn conformation ($\psi \approx 80°$) in different peptide structures.[11,13,14] The influence of the 3-position substituent is in part contingent on its relative stereochemistry. For example, the *trans*-diastereomer was much less effective at perturbing the γ-turn conformation relative to the *cis*-diastereomer in *N*-acetyl-3-methylproline *N'*-methylamides as demonstrated by circular dichroism (CD) and FT-IR spectroscopy.[13a] Although the consequence of a single 3-position substituent on prolyl geometry had been studied prior to our investigation, the influence of 3-position substituents on the rate of amide isomerization *N*-terminal to proline had not been reported. We have thus compared *N*-(acetyl)proline *N'*-methylamide (**1**) with its 3,3-dimethylproline analog **4** in order to ascertain the steric

effects of two methyl substituents on the proline conformation and the rate of prolyl amide isomerization.

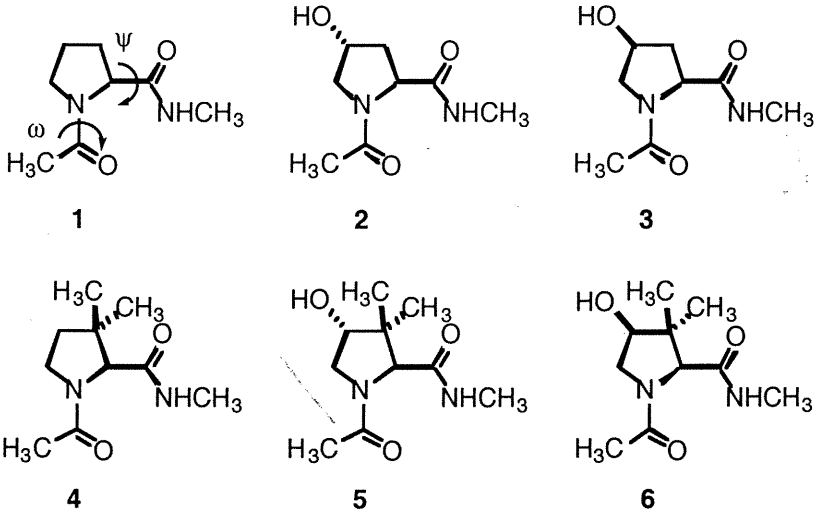
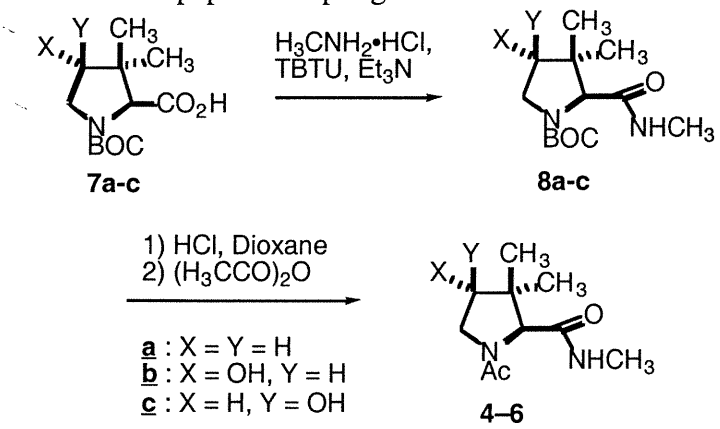


Figure 2. *N*-(Acetyl)Proline *N'*-Methylamides 1-6 (amide *trans*-isomers shown)

Electron withdrawing groups at the 4-position of proline have also been suggested to accelerate amide isomerization by inductive effects that increase sp^3 pyramidalization of the prolyl nitrogen.[18] Such an effect has been observed in dioxane, where the *trans*-diastereomer of *N*-acetyl 4-fluoroproline methyl ester exhibited a higher rate constant for amide isomerization relative to *N*-(acetyl)proline methyl ester.[18] On the other hand, the contrary was observed in water, when *cis–trans* isomerization of (2*S*,4*R*)-*N*-acetyl-4-hydroxyproline methyl ester was found to be slower than *N*-(acetyl)proline methyl ester.[18] In order to further examine this paradox, we have also investigated the consequence of a 4-position hydroxyl group on prolyl geometry and amide isomerization by studying (2*S*,4*R*)- and (2*S*,4*S*)-*N*-acetyl-4-hydroxyproline *N'*-methylamides (**2** and **3**) and their respective 3,3-dimethyl analogs **5** and **6**.

Results

Synthesis of *N*-(Acetyl)proline and *N*-Acetyl-4-hydroxyproline *N'*-Methylamides Enantiopure (2*S*)-*N*-BOC-3,3-dimethylproline (**7a**) as well as (2*S*, 4*R*)-and (2*S*, 4*S*)-*N*-BOC-3,3-dimethyl-4-hydroxyprolines (**7b** and **7c**) were synthesized from (2*S*,4*R*)-hydroxyproline as described.[15] Regioselective enolization of (2*S*)-4-oxo-*N*-(9-phenylfluoren-9-yl)proline benzyl ester and bis-*C*-alkylation with iodomethane at the 3-position provided the 3,3-dimethyl analog. Reduction of the ketone followed by a one-pot hydrogenolysis of both the *N*- and *O*-protecting groups and subsequent *N*-acylation with di-*tert*-butyldicarbonate gave *N*-(BOC)amino acids **7a-c** that were suitable for peptide coupling.



Scheme 1. Synthesis of *N*-Acetyl *N'*-Methylamides 4-6

(2*S*)-*N*-acetyl-3,3-dimethylproline *N'*-methylamide (**4**), (2*S*,4*R*)-*N*-acetyl-3,3-dimethyl-4-hydroxyproline *N'*-methylamide (**5**) and (2*S*,4*S*)-*N*-acetyl-3,3-dimethyl-4-hydroxyproline *N'*-methylamide (**6**), all were synthesized from their respective *N*-(BOC)amino acids **7a-c** (Scheme 1). Initially, *N*-(BOC)proline *N'*-methylamides **8a-c** were synthesized in good yields by coupling **7** to methylamine using benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in acetonitrile.[19] Solvolysis of the BOC group with HCl in dioxane and evaporation of the volatiles then gave the

hydrochloride salts that were acetylated in neat acetic anhydride. In the case of the hydroxyproline analogs, acetylation of the nitrogen was sometimes accompanied by *O*-acetylation. Selective hydrolysis of the *O*-acetyl group was then performed using potassium carbonate in methanol to furnish the desired *N*-acetyl-4-hydroxyproline *N'*-methylamides. Amide **1** was synthesized as previously described.[12] Amides **2** and **3** were prepared using the general procedure for the synthesis of **4** as described in the Experimental Section.[20]

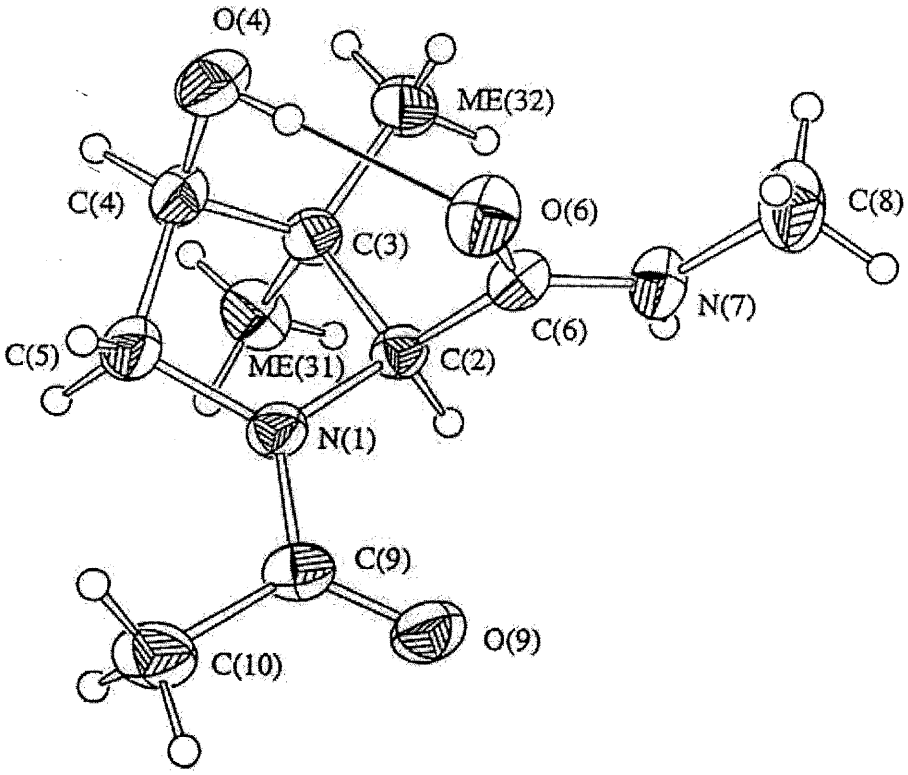


Figure 3. ORTEP Drawing of (2*S*, 4*S*)-*N*-Acetyl-3,3-dimethyl-4-hydroxyproline *N'*-Methylamide (6). Non hydrogen atoms are represented by ellipsoids corresponding to 40% probability. Hydrogen atoms are represented by spheres of arbitrary size.[21]

Crystals of (2*S*,4*S*)-*N*-acetyl-3,3-dimethyl-4-hydroxyproline *N'*-methylamide (**6**) were grown from methanol and subjected to X-ray crystallographic analysis.[21] The amide *trans*-isomer (ω = 173°) was observed in the crystal structure of **6** (Figure 3). The values for the ψ and ϕ dihedral angles were respectively 145° and –78° and the proline ring-puckering was of C4-endo conformation.[22] An intramolecular hydrogen bond between the 4-hydroxyl group and the *C*-terminal amide carbonyl was also inferred from the X-ray analysis. The distance between the alcohol proton and carbonyl oxygen was determined to be 1.91 Å and the O-H–O angle was 166°.

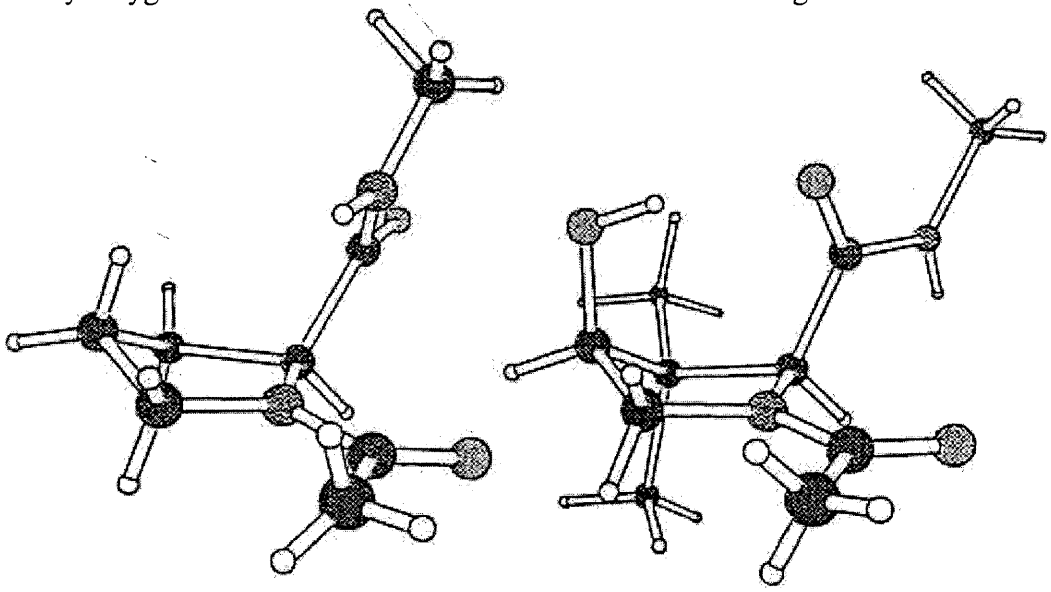


Figure 4. Structures of Amides 1 (ψ = –16°) and 6 (ψ = 145°) from X-ray Crystallography[23,24a]

The conformation observed for **6** was similar to the crystal structure geometry of *N*-(acetyl)proline *N'*-methylamide (**1**),[23] with respect to its amide *trans*-isomer (ω = 173°), ϕ dihedral angle value (ϕ = –76°) and C4-endo ring-puckering. On the other hand, the ψ dihedral angle for **6** (ψ = 145°) was significantly different from that of **1** (ψ = –16°) in their respective crystal structures, as illustrated in Figure 4.[24a] Besides crystal packing forces,[24b] the different orientations about the ψ dihedral angle in the structures of **1** and **6** may likely be due to a combination of steric interactions in the

latter between the 3-position methyl groups and *C*-terminal amide as well as the hydrogen bond between the 4-position hydroxyl and *C*-terminal carbonyl groups.

FT-IR Analysis of *N*-(Acetyl)proline *N'*-Methylamides 1 and 4 was performed in chloroform at 1×10^{-4} M concentration at 25°C in order to examine for the presence of a γ-turn conformation in which a seven-membered-ring, intramolecular hydrogen bond exists between the *C*-terminal amide NH and the carbonyl of the *N*-terminal amide *trans*-isomer.[25] In the spectrum of *N*-(acetyl)proline *N'*-methylamide (**1**) in chloroform,[12,13] the presence of the free N–H stretch band was observed at 3451 cm^{-1} for the non-hydrogen bonded amide. The predominant N–H stretch band was observed at 3328 cm^{-1} in the spectrum of **1** as the result of hydrogen bonding in the γ-turn conformation, and the ratio of the relative areas of the lower vs higher energy absorbance for the N–H stretches was 79:21. On the other hand, only a free N–H stretch band was observed at 3455 cm^{-1} for *N*-acetyl-3,3-dimethylproline *N'*-methylamide (**4**).[11] No second lower energy N–H stretch band was observed for **4** indicating the absence of a γ-turn conformation in chloroform. Similar FT-IR spectra to that of **4** have been reported with other 3-alkylproline analogs such as the *cis*-diastereomer of *N*-acetyl-3-methylproline *N'*-methylamide[13] and BOC-Dtc-Ile-OMe[14] (where Δtc equals 5,5-dimethylthiazolidine-4-carboxylate) in chloroform.

Table 1. Amide Isomer Equilibrium and Isomerization Rates of 1-6 in Water

Amide	$\delta\ ^{13}C^{\alpha}$ *cis*	$\delta\ ^{13}C^{\alpha}$ *trans*	$\delta\ ^{13}C^{\delta}$ *cis*	$\delta\ ^{13}C^{\delta}$ *trans*	*cis*-rotamer % Calcd[a]	*cis*-rotamer % ± 3%[b]	$k_{cis-trans}$ (s^{-1})	$k_{trans-cis}$[c] (s^{-1})
1	62.3	60.8	47.5	49.1	15	28 (29)	2.0 1 ± 0.09	0.82 ± 0.09
2	60.1	58.8	54.2	55.9	27	21 (24)	1.46 ± 0.13	0.47 ± 0.07
3	62.0	60.9	54.7	55.9	28	21 (29)	2.05 ± 0.50	0.82 ± 0.22
4	72.2	70.6	46.2	47.7	24	30 (30)	0.32 ± 0.07	0.12 ± 0.04
5	66.9	65.1	47.7	49.0	27	28 (25)	0.81 ± 0.19	0.27 ± 0.08
6	70.7	69.0	53.2	53.8	23	21 (25)	1.39 ± 0.16	0.47 ± 0.09

[a]Calculated with Macromodel 5.5x and the AMBER 94 force field. [b]Determined by 300 MHz NMR in D_2O at 25°C (60°C). [c]Calculated from K_{ct} and equilibrium at 60°C as described in text.

The amide *cis*- and *trans*-isomer populations and the rates for amide isomerization of *N*-(Acetyl)proline and Hydroxyproline *N*'-Methylamides 1-6 were determined by NMR analysis. Water (H_2O and D_2O) was chosen as solvent because of its physiological importance and for comparison with literature examples.[12,26] In comparison to reaction rates in non-protic and non-polar solvents, amide isomerization *N*-terminal to proline proceeds slower in water which stabilizes the polar amide ground states relative to the less polar transition state.[6,27,28] The assignments of the isomer geometry were made based on the chemical shift values of the α- and δ-carbons in D_2O as previously described for **1** (Table 1).[12,29] The populations of the amide isomers were measured in the proton NMR spectra by integration of the *N*'-methyl and α-proton signals in D_2O at 25° and at 60°C. Amide isomer populations measured in water varied, to a limited extent, upon addition of methyl and hydroxyl group substituents. Dimethyl analogs **4-6** tended to exhibit 3-7% more *cis*-isomer than the natural series **1-3** at 25°C. The additional 4-hydroxyl group resulted generally in a 3-9% decrease of *cis*-isomer population (Table 1). These percentages were not statistically significant.

The rates of *cis* to *trans* isomerization of *N*-(acetyl)proline *N'*-methylamides **1-6** were measured using ^{13}C NMR magnetization transfer experiments (Table 1). In these experiments, the signal of the α-carbon of the major amide isomer was selectively inverted and the rates of magnetization transfer between the major and minor isomer α-carbon signals were measured at different temperatures. Optimal magnetization transfer rates for **1-6** were observed at 60°C, and we therefore used data collected at this temperature for model fitting. This experiment was used to determine the amide isomerization rate constant, k_{ct}. The *trans* to *cis* amide isomerization rate constant (k_{tc}) was calculated using the values for k_{ct} and the ratio of the *cis*- and *trans*-isomer populations at 60°C. The amides displayed rate constants for *cis* to *trans* isomerization in water at 60°C in the decreasing order **1** ≈ **3** > **2** > **6** > **5** > **4**.

The presence of two methyl substituents at the 3-position was found to significantly diminish the rate for prolyl amide isomerization. For example, the rate for *cis* to *trans* isomerization of dimethylproline amide **4** was nearly seven-fold slower than that of *N*-(acetyl)proline *N'*-methylamide (**1**). A nearly two-fold reduction in the rate for amide isomerization was observed with (4*R*)-dimethyl-4-hydroxyproline amide **5** relative to (4*R*)-4-hydroxyproline amide **2**. In addition, the rate for isomerization of (4*S*)-dimethyl-4-hydroxyproline amide **6** was moderately slower than (4*S*)-4-hydroxyproline amide **3**.

In the case of the hydroxyproline amides, the isomerization rates were slower for the *trans*-diastereomers relative to the *cis*-diastereomer counterparts: (4*R* < 4*S*), **2** < **3** and **5** < **6**. In the natural series, a moderate reduction of the rate of amide isomerization was observed for (4*R*)-hydroxyproline amide **2** relative to proline amide **1**, and identical rates were observed for (4*S*)-hydroxyproline amide **3** and **1**. In contrast to the natural series, the dimethyl series exhibited a significant rate acceleration upon addition of a 4-position hydroxyl group. Relative to the case of dimethylproline

amide **4**, rates for isomerization were observed to be 2.7 fold-faster with (4*R*)-hydroxyproline amide **5** and 4.7 fold-faster with (4*S*)-hydroxyproline amide **6** (Table 1).

Molecular Mechanics Calculations The energy differences between the amide *cis*- and *trans*-isomers in **1-6** were compared and the minimum for each isomer was calculated using the MacroModel 5.5x program and the AMBER 94 force field with the GB/SA solvent model for water.[30,31] Systematic analyses of **1-6** were then performed in which the ψ- and ω-dihedral angles were rotated at 30° intervals and the energy of the local minimum was determined for each conformation and plotted against the values for the ψ- and ω-dihedral angles.[12,30] The results of the systematic analysis illustrated that relative to the natural analogs **1-3**, the conformational freedom about the ψ dihedral angle was more restricted in 3,3-dimethyl substituted analogs **4-6**. For example, the ideal γ-turn conformation at ω = 180° and ψ = 80° was located at an energy minimum in the analyses of **1-3**,[12] yet appeared at higher energy (~2 kcal/mole relative energy difference) in the analyses of **4-6**. Significant steric effects of the methyl substituents were observed as increases in energy at the ψ dihedral angle values centered at $\psi \approx 50°$ and $\psi \approx -130°$. These effects appeared to restrict the rotational liberty of the *C*-terminal carboxamide such that ψ dihedral angle values around $\psi \approx 150°$ were favored over values around $\psi \approx 0°$ for both the amide *cis*- and *trans*-isomers in the 3,3-dimethyl substituted analogs **4-6**.

Except in the case of **2**, the ring puckering for the minima of the amide *cis*- and *trans*-isomers was consistent with a C4-endo conformation. The ring puckering for the energy minimum conformers of the *cis*- and *trans*-isomers of (4*R*)-4-hydroxyproline amide **2**, both were of C4-exo conformation. The C4-exo ring puckering was the same as observed for (4*R*)-hydroxyproline inside collagen as determined by X-ray

diffraction,[32] as well as inside collagen triple-helical peptides as shown by NMR[33] and X-ray crystallographic analysis.[34]

Discussion

We have examined the influences of 3-position methyl and 4-position hydroxyl substituents on the conformation of *N*-(acetyl)proline *N'*-methylamides **1**-**6** by a combination of X-ray crystallography, FT-IR spectroscopy, NMR techniques and molecular mechanics. We have also used magnetization transfer experiments to determine the rates for *cis* to *trans* amide isomerization of these prolyl peptides, which decreased in the order **1** ≈ **3** > **2** > **6** > **5** > **4**. In light of proposed mechanisms for amide isomerization,[6,8] we may begin to interpret this order based on the results of our conformational analysis. Previous studies have noted the importance of the ψ-dihedral angle geometry and electron withdrawing substituents on prolyl amide isomerization. For example, at a ψ-dihedral angle around $\psi \approx 0°$, interactions between the *C*-terminal amide NH with the nitrogen lone pair of the rotating *N*-terminal amide have been suggested to stabilize the pyramidalized transition state and accelerate isomerization.[8-10] On the other hand, at ψ dihedral angles between 150° and 210° (–150°), an unfavorable Coulomb interaction between the *C*-terminal carbonyl oxygen and the developing lone pair on the pyramidalized nitrogen has been suggested to reduce the rate of prolyl amide isomerization.[8] The observed acceleration of isomerization on introduction of a fluoride at the proline 4-position has been implied to arise from inductive effects that facilitate sp^3 pyramidalization of the prolyl nitrogen.[18] Electron withdrawing substituents have also been noted to increase the acidity of the *C*-terminal amide NH and thereby accelerate *N*-terminal amide isomerization in $CHCl_3$ by increasing the favorable interactions at $\psi \approx 0$.[10]

Cis to *trans* isomerization rates for the dimethyl series **4**-**6** were always lower than those for the natural series **1**-**3**. This decrease in rate for the dimethyl analogues

may arise from steric interactions between the 3-position methyl groups and the *C*-terminal amide that restrict the ψ dihedral angle to values away from $\psi \approx 0°$. For example, the ψ dihedral angle in dimethyl hydroxyproline amide **6** was found to be 145° in the X-ray crystal structure. Methyl substituents at the 3-position prevented amide **4** from adopting a γ-turn ($\psi = 80°$) which was the preferred conformation for **1** in $CHCl_3$ as determined by examination of the N-H stretch region in their respective FT-IR spectra. In addition, conformational analyses of amides **1-6** revealed that the 3-position methyl substituents induced ψ dihedral angle values around 150° relative to values around $\psi \approx 0°$ for the parent series. The steric effects of the methyl substituents at the 3-position appear to favor ψ dihedral angle geometries that position the *C*-terminal carbonyl oxygen in a position which disfavors amide pyramidalization by Coulomb interactions. This geometry, which has been suggested to impede prolyl amide isomerization,[8] may account for *N*-acetyl-3,3-dimethylproline *N'*-methylamide (**4**) exhibiting the slowest rate of isomerization relative to **1**.

Inductive effects of the 4-position hydroxyl group may be responsible for the increased isomerization rates of **5** and **6** relative to *N*-acetyl-3,3-dimethylproline *N'*-methylamide (**4**). The greater acceleration exhibited by (4*S*)-hydroxyproline amide **6** may be due to the added effect of the intramolecular hydrogen bond between the hydroxyl and *C*-terminal amide carbonyl groups which should reduce Coulomb repulsion between the *C*-terminal carbonyl and the pyramidalized amide by diminishing the electronic density at the oxygen. The combination of steric effects from the methyl groups at the 3-position and inductive and hydrogen-bonding effects from the hydroxyl group at the 4-position may thus account for the observed order (**6** > **5** > **4**) of isomerization rate constants of the 3,3-dimethyl series.

Although the inductive effect of a 4-position electron withdrawing group has been suggested to accelerate prolyl amide isomerization,[18] we did not observe such an

effect on the rate constants in the natural series which decreased in the order of **1** ≈ **3** > **2**. As mentioned, *cis–trans* isomerization of (2*S*,4*R*)-*N*-acetyl-4-hydroxyproline methyl ester has also been found to be slower than *N*-(acetyl)proline methyl ester in water.[18] Amides **1**-**3** exhibit greater flexibility about the ψ dihedral angle relative to their dimethyl counterparts **4**-**6**. For example, studies of *N*-(acetyl)proline *N'*-methylamide (**1**) in water have indicated the presence of a γ-turn (ψ = 80°) based on the pH dependencies of the *N'*-methyl proton resonances,[35] as well as α-helical (ψ = –50°) and polyproline II-like (ψ = 150°) conformations based on CD and carbon NMR chemical shift data.[26] Since the potential for the *C*-terminal amide to either accelerate or retard isomerization about the *N*-terminal amide is regulated by the ψ dihedral angle, the inductive effects of the 4-position hydroxyl group in amides **2** and **3** should be considered with their influence on the *C*-terminal amide geometry. Rate acceleration may not be observed if the hydroxyl group influences the ψ dihedral angle to favor an orientation predisposed to Coulomb repulsion.[36] As in the case with dimethylhydroxyproline amides **5** and **6**, the faster rates of **3** relative to **2** may be due to the (4*S*)-hydroxyl group's ability to hydrogen-bond with the *C*-terminal carbonyl oxygen and reduce the effects of Coulomb repulsion at $\psi \approx 150°$. Therefore, a combination of ψ dihedral angle geometry, inductive effects and intramolecular hydrogen-bonding may also be responsible for the measured decreasing order **1** ≈ **3** > **2** for the rate constants in the natural series.

Conclusion

Our studies have illustrated the influence of 3-alkyl and 4-hydroxyl substituents on the conformation and isomerization of prolyl amides. In particular, methyl substituents at the proline 3-position were shown to reduce the rate of prolyl amide isomerization in water, presumably by their steric effects on the ψ dihedral angle which may place the *C*-terminal carbonyl oxygen in position to repel the developing lone pair

of the pyramidalized nitrogen by Coulomb interactions. Since these steric interactions should increase with larger *C*-terminal substituents, the replacement of proline by 3,3-dimethylproline is potentially a general method for reducing the rate of *N*-terminal amide isomerization in peptides. Our results with prolyl amides **1-6** may thus help guide future efforts involving the introduction of 3-alkylprolines into protein structures in order to explore the conformation and dynamics of prolyl residues in peptides.

Experimental Section

General: Unless otherwise noted all reactions were run under nitrogen atmosphere and distilled solvents were transferred by syringe. Dioxane, acetic anhydride and CH_3CN were distilled from CaH_2; Et_3N was distilled from BaO; MeOH was distilled from $Mg(OCH_3)_2$. 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 (EI), were obtained by the Université de Montréal Mass Spec. facility. ^1H NMR (300/400 MHz) and ^{13}C NMR (75/100 MHz) spectra were recorded in $CDCl_3$ except as specified. Chemical shifts are reported in ppm (δ units) downfield of internal tetramethylsilane ($(CH_3)_4Si$). The chemical shifts for the carbons of the minor rotamer are reported in parentheses. FT-IR data collection and molecular mechanics calculations with the MacroModel program 5.5x were performed using the protocols summarized in the Experimental Section of reference 12.

Syntheses of (2*S*)-*N*-Acetyl-3,3-dimethylproline, (2*S*,4*R*)- and (2*S*,4*S*)-*N*-Acetyl-3,3-dimethyl-4-hydroxyproline *N*'-Methylamides (4-6) were accomplished using the general protocol outlined below for the synthesis of (2*S*)-*N*-acetyl-3,3-dimethylproline *N*'-methylamide.

(2*S*)-*N*-BOC-3,3-Dimethylproline *N*'-Methylamide (8a) A suspension of (2*S*)-*N*-BOC-3,3-dimethylproline (**7a**, 24 mg, 0.1 mmol), methylamine hydrochloride

(6.7 mg, 0.1 mmol) and triethylamine (0.04 mL, 0.3 mmol) in acetonitrile (2 mL) was treated with TBTU (35 mg, 0.11 mmol), stirred 24 h and evaporated to a residue that was partitioned between EtOAc (2 mL) and brine (2 mL). The aqueous layer was extracted with EtOAc (3 × 3 mL) and the combined organic layers were washed with water (3 × 2 mL), dried, and evaporated to a residue that was purified by silica gel chromatography using 2% MeOH in $CHCl_3$ as eluant. Evaporation of the collected fractions gave 23 mg (90%) of (2*S*)-*N*-BOC-3,3-dimethylproline *N*'-methylamide (**8a**): ^{1}H NMR δ 1.03 (s, 3 H), 1.16 (s, 3 H), 1.43 (s, 9 H), 1.63 (m, 1 H), 1.85 (m, 1 H), 2.8 (d, 3 H, J = 4.5), 3.55 (m, 2 H), 3.73 (s, 1 H), 5.83 (br s, 1 H); ^{13}C NMR δ 23.5, 25.8, 28.1, 28.3, 37.7, 42.1, 45.1, 70.9, 80.3, 154.8, 171.9; HRMS calcd for $C_{13}H_{25}N_2O_3$ (MH^+) 257.1865, found 257.1854.

(2*S*)-*N*-Acetyl-3,3-dimethylproline *N*'-Methylamide (4). (2*S*)-*N*-BOC-3,3-dimethylproline *N*'-methylamide (23 mg, 0.09 mmol) was dissolved in a 2.7 M solution of HCl in dioxane (0.6 mL), stirred 2 h, and evaporated to provide 16 mg (98 %) of the corresponding amino amide hydrochloride: $[\alpha]^{22}_D$ 20.6° (*c* 5.0, MeOH); ^{1}H NMR (CD_3OD) δ 1.0 (s, 3 H), 1.28 (s, 3 H), 1.96 (t, 2 H, J = 7.4), 2.81 (d, 3 H, J = 4.6), 3.43 (m, 2 H), 3.8 (s, 1 H); ^{13}C NMR δ 22.7, 26.5, 26.8, 39.7, 43.4, 44.8, 69.5, 168.1; HRMS calcd for $C_8H_{17}N_2O$ (MH^+) 157.1341, found 157.1350. The amino amide hydrochloride (16 mg, 0.09 mmol) was dissolved in acetic anhydride (1 mL) and stirred at room temperature (heated to 40°C if necessary) until TLC showed complete consumption of the starting material. The volatiles were removed by evaporation under vacuum and the resulting residue was chromatographed with 20% MeOH : EtOAc as eluant. Evaporation of the collected fractions gave 10 mg (60 %) of (2*S*)-*N*-acetyl-3,3-dimethylproline *N*'-methylamide (**4**) as an oil: $[\alpha]^{22}_D$ –14.6° (*c* 0.1, CH_3OH); ^{1}H NMR (major isomer) δ 1.07 (s, 3 H), 1.08 (s, 3 H), 1.65 (m, 1 H), 2.08 (s, 3 H), 2.28 (m, 1 H), 2.79 (d, 3 H, J = 4.8), 3.54 (m, 1 H), 3.72 (m, 1 H), 3.93

(s, 1 H), 6.17 (br s, 1 H); ^{13}C NMR (major isomer) δ 22.1, 23.2, 26.0, 28.1, 37.6, 40.7, 46.7, 69.6, 170.2, 171.4; HRMS calcd for $C_{10}H_{19}N_2O_2$ (MH^+) 199.1447, found 199.1456.

(2*S*, 4*R*)-*N*-Acetyl-3,3-dimethyl-4-hydroxyproline *N*'-Methylamide (5) Amide **5** was prepared from **7b** using the protocol described above. Acylation of methylamine gave (2*S*, 4*R*)-*N*-BOC-3,3-dimethyl-4-hydroxyproline *N*'-methylamide (**8b**): 80%; oil; $[\alpha]^{22}_D$ 19.7° (*c* 0.7, CH_3OH); ^1H NMR δ 0.97 (s, 3 H), 1.16 (s, 3 H), 1.42 (s, 9 H), 2.56 (m, 1 H), 2.8 (s, 3 H), 3.45 (m, 1 H), 3.74 (dd, 1 H, *J* = 5.4, 11), 3.9 (s, 1 H); ^{13}C NMR δ 21, 25.8, 28.3, 38.5, 51.6, 69.5, 77.2, 80.3, 155, 171.6; HRMS calcd for $C_{13}H_{25}N_2O_4$ (MH^+) 273.1814, found 273.1803. Acid solvolysis removed the BOC group to give (2*S*, 4*R*)-3,3-dimethyl-4-hydroxyproline *N*'-methylamide hydrochloride (90%): ^1H NMR δ (CD_3OD) 0.89 (s, 3 H), 1.25 (s, 3 H), 2.82 (s, 3 H), 3.2 (d, 1 H, *J* = 12.6), 3.7 (dd, 1 H, *J* = 3.3, 12.6), 4.0 (d, 1 H, *J* = 3.5), 4.05 (s, 1 H), 8.3 (br s, 1 H). Acetylation then provided **5** in 97% yield as an oil: $[\alpha]^{22}_D$ 34.6° (*c* 1.7, MeOH); ^1H NMR (CD_3OD) δ 0.96 (s, 2.6 H), 0.97 (s, 0.4 H), 1.07 (s, 2.6 H), 1.11 (s, 0.4 H), 1.9 (s, 0.4 H), 2.06 (s, 2.6 H), 2.71 (s, 2.6 H), 2.76 (s, 0.4 H), 3.35 (dd, 1 H, *J* = 6.5, 10.3), 3.79 (dd, 0.1 H, *J* = 6.7, 12), 3.89 (dd, 0.9 H, *J* = 6.7, 10.3), 4.01 (s, 1 H), 4.07 (t, 1 H, *J* = 6.6); ^{13}C NMR (CD_3OD) δ 21.4, 21.55 (21.62), 22.1 (22.3), 26.1 (26.2), 44.6 (46.6), (52.4) 53.6, 70.4 (72), (75.6) 76.6, 172.7 (172.9), (173) 173.3; HRMS calcd for $C_{10}H_{19}N_2O_3$ (MH^+) 215.1396, found 215.1386.

(2*S*, 4*S*)-*N*-Acetyl-3,3-Dimethyl-4-hydroxyproline *N*'-Methylamide (6) Amide **6** was synthesized using the above protocol on **7c**. Acylation of methylamine gave (2*S*, 4*S*)-*N*-BOC-3,3-dimethyl-4-hydroxyproline *N*'-methylamide (**8c**): mp 199-200°C; $[\alpha]^{22}_D$ 33.2° (*c* 1, CH_3OH); ^1H NMR δ 1.07 (s, 3 H), 1.11 (s, 3 H), 1.47 (s, 9 H), 2.81 (d, 3 H, *J* = 4.7), 3.63 (m, 2 H), 3.75 (dd, 1 H, *J* = 5, 12), 3.83 (s, 1 H),

4.4 (br s, 1 H), 7.0 (br s, 1 H); ^{13}C NMR δ 18.4, 26.2, 27.7, 28.7, 46.7, 54.8, 71.4, 78.5, 81.8, 156.2, 175.5; HRMS calcd for $C_{13}H_{25}N_2O_4$ (MH^+) 273.1814, found 273.1806. Solvolysis of the BOC group gave a 98% yield of (2*S*, 4*S*)-3,3-dimethyl-4-hydroxyproline *N*'-methylamide hydrochloride: ^1H NMR δ (CD_3OD) 0.87 (s, 3 H), 1.23 (s, 3 H), 2.82 (s, 3 H), 3.14 (dd, 1 H, *J* = 8, 11), 3.55 (dd, 1 H, *J* = 7, 11), 3.91 (s, 1 H), 4.09 (t, 1 H, *J* = 7), 8.2 (br s, 1 H); ^{13}C NMR δ (CD_3OD) 16.1, 24.7, 26.7, 26.8, 45.4, 68, 77.5, 167.7. Acetylation then provided **6** in 83% yield as a white solid: mp 178°C; $[\alpha]^{22}_D$ 41.4° (*c* 1.6, MeOH); ^1H NMR δ (major isomer) 1.1 (s, 3 H), 1.15 (s, 3 H), 2.17 (s, 3 H), 2.83 (d, 3 H, *J* = 4.9), 3.7 (d, 1 H, *J* = 11.3), 3.76 (d, 1 H, *J* = 4.7), 3.92 (dd, 1 H, *J* = 11.3, 4.7), 4.0 (s, 1 H), 6.83 (s, 1 H); ^{13}C NMR (CD_3OD) δ 18.4 (18.7), (22) 22.1, 26.3 (26.4), 27.7 (27.9), 45.6 (47.7), (55.5) 56.6, 70.8 (72.4), (78.1) 79.6, 173, (174.4) 174.6; HRMS calcd for $C_{10}H_{19}N_2O_3$ (MH^+) 215.1387, found 215.1396. In the acetylation step to synthesize hydroxyprolyl amides **2**, **3**, **5** and **6**, some amounts of *O*-acetylation were detected and a selective hydrolysis was performed using potassium carbonate in methanol at room temperature to furnish the desired *N*-acetyl-4-hydroxyproline *N*'-methylamides after chromatography with MeOH in EtOAc as eluant.

NMR Experiments and Data Analysis

Samples were prepared by dissolving compounds in distilled and deionized H_2O and/or D_2O at concentrations between 1×10^{-2} and 5×10^{-2} M and purged with nitrogen gas in NMR tubes used for the experiments. All experiments were carried out on either Bruker AMX600 or DMX600 MHz spectrometers, both equipped with selective excitation units and ^1H broadband heteronuclear probes. Selective inversion of Cα *trans* or *cis* peaks were done with gaussian pulses centered on resonance (+/-) 25 Hz. Relaxation delays of 20 seconds and inversion-recovery delays of between 1 msec and 20 seconds were used. Data for each inversion recovery point was averaged over

32 to 128 points, depending on the concentration of the compound used. For each compound at 60°C, inversion-recovery results were collected with selective inversion of the Cα *trans* peak. Data were fitted according to the protocol described in the Experimental Section of reference 12.

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Supporting Information Available: The ^{1}H, ^{13}C and DEPT NMR spectra of **2-6** and **8**; plots of intensity vs mixing time for the magnetization transfer experiments on **1-6**; the FT-IR spectrum of the N–H stretch region of **4**; crystallographic data for **6**; maps of energy vs conformation for **1-6** (29 pages). This material is available on microfiche and 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), 3.73 (m, 1 H), 4.38 (m, 2 H); ^{13}C NMR (minor isomer is in parentheses) δ (22) 22.3, 26.4 (26.5), 38.6 (40.5), (56.2) 57.5, 60.6 (62.1), (69.8) 71.2, 172.9 (173.1), (175.1) 175.3.

21. The structure of **6** was solved and refined at l'Université de Montréal X-ray facility by the SHELX programs (SHELXS86 and SHELXL93): $C_{10}H_{18}N_2O_3$; M_r = 214.26; triangular prism, colorless crystal; space group $P2_1$; unit cell dimensions (Å) a = 6.346(1), b = 8.737(2), c = 10.821(2); β = 105.72(2)°; volume of unit cell 577.5(2) $Å^3$; Z = 2; R_1 = 0.0264 for I>2 sigma(I), wR_2 = 0.0737 for all data; GOF = 1.060. The author has deposited the atomic coordinates for the structure of **6** with the Cambridge Crystallographic Data Center. The coordinates can be obtained, on request, from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK.

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30. The ψ, ϕ and ω values for the calculated energy minima of the *trans*- and *cis*-isomers in **1-6** were as follows: **1** *trans*-isomer; 128°, –57°, –179°; **1** *cis*-isomer; 131°, –59°, 0°; **2** *trans*-isomer; 162°, –57°, 177°; **2** *cis*-isomer; 164°, –60°, –6°; **3** *trans*-isomer; 152°, –72°, 180°; **3** *cis*-isomer; 154°, –72°, –1°; **4** *trans*-isomer; 154°, –71°, 180°; **4** *cis*-isomer; 155°, –72°, –2°; **5** *trans*-isomer; 151°, –72°, 180°; **5** *cis*-isomer; 152°, –73°, –2°; **6** *trans*-isomer; 141°, –71°, 180°; **6** *cis*-isomer; 142°, –72°, –2°. Maps constructed for *N*-acetylproline *N'*-methylamides **1-6** by plotting the minimum energy value at each 30° interval against the values for the ψ- and ω-dihedral angles are included in the Supporting Information.

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36. It is interesting to note that the presence of a 4-position hydroxyl group increased the magnitude of the ψ dihedral angle from $\psi \approx 130°$ to $\psi \approx 153°$ to $\psi \approx 163°$ in the minimum energy conformations of the proline amides going from **1** to **3** to **2**.

Troisième partie:
L'utilisation des 5-*tert*-butylprolines afin de contrôler la nucléation de l'hélice PPI en milieu aqueux

Chapitre 5

Ce chapitre est constitué d'une brève introduction des articles 6 et 7 qui est suivie de la version intégrale des articles 6 et 7.

Introduction

L'influence des effets stériques sur la géométrie hélicoïdale et l'interconversion de polyproline type II (PPII) à polyproline type I (PPI) a été examinée par la synthèse et l'analyse de dimères et d'hexamères de proline contenant jusqu'à trois résidus (2*S*, 5*R*)-5-*tert*-butylprolines. Dans le cadre des dimères, le substituant stériquement encombrant 5-*tert*-butyle a exercé une influence significative sur la géométrie locale de l'amide de proline de telle sorte que l'isomère *trans* prédominant dans le *N*-(acétyl)prolyl-prolinamide (**1**) a été converti à 63% d'isomère *cis* dans *N*-(acétyl)prolyl-5-*tert*-butylprolinamide (**2**) telle que mesuré par spectroscopie RMN ^{1}H. Similairement la présence d'un groupe 5-*tert*-butyle sur le résidu en position *C*-terminale de l'hexamère Ac-Pro_5-5-*tert*-BuPro-NH_2 **4** a produit, localement au niveau de l'amide de 5-*tert*-butylprolyle, une population de 61% d'isomère *cis* dans l'eau. En dépit de la présence de la géométrie locale d'amide *cis*, les amides de proline se trouvant en direction de la position *N*-terminale de **4** ont conservé leur géométrie *trans* telle que déterminée par spectroscopie NOESY. L'analyse conformationnelle par RMN ^{13}C et le dichroïsme circulaire a indiqué que Ac-Pro_6-NH_2 **3** adopte une hélice polyproline type II contenant que des amides *trans* dans l'eau. L'augmentation de la quantité de 5-*tert*-butylproline de un à trois résidus dans les hexamères **4-6** a produit une déstabilisation graduelle de la géométrie hélicoïdale polyproline type II telle qu'observé par dichroïsme circulaire dans l'eau. Cependant aucun des spectres obtenus n'indique une conversion complète à une hélice polyproline de type I. Les implications de ces résultats sont discutées en regard des mécanismes théoriques d'interconversion hélicoïdale de

polyproline précédemment proposés qui ont suggéré que cette interconversion se produit soit par le biais d'une isomérisation coopérative des amides de proline de la position *C* vers la position *N* terminale, soit par le biais d'intermédiaires conformationnels composés de séquences disperses d'isomères *cis* et *trans* d'amides de proline.

Publication 6 :

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The use of 5-*tert*-butylproline to study nucleation of polyproline type I conformation.

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Introduction

Prolyl amide isomer geometry dictates two kinds of helical conformations in polyproline [1]. The amides *N*-terminal to proline are all *cis*-isomers in type I polyproline (PPI) which is a right handed helix composed of 3.3 residues per turn with an axial translation of 190 pm. Type II polyproline (PPII) is a left handed helix

comprised of 3 residues per turn with an axial translation of 320 pm due to the prolyl amides existing as *trans*-isomers. Peptides adopting PPII conformations have been shown to bind as ligands to kinases, SH2 and SH3 domains, and MHC class II proteins as observed by NMR spectroscopy and X-ray crystallography [2]. Because of its conformational lability, PPI has been mostly studied in the solid state by X-ray diffraction of amorphous precipitate and little is known about the importance of this peptide structure and its relevance in protein biology [1-3]. In order to better understand the physical and biological properties of PPI, we are using the steric interactions of 5-alkylprolines to augment *cis*-isomer populations in polyproline helices. We report attempts to induce PPI geometry in water by incorporating 5-*tert*-butylprolines (tBuPros) into polyproline hexamers as studied by NMR and CD spectroscopy.

Results and Discussion

Ac-Pro-Pro-NH_2 (**1**) Ac-Pro-tBuPro-NH_2 (**2**)

Ac-Pro-Pro-Pro-Pro-Pro-Pro-NH_2 (**3**)

Ac-Pro-Pro-Pro-Pro-Pro-tBuPro-NH_2 (**4**)

Ac-Pro-Pro-tBuPro-Pro-Pro-tBuPro-NH_2 (**5**)

Ac-Pro-tBuPro-Pro-tBuPro-Pro-tBuPro-NH_2 (**6**)

Proline oligomers **1**-**6** were prepared to examine both the local and global effects of tBuPro on polyproline conformation. Fmoc-Pro-tBuPro (**7**) was first synthesized from (2S,5R)-*N*-Boc-5-*tert*-butylproline methyl ester (**8**) [4]. Boc group removal with TFA in DCM, transesterification with allyl alcohol and *p*-TsOH in benzene, liberation of the amine with $NaHCO_3$, coupling to Fmoc-Pro using BOP-Cl and DIPEA in DCM, and

finally allyl ester removal with $Pd(Ph_3)_4$ and *N*-methylanaline in THF/DMSO/HCl afforded **7** in xx % overall yield from **8**. Proline oligomers **1**-**6** were synthesized on Rink MBHA resin (0.67 mmol/g) which was swollen in DCM for 20 min, then treated with 20% piperidine in DMF to effect Fmoc deprotection. Fmoc-Pro and **7** were introduced using TBTU and DIPEA in DMF for 2 h. Final oligomers were *N*-acetylated with excess Ac_2O and pyridine in DMF for 2 h, cleaved with TFA/H_2O 95:5 for 30 min, and purified by semi-preparative RPHPLC on a C18 column with a linear gradient of 0-25% CH_3CN in H_2O with 0.1% TFA.

Dipeptides **1** and **2** were first examined by proton NMR in water to dissect the influence of the *t*-butyl group on the prolyl amide equilibrium. The Pro-Pro amide was primarily in the *trans*-isomer in the spectrum of **1** as assigned based on a strong nOe between Hα of the *i* and Hδ of the *i*+1 residues. The spectrum of **2** indicated a 37:63 ratio of *trans*- and *cis*-isomers as assigned based on nOes between the Pro Hα and tBuPro Hδ for the *trans* and between the Pro Hα and tBuPro Hα for the *cis*-isomer.

Hexapeptides **3**-**6** were studied using CD and NMR spectroscopy in water. The proton and carbon NMR spectra of **3** were representative of PPII conformation [3,5]. A strong nOe was apparent between Hα and Hδ signals in the proton spectrum of **3**. The spectrum of **4** depicted a PPII-like conformation in which a *cis–trans* amide equilibrium was present *N*-terminal to the tBuPro residue. An nOe between the Pro Hα and tBuPro Hδ for the *trans*-isomer was observed for **4** and a 39:61 ratio of *trans*- and *cis*-isomers was ascertained. The CD spectra of **3** confirmed a PPII structure with a strong negative band at 204 nm and a weak positive band at 229 nm [1] (Figure 1). The CD spectra for **4**-**6** deviated from PPII-like curves and as more tBuPro residues were introduced into the hexapeptide, the negative band diminished in intensity shifting to higher wavelength and the positive band disappeared; however, PPI-like spectra were not observed in water.

In conclusion, tBuPro was readily introduced into polyproline oligomers. The *tert*-butyl substituent augmented prolyl amide *cis*-isomer in dimer **2** and hexamer **4**; however, the global conformation of **4** remained mostly PPII in water. As tBuPro content increased PPII geometry was disturbed, yet PPI was not favored in water. Thus, tBuPro exerted mostly local effects on prolyl amide geometry and not global effects on polyproline helical conformation in water. Alternative solvents and tBuPro 5-position stereochemistry are now being examined because they may enhance nucleation of PPI geometry [1,3].

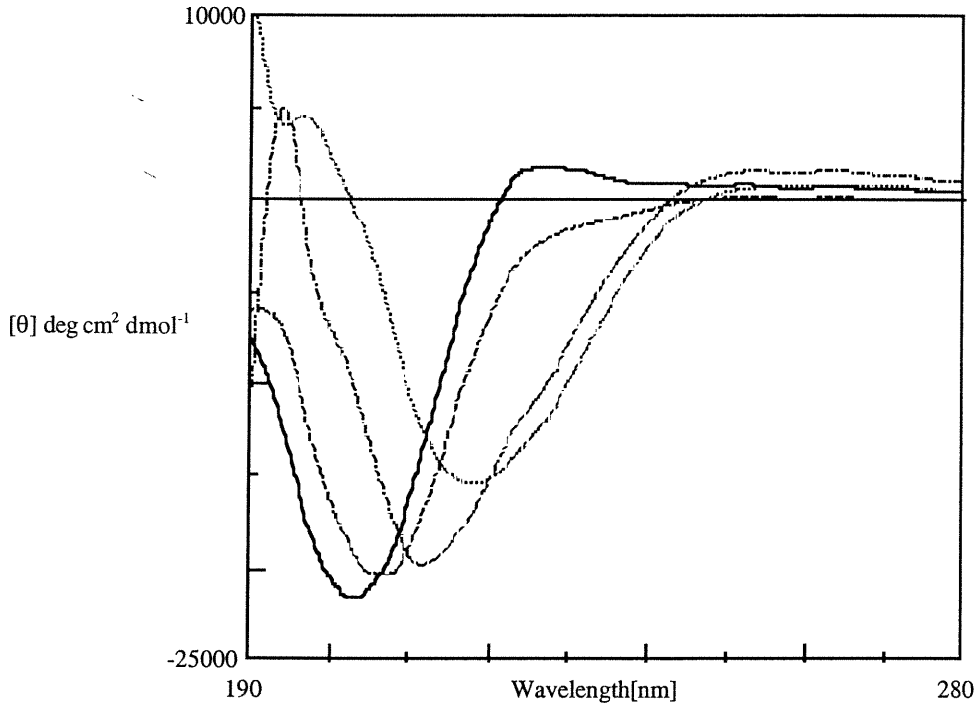

Figure 1. CD spectra of **3** (—), **4** (---), **5** (···) and **6** (···).

Acknowledgments

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Publication 7:

Eric Beausoleil; William D. Lubell

Biopolymers **2000**, *53*, 249-256

An Examination of the Steric Effects of 5-*tert*-Butylproline on the Conformation of Polyproline and the Cooperative Nature of Type II to Type I Helical Interconversion.

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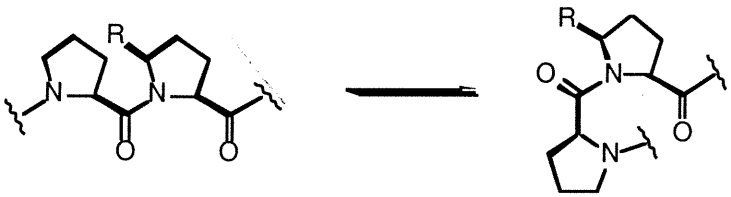
Abstract

The influence of steric effects on the helical geometry and the interconversion of type II to type I polyproline in water was examined by the synthesis and analysis of proline dimers and hexamers containing up to three (2*S*, 5*R*)-5-*tert*-butylproline residues. In the dimers, the bulky 5-*tert*-butyl substituent was found to exert a significant influence on the local prolyl amide geometry such that the predominant *trans*-isomer in *N*-(acetyl)prolyl-prolinamide (**1**) was converted to 63% *cis*-isomer in *N*-(acetyl)prolyl-5-*tert*-butylprolinamide (**2**) as measured by ^{1}H NMR spectroscopy. Similarly, the presence of a 5-*tert*-butyl group on the *C*-terminal residue in the polyproline hexamer Ac-Pro$_5$-*t*-BuPro-NH$_2$ **4** produced a local 5-*tert*-butylprolyl amide population containing 61% *cis*-isomer in water. Inspite of the presence of a local prolyl *cis*-amide geometry, the down-stream prolyl amides in **4** remained in the *trans*-isomer as determined by NOESY spectroscopy. Conformational analysis by ^{13}C NMR and

circular dichroism spectroscopy indicated that Ac-Pro_6-NH_2 **3** adopt the all *trans*-amide polyproline type II helix in water. As the amount of 5-*tert*-butylproline increased from one to three residues in hexamers **4-6** a gradual destabilization of the polyproline type II helical geometry was observed by circular dichroism spectroscopy in water; however, no spectrum was obtained indicative of a complete conversion to a polyproline type I helix. The implications of these results are discussed with respect to the previously proposed theoretical mechanisms for the helical interconversion of polyproline which has been suggested to occur by either a cooperative *C*- to *N*-terminal isomerization of the prolyl amide bonds or via a conformational intermediate composed of dispersed sequences of prolyl amide *cis*- and *trans*-isomers.

Introduction

Poly-L-proline adopts two kinds of helical conformations subject to prolyl amide geometry. Type I polyproline is a right handed helix with an axial translation of 190 pm composed of 3.3 prolyl residues per turn, linked by *cis*-amide bonds.[1] Type II polyproline is a left handed helix with an axial translation of 320 pm comprised of 3 prolyl residues per turn, joined by *trans*-peptide bonds.[2]



***trans*-isomer** R = H, *t*-Bu ***cis*-isomer**

Figure 1. Proline–Proline Peptide Bond Isomer Equilibrium.

Interconversion between type I and type II polyproline involves isomerization of prolyl amide geometry and is usually effected by dissolving oligomer in solvent.[3-12] Often termed mutarotation, helical interconversion has been suggested to involve either cooperative motions of the peptide bonds with amide isomerization progressing from the helix terminus proceeding inward down the sequence,[3-6] or an intermediate conformation composed of discontinuous sequences of prolyl amide *cis*- and *trans*-isomers.[13,14] For example, the *cis*-to-*trans* transition was proposed to prefer to start at the *N*-terminal end and the *trans*-to-*cis* transition to nucleate at the *C*-terminal end.[6,7] The cooperative transitions from type I-to-type II and from type II-to-type I polyproline in a benzyl alcohol : *n*-butanol system have also been described using the Zimm-Bragg analysis in which type I helix initiation is represented by an equilibrium constant σ, the nucleation parameter, which in the case of poly-L-proline of molecular weights between 3300 and 21000 g/mole, was estimated to be in the order of 10^{-5} and represented an unlikely event.[6] The propagation step, in which a type I helix unit is

added to an existing type I helix segment, was predicted to be less difficult than the nucleation step.[6] In addition, an analysis using molecular mechanics and a Gaussian coupling function in the absence of solvent,[7] suggested that the free energy barrier between type II and type I is at a minimum when at least three prolyl residues act in a cooperative motion. On the contrary, an examination of the type I-to-type II transition in D_2O by FTIR and vibrational circular dichroism spectroscopy has recently provided evidence to suggest an intermediate conformation consisting of discontinuous sequences of prolyl amide *cis*- and *trans*-isomers.[13] In addition to being at odds with the cooperative mechanism, the spectroscopic data support older studies that used viscosity measurements to advocated a similar transitional conformation during mutarotation.[14]

Alkyl groups attached at different positions on the proline ring can exhibit steric effects that alter the prolyl amide geometry and the rate of prolyl amide isomerization.[15-22] In proline oligomers, 4-position ring substituents have been shown to interact minimally with the helical structure.[23-26] Alkyl 2- and 5-position substituents are predisposed to interact with the proline ψ and ω dihedral angle geometries and were initially analyzed in pioneering studies of high molecular weight polyproline synthesized by polymerization of amino acid *N*-carboxyanhydrides.[27-31] A comparison of studies on high molecular weight polyprolines and model prolyl peptides, shows the effect of small alkyl groups on the helical nature of polyproline[27-29] to parallel the influence of similar 2- and 5-position substituents on the amide geometry of *N*-(acetyl)alkylproline *N'*-methylamides.[15,16] For example, in the same manner that poly(2-methylproline) was found to be "locked" into a type II helix that did not interconvert into a type I conformation,[27] only the *trans*-amide isomer (>98%) was observed in the ^{13}C NMR spectrum of *N*-acetyl-2-methylproline *N'*-methylamide.[15] Inasmuch as 5-methyl and 5-ethyl pyrrolidine ring substituents did not perturb

polyproline helicity,[28,29] a 5-position methyl group had no influence on the ratio of amide isomers in *N*-acetyl-*cis*-5-methylproline *N'*-methylamide and caused only a 5% increase in the amide *cis*-isomer population in water for *N*-acetyl-*trans*-5-methylproline *N'*-methylamide.[16] On the other hand, bulkier 5-position substituents appeared to impose more pronounced steric effects in polyproline than in *N*-(acetyl)alkylproline *N'*-methylamide. For example, the type I-like CD spectrum of poly(*cis*-5-*iso*-propylproline) was not effeced by conditions expected to catalyze conversion to a type II conformation;[30] however, equal amide *cis*- and *trans*-isomer populations were observed in the NMR spectrum of *N*-acetyl-*cis*-5-*tert*-butylproline *N'*-methylamide in water.[20]

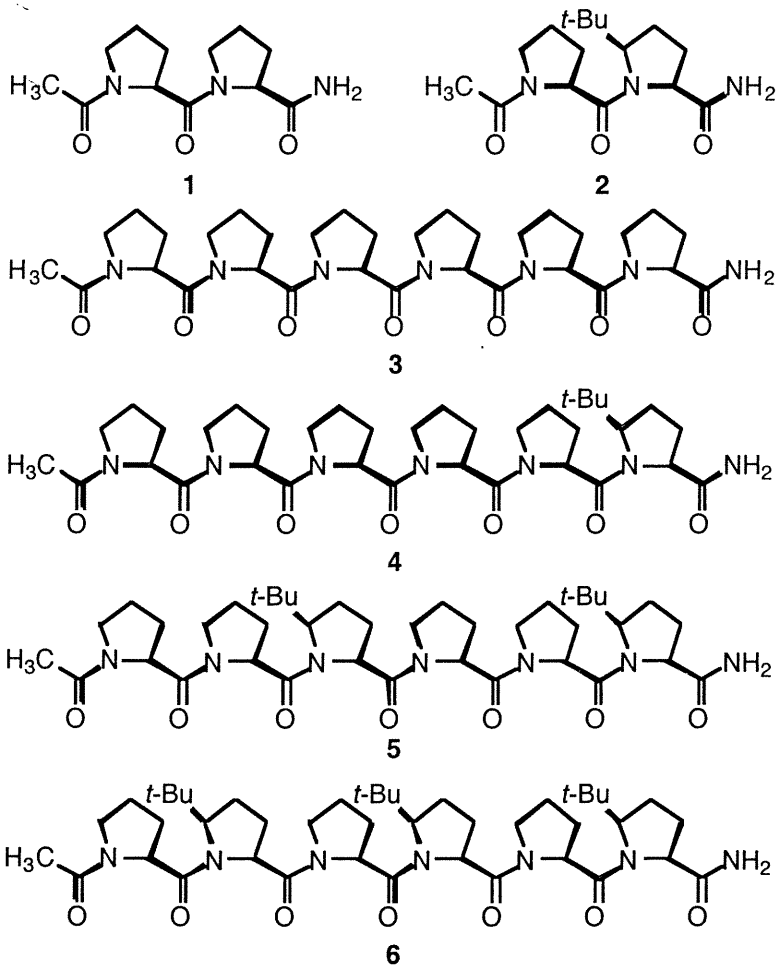
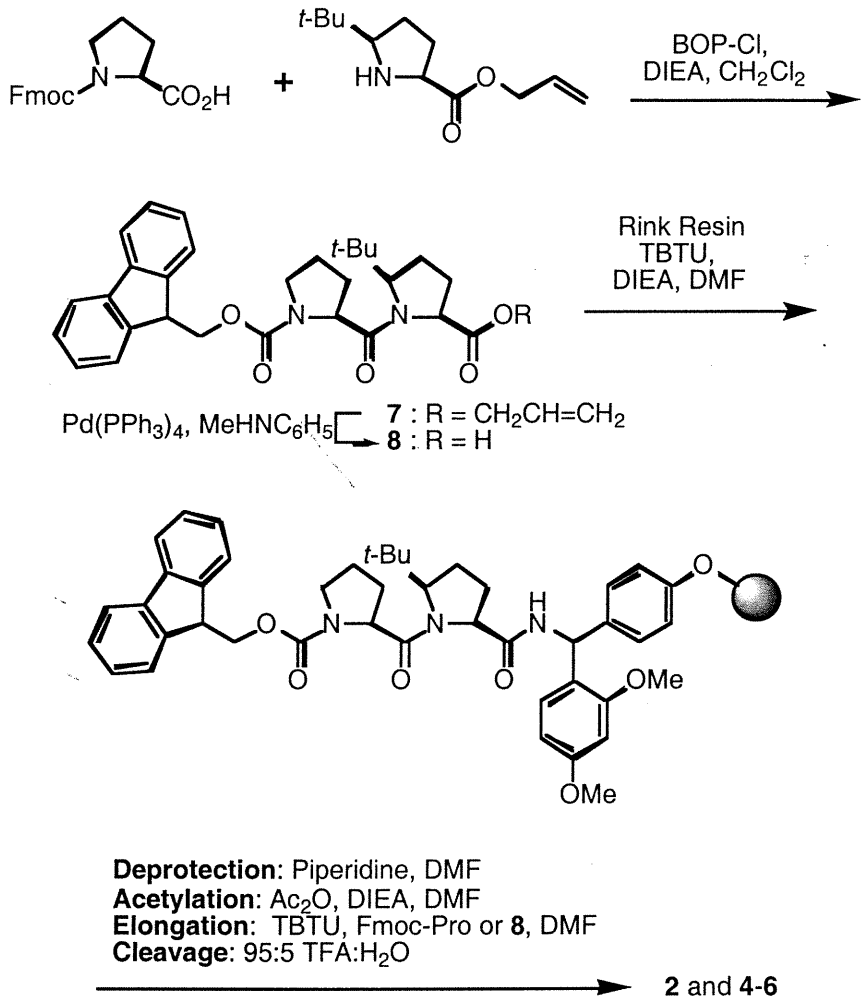


Figure 2. Proline Oligomers 1-6.

In this report, the effect of a 5-*tert*-butyl substituent on the amide isomer equilibrium of proline oligomers was used to examine if local influences of steric bulk on amide bond geometry have global consequences on the helical conformation of poly-L-proline. We have probed the hypothesis that helical interconversion is a cooperative process by examining the capacity of 5-*tert*-butylproline to nucleate the helical conformation of type I polyproline. We conducted our examination using enantiopure (2*S*, 5*R*)-5-*tert*-butylproline,[32] the *cis*-diastereomer, because poly(*cis*-5-*iso*-propylproline) of 2000-7000 g/mole was observed by CD to adopt a conformationally fixed type I-like helix,[30] and because copolymers containing proline and *cis*-5-*iso*-propylproline were found to be incapable of the "usual mutarotation process".[30b] Instead of studying high molecular weight polymer,[27-31] a systematic approach was taken to evaluate the influence of a 5-position substituent on proline oligomer conformation. Initially, *N*-(acetyl)prolyl-prolinamide (**1**) and *N*-(acetyl)prolyl-*cis*-5-*tert*-butylprolinamide (**2**) were synthesized and examined by NMR spectroscopy to ascertain the local influence of the 5-*tert*-butyl substituent on the Pro–Pro peptide bond (Figure 2). The outcome of the bulky 5-position substituent on polyproline helicity was subsequently studied on hexamers **3**-**6** in which proline was replaced by (2*S*, 5*R*)-5-*tert*-butylproline at the peptide *C*-terminus, at the *C*-terminals of each triad, a complete turn, and at the *C*-terminal of every two residues. Poly-L-proline hexamers were examined to guarantee an initial helical conformation because both spectroscopic[33,34] and computational[35] studies have indicated that polyproline begins to adopt a helical geometry at >4 residues. Because the *trans*-to-*cis* transition was suggested to nucleate at the *C*-terminal end and proceed inward,[6,7] we positioned (2*S*, 5*R*)-5-*tert*-butylproline at the *C*-terminals of the hexamer and repeating trimer and dimer units to induce cooperative nucleations of type I helicity. The *N*-terminal residue was capped as an acetamide and the *C*-terminal as a carboxamide in peptides **1**-**6**, because

ionic end groups were previously suggested to favor the *cis*-amide isomer by end-to-end intramolecular hydrogen bonding in short proline oligomers.[12,36,37] Water (H_2O and D_2O) was chosen as solvent for the conformational analyses of **1-6** because of its physiological importance and propensity to favor type II polyproline.[3] Since 5-*tert*-butyl substituents are expected to augment the *cis*-amide isomer population,[20] water serves as a rigorous environment for testing their potential to induce type I polyproline geometry. Our approach was thus designed to evaluate the consequence of the 5-*tert*-butyl substituent on the local prolyl amide equilibrium and overall helical conformation of the proline oligomer. Asuming the transition from type II-to-type I polyproline is cooperative,[6,7] then formation of a type I helix should be initiated in water by the 5-*tert*-butyl substituent favoring the *cis*-amide bond and thereby lowering the energy of activation for the *trans*-to-*cis* isomerisation of the down-stream prolyl amides. Alternatively, in a non-cooperative process, the 5-*tert*-butyl substituent may simply favor local prolyl *cis*-amide bonds without inducing helical interconversion.

Results



Scheme 1. Synthesis of Proline Oligomers 2 and 4-6 with 5-*tert*-Butylproline (amide *trans*-isomers shown).

Prolyl peptides **1-6** were synthesized by a combination of solid- and solution-phase methods. Automated peptide synthesis was employed to prepare *N*-Ac-(Pro)$_2$-NH_2 (**1**) and *N*-Ac-(Pro)$_6$-NH_2 (**3**) on deprotected 4-(2',4'-di-methoxyphenyl-Fmoc-aminomethyl)-phenoxymethyl-polystyrene resin (Rink resin)[38] by sequential couplings of *N*-(Fmoc)proline[39] using the onium salt-based coupling reagent TBTU[40] in DMF, followed by acetylation of *N*-terminal position with acetic anhydride and peptide cleavage with TFA.[41] *N*-Acylation of 5-*tert*-butylproline was achieved by coupling of

5-*tert*-butylproline allyl ester[42] with *N*-(Fmoc)proline using *N*,*N*'-bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOPCl)[43] in CH_2Cl_2 at 5 °C and afforded protected dipepeptide **7** in 77% yield after silica gel chromatography. Palladium-catalyzed cleavage of allyl ester **7** furnished *N*-(Fmoc)prolyl-*cis*-5-*tert*-butylproline (**8**) in 90% yield (Scheme 1).[44] Dipeptide **8** was then coupled to Rink resin using TBTU and prolyl peptides **2** and **4**-**6** were synthesized by employing both *N*-(Fmoc)proline and dipeptide **8** in the solid-phase protocol described for **1** and **3**. After resin cleavage, the final prolyl peptides **1**-**6** were purified on a reverse phase C_{18} column by HPLC and their composition was verified by fast atom bombardment mass spectrometry as described in the experimental section.

The influence of the *tert*-butyl group on the prolyl amide equilibrium was first examined by comparing the ^{1}H NMR spectra of prolyl dipeptides **1** and **2** in D_2O. The amide bond between the two prolyl residues in Ac-Pro-Pro-NH_2 (**1**) existed predominantly in the *trans* isomer exhibiting strong nOe between the α-proton of the *N*-terminal residue and the δ-protons of the *C*-terminal proline in the NOESY spectrum of **1**. On the other hand, a 37:63 ratio of amide *trans*- and *cis*-isomers was measured in the spectrum of Ac-Pro-5-*t*-BuPro-NH_2 (**2**) by integration of the *tert*-butylproline δ-proton signals at 4.25 and 4.15 ppm for the respective *trans*- and *cis*-isomers. Strong nOes were observed between the proline α-proton and the δ-proton of 5-*tert*-butylproline in the *trans*-amide isomer and between the proline and 5-*tert*-butylproline α-protons in the *cis* isomer in the NOESY spectrum of **2**. The larger steric bulk of the pyrrolidine ring resulted in a greater increase of the *cis*-amide isomer population in **2** relative to that observed for (2*S*, 5*R*)-*N*-actetyl-5-*tert*-butylproline *N*'-methylamide.[20]

The β- and γ-carbon NMR signals of the non-terminal pyrrolidine rings appear respectively at 32.2 and 22.5 ppm in the spectrum of type I polyproline and at 28.6 and 25.4 ppm in the spectrum of type II polyproline in water.[11] In the ^{13}C NMR spectrum

of Ac-$(Pro)_6$-NH_2 **3** in D_2O, signals for the β- and γ-carbons were consistent with a poly-proline type II structure and observed respectively at 29.3-29.5 and 25.9-26.1 ppm as described previously for the longer proline oligomer Ac-$(Pro)_9$-NH_2 that was also found to adopt a type II conformation in water.[25] A strong nOe was observed between the Hα and Hδ signals in the NOESY spectrum of **3** and indicated the all *trans*-amide conformation. This NMR data combined with the CD spectrum described below demonstrated that Ac-$(Pro)_6$-NH_2 **3** adopted a type II polyproline helix in D_2O.

Since the 5-*tert*-butyl group had significantly increased the *cis*-amide isomer population in dipeptide **2**, we examined next the NMR spectra of oligomers **4-6** to study the consequence of 5-*tert*-butylproline on poly-proline conformation. Although we could not interpret the NMR spectra of Ac-(Pro-Pro-5-*t*-BuPro)$_2$-NH_2 (**5**) and Ac-(Pro-5-*t*-BuPro)$_3$-NH_2 (**6**) due to multiple conformers, the ^1H NMR spectrum of Ac-$(Pro)_5$-5-*t*-BuPro-NH_2 (**4**) depicted a poly-proline type II conformation in which a *cis-trans* amide isomer equilibrium was present *N*-terminal to the 5-*tert*-butylproline residue. Assignment of the amide *trans*-isomer was made on the observation of a strong nOe between the δ-proton of the 5-*tert*-butylprolyl residue (4.27 ppm) and the α-proton of the neighboring prolyl residue (4.75 ppm) in the NOESY spectrum of **4**. Integration of the 5-*tert*-butylproline δ-proton signals at 4.12 and 4.27 ppm in the spectrum of **4** indicated a 39:61 ratio of *trans*- and *cis*-isomers about the Pro–5-*t*-BuPro amide bond. Strong nOes between the α-proton signals at 4.65 ppm and the δ-proton signals at 3.75 ppm indicated that the other amide bonds between the prolyl residues in poly-proline **4** existed as amide *trans*-isomers.

Figure 3. Circular Dichroism Spectra of Proline Hexamers 3-6.

Because the NMR spectra of Ac-(Pro-Pro-5-*t*-BuPro)2-NH_2 (**5**) and Ac-(Pro-5-*t*-BuPro)3-NH_2 (**6**) in water proved difficult to interpret, we used instead circular dichroism (CD) spectroscopy to analyze the conformations of hexamers **3-6** in water at

5°C. The CD spectrum of type I poly-L-proline is charaterized by a medium intensity band at 199 nm, a strong positive band at 215 nm and a weak negative band at 232 nm.[9] The CD spectrum of type II poly-L-proline exhibits a strong negative band at 206 nm and a weak positive band at 226 nm.[9] The CD spectrum of Ac-$(Pro)_6$-NH_2 (**3**) was characteristic of the poly-proline type II helix exhibiting a strong negative band at 204 nm and a weak positive band at 229 nm (Figure 2). The CD spectra of **4-6** deviated from that of **3** on incorporation of 5-*tert*-butylprolines into the hexamer indicating a perturbation of the type II polyproline helical conformation. The negative band diminished in intensity shifting to higher wavelength and the positive band disappeared as more 5-*tert*-butylprolines were introduced into the hexapeptide. Finally, the CD spectrum of Ac-(Pro-5-*t*-BuPro)$_3$-NH_2 **6** exhibited a weak positive band at 195 nm and a negative band at 221 nm (Figure 2). This spectrum was similar to that exhibited by poly(*trans*-5-*iso*-propylproline) of 2000-5600 g/mole in a solution of 10% TFA in TFE and was previously attributed to a type I helix possessing amide *trans*-isomers at the *N*-terminus.[30b]

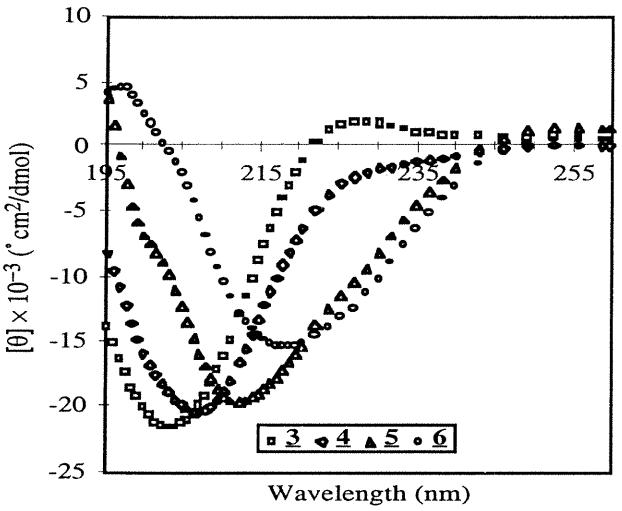


Figure 3. CD Spectra in H_2O of **3**, **4**, **5**, **6**.

Discussion

Proline-rich peptide sequences play imporant structural roles in many natural proteins such as collagen,[45] the nucleocapsid of herpes virus,[46] and the repetetive domains of maize Glutelin-2.[9] Peptides adopting polyproline type II conformation function as recognition elements that bind to kinases, SH2 and SH3 domains, and MHC class II proteins as observed by NMR spectroscopy and X-ray crystallography.[47] Oligoproline assemblies have also served as frameworks for the construction of molecular devices that perform electron transfer and photochemical energy conversion.[24-26,48] Moreover, polyprolines are constained examples of polypeptoids, oligo-*N*-substituted-glycines, that are suggested to adopt secondary structures similar to type I polyproline and have been recently employed as materials for gene delivery.[49,50] These examples illustrate the need for greater understanding of the factors controling polyproline conformation to construct proline oligomers having enhanced biological activity, improved chemical reactivity and refined physical attributes.

Steric interactions play a critical role in controlling the helical conformation of polyproline in solution due to the absence of hydrogen bonding. Earlier work on the synthesis and analysis of high molecular weight poly(2-methylproline) and poly(*cis*-5-*iso*-propylproline) demonstrated by CD that these continuous arrays of alkylprolines adopt respectively type II and type I-like helical conformations that do not interconvert.[27,30] In the present work, we have evaluated the effect of introducing single alkylproline residues at different positions in polyproline by the synthesis of oligoproline hexamers possessing one to three *tert*-butylproline residues and their analysis in water. Both ^{13}C NMR and circular dichroism spectroscopy confirmed that Ac-Pro_6-NH_2 (**3**) existed in a polyproline type II conformation in water. The local

influence of the 5-*tert*-butyl substituent on the *N*-terminal amide was similar in the dimer and hexamer and produced 63% *cis*-isomer in Ac-Pro-*t*-BuPro-NH_2 (**2**) and 61% *cis*-isomer in Ac-Pro-5-*t*-BuPro-NH_2 (**4**) as determined by ^1H NMR spectroscopy. However, the other prolyl amides in **4** remained in the amide *trans*-isomer as determined by NOESY spectroscopy. Furthermore, the circular dichroism spectra of **4**-**6** indicated that although the polyproline type II conformation was destabilized on addition of 5-*tert*-butylprolyl residues, no complete interconversion to type I polyproline had occurred. These results demonstrate that initiation of an amide *cis*-isomer at the *C*-terminus of polyproline is not sufficient to initiate the *trans*-to-*cis*-conversion of the neighboring proline residues in water. Therefore, a single bulky 5-*tert*-butyl substituent at the polyproline *C*-terminus was insufficient to promote a cooperative conversion from type II-to-type I polyproline in water, nor were two and three 5-*tert*-butylproline residues sufficient to stabilize type I polyproline in **5** and **6** in water. Polyprolines **4**-**6** may thus mimic the transitional intermediate proposed to form during helical interconversion.[13,14] Alternatively, the enhanced hydrophobic character of the amide *cis*- relative to the *trans*-isomer may have stabilized the type II polyproline structure and prevented the cooperative interconversion in water despite the disfavoring steric interactions of the 5-*tert*-butylproline residues.[8] Our results demonstrate that surmounting the barrier for *trans*-to-*cis* isomerization of the first prolyl amide is inadequate for propagating helical interconversion in a solvent that strongly disfavors the adjoining *N*-terminal amides from adopting a polyproline type I conformation. Although the influence of less hydrophobic solvents and consecutive 5-*tert*-butylproline sequences[30b,51] remain to be investigated to promote the nucleation of type I helical geometry, our study demonstrates the effective use of peptide synthesis to insert sterically demanding 5-alkylprolines into model peptides to probe the conformational features of polyproline.

Experimental

General. Unless otherwise noted all reactions were run under a nitrogen atmosphere. Acetonitrile and dichloromethane were distilled from calcium hydride, triethylamine from BaO, piperidine from NaOH, and diisopropylethylamine (DIEA) was distilled sequentially from ninhydrin and CaH_2. Rink resin[38] (0.65 mmol/g) was purchased from Advanced Chemtech (Louisville, KT, USA). Final reaction mixtures 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 (EI and FAB), were obtained by the Université de Montréal Mass Spectrometry facility. ^1H NMR (300/400 MHz) and ^{13}C NMR (75/100 MHz) were recorded in $CDCl_3$ and data for minor isomers are reported in brackets. Chemical shifts are reported in ppm (δ units) downfield of internal tetramethylsilane ($(CH_3)_4Si$).

Circular Dichroism Spectra were recorded at 5°C on a Jasco spectropolarimeter using a quartz cell of 0.05 cm path length at a spectral bandwith of 1 nm with a time constant of 4 s (scan speed 10 m/min) and a step resolution of 0.2 nm. The samples were prepared by dissolving the peptides in a buffered solution of KCl (100 mM), potassium phosphate buffer 100 mM at pH 7.0 in a concentration (7-17 μM) 24 h prior to the acquisition of the CD spectra. The CD spectra are reported as mean residue ellipticity ([θ], in ° cm^2 $dmol^{-1}$ calculated by the equation $[\theta](\lambda, T) = \theta_{obs} (\lambda, T) / C d n$) where $\theta_{obs} (\lambda, T)$ is the osbserved ellipticity (in °) at a given wavelength and temperature T, C is the peptide concentration in (mol/L), d is the optical pathlength (in mm) and n is the number of residues in the peptide.

NMR Spectroscopy The ^1H, ^{13}C, NOESY spectra of peptides **1-6** were recorded in 10 % [v/v] D_2O in H_2O at 25 °C. NOESY spectra were recorded with mixing times of 250 ms in the phase sensitive mode using States-TPPI method. Water peak

suppression was achieved by irradiation of the solvent resonnance during the relaxation delay and during the mixing time.

***N*-(Fmoc)Prolyl-5-*tert*-butylproline Allyl Ester (7)** A solution of *N*-(Fmoc)proline (1.23 g, 3.65 mmol, synthesized according to the procedure in ref 36), (2*S*, 5*R*)-5-*tert*-butylproline allyl ester (0.77 g, 3.65 mmol, prepared according to ref. 42) and DIEA (1.27 mL, 7.3 mmol) in CH_2Cl_2 (30 mL) was cooled to 4 °C, treated with Bop-Cl (1.02 g, 4.0 mmol) and stirred for 16 h. The volatiles were removed by evaporation and the residue was partitioned between brine (20 mL) and EtOAc (20 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL) and the combined organic layers were washed with 2 N HCl (3 × 20 mL) and sat. $NaHCO_3$ (3 × 20 mL), dried, filtered and evaporated to an oil that was chromatographed on silica gel with 0-10% MeOH in $CHCl_3$ as eluant. Evaporation of the collected fractions gave dipeptide **7** (1.49 g, 77 %): TLC (1:1, hexane:EtOAc) R_f = 0.8; ^{1}H NMR δ ($CDCl_3$) [0.9 (s, 2.4 H)] 1.0 (s, 6.6), 1.9-2.4 (m, 8 H), 3.6 (m, 1H), 3.7 (m, 1H), 4.2-4.4 (m, 4H), 4.6-4.8 (m, 4H), 5.2 (dd, 1 H, *J* = 1.4, 10.4), 5.3 (dd, 1H *J* = 1.5, 17.2), 5.89 (m, 1H), 7.3 (d, 2H, *J* = 7.4), 7.4 (t, 2H, *J* = 7.4), 7.7 (dd, 2H, *J* = 5.5, 6.8), 7.8 (d, 2H, *J* = 7.5); ^{13}C NMR δ ($CDCl_3$) 24.82, 27.61, 27.70, 27.80, 30.58, 35.71, 47.11, 47.32, 57.02, 60.69, 65.30, 6.36, 118.04, 119.87, 125.17, 125.41, 126.93, 126.99, 127.62, 132.05, 141.24, 143.82, 144.00, 154.83, 172.04, 175.30. HRMS calcd for $C_{32}H_{39}O_5N_2$ (MH^+) 531.28589, found 531.28830.

***N*-(Fmoc)Prolyl-5-*tert*-butylproline (8)** A solution of *N*-(Fmoc)prolyl-5-*tert*-butylproline allyl ester (**7**, 120 mg, 0.23 mmol) in 1:1, DMSO:THF (18 mL) was treated with a solution of 0.5 M HCl (9 mL, 4.52 mmol), *N*-methylaniline (1.2 mL, 11.3 mmol) and $Pd(PPh_3)_4$ (18.3 mg, 0.34 mmol), stirred for 1 h and poured into EtOAc (20 mL). The organic layer was washed with 2N HCl (3 × 5 mL) and brine (3 × 5 mL), dried, filtered and chromatographed on silica gel with 0-20% EtOAc in hexanes

containing 2% AcOH as eluant. Evaporation of the collected fractions gave **8** (100 mg, 90 %) as a crystalline solid: mp 174 °C; TLC (40:58:2 EtOAc:hexanes:AcOH) R_f = 0.3; ^{1}H NMR δ ($CDCl_3$) δ 0.85 (s, 1.4 H) [0.92 (s,7.6 H)], 1.9-2.6 (m, 8 H), 3.5 (m, 1 H), 3.7 (m, 1 H), 4.2-4.4 (m, 4H), 4.7 (m 2H), 7.28 (d, 2H *J* = 6.4), 7.3 (dd, 2H, *J* = 1.1, 7.4), 7.58 (dd, 2H, *J* = 7.0, 4.5) 7.75 (d, 2H, *J* = 7.5). ^{13}C RMN δ 20.6, 24.8, 25.6, 26.2, 27.5, 30.6, 35.2, 47.0, 47.2, 57.0, 60.3, 67.5, 68.5, 119.9, 125.0, 126.9, 127.6, 141.2, 143.7, 154.9, 172.5, 176.3, 178.4.

General Protocols for Peptide Synthesis Peptides **1-6** were synthesised in a semi-automatic Advanced Chemtech ACT 90 peptide synthesiser (Louisville, KT, USA). Reaction vessel volumes were 25-50 mL/g of resin. Total DMF volume for each coupling and acetylation step was ≤ 20 mL/g of resin. Before starting a peptide synthesis, the Rink resin (0.65 mmol/g) was swollen in dichloromethane (15 mL/g) for 30 min and then washed with DMF (3 × 15 mL/g of resin). Before and after each coupling, deprotection and acetylation step, the resin was washed with DMF (3 × 15 mL/g of resin). *N*-(Fmoc)Prolyl-5-*tert*-butylproline (**8**, 110 mol%) was coupled using DIEA (330 mol %) and TBTU (110 mol %) in DMF for 16 h. *N*-(Fmoc)proline (200 mol%) was coupled using DIEA (400 mol%) and TBTU (200 mol%) in DMF for 2 h. Deprotection of the Fmoc group was accomplished using 20 % piperidine (2000 mol%) in DMF for 7 min. Acetylation of the *N*-terminal residue was achieved with acetic anhydride (1500 mol %) and DIEA (1500 mol %) in DMF for 2 h, followed by washing with volumes of CH_2Cl_2, EtOH, and Et_2O (3 × 15 mL/g of resin). Acetylated peptides were then cleaved from the resin using a mixture of 95:5 TFA:H_2O (20 mL/g of resin) for 30 min, followed by filtration and resin washing with 95:5 TFA:H_2O (3 × 5 mL/g of resin). Evaporation of the combined TFA:H_2O solutions gave crude peptides that were shown by analytical HPLC to be of >90% purity in the cases of **1**, **2** and **4-6**, and of >50% purity for **3**. A portion of the crude products was then purified

by reversed phase HPLC using a Radial Nova pak C_{18} column with a linear gradient of 0-25% B in A over 30 min for **1** and **3**, and 0-50% B in A over 30 min for **2** and **4-6**, where A = H_2O:0.1% TFA and B = CH_3CN:0.1% TFA, at a flow rate of 1.5 mL/min and the detector centered at λ = 214 nm. The retention times and mass spectral data for **1-6** are as follows: **Ac-Pro-Pro-NH_2**.(**1**): 11.9 min; HRMS calcd for $C_{12}H_{19}N_3O_3$ (MH^+) 254.1505, found 254.1509; **Ac-Pro-tBuPro-NH_2**.(**2**): 17.6 min; HRMS calcd for $C_{16}H_{27}N_3O_3$ (MH^+) 310.2131, found 310.2116; **Ac-(Pro)$_6$-NH_2**.(**3**): 19.8 min; HRMS calcd for $C_{32}H_{47}N_7O_7$ (MH^+) 642.3615, found 642.3603; **(Ac-(Pro)$_5$-tBuPro-NH_2**.(**4**): 23.2 min; HRMS calcd for $C_{36}H_{55}N_7O_7$ (MH^+) 698.4241, found 698.4250; **Ac-((Pro)$_2$-tBuPro)$_2$-NH_2**.(**5**): 23.7 min; HRMS calcd for $C_{40}H_{64}N_7O_7$ (MH^+) 754.4867, found 754.4889; **Ac-(Pro-tBuPro)$_3$-NH_2**.(**6**): 31.23 min; HRMS calcd for $C_{44}H_{71}N_7O_7$ (MH^+) 810.5493, found 810.5465.

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Supporting Information Available: ^{13}C NMR spectrum of **3**, as well as NOESY spectra for **2** and **4** (3 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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solvents such as *n*-propanol have favored type I geometry (refs 9 and 10), and high salt concentration in water has caused random sequences of *cis* and *trans* amide bonds in polyproline (refs 4, 11 and 12).

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CHAPITRE 6

Discussion

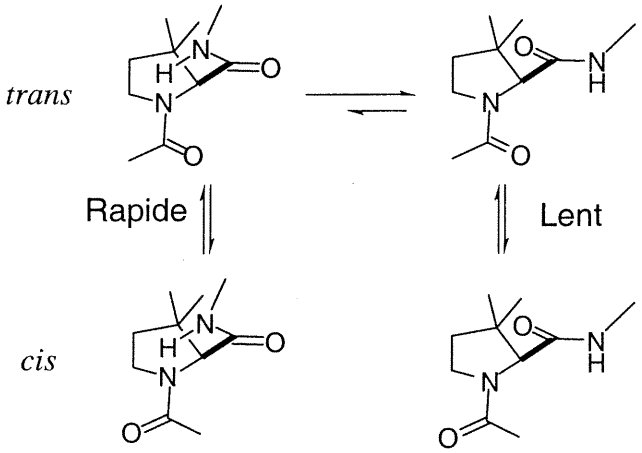
Les objectifs de la recherche décrite dans cette thèse couvrent deux aspects de la chimie organique moderne telle que la synthèse d'acides aminés énantiomériquement purs et la détermination de la conformation de peptides en solution. Il est alors important à ce point de mettre en perspective l'ensemble des résultats obtenus.

Dans un premier temps, nous avons développé de nouvelles méthodologies de synthèse d'acides aminés afin de pouvoir utiliser de nouveaux peptidomimétiques et ainsi étudier les effets stériques et la conformation des peptides. À ce titre, différentes méthodologies ont été décrites dans le chapitre 2. Brièvement, elles ont permis d'obtenir les quatre diastéréoisomères de la 5-*tert*-butylproline de façon énantiomériquement pure et en quantité suffisante pour explorer les effets stériques de ces nouveaux acides aminés à l'intérieur de peptides modèles.

Ensuite nous avons étudié expérimentalement et théoriquement les effets stériques causés par la présence d'un groupe *tert*-butyle en position 5 du cycle de proline. Nous avons ainsi observé expérimentalement les conséquences des effets stériques sur la conformation de peptides modèles, sur la formation de ponts hydrogènes ainsi que sur la cinétique d'isomérisation de ces conformations. En utilisant la mécanique moléculaire nous avons modélisé ces effets et nous avons construit des cartes conformationnelles qui montrent clairement et prédisent qualitativement les effets stériques observés expérimentalement.

Par la suite nous avons pu montrer que les effets stériques en position 3- de proline mettent en évidence les interactions de Coulomb et défavorisent certaines conformations durant l'état de transition de l'isomérisation. Par conséquent, la répulsion stérique générée par les substituants méthyles en position 3 influence la vitesse d'isomérisation d'amides en position *N*-terminales de proline. En effet, les substituants en position 3 exercent une répulsion stérique avec l'amide en position *C*-terminale qui défavorise l'adoption d'une conformation où le proton de l'amide pourrait stabiliser l'état de transition de l'isomérisation favorisant ainsi un équilibre rapide entre la forme *cis* et la forme *trans* (Figure 6.1). Au contraire, la conformation favorisée est celle où l'oxygène de l'amide en *C*-terminale est à proximité de l'azote de l'amide en *N*-terminale. La proximité de l'oxygène et de l'azote résulte en une interaction défavorable de Coulomb et a pour conséquence de retarder la pyramidalistaion de l'amide et ainsi diminue la vitesse d'isomérisation.

Figure 6.1 Influence de l'angle ψ sur la vitesse d'isomérisation *cis-trans*.



Par ailleurs, nous avons pu observer l'influence de groupes hydroxyles sur la vitesse d'isomérisation et proposer un modèle qui explique l'augmentation de la vitesse d'isomérisation par la diminution de la déstabilisation de l'état de transition via la formation des ponts hydrogènes. Enfin nous avons fait ressortir que les effets

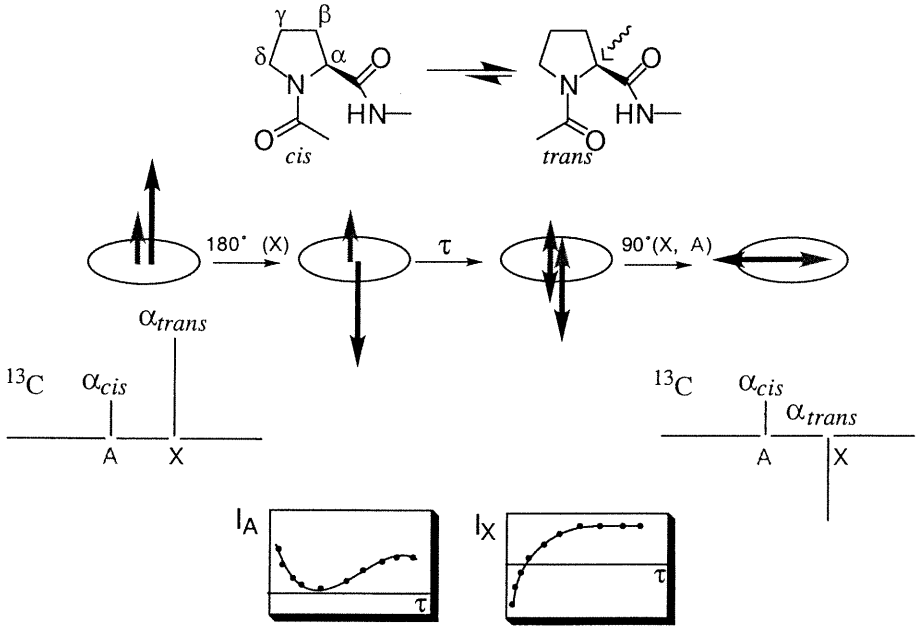
inductifs des groupes hyroxyles sur l'isomérisation de l'amide en position *N*-terminale doivent être pris en considération en tenant compte de leur influence sur la géométrie de l'amide en position *C*-terminale.

Enfin, en insérant la 5-*tert*-butylproline, nous avons pu montrer que la nucléation de la conformation hélicale polyproline type I (PPI) en position *C*-terminale est difficile en solvant aqueux. Nous avons montré que l'insertion de un, deux et trois *tert*-butylprolines dans un hexapeptide n'exerce d'effets que sur ses voisins immédiats sans agir sur la conformation globale du peptide. À la lumière de ces résultats, il semble que le processus de conversion coopérative de la conformation polyproline type II (PPII) vers PPI ainsi que la stabilisation coopérative de PPI ne soient pas suffisamment important pour induire la conformation hélicale polyproline type I en milieu aqueux.

Afin d'obtenir ces résultats deux problèmes majeurs ont dû être surmontés. Le premier se trouve dans la synthèse d'acide aminé stériquement encombrés. Les effets stériques ont aussi eu pour effet de diminuer les vitesses de réaction et dans certains cas empêcher complètement certaines réactions de se produire. A titre d'exemple représentatif, il est possible de mentionner la réaction de couplage peptidique. Dans le cas de la réaction de couplage en position *N*-terminale de la 5-*tert*-butylproline avec FMOC-Pro-OH, nous avons observé une très faible réactivité avec des réactifs de couplage telle que la combinaison TBTU et DIEA. Cette faible réactivité peut s'expliquer par la présence des effets de répulsion stérique importants occasionnés par le groupe *tert*-butyle. Par conséquent, nous avons utilisé la combinaison beaucoup plus efficace avec BOP-Cl et DIEA.

En dehors de nombreux autres problèmes de synthèse telle que la nécessité de disposer de quantités suffisantes d'acides aminés permettant la synthèse de peptides ainsi que la conservation de la pureté énantiomérique, la caractérisation de ces acides aminés à l'intérieur de peptides a été la deuxième défi majeur. Par exemple, la détermination de l'énergie d'activation de l'isomérisation de la liaison amide via l'observation en RMN de la coalescence des signaux de protons qui changent d'environnement magnétique lors de l'isomérisation est limitée à une échelle restreinte d'énergie d'activation. Pour calculer des énergies plus élevées nous avons dû utiliser une deuxième expérience qui se nomme transfert de magnétisation. Cette expérience consiste à étiqueter l'un des signaux du carbone qui se trouve dans deux environnements magnétiques différents par une irradiation sélective. Cette irradiation sélective permet d'inverser la phase de ce carbone. Ainsi, lorsque ce carbone change d'environnement magnétique, il diminue l'intensité du deuxième signal qui participe à l'échange et il est possible de mesurer le taux d'échange.

Figure 6.3 Détermination de l'énergie d'activation(ΔG‡) par l'expérience de transfert de magnétisation du ^{13}C en RMN.



Le taux d'échange étant proportionnel à l'énergie d'activation, il est possible de calculer indirectement l'énergie d'activation pour autant que l'isomérisation se produise à l'intérieur de l'échelle de temps RMN[1].

Par ailleurs, la caractérisation de structures secondaires peptidiques par RMN est simplifiée lorsqu'il existe une dispersion des déplacements chimiques des signaux des protons. Cependant, l'étude des homopolymères de proline devient difficile puisque l'utilisation de l'effet nOe devient difficile en raison de la faible disparité des signaux de protons. Par conséquent, l'utilisation d'une autre technique spectroscopique telle que le dichroïsme circulaire a été nécessaire pour déterminer la structure secondaire de ces homopolymères peptidiques.

Conclusion

Ces études ont montré qu'il est possible d'utiliser la synthèse organique pour construire de nouveaux peptidomimétiques et les utiliser comme des outils permettant de sonder les facteurs influençant la formation et la stabilisation de la structure secondaire des peptides telle que l'hélice polyproline type I et le repliement β type VIa[2].

Les résultats obtenus montrent que l'insertion de peptidomimétiques qui peuvent stabiliser certaines conformations sont utiles pour confirmer certaines hypothèses qui concernent la relation entre la structure et l'activité biologique de peptides[3]. En ayant un meilleur contrôle et une meilleure compréhension de ces structures, il sera possible d'approcher la création de structures non-peptidiques qui seront plus propices à se comporter comme drogue in-vivo.

En dehors de la conception de nouvelles drogues, l'importance de ces résultats

réside dans le développement de nouvelles méthodologies de synthèse énantiosélective, dans la conception rationnelle de nouvelles architectures peptidiques pouvant conduire à de nouvelles structures supramoléculaires, et enfin dans la caractérisation dynamique de structures moléculaires.

Références

(1) Freeman, R. *A Handbook of Nuclear Magnetic Resonance*, John Wiley & Sons, Inc. New York, 1988, pp. 198-202.
(2) Halab, L.; Lubell, W. D. *J. Org. Chem.* **1999**, *64*, 3312.
(3) Belec, L; Slavinova, J.; Lubell, W. D. *J. Med. Chem.* **2000**, *43*, 1448-1455.

The structure was solved by direct method using SHELXS-86 (Sheldrick, 1990) and difference Fourier synthesis using SHELXL-93 (Sheldrick, 1994). Full-matrix least-squares refinement based on F^2 using all reflections, all non-hydrogen atoms anisotropic, hydrogen atoms isotropic.

Hydrogen atoms were calculated at idealized positions and refined in the last cycles. Hydrogens of methyls were refined with displacement factors of H fixed to $U(H) = 1.5U_{eq}(C_{methyls})$ using a riding model with $d_{C-H} = 0.96$Å.

The last least-squares cycle was calculated with 165 parameters, 1 restraint (floating origin) and 2195 reflections.

Weights based on counting statistics were used. Function minimized: $\Sigma w(Fo^2-Fc^2)$; calc $w=1/[\sigma^2(Fo^2)+(0.0402P)^2+0.0317P]$ where $P=(Fo^2+2Fc^2)/3$.

The residuals are as follows :

Final R indices [I>2sigma(I)]	R1 = 0.0264, wR2 = 0.0736
R indices (all data)	R1 = 0.0266, wR2 = 0.0737
Goodness-of-fit on F^2	1.060

where $R1 = \Sigma(\|Fo| - |Fc\|)/\Sigma(|Fo|)$,
$wR2 = [\Sigma[w(Fo^2-Fc^2)^2]/\Sigma[w(Fo^2)^2]]^{1/2}$ and
$GoF = [\Sigma[w(Fo^2-Fc^2)^2]/(\text{No. of reflns - No. of params.})]^{1/2}$

The maximum shift/σ ratio was 0.007.

In the last ΔF map, the deepest hole was -0.105e/Å^3 and the highest peak 0.125 e/Å^3.

Extinction coefficient refined:	0.093(4) (SHELXL-93 (Sheldrick, 1994))
Absolute structure parameter:	0.0(2) (Flack, 1983; Flack and Schwarzenbach, 1988). The expected values for Flack x parameter are 0 (within 3 esd's) for correct and +1 for inverted absolute structure.

The scattering curves and anomalous dispersion contribution (df' and df'') for all atoms were taken from International Tables Vol C Tables 4.2.6.8 and 6.1.1.4 (1992).

Table 1. Crystal data and structure refinement for $C_{10}H_{18}N_2O_3$.

Space Group and Cell Dimensions

Monoclinic	$P2_1$	
a = 6.346(1)Å	b = 8.737(2)Å	c = 10.821(2)Å
β = 105.72(2)°	Volume = 577.5(2)Å^3	

Empirical formula $C_{10}H_{18}N_2O_3$

Cell dimensions were obtained from 25 reflections, 2θ angle in the range 45-50°.

Crystal description : grown from .... solution,
triangular prism

Crystal dimensions : 0.28 {0 0 1} x 0.34 {0 1 1} x 0.49 {1 0 0} mm

FW = 214.26	Z = 2	F(000) = 232
Dcalc = 1.232 Mg/m^3	μ = 0.751 mm^{-1}	λ = 1.54056Å
θ range fo data collection = 4.24 to 69.86°		T = 295K

The intensity data were collected on a Nonius CAD4 diffractometer (Enraf-Nonius, 1989) using the ω/2θ scan mode {0.80 + 0.14 tan(θ)°}, graphite monochromatized CuKα$_1$ radiation and scan rate of 16.5°min^{-1}. Orientation monitored every 400 measurements, intensity checked every 1800s using 5 standard reflections, largest intensity fluctuation: 1.6%.

No. of reflections measured	4307
The h,k,l ranges are :	0<=h<=7, -10<=k<=10, -13<=l<=12
No. of independent reflections, (R_{int} = 0.031)	2195
No. of reflections with I>2σ(I),	2165

Lp correction done. No correction was made for absorption.

Table 2. Atomic coordinates and equivalent isotropic temperature factor for the non-hydrogen atoms for $C_{10}H_{18}N_2O_3$.

ATOM	X	Y	Z	U_{eq}
N(1)	0.8718(2)	0.61149(11)	0.73138(9)	0.0403(2)
C(2)	0.9891(2)	0.48123(12)	0.80269(10)	0.0357(2)
C(3)	0.9301(2)	0.34919(13)	0.70269(12)	0.0412(3)
Me(31)	0.7045(2)	0.2873(2)	0.7032(2)	0.0589(4)
Me(32)	1.0941(2)	0.2188(2)	0.72741(14)	0.0514(3)
O(4)	1.1141(2)	0.46433(14)	0.54998(10)	0.0629(3)
C(4)	0.9101(2)	0.4353(2)	0.57544(11)	0.0507(3)
C(5)	0.7931(3)	0.5828(2)	0.59236(12)	0.0532(3)
O(6)	1.3334(2)	0.58352(13)	0.78441(9)	0.0563(3)
C(6)	1.2349(2)	0.51176(13)	0.85029(11)	0.0393(3)
N(7)	1.3322(2)	0.45105(13)	0.96345(10)	0.0466(3)
C(8)	1.5669(2)	0.4552(3)	1.0190(2)	0.0706(5)
O(9)	0.9041(2)	0.75022(11)	0.90956(9)	0.0550(3)
C(9)	0.8306(2)	0.73690(14)	0.79265(12)	0.0432(3)
C(10)	0.6864(3)	0.8554(2)	0.7110(2)	0.0628(4)

- $U_{eq} = (1/3)\Sigma_i\Sigma_j U_{ij} a^*_i a^*_j a_i \cdot a_j$

Table 3. Bond lengths [Å] and angles [°] for $C_{10}H_{18}N_2O_3$.

N(1)-C(9)	1.343(2)	N(1)-C(2)	1.4608(14)
N(1)-C(5)	1.472(2)	C(2)-C(6)	1.528(2)
C(2)-C(3)	1.556(2)	C(3)-Me(32)	1.517(2)
C(3)-Me(31)	1.532(2)	C(3)-C(4)	1.544(2)
O(4)-C(4)	1.418(2)	C(4)-C(5)	1.523(2)
O(6)-C(6)	1.238(2)	C(6)-N(7)	1.324(2)
N(7)-C(8)	1.448(2)	O(9)-C(9)	1.230(2)
C(9)-C(10)	1.501(2)		
C(9)-N(1)-C(2)	120.97(10)	C(9)-N(1)-C(5)	125.86(11)
C(2)-N(1)-C(5)	112.98(10)	N(1)-C(2)-C(6)	111.71(9)
N(1)-C(2)-C(3)	102.59(9)	C(6)-C(2)-C(3)	113.28(9)
Me(32)-C(3)-Me(31)	109.37(11)	Me(32)-C(3)-C(4)	114.18(11)
Me(31)-C(3)-C(4)	108.92(11)	Me(32)-C(3)-C(2)	114.17(10)
Me(31)-C(3)-C(2)	107.97(10)	C(4)-C(3)-C(2)	101.83(9)
O(4)-C(4)-C(5)	111.89(12)	O(4)-C(4)-C(3)	113.71(11)
C(5)-C(4)-C(3)	103.30(9)	N(1)-C(5)-C(4)	103.01(10)
O(6)-C(6)-N(7)	123.62(11)	O(6)-C(6)-C(2)	121.52(11)
N(7)-C(6)-C(2)	114.82(9)	C(6)-N(7)-C(8)	122.95(12)
O(9)-C(9)-N(1)	121.00(11)	O(9)-C(9)-C(10)	122.78(11)
N(1)-C(9)-C(10)	116.20(11)		

Table 4. Bond distances [Å] and angles [°] related to the hydrogen bonding for the $C_{10}H_{18}N_2O_3$ compound.

(A-H···B)	A-B	A-H	H-B	A-H-B
N(7)-H(N7)···O(9)$_a$	2.887(2)	0.88(2)	2.01(2)	169(2)
O(4)-H(O4)···O(6)	2.746(2)	0.85(2)	1.91(2)	166(2)

a : 2-x, -1/2+y, 2-z

Table 5. Torsion angles [°] for $C_{10}H_{18}N_2O_3$.

C(9)-N(1)-C(2)-C(6)	-76.82(13)
C(5)-N(1)-C(2)-C(6)	107.86(12)
C(9)-N(1)-C(2)-C(3)	161.53(10)
C(5)-N(1)-C(2)-C(3)	-13.79(13)
N(1)-C(2)-C(3)-Me(32)	156.42(10)
C(6)-C(2)-C(3)-Me(32)	35.84(14)
N(1)-C(2)-C(3)-Me(31)	-81.73(12)
C(6)-C(2)-C(3)-Me(31)	157.70(10)
N(1)-C(2)-C(3)-C(4)	32.87(11)
C(6)-C(2)-C(3)-C(4)	-87.71(11)
Me(32)-C(3)-C(4)-O(4)	-42.6(2)
Me(31)-C(3)-C(4)-O(4)	-165.12(12)
C(2)-C(3)-C(4)-O(4)	80.99(12)
Me(32)-C(3)-C(4)-C(5)	-164.03(11)
Me(31)-C(3)-C(4)-C(5)	73.40(13)
C(2)-C(3)-C(4)-C(5)	-40.49(12)
C(9)-N(1)-C(5)-C(4)	173.50(11)
C(2)-N(1)-C(5)-C(4)	-11.45(14)
O(4)-C(4)-C(5)-N(1)	-90.60(12)
C(3)-C(4)-C(5)-N(1)	32.10(14)
N(1)-C(2)-C(6)-O(6)	-37.6(2)
C(3)-C(2)-C(6)-O(6)	77.64(14)
N(1)-C(2)-C(6)-N(7)	144.78(10)
C(3)-C(2)-C(6)-N(7)	-99.96(12)
O(6)-C(6)-N(7)-C(8)	-3.5(2)
C(2)-C(6)-N(7)-C(8)	174.09(14)
C(2)-N(1)-C(9)-O(9)	5.3(2)
C(5)-N(1)-C(9)-O(9)	-179.97(12)
C(2)-N(1)-C(9)-C(10)	-173.04(11)
C(5)-N(1)-C(9)-C(10)	1.6(2)

Table 6. Anisotropic displacement parameters ($Å^2$) for $C_{10}H_{18}N_2O_3$.

ATOM	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1)	0.0512(6)	0.0342(5)	0.0372(5)	0.0012(4)	0.0149(4)	0.0060(4)
C(2)	0.0411(5)	0.0338(5)	0.0340(5)	0.0013(4)	0.0132(4)	0.0007(4)
C(3)	0.0434(6)	0.0356(6)	0.0427(6)	-0.0062(5)	0.0087(4)	0.0011(5)
Me(31)	0.0471(6)	0.0467(7)	0.0774(10)	-0.0072(7)	0.0075(6)	-0.0080(5)
Me(32)	0.0569(7)	0.0400(6)	0.0555(7)	-0.0047(6)	0.0122(6)	0.0083(5)
O(4)	0.0769(7)	0.0747(7)	0.0440(5)	-0.0003(5)	0.0283(5)	0.0099(6)
C(4)	0.0615(8)	0.0520(7)	0.0358(6)	-0.0062(6)	0.0083(5)	0.0083(6)
C(5)	0.0689(9)	0.0515(7)	0.0379(6)	0.0033(6)	0.0125(6)	0.0147(7)
O(6)	0.0563(5)	0.0658(6)	0.0506(5)	0.0088(5)	0.0212(4)	-0.0169(5)
C(6)	0.0453(6)	0.0373(5)	0.0372(5)	-0.0022(4)	0.0145(4)	-0.0066(5)
N(7)	0.0430(5)	0.0566(6)	0.0383(5)	0.0041(5)	0.0079(4)	-0.0119(5)
C(8)	0.0467(7)	0.1031(14)	0.0558(8)	0.0046(9)	0.0035(6)	-0.0171(8)
O(9)	0.0664(6)	0.0482(5)	0.0522(5)	-0.0124(4)	0.0194(4)	0.0032(4)
C(9)	0.0523(6)	0.0336(6)	0.0499(7)	-0.0008(5)	0.0243(5)	-0.0016(5)
C(10)	0.0870(10)	0.0407(7)	0.0692(9)	0.0100(6)	0.0356(8)	0.0175(7)

The anisotropic thermal parameters are the coefficients of the expression:
$T = \exp[-2\pi^2(U_{11}a^{*2}h^2 + \ldots + 2U_{12}a^*b^*hk + \ldots)]$.

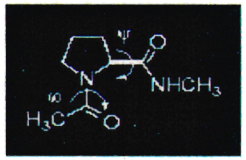
Table 7. Hydrogen coordinates and isotropic displacement parameters ($Å^2$) for $C_{10}H_{18}N_2O_3$.

ATOM	X	Y	Z	U_{eq}
H(2)	0.937(2)	0.466(2)	0.8757(12)	0.031(3)
H(31A)	0.652	0.220	0.631	0.088
H(31B)	0.604	0.371	0.698	0.088
H(31C)	0.716	0.232	0.781	0.088
H(32A)	1.107	0.177	0.811	0.077
H(32B)	1.234	0.257	0.723	0.077
H(32C)	1.045	0.141	0.664	0.077
H(O4)	1.190(3)	0.513(3)	0.615(2)	0.075(6)
H(4)	0.817(2)	0.366(2)	0.498(2)	0.049(4)
H(5A)	0.830(3)	0.664(3)	0.547(2)	0.067(5)
H(5B)	0.626(3)	0.568(2)	0.562(2)	0.070(5)
H(N7)	1.246(3)	0.398(2)	0.999(2)	0.055(4)
H(8A)	1.630	0.361	1.001	0.106
H(8B)	1.598	0.469	1.110	0.106
H(8C)	1.628	0.539	0.983	0.106
H(10A)	0.538	0.820	0.686	0.094
H(10B)	0.735	0.874	0.636	0.094
H(10C)	0.695	0.949	0.759	0.094

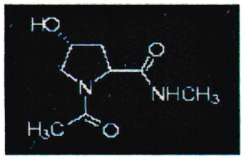
Table 8. Bond lengths [Å] and angles [°] for the refined hydrogen atom of compound $C_{10}H_{18}N_2O_3$.

C(2)-H(2)	0.944(13)	N(1)-C(2)-H(2)	107.9(8)
		C(6)-C(2)-H(2)	107.4(7)
		C(3)-C(2)-H(2)	113.9(8)
O(4)-H(O4)	0.85(2)	C(4)-O(4)-H(O4)	105.5(13)
C(4)-H(4)	1.07(2)	O(4)-C(4)-H(4)	106.6(8)
		C(5)-C(4)-H(4)	113.1(9)
		C(3)-C(4)-H(4)	108.4(9)
C(5)-H(5A)	0.93(2)	N(1)-C(5)-H(5A)	110.2(12)
C(5)-H(5B)	1.03(2)	C(4)-C(5)-H(5A)	112.6(12)
		N(1)-C(5)-H(5B)	112.5(10)
		C(4)-C(5)-H(5B)	110.5(12)
		H(5A)-C(5)-H(5B)	108(2)
N(7)-H(N7)	0.88(2)	C(6)-N(7)-H(N7)	115.3(11)
		C(8)-N(7)-H(N7)	121.4(11)

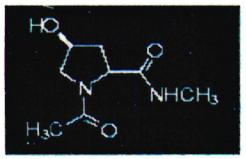
Conformation vs Energy of Prolyl Amides 1–6



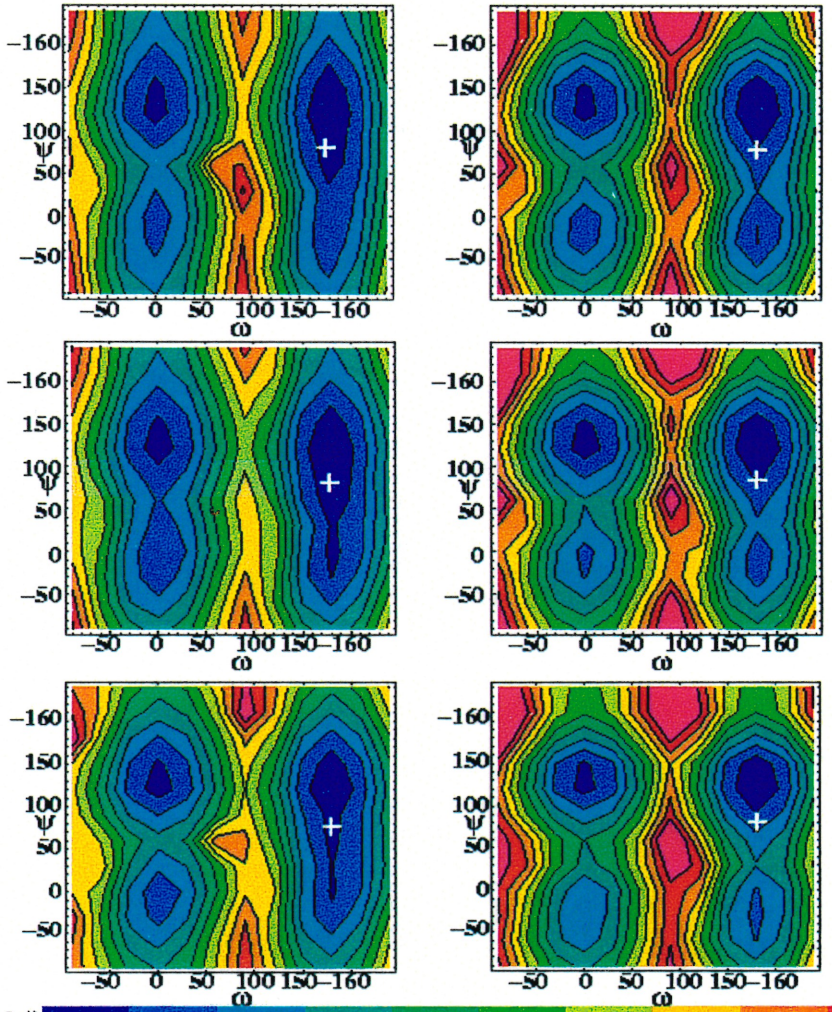
(2*S*)–*N*–Acetyl–proline *N*'–Methylamide (1)

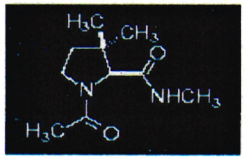


(2*S*, 4*R*)–*N*–Acetyl–4–hydroxyproline *N*'–Methylamide (2)

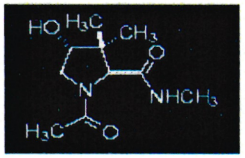


(2*S*, 4*S*)–*N*–Acetyl–4–hydroxyproline *N*'–Methylamide (3)

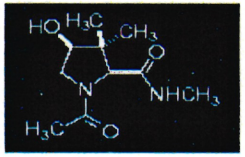




(2*S*)–*N*–Acetyl–3,3–dimethylproline *N*'–Methylamide (4)



(2*S*, 4*R*)–*N*–Acetyl–3,3–dimethyl–4–hydroxyproline *N*'–Methylamide (5)



(2*S*, 4*S*)–*N*–Acetyl–3,3–dimethyl–4–hydroxyproline *N*'–Methylamide (6)