ピロリジン骨格を有する新規Dipeptidyl peptidase IV阻害剤に関する研究

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略語表

AcCl: acetyl chloride、塩化アセチル
Ac_2O: Acetic anhydride、無水酢酸
AcOH : acetic acid、酢酸
AMC: 7-amino-4-methyl coumarin, 7-アミノ-4-メチルクマリン
Bn: benzyl、ベンジル
Boc: *tert*-Butoxycarbonyl, *tert*-ブトキシカルボニル
Bu: butyl、ブチル
BzOAg: silver benzoate、安息香酸銀
Cbz: benzyloxycarbonyl, ベンジルオキシカルボニル
CSA: (1*S*)-(+)-10-camphorsulfonic acid、(1*S*)-(+)-10-カンファースルホン酸
DHP: 3,4-dihydro-2*H*-pyran、3,4- ジヒドロ-2*H*-ピラン
DMAP: 4-(dimethylamino)pyridine、4-ジメチルアミノピリジン
DMF : dimethylformamide ジメチルホルムアミド
DMSO: dimethylsulfoxide、ジメチルスルホキシド
DPP-IV: Dipeptidyl peptidase IV、ジペプチジルペプチダーゼ IV
EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1-エチル-3-(3'-ジメチルアミドプロピル)カルボジイミド塩酸塩
ESI: Electrospray ionization、エレクトロスプレー法
Et: ethyl、エチル
Et_2O: diethyl ether、ジエチルエーテル
EtOAc: ethyl acetate、酢酸エチル
EtOH : ethanol、エタノール
Et_3N: triethylamine、トリエチルアミン
GLP-1: Glucagon-like peptide-1、グルカゴン様ペプチド-1
HMPA: hexamethylphosphoric Triamide、ヘキサメチルりん酸トリアミド
HOBt: 1-hydroxybenzotriazole、1-ヒドロキシベンゾトリアゾール
HPLC: high-performance liquid chromatography、高速液体クロマトグラフィー
LC/MS: Liquid Chromatography / Mass Spectrometry、液体クロマトグラフィー/質量分析法
LDA: lithium diisopropylamide、リチウムジイソプロピルアミド
LiHMDS: lithium bis(trimethylsilyl)amide、リチウムビストリメチルシリルアミド
Me: methyl、メチル
MeOH : methanol、メタノール
MOM: methoxy methyl、メトキシメチル
MsCl: methanesulfonyl chloride、メタンスルホニルクロリド
MsOBt: 1-methanesulfonyloxy-1*H*-benzotriazole, 1 -メタンスルホニルオキシベンゾト

リアゾール
NADPH: nicotinamide adenine dinucleotide phosphate、ニコチンアミドアデニンジヌクレオチドリン酸
NaHMDS: sodium bis(trimethylsilyl)amide、ナトリウムビストリメチルシリルアミド
NMM: *N*-methylmorpholine、*N*-メチルモルホリン
Ph: Phenyl、フェニル
po: *per os*, 経口
PPTS: pyridinium *p*-toluenesulfonate、ピリジニウム *p*-トルエンスルホナート
QOL: Quality of life、生活の質
TEMPO: 2,2,6,6-tetramethylpiperidine 1-oxy、2,2,6,6-テトラメチルピペリジン 1-オキシル
Tf: trifluoromethanesulfonyl、トリフルオロメタンスルホニル
TFA: trifluoroacetic acid、トリフルオロ酢酸
TFAA: trifluoroacetic anhydride、トリフルオロ酢酸無水物
THF : tetrahydrofuran、テトラヒドロフラン
THP: tetrahydropyranyl、テトラヒドロピラニル
TLC: thin-layer chromatography、薄層クロマトグラフィー
Tris: 2-Amino-2-(hydroxymethyl)propane-1,3-diol、2-アミノ-2-ヒドロキシメチルプロパン-1,3-ジオール
TsCl: *p*-toluenesulfonyl chrolide、*p*-トルエンスルホニルクロリド
TsOH: *p*-toluenesulfonic acid、*p*-トルエンスルホン酸
UDPGA: uridine diphosphate glucuronic acid、ウリジン二リン酸-α-グルクロニド

第一章　緒言

１-１．糖尿病[1, 2]

糖尿病とはインスリン作用不足による慢性の高血糖状態を主徴とする代謝疾患群である。糖尿病は生活習慣と社会環境の変化に伴って増加しており、平成14年の厚生労働省の調査結果によると、「糖尿病が強く疑われる人」は約740万人、「糖尿病の可能性を否定できない人」を合わせると約1, 620万人となっている。

糖尿病はその成因により2種類に分類される。1型糖尿病は、主に自己免疫を基礎にした膵ランゲルハンス島β細胞の破壊、消失がインスリン作用不足の主要な原因である。日本人の糖尿病の５％前後であり、小児での発症が多く、治療にはインスリン注射による血糖コントロールが不可欠である。2型糖尿病は、インスリン分泌低下やインスリン抵抗性をきたす複数の遺伝因子に、過食、運動不足、肥満、ストレスなどの環境因子および加齢が加わり発症する。日本人の糖尿病の90％以上を占め、40歳以上での発症が多い。2型糖尿病においては食事療法、運動療法により血糖コントロールが達成できない場合、経口血糖降下薬が用いられる。

糖尿病の症状が進行すると、網膜症、腎臓障害、神経障害などの合併症を引き起こし、患者の生活の質（QOL）を著しく低下させる。また、糖尿病は動脈硬化症を促進し、心筋梗塞、脳梗塞など生命に関わる疾患を引き起こすリスクが高まる。最近の大規模臨床試験では、空腹時の高血糖よりも食後の高血糖が動脈硬化性疾患のリスクであることが明らかにされており、動脈硬化性疾患の予防のためにも、早期糖尿病の段階から食後高血糖の治療を開始することが求められている。

１-２．Glucagon-like peptide-1（GLP-1）[3, 4]

グルコースを経口投与した場合と静脈内投与した場合を比較すると、インスリン分泌は前者の場合が多く、血糖上昇は軽度である。グルコース経口負荷によるインスリン分泌反応の増強は消化管から分泌されるインクレチンの関与を示唆するものである。インクレチンとは栄養素の刺激により消化管から分泌され、インスリン分泌を増強させるホルモンの総称である。Glucagon-like peptide-1（GLP-1）は1984年に発見されたインクレチンホルモンであり、近年、糖尿病治療、特に食後高血糖治療の側面から注目されている。

GLP-1は摂食に伴い小腸のL細胞より分泌され、膵ランゲルハンス島β細胞において血糖濃度依存的にインスリン分泌を促進する。そのメカニズムは以下のように説明される（Figure 1-1）。すなわち、膵β細胞に糖輸送担体を経由してグルコースが取り込まれると、adenosine 5’-triphosphate（ATP）が産生される。その結果、ATP濃度とadenosine 5’-diphosphate（ADP）濃度の比が増加し、細胞膜上のATP依存性K^+チャネルが閉鎖し、膜の脱分極が生じる。これに伴い、電位依存性Ca^{2+}チャネルが活性化し、細胞外から細胞内へCa^{2+}を流入させる。細胞内Ca^{2+}濃度の増加はインスリン分泌顆粒のエキソサイトーシスを惹起し、インスリンを分泌する。一方、GLP-1はβ細胞膜上

の７回膜貫通性のG蛋白共役型受容体であるGLP-１受容体に結合し、アデニル酸シクラーゼを活性化し細胞内cyclic AMP（cAMP）が増加する。cAMPの上昇はCa^{2+}によるインスリンのエキソサイトーシスを増強する。その他、GLP-1には、β細胞の保護・新生作用[5, 6]、グルカゴン分泌抑制による糖新生抑制作用[7]、胃排泄抑制による摂食抑制作用[8]が知られており、これら一連の作用は糖尿病治療において有益と考えられる。そのため、GLP-1の作用増強をターゲットとした創薬は次世代の2型糖尿病治療薬として注目されている。

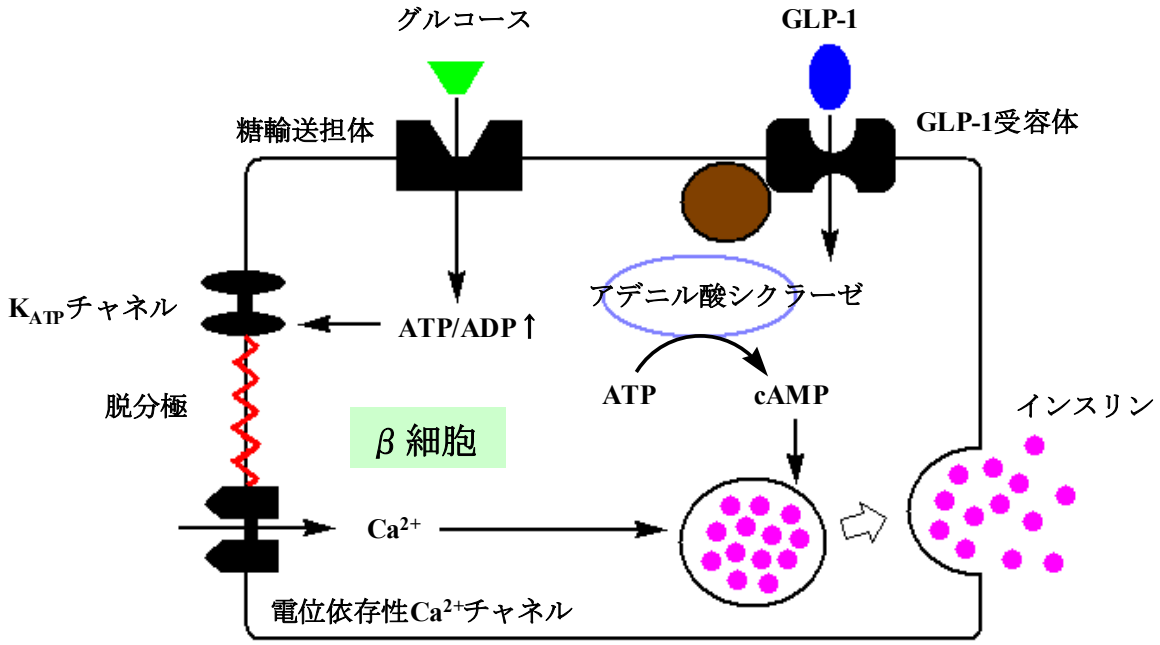


Figure 1-1. GLP-1によるインスリン分泌機序

活性型のGLP-1はGLP-1(7-37)あるいはGLP-1(7-36)amideと表される31あるいは30アミノ酸からなるペプチドである（Figure 1-2）。GLP-1は分泌後、血中のDipeptidyl peptidase IV（DPP-IV）により不活性型のGLP-1(9-37)あるいはGLP-1(9-36)amideに分解される。GLP-1の血中半減期は2分以下とされている。GLP-1の分解酵素として、DPP-IVの他にNeutral endopeptidase 24.11（NEP-24.11）が知られている[9]。生体内でのGLP-1の分解にNEP-24.11がどの程度寄与しているか十分に解明されていない。

DPP-IVに対する代謝安定性の増したGLP-1アナログが開発され、Byetta（一般名Exenatide）として2005年4月にFDA（アメリカ食品医薬品局）より承認され臨床において2型糖尿病治療薬としての有用性が明らかにされている。しかしながらByettaはペプチド製剤であることから、経口投与は不可能で1日2回の注射を必要とする。また、Byettaには吐き気などの副作用も報告されている。一方、低分子のDPP-IV阻害剤は、経口投与により内因性のGLP-1の作用を増強しうる事から、創薬ターゲットとして注目され、数多くの製薬会社が研究開発競争を行っている。

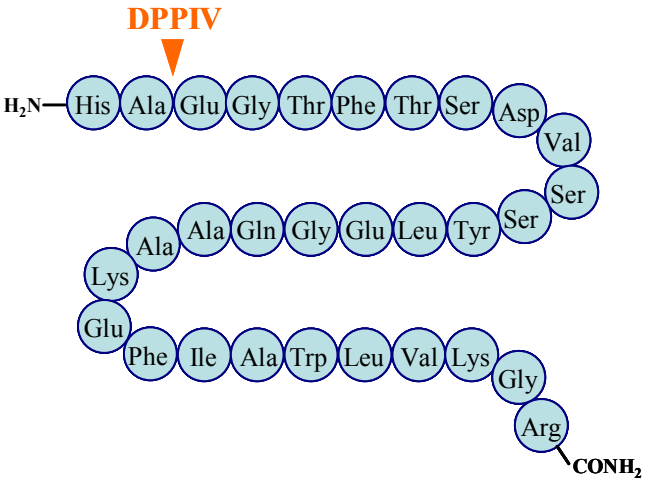


Figure 1-2. GLP-1(7-36)amideの構造とDPP-IVによる切断部位

１-３．DPP-IV阻害剤の創薬ターゲットとしての妥当性

DPP-IV欠損マウスやDPP-IV欠損ラットでは、対照動物と比較して、有意な血中GLP-1の増加と良好な耐糖能が観察され、さらに高脂肪食負荷によるインスリン抵抗性および肥満の進展抑制が認められた[10, 11, 12]。また、DPP-IV欠損マウスは健康に生存することが知られている。以上のことから、DPP-IVを阻害することに基づく2型糖尿病治療における有効性が期待され、副作用発現の可能性も低いと考えられる.

既存の食後高血糖を是正する薬剤（食後過血糖改善薬）にはα-グルコシダーゼ阻害薬、速効型インスリン分泌促進薬が有る[1]。これら薬剤はともに毎食直前に服用する必要があり、食後では効果を示さない。速効型インスリン分泌促進薬は食前30分での服用では低血糖を起こす可能性があり、より厳密な服薬コントロールが必要である。また、α-グルコシダーゼ阻害薬は副作用として腹部膨満感、放屁、下痢などが認められている。

DPP-IV阻害剤は、既存の食後高血糖改善薬を上回る有用性と既存薬の欠点の克服が期待される。すなわち、食後高血糖是正効果に加え、β細胞の保護・新生作用、糖新生抑制作用、摂食抑制作用に基づく糖尿病治療効果が期待される。またGLP-1の作用は血糖濃度に依存しているため、GLP-1の作用を延長させるDPP-IV阻害剤は低血糖のリスクが極めて低いと考えられる。また、持続性のあるDPP-IV阻害剤が見出されれば、一日一回の服薬で食後血糖値をコントロールすることが可能になり患者のQOLの改善に大きく貢献すると考えられる。

１-４．DPP-IV

DPP-IV (EC3.4.14.5) はリンパ球の細胞表面に存在するCD26として知られている。DPP-IVは110kDaの糖タンパクとして腎臓、肝臓、腸管、胎盤、皮膚、リンパ球、内皮細胞などの細胞表面に広く分布する。血中には約100kDaの可溶性DPP-IVが存在する。DPP-IVはセリンプロテアーゼの一つであり、N末端から2番目のプロリンあるい

はアラニンを認識し切断する。DPP-IVの基質としては、インクレチンの他、ケモカイン、神経ペプチドなど多数知られている[13, 14]。しかしながら、これら基質のDPP-IVによる分解の生理学的な意味は未だ十分に解明されていない。

DPP-IVによる基質の認識は以下の通りである（Figure 1-3）[15, 16, 17]。N末端のアミノ基は２つのグルタミン酸（Glu205, Glu206）とチロシン（Tyr662）の側鎖によって認識される。アミドのカルボニル基はアルギニン（Arg125）とアスパラギン（Asn710）の側鎖NH基と水素結合している。またP1部分のプロリンまたはアラニンは親油性のアミノ酸（Val656, Tyr631, Tyr662, Trp659, Tyr666, Val711）で形成されるS1ポケットにより厳密に認識される。

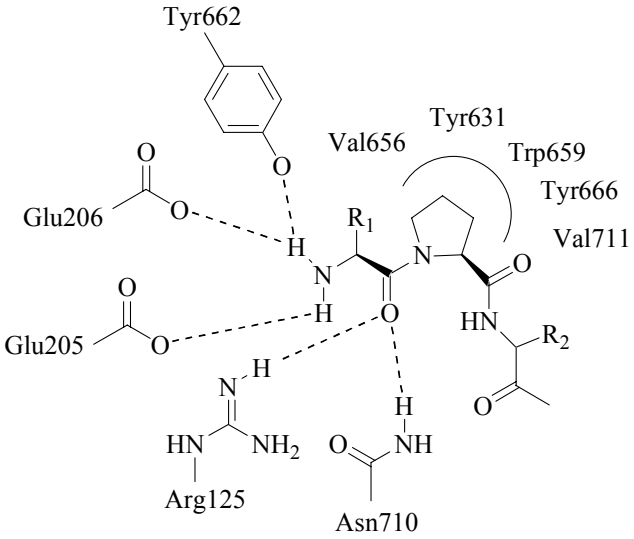


Figure 1-3. DPP-IV による基質の認識

アミド結合を切断するセリンはヒスチジンとアスパラギン酸によりcatalytic triad（Ser630, His740, Asp708）を形成し活性化されている（Figure 1-4.a）。すなわち，アスパラギン酸のカルボキシラートイオンがヒスチジンのイミダゾールの水素と水素結合することによりイミダゾールの塩基性が増し、セリンの水酸基の水素が引き抜かれ、求核性が増大している。基質が求核攻撃を受け生じたオキシアニオンはチロシン（Tyr547）の側鎖水酸基とセリン（Ser630）主鎖のアミドNHにより安定化される（Figure 1-4.b）。ついで、基質とセリンがエステル結合で共有結合したアシル中間体が生成し（Figure 1-4.c）、最後にcatalytic triad により活性化された水の求核攻撃を受けカルボン酸を遊離し、基質の切断が終了し、酵素が再生する（Figure 1-4.d,e）。

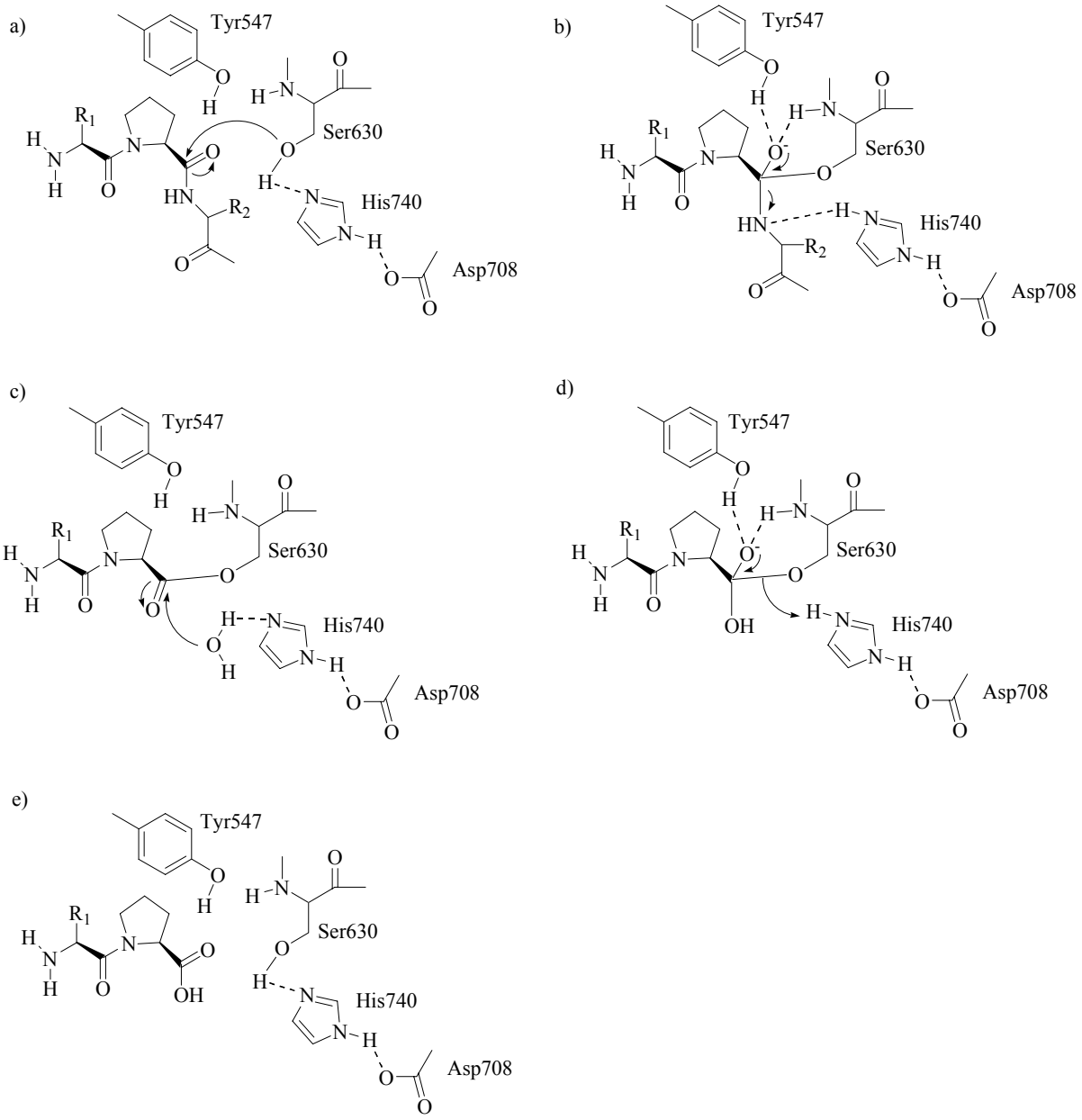
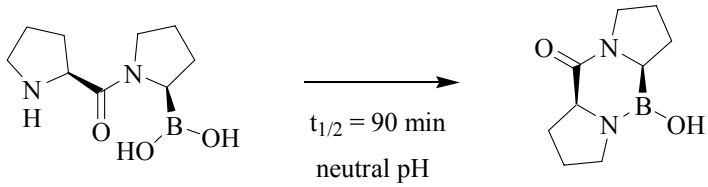


Figure 1-4. DPP-IV による基質の切断過程

1-5．DPP-IV阻害剤

1980年代後半より、DPP-IV阻害剤が報告され始めている。それらの多くは基質の構造を模倣し、N-末端にアミノ基とC-末端にセリンの水酸基と相互作用する求電子性基を持つ構造を有している。アミノ基と求電子基は分子内で6員環を形成する位置に配置されているため、報告された阻害剤の多くは分子内環化反応により分解することが知られている。Flentkeらが報告したボロン酸誘導体**1a**は強力なDPP-IV阻害作用（Ki = 3 nM）を有していたが、中性水溶液中ですみやかに分子内環化反応が進行し、不活性な**1b**を生成する（$t_{1/2}$ = 90 min）[18]。



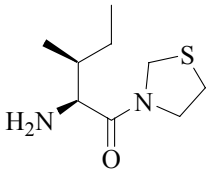
1a

Ki = 3 nM

1b

不活性

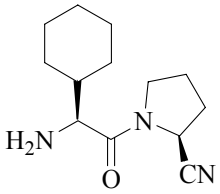
求電子基を有さないP32/98（**2**）はDPP-IV阻害作用はそれほど強力ではないが、臨床試験においてDPP-IV阻害剤の臨床上での有用性が確認された[19]。



P32/98 (**2**)

IC_{50} 1.7 μM

1995年にAshworthらは強力なDPP-IV阻害作用と分子内環化反応に対する安定性を併せ持つ阻害剤として2-シアノピロリジン類を報告した[20]。彼らの報告した化合物**3**はIC_{50} = 1.4 nM と強力で、安定性も優れている（$t_{1/2}$ = >48 h）。

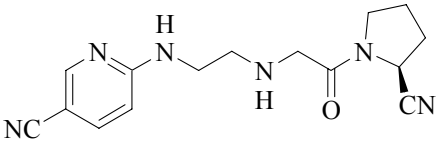


3

IC_{50} = 1.4 nM

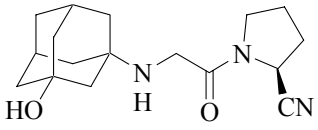
$t_{1/2}$ = >48 h

Ashworthらの報告の後、数多くの2-シアノピロリジン類が報告されるようになった。ノバルティス社の開発したNVP-DPP728（**4a**）は2型糖尿病患者に対する150 mg（1日2回）または100 mg（1日3回）の4週間投与において、食後血糖の改善およびHbA1cの低下を示し、副作用は軽度な掻痒や鼻咽喉炎などであり、DPP-IV阻害剤の糖尿病治療における有用性および安全性の高さが初めて明示された[21]。ノバルティス社は、その後、持続性の改善したNVP-LAF237 (**4b**) を開発し、最近上市に至っている[22]。



NVP-DPP728 (**4a**)

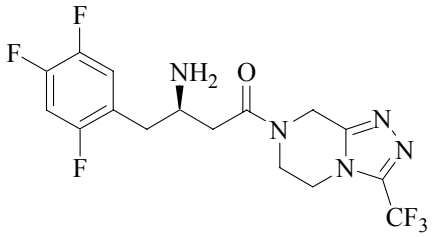
IC_{50} = 22 nM



NVP-LAF237 (**4b**)

IC_{50} = 3.5 nM

また、Merck社はシアノピロリジン骨格を持たないMK-0431 (**5**) を開発し、最近上市に至っている[23]。



5

IC_{50} = 18 nM

１-６．本研究の目的

本研究が開始されたのは2000年であるが、低投与量で1日１回投与を実現可能な持続性を示すDPP-IV阻害剤は見出されていなかった。本研究は、糖尿病患者の生活の質（QOL）の改善につながる、長時間作用の持続するDPP-IV阻害剤の創製を目的として行われた。DPP-IV阻害剤の持つ課題である分子内環化反応に対する安定化と作用持続性について、構造との相関を分子レベルで考察し、これらの課題を克服したDPP-IV阻害剤の創製を目指した。

１-７．参考文献

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第二章　4位置換ピロリジン構造を有する新規DPP-IV阻害剤のリード化合物の創出

２-１．序論

医薬品開発において、医薬品化学は①ターゲット蛋白と相互作用する低分子（ヒット化合物）を見出し、②構造修飾により目的とする性質（活性、体内動態、安全性、特許性など）を付与可能な化合物（リード化合物）へと導き、③構造最適化により目的とする性質の最大化された臨床開発候補化合物へと導く、という経過を辿る。

医薬品として備えるべき化合物の性質とは、ターゲット蛋白に対する親和性（活性）に加え、体内動態、安全性、安定性など様々である。製薬企業にとっては特許取得可能性も重要な要素の一つである。

ヒット化合物の探索には、研究機関の持つ化合物ライブラリーのスクリーニングが行われることが多い。様々な骨格を有する化合物ライブラリーの中から独自性の高いヒット化合物が得られれば、特許的にも新規性のあるリード化合物が得られる可能性がある。本プロジェクトにおいても自社化合物ライブラリーのスクリーニングを行ったが、リード化合物となりうるヒット化合物は見出されなかった。

このような場合、酵素阻害剤の研究においては酵素の基質の構造を基に阻害剤（基質アナログ）が設計される。第一章で述べた通り、DPP-IV阻害剤の分野において設計された基質アナログのうち、目的とする阻害活性と安定性を実現しうるものはシアノピロリジン類のみであった。DPP-IVは基質としてP1部分のアラニンも認識するが、阻害剤としてP1部分にアラニン骨格を用いた検討は報告が少ない。アラニン骨格でシアノ基を有する阻害剤は2-シアノピロリジンと比較し酵素阻害活性が大幅に低いことが報告されている[1]。そこで、我々もシアノピロリジン類を基にDPP-IV阻害剤を創製する事とした。Figure 2-1に示すようにP1部分に2-シアノピロリジン構造を有するDPP-VI阻害剤として、P2部分には様々な構造が報告されていた。

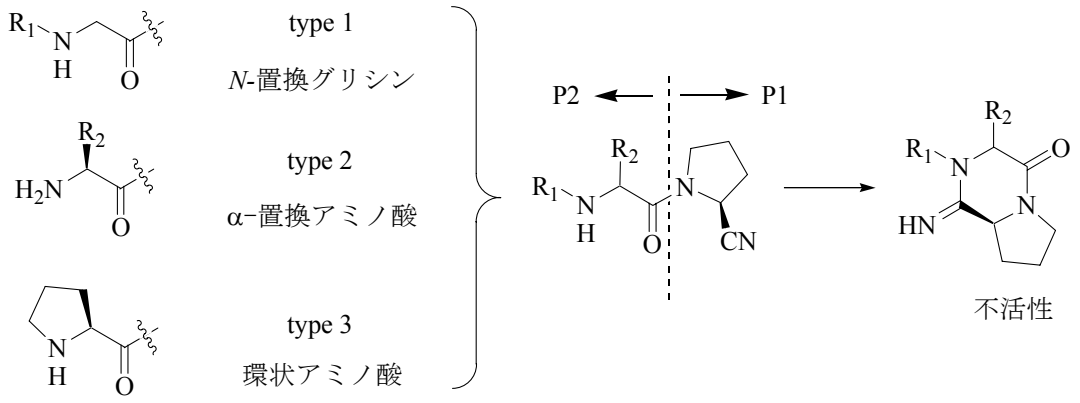
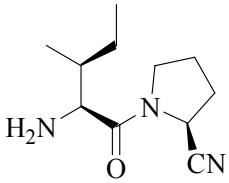
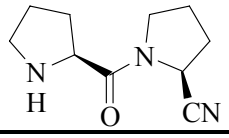


Figure 2-1. シアノピロリジン類の構造分類

P2部分として、type 1についてはNovartis社が特許出願をしており、その請求範囲は

広いものであった[2]。Ferring社はtype 2とtype 3の阻害剤について酵素阻害活性、分子内環化反応に対する安定性を報告している[3]。Table 2-1に示すように、代表化合物を比較するとtype2のものは阻害活性が高く、分子内環化反応に対する安定性が高い傾向が見られた。この報告を受け、type2のDPP-IV阻害剤に関する数多くの特許出願がなされている。一方、type3の構造を持つ化合物は阻害活性、安定性が低く、DPP-IV阻害剤の特許出願は少なかった。

Table 2-1. 2-シアノピロリジン骨格のDPP-IV阻害剤の酵素阻害活性と安定性

type	compound	structure	*in vitro* DPP-IV Ki (nM)	solution stability (pH 7.4) $t_{1/2}$ (h)
2	**6**		2.2	48
3	**7**		22	7.5

本研究では特許取得性を考慮し、type 3の阻害剤の可能性を検討することとした。化合物**7**が低投与量で作用持続性のあるDPP-IV阻害剤の創薬の出発点として妥当であるか判断するために、化合物**7**への置換基導入により以下の３点を検証することとした。

① 低投与量を実現するために酵素阻害活性が高まる可能性。
② 経口吸収性、作用持続性が高まる可能性。
③ 分子内環化反応に対する安定性が高まる可能性。

この3点を検証するにあたり、化合物**7**のどの位置にどのような置換基を導入するのが妥当か考察した。①の検証課題に対して、研究開始当時DPP-IVの蛋白質三次元立体構造は不明であったため、蛋白質の構造に基づく合理的な阻害剤の設計は不可能であった。従って、さまざまな置換基を立体選択的に導入可能な置換位置が好ましい。P1部分はDPP-IVの基質特異性を考慮するとあまり大きな変換は許容されないと考えられ、P2部分の変換が妥当と考えられる。プロリン環の4位への立体選択的な置換基導入法は数多く報告されている。②の検証課題については、経口吸収性、作用持続性に寄与する要因として膜透過性、組織分布、代謝、排泄など様々考えられる。これらに影響を与える薬物の構造要因としては、分子の親油性、親水性、置換基導入による代謝阻害が考えられる。プロリン環の代謝としては4位の水酸化が知られている。③の検証課題に対しては置換基導入による立体障害が安定性に寄与すると考えられる。従

って、ある程度嵩高い置換基が導入可能な置換位置が望ましい。

以上の考察から、化合物 **7** のP2部分のピロリジン環の4位に置換基を導入し、*in vitro* 酵素阻害活性、作用持続性、分子内環化反応に対する安定性がどのように変動するか検討を行うこととした（Figure 2-2）。

GLP-1は分泌後、血中のDPP-IVにより分解され不活性化されるため、作用持続性はラット経口投与後の血漿中DPP-IV阻害活性（*ex vivo*酵素阻害活性）により評価することとした。また、分子内環化反応に対する安定性は、pH 7.4バッファー溶液中での安定性により評価することとした。

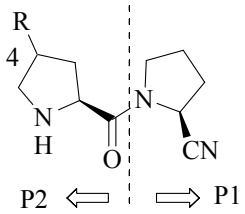
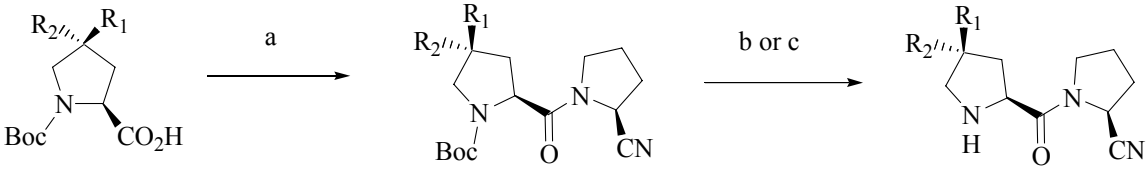


Figure 2-2. プロリル-2-シアノピロリジンからの合成展開

２-２．4位置換ピロリジン誘導体の合成

Scheme 2-1に示すように、4-フェニルピロリジン誘導体**8**、**9**の合成を行った。Krapchoらの報告[4]に従い合成した**19a**と(2*S*)-2-シアノピロリジンを１-メタンスルホニルオキシベンゾトリアゾール（MsOBt）を用いて縮合し、アミド体**20a**を得た。アミド体**20a**のBoc基を、ベンゼンスルホン酸を用いて脱保護し、目的とする(4*R*)-フェニル体**8**をベンゼンスルホン酸塩として得た。市販の**19b**を用い、**8**の合成と同様の方法で(4*S*)-フェニル体**9**を塩酸塩として得た。



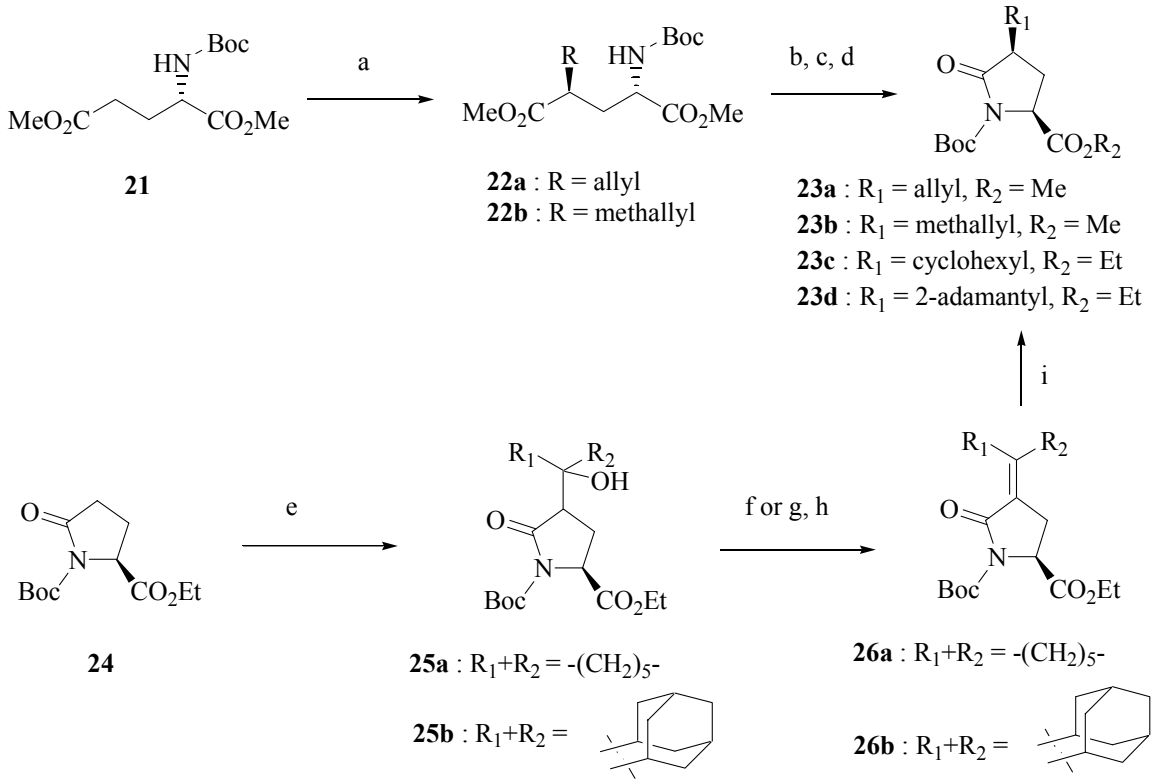
19a : R_1 = Ph, R_2 = H
19b : R_1 = H, R_2 = Ph

20a : R_1 = Ph, R_2 = H
20b : R_1 = H, R_2 = Ph

8-9

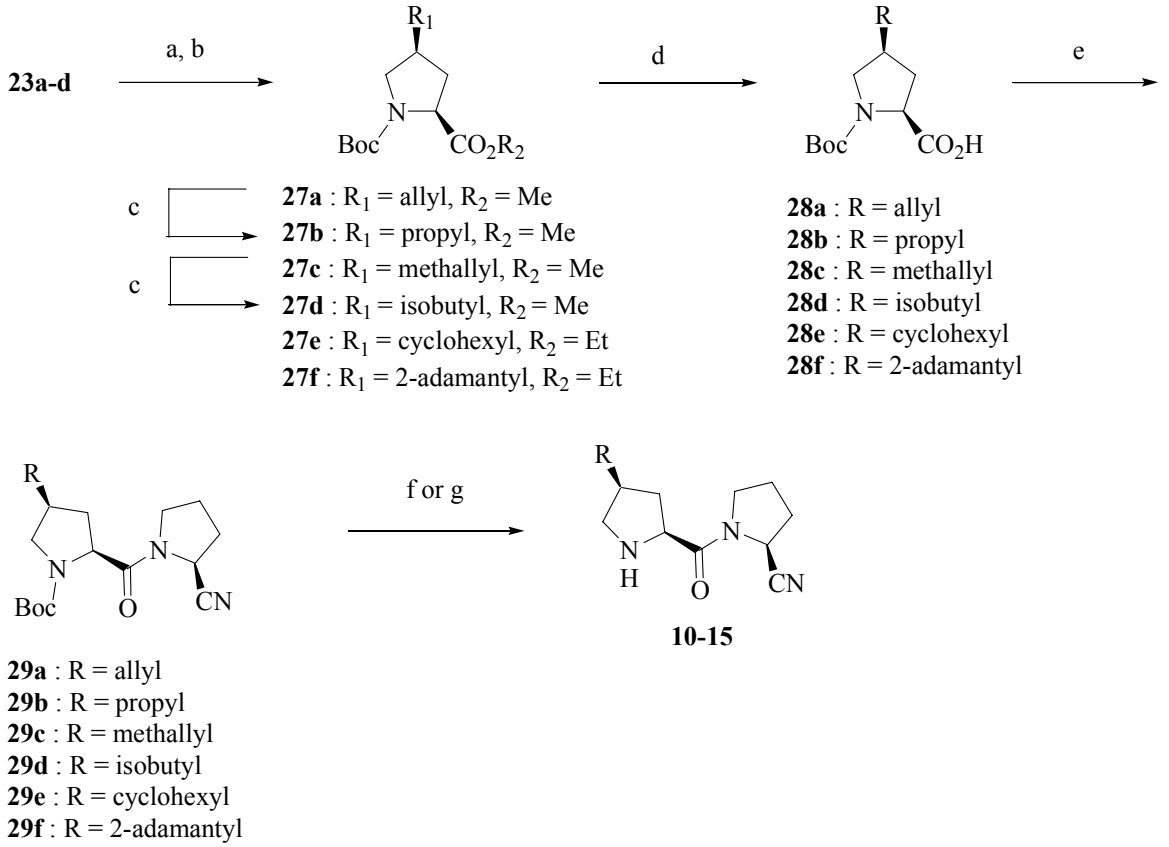
Scheme 2-1. Synthesis of **8-9**. Reagents: (a) (2*S*)-2-cyanopyrrolidine, MsOBt, Et_3N, DMF; (b) $PhSO_3H$, EtOH; (c) 4N HCl/EtOAc.

4-アルキル置換体の合成の鍵中間体である**23a-d**をScheme 2-2に示す。Hanessianらの方法[5]に従い、鍵中間体である**23a-b**は*N*-Boc-L-グルタミン酸ジメチル**21**より立体選択的に合成した。すなわち、*N*-Boc-L-グルタミン酸ジメチル**21**をリチウムビストリメチルシリルアミドによりエノラートとし、アリルブロミドまたはメタリルブロミドを反応させ、グルタミン酸エステルのγ位に*S*配置でアリル基、メタリル基を導入し**22a-b**を得た。トリフルオロ酢酸で**22a-b**のBoc基を除去し、後処理でフリーのアミンとし、トルエン中還流することにより**23a-b**を得た。鍵中間体である**23c-d**はEzquerraらの方法[6]に従い*N*-Boc-L-ピログルタミン酸エチル**24**より立体選択的に合成した。すなわち、*N*-Boc-L-ピログルタミン酸エチル**24**をリチウムビストリメチルシリルアミドによりエノラートとし、BF_3-OEt_2存在下、シクロヘキサノンまたは2-アダマンタノンを反応させ、アルコール**25a-b**を得た。メタンスルホニルクロリドまたは*p*-トルエンスルホン酸を用い、アルコール**25a-b**を脱水し、酸化白金(IV)の存在下、立体選択的に水素化し、**23c-d**を得た。



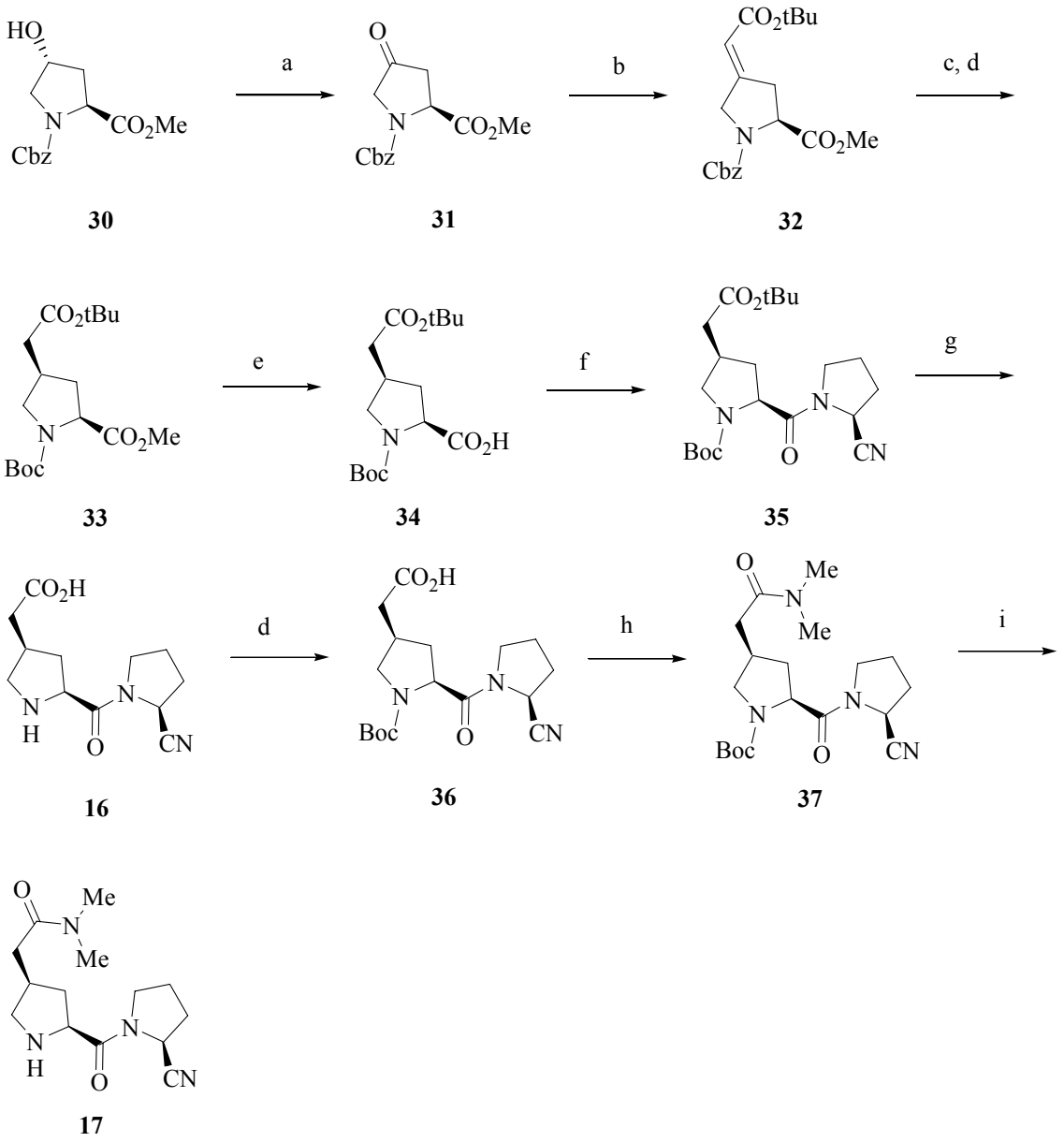
Scheme 2-2. Synthesis of **23a-d**. Reagents: (a) LiHMDS, RBr, THF; (b) TFA, CH_2Cl_2; (c) toluene, reflux; (d) Boc_2O, DMAP, CH_3CN; (e) LiHMDS, R_1R_2C=O, BF_3-OEt_2, THF; (f) MsCl, Et_3N, CH_2Cl_2; (g) *p*-TsOH-H_2O, toluene; (h) Boc_2O, DMAP, CH_2Cl_2; (i) H_2, PtO_2.

ついで、**23a-d** より4-アルキル置換体をScheme 2-3に示す方法により合成した。Pedregal らの方法[7]に従い**23a-d**を還元し**27a**、 **27c**、 **27e-f**に導いた。すなわち、**23a-d**を水素化トリエチルホウ素リチウムで還元後、BF_3-OEt_2存在下トリエチルシランでさらに還元し、**27a**、 **27c**、 **27e-f**を得た。**27a**及び**27c**の二重結合を10%パラジウム炭素の存在下、水素化し**27b**、 **27d**を得た。**27a-27f**を加水分解しカルボン酸**28a-f**を得た。得られたカルボン酸**28a-f**と(2*S*)-2-シアノピロリジンを１-メタンスルホニルオキシベンゾトリアゾールを用いて縮合し、アミド体**29a-f**を得た。アミド体**29a-f**のBoc基を4N塩化水素ジオキサン溶液または*p*-トルエンスルホン酸を用いて脱保護し、目的とする4-アルキル置換体**10-15**を塩酸塩または*p*-トルエンスルホン酸塩として得た。



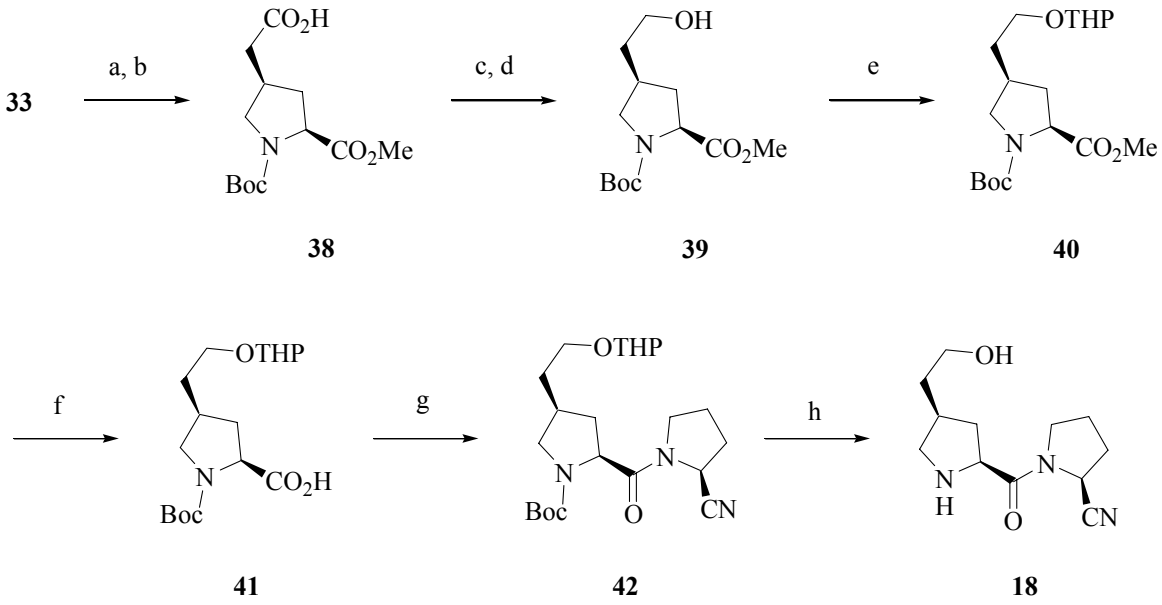
Scheme 2-3. Synthesis of **10-15**. (a) $LiEt_3BH$, THF; (b) Et_3SiH, BF_3-OEt_2, CH_2Cl_2; (c) H_2, 10% Pd-C, MeOH; (d) NaOH aq, MeOH; (e) (2*S*)-2-cyanopyrrolidine, MsOBt, Et_3N, DMF; (f) *p*-TsOH-H_2O, EtOH; (g) 4N HCl/1,4-dioxane.

4-酢酸体**16**または4-*N,N*-ジメチルアセトアミド体**17**の合成をScheme 2-4に示す。*N*-Cbz-4-ヒドロキシプロリンメチルエステル**30**をDMSOとピリジン-SO_3錯体を用いて酸化し、ケトン**31**を得た。Kempらの方法に従い[8]ケトン**31**に対しPeterson反応を行い２炭素増炭し共役エステル**32**を得た。共役エステル**32**を酸化白金(IV)の存在下、立体選択的に水素化した後、Cbz基をBoc基に掛けかえ、メチルエステルを加水分解し、カルボン酸**34**を得た。カルボン酸**34**と(2*S*)-2-シアノピロリジンを、1-エチル-3-(3'-ジメチルアミドプロピル)カルボジイミド塩酸塩（EDC）を用いて縮合し、アミド体**35**を得た。アミド体**35**を*p*-トルエンスルホン酸を用いて、Boc基および*t*-ブチル基を除去し、4-酢酸体**16**を*p*-トルエンスルホン酸塩として得た。 4-酢酸体**16**を再びBoc基で保護し、EDCを用いてジメチルアミンと縮合し、ジメチルアミド**36**を得た。 *p*-トルエンスルホン酸を用いて、ジメチルアミド**36**のBoc基を除去し、4-*N,N*-ジメチルアセトアミド体**17**を*p*-トルエンスルホン酸塩として得た。



Scheme 2-4. Synthesis of **16** and **17**. Reagents: (a) SO_3-pyridine, Et_3N, DMSO; (b) $Me_3SiCH_2CO_2{}^tBu$, LDA, THF; (c) H_2, PtO_2, AcOH; (d) Boc_2O, $NaHCO_3$ aq, THF; (e) NaOH aq, MeOH, THF; (f) (2*S*)-2-cyanopyrrolidine, EDC, HOBt, Et_3N, DMF; (g) *p*-TsOH, CH_3CN; (h) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (i) *p*-TsOH, EtOH.

アルコール**18**の合成をScheme 2-5に示す。4-酢酸体**16**の合成中間体**33**のBoc基および*t*-ブチル基を除去し、Boc基で保護し、カルボン酸**38**とした。カルボン酸**38**を、クロロギ酸エチルを用いて混合酸無水物とした後、水素化ホウ素ナトリウムで還元しアルコール**39**を得た。水酸基をTHP基で保護し、メチルエステルを加水分解し、カルボン酸**41**を得た。カルボン酸**41**と(2*S*)-2-シアノピロリジンを、EDCを用いて縮合し、アミド体**42**を得た。アミド体**42**を(+)-カンファースルホン酸を用いてBoc基を除去し、アルコール**18**を(+)-カンファースルホン酸塩として得た。

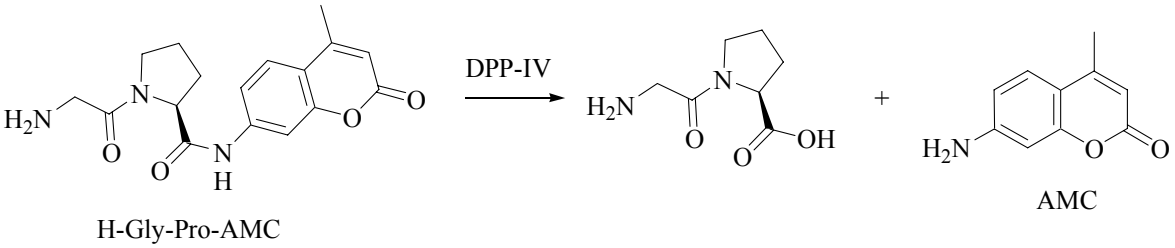


Scheme 2-5. Synthesis of **18**. Reagents: (a) TFA, PhOMe; (b) Boc_2O, $NaHCO_3$ aq, THF; (c) $ClCO_2Et$, Et_3N, CH_2Cl_2; (d) $NaBH_4$, THF, H_2O; (e) DHP, PPTS, CH_2Cl_2; (f) NaOH aq, MeOH, THF; (g) (2*S*)-2-cyanopyrrolidine, EDC, HOBt, Et_3N, DMF; (h) (+)-CSA, EtOH.

２-３．4位置換ピロリジン誘導体の*in vitro*酵素阻害活性の評価

２-３-１．方法

合成した阻害剤のDPP-IV酵素阻害活性は、ヒト血漿より文献記載の方法にて精製したDPP-IVを用いて評価した[9]。酵素阻害活性は、合成基質であるH-Gly-Pro-AMCのDPP-IVによる分解を阻害剤がどれだけ抑制するかを、7-amino-4-methyl coumarin (AMC)の生成速度を測定することにより求めた[10]。評価は複数の化合物濃度において行い、50%抑制する濃度をIC_{50}値として求めた。



Scheme 2-6. DPP-IV酵素阻害活性の測定

２-３-２．結果と考察

合成した4位置換ピロリジン誘導体の*in vitro*酵素阻害活性の評価結果をtable 2-2 に示した。

フェニル基を*R*配置（２位カルボニル基と*cis*配置）に導入した化合物**8**においては、無置換の化合物**7**と比較し、約6倍の酵素阻害活性の向上が見られた。一方、*S*配置（２位カルボニル基と*trans*配置）に導入した化合物**9**は、無置換の化合物**7**と同等であった。置換基の導入は2位カルボニル基と*cis*配置が好ましいと考えられた。

2位カルボニル基と*cis*配置で様々な嵩高さのアルキル置換基を導入した。アリル基**10**とプロピル基**11**、メタリル基**12**とイソブチル基**13**は同等の酵素阻害活性を示した。また、シクロヘキシル基**14**とフェニル基**8**は同等の酵素阻害活性を示した。これらの結果より、置換基のπ電子の酵素阻害活性への寄与が少ないと考えられる。また、非常に嵩高い2-アダマンチル基**15**も高い酵素阻害活性を示し、酵素のポケットはかなり広いと考えられる。

アルキル鎖に親水性の置換基であるカルボキシル基**16**、ジメチルアミノカルボニル基**17**、水酸基**18**を導入したところ、カルボキシル基以外の化合物において、高い酵素阻害活性を示した。

以上の結果から下記の構造活性相関が得られた。

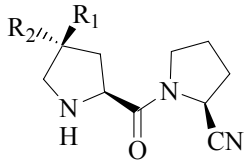
① 4位には2位カルボニル基と*cis*配置であらゆる嵩高さの置換基が導入可能である。

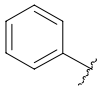
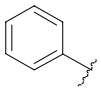
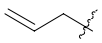
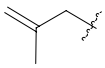
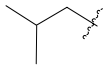
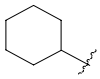
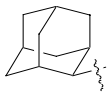
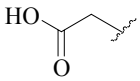
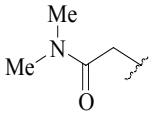
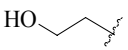
② 親油性、親水性のどちらの置換基も導入可能である。

また、①、②の結果より、置換基の導入による酵素阻害活性の向上は酵素との直接的な相互作用（酵素のアミノ酸残基との親油性の相互作用や水素結合など）ではなく、

阻害剤を酵素阻害に適した立体配座に固定する、または阻害剤と酵素が結合した際に阻害剤の運動を規制している、などの間接的な効果によるものと考えられる。

Table 2-2. 4位置換ピロリジン誘導体の*in vitro*酵素阻害活性



compound	R_1	R_2	human DPP-IV IC_{50} (nM)
7	H	H	20
8		H	3.5
9	H		16
10		H	3.5
11		H	3.4
12		H	5.7
13		H	2.9
14		H	2.4
15		H	7.8
16		H	16
17		H	4.5
18		H	3.8

２-３-３．DPP-IVの三次元構造に基づく考察

最近、シアノピロリジン類のDPP-IV阻害剤とDPP-IVとの複合体のX線結晶構造解析の結果が報告されている。このうち、P2位にピロリジン環を有する阻害剤についてもPeiらにより報告され、複合体のX線結晶構造解析がProtein Data Bank に登録されている（2G63）[11]。この解析結果を基に、4位置換ピロリジン誘導体の構造活性相関について考察した。

ピロリジン環の4位置換基の立体化学について、カルボニルと*cis*配置での置換基の導入が好ましいという構造活性相関が得られている。DPP-IVの3次元構造からは、ピロリジン環の2位のカルボニル基と*cis*配置の方向は大きな空間があることが分かった。一方、2位のカルボニル基と*trans*配置の方向には酵素のアミノ酸残基で塞がれており、“壁”があることがわかり、構造活性相関と一致する。

大きな空間が*cis*配置の方向にあるというDPP-IVの3次元構造から、あらゆる嵩高さ、種類の置換基が導入可能な事も説明可能と考えられる。

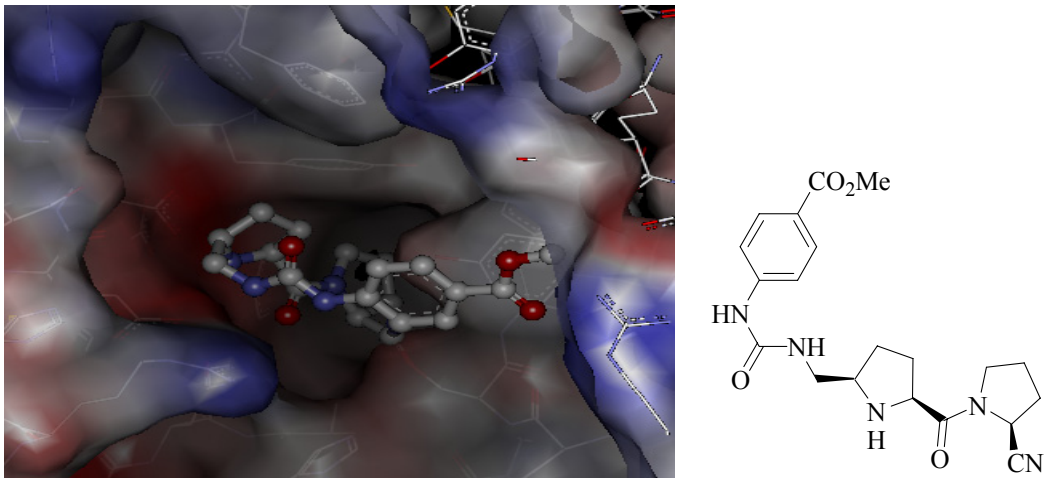


Figure 2-3. 5位置換プロリル-2-シアノピロリジンのDPP-IVとの複合体（2G63）

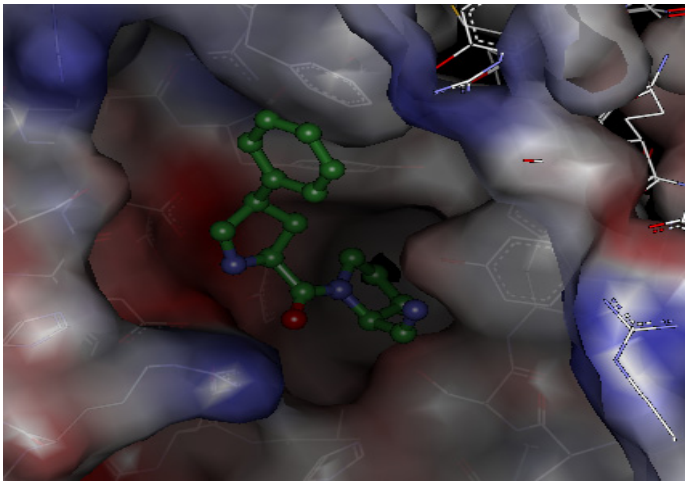


Figure 2-4. 4位置換プロリル-2-シアノピロリジンの2G63への重ね合わせ

２-４．4位置換ピロリジン誘導体のラットにおける経口吸収性と作用持続性の評価

２-４-１．方法

合成した阻害剤の経口吸収性と作用持続性を、正常ラット（Sprague-Dawley rats、SDラット）を用い、経口投与後の血漿中DPP-IV阻害活性（*ex vivo*酵素阻害活性）を測定することにより評価した。0.5%メチルセルロース水溶液に溶解した阻害剤を、1 mg/kgの投与量で経口投与後、経時的に頚静脈より採血し、血漿を調製した。得られた血漿に合成基質であるH-Gly-Pro-AMCを加え、血漿中のDPP-IVの阻害活性を測定した。酵素阻害活性は、投与前の血漿のDPP-IV活性に対する阻害率（%阻害率）で示した。なお、*ex vivo*酵素阻害活性において約50%以上の阻害率を示す時、経口糖負荷試験において血糖効果作用が見られることがわかっている。また、上市品である化合物**4b**、化合物**5**は投与9時間後において62%、59%の阻害率を示す。

２-４-２．結果と考察

合成した4位置換ピロリジン誘導体の*ex vivo*酵素阻害活性の評価結果をtable 2-3 に示した。

カルボキシル基を有する化合物**16**を除いて、投与15分後より血中DPP-IV活性を90%近く抑制し、本骨格の化合物が良好な経口吸収性を示すことが分かった。Lipinskiらは、経口投与の医薬品と物理化学的性質の相関について検討し、Rule of Five（Ro5）という経験則を示している[12, 13]。この経験則は、分子量500以下、cLogP（水とオクタノール間の化合物の分配率の計算値）5以下、水素結合供与基の数（NH, OHの数）が5以下、水素結合受容基の数（N、Oの数）が10以下の化合物が高い経口吸収性を示すというものである。table 2-3に示した化合物はいずれのパラメーターをも満たしていることから、いわゆる“薬らしい構造”と言える。一方、カルボキシル基を有する化合物**16**は塩基性のアミノ基と酸性のカルボキシル基が分子内に共存し、イオン型で存在していると考えられる。薬物が消化管膜を通過し吸収されるためにはイオン型ではなく電荷をもたない分子型をとる必要があると言われている。そのため、化合物**16**は経口吸収性が低く、低い*ex vivo*酵素阻害活性を示したと考えられる。

*ex vivo*酵素阻害活性の経時変化は化合物によって差が見られた。フェニル基を*cis*配置で有する化合物**8**は、投与1時間後に減弱した阻害活性が2時間後に再び強くなり、6時間後においても60%の阻害率を示した。この現象はフェニル基を*trans*配置で有する化合物**9**においては見られていない。詳細は第三章にて議論するが、化合物**8**がラット生体内で活性を有する代謝物（活性代謝物）を生成し、持続性を示していると考えられる。

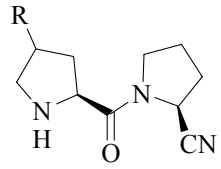
アルキル置換基についてはプロピル基**11**、イソブチル基**13**が4時間後に50%以上の阻害活性を示したが、アリル基**10**、メタリル基**12**、シクロヘキシル基**14**、アダマンチル基**15**は阻害活性の消失が速やかであった。

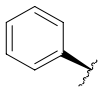
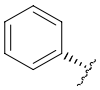
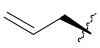
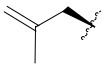
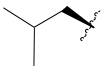
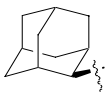
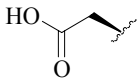
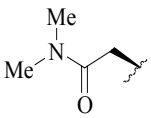
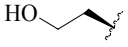
一方、アルキル鎖に親水性の置換基であるジメチルアミノカルボニル基**17**、水酸基

18を導入した化合物では6時間後においても60%近い阻害率を示し、作用持続性が見られた。

親水性の高い化合物が持続性を示す理由は明らかではないが、以下のような可能性が推測される。生体内に吸収された化合物は異物であるため、生体は代謝により水溶性を増加させ排泄する。生成する代謝物は多くの場合、元々有していた生理活性を失う。例えば、DPP-IV阻害剤ではP1部のピロリジン環の4位に水酸化を受けると阻害活性が大幅に減弱することが知られている[14]。親油性の高い阻害剤ではより速やかに阻害活性を失う代謝が進行し、親水性が高い化合物では代謝の進行が遅く持続性を示した可能性がある。

Table 2-3. 4位置換ピロリジン誘導体の*ex vivo*酵素阻害活性



compound	R	human DPP-IV IC_{50} (nM)	*ex vivo* DPP-IV inhibition (%) at 1 mg/kg po, normal rats					
			time after administraton (h)					
			0.25	0.5	1	2	4	6
7	H	20	94	94	89	68	26	20
8		3.5	88	81	68	93	88	60
9		16	90	80	57	36	12	17
10		3.5	97	97	96	77	46	26
11		3.4	96	96	94	83	56	50
12		5.7	93	94	90	69	46	27
13		2.9	95	96	93	77	54	40
14		2.4	97	97	94	70	36	38
15		7.8	95	87	66	47	38	47
16		16	40	46	53	50	47	38
17		4.5	96	97	96	89	78	66
18		3.8	93	95	94	87	75	58

２-５．4位置換ピロリジン誘導体の分子内環化反応に対する安定性

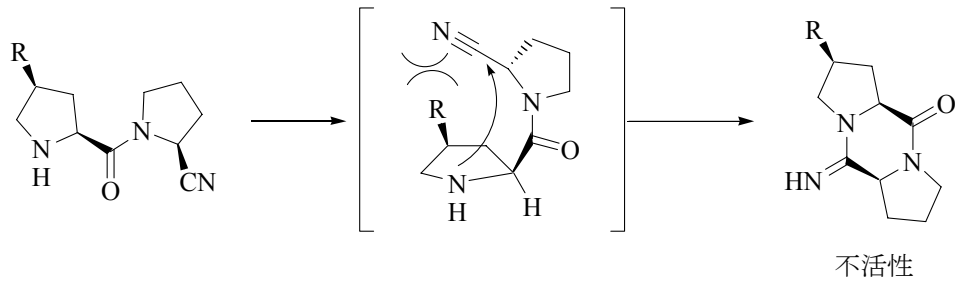
２-５-１．方法

合成した4位置換ピロリジン誘導体のうち、代表化合物の分子内環化反応に対する安定性を、pH 7.4バッファーに溶解し経時的にHPLCを測定し評価した。pH 7.4 Tris buffer（100 mM）に阻害剤を1 mg/mLになるよう溶解し、室温にて反応した。最初にHPLCに注入した時間を0時とし、2、6、24時間後のサンプルを分析した。残存している化合物のピークの面積をLC/MSにて算出し、時間に対しlog(ピーク面積/0時のピーク面積)をプロットし、線形近似直線の傾きより半減期を求めた。

２-５-２．結果と考察

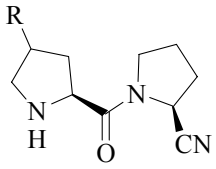
4位置換ピロリジン誘導体の安定性をtable 2-4 に示した。

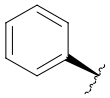
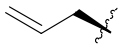
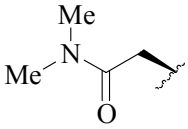
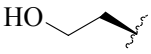
評価した化合物はいずれも無置換の化合物**7**と比較しわずかながら安定性が改善していた。分子内環化反応が進行する際、*cis*配置に置換基がある場合ニトリル基が窒素原子に近づく際に4位の置換基により立体障害を受けるため、安定性が増したと考えられる。従って、4位置換ピロリジン誘導体をリード化合物として最適化を行う際には、4位に嵩高い置換基を導入する事が阻害剤の設計方針の一つと考えられた。



Scheme 2-7. 4位置換ピロリジン誘導体の分子内環化反応に対する安定化

Table 2-4. 4位置換ピロリジン誘導体の分子内環化反応に対する安定性



compound	R	solution stability (pH 7.4) $t_{1/2}$ (h)
7	H	7.5
8		13
10		12
17		24
18		19

２-６．小括

プロリル2-シアノピロリジン**7**のP2位ピロリジン環の4位に置換基導入を行い、4位置換ピロリジン誘導体から最適化すべきリード化合物が得られるか検証した。検証課題は下記の3点であった。

① 低投与量を実現するために酵素阻害活性が高まる可能性。

② 経口吸収性、作用持続性が高まる可能性。

③ 分子内環化反応に対する安定性が高まる可能性。

①の検証課題に対して、ピロリジン環4位にカルボニル基と*cis*配置で置換基を導入すると酵素阻害活性が高まる可能性があることを見出した。

②の検証課題に対して、4位置換ピロリジン誘導体が高い経口吸収性を示す可能性を見出した。また、置換基としてフェニル基、またはジメチルアミノカルボニルメチル基、水酸基を導入することにより、作用持続性が高まる可能性があることを見出した。

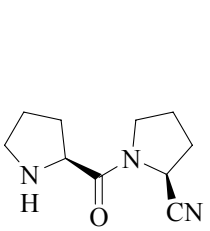
③の検証課題に対して、4位置換基導入によりわずかながら安定性が高まることを見出した。安定性が高まった理由としては4位置換基による立体障害と考えられた。

以上の検証結果に基づき、最適化すべきリード化合物として4位フェニル誘導体**8**と4位アミド誘導体**17**を選択することとした。

フェニル誘導体**8**に関しては、置換基導入により分子内環化反応に対する安定性が高まる可能性が考えられる。また、活性代謝物による作用持続性の発現のメカニズムを解明することは科学的にも興味深い。

アミド誘導体**17**に関しては、アミノ基を幅広く変換可能であり、分子内環化反応に対する安定性や作用持続性を最適化できる可能性が考えられた。

アルコール誘導体**18**に関しては、高い作用持続性を示したが、構造変換の可能性が低いと考え、最適化すべきリード化合物として選択しなかった。



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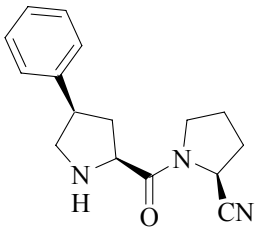

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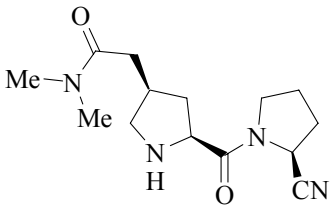

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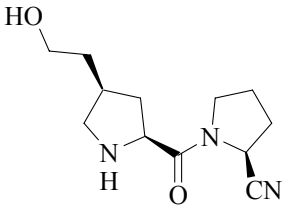

18

２-７．実験の部

２-７-１．Chemistry

General directions

Analytical samples were homogeneous as confirmed by TLC and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were taken on a Varian Mercury 300 spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. The chemical shift values are reported in parts per million (δ) and coupling constants (*J*) in hertz (Hz). Fast atom bombardment mass spectra (FAB-MS, HRMS) and electron ionization (EI) were obtained on a JEOL JMS-700 spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a HITACHI M-1200H spectrometer. Matrix-assisted laser desorption ionization (MALDI) mass spectra were obtained on a PerSeptive Biosystems VoyagerTM Elite spectrometer. Infrared spectra (IR) were measured in a JASCO FT/IR-430 spectrometer. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063-0.200 mm), Wako gel C200 or Fuji Silysia FL60D]. Thin-layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, silica gel 60 F254). The following abbreviations for solvents and reagents are used; tetrahydrofuran (THF), diethyl ether (Et_2O), dimethylsulfoxide (DMSO), ethyl acetate (EtOAc), dimethylformamide (DMF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), methanol (MeOH), ethanol (EtOH), acetic acid (AcOH), hydrochloric acid (HCl).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-phenyl-2-pyrrolidinecarboxylic acid (19a)

To a stirred solution of methyl (2*S*,4*R*)-4-phenyl-2-pyrrolidinecarboxylate[4] (524 mg, 2.56 mmol) in EtOH (3 mL) was added di-*tert*-butyl-dicarbonate (655 mg, 3.00 mmol) at room temperature. After being stirred for 15 h, the reaction mixture was diluted with EtOAc. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. To a stirred solution of the resulting residue in MeOH (5 mL) was added 1 M NaOH (3 mL) at 0 ºC. After being stirred at room temperature for 3 h, the reaction mixture was quenched with 1 M HCl (3 mL) and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to yield **19a** (635 mg, 85%) as a white powder. TLC R_f = 0.36 ($CHCl_3$/MeOH, 9/1); MS (APCI, Neg. 20 V) *m/z* 290 $(M-H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 and 1.49 (s, 9H), 2.01−2.47 (m, 1H), 2.54−2.87 (m, 1H), 3.16−3.56 (m, 2H), 3.91−4.21 (m, 1H), 4.24−4.64 (m, 1H), 7.02−7.48 (m, 5H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-phenyl-1-pyrrolidinecarboxylate (20a)**

To a stirred solution of **19a** (590 mg, 2.03 mmol) in DMF (10 mL) were added (2*S*)-2-pyrrolidinecarbonitrile hydrochloride (268 mg, 2.01 mmol), 1-methanesulfonyloxy-1*H*-benzotriazole (433 mg, 2.10 mmol), triethylamine (0.57 mL, 4.1 mmol) at 0 ºC. After being stirred at room temperature for 15 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/2) as an eluant to yield **20a** (562 mg, 75%) as a white powder. TLC R_f = 0.61 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 370 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 and 1.44 (s, 9H), 2.06−2.43 (m, 5H), 2.51−2.71 (m, 1H), 3.27−3.44 (m, 1H), 3.44−3.56 (m, 1H), 3.56−3.72 (m, 1H), 3.74−4.24 (m, 2H), 4.39−4.65 (m, 1H), 4.79−4.99 (m, 1H), 7.10−7.50 (m, 5H).

According to the same procedure as described above, **20b** was prepared from **19b**.

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-phenyl-1-pyrrolidinecarboxylate (20b)**

Yield 66%. A white powder. TLC R_f = 0.64 (CH_2Cl_2/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 370 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 2.09−2.43 (m, 6H), 3.32−3.54 (m, 1H), 3.56−3.91 (m, 3H), 3.98−4.16 (m, 1H), 4.51 and 4.62 (dd, *J* = 8.1, 2.3 Hz, 1H), 4.83−4.91 (m, 1H), 7.16−7.44 (m, 5H).

(2*S*)-1-{[(2*S*,4*R*)-4-Phenyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile benzenesulfonate (8)

A solution of **20a** (1.61 g, 4.37 mmol) and benzenesulfonic acid (1.04 g, 6.56 mmol) in EtOH (8 mL) was refluxed for 3 h. The reaction mixture was concentrated in vacuo. The resulting crystalline solid was collected by filtration and washed with hexane-EtOAc to yield **8** (1.28 g, 69%) as a white powder. TLC R_f = 0.45 ($CHCl_3$/MeOH/H_2O, 10/2/0.1); MS (APCI, pos. 20 V) *m/z* 270 $(M+H)^+$; IR (KBr) 3448, 3085, 2244, 1660, 1226, 1161, 1148, 1123, 1015, 608 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.74–1.89 (m, 1H), 1.95–2.09 (m, 2H), 2.09–2.34 (m, 2H), 2.89–3.03 (m, 1H), 3.18–3.29 (m, 1H), 3.49–3.66 (m, 3H), 3.66–3.77 (m, 1H), 4.61 (dd, *J* = 10.6, 7.6 Hz, 1H), 4.86 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.23–7.40 (m, 8H), 7.55–7.62 (m, 2H), 9.21 (s, 2H); HRMS (FAB) calcd for $C_{16}H_{20}N_3O$: 270.1606. Found: 270.1606.

(2*S*)-1-{[(2*S*,4*S*)-4-Phenyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile hydrochloride (9)

To a solution of **20b** (421 mg, 1.14 mmol) in EtOAc (4 mL) was added 4M HCl in EtOAc (2 mL). After 3 h, the reaction mixture was concentrated in vacuo. The resulting crystalline solid was washed with EtOAc to yield **9** (205 mg, 59%) as a white powder. TLC R_f = 0.43

(CH_2Cl_2/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 270 $(M+H)^+$; IR (KBr) 3440, 2242, 1654, 1495, 1455, 1446, 1348, 763, 704, 523 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.96–2.34 (m, 4H), 2.41–2.48 (m, 1H), 3.07–3.29 (m, 1H), 3.34–3.51 (m, 1H), 3.52–3.85 (m, 4H), 4.75 (s, 1H), 4.84 (dd, *J* = 7.9, 4.6 Hz, 1H), 7.13–7.54 (m, 5H), 8.93 (s, 1H), 10.76 (s, 1H); HRMS (FAB) calcd for $C_{16}H_{20}N_3O$: 270.1606. Found: 270.1604.

Dimethyl (2*S*,4*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-(2-methyl-2-propen-1-yl)pentanedioate (22b)

Compound **22b** was prepared from **21** according to the method reported by Hanessian et al.[5] To a stirred solution of lithium bis(trimethylsilyl)amide in THF (32 mL ,1.0 M) was added dropwise a solution of **21** (4.13 g, 15.0 mmol) in THF (45 mL) at −78 ºC. After being stirred for 30 min, a solution of methallyl bromide (4.05 g, 30 mmol) in THF (45 mL) was added and the reaction mixture was stirred at −78 ºC for additional 3 h. The reaction mixture was quenched with 1 M HCl, and extracted with EtOAc. The organic layer was successively washed with aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/5) as an eluant to yield **22b** (4.41 g, 89%) as a colorless oil. TLC R_f = 0.55 (EtOAc/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 330 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.71 (s, 3H), 1.93−2.03 (m, 2H), 2.20 (dd, *J* = 14.0, 7.1 Hz, 1H), 2.29−2.41 (m, 1H), 2.64−2.76 (m, 1H), 3.65 (s, 3H), 3.73 (s, 3H), 4.31−4.42 (m, 1H), 4.68−4.72 (m, 1H), 4.76−4.82 (m, 1H), 4.91−5.02 (m, 1H)

1-*tert*-Butyl 2-methyl (2*S*,4*S*)-4-(2-methyl-2-propen-1-yl)-5-oxo-1,2-pyrrolidinedicarboxylate (23b)

Compound **23b** was prepared from **22b** according to the method reported by Hanessian et al.[5] To a stirred solution of **22b** (4.41 g, 13.4 mmol) in CH_2Cl_2 (13 mL) was added trifluoroacetic acid (13 mL) at room temperature. After being stirred for 50 min, the reaction mixture was concentrated in vacuo and diluted with EtOAc. The organic layer was successively washed with aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was diluted with toluene (40 mL), refluxed for 2 h, and evaporated. To a stirred solution of the resulting residue in CH_3CN (55 mL) were added 4-(dimethylamino)pyridine (1.71 g, 14.0 mmol) and di-*tert*-butyl-dicarbonate (2.55 g, 11.7 mmol) at room temperature. After 18 h, the reaction mixture was diluted with EtOAc. The organic layer was successively washed with 1M HCl, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/5) as an eluant to yield **23b** (1.98 g, 49%) as a coloeless oil. TLC R_f = 0.42 (EtOAc/hexane, 1/3); MS (APCI, pos. 20 V) *m/z* 298 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 1.64−1.77 (m, 1H), 1.71 (s, 3H), 2.12 (dd, *J* = 14.1, 10.8 Hz, 1H), 2.38−2.52 (m, 1H), 2.61−2.70 (m,

1H), 2.70−2.80 (m, 1H), 3.78 (s, 3H), 4.52 (dd, *J* = 9.0, 6.6 Hz, 1H), 4.66 (s, 1H), 4.80 (s, 1H).

1-*tert*-Butyl 2-ethyl (2*S*)-4-(1-hydroxycyclohexyl)-5-oxo-1,2-pyrrolidinedicarboxylate (25a)

Compound **25a** was prepared from **24** according to the method reported by Ezquerra et al.[6] To a stirred solution of **24** (4.77 g, 18.5 mmol) in THF (50 mL) was added dropwise a solution of lithium bis(trimethylsilyl)amide in THF (20 mL, 1.0 M) at −78 ºC. The reaction mixture was stirred for 1 h at −78 ºC prior to the addition of a solution of cyclohexanone (1.99 g, 20.2 mmol) and boron trifluoride etherate (2.6 mL, 21 mmol) in THF (50 mL). After being stirred for 2.5 h, the reaction mixture was quenched with aqueous NH_4Cl and concentrated in vacuo. The aqueous layer was extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/3) as an eluant to yield **25a** (5.32 g, 80%) as a yellow oil. TLC R_f = 0.52 (Acetone/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 356 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.08−1.90 (m, 10H), 1.29 (t, *J* = 6.9 Hz, 3H), 2.05−2.25 (m, 2H), 2.77 (dd, *J* = 12.0, 9.0 Hz, 1H), 3.21 (s, 1H), 4.24 (q, *J* = 6.9 Hz, 2H), 4.54 (dd, *J* = 9.6, 1.8 Hz, 1H).

According to the same procedure as described above, **25b** was prepared from **24**.

1-*tert*-Butyl 2-ethyl (2*S*)-4-(2-hydroxy-2-adamantyl)-5-oxo-1,2-pyrrolidinedicarboxylate (25b)

Yield 66%. A colorless oil. R_f = 0.54 (Acetone/hexane, 1/3); MS (APCI, pos. 20 V) *m/z* 408 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.22−1.34 (m, 3H), 1.34−2.03 (m, 13H), 1.50 (s, 9H), 2.07−2.27 (m, 2H), 2.36−2.50 (m, 1H), 2.84 (s, 1H), 3.34 (dd, *J* = 12.1, 8.5 Hz, 1H), 4.25 (q, *J* = 6.9 Hz, 2H), 4.56 (dd, *J* = 9.8, 1.2 Hz, 1H).

1-*tert*-Butyl 2-ethyl (2*S*)-4-cyclohexylidene-5-oxo-1,2-pyrrolidinedicarboxylate (26a)

Compound **26a** was prepared from **25a** according to the method reported by Ezquerra et al.[6] To a stirred solution of **25a** (5.32 g, 14.9 mmol) in CH_2Cl_2 (30 mL) were added methanesulfonyl chloride (1.88 g, 16.4 mmol) and triethylamine (23 mL, 165 mmol) at room temperature. After being stirred for 2 days, the reaction mixture was quenched with water, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/9) as an eluant to yield a mixture of **26a** and *endo*−olefin isomer (1.43 g). This compound was used for the next reaction without further purification.

1-*tert*-Butyl 2-ethyl (2*S*)-5-oxo-4-tricyclo[3.3.1.1~3,7~]dec-2-ylidene-1,2-pyrrolidinedica

rboxylate (26b)

To a stirred solution of **25b** (1.23 g, 3.02 mmol) in toluene (10 mL) was added *p*-toluenesulfonic acid (780 mg, 4.10 mmol). The reaction mixture was refluxed for 15 h, cooled to room temperature, and diluted with EtOAc. The organic layer was successively washed with 1M HCl, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. To a stirred solution of the residue in CH_2Cl_2 (5 mL) were added 4-(dimethylamino)pyridine (54 mg, 0.44 mmol) and di-*tert*-butyl-dicarbonate (4.82 g, 22 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was diluted with EtOAc. The organic layer was successively washed with 1M HCl, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/3) as an eluant to yield **26b** (825 mg, 69%) as a colorless oil. TLC R_f = 0.92 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 390 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, *J* = 7.1 Hz, 3H), 1.45−1.55 (m, 9H), 1.70−2.02 (m, 12H), 2.48−2.57 (m, 1H), 2.57 (dd, *J* = 16.3, 4.0 Hz, 1H), 2.95 (dd, *J* = 16.3, 10.6 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.49−4.55 (m, 1H), 4.51−4.59 (m, 1H).

1-*tert*-Butyl 2-ethyl (2*S*,4*R*)-4-cyclohexyl-5-oxo-1,2-pyrrolidinedicarboxylate (23c)

Compound **23c** was prepared from **26a** according to the method reported by Ezquerra et al.[6] To a mixture of **26a** and *endo*−olefin isomer (1.43 g, 4.24 mmol) in EtOAc (20 mL) was added platinum(IV) oxide (96 mg, 0.42 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 18 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/8) as an eluant to yield **23c** (527 mg, 36%) as a colorless oil. TLC R_f = 0.56 (EtOAc/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 340 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96−1.20 (m, 4H), 1.29 (t, *J* = 7.2 Hz, 3H), 1.49 (s, 9H), 1.60−1.95 (m, 8H), 2.30−2.40 (m, 1H), 2.45−2.53 (m, 1H), 4.22 (q, *J* = 7.2 Hz, 2H), 4.51 (dd, *J* = 8.4, 7.5 Hz, 1H).

According to the same procedure as described above, **23d** was prepared from **26b**.

1-*tert*-Butyl 2-ethyl (2*S*,4*R*)-4-(2-adamantyl)-5-oxo-1,2-pyrrolidinedicarboxylate (23d)

Yield 79%. A colorless oil. TLC *R*f = 0.63 (EtOAc/toluene, 1/5); MS (APCI, pos. 20 V) *m/z* 392 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25−1.34 (m, 3H), 1.45−1.55 (m, 9H), 1.56−2.00 (m, 15H), 2.37−2.50 (m, 1H), 2.57 (s, 1H), 2.76−2.97 (m, 1H), 4.16−4.30 (m, 2H), 4.46 (dd, *J* = 8.2, 7.1 Hz, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*S*)-4-allyl-1,2-pyrrolidinedicarboxylate (27a)

Compound **27a** was prepared from **23a** according to the method reported by Pedregal et al.[7] To a stirred solution of **23a** (2.88 g, 10.2 mmol) in THF (55 mL) was added a solution of

lithium triethylborohydride in THF (12.2 mL, 1.0 M) at −78 ºC. After being stirred for 40 min, the reaction mixture was quenched with aqueous $NaHCO_3$ and warmed to 0 ºC. After the addition of 35% H_2O_2 (2 mL), the reaction mixture was stirred at 0 ºC. After being stirred for 20 min, the reaction mixture was evaporated to remove organic solvent, and the aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was used for the next reaction without further purification. To a stirred solution of the residue in CH_2Cl_2 (55 mL) were added triethylsilane (3.4 mL, 21 mmol) and boron trifluoride etherate (3.0 mL, 24 mmol) at −78 ºC. After being stirred for 3 h, the reaction mixture was quenched with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/9) as an eluant to yield **27a** (2.45 g, 89%) as a colorless oil. TLC R_f = 0.42 (EtOAc/hexane, 1/4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 and 1.40 (s, 9H) , 1.60 (m, 1H) , 2.43−2.08 (m, 4H) , 3.05 (m, 1H) , 3.73 and 3.72 (s, 3H), 3.79−3.63 (m, 1H), 4.28−4.16 (m, 1H), 5.07−4.99 (m, 2H), 5.74 (m, 1H).

According to the same procedure as described above, **27c**, **27e−f** were prepared from **23b−d**, respectively.

1-*tert*-Butyl 2-methyl (2*S*,4*S*)-4-(2-methyl-2-propen-1-yl)-1,2-pyrrolidinedicarboxylate (27c)

Yield 77%. A colorless oil. TLC R_f = 0.35 (Acetone/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37−1.49 (m, 9H), 1.53−1.66 (m, 1H), 1.71 (s, 3H), 2.11 (d, J = 6.6 Hz, 2H), 2.27−2.47 (m, 2H), 2.97−3.09 (m, 1H), 3.69−3.77 (m, 3H), 4.15−4.32 (m, 1H), 4.69 (s, 1H), 4.74 (s, 1H).

1-*tert*-Butyl 2-ethyl (2*S*,4*R*)-4-cyclohexyl-1,2-pyrrolidinedicarboxylate (27e)

Yield 98%. A colorless oil. TLC R_f = 0.68 (Acetone/hexane, 1/3); MS (APCI, pos. 20 V) *m/z* 326 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.30 (m, 6H), 1.22 (m, 3H), 1.40 and 1.45 (s, 9H), 1.50−1.95 (m, 7H), 2.40 (m, 1H), 3.01 (t, J = 10.5 Hz, 1H), 3.63−3.83 (m, 1H), 4.10−4.30 (m, 3H).

1-*tert*-Butyl 2-ethyl (2*S*,4*R*)-4-(2-adamantyl)-1,2-pyrrolidinedicarboxylate (27f)

Yield 98%. A colorless oil. TLC R_f = 0.56 (EtOAc/hexane, 1/3); MS (APCI, pos. 20 V) *m/z* 378 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.60 (m, 1H), 0.97 (m, 2H), 1.15−1.34 (m, 3H), 1.35−1.48 (m, 9H), 1.48−2.00 (m, 13H), 2.28−2.66 (m, 2H), 2.89−3.05 (m, 1H), 3.57−3.89 (m, 1H), 4.06−4.32 (m, 3H).

1-*tert*-Butyl 2-methyl (2*S*,4*S*)-4-propyl-1,2-pyrrolidinedicarboxylate (27b)

To a solution of **27a** (538 mg, 2.0 mmol) in MeOH (4 mL) was added 10 % palladium on

carbon (200 mg). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 2 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/9) as an eluant to yield **27b** (505 mg, 93%) as a colorless oil. TLC R_f = 0.47 (EtOAc/hexane, 1/4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, J = 7.0 Hz, 3H), 1.40 and 1.46 (s, 9H), 1.24−1.62 (m, 5H), 2.06−2.22 (m, 1H), 2.33−2.49 (m, 1H), 2.99 (t, J = 10.3 Hz, 1H), 3.72 and 3.73 (s, 3H), 3.61−3.82 (m, 1H), 4.07−4.30 (m, 1H).

According to the same procedure as described above, **27d** was prepared from **27c**.

1-*tert*-Butyl 2-methyl (2*S*,4*S*)-4-isobutyl-1,2-pyrrolidinedicarboxylate (27d)
Yield 91%. A colorless oil. TLC R_f = 0.38 (EtOAc/hexane, 1/4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (d, J = 6.6 Hz, 6H), 1.20−1.33 (m, 2H), 1.36−1.49 (m, 9H), 1.49−1.66 (m, 2H), 2.11−2.31 (m, 1H), 2.33−2.46 (m, 1H), 2.97 (t, J = 10.3 Hz, 1H), 3.61−3.84 (m, 1H), 3.70−3.75 (m, 3H), 4.10−4.31 (m, 1H).

(2*S*,4*S*)-4-Allyl-1-(*tert*-butoxycarbonyl)-2-pyrrolidinecarboxylic acid (28a)
To a stirred solution of **27a** (538 mg, 2.0 mmol) in MeOH (7 mL) was added 1 M NaOH (3 mL) at 0 ºC. After being stirred at room temperature for 6 h, the reaction mixture was quenched with 1 M HCl (3 mL). The organic solvent was removed by evaporation, and the aqueous layer was extracted with EtOAc. The organic layer was dried over $MgSO_4$, concentrated in vacuo. The resulting residue was solidified by hexane yielding **28a** (440 mg, 86%) as a white powder. TLC R_f = 0.24 ($CHCl_3$/MeOH, 19/1); MS (APCI, Neg. 20 V) *m/z* 255 $(M-H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.45−1.59 (m, 1H), 2.05−2.46 (m, 4H), 2.91 (dd, J = 10.4, 8.5 Hz, 1H), 3.59 (dd, J = 10.4, 7.1 Hz, 1H), 4.08 (t, J = 8.0 Hz, 1H), 4.94−5.10 (m, 2H), 5.63−5.90 (m, 1H).

According to the same procedure as described above, **28b**−**f** were prepared from **27b**−**f**, respectively.

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-propyl-2-pyrrolidinecarboxylic acid (28b)
Yield 67%. A white powder. TLC R_f = 0.27 (CH_2Cl_2/MeOH, 19/1); MS (APCI, Neg. 20 V) *m/z* 257 $(M-H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, J = 6.9 Hz, 3H), 1.28−1.39 (m, 4H), 1.42 and 1.48 (s, 9H), 1.55−2.58 (m, 3H), 2.82−3.13 (m, 1H), 3.56−3.88 (m, 1H), 4.13−4.43 (m, 1H).

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(2-methyl-2-propen-1-yl)-2-pyrrolidinecarboxylic acid (28c)
Yield 96%. A white powder. TLC R_f = 0.43 (CH_2Cl_2/MeOH, 9/1); MS (APCI, neg. 20 V) *m/z*

268 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.38−1.52 (m, 9H), 1.72 (s, 3H), 1.60−2.01 (m, 1H), 2.28−2.52 (m, 2H), 3.60−3.83 (m, 1H), 4.17−4.40 (m, 1H), 4.70 (s, 1H), 4.77 (s, 1H).

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-isobutyl-2-pyrrolidinecarboxylic acid (28d)

Yield 100%. A white powder. TLC R_f = 0.52 (CH_2Cl_2/MeOH, 9/1); MS (APCI, neg. 20 V) *m/z* 270 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85−0.93 (m, 6H), 1.22−1.33 (m, 2H), 1.38−1.50 (m, 9H), 1.51−1.84 (m, 2H), 2.13−2.53 (m, 2H), 2.95 (q, *J* = 10.6 Hz, 1H), 3.63−3.86 (m, 1H), 4.15−4.35 (m, 1H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-cyclohexyl-2-pyrrolidinecarboxylic acid (28e)

Yield 89%. A white powder. TLC R_f = 0.15 (EtOAc/hexane, 1/3); MS (APCI, neg. 20 V) *m/z* 296 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85−1.30 (m, 6H), 1.42 and 1.48 (s, 9H), 1.60−1.97 (m, 7H), 2.30−2.50 (m, 1H), 2.90−3.10 (m, 1H), 3.70 − 3.83 (m, 1H), 4.15−4.35 (m, 1H).

(2*S*,4*R*)-4-(2-Adamantyl)-1-(*tert*-butoxycarbonyl)-2-pyrrolidinecarboxylic acid (28f)

Yield 82%. A white powder. TLC R_f = 0.69 ($CHCl_3$/MeOH, 9/1); MS (APCI, neg. 20 V) *m/z* 348 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.38−1.53 (m, 9H), 1.17−1.96 (m, 16H), 2.24−2.61 (m, 2H), 2.81−3.05 (m, 1H), 3.64−3.90 (m, 1H), 4.17−4.45 (m, 1H).

According to the same procedure as described for the preparation of **20a** from **19a**, **29a−f** were prepared from **28a−f**, respectively.

***tert*-Butyl (2*S*,4*S*)-4-allyl-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (29a)**

Yield 76%. A white powder. TLC R_f = 0.24 (EtOAc/hexane, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.38 and 1.44 (s, 9H), 1.54−1.74 (m, 1H), 1.98−2.44 (m, 8H), 3.11 (t, *J* = 9.8 Hz, 1H), 3.47−3.88 (m, 3H), 4.26−4.44 (m, 1H), 4.84 (t, *J* = 9.6 Hz, 1H), 4.92−5.19 (m, 2H), 5.65−5.87 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-propyl-1-pyrrolidinecarboxylate (29b)**

Yield 61%. A white powder. TLC R_f = 0.29 (EtOAc/hexane, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.0 Hz, 3H), 1.18−1.49 (m, 4H), 1.38 and 1.37 (s, 9H), 1.50−1.72 (m, 1H), 2.03−2.43 (m, 6H), 2.92−3.16 (m, 1H), 3.51−3.89 (m, 3H), 4.23−4.45 (m, 1H), 4.71−4.95 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(2-methyl-2-propen-1-yl)-1-pyrrolidinecarboxylate (29c)**

Yield 64%. A white powder. TLC R_f = 0.74 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 348 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.35 (s, 9H), 1.36−1.47 (m, 1H), 1.71 (s, 3H), 1.99−2.24 (m, 6H), 2.29−2.48 (m, 2H), 2.89−2.99 (m, 1H), 3.50−3.68 (m, 3H), 4.39 (t, *J* = 7.9 Hz, 1H), 4.68−4.76 (m, 2H), 4.74−4.82 (m, 1H).

tert-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-isobutyl-1-pyrrolidinecarboxylate (29d)

Yield 59%. A white powder. TLC R_f = 0.78 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 350 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 0.89 (dd, *J* = 6.6, 1.5 Hz, 6H), 1.28 (t, *J* = 6.8 Hz, 3H), 1.35 (s, 9H), 1.49−1.65 (m, 1H), 1.99−2.11 (m, 2H), 2.11−2.28 (m, 3H), 2.40 −2.47 (m, 1H), 2.87 (t, *J* = 10.2 Hz, 1H), 3.51−3.62 (m, 2H), 3.65 (dd, *J* = 10.2, 7.4 Hz, 1H), 4.37 (t, *J* = 8.1 Hz, 1H), 4.71−4.83 (m, 1H).

tert-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-cyclohexyl-1-pyrrolidine carboxylate (29e)

Yield 60%. A colorless oil. TLC R_f = 0.76 (EtOAc/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 376 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 0.90−1.20 (m, 2H), 1.10−1.42 (m, 4H), 1.35 (s, 9H), 1.52−1.75 (m, 5H), 1.85−2.40 (m, 7H), 2.89−2.99 (m, 1H), 3.30 − 3.63 (m, 3H), 4.37 (t, *J* = 8.1 Hz, 1H), 4.71−4.83 (m, 1H).

tert-Butyl (2*S*,4*R*)-4-(2-adamantyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (29f)

Yield 49%. A white powder. TLC R_f = 0.41 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 428 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 1.24−1.32 (m, 9H), 1.20−2.28 (m, 20H), 2.38 −2.54 (m, 1H), 2.70−2.90 (m, 1H), 3.35−3.69 (m, 4H), 4.31−4.41 (m, 1H), 4.73−4.86 (m, 1H).

(2*S*)-1-{[(2*S*,4*S*)-4-Allyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (10)

A solution of **29a** (866 mg, 2.60 mmol) and *p*-toluenesulfonic acid (740 mg, 3.9 mmol) in EtOH (5 mL) was refluxed for 3 h. After cooling to room temperature, the resulting precipitates were collected by filtration and dried under reduced pressure to yield **10** (891 mg, 84%) as a white powder. TLC R_f = 0.35 (CH_2Cl_2/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 234 $(M+H)^+$; IR (KBr) 3852, 3152, 3083, 3002, 2603, 2464, 2241, 1916, 1809, 1667, 1492, 1460, 1383, 1268, 1236, 1162, 1118, 1032, 1010, 996, 920, 880 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.32−1.50 (m, 1H) 1.95−2.07 (m, 2H) 2.09−2.25 (m, 4H) 2.28 (s, 3H) 2.32−2.45 (m, 1H) 2.54−2.69 (m, 1H) 2.82−2.99 (m, 1H) 3.28−3.35 (m, 1H) 3.46−3.66 (m, 2H) 4.34−4.57 (m, 1H) 4.82 (dd, *J* = 7.8, 4.7 Hz, 1H) 4.95−5.16 (m, 2H) 5.65−5.87 (m, 1H) 7.11 (d, *J* = 8.0 Hz, 2H) 7.47 (d, *J* = 8.0 Hz, 2H) 8.72 (s, 1H) 9.35 (s, 1H); Anal. Calcd for $C_{20}H_{27}N_3O_4S$: C, 59.24; H, 6.71; N, 10.36. Found: C, 59.34; H, 6.70; N, 10.07.

(2*S*)-1-{[(2*S*,4*S*)-4-Propyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile hydrochloride (11)

Compound **11** was obtained as a white powder in 91% yield from **29b** according to the same procedure as described for the preparation of **9** from **20b**. TLC R_f = 0.34 (CH_2Cl_2/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 236 $(M+H)^+$; IR (KBr) 3434, 2959, 2874, 2739, 2243, 1658, 1454, 1348, 1265, 1189, 739 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.18–1.49 (m, 5H), 1.87–2.38 (m, 5H), 2.56–2.68 (m, 1H), 2.80 (s, 1H), 3.24–3.40 (m, 1H), 3.50–3.72 (m, 2H), 4.43 (s, 1H), 4.82 (dd, *J* = 7.8, 4.8 Hz, 1H), 8.66 (s, 1H), 10.38 (s, 1H); HRMS (FAB) calcd for $C_{13}H_{22}N_3O$: 236.1763. Found: 236.1767.

(2*S*)-1-{[(2*S*,4*S*)-4-(2-Methyl-2-propen-1-yl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (12)

Compound **12** was obtained as a white powder in 45% yield from **29c** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.16 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 248 $(M+H)^+$; IR (KBr) 3438, 2984, 2594, 2242, 1664, 1235, 1164, 1121, 1034, 1011, 683, 569 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.30–1.48 (m, 1H) 1.67 (s, 3H), 1.90–2.27 (m, 6H), 2.28 (s, 3H), 2.49–2.67 (m, 2H), 2.89 (dd, *J* = 11.0, 8.3 Hz, 1H), 3.24–3.40 (m, 1H), 3.48–3.63 (m, 2H), 4.42–4.55 (m, 1H), 4.71 (s, 1H), 4.75 (s, 1H), 4.82 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 8.85–9.24 (m, 2H); HRMS (FAB) calcd for $C_{14}H_{22}N_3O$: 248.1763. Found: 248.1762.

(2*S*)-1-{[(2*S*,4*S*)-4-Isobutyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (13)

Compound **13** was obtained as a white powder in 60% yield from **29d** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.16 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 250 $(M+H)^+$; IR (KBr) 3442, 2956, 2870, 2239, 1661, 1161, 1010, 683 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.85 (d, *J* = 6.5 Hz, 6H), 1.20–1.41 (m, 3H), 1.45–1.61 (m, 1H), 1.93–2.07 (m, 2H), 2.08–2.42 (m, 3H), 2.28 (s, 3H), 2.58–2.73 (m, 1H), 2.81 (t, *J* = 10.5 Hz, 1H), 3.31–3.41 (m, 1H), 3.56 (t, *J* = 6.6 Hz, 2H), 4.45 (dd, *J* = 9.7, 7.6 Hz, 1H), 4.82 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 8.49–9.47 (m, 2H); HRMS (FAB) calcd for $C_{14}H_{24}N_3O$: 250.1919. Found: 250.1921.

(2*S*)-1-{[(2*S*,4*R*)-4-Cyclohexyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile hydrochloride (14)

Compound **14** was obtained as a white powder in 28% yield from **29e** according to the same procedure as described for the preparation of **9** from **20b**. TLC R_f = 0.37 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 276 $(M+H)^+$; IR (KBr) 3380, 2926, 2852, 2239, 1655, 1565, 1541,

1451, 1185 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.83–1.01 (m, 2H), 1.09–1.29 (m, 4H), 1.32–1.47 (m, 1H), 1.53–1.76 (m, 5H), 1.93–2.08 (m, 3H), 2.08–2.32 (m, 2H), 2.53–2.67 (m, 1H), 2.78–2.94 (m, 1H), 3.29–3.40 (m, 1H), 3.59 (t, *J* = 6.5 Hz, 2H), 4.41 (dd, *J* = 10.0, 7.8 Hz, 1H), 4.82 (dd, *J* = 7.6, 4.6 Hz, 1H); HRMS (FAB) calcd for $C_{16}H_{26}N_3O$: 276.2076. Found: 276.2077.

(2*S*)-1-{[(2*S*,4*R*)-4-(2-Adamantyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (15)

Compound **15** was obtained as a white powder in 57% yield from **29f** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.60 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 328 $(M+H)^+$; IR (KBr) 3448, 2906, 2239, 1662, 1455, 1216, 1190, 1181, 1171, 1123, 1033, 1010, 684 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.21–1.39 (m, 1H), 1.44–1.91 (m, 15H), 1.94–2.08 (m, 2H), 2.08–2.26 (m, 2H), 2.28 (s, 3H), 2.55–2.74 (m, 2H), 2.82 (t, *J* = 10.5 Hz, 1H), 3.34–3.42 (m, 1H), 3.48–3.67 (m, 2H), 4.42–4.53 (m, 1H), 4.82 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 7.8 Hz, 2H), 8.96 (s, 2H); HRMS (FAB) calcd for $C_{20}H_{30}N_3O$: 328.2389. Found: 328.2395.

Methyl (2*S*)-1-(benzyloxycarbonyl)-4-oxo-2-pyrrolidinecarboxylate (31)

To a stirred solution of **30** (15.5 g, 55 mmol) in EtOAc (40 mL) were added triethyamine (15.4 mL, 110 mmol), DMSO (20 mL), sulfur trioxide-pyridine complex (17.5 g, 110 mmol) at 0 ºC. After being stirred for 88 h at room temperature, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/2) as an eluant to yield **31** (8.81 g, 58%) as a colorless oil. TLC R_f = 0.35 (hexane/EtOAc, 2/1); ^1H NMR (300 MHz, $CDCl_3$) δ 2.57−2.64 (m, 1H), 2.88−3.02 (m, 1H), 3.63 and 3.77 (s, 3H), 3.95 (s, 2H), 4.80−4.92 (m, 1H), 5.08−5.26 (m, 2H), 7.26−7.42 (m, 5H).

Methyl (2*S*,4*E*)-1-(benzyloxycarbonyl)-4-(2-*tert*-butoxy-2-oxoethylidene)-2-pyrrolidinecarboxylate (32)

To a stirred solution of diisopropylamine (72 mL, 510 mmol) in THF (1 L) was added a solution of *n*-butyllithum in hexane (325 mL, 1.58 M) at −10 ºC. After being stirred for 30 min, the reaction mixture was cooled to −78 ºC. To the reaction mixture was added (trimethylsilyl)acetic acid *tert*-butyl ester (96.7 g, 513 mmol) and stirred for 15 min. To the reaction mixture was added a solution of **31** (119 g, 428 mmol) in THF (500 mL). After being stirred for 3 h at −78 ºC, the reaction mixture was quenched with 1M HCl and extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/3) as an eluant to yield **32** (62.5 g, 39%) as a colorless oil. TLC R_f = 0.35

(hexane/EtOAc, 3/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44−1.46 (m, 9H), 2.76−2.84 (m, 1H), 3.07−3.20 (m, 1H), 3.58−3.75 (m, 3H), 4.30−4.60 (m, 3H), 5.03−5.24 (m, 2H), 5.57−5.74 (m, 1H), 7.26−7.42 (m, 5H).

Methyl (2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-(2-*tert*-butoxy-2-oxoethyl)-2-pyrrolidinecarboxylate (33)

To a solution of **32** (2.66 g, 7.1 mmol) in AcOH (25 mL) was added platinum(IV) oxide (250 mg, 1.1 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 24 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. To the resulting residue were added AcOH (25 mL) and 10 % palladium on carbon (250 mg). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 3 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. To a solution of the residue in THF (30 mL) were added aqueous $NaHCO_3$ and di-*tert*-butyl-dicarbonate (2.18 g, 10 mmol) at room temperature. After being stirred for 10 min, the reaction mixture was extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/3) as an eluant to yield **33** (2.02 g, 83%). TLC R_f = 0.30 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40−1.44 (m, 18H), 1.52−1.70 (m, 1H), 2.25−2.60 (m, 4H), 3.03−3.12 (m, 1H), 3.72 and 3.74 (s, 3H), 3.75−3.85 (m, 1H), 4.15−4.30 (m, 1H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(2-*tert*-butoxy-2-oxoethyl)-2-pyrrolidinecarboxylic acid (34)

To a stirred solution of **33** (12 g, 34.9 mmol) in MeOH (70 mL) and THF (35 mL) was added 1 M NaOH (70 mL) at 0 ºC. After being stirred for 15 h at room temperature, the reaction mixture was quenched with 2 M HCl (35 mL). The organic solvent was removed by evaporation, and the aqueous layer was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to yield **34** (8.54 g), which was used for the next reaction without further purification.

***tert*-Butyl (2*S*,4*R*)-4-(2-*tert*-butoxy-2-oxoethyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (35)**

To a stirred solution of **34** (8.54 g, 26 mmol) in DMF (30 mL) were added (2*S*)-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (6.98 g, 26 mmol), 1-hydroxybenzotriazole (3.18 g, 26 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.96 g, 26 mmol) and triethylamine (3.6 mL, 26 mmol) at 0 ºC. After being stirred for 3 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 5% $KHSO_4$, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel

chromatography using acetone/hexane (1/2) as an eluant to yield **35** (8.65 g, 61%) as a colorless oil. TLC R_f = 0.33 (acetone/hexane, 1/2); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.30−1.44 (m, 18H), 1.90−2.50 (m, 8H), 2.90−3.03 (m, 1H), 3.30−3.72 (m, 4H), 4.37−4.43 (m, 1H), 4.72−4.80 (m, 1H).

((3*R*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinyl)acetic acid 4-methyl benzenesulfonate (16)

A solution of **35** (106 mg, 0.26 mmol) and *p*-toluenesulfonic acid (74 mg, 0.39 mmol) in CH_3CN (2 mL) was refluxed for 5 h. The reaction mixture was evaporated. The resulting crystalline solid was washed with ^{t}BuOMe, collected by filtration, and dried under reduced pressure to yield **16** (104 mg, 95%) as an ivory powder. TLC R_f = 0.17 ($CHCl_3$/MeOH/AcOH, 5/1/1); MS (APCI, neg. 20 V) *m/z* 250 $(M-H)^-$; IR (KBr) 3063, 2983, 2244, 1728, 1662, 1455, 1372, 1221, 1156, 1122, 1033, 1008, 682, 566 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37−1.54 (m, 1H), 1.94−2.26 (m, 4H), 2.28 (s, 3H), 2.37−2.75 (m, 4H), 2.85−3.04 (m, 1H), 3.33−3.47 (m, 1H), 3.47−3.65 (m, 2H), 4.40−4.55 (m, 1H), 4.82 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.42−7.50 (m, 2H), 8.70 (s, 1H), 9.34 (s, 1H); HRMS (FAB) calcd for $C_{12}H_{18}N_3O_3$: 252.1348. Found: 252.1347.

((3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinyl)acetic acid (36)

A solution of **35** (7.50 g, 18.4 mmol) and *p*-toluenesulfonic acid (5.25 g, 27.6 mmol) in CH_3CN (100 mL) was refluxed for 5 h. The reaction mixture was evaporated. To a solution of the residue in THF (50 mL) were added 1M $NaHCO_3$ (50 mL) and di-*tert*-butyl-dicarbonate (5.89 g, 27 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was diluted with hexane (100 mL) and extracted with aqueous $NaHCO_3$. The aqueous layer was acidified with 1M HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting crystalline solid was washed with EtOAc/hexane (1/1), collected by filtration, and dried under reduced pressure to yield **36** (4.48 g, 69%) as a white powder. TLC R_f = 0.16 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos.) *m/z* 352 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.37 (s, 9H), 1.39−1.50 (m, 1H), 2.00−2.20 (m, 4H), 2.30−2.77 (m, 4H), 2.90−3.00 (m, 1H), 3.50−3.74 (m, 3H), 4.37 −4.43 (m, 1H), 4.72−4.82 (m, 1H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[2-(dimethylamino)-2-oxoethyl]-1-pyrrolidinecarboxylate (37)**

To a stirred solution of **36** (250 mg, 0.71 mmol) in CH_2Cl_2 (7 mL) were added a solution of dimethyl amine in THF (2M, 0.53 mL), triethylamine (0.10 mL, 0.71 mmol), 1-hydroxybenzotriazole (96 mg, 0.71 mmol) and 1-(3-dimetylaminopropyl)-3-ethylcarbodiimide hydrochloride (164 mg, 0.85 mmol) at room

temperature. After being stirred for 6 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with 1M HCl, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (40/1) as an eluant to yield **37** (174 mg, 64%) as a white powder. TLC R_f = 0.43 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 and 1.36 (s, 9H), 1.30−1.40 (m, 1H), 1.90−2.26 (m, 4H), 2.35−2.58 (m, 3H), 2.79 (s, 3H), 2.80−2.90 (m, 1H), 2.91 and 2.92 (s, 3H), 3.25−3.30 (m, 1H), 3.48−3.60 (m, 2H), 3.64−3.70 (m, 1H), 4.30−4.40 (m, 1H), 4.76−5.00 (m, 1H).

2-((3*R*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinyl)-*N*,*N*-dimethylacetamide 4-methylbenzenesulfonate (17)

Compound **17** is obtained as a white powder in 77% yield from **37** according to the same procedures as described for the preparation of **10** from **29a**. TLC R_f = 0.27 (EtOAc/AcOH/H_2O, 3/1/1); MS (FAB, Pos.) *m/z* 279 $(M+H)^+$; IR (KBr) 3447, 2959, 2244, 1662, 1649, 1456, 1373, 1221, 1170, 1122, 1035, 1011 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37−1.54 (m, 1H), 1.93−2.07 (m, 2H), 2.08−2.33 (m, 3H), 2.28 (s, 3H), 2.46−2.57 (m, 1H), 2.57−2.73 (m, 2H), 2.80 (s, 3H), 2.83−2.99 (m, 1H), 2.91 (s, 3H), 3.33−3.67 (m, 3H), 4.41−4.54 (m, 1H), 4.82 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 7.8 Hz, 2H), 8.66 (s, 1H), 9.30 (s, 1H).

[(3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-(methoxycarbonyl)-3-pyrrolidinyl]acetic acid (38)

A solution of **33** (1.37 g, 3.99 mmol) and anisole (0.8 mL) in trifluoroacetic acid (8 mL) was stirred for 1 h at room temperature. The reaction mixture was evaporated. To a stirred solution of the residue in THF (10 mL) were added aqueous $NaHCO_3$ (10 mL) and di-*tert*-butyl-dicarbonate (1.33 g, 6.10 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was quenched with 1 M HCl and extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using $CHCl_3$/MeOH (9/1) as an eluant to yield **38** (1.16 g, 100%) as a colorless oil. TLC R_f = 0.38 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.44 (s, 9H), 1.60−1.70 (m, 1H), 2.40−2.63 (m, 4H), 3.07−3.14 (m, 1H), 3.73 and 3.74 (s, 3H), 3.75−3.85 (m, 1H), 4.20−4.34 (m, 1H).

Methyl (2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(2-hydroxyethyl)-1,2-pyrrolidinecarboxylate (39)

To a stirred solution of **38** (575 mg, 2.00 mmol) in THF (10 mL) were added triethylamine (0.42 mL, 3.0 mmol) and ethyl chloroformate (0.23 mL, 2.4 mmol) at 0 ºC. After being stirred for 2 h at room temperature, the reaction mixture was filtered and the filtrate was concentrated in vacuo. To a stirred solution of the residue in THF (5 mL) was added a solution of sodium borohydride (378 mg, 10 mmol) in water (5 mL) at 0 ºC. After being stirred for 30 min, the

reaction mixture was extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and evaporated to yield **39** (546 mg, 100%) as a colorless oil. TLC R_f = 0.50 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.57−1.72 (m, 3H), 2.20−2.51 (m, 2H), 3.05 (t, *J* = 11.2 Hz, 1H), 3.63−3.84 (m, 3H), 3.72 and 3.73 (s, 3H), 4.13−4.30 (m, 1H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]-2-pyrrolidine carboxylic acid (41)

To a stirred solution of **39** (546 mg, 2 mmol) in CH_2Cl_2 (4 mL) was added 3,4-dihydro-2*H*-pyran (0.27 mL, 3.0 mmol) and pyridinium *p*-toluenesulfonate (50 mg, 0.20 mmol) at room temperature. After being stirred for 3 h, the reaction mixture was quenched with aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to yield **40**, which was used for the next reaction without further purification. To a stirred solution of **40** in MeOH (4 mL) and THF (4 mL) was added 1 M NaOH (4 mL) at room temperature. After being stirred for 15 h, the reaction mixture was quenched with 1 M HCl (4 mL). The organic solvent was removed in vacuo, and the aqueous layer was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to yield **41**, which was used for the next reaction without further purification.

(2*S*)-1-({(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]-2-pyrrolidinyl}carbonyl)-2-pyrrolidinecarbonitrile (42)

Compound **42** was obtained as a colorless oil in 65% yield from **41** according to the same procedures as described for the preparation of **35** from **34**. TLC R_f = 0.50 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 and 1.43 (s, 9H), 1.50−1.90 (m, 9H), 2.05−2.46 (m, 6H), 3.00−3.17 (m, 1H), 3.35−3.62 (m, 4H), 3.70−3.90 (m, 4H), 4.30−4.60 (m, 1H), 4.65−4.90 (m, 1H).

(2*S*)-1-{[(2*S*,4*R*)-4-(2-Hydroxyethyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile [(1*S*,4*R*)-7,7-dimethyl-2-oxobicyclo[2.2.1]hept-1-yl]methanesulfonate (18)

A solution of **42** (6.0 g, 17.8 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (4.55 g, 19.6 mmol) in EtOH (20 mL) was refluxed for 4 h. After cooling to 0 °C, the resulting precipitates were collected by filtration and dried under reduced pressure to yield **18** (6.80 g, 81%) as a white powder. TLC R_f = 0.22 ($CHCl_3$/MeOH/AcOH, 8/2/1); MS (APCI, pos. 20 V) *m/z* 238 $(M+H)^+$; IR (KBr) 3467, 3166, 2991, 2954, 2239, 1739, 1670, 1387, 1374, 1163, 1033 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.73 (s, 3H), 1.03 (s, 3H), 1.18−1.33 (m, 2H), 1.32−1.46 (m, 1H), 1.46−1.64 (m, 2H), 1.78 (d, *J* = 18.1 Hz, 1H), 1.82−1.89 (m, 1H), 1.92 (t, *J* = 4.4 Hz, 1H), 1.95−2.09 (m, 2H), 2.08−2.29 (m, 3H), 2.29−2.44 (m, 2H), 2.56−2.76 (m, 2H), 2.78−2.95 (m, 2H), 3.33−3.50 (m, 3H), 3.50−3.69 (m, 2H), 4.20−4.63 (m, 2H), 4.83 (dd, *J* = 7.8, 4.7 Hz, 1H), 8.66 (s, 1H), 9.33 (s, 1H); Anal. Calcd for $C_{22}H_{35}N_3O_6S$: C, 56.27; H, 7.51; N, 8.95. Found: C, 56.25; H, 7.45; N, 8.81.

２-７-２． Biological methods

Purification of human DPP-IV

Human DPP-IV was purified according to the published procedure with some modifications.[9] Briefly, the enzyme was prepared from pooled plasma obtained from healthy volunteers by ammonium sulfate precipitation (50–70%). After extensive dialysis against 25 mM Tris-HCl (pH 7.4), the material was mixed with DEAE cellulose, DE52 (Whatman Chemical Separation, Inc., USA) for 60 min, and eluted with buffer containing 100 mM NaCl. Fractions of 10 mL were collected, and the fraction with maximal DPP-IV activity was dialyzed against 25 mM MES-NaOH (pH 6.0). DPP-IV-containing fractions were detected by the ability to hydrolyze Gly-Pro-7-amido-4-methyl-coumarin (Gly-Pro-AMC) (Sigma-Aldrich, USA) using the standard method described below. The DE52 elute was loaded onto a SP Sepharose Fast Flow column (GE Healthcare, Sweden), and the flow-through fraction containing DPP-IV was then applied to a DEAE cellulose column (Whatman DE52). Bounded proteins were eluted with 25 mM Tris-HCl (pH 7.8) containing 150 mM NaCl. Fractions of 10 ml were collected, and the fraction with maximum DPP-IV activity was concentrated using polyethylene glycol 20000 (PEG20000). The concentrated material was applied to a Sephacryl S-300 High Resolution 26/60 column (GE Healthcare, Sweden), and was eluted at a flow rate of 0.1 ml/min. Fractions of 1 ml were collected, and the fractions containing DPP-IV activity were pooled.

Enzyme assays

Enzymatic activity was determined at 37 °C by the cleavage rate of a substrate, Gly-Pro-AMC (30μM) (Sigma-Aldrich, USA).[10] Briefly, 10 μL of DPP-IV solution was added to each well of a 96-well flat-bottomed microtiter plate , followed by the addition of 50 μL of 60 μM Gly-Pro-AMC, 10 μL of 500 mM Tris-HCl (pH 7.4), 20 μL of distilled water, and 10 μL of a test compound. The change of fluorescence was monitored at 37 °C using a spectrofluorometer (excitation at 355 nm/ emission at 460 nm) (fmax, Molecular Devices, USA). The initial rate of DPP-IV enzyme activity was calculated over the first 15 min of the reaction, with units/mL being defined as the rate of increase in the fluorescence intensity (arbitrary units) under these conditions. The percent inhibition relative to addition of the solvent alone was calculated and IC_{50} values were determined by logistic analysis.

DPP-IV Inhibition in rats

Male Sprague-Dawley (SD) rats were purchased from Charles River Laboratories Japan. The rats were housed in an air-conditioned animal room with a controlled temperature (24 ± 2 °C), humidity (55 ± 5%), and lighting (12:12 h light/dark cycle), and were provided with standard pellet food for rodents CRF-1 (Oriental Yeast, Japan) and water ad libitum. All

procedures were conducted according to the ONO Pharmaceutical Animal Care Committee guidelines. After at least 8 h fast, male SD rats (6-7 weeks of age) were orally administered test compounds dissolved in 0.5% methyl cellulose at a single dose of 1 mg/kg. Blood samples were collected from the jugular vein before, and 0.25, 0.5, 1, 2, 4, 6 and 10 h after administration. Blood was centrifuged immediately to obtain plasma and its DPP-IV activity was determined. Then 50 μL of plasma was added to each well of a 96-well flat-bottomed microtiter plate, followed by the addition of 50 μL of 60 μM substrate. The initial rate of DPP-IV enzyme activity was calculated using the standard method described above. The percent inhibition relative to basal DPP-IV activity was calculated.

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第三章　4-フェニルピロリジン骨格の持続性発現のメカニズムの検討とリード最適化

３-１．序論

第二章において最適化すべきリード化合物として4-フェニルピロリジン化合物**8**を見出した。化合物**8**は高い酵素阻害活性と高い持続性が期待される骨格であった。フェニル基上への置換基導入も可能であり、最適化の余地が残された化合物である。

第二章で述べたとおり、化合物**8**を1 mg/kgラットに経口投与し、血中DPP-IV活性の経時変化を観察すると1時間後に弱くなった阻害活性が、2時間後に再び強くなることが分かった。経口投与後に体内で活性代謝物が生成され2時間後以降の作用持続が見られているのではないかと考えた。そこで、化合物**8**の血中動態および代謝物の探索を開始した。

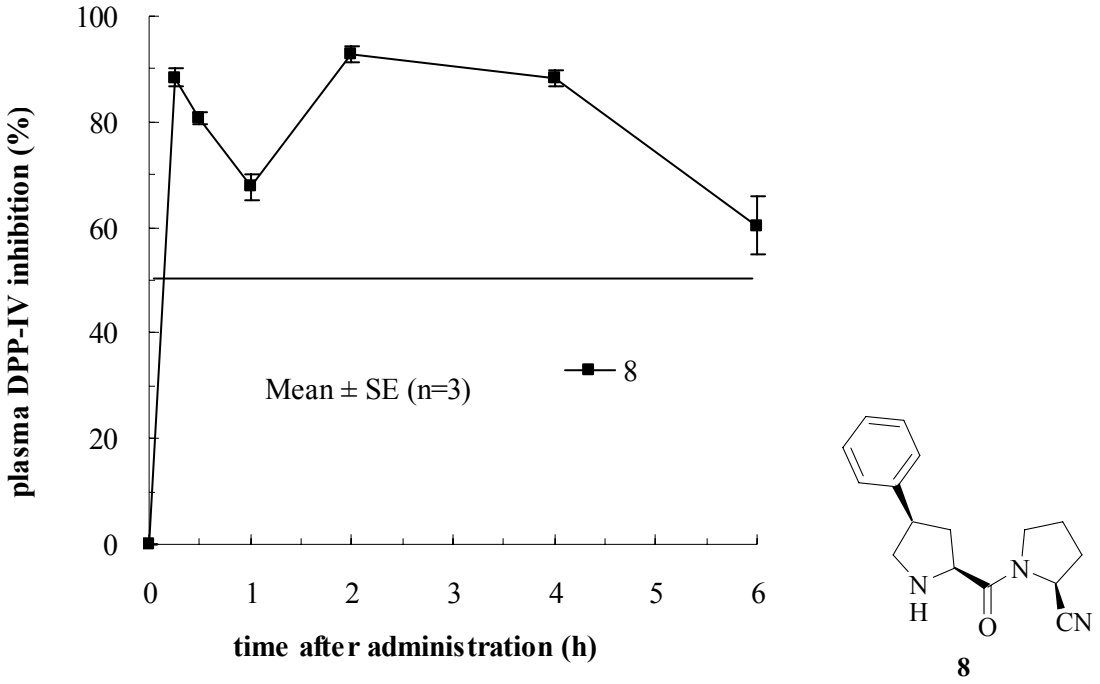


Figure 3-1. 化合物**8**のラット1 mg/kg経口投与時の血中DPP-IV阻害活性の経時変化

３-２．化合物**8**の血中動態と代謝物の探索

３-２-１．方法（化合物**8**の血漿中濃度測定）

化合物**8**の正常ラット（Sprague-Dawley rats、SDラット）経口投与後の血漿中濃度を測定した。0.5%メチルセルロース水溶液に溶解した化合物**8**を、1 mg/kgの投与量で経口投与後、経時的に頚静脈より採血し、血漿を調製した。得られた血漿を除蛋白後、LC/MS/MSにて分析し、血漿中濃度を算出した（Figure 3-2）。

３-２-２．結果と考察（化合物**8**の血漿中濃度測定）

化合物**8**の血漿中濃度推移をFigure 3-2に示した。化合物**8**のラット血漿におけるDPP-IV阻害活性のIC_{50}値は18 nMであり、4.8 ng/mLに相当し（Figure 3-2のグラフ中の横線）、2時間後以降の血漿中濃度はこの値を下回ることが分かった。従って、Figure 3-1に示した2時間後以降の血中DPP-IV阻害活性は化合物**8**の血中濃度からは説明できず、活性代謝物によるものと考えられる。そこで、投与2時間後の血漿中の代謝物を探索する事とした。

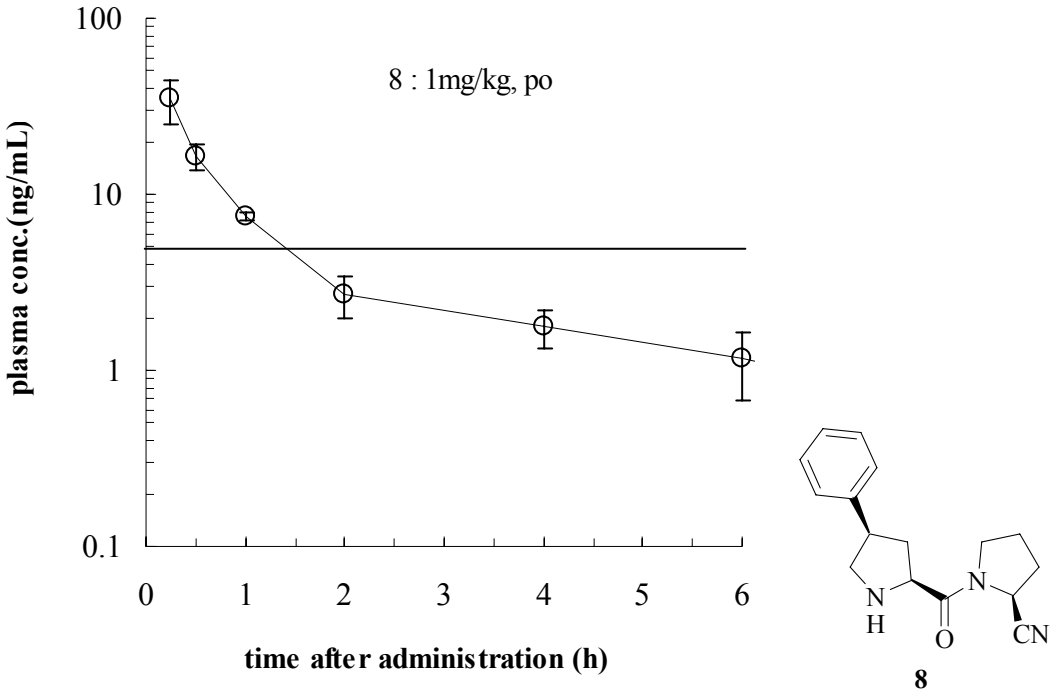


Figure 3-2. 化合物**8**のラット1 mg/kg経口投与時の血漿中濃度の経時推移

３-２-３．方法（化合物**8**の経口投与後の血漿中代謝物の探索）

化合物**8**を正常ラット（Sprague-Dawley rats、SDラット）に経口投与し、血漿中の代謝物を探索した。0.5%メチルセルロース水溶液に溶解した化合物**8**を、100 mg/kgの投与量で経口投与後、2時間後に頚静脈より採血し、血漿を調製した。得られた血漿を

除蛋白後、LC/MS（カラム: YMC-Pack ODS-A,　4.6 x 150 mm, 5 μm; 溶離液 A: 0.1 % ギ酸水溶液; 溶離液B: CH_3CN; 25分で溶離液B 5%から20%へ線形グラジエント; 送液速度: 1 mL/min）にて１分間隔で分取し、各フラクションにおけるDPP-IV阻害活性を測定した。

３-２-４．結果と考察（化合物**8**の経口投与後の血漿中代謝物の探索）

Figure 3-3に示すように、HPLC保持時間が21-23分の化合物**8**を含むフラクションの他に、HPLC保持時間が5-6分のフラクションにおいて高い阻害活性が見られた。このフラクションをLC/MS/MS（イオン化法ESI）にて分析した結果、*m/z*が462（M+H）を示した。この分子量は化合物**8**が水酸化を受け、さらにグルクロン酸抱合を受けた化合物に相当する（Figure 3-４）。

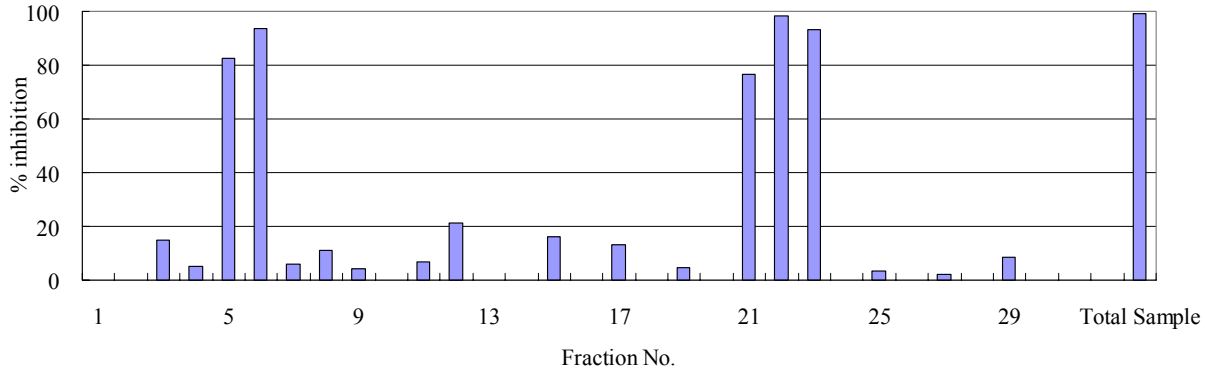


Figure 3-3. 化合物**8**の投与後血漿をHPLCで分離した各フラクションのDPP-IV阻害活性（ラット100 mg/kg経口投与2時間後）

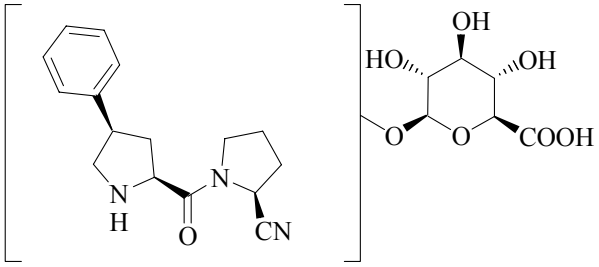
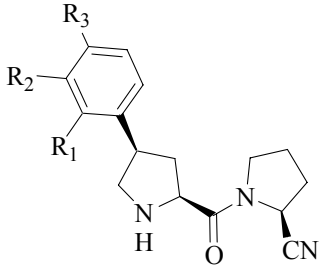


Figure 3-4. 化合物**8**のラット経口投与後の血漿に含まれる代謝物の推定構造

LC/MS/MSの分析からは水酸化の位置は特定できなかった。第二章で得られた構造活性相関より、P2位のピロリジン環の4位置換基は立体的に嵩高いものが許容である事から、フェニル基部分が水酸化を受けグルクロン酸抱合されてもDPP-IV阻害活性を示すと考えられる。そこで、本代謝物はフェノールのグルクロン酸抱合体と推定し、フェノール誘導体を合成し、代謝物の構造を特定する事とした。

３-３．フェノール体の合成

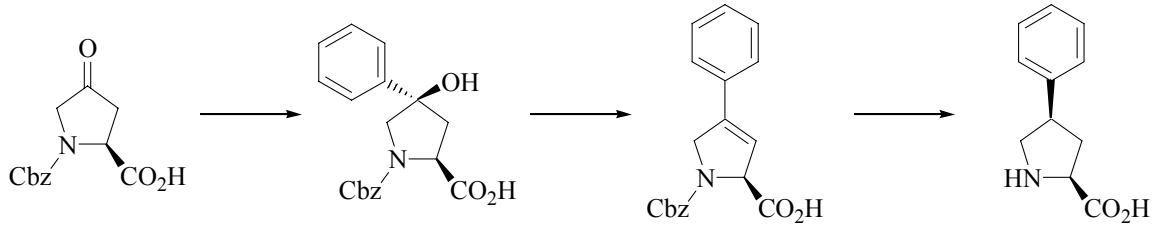


43 : R_1 = OH, R_2 = R_3 = H
44 : R_1 = H, R_2 = OH, R_3 = H
45 : R_1 = R_2 = H, R_3 = OH

Figure 3-5. フェノール誘導体

4位に*cis*配置でフェノール基を有するプロリン誘導体**43-45**を合成した。

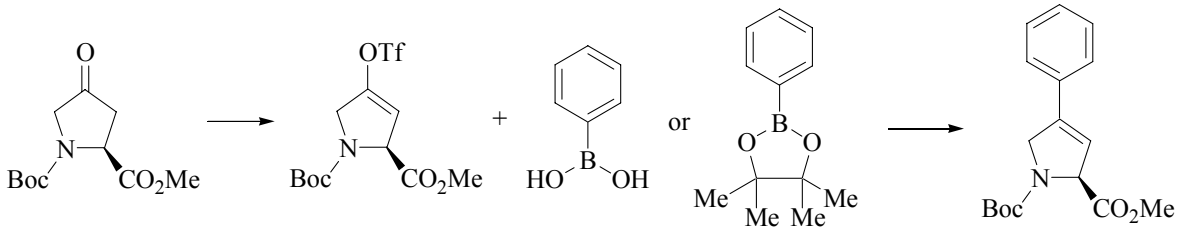
2,4-*cis*-4-フェニルプロリンの合成法としては、Krapchoらの方法[1]やHorikawaらの方法[2]が知られていた（Scheme 3-1)。本方法では、*N*-Cbz-4-オキソプロリン**46**に対し、フェニルグリニヤール試薬またはフェニルリチウム試薬を反応し、生成したアルコール**47**を脱水しオレフィン体**48**を得た後、二重結合の水素化により2,4-*cis*-4-フェニルプロリン**49**を合成している．しかしながら、本方法はグリニヤール試薬の付加の工程の収率があまり高くなく、また置換基を持つグリニヤール試薬は対応するハロゲン化物から調製する必要がある。また、オレフィン体**48**を得るために脱水反応が必要であり、共通中間体の**46**から2工程必要であり、誘導体合成を行う上では操作が煩雑である。



46 **47** **48** **49**

Scheme 3-1. Krapchoらの方法

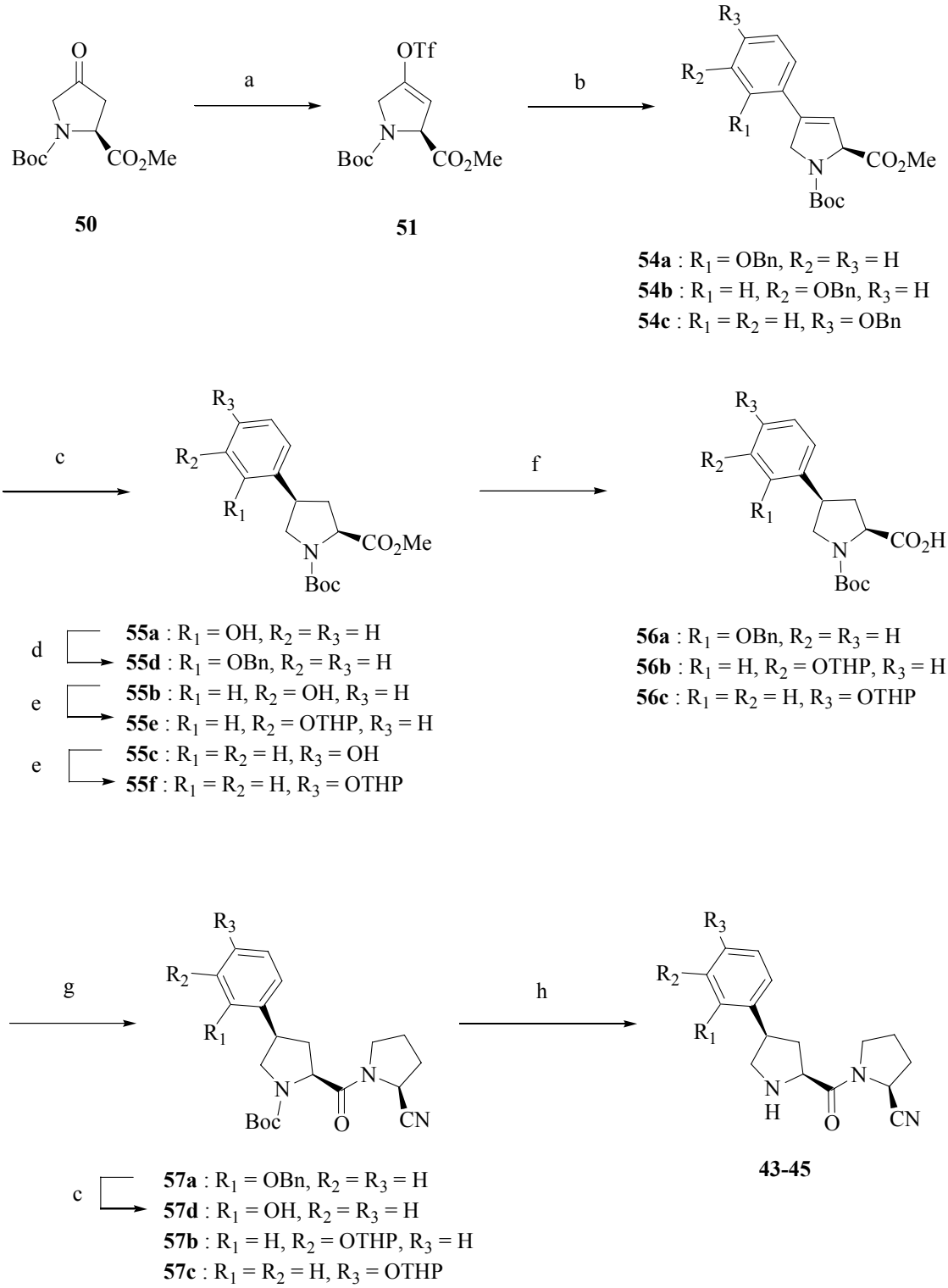
そこで、Krapchoらの中間体であるオレフィン体**48**の改良合成法を構築する事とした（Scheme 3-2)。すなわち、Koskinenらの方法[3]に従い、*N*-Boc-4-オキソプロリンメチルエステル**50**を、エノールトリフラート**51**に変換し、アリールボロン酸**52**またはボロン酸エステル**53**と鈴木カップリング[4]を行い、オレフィン体**54**を得るというものである。アリールボロン酸**52**は対応するハロゲン化物より、アリールボロン酸エステル**53**はハロゲン化物またはフェノールトリフラートより調製可能である。また共通中間体**51**より1工程でオレフィン体が合成でき、フェニル基上への置換基の導入に多様性を持たせる事が容易である。



50 **51** **52** **53** **54**

Scheme 3-2. 2,4-*cis*-4-フェニルプロリン誘導体の改良合成法

実際のフェノール体の合成をScheme 3-3に示した。*N*-Boc-4-オキソプロリンメチルエステル**50**を、ナトリウムビストリメチルシリルアミドでナトリウムエノラートとし*N*-フェニルビストリフルオロメタンスルホンイミドと反応し、エノールトリフラート**51**を得た。エノールトリフラート**51**とアリールボロン酸と鈴木カップリングを行い、オレフィン体**54a-c**を得た。オレフィン体**54a-c**を10%パラジウム炭素の存在下、水素化し2,4-*cis*フェニル体**55a-c**を得た。2,4-*cis*フェニル体**55a-c**をベンジルエーテルまたはテトラヒドロピラニルエーテルとして保護した後、メチルエステルを加水分解し、カルボン酸**56a-c**を得た。得られたカルボン酸**56a-c**と(2*S*)-2-シアノピロリジンを１-メタンスルホニルオキシベンゾトリアゾールを用いて縮合し、アミド体**57a-c**を得た。アミド体**57a**のベンジルエーテルを水素雰囲気下、10%パラジウム炭素を用いて脱保護し、フェノール体**57d**を得た。**57a**, **57d**, **57c**のBoc基を*p*-トルエンスルホン酸を用いて脱保護し、目的とするフェノール体**43-45**を*p*-トルエンスルホン酸塩として得た。



Scheme 3-3. Synthesis of **43-45**. Reagents: (a) NaHMDS, $PhNTf_2$, THF; (b) $ArB(OH)_2$, Na_2CO_3 aq, $Pd(Ph_3P)_4$, 1,4-dioxane; (c) H_2, 10% Pd-C, MeOH; (d) BnBr, K_2CO_3, DMF; (e) DHP, PPTS, CH_2Cl_2; (f) NaOH aq, MeOH; (g) (2*S*)-2-cyanopyrrolidine, MsOBt, Et_3N, DMF; (h) *p*-TsOH-H_2O, EtOH.

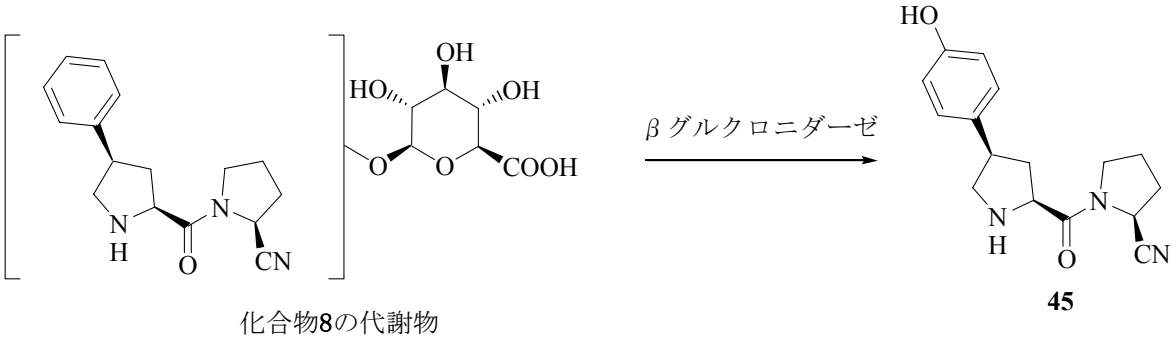
３-４．*in vitro*代謝実験による化合物**8**の代謝物の構造特定

３-４-１．方法（代謝物のグルクロニダーゼ処理）

化合物**8**の代謝物であるグルクロン酸抱合体のグリコシド結合をグルクロニダーゼで処理することにより切断し、合成したフェノール体**43-45**とLC/MSにて比較した（Scheme 3-4.）。化合物**8**を0.5%メチルセルロース水溶液に溶解し、100 mg/kgの投与量で経口投与後、2時間後に頚静脈より採血し、血漿を調製した。得られた血漿を、除蛋白後、乾固し、β-Glucuronidase/arylsulfatase（Roche Diagnostics Corporation）で37℃にて2時間反応した。生成物を、除蛋白後、LC/MSにて分析した（カラム: YMC-Pack ODS-A, 4.6 x 150 mm, 5 μm; 溶離液 A: 0.1 % ギ酸水溶液; 溶離液B: CH_3CN; 25分で溶離液B 5%から20%へ線形グラジエント; 送液速度: 1 mL/min）。

３-４-２．結果と考察（代謝物のグルクロニダーゼ処理）

化合物**8**の代謝物のグルクロニダーゼ処理生成物は保持時間（11分）、*m/z*が286（M+H）を示し、パラ位フェノール体**45**と一致した。立体障害が無く、電子密度の高いパラ位が水酸化を受けたと考えられる。



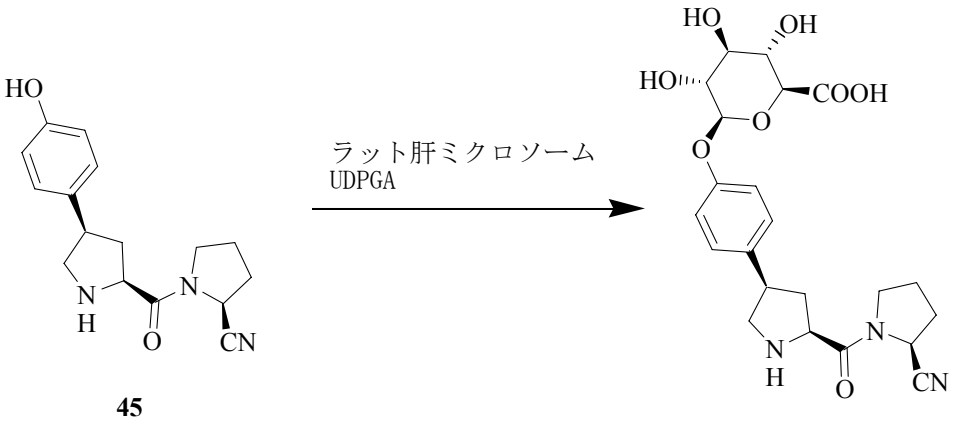
Scheme 3-4. 代謝物の構造推定

３-４-３．方法（*in vitro*グルクロン酸抱合反応）

パラ位フェノール体**45**をラット肝ミクロソームとグルクロン酸抱合代謝の補酵素であるUDP-グルクロン酸（UDPGA、ウリジン二リン酸にグルクロン酸がグリコシド結合したもの）を用い、*in vitro*にて代謝反応を行った（Scheme 3-5.）。ラット肝ミクロソーム（Xenotech Corporation、1 mg/mL）とパラ位フェノール体（100 ng/mL）に2 mM UDPGAを加え、37℃にて1時間反応した。生成物を、除蛋白後LC/MSにて分析した（カラム: YMC-Pack ODS-A, 4.6 x 150 mm, 5 μm; 溶離液 A: 0.1 % ギ酸水溶液; 溶離液B: CH_3CN; 25分で溶離液B 5%から20%へ線形グラジエント; 送液速度: 1 mL/min）。

３-４-４．結果と考察（*in vitro*グルクロン酸抱合反応）

*in vitro*代謝反応生成物は保持時間（5分）、*m/z*が462（M+H）を示し、ラットに化合物**8**を経口投与して生成したグルクロン酸抱合体と一致した。



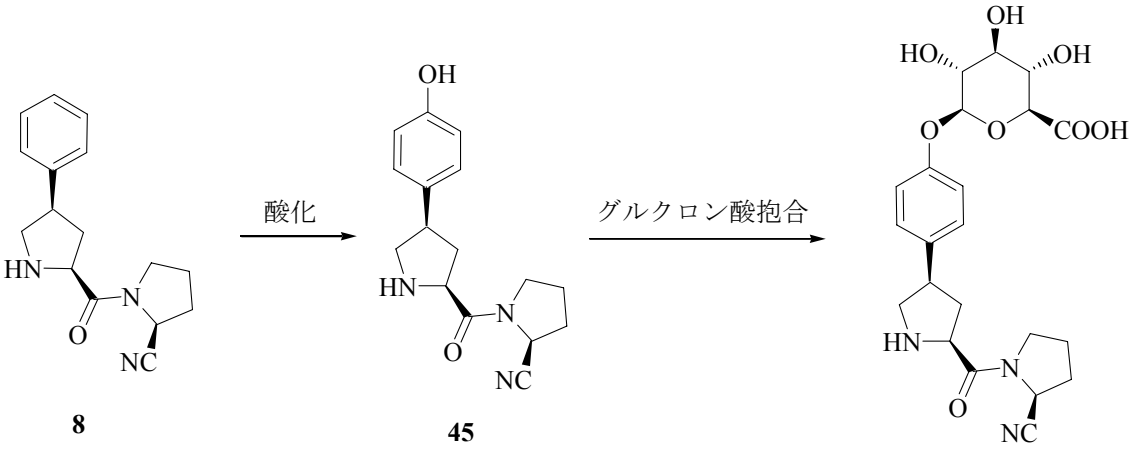
45

II

化合物**8**の代謝物

Scheme 3-5. *in vitro*代謝反応

以上の結果より、ラットに化合物**8**を経口投与し生成した代謝物の構造は、パラ位が水酸化を受け、さらにグルクロン酸抱合された化合物と考えられる。



8 **45**

Scheme 3-6. 化合物**8**のラットにおける代謝経路

３-５．フェノール誘導体のDPP-IV阻害活性の評価

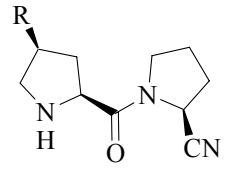
３-５-１．方法

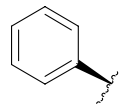
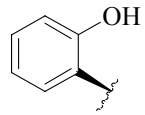
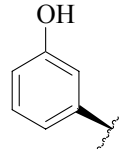
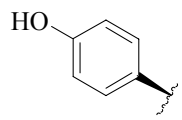
合成したフェノール誘導体**43-45**のヒト精製酵素に対する*in vitro*酵素阻害活性を２-３-１と同様の方法により評価した。また、２-４-１と同様の方法によりSDラットにフェノール誘導体**43-45**を1 mg/kg経口投与した際の*ex vivo*酵素阻害活性を評価した。

３-５-２．結果と考察

フェノール誘導体**43-45**の*in vitro*酵素阻害活性は水酸基の位置に関わらず、高い値を示した。一方、ラット1 mg/kg経口投与後の*ex vivo*酵素阻害活性では異性体間で差が見られた。すなわち、オルト体**43**は6時間後には43%の阻害率を示したのに対し、メタ体**44**、パラ体**45**は77%、85％の阻害率を示しており、持続性が見られた。

Table 3-1. フェノール誘導体のDPP-IV阻害活性



Compound	R	human DPP-IV IC_{50} (nM)	*ex vivo* DPP-IV inhibition (%) at 1 mg/kg po, normal rats					
			time after administraton (h)					
			0.25	0.5	1	2	4	6
8		3.5	88	81	68	93	88	60
43		9.1	83	90	84	69	47	43
44		3.5	98	98	97	94	80	77
45		2.5	98	99	99	98	88	85

メタ体**44**、パラ**45**体がフェニル体**8**以上の*ex vivo*阻害活性の持続性を示したのは、フェニル体**8**では活性代謝物であるグルクロン酸抱合体を生成するために酸化代謝を受ける必要があるのに対し、フェノール体である**44**や**45**は活性代謝物であるグルクロン酸抱合体を効率よく生成するためと考えられる。一方、オルト体**43**は立体障害のためグルクロン酸抱合体が生成しにくく、DPP-IV阻害活性を失う代謝または排泄により持続性が短くなったと考えられる。

３-６．フェノール誘導体の分子内環化反応に対する安定性の評価

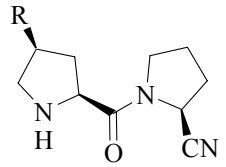
３-６-１．方法

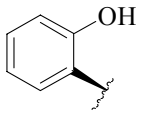
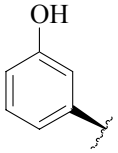
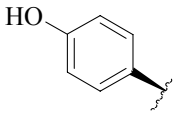
合成したフェノール誘導体**43-45**の分子内環化反応に対する安定性を、第二章２-５-１と同様の方法により評価した。

３-６-２．結果と考察

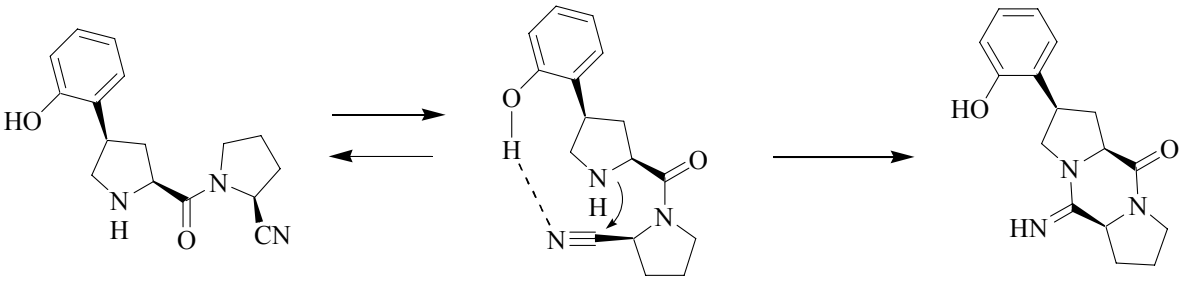
フェノール誘導体**43-45**の分子内環化反応に対する安定性を評価したところ、オルト体**43**の半減期は3.2時間であり、メタ体**44**、パラ体**45**と比較し、大幅に短かった。

Table 3-2. フェノール誘導体の分子内環化反応に対する安定性



Compound	R	solution stability (pH 7.4) $t_{1/2}$ (h)
43		3.2
44		20
45		21

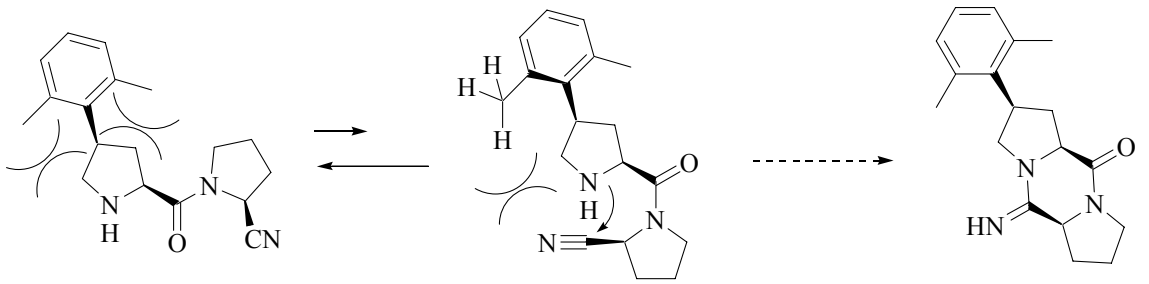
オルト体**43**が不安定化された理由を次のように考察した。分子内環化反応が進行する際、シアノ基は4位のフェニル基や2位のカルボニル基と同じ側からアミノ基に近づく。フェニル基上のオルト位の水酸基はシアノ基の近傍に位置することが可能で、フェノールの酸性プロトンがシアノ基の窒素と水素結合し、酸触媒として働き分子内環化反応を促進したと考えられる。メタ体**44**、パラ体**45**では酸性プロトンがシアノ基と水素結合するコンフォメーションを取りえないため安定で、その程度はメタ体**44**とパラ体**45**で同程度であったと考えられる。



43

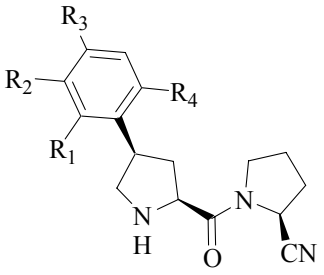
Scheme 3-7 オルトフェノール体**43**の分子内環化反応促進メカニズム

以上の考察より、オルト位の置換基は環化反応の際にシアノ基の近傍に存在しうると考えられた。そこで、オルト位に立体障害となる置換基を導入すれば、分子内環化反応が抑制できると考えられる。シアノ基とアミノ基が近づくのを抑制するためには、ベンゼン環がピロリジン環と直交し、ピロリジン環上にオルト位の置換基を配置できれば良いと考えられる。両方のオルト位に立体障害となる置換基を導入すれば、ピロリジン環とフェニル基が直交すると考え、両方のオルト位に置換基を持つ阻害剤を設計し、合成することとした（**Scheme 3-8**）。



Scheme 3-8 オルト位への置換基導入による分子内環化反応の抑制

３-７．2,6置換フェニル誘導体の合成

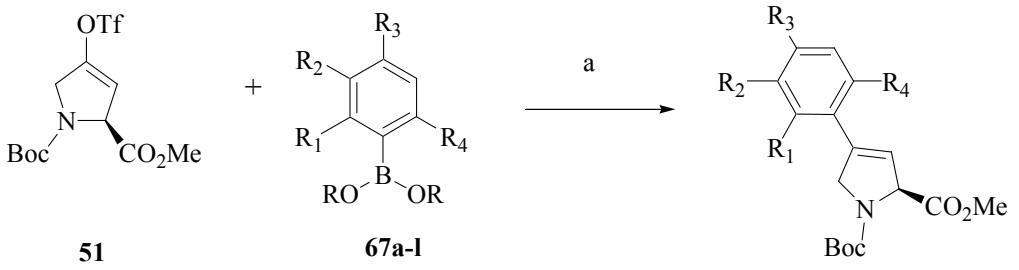


58 : R_1 = Me, R_2 = H, R_3 = H, R_4 = Me
59 : R_1 = Me, R_2 = H, R_3 = OH, R_4 = Me
60 : R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe
61 : R_1 = OMe, R_2 = H, R_3 = OH, R_4 = OMe
62 : R_1 = Me, R_2 = H, R_3 = OH, R_4 = Et
63 : R_1 = OEt, R_2 = H, R_3 = OH, R_4 = OEt
64 : R_1 = Me, R_2 = OH, R_3 = H, R_4 = Me
65 : R_1 = Me, R_2 = OH, R_3 = H, R_4 = OMe
66 : R_1 = Me, R_2 = OH, R_3 = Me, R_4 = Me

Figure 3-6. 2,6置換フェニル誘導体

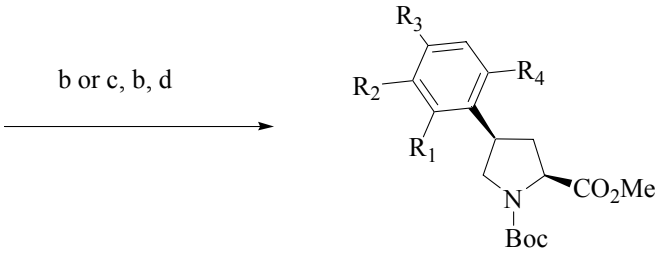
2,6置換フェニル誘導体**58-66**の合成をScheme 3-9、3-10に示した。

エノールトリフラート**51**とボロン酸またはボロン酸エステル**67a-l**を鈴木カップリングしオレフィン体**68a-l**を得た。オレフィン体**68a-d**, **68f**, **68i**は10%パラジウム炭素の存在下、水素化し2,4-*cis*フェニル体**69a-d**, **69f**, **69i**を得た。一方、オレフィン体**68e**, **68g-h**は立体障害の影響で水素化反応の進行が遅いため、Boc基を除去し、水素化した後、再びBoc化し、2,4-*cis*フェニル体**69e**, **69g-h**を得た。**69b**, **69h**はフェノールをベンジルエーテルとして保護し**69j-k**を得た。2,4-*cis*フェニル体**69a, 69j, 69c-g, 69k, 69i**を加水分解し、カルボン酸**70a-i**とした。カルボン酸**70a-b, 70d, 70f-g, 70i**と(2*S*)-2-シアノピロリジンを、1-メタンスルホニルオキシベンゾトリアゾールを用いて縮合し、アミド体**71a-b, 71d, 71f-g, 71i**を得た。**71c**, **71e**, **71h**は１-メタンスルホニルオキシベンゾトリアゾールの代わりにEDCを縮合剤として用い合成した。アミド体**71b**, **71h**のベンジルエーテルを水素雰囲気下、10%パラジウム炭素または20%水酸化パラジウム炭素を用いて脱保護し、フェノール体**71j**, **71k**を得た。**71a**, **71j 71c-g**, **71k**, **71i**のBoc基を4N 塩化水素ジオキサン溶液または*p*-トルエンスルホン酸を用いて脱保護し、目的とする2,6置換フェニル誘導体**58-66**を塩酸塩または*p*-トルエンスルホン酸塩として得た。



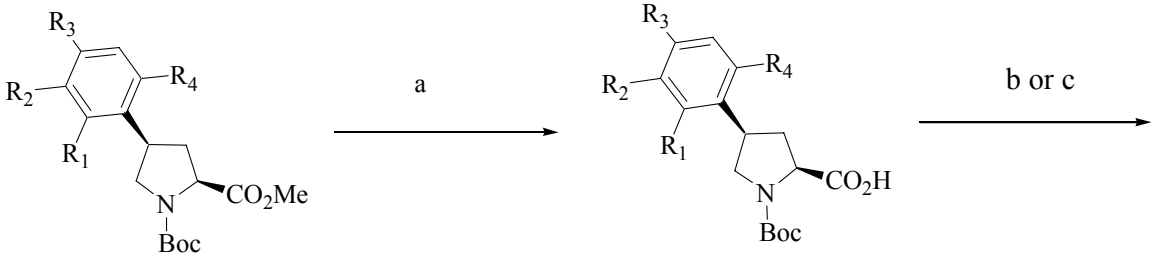
51 **67a-l**

68a : R_1 = Me, R_2 = R_3 = H, R_4 = Me
68b : R_1 = Me, R_2 = H, R_3 = OBn, R_4 = Me
68c : R_1 = Me, R_2 = H, R_3 = OBn, R_4 = OMe
68d : R_1 = OMe, R_2 = H, R_3 = OMOM, R_4 = OMe
68e : R_1 = Me, R_2 = H, R_3 = OTHP, R_4 = Et
68f : R_1 = OEt, R_2 = H, R_3 = OMOM, R_4 = OEt
68g : R_1 = Me, R_2 = OH, R_3 = H, R_4 = Me
68h : R_1 = Me, R_2 = OBn, R_3 = H, R_4 = OMe
68i : R_1 = Me, R_2 = OH, R_3 = Me, R_4 = Me



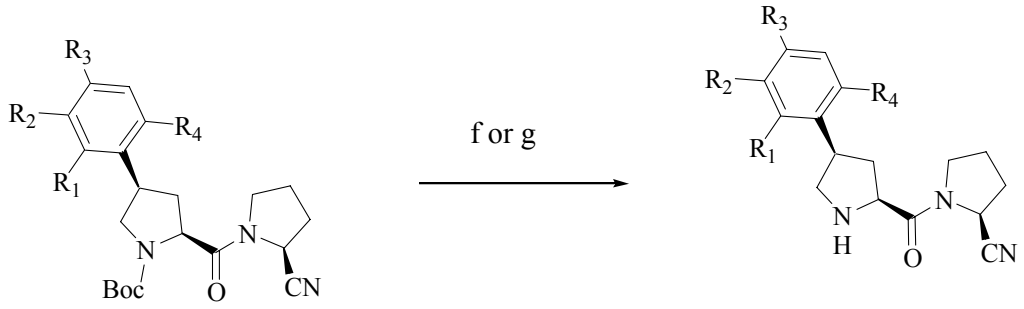
69a : R_1 = Me, R_2 = R_3 = H, R_4 = Me
e → **69b** : R_1 = Me, R_2 = H, R_3 = OH, R_4 = Me
e → **69j** : R_1 = Me, R_2 = H, R_3 = OBn, R_4 = Me
69c : R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe
69d : R_1 = OMe, R_2 = H, R_3 = OMOM, R_4 = OMe
69e : R_1 = Me, R_2 = H, R_3 = OH, R_4 = Et
69f : R_1 = OEt, R_2 = H, R_3 = OMOM, R_4 = OEt
69g : R_1 = Me, R_2 = OH, R_3 = H, R_4 = Me
e → **69h** : R_1 = Me, R_2 = OH, R_3 = H, R_4 = OMe
e → **69k** : R_1 = Me, R_2 = OBn, R_3 = H, R_4 = OMe
69i: R_1 = Me, R_2 = OH, R_3 = Me, R_4 = Me

Scheme 3-9. Synthesis of **58-66**. Reagents: (a) Na_2CO_3 aq, $Pd(Ph_3P)_4$, 1,4-dioxane; (b) H_2, 10% Pd-C, MeOH; (c) 4N HCl/1,4-dioxane ; (d) Boc_2O, $NaHCO_3$ aq, THF; (e) BnBr, K_2CO_3, DMF



69a : R_1 = Me, R_2 = R_3 = H, R_4 = Me
69j : R_1 = Me, R_2 = H, R_3 = OBn, R_4 = Me
69c : R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe
69d : R_1 = OMe, R_2 = H, R_3 = OMOM, R_4 = OMe
69e : R_1 = Me, R_2 = H, R_3 = OH, R_4 = Et
69f : R_1 = OEt, R_2 = H, R_3 = OMOM, R_4 = OEt
69g : R_1 = Me, R_2 = OH, R_3 = H, R_4 = Me
69k : R_1 = Me, R_2 = OBn, R_3 = H, R_4 = OMe
69i : R_1 = Me, R_2 = OH, R_3 = Me, R_4 = Me

70a : R_1 = Me, R_2 = R_3 = H, R_4 = Me
70b : R_1 = Me, R_2 = H, R_3 = OBn, R_4 = Me
70c : R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe
70d : R_1 = OMe, R_2 = H, R_3 = OMOM, R_4 = OMe
70e : R_1 = Me, R_2 = H, R_3 = OH, R_4 = Et
70f : R_1 = OEt, R_2 = H, R_3 = OMOM, R_4 = OEt
70g : R_1 = Me, R_2 = OH, R_3 = H, R_4 = Me
70h : R_1 = Me, R_2 = OBn, R_3 = H, R_4 = OMe
70i : R_1 = Me, R_2 = OH, R_3 = Me, R_4 = Me



58-66

d
71a : R_1 = Me, R_2 = R_3 = H, R_4 = Me
71b : R_1 = Me, R_2 = H, R_3 = OBn, R_4 = Me
71j : R_1 = Me, R_2 = H, R_3 = OH, R_4 = Me
71c : R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe
71d : R_1 = OMe, R_2 = H, R_3 = OMOM, R_4 = OMe
71e : R_1 = Me, R_2 = H, R_3 = OTHP, R_4 = Et
71f : R_1 = OEt, R_2 = H, R_3 = OMOM, R_4 = OEt
71g : R_1 = Me, R_2 = OH, R_3 = H, R_4 = Me
e
71h : R_1 = Me, R_2 = OBn, R_3 = H, R_4 = OMe
71k : R_1 = Me, R_2 = OH, R_3 = H, R_4 = OMe
71i : R_1 = Me, R_2 = OH, R_3 = Me, R_4 = Me

Scheme 3-10. Synthesis of **58-66**. Reagents: (a) NaOH aq, MeOH; (b) (2*S*)-2-cyanopyrrolidine, MsOBt, Et_3N, DMF; (c) (2*S*)-2-cyanopyrrolidine, EDC, HOBt, NMM, DMF; (d) H_2, 10% Pd-C, MeOH; (e) H_2, 20% $Pd(OH)_2$-C, EtOAc; (f) 4N HCl/1,4-dioxane ; (g) *p*-TsOH-H_2O, EtOH.

３-８．2,6置換フェニル誘導体のDPP-IV阻害活性および分子内環化反応に対する安定性の評価

３-８-１．方法

合成した2,6置換フェニル誘導体**58-66**のヒト精製酵素に対する*in vitro*酵素阻害活性を２-３-１と同様の方法により評価した。2,6置換フェニル誘導体**58-66**の分子内環化反応に対する安定性を、２-５-１と同様の方法により評価した（table 3-3）。

また、２-４-１と同様の方法によりSDラットに2,6置換フェニル誘導体**58-66**を1 mg/kg経口投与した際の*ex vivo*酵素阻害活性を評価した（table 3-4）。

３-８-２．結果と考察

両方のオルト位にメチル基を導入した化合物**58**, **59**, **64**は高い*in vitro*酵素阻害活性を示した。オルト位の一方の置換基をメチル基からメトキシ基**60**, **65**、エチル基**62**に変換しても高い酵素阻害活性を保持したが、両方の置換基をメトキシ基**61**、エトキシ基**63**に変換すると数倍の阻害活性の減弱がみられ、立体障害によるものと考えられる。

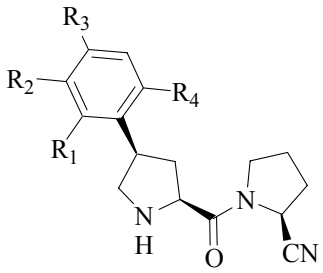
オルト位の置換基を大きくするに従い（**59**→**60**→**61**）、分子内環化反応に対する安定性が高まっていった。化合物**60**のX線結晶構造解析よりピロリジン環とフェニル基はほぼ直行しており、オルト位の置換基は、環化反応の際にニトリルが近づくのを阻害していると考えられる（Figure 3-7）。

水酸基を持たない**58**はラット1 mg/kg経口投与6時間後に62％の阻害率を示したのに対し、パラ位**59**、メタ位**64**に水酸基を導入すると、6時間後の阻害率が88％、95％になり、持続性が長くなった。

パラ位フェノール体**59**, **60**とメタ位フェノール体**64**, **65**の持続性を比較すると、メタ位フェノール体はラット1 mg/kg経口投与10時間後においても80%以上の阻害率を示し、パラ位フェノール体より持続性を示した。

化合物**64**の水酸基のオルト位にメチル基を導入した化合物**66**は、ラット1 mg/kg経口投与6時間後において阻害率が39％と持続性を示さなかった。フェノールの両方のオルト位に置換基が入ることで、立体障害によりグルクロン酸抱合を受けにくくなり、持続性を示さなくなったと考えられる。

Table 3-3. 2,6置換フェニル誘導体のDPP-IV阻害活性および分子内環化反応に対する安定性



compound	R_1	R_2	R_3	R_4	human DPPIV IC_{50} (nM)	solution stability (pH 7.4) $t_{1/2}$ (h)
58	Me	H	H	Me	6.1	NT*
59	Me	H	OH	Me	3.3	31
60	Me	H	OH	OMe	8.3	49
61	OMe	H	OH	OMe	23	59
62	Me	H	OH	Et	8.9	39
63	OEt	H	OH	OEt	22	NT*
64	Me	OH	H	Me	4.9	65
65	Me	OH	H	OMe	7.1	NT
66	Me	OH	Me	Me	5.3	40

*NT : Not tested.

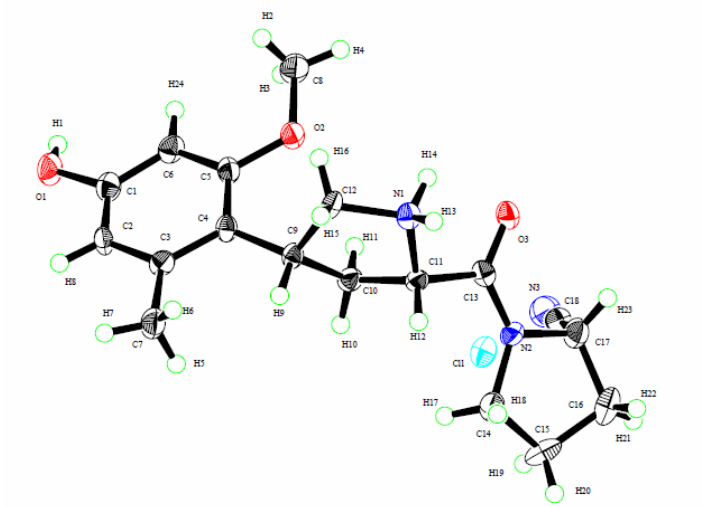


Figure 3-7. 化合物**60**のX線結晶構造解析

Table 3-4. 2,6置換フェニル誘導体の*ex vivo* DPP-IV阻害活性

compound	*ex vivo* DPP-IV inhibition (%) at 1 mg/kg po, normal rats							
	time after administraton (h)							
	0.25	0.5	1	2	4	6	8	10
58	89	93	92	83	81	62	NT*	NT*
59	98	98	97	93	90	88	79	57
60	96	97	98	95	94	90	81	73
61	89	92	92	87	85	81	78	66
62	98	98	97	91	87	90	NT*	NT*
63	78	81	79	71	81	79	68	50
64	99	99	99	98	97	95	91	83
65	99	99	98	96	93	93	92	86
66	93	95	91	76	53	39	16	NT*

*NT : Not tested.

３-９．化合物**60**の*in vitro*代謝実験によるグルクロン酸抱合体の生成と評価

これまでの検討で、経口投与後の血漿にフェノールのグルクロン酸抱合体が含まれ、持続性に寄与している事を示唆するデータが得られている（第三章３-２-４、３-４-２、３-４-４）。フェノールのグルクロン酸抱合体が活性代謝物であり、その生成が容易な化合物がラットにおいて持続性を示すという仮説が立てられる。この仮説を確認するため、まず、フェノールのグルクロン酸抱合体が活性代謝物であることを確認する事とした。そこで、化合物**60**の*in vitro*代謝実験を行い、代謝反応生成物の酵素阻害活性を測定する事とした。

３-９-１．方法

化合物**60**をラット肝ミクロソームとグルクロン酸抱合代謝の補酵素であるUDP-グルクロン酸（UDPGA）またはニコチンアミドアデニンジヌクレオチドリン酸（NADPH）を用い、*in vitro*にて代謝反応を行い、代謝反応生成物の*in vitro*酵素阻害活性を測定した。ラット肝ミクロソーム（Xenotech Corporation、1 mg/mL）と化合物**60**（100 ng/mL）に2 mM UDPGAまたは 2 mM NADPHを加え、37℃にて1時間反応した。生成物を、除蛋白後LC/MSにて分析し、化合物**60**の残存率を求めた。また、この生成物のDPP-IV阻害活性を測定した。

３-９-２．結果と考察

肝ミクロソームとNADPHを用いた代謝反応ではシトクロムP450（CYP）による酸化代謝が評価されるが、化合物**60**においては酸化代謝があまり進行しないことが分かった（Figure 3-8）。一方、肝ミクロソームとUDPGAを用いた代謝反応ではグルクロン酸抱合反応が進行し、60分後には化合物**60**はほぼ消失していた（Figure 3-8）。グルクロン酸抱合体は標品がないため定量できなかったが、HPLCにおける内部標準化合物のピーク面積との比より経時的にグルクロン酸抱合体が生成しているのを確認した（Figure 3-9）。このグルクロン酸抱合反応生成物のDPP-IV阻害活性は反応の進行に関わらず、高い阻害活性を維持したことから、グルクロン酸抱合体にも高いDPP-IV阻害活性があることが確認された（Figure 3-10）。

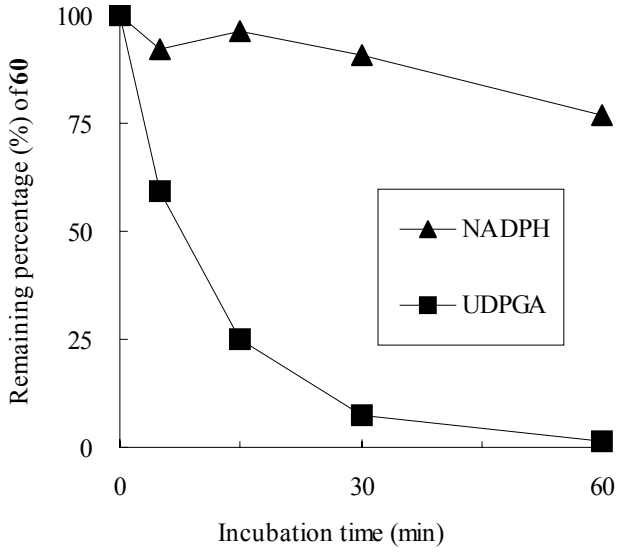


Figure 3-8. 化合物**60**のラット肝ミクロソームを用いた代謝反応。X軸はインキュベーション時間、Y軸は化合物**60**の残存率を示す。

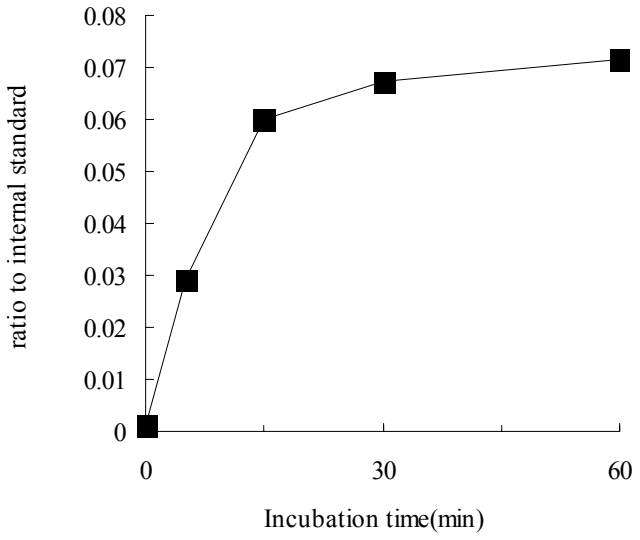


Figure 3-9. 化合物**60**のラット肝ミクロソームを用いた代謝反応。X軸はインキュベーション時間、Y軸はグルクロン酸抱合体の生成量（内部標準に対する比）を示す。

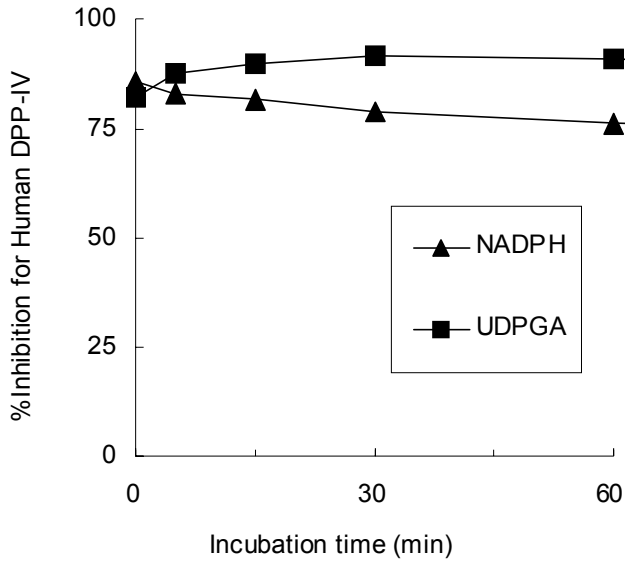


Figure 3-10. 化合物**60**のラット肝ミクロソームを用いた代謝反応生成物のDPP-IV阻害活性。X軸はインキュベーション時間、Y軸はヒト精製酵素に対する阻害率。

３-１０．化合物**64**と化合物**66**のラット経口投与時の血中グルクロン酸抱合体濃度の検討

持続性を示した化合物**64**と持続性を示さなかった化合物**66**のラット血中グルクロン酸抱合体濃度を検討し、グルクロン酸抱合能と持続性の関係を明らかにすることとした。

３-１０-１．方法

化合物**64**と化合物**66**の正常ラット（Sprague-Dawley rats、SDラット）経口投与後の血漿中濃度を測定した。0.5%メチルセルロース水溶液に溶解した化合物**64**または化合物**66**を、1 mg/kgの投与量で経口投与後、経時的に頚静脈より採血し、血漿を調製した。得られた血漿を除蛋白後、LC/MS/MSにて分析し、化合物**64**または化合物**66**の血漿中濃度（未変化体）を算出した。一方、調製した血漿を除蛋白後、乾固し、β-Glucuronidase/arylsulfatase（Roche Diagnostics Corporation）で37℃にて2時間反応した。反応性生物を除蛋白後、LC/MS/MSにて分析し、化合物**64**または化合物**66**の濃度を算出し、グルクロン酸抱合体の濃度とした（table 3-5）。

３-１０-２．結果と考察

持続性を示した化合物**64**と化合物**66**の血漿中未変化体濃度は化合物間であまり差が見られなかった。一方、血漿のグルクロニダーゼ処理を行い、グルクロン酸抱合体を未変化体として検出したグルクロン酸抱合体濃度については、化合物**64**は化合物**66**と比較し高い値を示した。未変化体とグルクロン酸抱合体の比も化合物**64**が約38倍から122倍であったのに対し、化合物**66**は約10倍から15倍と、化合物**64**がより高い割合でグルクロン酸抱合を受けていることがわかった。この結果は、化合物**66**にはフェノールの両方のオルト位にメチル基が存在し、その立体障害の影響でグルクロン酸抱合を受けにくいためと考えられる。

Table 3-5. 化合物**64**、**66**の経口投与（1 mg/kg）後の血漿中濃度

compound	time after administration (h)	plasma concentrarion (ng/mL)	
		without glucuronidase treatment	with glucuronidase treatment
64	0.25	6.89	266
	2	0.31	38
	6	ND*	14.7
66	0.25	9.7	100
	2	0.65	9.5
	6	0.95	ND*

*ND : Not detected.

以上の結果より、グルクロン酸抱合を受けやすい化合物において、持続性を示す可能性があることが示唆された。この理由については、結論付けるための十分なデータが無いが、以下のような可能性が考えられる。まず、活性代謝物であるグルクロン酸抱合体を効率良く生成する化合物は、その他の活性を失う代謝経路（例えばP1部ピロリジン環の4位の水酸化など[5]）の寄与が少なくなることが考えられる。生成したグルクロン酸抱合体は一般的には胆汁排泄により腸管に排泄される。腸管に排泄されたグルクロン酸抱合体は腸内のグルクロニダーゼにより元のフェノール体へ戻り、再び吸収される（腸肝循環）[6]。このようなメカニズムにより、活性代謝物であるグルクロン酸抱合体を生成しやすい化合物が持続性を示したと考えられる。同様のメカニズムにより持続性を示す薬剤の例として、鎮痛薬のモルヒネが知られている[7]。グルクロン酸抱合を受けにくい化合物は、活性を失う代謝や排泄により持続性を示さなかった可能性が考えられるが、それを明らかにするためには、血漿中、尿中、糞中などに含まれる代謝物、化合物の体内動態を詳細に検討する必要がある。

代謝反応には種差、個体差があることが知られている。したがって、代謝物に基づいて持続性を示す化合物では薬効に個人差が出やすく、投与量の設定に困難が伴う場合があると考えられる。

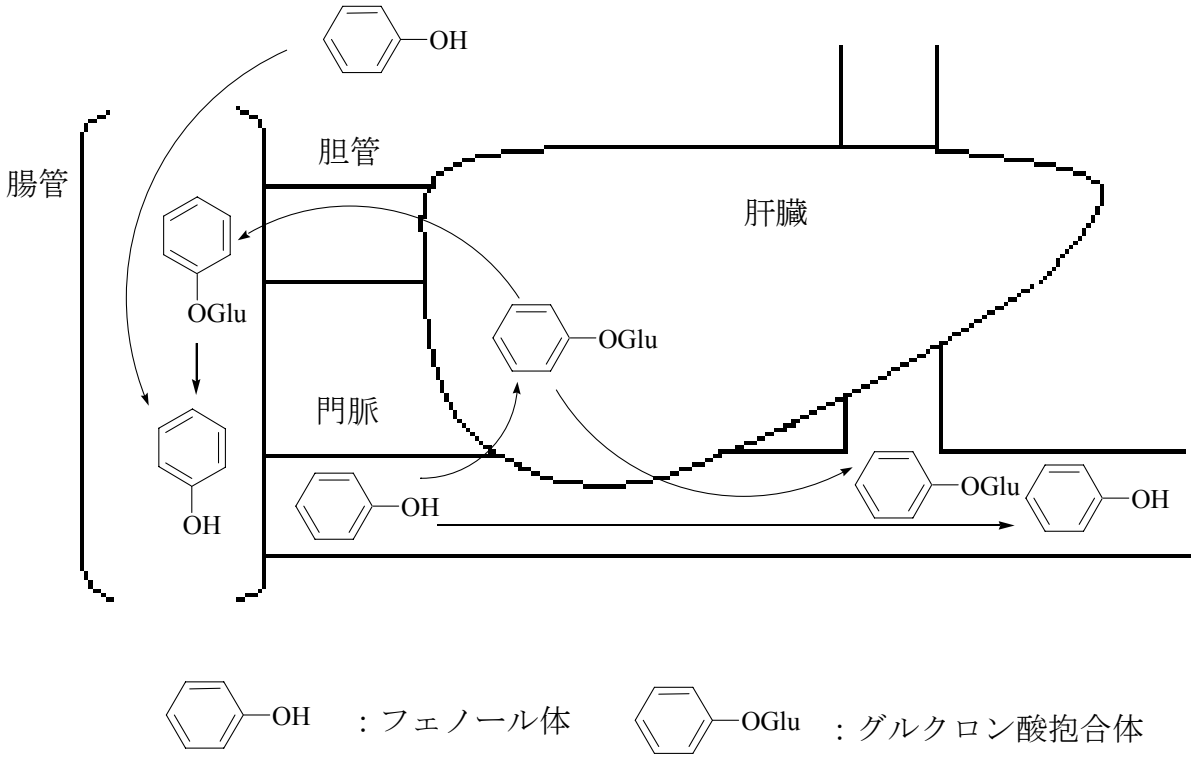
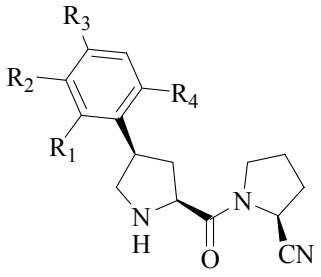


Figure 3-11. 腸肝循環による持続性

３-１１．小括

リード化合物である4-フェニルプロリル-2-シアノピロリジン**8**の最適化を行うにあたり、まず化合物**8**のラットにおける持続性のメカニズムを検討した。その結果、化合物**8**のラットにおける持続性には活性代謝物の関与が示唆され、その代謝物は化合物**8**が水酸化され、さらにグルクロン酸抱合を受けた構造であることを明らかにした。第二章で得られた構造活性相関より、水酸化の位置をベンゼン環上と推定し、フェノール誘導体を合成した。代謝物のグルクロニダーゼ処理により、パラ位のフェノール体**45**が化合物**8**の代謝の中間体であることが明らかとなった。化合物**45**はラットにおいて持続性を示した。また、オルト位のフェノール体**43**においてフェノールの酸性プロトンが分子内環化反応を促進していることを見出し、オルト位の置換基が分子内環化反応の際にニトリル基の近傍に存在しうると考察した。その考察を基に、分子内環化反応に対する安定性の向上を目的とし、２ヶ所のオルト位に置換基を導入したフェノール誘導体を設計し、合成した。その結果、2ヶ所のオルト位にメチル基またはメトキシ基を導入した化合物**60**、**61**、**64**、**65**において高い酵素阻害活性と持続性を示すことが明らかとなった。特に、メタ位フェノール体である化合物**64**、**65**はラット1 mg/kg経口投与10時間後においても80%以上の阻害率を示した。化合物**60**の*in vitro*グルクロン酸抱合反応により、グルクロン酸抱合体が活性代謝物であることが確認された。また、立体障害によりグルクロン酸抱合を受けにくい化合物**66**より、グルクロン酸抱合を受けやすい化合物**64**が持続性を示したことから、グルクロン酸抱合を受けやすい化合物が持続性を示すと考察した。これらのフェノール誘導体は活性代謝物であるグルクロン酸抱合体の生成、胆汁排泄、再吸収を繰り返すことにより、持続性が発現していると推定している。



8 : $R_1 = R_2 = R_3 = R_4 = H$
43 : $R_1 = OH$, $R_2 = R_3 = R_4 = H$
45 : $R_1 = R_2 = H$, $R_3 = OH$, $R_4 = H$
60 : $R_1 = Me$, $R_2 = H$, $R_3 = OH$, $R_4 = OMe$
61 : $R_1 = OMe$, $R_2 = H$, $R_3 = OH$, $R_4 = OMe$
64 : $R_1 = Me$, $R_2 = OH$, $R_3 = H$, $R_4 = Me$
65 : $R_1 = Me$, $R_2 = OH$, $R_3 = H$, $R_4 = OMe$
66 : $R_1 = Me$, $R_2 = OH$, $R_3 = Me$, $R_4 = Me$

３-１２．実験の部

３-１２-１．Chemistry

General directions

Analytical samples were homogeneous as confirmed by TLC and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were taken on a Varian Mercury 300 spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. The chemical shift values are reported in parts per million (δ) and coupling constants (*J*) in hertz (Hz). Fast atom bombardment mass spectra (FAB-MS, HRMS) and electron ionization (EI) were obtained on a JEOL JMS-700 spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a HITACHI M-1200H spectrometer. Matrix-assisted laser desorption ionization (MALDI) mass spectra were obtained on a PerSeptive Biosystems VoyagerTM Elite spectrometer. Infrared spectra (IR) were measured in a JASCO FT/IR-430 spectrometer. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063-0.200 mm), Wako gel C200 or Fuji Silysia FL60D]. Thin-layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, silica gel 60 F254). The following abbreviations for solvents and reagents are used; tetrahydrofuran (THF), diethyl ether (Et_2O), dimethylsulfoxide (DMSO), ethyl acetate (EtOAc), dimethylformamide (DMF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), methanol (MeOH), ethanol (EtOH), acetic acid (AcOH), hydrochloric acid (HCl).

1-*tert*-Butyl 2-methyl (2*S*)-4-{[(trifluoromethyl)sulfonyl]oxy}-2,5-dihydro-1H-pyrrole-1, 2-dicarboxylate (51)

To a stirred solution of sodium bis(trimethylsilyl)amide (2.02 g, 11 mmol) in THF (20 mL) was added dropwise a solution of **50** (2.43 g, 10 mmol) in THF (7 mL) at −78 ºC. After being stirred for 15 min, *N*-phenyl-bis(trifluoromethanesulfonimide) (3.57 g, 10 mmol) in THF (12 mL) was added and the reaction mixture was stirred at −78 ºC for additional 3 h. The reaction mixture was quenched with aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/15) as an eluant to yield **51** (2.89 g, 77%) as a colorless oil. TLC R_f = 0.37 (EtOAc/hexane, 1/4); MS (APCI, pos. 20 V) *m/z* 398 $(M+Na)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 and 1.49 (s, 9H), 3.77 (s, 3H), 4.16−4.53 (m, 2H), 4.89−5.20 (m, 1H), 5.67−5.79 (m, 1H).

1-*tert*-Butyl 2-methyl (2*S*)-4-(2,6-dimethylphenyl)-2,5-dihydro-1H-pyrrole-1,2-dicarbox ylate (68a)

To a heterogeneous mixture of **51** (1.12 g, 3.0 mmol), 2,6-dimethylphenylboronic acid (540

mg, 3.6 mmol) and 2M Na_2CO_3 (3.5 mL), in 1,4-dioxane (28 mL) was added tetrakis(triphenylphosphine)palladium(0) (86 mg, 0.074 mmol). The reaction mixture was refluxed for 1.5 h under argon atmosphere. The reaction mixture was cooled to room temperature and diluted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/7) as an eluent to yield **68a** (933 mg, 100%) as a colorless oil. TLC R_f = 0.60 (EtOAc/hexane, 1/3); MS (APCI, pos. 20 V) *m/z* 332 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 1.35−1.44 (m, 9H), 2.16 (s, 6H), 3.65−3.73 (m, 3H), 4.18−4.24 (m, 2H), 5.04−5.11 (m, 1H), 5.59−5.67 (m, 1H), 7.04 (d, *J* = 6.6 Hz, 2H), 7.07−7.15 (m, 1H).

According to the same procedure as described above, **54a-c** and **68b−i** were prepared from **51.**

1-*tert*-Butyl 2-methyl (2*S*)-4-[2-(benzyloxy)phenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (54a)

Yield 72%. A colorless oil. TLC R_f = 0.63 (EtOAc/hexane, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.41 - 1.52 (m, 9H), 3.67 - 3.77 (m, 3H), 4.52 - 4.77 (m, 2H), 5.05 - 5.18 (m, 3H), 6.35 - 6.41 (m, 1H), 6.92 - 7.02 (m, 2H), 7.19 - 7.30 (m, 2H), 7.30 - 7.46 (m, 5H).

1-*tert*-Butyl 2-methyl (2*S*)-4-[3-(benzyloxy)phenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (54b)

Yield 89%. A colorless oil. TLC R_f = 0.28 (EtOAc/hexane, 1/4); ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 and 1.52 (s, 9H), 3.75 and 3.76 (s, 3H), 4.44−4.73 (m, 2H), 5.03−5.09 (m, 2H), 5.09−5.22 (m, 1H), 5.99−6.11 (m, 1H), 6.89−7.04 (m, 3H), 7.23−7.48 (m, 6H).

1-*tert*-Butyl 2-methyl (2*S*)-4-[4-(benzyloxy)phenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (54c)

Yield 75%. A colorless oil. TLC R_f = 0.39 (EtOAc/hexane, 1/3); ^1H NMR (300 MHz, $CDCl_3$) δ 1.42−1.54 (m, 9H), 3.71−3.79 (m, 3H), 4.44−4.70 (m, 2H), 5.05−5.21 (m, 3H), 5.87−5.98 (m, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 7.27−7.52 (m, 7H).

1-*tert*-Butyl 2-methyl (2*S*)-4-[4-(benzyloxy)-2,6-dimethylphenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (68b)

Yield 90%. A colorless oil. TLC R_f = 0.52 (EtOAc/hexane, 1/3); ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 and 1.49 (s, 9H), 2.20 and 2.21 (s, 6H), 3.77 and 3.78 (s, 3H), 4.15−4.44 (m, 2H), 5.03 (s, 2H), 5.08−5.22 (m, 1H), 5.43−5.55 (m, 1H), 6.68 (s, 2H), 7.28−7.47 (m, 5H).

1-*tert*-Butyl 2-methyl (2*S*)-4-[4-(benzyloxy)-2-methoxy-6-methylphenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (68c)

Yield 83%. A pale yellow powder. TLC R_f = 0.54 (EtOAc/hexane, 1/2); ^1H NMR (300 MHz,

$CDCl_3$) δ 1.45 and 1.49 (s, 9H), 2.21 (s, 3H), 3.73 and 3.75 (s, 3H), 3.77 (s, 3H), 4.31−4.48 (m, 2H), 5.04 (s, 2H), 5.11−5.18 (m, 1H), 5.50−5.53 (m, 1H), 6.45 (d, *J* = 2.0 Hz, 1H), 6.93 (d, *J* = 2.0 Hz, 1H), 7.30−7.46 (m, 5H).

1-*tert*-Butyl 2-methyl (2*S*)-4-[2,6-dimethoxy-4-(methoxymethoxy)phenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (68d)

Yield 23%. A pale yellow oil. TLC R_f = 0.31 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 and 1.52 (s, 9H), 3.49 (s, 3H), 3.70−3.80 (m, 9H), 4.28−4.76 (m, 2H), 5.05−5.13 (m, 1H), 5.18 (s, 2H), 5.84−5.98 (m, 1H), 6.27 (s, 2H).

1-*tert*-Butyl 2-methyl (2*S*)-4-[2-ethyl-6-methyl-4-(tetrahydro-2H-pyran-2-yloxy)phenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (68e)

Yield 88%. A colorless oil. TLC R_f = 0.50 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 and 1.15 (t, *J* = 7.5 Hz, 3H), 1.46 and 1.48 (s, 9H), 1.55−1.74 (m, 3H), 1.81−1.88 (m, 2H), 2.00 (m, 1H), 2.20 and 2.21 (s, 3H), 2.50 (q, *J* = 7.5 Hz, 2H), 3.61 (m, 1H), 3.77 (s, 3H), 3.92 (m, 1H), 4.15−4.42 (m, 2H), 5.11−5.18 (m, 1H), 5.42 (t, *J* = 3.0 Hz, 1H), 5.48−5.42 (m, 1H), 6.77 (m, 2H).

1-*tert*-Butyl 2-methyl (2*S*)-4-[2,6-diethoxy-4-(methoxymethoxy)phenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (68f)

Yield 36%. An orange oil. TLC R_f = 0.29 (EtOAc/hexane, 1/4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34−1.43 (m, 6H), 1.43−1.53 (m, 9H), 3.48 (s, 3H), 3.72−3.76 (m, 3H), 3.94−4.04 (m, 4H), 4.54−4.63 (m, 2H), 5.05−5.12 (m, 1H), 5.15 (s, 2H), 5.93−6.11 (m, 1H), 6.25 (s, 2H).

1-*tert*-Butyl 2-methyl (2*S*)-4-(3-hydroxy-2,6-dimethylphenyl)-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (68g)

Yield 35%. A colorlesss oil. TLC R_f = 0.44 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 and 1.49 (s, 9H), 2.11−2.16 (m, 6H), 3.78 (s, 3H), 4.20−4.41 (m, 2H), 4.96 and 5.06 (s, 1H), 5.15−5.25 (m, 1H), 5.47−5.53 (m, 1H), 6.69 (d, *J* = 8.0 Hz, 1H), 6.91 (d, *J* = 8.0 Hz, 1H).

1-*tert*-Butyl 2-methyl (2*S*)-4-[3-(benzyloxy)-2-methoxy-6-methylphenyl]-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (68h)

Yield 61%. A pale yellow oil. TLC R_f = 0.45 (EtOAc/hexane, 1/4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45−1.50 (m, 9H), 2.14−2.20 (m, 3H), 3.75−3.83 (m, 6H), 4.29−4.55 (m, 2H), 5.09 (s, 2H), 5.11−5.26 (m, 1H), 5.52−5.64 (m, 1H), 6.80−6.90 (m, 2H), 7.28−7.47 (m, 5H).

1-*tert*-Butyl 2-methyl (2*S*)-4-(3-hydroxy-2,4,6-trimethylphenyl)-2,5-dihydro-1H-pyrrole-1,2-dicarboxylate (68i)

Yield 35%. A colorless oil. TLC R_f = 0.44 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$)

δ 1.46 and 1.48 (s, 9H), 2.12 (s, 3H), 2.14 (s, 3H), 2.22 (s, 3H), 3.78 (s, 3H), 4.20−4.41 (m, 2H), 4.56 and 4.58 (s, 1H), 5.10−5.20 (m, 1H), 5.45−5.55 (m, 1H), 6.84 (s, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(2,6-dimethylphenyl)-1,2-pyrrolidinedicarboxylate (69a)
To a solution of **68a** (933 mg, 2.81 mmol) in MeOH (8 mL) was added 10% palladium on carbon (200 mg). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 13 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/7) as an eluant to yield **69a** (528 mg, 56%) as a colorless oil. TLC R_f = 0.59 (EtOAc/hexane, 1/3); MS (APCI, pos. 20 V) *m/z* 334 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31−1.42 (m, 9H), 2.12−2.31 (m, 2H), 2.34 (s, 6H), 3.50−3.63 (m, 2H), 3.63−3.73 (m, 3H), 3.76−3.97 (m, 1H), 4.30 (t, *J* = 8.5 Hz, 1H), 6.93−7.05 (m, 3H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(2-hydroxyphenyl)-1,2-pyrrolidinedicarboxylate (55a)
Compound **55a** was obtained as a colorless oil in 98% yield from **54a** according to the same procedure as described for the preparation of **69a** from **68a**. TLC R_f = 0.63 (EtOAc/hexane, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 − 1.52 (m, 9H), 3.67−3.77 (m, 3H), 4.52−4.77 (m, 2H), 5.05−5.18 (m, 3H), 6.35−6.41 (m, 1H), 6.92−7.02 (m, 2H), 7.19−7.30 (m, 2H), 7.30−7.46 (m, 5H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(3-hydroxyphenyl)-1,2-pyrrolidinedicarboxylate (55b)
Compound **55b** was obtained as a colorless oil in 91% yield from **54b** according to the same procedure as described for the preparation of **69a** from **68a**. TLC R_f = 0.37 (EtOAc/hexane, 2/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 and 1.46 (s, 9H), 1.96−2.17 (m, 1H), 2.54−2.72 (m, 1H), 3.19−3.52 (m, 2H), 3.76 (s, 3H), 3.89−4.07 (m, 1H), 4.27−4.45 (m, 1H), 5.61 and 5.88 (s, 1H), 6.65−6.85 (m, 3H), 7.08−7.22 (m, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(4-hydroxyphenyl)-1,2-pyrrolidinedicarboxylate (55c)
Compound **55c** was obtained as a colorless oil in 100% yield from **54c** according to the same procedure as described for the preparation of **69a** from **68a**. TLC R_f = 0.43 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39−1.51 (m, 9H), 1.92−2.12 (m, 1H), 2.55−2.68 (m, 1H), 3.18−3.43 (m, 2H), 3.76 (s, 3H), 3.85−4.04 (m, 1H), 4.26−4.44 (m, 1H), 6.80 (d, *J* = 8.6 Hz, 2H), 5.73−8.14 (m, 1H), 7.03−7.12 (m, 2H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(4-hydroxy-2,6-dimethylphenyl)-1,2-pyrrolidinedicarboxylate (69b)
Compound **69b** was obtained as a colorless oil in 92% yield from **68b** according to the same procedure as described for the preparation of **69a** from **68a**. TLC R_f = 0.42 (EtOAc/hexane,

2/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40−1.52 (m, 9H), 2.25−2.54 (m, 8H), 3.77 (s, 6H), 4.26 −4.45 (m, 1H), 4.80−5.03 (m, 1H), 6.51 (s, 2H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(4-hydroxy-2-methoxy-6-methylphenyl)-1,2-pyrrolidine dicarboxylate (69c)

Compound **69c** was obtained as a colorless oil in 100% yield from **68c** according to the same procedure as described for the preparation of **69a** from **68a**. TLC R_f = 0.49 (EtOAc/hexane, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 and 1.46 (s, 9H), 2.27 and 2.29 (s, 3H), 2.50−2.71 (m, 1H), 3.48−3.68 (m, 2H), 3.73 (s, 3H), 3.77 (s, 3H), 3.84 (m, 1H), 4.31 and 4.40 (t, J = 8.4 Hz, 1H), 4.79 and 4.87 (brs, 1H), 6.25 (d, J = 2.4 Hz, 1H), 6.28 (d, J = 2.4 Hz, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-[2,6-dimethoxy-4-(methoxymethoxy)phenyl]-1,2-pyrroli dinedicarboxylate (69d)

Compound **69d** was obtained as a beige powder in 100% yield from **68d** according to the same procedure as described for the preparation of **69a** from **68a**. TLC R_f = 0.38 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35−1.51 (m, 9H), 2.17−2.42 (m, 1H), 2.46−2.68 (m, 1H), 3.45−3.53 (m, 3H), 3.72−3.80 (m, 9H), 3.80−3.97 (m, 3H), 4.20−4.45 (m, 1H), 5.16 (s, 2H), 6.27 (s, 2H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(2-ethyl-4-hydroxy-6-methylphenyl)-1,2-pyrrolidinedic arboxylate (69e)

To a stirred solution of **68e** (933 mg, 2.81 mmol) in MeOH (3 mL) was added 4M HCl in 1,4-dioxane (2 mL). After being stirred for 3.5 h, the reaction mixture was concentrated in vacuo. To a solution of the residue in MeOH (5 mL) was added 10% palladium on carbon (30 mg). The reaction mixture was vigorously stirred under an atmospheric pressure of hydrogen for 17 h at room temperature and additional 24 h at 50 ºC. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. To a stirred solution of the residue in THF (2.5 mL) and water (2 mL) were added triethylamine (0.11 mL, 0.79 mmol) and di-*tert*-butyl-dicarbonate (171 mg, 0.79 mmol) at room temperature. After 30 min, the reaction mixture was diluted with EtOAc. The organic layer was successively washed with water, 1M HCl, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/6) as an eluant to yield **69e** (204 mg, 75%) as a colorless oil. TLC R_f = 0.64 (EtOAc/hexane, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, J = 7.3 Hz, 3H), 1.44 and 1.46 (s, 9H), 2.35 and 2.38 (s, 3H), 2.25−2.50 (m, 2H), 2.64 (q, J = 7.3 Hz, 2H), 3.70 (m, 3H), 3.77 (s, 3H), 4.34 and 4.40 (t, J = 8.4 Hz, 1H), 4.80 and 4.90 (s, 1H), 6.51 (d, J = 3.0 Hz, 1H), 6.54 (d, J = 3.0 Hz, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-[2,6-diethoxy-4-(methoxymethoxy)phenyl]-1,2-pyrrolidi nedicarboxylate (69f)

Compound **69f** was obtained as a pale yellow oil in 69% yield from **68f** according to the same procedure as described for the preparation of **69a** from **68a**. TLC R_f = 0.24 (EtOAc/hexane, 1/4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35−1.51 (m, 15H), 2.17−2.32 (m, 1H), 2.61−2.81 (m, 1H), 3.46−3.51 (m, 3H), 3.72−3.78 (m, 3H), 3.80−3.91 (m, 2H), 3.91−4.05 (m, 5H), 4.24−4.41 (m, 1H), 5.13 (s, 2H), 6.24 (s, 2H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(3-hydroxy-2,6-dimethylphenyl)-1,2-pyrrolidinedicarboxylate (69g)

Compound **69g** was obtained as a colorless oil in 70% yield from **68g** according to the same procedure as described for the preparation of **69e** from **68e**. TLC R_f = 0.42 (EtOAc/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 350 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 and 1.46 (s, 9H), 2.28−2.32 (m, 6H), 2.40−2.54 (m, 2H), 3.72−3.88 (m, 6H), 4.44−4.45 (m, 1H), 4.70−4.79 (m, 1H), 6.61 (d, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(3-hydroxy-2-methoxy-6-methylphenyl)-1,2-pyrrolidinedicarboxylate (69h)

Compound **69h** was obtained as a colorless oil in 87% yield from **68h** according to the same procedure as described for the preparation of **69e** from **68e**. TLC R_f = 0.37 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42−1.49 (m, 9H), 2.28−2.35 (m, 3H), 2.39−2.61 (m, 2H), 3.69−3.89 (m, 9H), 4.29−4.46 (m, 1H), 5.30 (s, 1H), 6.77 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 8.2 Hz, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-(3-hydroxy-2,4,6-trimethylphenyl)-1,2-pyrrolidinedicarboxylate (69i)

Compound **69i** was obtained as a colorless oil in 77% yield from **68i** according to the same procedure as described for the preparation of **69a** from **68a**. TLC R_f = 0.52 (EtOAc/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 364 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 and 1.46 (s, 9H), 2.10−2.30 (m, 9H), 2.36−2.53 (m, 2H), 3.60−3.90 (m, 6H), 4.32−4.43 (m, 1H), 4.55−4.62 (m, 1H), 6.80 (s, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-[2-(benzyloxy)phenyl]-1,2-pyrrolidinedicarboxylate (55d)

To a stirred solution of **55a** (466 mg, 1.45 mmol) in DMF (15 mL) were added K_2CO_3 (201 mg, 1.45 mmol) and benzyl bromide (0.18 mL, 1.5 mmol) at room temperature. After being stirred for 15 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/8) as an eluant to yield **55d** (586 mg, 98%) as a colorless oil. TLC R_f = 0.24 (EtOAc/hexane, 1/3); ^{1}H

NMR (300 MHz, $CDCl_3$) δ 1.41−1.47 (m, 9H), 2.03−2.15 (m, 1H), 2.48−2.76 (m, 1H), 3.33−3.57 (m, 1H), 3.64−3.85 (m, 4H), 3.89−4.08 (m, 1H), 4.24−4.44 (m, 1H), 5.09 (s, 2H), 6.84−7.01 (m, 2H), 7.15−7.28 (m, 2H), 7.29−7.47 (m, 5H).

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-[3-(tetrahydro-2H-pyran-2-yloxy)phenyl]-1,2-pyrrolidinedicarboxylate (55e)

To a stirred solution of **55b** (466 mg, 1.45 mmol) in CH_2Cl_2 (3 mL) were added 3,4-dihydro-2*H*-pyran (0.41 mL, 4.5 mmol) and pyridinium *p*-toluenesulfonate (80 mg, 0.31 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was diluted with Et_2O, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/7) as an eluant to yield **55e** (1.28 g, 98%) as a colorless oil. TLC R_f = 0.58 (EtOAc/hexane, 2/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 and 1.47 (s, 9H), 1.55−1.74 (m, 3H), 1.85 (dd, J = 8.4, 3.3 Hz, 2H), 1.94−2.15 (m, 2H), 2.57−2.72 (m, 1H), 3.22−3.50 (m, 2H), 3.54−3.67 (m, 1H), 3.72 and 3.77 (s, 3H), 3.84−4.11 (m, 2H), 4.26−4.44 (m, 1H), 5.35−5.49 (m, 1H), 6.77−7.02 (m, 3H), 7.17−7.31 (m, 1H).

According to the same procedure as described above, **55f** was prepared from **55c**.

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-[4-(tetrahydro-2H-pyran-2-yloxy)phenyl]-1,2-pyrrolidinedicarboxylate (55f)

Yield 95%. A colorless oil. TLC R_f = 0.58 (EtOAc/hexane, 2/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 and 1.46 (s, 9H), 1.52−1.78 (m, 3H), 1.80−2.10 (m, 4H), 2.58−2.68 (m, 1H), 3.22−3.44 (m, 2H), 3.56−3.63 (m, 1H), 3.74 and 3.76 (s, 3H), 3.84−4.05 (m, 2H), 4.28−4.40 (m, 1H), 5.39 (t, J = 3.0 Hz, 1H), 6.99−7.01 (m, 2H), 7.12−7.20 (m, 2H).

According to the same procedure as described for the preparation of **55d** from **55a**, **69j**−**k** were prepared from **69b** and **69h**, respectively.

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-[4-(benzyloxy)-2,6-dimethylphenyl]-1,2-pyrrolidinedicarboxylate (69j)

Yield 79%. A colorless oil. TLC R_f = 0.40 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 and 1.46 (s, 9H), 2.31−2.49 (m, 8H), 3.63−3.83 (m, 6H), 4.27−4.45 (m, 1H), 5.01 (s, 2H), 6.66 (s, 2H), 7.28−7.47 (m, 5H)

1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-[3-(benzyloxy)-2-methoxy-6-methylphenyl]-1,2-pyrrolidinedicarboxylate (69k)

Yield 90%. A colorless oil. TLC R_f = 0.34 (EtOAc/hexane, 1/4); ^{1}H NMR (300 MHz, $CDCl_3$)

δ 1.42−1.49 (m, 9H), 2.27−2.32 (m, 3H), 2.33−2.73 (m, 2H), 3.58−3.93 (m, 9H), 4.25−4.48 (m, 1H), 5.06 (s, 2H), 6.75−6.84 (m, 2H), 7.27−7.49 (m, 5H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(2,6-dimethylphenyl)-2-pyrrolidinecarboxylic acid (70a)

Compound **70a** was obtained as a white powder in 56% yield from **69a** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.59 (EtOAc/hexane, 1/3); MS (APCI, Neg. 20 V) *m/z* 318 $(M-H)^-$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 - 1.42 (m, 9H), 2.12 - 2.31 (m, 2H), 2.34 (s, 6H), 3.50 - 3.63 (m, 2H), 3.63 - 3.73 (m, 3H), 3.76 - 3.97 (m, 1H), 4.30 (t, *J* = 8.5 Hz, 1H), 6.93 - 7.05 (m, 3H).

(2*S*,4*R*)-4-[2-(Benzyloxy)phenyl]-1-(*tert*-butoxycarbonyl)-2-pyrrolidinecarboxylic acid (56a)

Compound **56a** was obtained as a white powder in 91% yield from **55d** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.36 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 and 1.53 (s, 9H), 2.41−2.79 (m, 2H), 3.14−3.86 (m, 2H), 3.94−4.16 (m, 1H), 4.22−4.54 (m, 1H), 5.09 (s, 2H), 6.87−7.03 (m, 2H), 7.12−7.30 (m, 2H), 7.30−7.48 (m, 5H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[3-(tetrahydro-2H-pyran-2-yloxy)phenyl]-2-pyrrolidinecarboxylic acid (56b)

Compound **56b** was obtained as a white powder in 100% yield from **55e** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.41 (CH_2Cl_2/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 and 1.49 (s, 9H), 1.55−1.77 (m, 3H), 1.80−1.90 (m, 2H), 1.93−2.04 (m, 1H), 2.09−2.49 (m, 1H), 2.57−2.81 (m, 1H), 3.26−3.52 (m, 2H), 3.56−3.66 (m, 1H), 3.84−3.96 (m, 1H), 3.99−4.09 (m, 1H), 4.29−4.52 (m, 1H), 5.42 (s, 1H), 6.82−7.01 (m, 3H), 7.19−7.28 (m, 1H)

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[4-(tetrahydro-2H-pyran-2-yloxy)phenyl]-2-pyrrolidinecarboxylic acid (56c)

Compound **56c** was obtained as a white powder in 86% yield from **55f** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.35 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29−1.44 (m, 9H), 1.44−1.95 (m, 8H), 2.52−2.67 (m, 1H), 3.14 (m, 1H), 3.46−3.58 (m, 1H), 3.65−3.90 (m, 2H), 4.15 (t, *J* = 8.4 Hz, 1H), 5.41 (s, 1H), 6.95 (d, *J* = 8.6 Hz, 2H), 7.18 (d, *J* = 8.6 Hz, 2H), 12.57 (s, 1H).

(2*S*,4*R*)-4-[4-(Benzyloxy)-2,6-dimethylphenyl]-1-(*tert*-butoxycarbonyl)-2-pyrrolidinecarboxylic acid (70b)

Compound **70b** was obtained as a colorless oil in 100% yield from **69j** according to the same

procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.75 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 and 1.48 (s, 9H), 2.38 (s, 6H), 2.41−2.75 (m, 2H), 3.63−3.90 (m, 3H), 4.29−4.50 (m, 1H), 5.01 (s, 2H), 6.66 (s, 2H), 7.27−7.46 (m, 5H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(4-hydroxy-2-methoxy-6-methylphenyl)-2-pyrrolidine carboxylic acid (70c)

Compound **70c** was obtained as a white powder in 100% yield from **69c** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.35 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.26 and 2.27 (s, 3H), 2.34 (m, 1H), 2.68 (m, 1H), 2.70 (brs, 1H), 3.45−3.65 (m, 2H), 3.72 and 3.73 (s, 3H), 3.79 (m, 1H), 4.27 and 4.34 (t, J = 8.6 Hz, 1H), 6.28 (s, 2H), 8.66 (brs, 1H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[2,6-dimethoxy-4-(methoxymethoxy)phenyl]-2-pyrroli dinecarboxylic acid (70d)

Compound **70d** was obtained as a beige powder in 77% yield from **69d** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.22 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38−1.56 (m, 9H), 2.20−2.44 (m, 1H), 2.81−3.01 (m, 1H), 3.49 (s, 3H), 3.57−4.02 (m, 9H), 4.28−4.54 (m, 1H), 5.16 (s, 2H), 6.27 (s, 2H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(2-ethyl-4-hydroxy-6-methylphenyl)-2-pyrrolidinecar boxylic acid (70e)

Compound **70e** was obtained as a white powder in 91% yield from **69e** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.38 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, J = 7.5 Hz, 3H), 1.46 and 1.49 (s, 9H), 2.37 (s, 3H), 2.48 (m, 2H), 2.65 (q, J = 7.5 Hz, 2H), 2.72 (m, 1H), 3.62−3.83 (m, 2H), 4.38 and 4.48 (m, 1H), 6.51 (d, J = 3.0 Hz, 1H), 6.55 (d, J = 3.0 Hz, 1H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[2,6-diethoxy-4-(methoxymethoxy)phenyl]-2-pyrrolidi necarboxylic acid (70f)

Compound **70f** was obtained as a brown powder in 72% yield from **69f** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.04 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33−1.56 (m, 15H), 2.17−2.41 (m, 1H), 2.94−3.15 (m, 1H), 3.47 (s, 3H), 3.55−3.74 (m, 2H), 3.91−4.06 (m, 5H), 4.27−4.52 (m, 1H), 5.14 (s, 2H), 6.25 (s, 2H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(3-hydroxy-2,6-dimethylphenyl)-2-pyrrolidinecarboxy lic acid (70g)

Compound **70g** was obtained as a colorless oil in 92% yield from **69g** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.23 ($CHCl_3$/MeOH,

9/1); MS (APCI, neg. 20 V) *m/z* 334 $(M-H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 and 1.49 (s, 9H), 2.29 (s, 3H), 2.33 (s, 3H), 2.50−2.57 (m, 2H), 3.70−3.90 (m, 3H), 4.38−4.52 (m, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H).

(2*S*,4*R*)-4-[3-(Benzyloxy)-2-methoxy-6-methylphenyl]-1-(*tert*-butoxycarbonyl)-2-pyrrolidinecarboxylic acid (70h)

Compound **70h** was obtained as a white powder in 89% yield from **69k** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.75 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.31 (s, 3H), 2.39−2.97 (m, 2H), 3.55−3.87 (m, 3H), 3.91 (s, 3H), 4.33−4.52 (m, 1H), 5.06 (s, 2H), 6.75−6.86 (m, 2H), 7.28−7.51 (m, 5H).

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(3-hydroxy-2,4,6-trimethylphenyl)-2-pyrrolidinecarboxylic acid (70i)

Compound **70i** was obtained as a white powder in 100% yield from **69i** according to the same procedure as described for the preparation of **28a** from **27a**. TLC R_f = 0.52 ($CHCl_3$/MeOH, 9/1); MS (APCI, neg. 20 V) *m/z* 348 $(M-H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44−1.52 (m, 9H), 2.19 (s, 3H), 2.29 (s, 3H), 2.31 (s, 3H), 2.40−2.60 (m, 2H), 3.65−3.90 (m, 3H), 4.35−4.52 (m, 1H), 6.81 (s, 1H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(2,6-dimethylphenyl)-1-pyrrolidinecarboxylate (71a)**

Compound **71a** was obtained as a white powder in 99% yield from **70a** according to the same procedure as described for the preparation of **20a** from **19a**. TLC R_f = 0.33 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 398 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36−1.59 (m, 9H), 2.42 (s, 6H), 2.46−2.82 (m, 2H), 3.62−3.96 (m, 3H), 4.30−4.53 (m, 1H), 6.95−7.11 (m, 3H).

***tert*-Butyl (2*S*,4*R*)-4-[2-(benzyloxy)phenyl]-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (57a)**

Compound **57a** was obtained as a white powder in 59% yield from **56a** according to the same procedure as described for the preparation of **20a** from **19a**. TLC R_f = 0.61 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26−1.40 (m, 9H), 1.75−2.30 (m, 5H), 2.54−2.74 (m, 1H), 3.05−3.44 (m, 2H), 3.51−3.71 (m, 2H), 3.81−3.98 (m, 1H), 4.43−4.57 (m, 1H), 4.75−4.89 (m, 1H), 5.05−5.20 (m, 2H), 6.93 (t, *J* = 7.3 Hz, 1H), 7.08 (t, *J* = 8.8 Hz, 1H), 7.16−7.27 (m, 2H), 7.28−7.54 (m, 5H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[3-(tetrahydro-2H-pyran-2-yloxy)phenyl]-1-pyrrolidinecarboxylate (57b)**

Compound **57b** was obtained as a white powder in 60% yield from **56b** according to the same

procedure as described for the preparation of **20a** from **19a**. TLC R_f = 0.58 (CH_2Cl_2/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 and 1.44 (s, 9H), 1.54−1.76 (m, 3H), 1.80−1.91 (m, 2H), 1.95−2.39 (m, 6H), 2.52−2.68 (m, 1H), 3.26−3.41 (m, 1H), 3.43−3.55 (m, 1H), 3.57−3.68 (m, 2H), 3.76−4.15 (m, 3H), 4.41−4.57 (m, 1H), 4.80−4.94 (m, 1H), 5.37−5.47 (m, 1H), 6.85−7.01 (m, 3H), 7.19−7.28 (m, 1H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[4-(tetrahydro-2H-pyran-2-yloxy)phenyl]-1-pyrrolidinecarboxylate (57c)**

Compound **57c** was obtained as a white powder in 61% yield from **56c** according to the same procedure as described for the preparation of **20a** from **19a**. TLC R_f = 0.62 ($CHCl_3$/MeOH,10/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36−1.49 (m, 9H), 1.51−1.77 (m, 3H), 1.79−1.89 (m, 2H), 1.92−2.39 (m, 6H), 2.50−2.65 (m, 1H), 3.21−3.39 (m, 1H), 3.38−3.51 (m, 1H), 3.53−4.10 (m, 5H), 4.39−4.56 (m, 1H), 4.81−4.93 (m, 1H), 5.40 (t, J = 3.2 Hz, 1H), 6.94−7.06 (m, 2H), 7.18 (t, J = 8.0 Hz, 2H).

***tert*-Butyl (2*S*,4*R*)-4-[4-(benzyloxy)-2,6-dimethylphenyl]-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (71b)**

Compound **71b** was obtained as a white powder in 93% yield from **70b** according to the same procedure as described for the preparation of **20a** from **19a**. TLC R_f = 0.38 (EtOAc/hexane, 2/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.38 (s, 9H), 1.94−2.33 (m, 5H), 2.34 (s, 6H), 2.52−2.66 (m, 1H), 3.28−3.47 (m, 1H), 3.51−3.72 (m, 3H), 3.72−3.90 (m, 1H), 4.56 (t, J = 8.2 Hz, 1H), 4.73−4.86 (m, 1H), 5.04 (s, 2H), 6.66 (s, 2H), 7.26−7.46 (m, 5H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(4-hydroxy-2-methoxy-6-methylphenyl)-1-pyrrolidinecarboxylate (71c)**

To a stirred solution of **70c** (9.10 g, 23.8 mmol) in DMF (60 mL) were added (2*S*)-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (7.65 g, 28.6 mmol), 4-methylmorholine (5.8 mL, 52 mmol), 1-hydroxybenzotriazole (3.21 g, 23.8 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.47 g, 28.5 mmol) at room temperature. After being stirred for 15 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/1) as an eluant to yield **71c** (5.20 g, 51%) as a white powder. TLC R_f = 0.48 ($CHCl_3$/MeOH, 19/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 and 1.38 (s, 9H), 2.20 and 2.22 (s, 3H), 1.97−2.44 (m, 6H), 3.39−3.68 (m, 5H), 3.64 and 3.65 (s, 3H), 4.43 (m, 1H), 4.80 and 4.95 (m, 1H), 6.17 (m, 1H), 6.23 (m, 1H), 9.22 (s, 1H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[2,6-dimethoxy-4-(metho**

xymethoxy)phenyl]-1-pyrrolidinecarboxylate (71d)

Compound **71d** was obtained as a white powder in 53% yield from **70d** according to the same procedure as described for the preparation of **20a** from **19a**. TLC R_f = 0.23 (EtOAc/hexane, 2/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.37 (s, 9H), 1.98−2.11 (m, 2H), 2.11−2.25 (m, 2H), 2.29−2.39 (m, 2H), 3.41 (s, 3H), 3.43−3.67 (m, 4H), 3.74 (s, 6H), 3.77−3.87 (m, 1H), 4.47 (t, J = 8.3 Hz, 1H), 4.75−4.86 (m, 1H), 5.16 (s, 2H), 6.32 (s, 2H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(2-ethyl-4-hydroxy-6-methylphenyl)-1-pyrrolidinecarboxylate (71e)**

Compound **71e** was obtained as a brown oil in 63% yield from **70d** according to the same procedure as described for the preparation of **71c** from **70c**. TLC R_f = 0.47 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09 (t, J = 7.2 Hz, 3H), 1.30 and 1.37 (s, 9H), 1.97−2.30 (m, 4H), 2.25 and 2.26 (s, 3H), 2.26−2.45 (m, 4H), 3.40−3.80 (m, 4H), 4.51 and 4.54 (m, 1H), 4.81 and 4.98 (m, 1H), 6.39 (m, 2H), 9.05 and 9.06 (s, 1H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[2,6-diethoxy-4-(methoxymethoxy)phenyl]-1-pyrrolidinecarboxylate (71f)**

Compound **71f** was obtained as a brown powder in 51% yield from **70f** according to the same procedure as described for the preparation of **71c** from **70c**. TLC R_f = 0.32 (EtOAc/hexane, 2/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.28−1.44 (m, 15H), 1.99−2.34 (m, 5H), 2.41−2.52 (m, 1H), 3.40 (s, 3H), 3.41−3.88 (m, 5H), 3.94−4.09 (m, 4H), 4.43−4.52 (m, 1H), 4.74−4.85 (m, 1H), 5.13 (s, 2H), 6.29 (s, 2H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(3-hydroxy-2,6-dimethylphenyl)-1-pyrrolidinecarboxylate (71g)**

Compound **71g** was obtained as a colorless oil in 72% yield from **70g** according to the same procedure as described for the preparation of **20a** from **19a**. TLC R_f = 0.49 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 414 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.38 (s, 9H), 2.00−2.20 (m, 5H), 2.18 (s, 3H), 2.26 (s, 3H), 2.50−2.60 (m, 1H), 3.60−3.67 (m, 4H), 3.82−3.90 (m, 1H), 4.57 (t, J = 8.2 Hz, 1H), 4.75−4.85 (m, 1H), 6.61 (d, J = 8.1 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 8.55 (s, 1H).

***tert*-Butyl (2*S*,4*R*)-4-[3-(benzyloxy)-2-methoxy-6-methylphenyl]-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (71h)**

Compound **71h** was obtained as a white powder in 63% yield from **70h** according to the same procedure as described for the preparation of **71c** from **70c**. TLC R_f = 0.39 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40−1.50 (m, 9H), 2.09−2.72 (m, 9H), 3.55−3.99 (m, 8H), 4.44−4.63 (m, 1H), 4.81−4.97 (m, 1H), 5.06 (s, 2H), 6.74−6.85 (m, 2H), 7.28−7.51 (m, 5H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(3-hydroxy-2,4,6-trimethylphenyl)-1-pyrrolidinecarboxylate (71i)**

Compound **71i** was obtained as a white powder in 36% yield from **70i** according to the same procedure as described for the preparation of **20a** from **19a**. TLC R_f = 0.69 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 428 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.38 (s, 9H), 2.00−2.23 (m, 5H), 2.11 (s, 3H), 2.21 (s, 3H), 2.24 (s, 3H), 2.50−2.60 (m, 1H), 3.55−3.65 (m, 4H), 3.70−3.90 (m, 1H), 4.56 (t, *J* = 8.3 Hz, 1H), 4.75−4.85 (m, 1H), 6.70 (s, 1H), 7.49 (s, 1H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(2-hydroxyphenyl)-1-pyrrolidinecarboxylate (57d)**

To a solution of **57a** (294 mg, 0.62 mmol) in MeOH (6 mL) was added 10 % palladium on carbon (29 mg). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 5 h. The catalyst was removed by filtration and the filtrate was evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/2) as an eluant to yield **57d** (76 mg, 42%) as a white powder. TLC R_f = 0.39 (EtOAc/hexane, 2/1); MS (APCI, pos. 20 V) *m/z* 386 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28−1.41 (m, 9H), 1.75−2.31 (m, 5H), 2.53−2.78 (m, 1H), 3.10−3.23 (m, 1H), 3.42−3.71 (m, 3H), 3.86 (dd, *J* = 9.9, 7.3 Hz, 1H), 4.40−4.54 (m, 1H), 4.76−4.88 (m, 1H), 6.67−6.86 (m, 2H), 6.97−7.18 (m, 2H), 9.53 (s, 1H).

According to the same procedure as described above, **71j** was prepared from **71b**.

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(4-hydroxy-2,6-dimethylphenyl)-1-pyrrolidinecarboxylate (71j)**

Yield 56%. A white powder. TLC R_f = 0.60 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 2.07−2.51 (m, 12H), 3.51−3.87 (m, 5H), 4.41−4.60 (m, 1H), 4.80−4.92 (m, 1H), 6.50 (s, 2H).

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(3-hydroxy-2-methoxy-6-methylphenyl)-1-pyrrolidinecarboxylate (71k)**

To a solution of **71h** (142 mg, 0.273 mmol) in EtOAc (3 mL) was added 20 % palladium hydroxide on carbon (28 mg). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 2.5 h. The catalyst was removed by filtration and the filtrate was evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (2/1) as an eluant to yield **71k** (100 mg, 85%) as a white powder. TLC R_f = 0.28 (EtOAc/hexane, 2/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100ºC) δ

1.38 (s, 9H), 1.99−2.38 (m, 5H), 2.24 (s, 3H), 2.40−2.58 (m, 1H), 3.46−3.70 (m, 5H), 3.75 (s, 3H), 4.53 (t, J = 8.3 Hz, 1H), 4.75−4.86 (m, 1H), 6.65 (d, J = 8.1 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 8.64 (s, 1H).

(2*S*)-1-{[(2*S*,4*R*)-4-(2,6-Dimethylphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile hydrochloride (58)

Compound **58** was obtained as a white powder in 76% yield from **71a** according to the same procedure as described for the preparation of **9** from **20b**. TLC R_f = 0.43 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 298 $(M+H)^+$; IR (KBr) 3434, 2974, 2885, 2242, 1656, 1544, 1363, 1340, 1154, 556 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.94–2.08 (m, 2H), 2.08–2.27 (m, 4H), 2.33 (s, 6H), 2.68–2.85 (m, 1H), 3.43–3.71 (m, 4H), 3.88–4.20 (m, 1H), 4.68 (t, J = 8.6 Hz, 1H), 4.81–4.90 (m, 1H), 6.93–7.09 (m, 3H); HRMS (FAB) calcd for $C_{18}H_{24}N_3O$: 298.1919. Found: 298.1916.

(2*S*)-1-{[(2*S*,4*R*)-4-(2-Hydroxyphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (43)

Compound **43** was obtained as an ivory powder in 42% yield from **57d** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.24 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 286 $(M+H)^+$; IR (KBr) 3588, 2243, 1656, 1455, 1170, 1124, 1034, 1009, 683, 567 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.88–2.08 (m, 3H), 2.09–2.26 (m, 2H), 2.28 (s, 3H), 2.74–2.89 (m, 1H), 3.20–3.37 (m, 1H), 3.48–3.81 (m, 4H), 4.54–4.69 (m, 1H), 4.85 (dd, J = 7.7, 4.6 Hz, 1H), 6.73–6.86 (m, 2H), 7.05–7.14 (m, 3H), 7.14–7.20 (m, 1H), 7.46 (d, J = 8.0 Hz, 2H), 8.82 (s, 1H), 9.47 (s, 1H), 9.76 (s, 1H); HRMS (FAB) calcd for $C_{16}H_{20}N_3O_2$: 286.1556. Found: 286.1555.

(2*S*)-1-{[(2*S*,4*R*)-4-(3-Hydroxyphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (44)

Compound **44** was obtained as a white powder in 87% yield from **57b** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.20 (CH_2Cl_2/MeOH, 9/1); MS (MALDI, pos.) *m/z* 286 $(M+H)^+$; IR (KBr) 3165, 2243, 1661, 1601, 1589, 1454, 1161, 1122, 1033, 1009, 682, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.65–1.87 (m, 1H), 1.95–2.09 (m, 2H), 2.09–2.26 (m, 2H), 2.28 (s, 3H), 2.82–3.04 (m, 1H) 3.08–3.31 (m, 1H), 3.45–3.55 (m, 1H), 3.56–3.74 (m, 3H), 4.51–4.69 (m, 1H), 4.85 (dd, J = 7.7, 4.8 Hz, 1H), 6.61–6.81 (m, 3H), 7.06–7.19 (m, 3H), 7.46 (d, J = 8.0 Hz, 2H), 8.78–9.05 (m, 1H), 9.31–9.63 (m, 2H); HRMS (FAB) calcd for $C_{16}H_{20}N_3O_2$: 286.1556. Found: 286.1554.

(2*S*)-1-{[(2*S*,4*R*)-4-(4-Hydroxyphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (45)

Compound **45** was obtained as an ivory powder in 92% yield from **57c** according to the same

procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.25 ($CHCl_3$/MeOH, 2/1); MS (APCI, pos. 20 V) *m/z* 286 $(M+H)^+$; IR (KBr) 3290, 2242, 1660, 1615, 1519, 1449, 1213, 1164, 1122, 1009, 684 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.62–1.84 (m, 1H), 1.92–2.08 (m, 2H), 2.09–2.27 (m, 2H), 2.28 (s, 3H), 2.80–2.97 (m, 1H), 3.06–3.22 (m, 1H), 3.35–3.53 (m, 2H), 3.55–3.70 (m, 2H), 4.47–4.68 (m, 1H), 4.85 (dd, *J* = 7.8, 4.7 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 2H), 7.11 (dd, *J* = 8.2, 2.9 Hz, 4H), 7.47 (d, *J* = 8.0 Hz, 2H), 8.69–9.12 (m, 1H), 9.27–9.60 (m, 2H); HRMS (FAB) calcd for $C_{16}H_{20}N_3O_2$: 286.1556. Found: 286.1558.

(2*S*)-1-{[(2*S*,4*R*)-4-(4-Hydroxy-2,6-dimethylphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (59)

Compound **59** was obtained as an ivory powder in 100% yield from **71j** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.25 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 314 $(M+H)^+$; IR (KBr) 3169, 2243, 1662, 1611, 1593, 1453, 1150, 1122, 1033, 1009, 682, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.95–2.22 (m, 5H), 2.23 (s, 6H), 2.28 (s, 3H), 2.61–2.80 (m, 1H), 3.34–3.66 (m, 4H), 3.75–3.97 (m, 1H), 4.54–4.74 (m, 1H), 4.86 (dd, *J* = 7.8, 4.7 Hz, 1H), 6.42 (s, 2H), 7.07–7.13 (m, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 9.00 (s, 1H), 9.18 (s, 1H), 9.41 (s, 1H); HRMS (FAB) calcd for $C_{18}H_{24}N_3O_2$: 314.1869. Found: 314.187.

(2*S*)-1-{[(2*S*,4*R*)-4-(4-Hydroxy-2-methoxy-6-methylphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile hydrochloride (60)

Compound **60** was obtained as a white powder in 69% yield from **71c** according to the same procedure as described for the preparation of **9** from **20b**. TLC R_f = 0.19 ($CHCl_3$/MeOH, 9/1); MS (EI, pos.) *m/z* 329 $(M+H)^+$; IR (KBr) 3129, 2939, 2237, 1644, 1618, 1609, 1586, 1455, 1381, 1371, 1155 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.93–2.07 (m, 2H), 2.20 (s, 3H), 2.07–2.34 (m, 3H), 2.53–2.67 (m, 1H), 3.25–3.40 (m, 1H), 3.44–3.64 (m, 3H), 3.68 (s, 3H), 3.64–3.81 (m, 1H), 4.49–4.64 (m, 1H), 4.86 (dd, *J* = 7.8, 4.8 Hz, 1H), 6.21 (d, *J* = 2.2 Hz, 1H), 6.27 (d, *J* = 2.2 Hz, 1H), 8.64 (s, 1H), 9.39 (s, 1H), 10.15 (s, 1H); Anal. Calcd for $C_{18}H_{24}N_3O_3$: C, 59.09; H, 6.61; N, 11.49. Found: C, 58.79; H, 6.68; N, 11.03.

(2*S*)-1-{[(2*S*,4*R*)-4-(4-Hydroxy-2,6-dimethoxyphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (61)

Compound **61** was obtained as a beige powder in 100% yield from **71d** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.43 (CH_2Cl_2/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 346 $(M+H)^+$; IR (KBr) 3194, 2245, 1662, 1615, 1600, 1196, 1153, 1121, 1033, 1009, 682 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.91–2.06 (m, 2H), 2.07–2.33 (m, 3H), 2.28 (s, 3H), 2.45–2.62 (m, 1H), 3.19–3.81 (m, 4H), 3.69 (s, 6H), 3.86–4.08 (m, 1H), 4.49–4.72 (m, 1H), 4.85 (dd, *J* = 8.0, 4.7 Hz, 1H), 6.07 (s, 2H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 8.67 (s, 1H), 9.33 (s, 1H), 9.56 (s, 1H); HRMS (FAB)

calcd for $C_{18}H_{24}N_3O_4$: 346.1767. Found: 346.1768.

(2*S*)-1-{[(2*S*,4*R*)-4-(2-Ethyl-4-hydroxy-6-methylphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (62)

Compound **62** was obtained as a pale pink powder in 85% yield from **71e** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.25 ($CHCl_3$/MeOH, 5/1); MS (APCI, neg. 20 V) *m/z* 326 $(M-H)^-$; IR (KBr) 3377, 2967, 2245, 1661, 1611, 1454, 1146, 1123, 1033, 1009, 682 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.07–1.15 (m, 3H), 1.94–2.09 (m, 2H), 2.10–2.32 (m, 3H), 2.23 (s, 3H), 2.28 (s, 3H), 2.44–2.58 (m, 2H), 2.63–2.80 (m, 1H), 3.30–3.50 (m, 2H), 3.51–3.72 (m, 2H), 3.64 (s, 1H), 3.78–3.95 (m, 1H), 4.61–4.75 (m, 1H), 4.86 (dd, *J* = 7.6, 4.7 Hz, 1H), 6.40–6.47 (m, 2H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 9.05 (s, 1H), 9.42 (s, 1H); HRMS (FAB) calcd for $C_{19}H_{26}N_3O_2$: 328.2025. Found: 328.2023.

(2*S*)-1-{[(2*S*,4*R*)-4-(2,6-Diethoxy-4-hydroxyphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (63)

Compound **63** was obtained as a white powder in 93% yield from **71f** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.36 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 374 $(M+H)^+$; IR (KBr) 3186, 2978, 2243, 1662, 1599, 1461, 1159, 1121, 1033, 1009, 682 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.22–1.37 (m, 6H), 1.91–2.32 (m, 4H), 2.28 (s, 3H), 2.36–2.66 (m, 2H), 3.15–3.82 (m, 5H), 3.82–4.04 (m, 4H), 4.53–4.69 (m, 1H), 4.73–4.90 (m, 1H), 6.00–6.08 (m, 2H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 8.67 (s, 1H), 9.35 (s, 1H), 9.48 (s, 1H); HRMS (FAB) calcd for $C_{20}H_{28}N_3O_4$: 374.208. Found: 374.2082.

(2*S*)-1-{[(2*S*,4*R*)-4-(3-Hydroxy-2,6-dimethylphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (64)

Compound **64** was obtained as a white powder in 92% yield from **71g** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.28 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 314 $(M+H)^+$; IR (KBr) 3148, 2244, 1662, 1452, 1281, 1156, 1122, 1033, 1009, 682, 567 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.95–2.26 (m, 5H), 2.10 (s, 3H), 2.22 (s, 3H), 2.28 (s, 3H), 2.67–2.83 (m, 1H), 3.44–3.69 (m, 4H), 3.90–4.12 (m, 1H), 4.63–4.79 (m, 1H), 4.86 (dd, *J* = 7.8, 4.9 Hz, 1H), 6.63 (d, *J* = 8.0 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 9.07 (s, 2H), 9.20–9.70 (m, 1H); HRMS (FAB) calcd for $C_{18}H_{24}N_3O_2$: 314.1869. Found: 314.1868.

(2*S*)-1-{[(2*S*,4*R*)-4-(3-Hydroxy-2-methoxy-6-methylphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (65)

Compound **65** was obtained as a white powder in 97% yield from **71k** according to the same

procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.26 (CH_2Cl_2/MeOH/AcOH, 100/10/1); MS (APCI, pos. 20 V) *m/z* 330 $(M+H)^+$; IR (KBr) 3396, 2961, 2776, 2244, 1661, 1452, 1174, 1123, 1034, 1009, 683 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.92–2.35 (m, 4H), 2.20 (s, 3H), 2.28 (s, 3H), 2.43–2.59 (m, 1H), 2.64–2.84 (m, 1H), 3.38–3.75 (m, 5H), 3.77 (s, 3H), 4.56–4.73 (m, 1H), 4.80–5.14 (m, 1H), 6.64–6.75 (m, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 8.75–8.92 (m, 1H), 9.18–9.55 (m, 2H); HRMS (FAB) calcd for $C_{18}H_{24}N_3O_3$: 330.1818. Found: 330.1817.

(2*S*)-1-{[(2*S*,4*R*)-4-(3-Hydroxy-2,4,6-trimethylphenyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (66)

Compound **66** was obtained as a beige powder in 90% yield from **71i** according to the same procedure as described for the preparation of **10** from **29a**. TLC R_f = 0.24 (CHCl3/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 328 $(M+H)^+$; IR (KBr) 3408, 2976, 2244, 1662, 1574, 1452, 1122, 1033, 1009, 683, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.94–2.26 (m, 5H), 2.09 (s, 3H), 2.14 (s, 3H), 2.20 (s, 3H), 2.28 (s, 3H), 2.64–2.83 (m, 1H), 3.41–4.02 (m, 5H), 4.58–4.78 (m, 1H), 4.86 (dd, *J* = 7.6, 4.7 Hz, 1H), 6.73 (s, 1H), 7.10 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 8.94–9.15 (m, 1H), 9.33–9.65 (m, 1H); HRMS (FAB) calcd for $C_{19}H_{26}N_3O_2$: 328.2025. Found: 328.2021.

Chemical stability assay.

Test compounds were dissolved in pH 7.4 Tris buffer (100 mM) to produce a solution of 1 mg/mL. Samples were incubated at room temperature and analyzed by LC/MS, with the first sample injected being designated as the time zero sample. Then the peak area of the remaining samples was measured at the intervals of 0 h, 2 h, 6 h, 24 h. Reactions were analyzed using a first-order kinetics model, in which a plot of log(peak area /peak area at time zero) versus time was linear. The half-life was obtained by linear fitting to the plot using Microsoft Excel 2000.

3-12-2. Biological methods

Pharmacokinetic (PK) studies in rats.

Male Sprague-Dawley rats (5-7 weeks old) were purchased from Charles River Laboratories, and fasted for 24 h prior to dosing. Test compounds were prepared as solutions in 0.5% methylcellulose (0.2 mg/mL) for oral administration (po). An oral dose of 1 mg/5 mL/kg was administered to rats (n = 3 each).

Blood samples (250 μL) were collected from the jugular vein using a heparinized syringe at multiple times from 0 to 24 h. The blood was chilled on ice and then centrifuged at 12,000 rpm for 3 min at 4 °C to obtain plasma. Plasma protein was precipitated by acetonitrile and the supernatant was evaporated. Then the sample was reconstituted in the mobile phase and

analyzed by LC/MS/MS.

Plasma analysis after glucuronidase treatment.

β-Glucuronidase / arylsulfatase was purchased from Roche Diagnostics Corporation. Then 0.5 mol/L acetate buffer (pH 5.0) : β-glucuronidase / arylsulfatase = 40 : 1 was added to the evaporated supernatant prepared from a blood sample according to the procedure as described above and incubated for 2 h at 37 °C. Incubation was terminated by the addition of acetonitrile and the supernatant was evaporated. The sample was reconstituted in the mobile phase and analyzed by LC/MS/MS.

Assessment of hepatic microsomal metabolism.

Rat liver microsomes were purchased from Xenotech Corporation. Reaction mixtures contained 50 mmol/L sodium phosphate, 8 mmol/L magnesium chloride, 25 μg/mL alamethicin, 1 mg/mL microsomal protein, and 100 ng/mL test compound. Reactions were initiated by the addition of 2 mM NADPH or UDPGA and were carried out for up to 60 min in a water bath at 37 °C. Incubation was terminated by addition of acetonitrile and the supernatant was evaporated. Then the sample was reconstituted in the mobile phase and analyzed by LC/MS/MS.

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第四章　4位アミド誘導体のリード最適化と持続性発現のメカニズムの検討

４-１．序論

第二章において最適化すべきリード化合物として4位アミド誘導体**17**が見出された。化合物**17**は高い酵素阻害活性と持続性が期待される骨格であった。作用持続性と分子内環化反応に対する安定性のさらなる向上という2つの課題を克服することを目的に、化合物**17**の構造変換を行うこととした。

化合物**17**の最適化として次のような構造変換が考えられる。化合物**17**はピロリジン環の4位からメチレンを一つ挟んでジメチルアミノカルボニル基を持つ。第一に、ピロリジン環とジメチルアミノカルボニル基の間のメチレン鎖の長さと酵素阻害活性および作用持続性の相関に関して検討し、最適な長さを決定することが考えられる。第二に、最適な骨格を用いての、アミド部分のアミノ基の最適化が考えられる。第三章のフェノール誘導体と同様に4位の置換基のアミド部分を嵩高くすれば、立体障害により分子内環化反応が抑制できる可能性もあると考えられる。

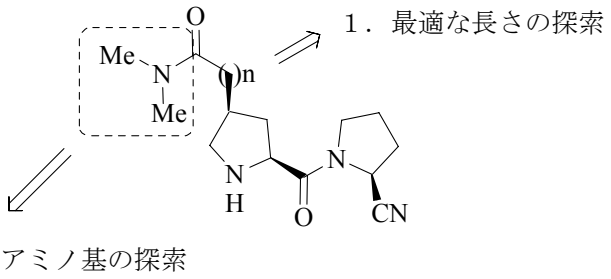
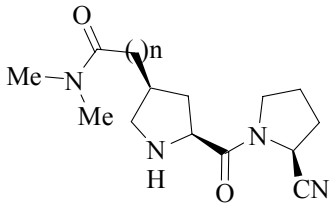


Figure 4-1. 4位アミド誘導体の最適化の合成方針

４-２．メチレン鎖の長さの異なるアミド体の合成

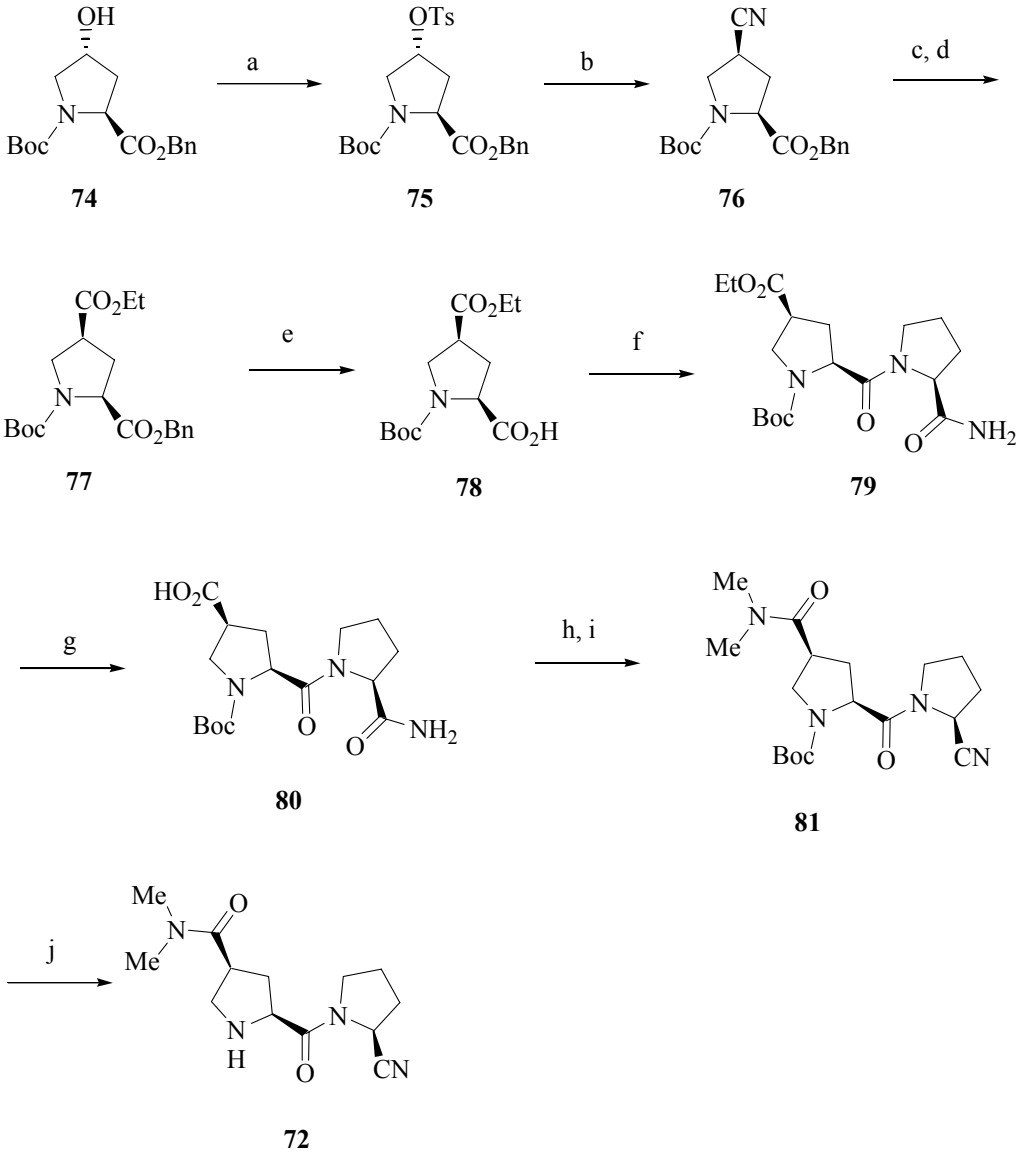


72 : n = 0
17 : n = 1
73 : n = 2

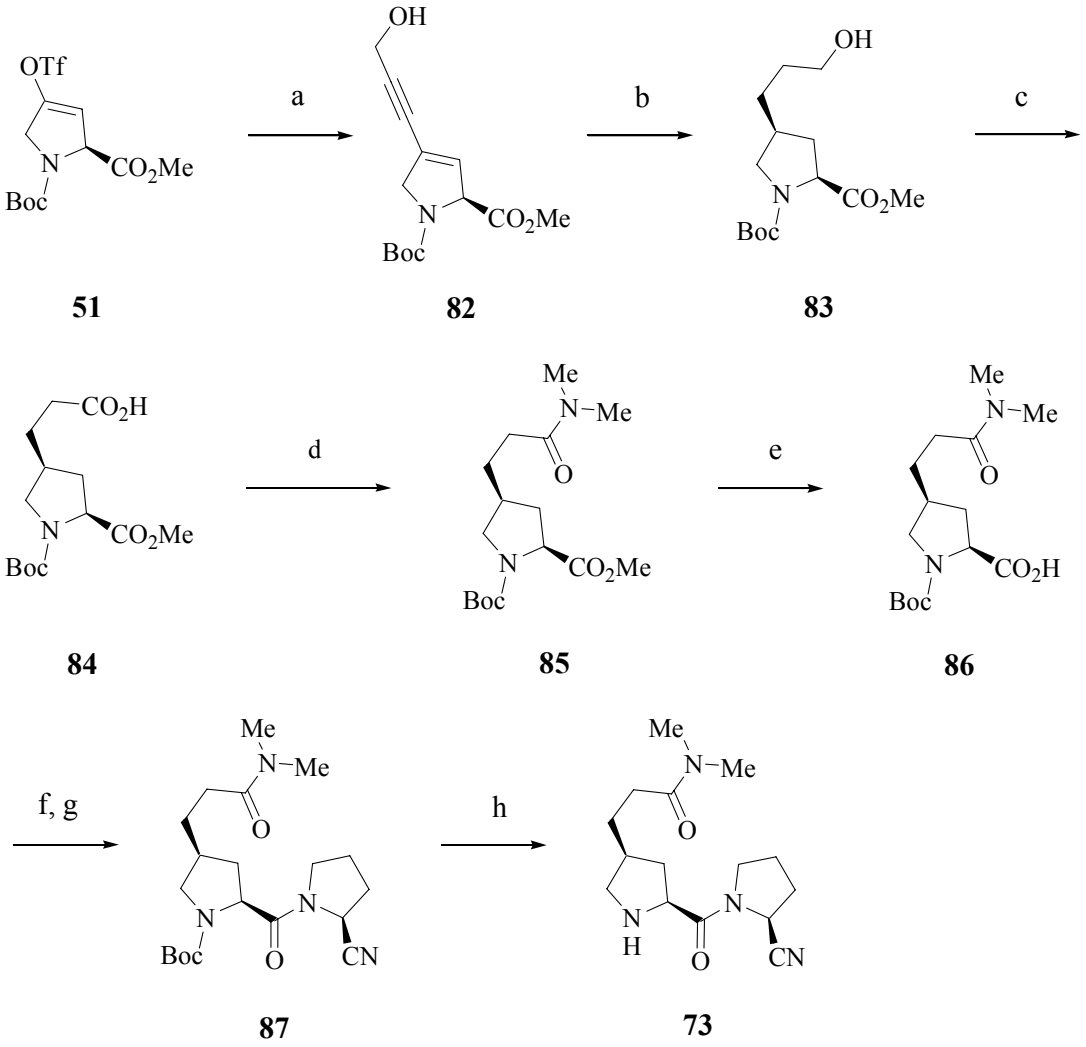
Figure 4-2. メチレン鎖の長さの異なるアミド体

化合物**17**を一炭素減炭した化合物**72**の合成をScheme 4-1に示した。Bridges らの方法[1]に従い、4-ヒドロキシプロリン**74**をトシル化し、シアノ体**76**へと変換した。シアノ体**76**を酸性条件下、エチルエステルへと変換し、再びBoc基で保護した。エチルエステル**77**のベンジルエステルを加水素分解にて脱保護し、カルボン酸**78**を得た。カルボン酸**78**とL-プロリンアミドを、EDCとHOBtを用いて縮合しアミド体**79**を得た。アミド体**79**のエチルエステルを加水分解しカルボン酸**80**を得た。カルボン酸**80**とジメチルアミンを、EDCとHOBtを用いて縮合した後、トリフルオロ酢酸無水物とピリジンを用いて脱水し、ニトリル**81**へと変換した。アミド**81**のBoc基を*p*-トルエンスルホン酸で脱保護し、目的物である化合物**72**を得た。

化合物**17**より一炭素増炭した化合物**73**の合成をScheme 4-2に示した。第三章で合成したトリフラート**51**を用い、2-プロピン1-オールと園頭カップリング反応を行い、プロパルギルアルコール**82**を得た。プロパルギルアルコール**82**を10% パラジウム炭素存在下、水素化しアルコール**83**を得た。アルコール**83**を酸化し、カルボン酸**84**とした後、ジメチルアミンとEDCとHOBtを用いて縮合し、ジメチルアミド**85**を得た。ジメチルアミド**85**を加水分解しカルボン酸**86**を得た。カルボン酸**86**とL-プロリンアミドを、EDCを用いて縮合した後、トリフルオロ酢酸無水物とピリジンを用いて脱水し、アミド**87**を得た。アミド**87**のBoc基を*p*-トルエンスルホン酸で脱保護し、化合物**73**を*p*-トルエンスルホン酸塩として得た。



Scheme 4-1. Synthesis of **72**. Reagents: (a) TsCl, pyridine, CH_2Cl_2; (b) NaCN, DMSO; (c) TMSCl, EtOH; (d) Boc_2O, $NaHCO_3$ aq, THF, H_2O; (e) H_2, 10% Pd/C, MeOH; (f) L-ProNH_2, EDC, HOBt, CH_2Cl_2; (g) NaOH aq, MeOH; (h) Me_2NH, EDC, HOBt, CH_2Cl_2; (i) TFAA, pyridine, THF, CH_2Cl_2; (j) *p*-TsOH, EtOH, .



51 **82** **83** **84** **85** **86** **87** **73**

Scheme 4-2. Synthesis of **73**. Reagents: (a) 2-propyn-1-ol, $PdCl_2(PPh_3)_2$, CuI, $^iPr_2NH$, THF; (b) H_2, 10% Pd-C, MeOH; (c) TEMPO, NaClO, $NaClO_2$, MeCN, sodium phosphate buffer; (d) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (e) NaOH aq, MeOH, THF; (f) L-ProNH_2, EDC, HOBt, Et_3N, CH_2Cl_2; (g) TFAA, pyridine, THF; (h) *p*-TsOH, EtOH.

４-３．アミド誘導体のDPP-IV阻害活性の評価

４-３-１．方法

合成したアミド誘導体**17, 72-73**のヒト精製酵素に対する*in vitro*酵素阻害活性を第二章２-３-１と同様の方法により評価した。また、第二章２-４-１と同様の方法によりSDラットにアミド誘導体**17, 72-73**を1 mg/kg経口投与した際の*ex vivo*酵素阻害活性を評価した。

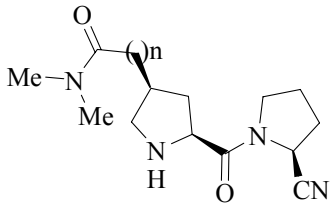
４-３-２．結果と考察

アミド誘導体**17, 72-73**の*in vitro*酵素阻害活性はメチレンの長さに関わらず、高い値を示した（table 4-1)。

ラット1 mg/kg経口投与時の血中DPP-IV阻害活性の持続性に関しては、メチレン鎖の長さにより差が見られた（table 4-1)。すなわち、ジメチルアミノカルボニル基がピロリジン環に直接結合した化合物**72**は投与10時間後においても50%近い阻害率を示し、持続が見られた。メチレン鎖の長さによって持続性に差が見られた理由については後に考察する。

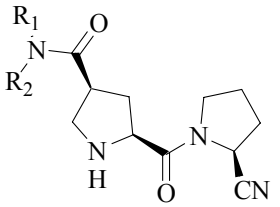
この結果を受け、カルボニル基がピロリジン環に直接結合した骨格を用い、4位のアミド部分の最適化を行うこととした。

Table 4-1. アミド誘導体のDPP-IV阻害活性



compound	n	human DPP-IV IC_{50} (nM)	*ex vivo* DPP-IV inhibition (%) at 1 mg/kg po, normal rats							
			time after administraton (h)							
			0.25	0.5	1	2	4	6	8	10
72	0	1.8	90	97	97	94	86	76	65	52
17	1	4.5	91	95	95	88	63	50	41	16
73	2	2.4	77	85	85	73	74	58	50	21

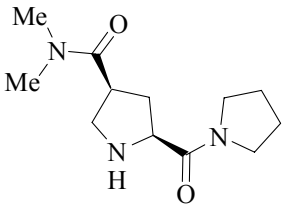
４-４．4位アミド体の最適化合成



88-98

	R_1	R_2
88	H	H
89	H	Me
90	H	Et
91	H	nPr
92	Me	Et
93	Et	Et

	R_1 R_2
94	$-(CH_2)_3-$
95	$-(CH_2)_4-$
96	-(CH2)5-
97	$-(CH_2)_6-$
98	$-(CH_2)_7-$



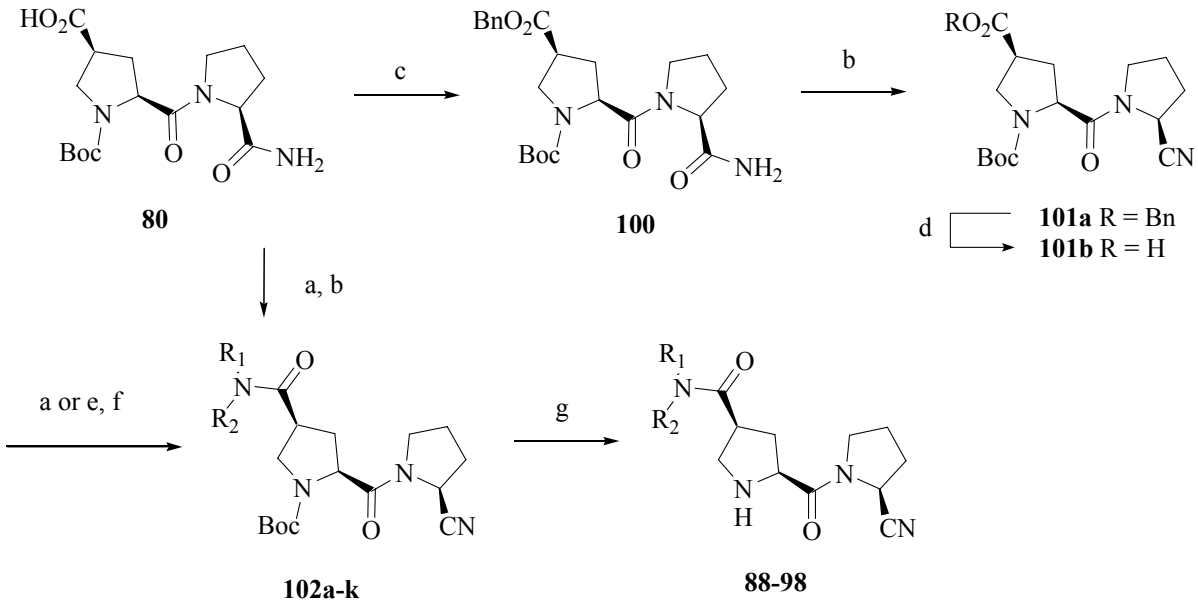
99

Figure 4-3. 4位アミド部の変換とシアノ基の除去

カルボニル基がピロリジン環に直接結合した骨格を用い、4位のアミド部分の最適化を行った。アミノ基の種類としては、無置換アミン、モノアルキルアミン、ジアルキルアミン、環状アミンを選択し、アミノ基の大きさに関して小さいものから一炭素ずつ大きくしていくこととした。また、分子内環化反応の原因構造の一つであるシアノ基を除去した化合物**99**を合成することとした。

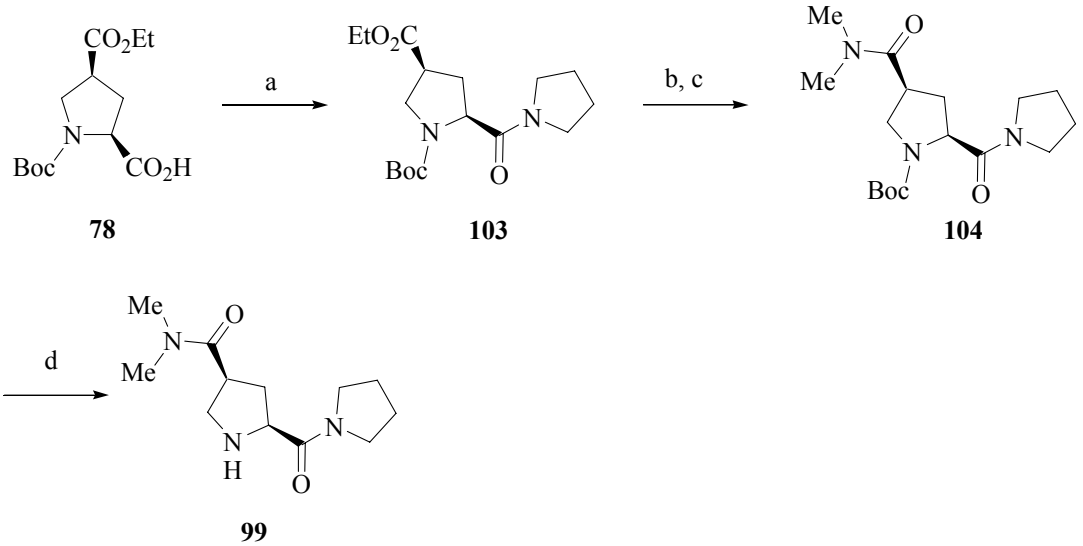
アミド体**88-98**は２通りの方法で合成した（Scheme 4-3）。カルボン酸**80**を炭酸カリウムとベンジルブロミドでベンジルエステル**100**とし、トリフルオロ酢酸無水物とピリジンを用いて脱水し、ニトリル**101a**へと変換した。ニトリル**101a**の水酸化パラジウム存在下ベンジルエステルを脱保護し、カルボン酸**101b**を得た。カルボン酸**101b**を様々なアミンと縮合し、アミド**102a–d**、**102j-k**を得た。または、カルボン酸**80**に対し、様々なアミンと縮合反応と脱水反応を行い、アミド**102e–i**を得た。また、アミド**102a-k**のBoc基を*p*-トルエンスルホン酸で脱保護し、目的物である化合物**88-98**を得た。

シアノ基を除去した化合物**99**の合成をScheme 4-4に示す方法で行った。カルボン酸**78**とピロリジンをEDC、HOBtを用いて縮合し、アミド体**103**を得た。得られたアミド体**103**を加水分解した後、ジメチルアミンと縮合し、ジメチルアミド**104**を得た。ジメチルアミド**104**のBoc基を*p*-トルエンスルホン酸で脱保護し、目的物である化合物**99**を得た。



	R_1	R_2		R_1	R_2
a	H	H	**g**	-$(CH_2)_3$-	
b	H	Me	**h**	-$(CH_2)_4$-	
c	H	Et	**i**	-$(CH_2)_5$-	
d	H	nPr	**j**	-$(CH_2)_6$-	
e	Me	Et	**k**	-$(CH_2)_7$-	
f	Et	Et			

Scheme 4-3. Synthesis of **88-98**. Reagents: (a) R_1R_2NH, EDC, HOBt, CH_2Cl_2; (b) TFAA, pyridine, THF, CH_2Cl_2; (c) BnBr, K_2CO_3, DMF; (d) H_2, $Pd(OH)_2$, EtOAc; (e) $ClCO_2Et$, Et_3N, THF; (f) R_1R_2NH, THF; (g) *p*-TsOH, EtOH.



Scheme 4-4. Synthesis of **99**. Reagents: (a) pyrrolidine, EDC, HOBt, CH_2Cl_2; (b) NaOH aq, MeOH; (c) Me_2NH, NMM, EDC, HOBt, CH_2Cl_2; (d) *p*-TsOH, EtOH.

４-５．4位アミド体のDPP-IV阻害活性の評価

４-５-１．方法

合成した4位アミド体**88-99**のヒト精製酵素に対する*in vitro*酵素阻害活性を第二章２-３-１と同様の方法により評価した。また、第二章２-４-１と同様の方法によりSDラットに4位アミド体**88-98**を1 mg/kg経口投与した際の*ex vivo*酵素阻害活性を評価した。

４-５-２．結果と考察

4位アミド体**88-98**の*in vitro*酵素阻害活性はアミノ基の構造に関わらず、高い値を示した。一方、分子内環化反応の原因構造の一つであるシアノ基を除去した化合物**99**は*in vitro*酵素阻害活性が大幅に減弱した。

ラット1 mg/kg経口投与時の血中DPP-IV阻害活性の持続性に関しては、アミノ基の構造で差が見られた。すなわち、無置換のアミド**88**、モノアルキルアミド**89-91**は、投与直後は高い阻害率を示すものの、10時間後ではジメチルアミド**72**と比較すると低い阻害率を示し、持続性が低かった。

鎖状のジアルキルアミドは、ジメチルアミド**72**、エチルメチルアミド**92**、ジエチルアミド**93**とアルキル鎖が長くなるにつれ、6時間後、10時間後の阻害率が低くなり、持続性が低くなった。

環状アミドについては4-8員環**94-98**を比較すると、6員環**96**が6時間後、10時間後の阻害率が最も高く、大きな環になるにつれ、6時間後の阻害率が低くなり、持続性が低くなった。

以上の結果から、*in vitro*酵素阻害活性はアミド部分を嵩高くしても高い値を示したが、アミド部分を嵩高くすると持続性が低くなることがわかり、この位置を嵩高くすることにより分子内環化反応に対する安定性を高める展開は不可能であると考えられた。

そこで、新たな合成方針としてピロリジン環の5位に置換基を導入することとした。

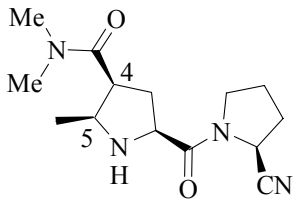
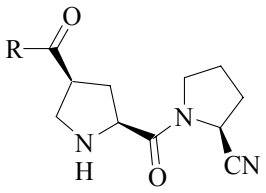
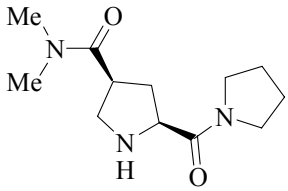


Figure 4-4. 4位アミド誘導体の最適化の新たな合成方針

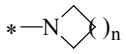
Table 4-2. 4位アミド体のDPP-IV阻害活性



88-98

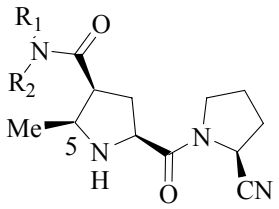


99

compound	R	human DPP-IV IC_{50} (nM)	*ex vivo* DPP-IV inhibition (%) at 1 mg/kg po, normal rats							
			time after administraton (h)							
			0.25	0.5	1	2	4	6	8	10
88	NH_2	7.7	91	96	96	91	73	47	40	20
89	NHMe	3.5	96	96	96	95	89	75	56	28
90	NHEt	4.4	96	95	95	91	80	60	38	19
91	NH^nPr	5.7	92	95	94	87	77	59	44	21
72	NMe_2	1.8	90	97	97	94	86	76	65	52
92	NMeEt	4.9	93	95	94	89	80	71	53	34
93	NEt_2	6.0	86	94	91	75	57	43	NT*	NT*
										
94	n = 1	4.0	83	92	94	91	82	75	63	46
95	n = 2	5.6	95	96	97	92	86	75	63	40
96	n = 3	4.9	96	97	97	92	82	74	65	50
97	n = 4	8.3	93	94	92	82	73	68	NT*	NT*
98	n = 5	11	92	93	86	77	55	63	NT*	NT*
99		670	NT*	NT*	NT*	NT*	NT*	NT*	NT*	NT*

*NT : not tested

４-６．5位への置換基導入



105-112

	R_1	R_2
105	Me	Me
106	Me	Et
107	-$(CH_2)_3$-	
108	-$(CH_2)_4$-	
109	-$(CH_2)_5$-	

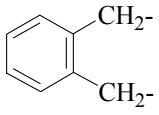
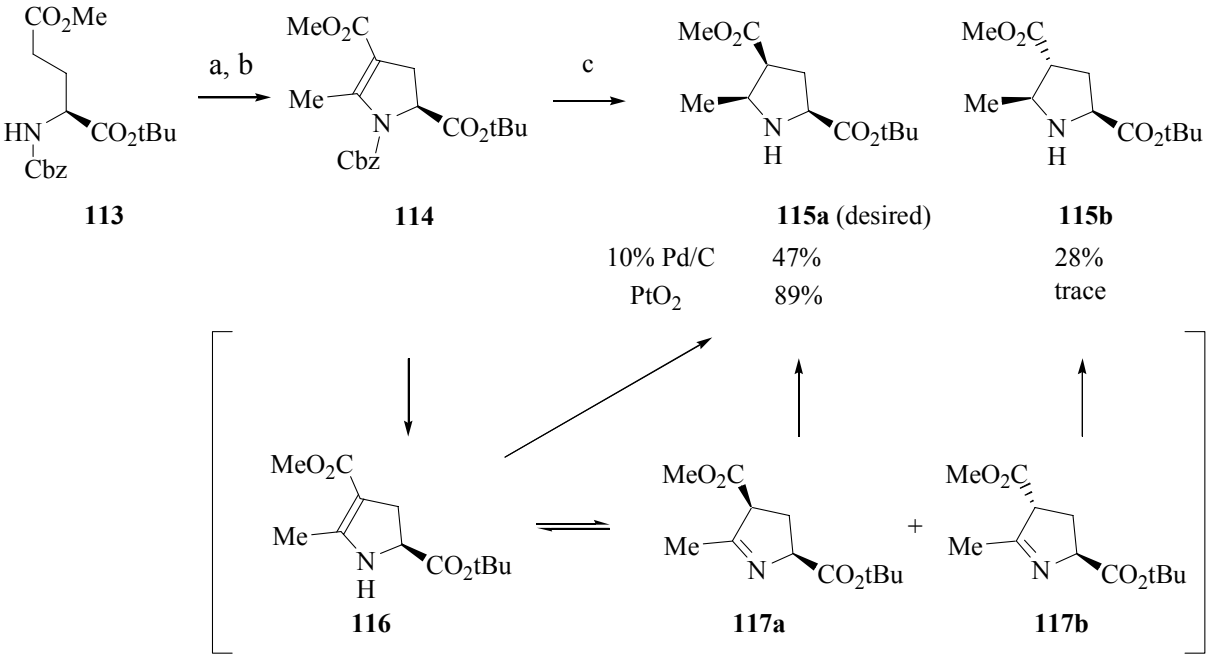
	R_1 R_2
110	-$CH_2CH_2OCH_2CH_2$-
111	-$CH_2CH_2SCH_2CH_2$-
112	

Figure 4-5. 5位メチル体

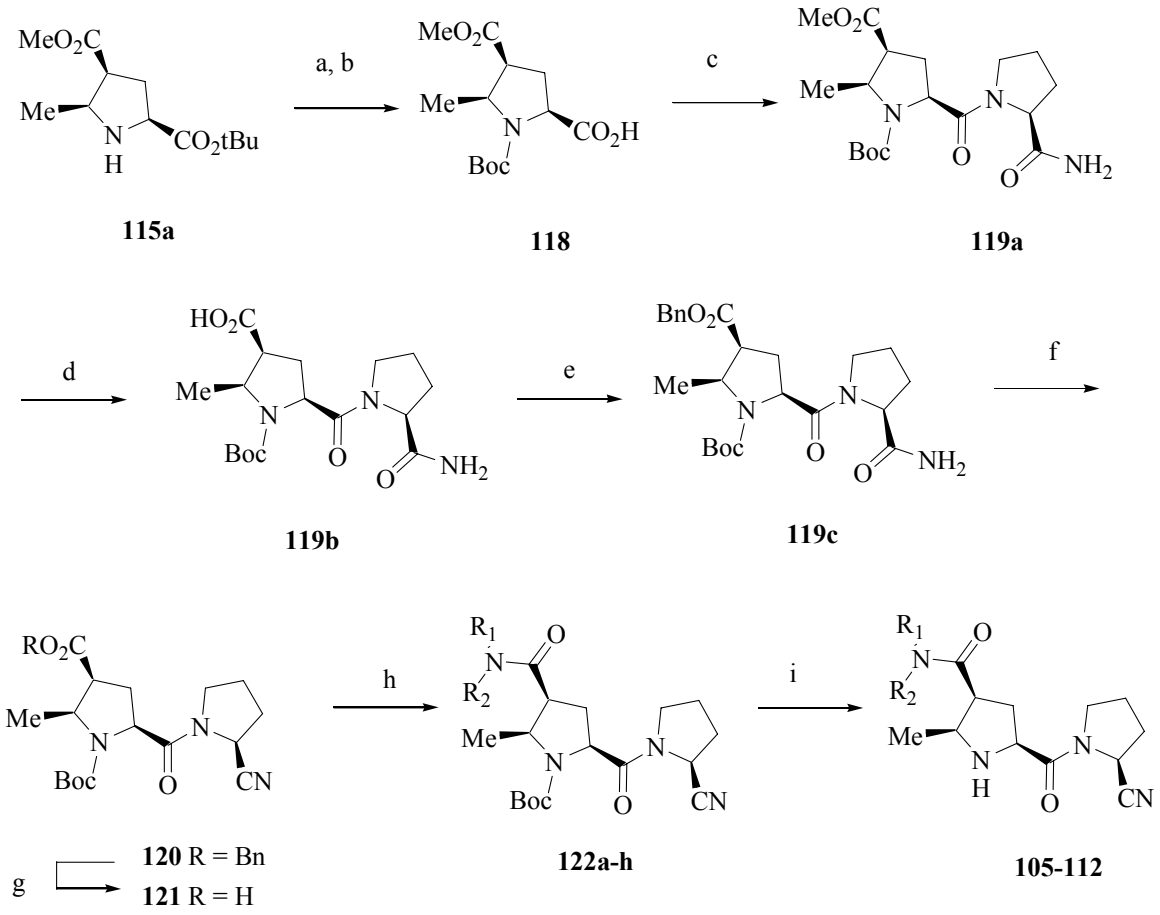
化合物**72**のピロリジン環の5位へ*cis*配置で置換基を導入すれば、置換基による立体障害のため分子内環化反応に対する安定性が高まると考えられる。そこで、ピロリジン環の5位にメチル基を導入することとした。アミド部分は鎖状または環状の2級アミンを導入することとした。

これまでに2、4、5位に*cis*配置で置換基を持つピロリジン環の合成法はあまり知られておらず、新しい合成法が必要であった[2]。Boscoらは保護されたグルタミン酸**113**のγ位をアシル化し、環化することによりジヒドロピロール誘導体**114**が得られることを報告している[3]。この化合物**114**を接触水素化すれば、2位の*tert*-ブトキシカルボニル基の立体障害により、水素化は*tert*-ブトキシカルボニル基と*trans*で進行し、目的とする2,4,5-*cis*置換体**115a**が得られると考えた（Scheme 4-5）。そこで化合物**114**を10%パラジウム炭素存在下、水素化反応を行ったところ、目的物である2*S*,4*S*,5*S*体**115a**と立体異性体の2*S*,4*R*,5*S*体**115b**が約３：２の比率で得られた。4位の立体化学が制御できなかった理由を以下のように考察している。すなわち、化合物**114**を10%パラジウム炭素存在下、水素化反応を行うと、まずCbz基の脱保護が進行する。生成したエナミン中間体**116**はイミン体と平衡にあると考えられる。イミン体は4位の立体化学に関して４*S*体**117a**と４*R*体**117b**の2種の異性体が存在し、エナミン中間体**116**および４*S*体**117a**からは目的物の2*S*,4*S*,5*S*体**115a**が生成し、４*R*体**117b**からは2*S*,4*R*,5*S*体**115b**が生成したと考えられる。一方、10%パラジウム炭素のかわりに酸化白金（IV）を用いると、目的物の2*S*,4*S*,5*S*体**115a**がより優先して生成することがわかった。この理由の詳細は不明であるが、Cbzの脱保護の前に水素化が進行する反応経路が存在するか、イミン体とエナミン体の水素化の反応速度がパラジウムと白金で異なる等の理由が考えられる。

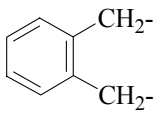


Scheme 4-5 Reagents: (a) AcCl, LiHMDS, THF; (b) AcOH; (c) H_2, 10% Pd/C or PtO_2, AcOH

このようにして得られた化合物**115a**を用い、Scheme 4-6に示す方法で5位メチル体を合成した。化合物**115a**の*tert*-ブチルエステルを、トリフルオロ酢酸を用いて脱保護し、アミノ基をBoc基で保護した。得られたカルボン酸**118**とL-プロリンアミドをEDCとHOB t を用いて縮合しアミド体**119a**を得た。アミド体**119a**のメチルエステルを、水酸化リチウムを用いて加水分解し、カルボン酸**119b**を得た。アミド体**119a**の加水分解の際に一部4位が異性化したため、一旦ベンジルエーテルとして保護し、アミドを脱水し、ニトリル**120**とし、シリカゲルカラムクロマトグラフィーにより精製し、4位の異性体を分離した。ニトリル**120**のベンジルエーテルを水酸化パラジウムの存在下、加水素分解により脱保護しカルボン酸**121**を得た。得られたカルボン酸**121**と様々なアミンをEDCとHOBtを用いて縮合しアミド体**122a-h**を得た。アミド体**122a-h**のBoc基を*p*-トルエンスルホン酸を用いて脱保護し、5位メチル体**105-112**を*p*-トルエンスルホン酸塩として得た。



	R_1	R_2
a	Me	Me
b	Me	Et
c	$-(CH_2)_3-$	
d	$-(CH_2)_4-$	
e	$-(CH_2)_5-$	

	R_1 R_2
f	$-CH_2CH_2OCH_2CH_2-$
g	$-CH_2CH_2SCH_2CH_2-$
h	

Scheme 4-6 Synthesis of **105-112**. Reagents: (a) TFA aq; (b) Boc_2O, $NaHCO_3$ aq, THF; (c) L-ProNH_2, EDC, HOBt, Et_3N, CH_2Cl_2; (d) LiOH aq, MeOH; (e) BnBr, K_2CO_3, DMF; (f) TFAA, pyridine, THF; (g) H_2, $Pd(OH)_2$, EtOAc; (h) R_1R_2NH, EDC, HOBt, NMM, CH_2Cl_2; (i) *p*-TsOH, EtOH.

４-７．5位メチル体のDPP-IV阻害活性、分子内環化反応に対する安定性の評価

４-７-１．方法

合成した5位メチル体**105-112**のヒト精製酵素に対する*in vitro*酵素阻害活性を第二章２-３-１と同様の方法により評価した。また、第二章２-４-１と同様の方法によりSDラットに4位アミド体**105-112**を1 mg/kg経口投与した際の*ex vivo*酵素阻害活性を評価した。

合成した5位メチル体**105-112**の分子内環化反応に対する安定性を、２-５-１と同様の方法により評価した。

４-７-２．結果と考察

5位メチル体**105**の*in vitro*酵素阻害活性は5位無置換体**72**と比較し、わずかながら減弱が見られた。後述するが、5位メチル体はslow bindingという阻害様式を有しており、酵素とプレインキュベーションした後に酵素阻害活性を測定すると5位無置換体と同等の*in vitro*酵素阻害活性を示すことがわかった。

ラット1 mg/kg経口投与時の血中DPP-IV阻害活性の持続性に関しては、5位にメチル基を導入することで著しい向上が見られた（table 4-3）。5位無置換体**72**が投与10時間後において52%の阻害率を示すのに対し、5位メチル体**105**では投与10時間後において84%の阻害率を示した。5位メチル骨格でアミド部分を変換した化合物**106-112**は約80%の阻害率を示し、5位メチル骨格では一様に持続性が高くなることがわかった。

一方、5位にメチル基を導入することで、分子内環化反応に対する安定性においても、著しい改善が見られた（table 4-4）。すなわち、5位無置換体**72**が半減期28時間を示したのに対し、5位メチル体**105**では24時間後においても92%残存しており、半減期約200時間に相当する安定性を示した。予期した通り、5位のメチル基が立体障害により分子内環化反応を抑制したと考えられる。

5位メチル体**105**は持続性が高く、分子内環化反応に対する安定性も高い化合物であったが、4位置換基、5位置換基、立体化学、P1部など最適化されているか、さらに合成展開を行い検討することとした。

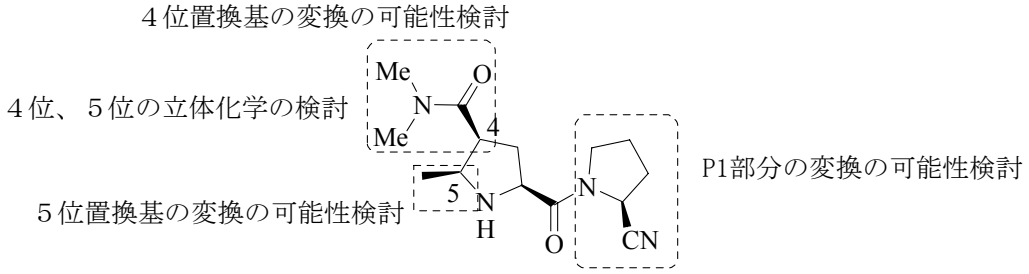
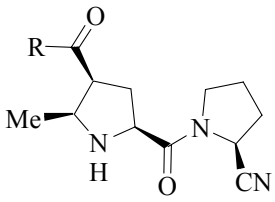


Figure 4-6. 5位メチル体の最適化の可能性検討

Table 4-3. 5位メチル体のDPP-IV阻害活性



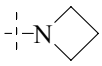
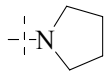
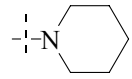
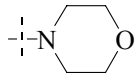
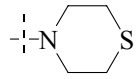
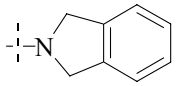
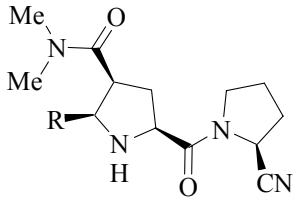
compound	R	human DPP-IV IC_{50} (nM)	*ex vivo* DPP-IV inhibition (%) at 1 mg/kg po, normal rats									
			time after administraton (h)									
			0.25	0.5	1	2	4	6	8	10	12	24
72		1.8	90	97	97	94	86	76	65	52	NT	NT
105	NMe_2	10	95	96	97	95	94	92	89	84	81	43
106	NMeEt	15	97	97	97	93	93	92	90	79	74	-5
107		13	95	97	97	96	95	95	93	88	NT	NT
108		6.5	96	97	96	95	93	94	92	88	82	13
109		13	98	98	97	95	94	91	86	81	NT	NT
110		20	94	96	96	95	95	94	91	85	NT	NT
111		6.2	96	97	97	95	94	92	94	89	85	30
112		5.6	98	98	97	92	90	86	81	77	71	13

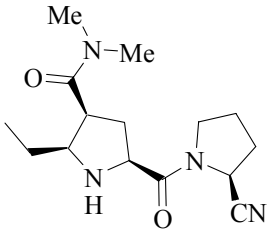
Table 4-4. 5位メチル体の分子内環化反応に対する安定性



compound	R	solution stability (pH 7.4) $t_{1/2}$ (h)
72	H	28
105	Me	92%* (24 h)

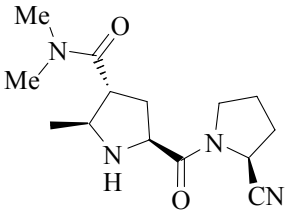
*remaining percentage

４-８．5位メチル体**105**の周辺化合物の合成

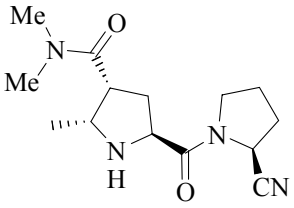


123

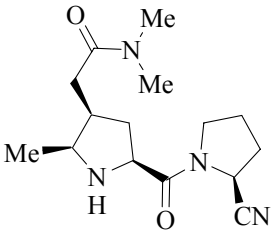
５位置換基の変換の可能性検討



124

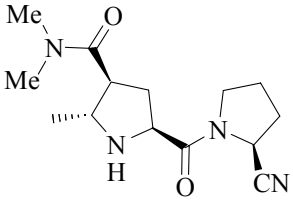


126



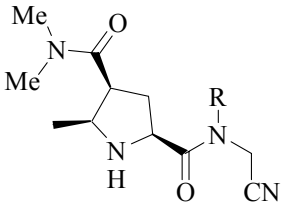
127

４位置換基の変換の可能性検討



125

４位、５位の立体化学の検討



128 : R = H
129 : R = Me
130 : R = Et
131 : R = *n*Pr

132 : R = allyl
133 : R = cyclopropyl
134 : R = propargyl

P1部分の変換の可能性検討

Figure 4-7. 5位メチル体

5位メチル体**105**が最適な構造であるか確認するために化合物**123-134**を合成することとした。すなわち、分子内環化反応に対する安定化に寄与しうる5位の置換基をさらに嵩高いものにできるか検討するため、エチル基に変換した**123**を合成することとした。また、4位、5位の立体化学が最適であることを確認するため、立体異性体**124-126**を合成することとした。また、4位の置換基を変換可能か検討するため、メチレン鎖を伸ばした**127**を合成することとした。また、不斉点を減らす可能性があるか検討するため**128-134**を合成することとした。

5位エチル体**123**は5位メチル体**105**と同様の方法にて合成した（Scheme 4-7）。

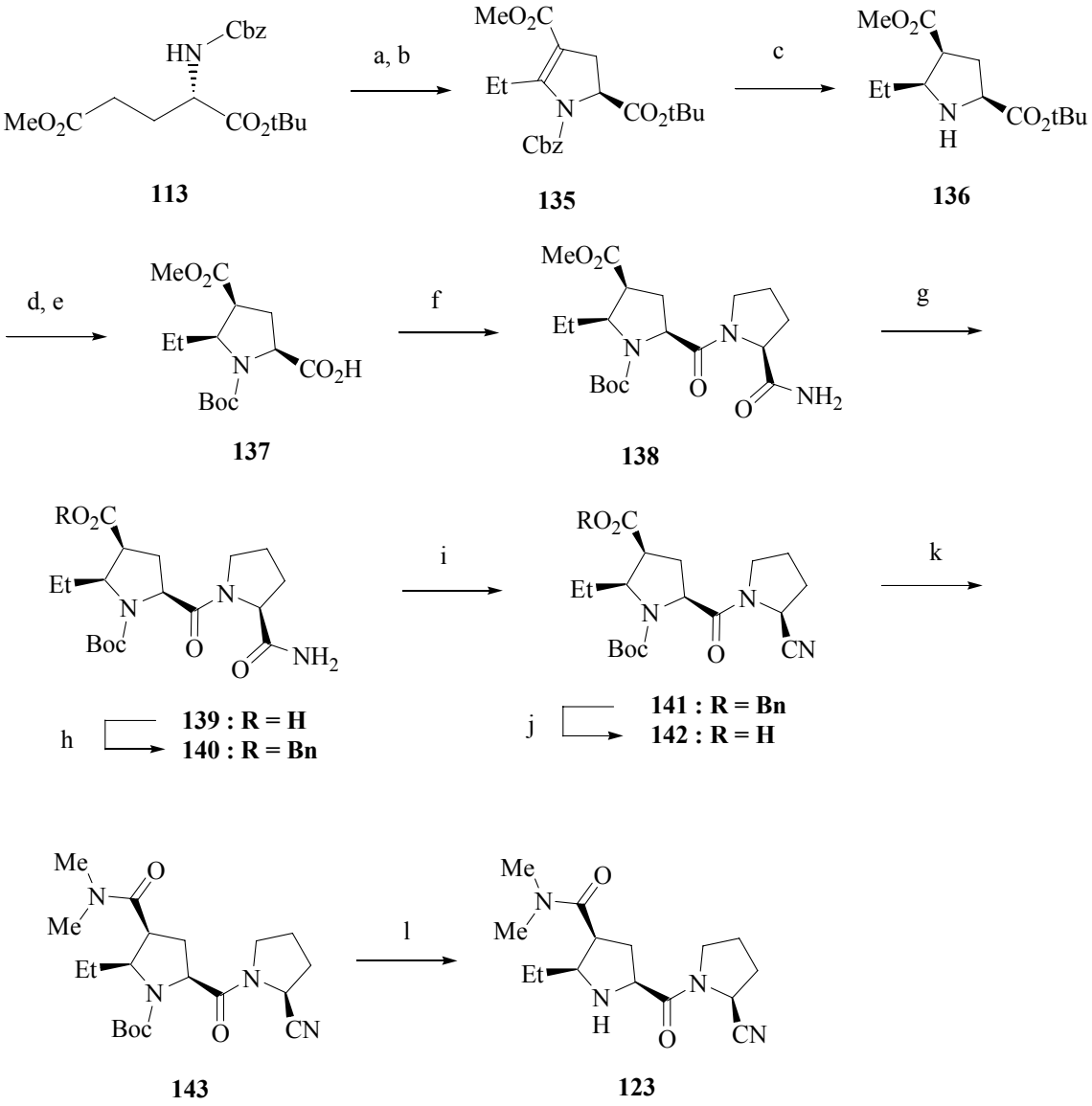
4位、5位の立体異性体の合成をScheme 4-8、4-9、4-10、に示した。4*R*, 5*S*体**124**は化合物**105**を合成した際に得られた立体異性体**115b**を用い、化合物**105**と同様の方法にて

合成した（Scheme 4-8）。4*S*, 5*R*体**125**、および4*R*, 5*R*体**126**の合成はPedregalらの方法[4]に従い、ピログルタミン酸エチル**24**のアルキル化により得られる**149a**、**149b**を用いて行った（Scheme 4-9、4-10）。すなわち、ピログルタミン酸エチル**24**をリチウムヘキサメチルジシラジドによりリチウムエノラートとし、ベンジルオキシメチルクロリドでアルキル化し、4*R*体**149a**、4*S*体**149b**を得た。5位へのメチル基の導入はColladoの方法[5]に基づいて行った。すなわち、4*R*体**149a**、を水素化トリエチルホウ素リチウムで還元し、酸性条件下メタノールを作用し、5-メトキシ体**150**を得た。5-メトキシ体**150**をメチルグリニヤール試薬によりメチル基を導入し、5*R*メチル体**151**を得た。この反応中に一部Boc基が脱保護されるため再びBoc基で保護した。5*R*メチル体**151**のベンジルエーテルを10%パラジウム炭素存在下、加水素分解しアルコール**152**を得た。アルコール**152**を酸化しカルボン酸**153**とした後、ジメチルアミンとEDCとHOBtを用いて縮合し、ジメチルアミド**154**を得た。ジメチルアミド**154**を加水分解しカルボン酸**155**を得た。カルボン酸**155**と(2*S*)-2-シアノピロリジンをEDCとHOBtを用いて縮合し、アミド体**156**を得た。アミド体**156**のBoc基を*p*-トルエンスルホン酸を用いて脱保護し、4*S*, 5*R*体**125**を*p*-トルエンスルホン酸塩として得た。4*R*, 5*R*体**126**の合成は4*S*, 5*R*体**125**と同様の方法にて行った。

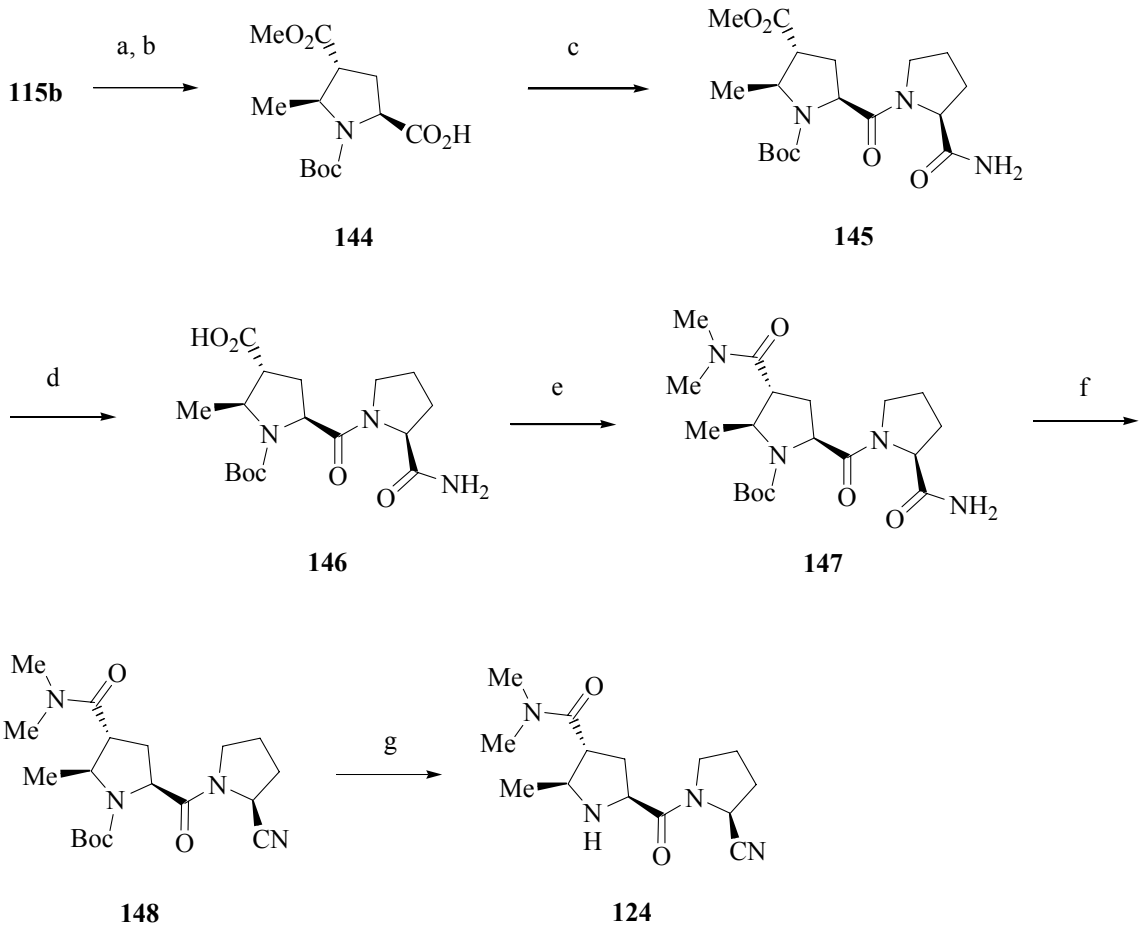
4位酢酸体**127**は5位メチル体**105**の合成中間体**119b**を用いて行った（Scheme 4-11）。化合物**119b**を、クロロギ酸エチルを用いて混合酸無水物とした後、ジアゾメタンと反応し、ジアゾケトン**164**を得た。ジアゾケトン**164**を安息香酸銀によりメチルエステル**165**へと変換した。メチルエステル**165**を加水分解しカルボン酸**166**を得た。カルボン酸**166**とジメチルアミンを、EDCを用いて縮合した後、トリフルオロ酢酸無水物とピリジンを用いて脱水し、ニトリル**168**を得た。ニトリル**168**のBoc基を*p*-トルエンスルホン酸で脱保護し、4位酢酸体**127**を*p*-トルエンスルホン酸塩として得た。

P1部の変換は化合物**105**の合成法を改良して行った（Scheme 4-12）。すなわち、Boc基で保護されたグルタミン酸エステル**169**を、リチウムヘキサメチルジシラジドを用いリチウムエノラートとし、無水酢酸によりγ位をアセチル化し、酸性条件下、環化し、ジヒドロピロール**170**を得た。ジヒドロピロール**170**のベンジルエステルを加水素分解により脱保護し、カルボン酸**171**を得た。カルボン酸**166**とジメチルアミンを、EDCを用いて縮合し、ジメチルアミド**172**を得た。ジメチルアミド**172**の二重結合を酸化白金により水素化し、2*S*, 4*S*, 5*S*体**173a**を得た。**173a**には2*S*, 4*R*, 5*R*体**173b**が含まれていたが（173a:173b = 約 8:1）、**173a**と**173b**の混合物を加水分解した後、再結晶により純粋なカルボン酸**174**を得た。カルボン酸**174**の酸塩化物を合成するためにBoc基をCbz基に付け替えた後、酸塩化物とし、*N*-メチルグリシンアミドと縮合し、アミド体**176**を得た。アミド体**176**のCbz基をBoc基に付け替えた後、トリフルオロ酢酸無水物とピリジンを用いて脱水し、ニトリル**178b**を得た。また、Carpinoらの方法[6]に従いカルボン酸**174**をフッ化シアヌルにより酸フッ化物とし、様々な*N*-アルキルアミノアセトニトリルと縮合しニトリル**178a**, **178c-g**を得た。この方法では保護基の付け替えは必要なかった。ニトリル**178a-g**のBoc基を*p*-トルエンスルホン酸で脱保護し、*N*-アルキルアミノア

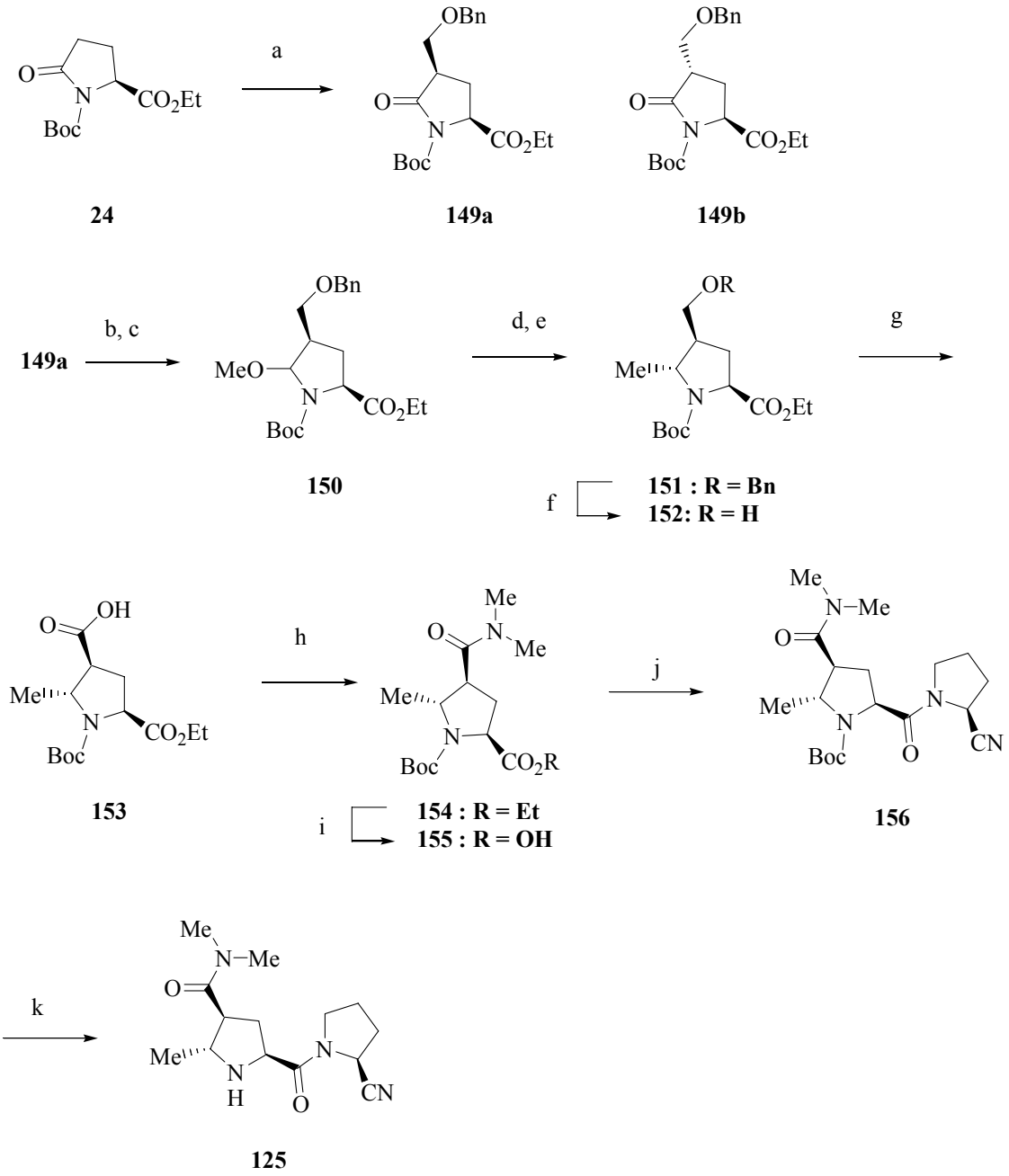
セトニトリル体**128-134**を*p*-トルエンスルホン酸塩として得た。



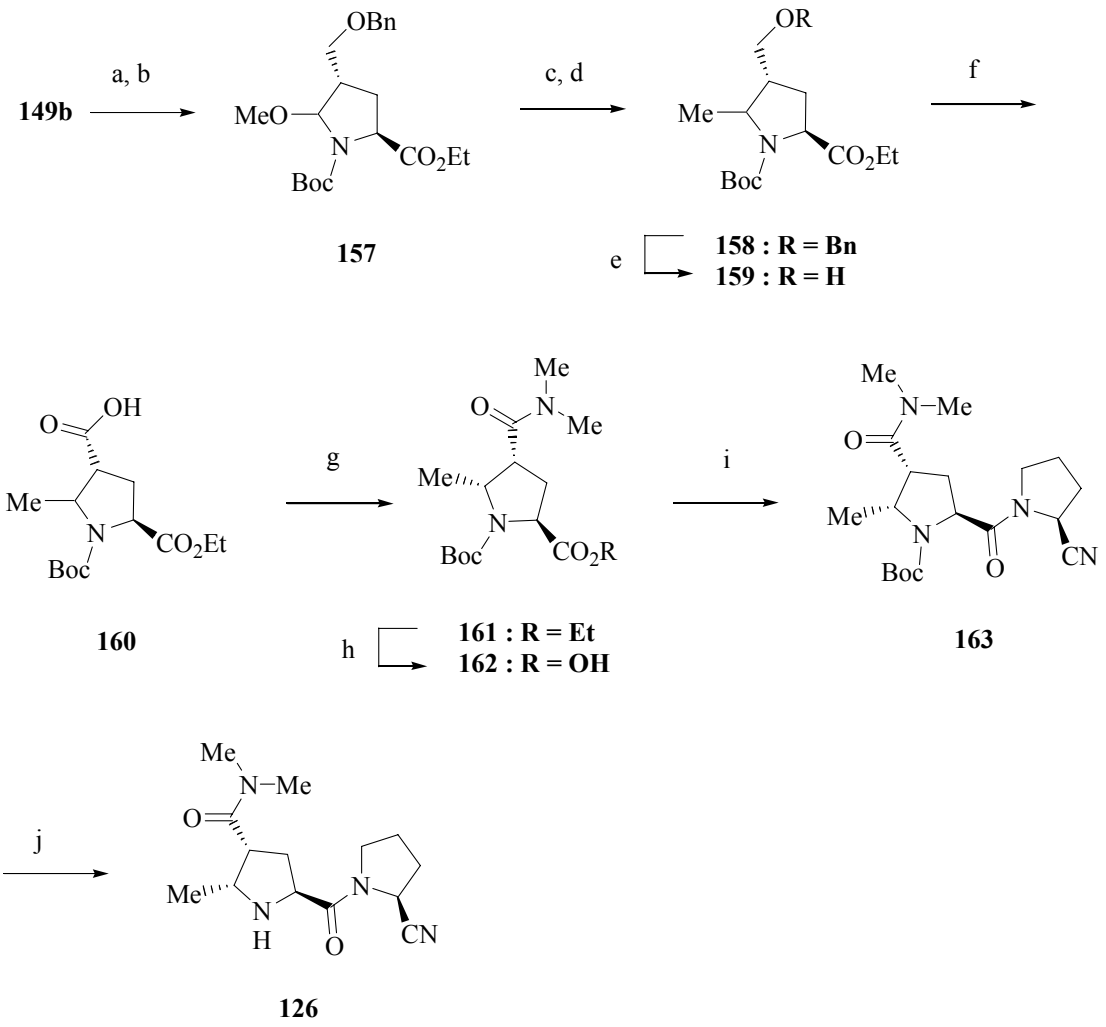
Scheme 4-7. Synthesis of **123**. Reagents: (a) EtCOCl, LiHMDS, THF; (b) TFA, CH_2Cl_2; (c) H_2, PtO_2, AcOH; (d) TFA aq; (e) Boc_2O, $NaHCO_3$ aq, THF; (f) L-ProNH_2, EDC, HOBt, Et_3N, CH_2Cl_2; (g) LiOH aq, MeOH; (h) BnBr, K_2CO_3, DMF; (i) TFAA, pyridine, THF; (j) H_2, $Pd(OH)_2$, EtOAc, THF; (k) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (l) *p*-TsOH, EtOH.



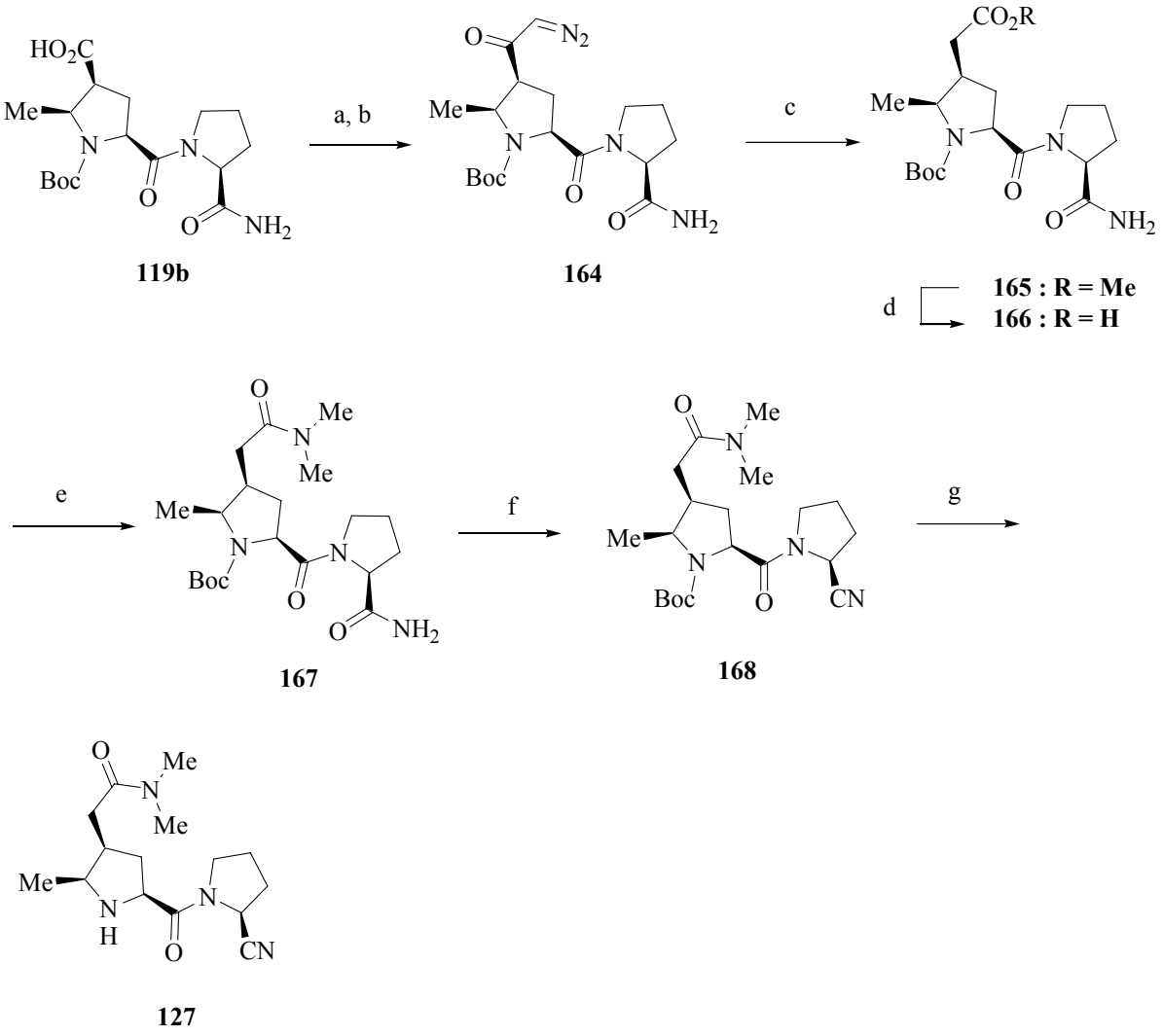
Scheme 4-8. Synthesis of **124**. Reagents: (a) TFA aq; (b) Boc_2O, $NaHCO_3$ aq, THF; (c) L-ProNH_2, EDC, HOBt, Et_3N, CH_2Cl_2; (d) NaOH aq, MeOH; (e) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (f) TFAA, pyridine, THF; (g) 4N HCl/EtOAc.



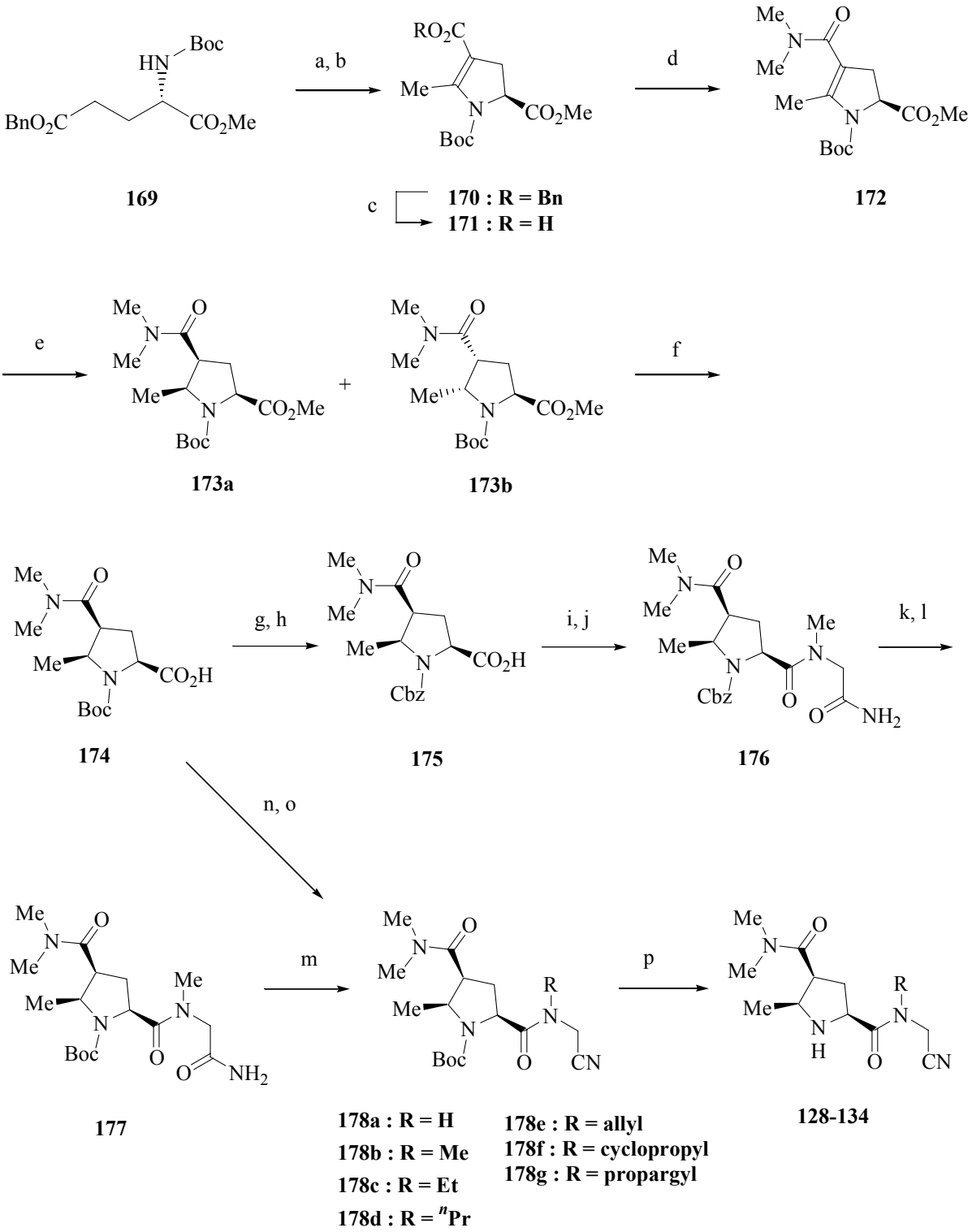
Scheme 4-9. Synthesis of **125**. Reagents: (a) LiHMDS, $BnOCH_2Cl$, HMPA, THF; (b) $LiBEt_3H$, THF; (c) *p*-TsOH, MeOH; (d) MeMgBr, CuBr-Me_2S, BF_3-OEt_2, Et_2O; (e) Boc_2O, $NaHCO_3$ aq, THF; (f) H_2, 10% Pd/C, EtOH; (g) Jones reagent, Acetone; (h) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (i) NaOH aq, MeOH; (j) (2*S*)-2-cyanopyrrolidine, EDC, HOBt, Et_3N, CH_2Cl_2; (k) *p*-TsOH, EtOH.



Scheme 4-10. Synthesis of **126**. Reagents: (a) $LiBEt_3H$, THF; (b) *p*-TsOH, MeOH; (c) MeMgBr, CuBr-Me_2S, BF_3-OEt_2, Et_2O; (d) Boc_2O, $NaHCO_3$ aq, THF; (e) H_2, 10% Pd/C, EtOH; (f) Jones reagent, Acetone; (g) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (h) NaOH aq, MeOH; (i) (2*S*)-2-cyanopyrrolidine, EDC, HOBt, Et_3N, CH_2Cl_2; (j) *p*-TsOH, EtOH.



Scheme 4-11. Synthesis of **127** Reagents: (a) $ClCO_2Et$, Et_3N, THF; (b) CH_2N_2, Et_2O, THF; (c) BzOAg, Et_3N, MeOH; (d) NaOH aq, MeOH; (e) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (f) TFAA, pyridine, THF; (g) *p*-TsOH, EtOH.



Scheme 4-12. Synthesis of **128-134**. Reagents: (a) Ac_2O, LiHMDS, THF; (b) TFA, CH_2Cl_2; (c) H_2, 10% Pd/C, MeOH; (d) Me_2NH, EDC, HOBt, NMM, CH_2Cl_2; (e) H_2, PtO_2, AcOH; (f) NaOH aq, THF; (g) 4N HCl/1,4-dioxane, CH_2Cl_2; (h) CbzCl, $NaHCO_3$ aq, THF; (i) $(COCl)_2$, DMF, CH_2Cl_2; (j) $MeNHCH_2CONH_2$, Et_3N, CH_2Cl_2; (k) H_2, Pd/C, THF; (l) Boc_2O, THF; (m) TFAA, pyridine, CH_2Cl_2 ; (n) Cyanuric fluoride, pyridine, CH_2Cl_2; (o) $RNHCH_2CN$, pyridine, $ClCH_2CH_2Cl$; (p) *p*-TsOH, EtOH.

４-９．5位メチル体**105**の周辺化合物のDPP-IV阻害活性の評価

４-９-１．方法

合成した5位メチル体**105**の周辺化合物**123-134**のヒト精製酵素に対する*in vitro*酵素阻害活性を第二章２-３-１と同様の方法により評価した。また、第二章２-４-１と同様の方法によりSDラットに化合物**123-134**を0.3 mg/kg経口投与した際の*ex vivo*酵素阻害活性を評価した。

４-９-２．結果と考察

化合物**105**の5位置換基をメチル基からエチル基に変換した**123**はメチル体**105**と同等の*in vitro*酵素阻害活性を示した。しかしながら、ラット*ex vivo*酵素阻害活性はメチル体**105**と比較し弱く、5位の置換基のこれ以上の変換は持続性を損なうと考えられた（table 4-5）。

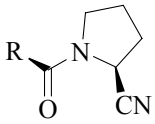
化合物**105**の３種の立体異性体は、いずれも化合物**105**と比較し、*in vitro*酵素阻害活性が減弱し、高い酵素阻害活性の発現には2*S*, 4*S*, 5*S*という立体化学が重要であることがわかった（table 4-5）。

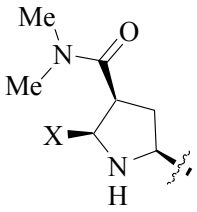
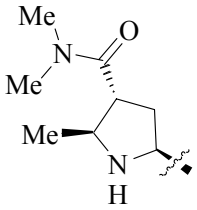
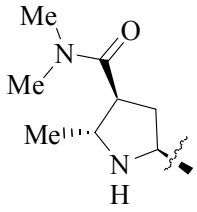
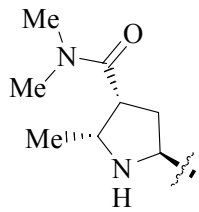
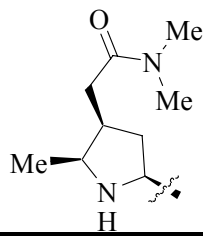
4位のジメチルアミノカルボニル基のメチレン鎖を一つ伸ばした酢酸体**127**は高い*in vitro*酵素阻害活性を示した。しかしながら、ラット*ex vivo*酵素阻害活性は化合物**105**と比較し弱く、持続性の発現には4位にカルボニル基が直接結合した構造が重要であることがわかった（table 4-5）。

不斉点を減らす目的で合成したP1部の*N*-アルキルアセトニトリル体のうち、NH体の**128**は*in vitro*酵素阻害活性が大幅に減弱した。メチル体**129**、エチル体**130**、アリル体**132**、シクロプロピル体**133**、プロパルギル体**134**は、化合物**105**とほぼ同等の*in vitro*酵素阻害活性を示した。しかしながら、プロピル体**131**は*in vitro*酵素阻害活性が大幅に減弱した。理由は不明であるが、この構造活性相関はMagninらの報告している*N*-アルキルアセトニトリル体の構造活性相関と一致する[7]。ラット*ex vivo*酵素阻害活性はアルキル基によって差が見られた。すなわち、エチル体**130**は9時間後においても59%の阻害率を示したのに対し、メチル体**129**、アリル体**132**、シクロプロピル体**133**、プロパルギル体**134**では14%から33％の阻害率で、持続性を示さなかった。最も阻害率の高いエチル体**130**においても、化合物**105**には及ばず、化合物**105**の持続性にはP1部の構造も重要であると考えられる（table 4-6）。

以上の検討により、化合物**105**は持続性、分子内環化反応に対する安定性の両面から最適化された化合物であると結論した（Figure 4-8）。

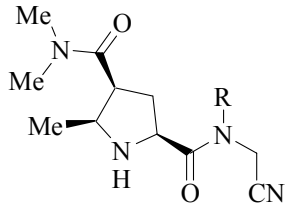
Table 4-5. 5位置換体のDPP-IV阻害活性



compound	R	human DPP-IV IC_{50} (nM)	*ex vivo* DPP-IV inhibition (%) at 1 mg/kg po, normal rats				
			time after administraton (h)				
			0.25	0.5	1	3	6
							
105	X = Me	10	95	97	97	96	95
123	X = Et	16	71	84	84	75	58
124		1100	NT*	NT*	NT*	NT*	NT*
125		70	NT*	NT*	NT*	NT*	NT*
126		110	NT*	NT*	NT*	NT*	NT*
127		5.6	83	87	83	NT*	40

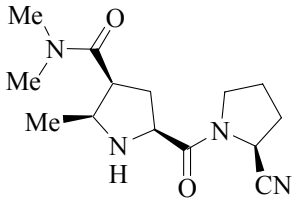
*NT : not tested.

Table 4-6. *N*-アルキルアセトニトリル体のDPP-IV阻害活性



compound	R	human DPP-IV IC_{50} (nM)	*ex vivo* DPP-IV inhibition (%) at 0.3 mg/kg po, normal rats					
			time after administraton (h)					
			0.25	0.5	1	3	6	9
105		10	90	96	96	94	89	84
128	H	13000	NT*	NT*	NT*	NT*	NT*	NT*
129	Me	25	64	77	80	67	55	33
130	Et	24	79	85	90	82	71	59
131	^{n}Pr	600	NT*	NT*	NT*	NT*	NT*	NT*
132	allyl	41	49	58	57	35	19	14
133	cyclopropyl	56	39	53	59	46	29	22
134	propargyl	60	54	64	62	31	18	17

*NT : not tested.



105

Figure 4-8. アミド誘導体の最適化構造

４-１０．アミド誘導体のDPP-IV類縁酵素に対する選択性の評価

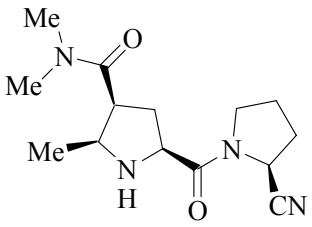
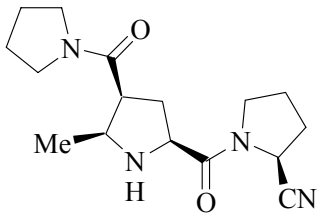
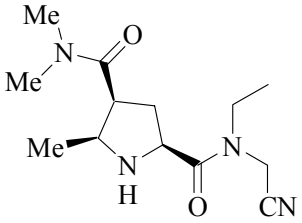
４-１０-１．方法

文献記載の方法にて調製したDPP-VIII、DPP-IXを用いて阻害活性を評価した[8,9]。酵素阻害活性は、合成基質であるH-Gly-Pro-AMCのDPP-VIII、DPP-IXによる分解を阻害剤がどれだけ抑制するかを、7-amino-4-methyl coumarin（AMC）の生成速度を測定することにより求めた。 評価は複数の化合物濃度において行い、50%抑制する濃度をIC_{50}値として求めた。

４-１０-２．結果と考察

P2部分の4位のアミド部分がジメチルアミノカルボニル基である化合物**105**、ピロリジニルカルボニル基である化合物**108**、P1部分が*N*-エチルアセトニトリル構造である化合物**130**のDPP-VIII、DPP-IXの阻害活性を評価した（table 4-7）。DPP-VIII、DPP-IXの選択的阻害剤がイヌにおいて消化器系の毒性を示すことが報告されており、安全性の面から回避すべき阻害活性とされている[10]。化合物**105**はDPP-VIII、DPP-IXに対し、1300倍、250倍と十分に乖離が見られている。4位アミド部分が立体的に嵩高く、疎水性の高くなった化合物**108**はDPP-VIII、DPP-IXに対し、750倍、110倍と乖離が小さくなる傾向が見られた。また、P1部分が*N*-エチルアセトニトリル構造である化合物**130**はDPP-VIII、DPP-IXに対し、4100倍以上、1300倍と乖離が大きくなる傾向が見られた。DPP-VIIIやDPP-IXのタンパク質3次元構造は未だ明らかにされていないが、P1部分を認識するS1ポケットの構造、P2部分を認識するS2ポケットの構造の違いを示唆する報告がある[11, 12]。阻害剤の構造のわずかな違いによる選択性の変化は、S1ポケット、S2ポケットの構造の違いによりもたらされていると考えられる。

Table 4-7. アミド誘導体のDPP-IV類縁酵素に対する選択性

compound	structure	human DPP-IV IC_{50} (nM)	human DPP-VIII IC_{50} (nM)	human DPP-IX IC_{50} (nM)
105		10	13000	2500
108		6.5	4900	740
130		24	>100000	32000

４-１１．アミド誘導体の経口糖負荷試験での評価

４-１１-１．方法

5位無置換体**72**および5位メチル体**105**の薬理学的評価を正常ラット（Sprague-Dawley rats、SDラット）を用い、経口糖負荷試験によりを行った。SDラットを20時間以上絶食した後、化合物**72**または**105**を1 mg/kg 経口投与した。化合物投与の30分後にグルコース（1 g/kg）を経口投与した。薬効の持続性を評価するため、最初の糖負荷の6時間後、12時間後にも同様にグルコース（1 g/kg）を経口投与した。糖負荷後、経時的に採血し、血漿中のグルコース濃度を測定した。

４-１１-２．結果と考察

化合物投与30分後の最初の糖負荷に対して、化合物**72**および化合物**105**は投与媒体群に対し有意に血中グルコース濃度上昇を抑制した（Figure 4-9）。*ex vivo*酵素阻害活性の評価において持続性が高かった化合物**105**は6時間後の糖負荷に対しても有意に血中グルコース濃度上昇を抑制し、12時間後の糖負荷に対しても抑制傾向を示した。一方、*ex vivo*酵素阻害活性の評価において化合物**105**と比較し持続性が短かった化合物**72**は、6時間後、12時間後の糖負荷に対しては血中グルコース濃度上昇を抑制しなかった。以上の結果より、化合物**105**は、低投与量で一日一回投与による血糖値の上昇抑制を実現可能な、持続性を有する化合物であると考えられる。

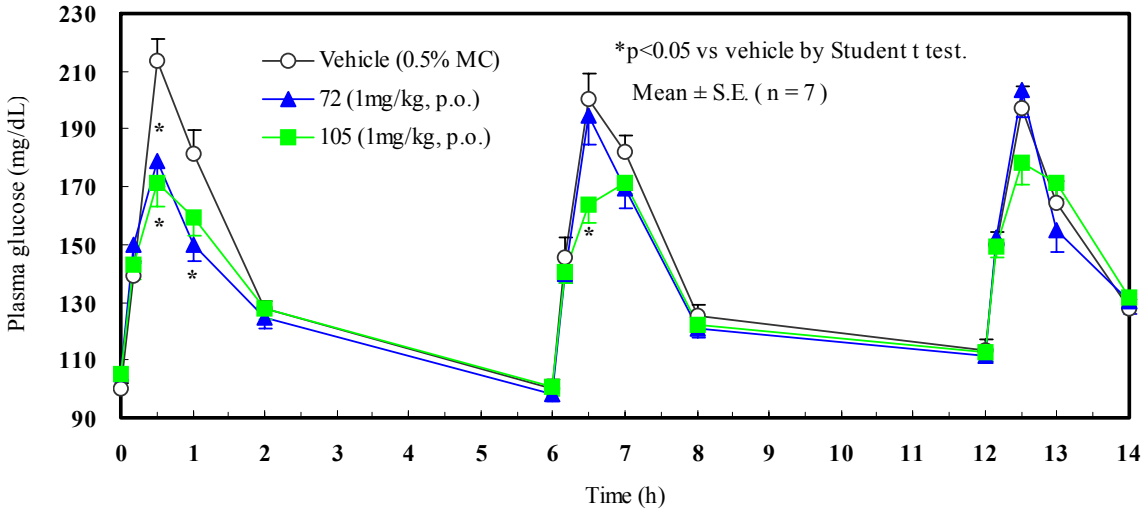
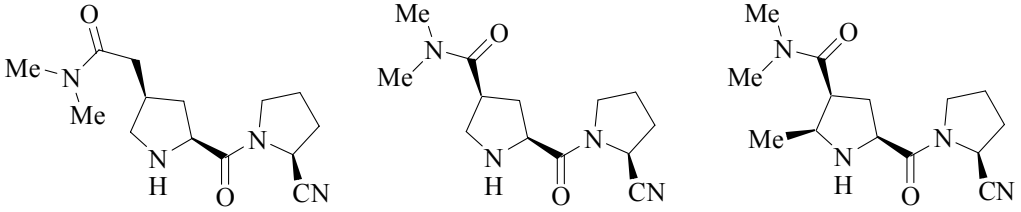


Figure 4-9. アミド誘導体**72**と**105**のラット経口糖負荷試験

４-１２．アミド誘導体のDPP-IV阻害活性の時間依存的な変化



17 **72** **105**

Figure 4-10. アミド誘導体**17**、**72**、**105**

Figure 4-11に、アミド誘導体**17**、**72**、**105**について、様々な化合物濃度で*in vitro*酵素阻害活性を測定した際の様子を示した。X軸はインキュベーション時間、Y軸は基質であるH-Gly-Pro-AMCのDPP-IVによる分解に伴う蛍光強度の変化を示している。アミド誘導体**72**、**105**においては、経時的に蛍光強度の増加の傾きが小さくなる様子がみられ、経時的に阻害活性が強くなる傾向が見られた。特に化合物**105**の際に顕著であった。一方、リード化合物の化合物**17**においてはそのような現象が見られなかった。このような阻害様式はslow binding inhibitionと呼ばれる。通常の酵素阻害活性の測定は、化合物と基質を混合しておき、そこに酵素を加えることにより反応を開始しているが、slow binding inhibitionを示す化合物においては阻害活性が低く見積もられる可能性がある。そこで、化合物と酵素をあらかじめ一定時間混合し（プレインキュベーション)、基質を加えることにより阻害活性に変化が見られるか測定することとした。

４-１２-１．方法

ヒト血漿より文献記載の方法にて精製したDPP-IVとアミド誘導体**17**、**72**、**105**を37℃にて10分、30分、60分、120分プレインキュベーションした後、基質であるH-Gly-Pro-AMCを加え、酵素阻害活性を測定した。評価は複数の化合物濃度において行い、50%抑制する濃度をIC_{50}値として求めた。

４-１２-２．結果と考察

プレインキュベーション時間に依存したIC_{50}値の変化をFigure 4-12に示した。化合物**17**、化合物**72**においてはIC_{50}値に大きな変動が見られなかったが、化合物**105**においては、30分以上のプレインキュベーションにおいて約10倍の活性の上昇が見られた。第四章４-７-２において通常の*in vitro*酵素阻害活性の測定においては、化合物**105**は化合物**72**と比較しわずかながらの減弱が見られていたが、プレインキュベーションにより、両化合物は同程度の酵素阻害活性を示すことが分かった。

以上の検討により、4位のアミド部分の構造、および5位のメチル基の有無で酵素との結合様式がことなることが分かってきた。

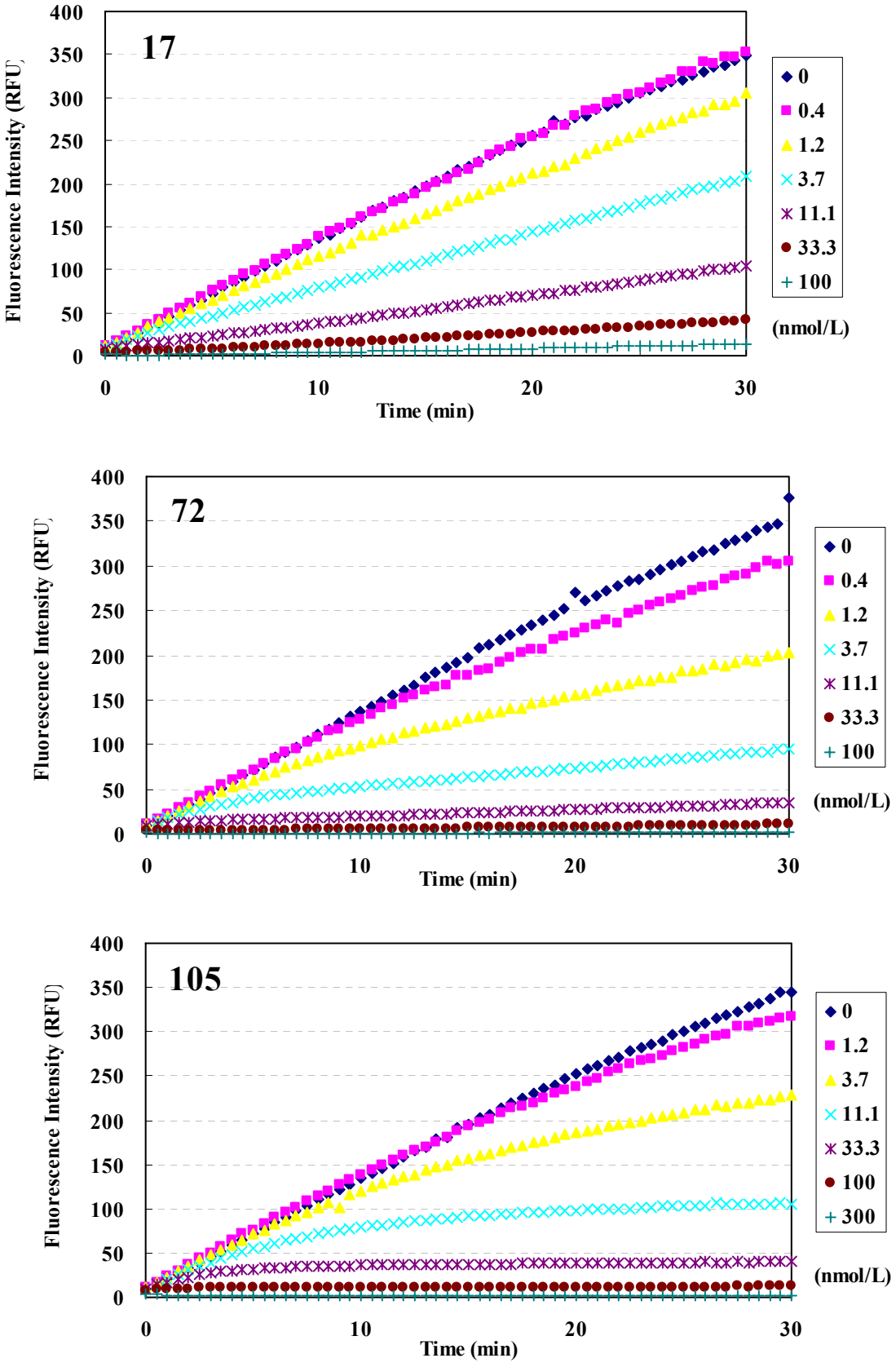


Figure 4-11. アミド誘導体**17**、**72**、**105**の*in vitro*酵素阻害活性

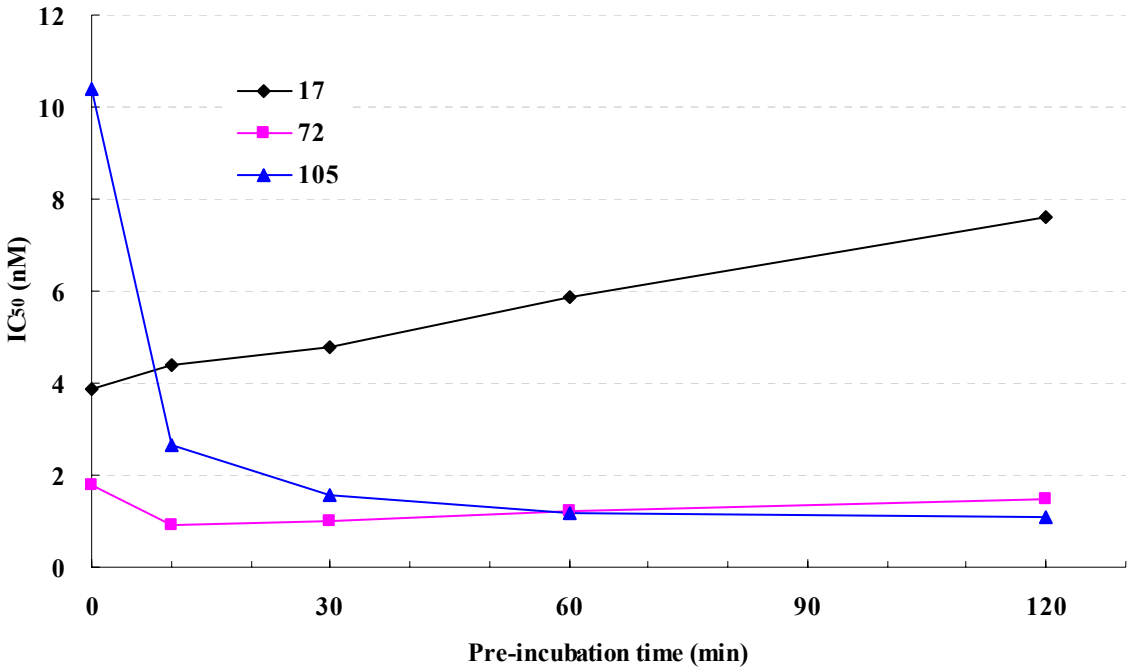


Figure 4-12. アミド誘導体**17**、**72**、**105**の時間依存的な*in vitro*酵素阻害活性の変化

４-１３．アミド誘導体とDPP-IVの複合体の解離速度の検討

アミド誘導体**17**、**72**、**105**をSDラットに1 mg/kg 経口投与した際の血漿中濃度推移をFigure 4-13に示した。化合物**17**、**72**、**105**は*ex vivo*活性の持続性には明確に差が見られるにも関わらず、ほぼ同様の血漿中濃度推移を示すことがわかった。よって、*ex vivo*活性の持続性の差は、血漿中濃度で説明できないと考えられる。

第四章４-１２で示した通り、化合物**17**、**72**、**105**の間には、DPP-IVとの結合様式に違いがあることがわかった。これら化合物とDPP-IVの複合体の安定性、すなわち酵素からの阻害剤の解離速度に差があると考えられ、検討することとした。

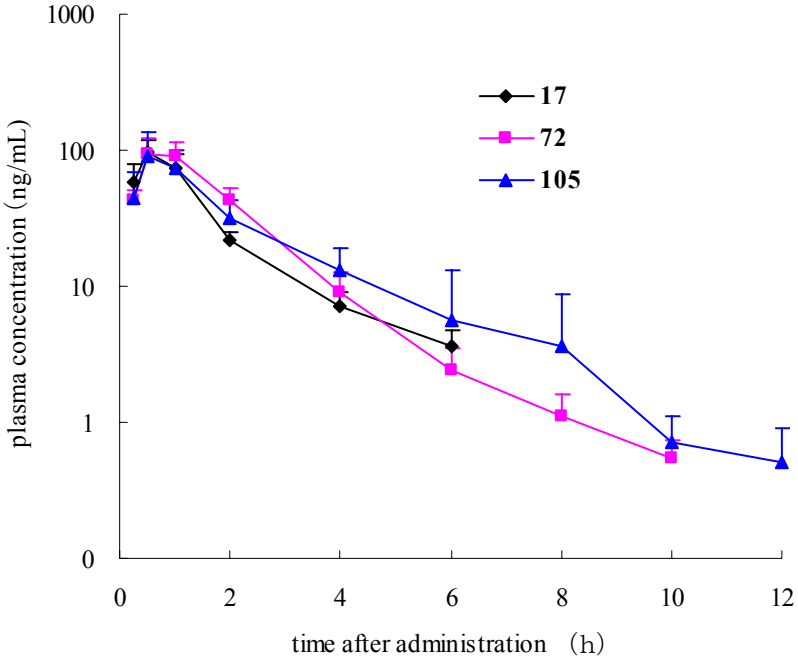


Figure 4-13. アミド誘導体**17**、**72**、**105**の経口投与後の血漿中濃度推移（1 mg/kg）

４-１３-１．方法

ヒト血漿より文献記載の方法にて精製したDPP-IVとアミド誘導体**17**、**72**、**105**を、ほぼ完全にDPP-IV活性を抑制する濃度、およびその1/100の濃度で37℃にて90分プレインキュベーションした。その後、基質であるH-Gly-Pro-AMCを加え、酵素阻害活性を測定した。また、ほぼ完全にDPP-IV活性を抑制する濃度で37℃にて90分プレインキュベーションした後、基質溶液で100倍に希釈し、酵素阻害活性を120分間測定した。ほぼ完全にDPP-IV活性を抑制する濃度として、化合物**17**、**105**は1 nM、化合物**72**は0.3 nMと設定した。

酵素との複合体からの阻害剤の解離が速ければ、100倍に希釈すると同時に、100倍希釈濃度に相当する酵素阻害活性を示すと考えられる。一方、酵素との複合体からの阻害剤の解離が遅ければ、100倍に希釈しても、徐々に100倍希釈濃度に相当する酵素

阻害活性になっていくと考えられる。

４-１３-２．結果と考察

化合物**17**、**72**、**105**とDPP-IVの複合体からの阻害剤の解離速度を検討した結果をFigure 4-14, 15, 16に示した。X軸には基質添加、また基質添加による100倍希釈後の時間（分）、Y軸には基質であるH-Gly-Pro-AMCのDPP-IVによる分解に伴う蛍光強度の変化を示している。曲線の傾きが小さいほど、阻害活性が強いことを示している。阻害曲線を15分間隔で線形近似し、傾き（ΔRFU/min）を求めた（Table 4-8）。この傾きの値が小さいほど、阻害活性が強いことを示している。

化合物**17**は希釈直後に大きな傾きを示し、経時的に変化が見られなかった（Table 4-8 、Figure 4-14）。

化合物**72**は、希釈直後は小さな傾きを示していたが、16分以降には大きな傾きを示し、経時的に変化が見られなくなった（Table 4-8 、Figure 4-15）。

化合物**105**は、希釈直後は極めて小さな傾きを示し、75分後までに徐々に大きな傾きに変化していった（Table 4-8 、Figure 4-16）。

以上の結果から、阻害剤とDPP-IVの複合体からの阻害剤の解離速度は化合物**17**、**72**、**105**の順で速いという結果であった。この解離速度の差が、*ex vivo*活性の持続性の差に表れていると考えられる。

Table 4-8. アミド誘導体のDPP-IVの複合体からの解離速度

time (min)	ΔRFU/min		
	17	**72**	**105**
0-15	2.4	1.0	0.1
16- 30	2.4	2.0	0.5
31- 45	2.3	2.1	0.6
46-60	2.2	2.2	0.8
61-75	2.3	2.2	0.9
76-90	2.2	2.2	1.0
91-105	2.0	2.1	1.0
106-120	2.2	2.1	1.0

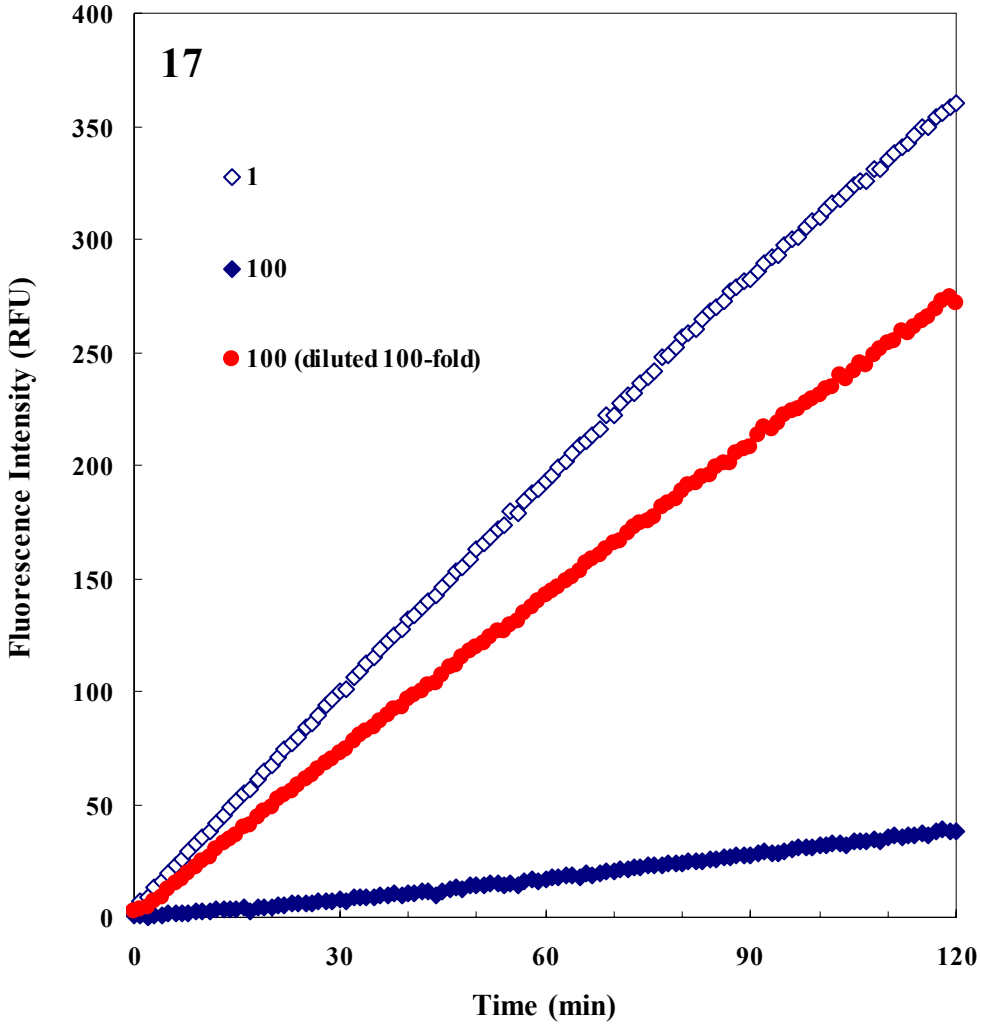


Figure 4-14. アミド誘導体**17**のDPP-IVからの解離速度

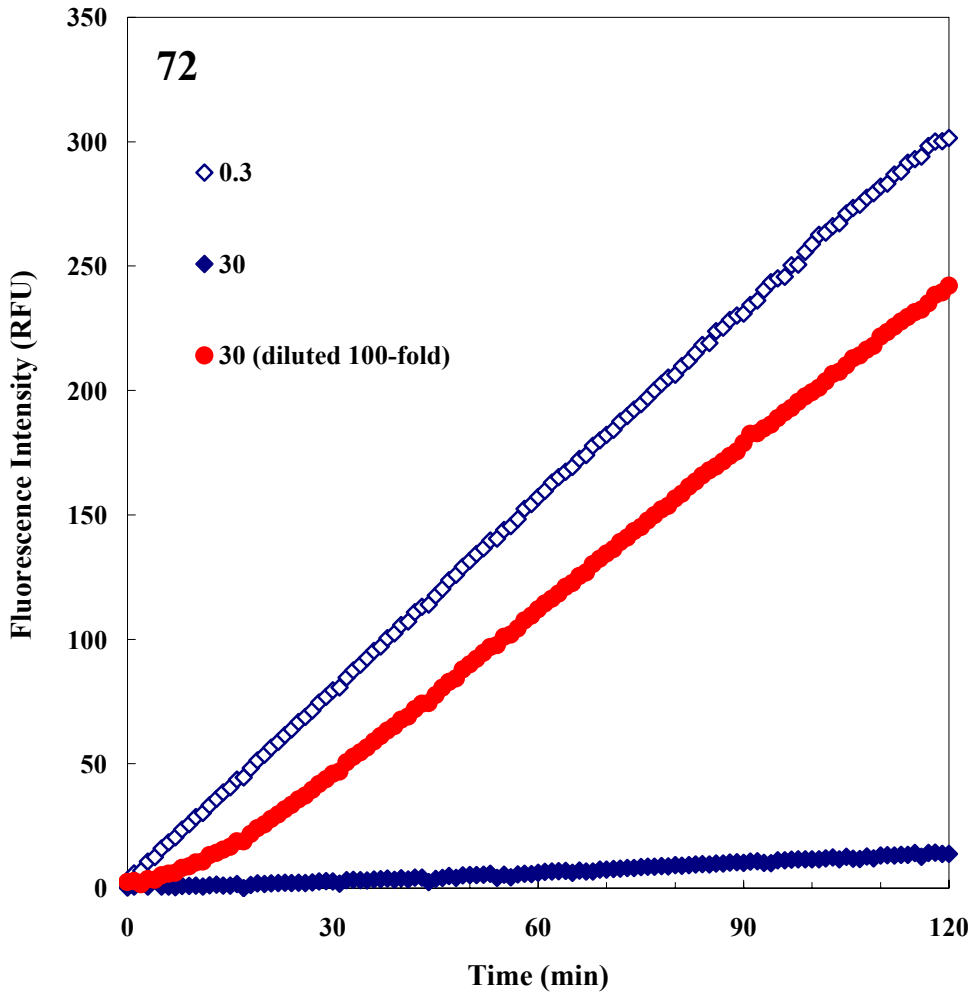


Figure 4-15. アミド誘導体**72**のDPP-IVからの解離速度

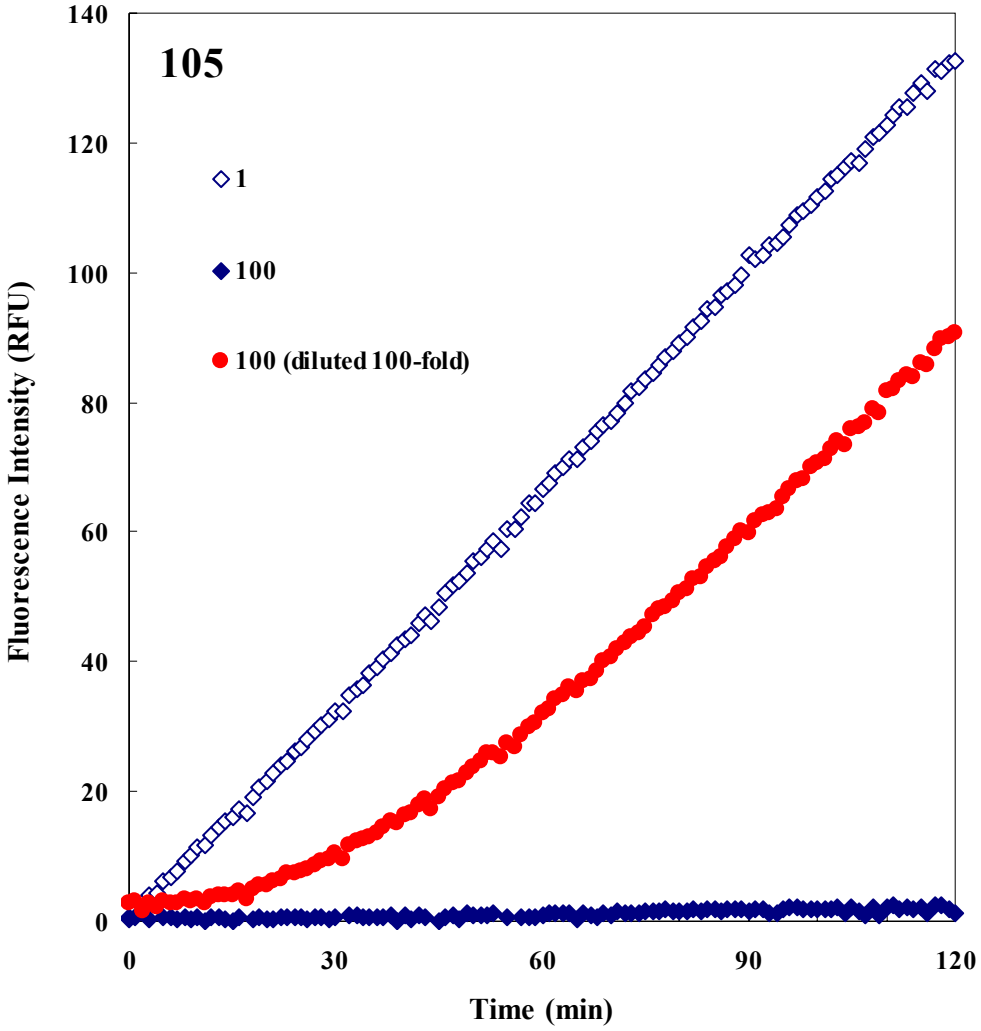
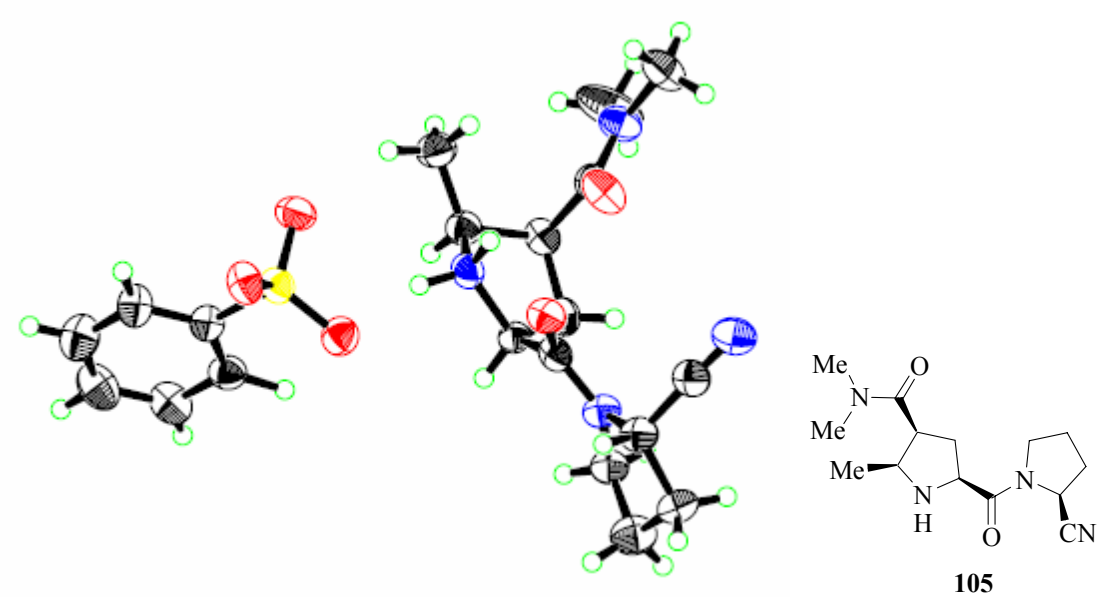


Figure 4-16. アミド誘導体**105**のDPP-IVからの解離速度

４-１４．DPP-IVの三次元構造に基づく考察

化合物**105**の単結晶の結晶構造解析を行った（Figure 4-17）。その結果、P2の炭素 - 窒素結合とカルボニル基がなす二面角は0.8度、P1の窒素 - 炭素結合とカルボニル基がなす二面角は5.8度であった。第一章で述べたとおり、アミノ基、カルボニル基、シアノ基はDPP-IVと阻害剤の相互作用の中でも最も重要な相互作用である。



105

Figure 4-17. アミド誘導体**105**のX線結晶構造解析

一方、P2位にピロリジン環を有する阻害剤**179**について、Peiらにより報告され[13]、複合体のX線結晶構造解析がProtein Data Bank に登録されている（2G5T, Figure 4-18, 19）。阻害剤**179**のP2のアミノ基は２つのグルタミン酸（Glu205, Glu206）の側鎖カルボキシル基と、カルボニル基はアルギニン125（Arg125）およびアスパラギン710（Asn710）と、P1のピロリジン環は疎水性アミノ酸からなるS1ポケットと相互作用している。シアノ基はセリン630（Ser630）と反応しイミダートを生成し、生成したイミダートはSer630の主鎖のNHおよびチロシン547（Tyr547）の側鎖水酸基と水素結合している。チロシン547（Tyr547）の側鎖水酸基は2つの水分子を介しチロシン666（Tyr666）と水素結合している。

本複合体における阻害剤のP2の炭素 - 窒素結合とカルボニル基がなす二面角は1.6度、P1の窒素 - 炭素結合とカルボニル基がなす二面角は0.3度であった。化合物**105**の単結晶の構造と、DPP-IVとの複合体中での化合物**179**の構造とを比較すると、DPP-IVとの相互作用において非常に重要な官能基が、非常に似た二面角で配置されていることが分かった。そこで、化合物**105**の単結晶の構造をこの化合物**179**とDPP-IVの複合体をコンピューター上で重ね合わせ、化合物**105**とDPP-IVの相互作用を予測した（Figure 4-20, 4-21）。

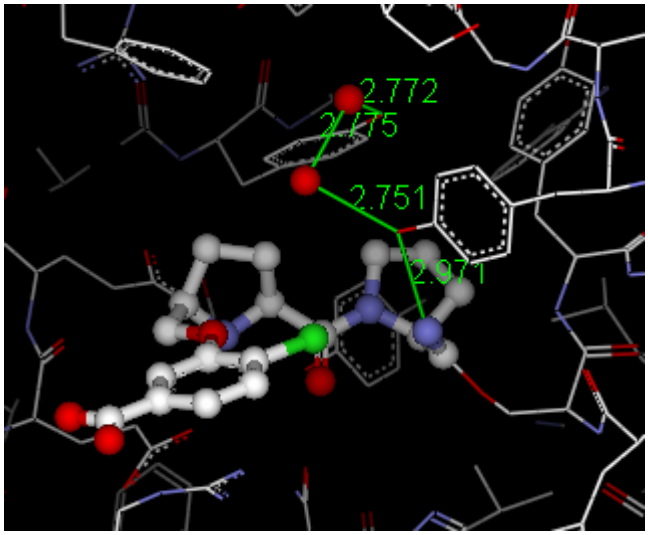
その結果、P2位のピロリジン環の4位のジメチルアミノカルボニル基の窒素の近傍（約3.5Å）に水分子（Figure 4-21中の赤丸）が存在することが分かった。この水は、

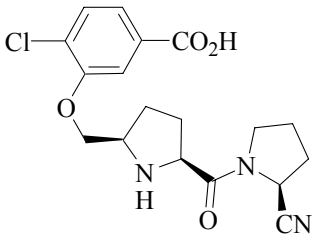
DPP-IVと化合物**179**との複合体中でのTyr547とTyr666の水を介した水素結合に関与している。この水は、別のグループが報告しているDPP-IVと阻害剤の複合体結晶構造においても見られている[14]。この水分子とP2位のジメチルアミノカルボニル基の水素結合は化合物**105**とDPP-IVの複合体の安定な結合（複合体からの解離速度の遅さ）になんらかの寄与をしていると推定される。

Tyr547は第一章で述べたが、基質がDPP-IVにより切断される過程において重要な役割を果たしているアミノ酸である[14]。すなわち、Tyr547は、基質のアミド結合にセリン（Ser630）が求核攻撃した際に生成する正四面体中間体を、フェノール性水酸基とSer630の主鎖の窒素でオキシアニオンホールを形成することによって安定化している。シアノ基を有する阻害剤とDPP-IVの複合体においては、生成するイミダートとTyr547のフェノール性水酸基は水素結合をしている。したがって、阻害剤がDPP-IVから解離する際には、チロシン側鎖が動き、水素結合が切れる必要がある。

第四章４-１３で述べた化合物**72**、**105**の複合体からの解離速度が遅い理由は、4位のジメチルアミノカルボニル基が水分子を介してTyr547のフェノール性水酸基と水素結合することにより、Tyr547の側鎖の運動を制限し、DPP-IVとの複合体を安定化しているためと推定される。メチレン鎖をひとつ挟んだ化合物**17**においては、Tyr547との水素結合の寄与がないため、DPP-IVからの解離速度が速いと推定される。

またP2位のピロリジン環の5位のメチル基は酵素の壁に沿うように配置される（Figure 4-23）。5位のメチル基は酵素の壁に沿って配置しているため、P2位のピロリジン環の運動を制限していると考えられる。そのため、化合物**105**のDPP-IVからの解離速度は化合物**72**と比較し非常に遅くなっていると推定される。





179

Figure 4-18. 5置換ピロリジン誘導体**179**とDPP-IVの複合体X線結晶構造解析（2G5T）

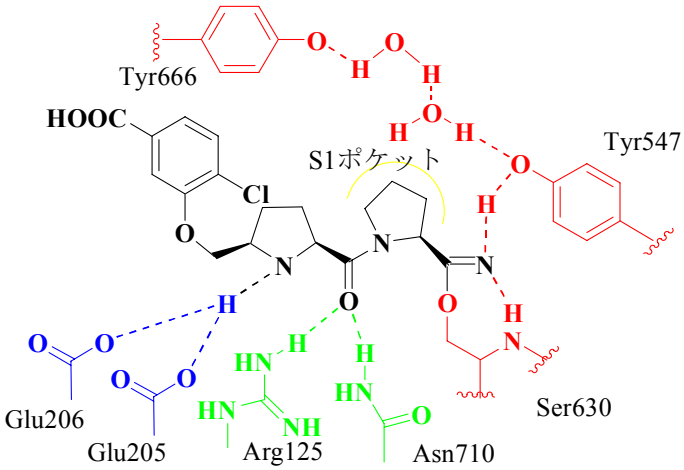
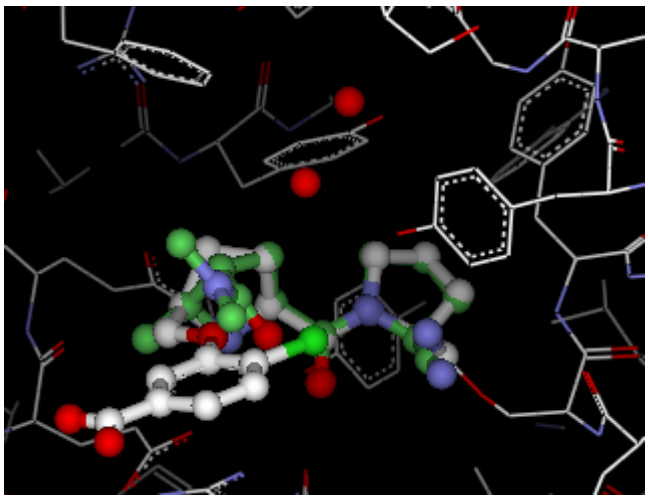
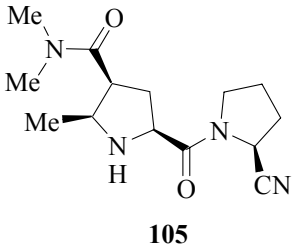


Figure 4-19. 5置換ピロリジン誘導体**179**とDPP-IVの相互作用の模式図





105

Figure 4-20. 5置換ピロリジン誘導体**179**とDPP-IVの複合体とアミド誘導体**105**の重ね合わせ

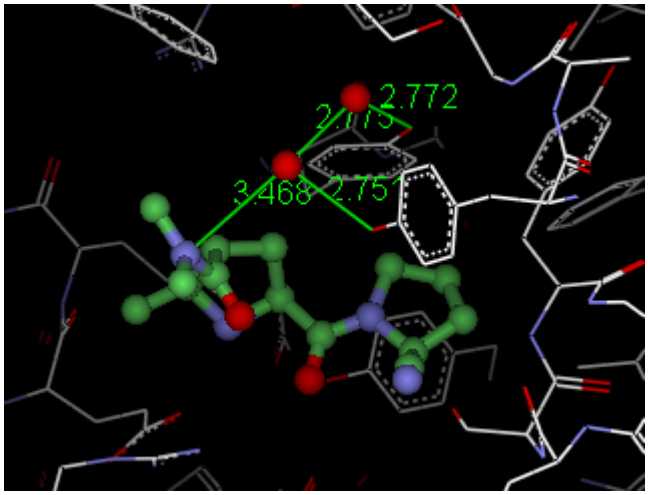


Figure 4-21. 5置換ピロリジン誘導体**179**とDPP-IVの複合体とアミド誘導体**105**の重ね合わせ（5置換ピロリジン誘導体**179**を抜いたもの）

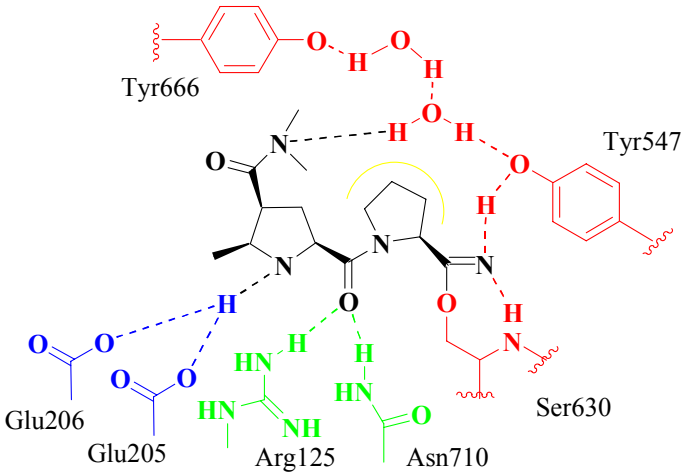


Figure 4-22. アミド誘導体**105**とDPP-IVの相互作用の予測

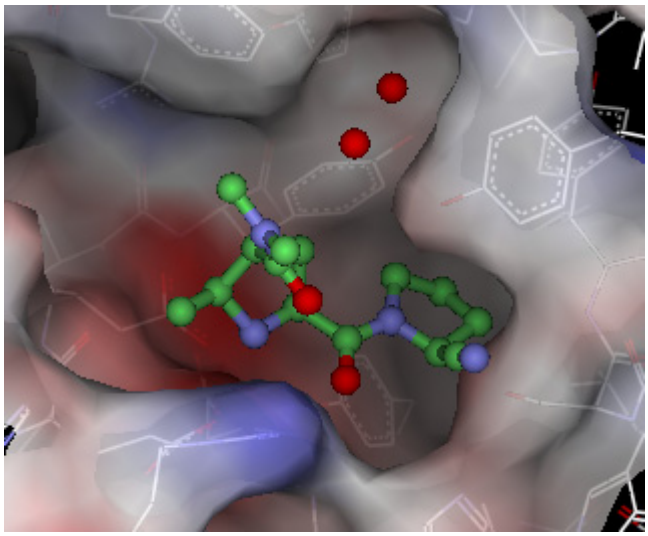
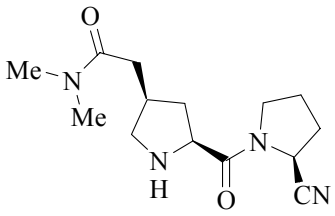


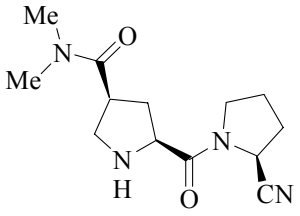
Figure 4-23. 5置換ピロリジン誘導体**179**とDPP-IVの複合体とアミド誘導体**105**の重ね合わせ（5置換ピロリジン誘導体**179**を抜き、タンパク質表面を描いたもの）

４-１５．小括

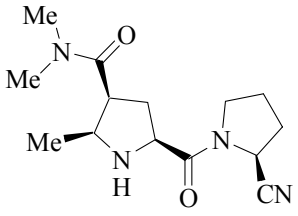
4位アミド誘導体**17**をリード化合物として最適化を行い、まずアミド部分のメチレン鎖の長さの検討を行った。その結果、ピロリジン環にジメチルアミノカルボニル基が直接結合した化合物**72**においてラットにおける持続性が見られた。化合物**72**のアミノ基部分の最適化を行った結果、比較的小さい二級アミンがラットにおける持続性の面で優れており、4位のアミド部分を嵩高くして分子内環化反応に対する安定性を高める方針を取る事は困難と考えられた。そこで、分子内環化反応に対する安定性を高める目的で5位にメチル基を導入した。その結果、化合物**105**においてラットにおける持続性が飛躍的に向上し、分子内環化反応に対する安定性も高まった。化合物**105**は経口糖負荷試験においても1 mg/kgという低投与量で、投与12時間後においても抑制傾向が見られ、持続性が確認された。化合物**105**はDPP-IVとの複合体からの解離速度が遅いことが、酵素阻害活性の測定により明らかとなった。この解離速度の遅さが、持続性の発現に寄与していると考えられる。また、解離速度が遅い原因は、DPP-IVの酵素三次元構造より、DPP-IVのチロシン547残基との水を介した水素結合に基づくと推察している。



17



72



105

４-１６．実験の部

４-１６-１．Chemistry

General directions

Analytical samples were homogeneous as confirmed by TLC and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were taken on a Varian Mercury 300 spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. The chemical shift values are reported in parts per million (δ) and coupling constants (*J*) in hertz (Hz). Fast atom bombardment mass spectra (FAB-MS, HRMS) and electron ionization (EI) were obtained on a JEOL JMS-700 spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a HITACHI M-1200H spectrometer. Matrix-assisted laser desorption ionization (MALDI) mass spectra were obtained on a PerSeptive Biosystems VoyagerTM Elite spectrometer. Infrared spectra (IR) were measured in a JASCO FT/IR-430 spectrometer. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063-0.200 mm), Wako gel C200 or Fuji Silysia FL60D]. Thin-layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, silica gel 60 F254). The following abbreviations for solvents and reagents are used; tetrahydrofuran (THF), diethyl ether (Et_2O), dimethylsulfoxide (DMSO), ethyl acetate (EtOAc), dimethylformamide (DMF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), methanol (MeOH), ethanol (EtOH), acetic acid (AcOH), hydrochloric acid (HCl).

Benzyl (2*S*,4*R*)-(1- *tert*-butoxycarbonyl)-4-[(4-methylbenzenesulfonyl)oxy]-2-pyrrolidinecarboxylate (75)

To a stirred solution of **74** (64.2 g, 200 mmol) in $CHCl_3$ (200 mL) were added pyridine (50 mL, 646 mmol) and *p*-toluenesulfonyl chrolide (76.3 g, 400 mmol) at room temperature. After being stirred for 88 h, the reaction mixture was quenched with 1M HCl and extracted with $CHCl_3$. The organic layer was successively washed with 1 M HCl, water, brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/4) as an eluant. The resulting crystalline solid was washed with ^{i}Pr$_2$O to yield **75** (77.7 g, 82%) as a white powder. TLC R_f = 0.63 (hexane/EtOAc, 1/1); MS (MALDI, pos. 20V) *m/z* 498 $(M+Na)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 and 1.42 (s, 9H), 2.10−2.20 (m, 1H), 2.37−2.60 (m, 1H), 2.46 (s, 3H), 3.54−3.68 (m, 2H), 4.37−4.50 (m, 1H), 4.97−5.28 (m, 3H), 7.28−7.42 (m, 7H), 7.78 (d, *J* = 7.5 Hz, 2H).

Benzyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-cyano-2-pyrrolidinecarboxylate (76)

To a stirred solution of **75** (77 g, 162 mmol) in DMSO (200 mL) was added NaCN (12 g, 244

mmol) at room temperature. After being stirred for 4 h at 80 ºC, the reaction mixture was cooled to room temperature and diluted with ^{t}BuOMe. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/4) as an eluant to yield **76** (31.5 g, 58%) as a colorless oil. TLC R_f = 0.32 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 and 1.47 (s, 9H), 2.21−2.37 (m, 1H), 2.60−2.77 (m, 1H), 3.03−3.17 (m, 1H), 3.60−3.73 (m, 1H), 3.84−4.03 (m, 1H), 4.30−4.50 (m, 1H), 5.05−5.37 (m, 2H), 7.30−7.42 (m, 5H).

2-Benzyl 4-ethyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-2,4-pyrrolidinediicarboxylate (77)

Trimethylsilyl chloride (150 mL, 1.18 mol) was added dropwise to EtOH (200 mL) at 0 ºC. To the reaction mixture was added a solution of **76** (20.9 g, 63 mmol) in CH_2Cl_2 (120 mL). The reaction mixture was stirred at room temperature for 20 h. The reaction mixture was cooled to 0 ºC, and quenched with H_2O. The aqueous layer was neutralized with aqueous $NaHCO_3$, and extracted with CH_2Cl_2 (1000 mL). To the organic layer was added di-*tert*-butyl-dicarbonate (13.7 g, 63 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/6) as an eluant to yield a mixture of **77** and the corresponding diethylester (20.0 g), which was used for the next reaction without further purification.

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(ethoxycarbonyl)-2-pyrrolidinecarboxylic acid (78)

To a solution of **77** and corresponding diethyl ester (20.0 g) in MeOH (150 mL) was added 10 % palladium on carbon (6.0 g). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 1 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was diluted with EtOAc/hexane (1/1) and extracted with aqueous $NaHCO_3$. The aqueous layer was acidified with 2M HCl and extracted with EtOAc. The organic layer was dried with Na_2SO_4 and concentrated in vacuo. The resulting crystalline solid was washed with $^{i}Pr_2O$ to yield **78** (9.25 g, 51%) as a white powder. TLC R_f = 0.40 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, J = 7.5 Hz, 3H), 1.44 and 1.50 (s, 9H), 2.30−2.67 (m, 2H), 3.00−3.12 (m, 1H), 3.60−3.92 (m, 2H), 4.14 (q, J = 7.5 Hz, 2H), 4.22−4.42 (m, 1H).

Ethyl (3*S*,5*S*)-5-{[(2*S*)-2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-3-pyrrolidinecarboxylate (79)

To a stirred solution of **78** (4.31 g, 15 mmol) in CH_2Cl_2 (30 mL) were added L-prolineamide (2.57 g, 22 mmol), 1-hydroxybenzotriazole (2.30 g, 15 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.45 g, 18 mmol) at 0 ºC. After being stirred for 4 h at room temperature, the reaction mixture was poured into water

and extracted with CH_2Cl_2. The organic layer was washed with 5% $KHSO_4$, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated to give **79** as a colorless oil. TLC R_f = 0.42 (CH_2Cl_2/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22−1.30 (m, 3H), 1.40 and 1.47 (s, 9H), 1.70−2.60 (m, 6H), 3.00−3.18 (m, 1H), 3.48−3.94 (m, 4H), 4.10−4.20 (m, 2H), 4.22-4.70 (m, 2H), 5.35 and 5.59 (brs, 1H), 7.01 and 7.94 (brs, 1H).

(3*S*,5*S*)-5-{[(2*S*)-2-(Aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-3-pyrrolidinecarboxylic acid (80)

To a stirred solution of **79** (5.75 g, 15 mmol) in MeOH (40 mL) was added 1 M NaOH (20 mL) at 0 ºC. After being stirred for 2 h at room temperature, the reaction mixture was quenched with 1 M HCl (20 mL). The organic solvent was removed in vacuo. The resulting crystalline solid was collected by filtration, washed with H2O, and dried in vacuo to yield **80** (4.20 g, 78%) as a white powder. TLC R_f = 0.20 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 and 1.46 (s, 9H), 1.83−2.08 (m, 3H), 2.25−2.45 (m, 2H), 2.57−2.64 (m, 1H), 3.10−3.23 (m, 1H), 3.54−3.63 (m, 2H), 3.73−3.84 (m, 1H), 4.00−4.20 (m, 1H), 4.43−4.66 (m, 2H), 7.15 and 7.22 (s, 1H), 7.75 and 7.81 (s, 1H).

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(dimethylamino)carbonyl]-1-pyrrolidinecarboxylate (81)**

To a stirred solution of **80** (300 mg, 0.85 mmol) in CH_2Cl_2 (2 mL) were added dimethylamine hydrochloride (104 mg, 1.27 mmol), triethylamine (0.36 mL, 2.5 mmol), 1-hydroxybenzotriazole (114 mg, 0.85 mmol) and 1-(3-dimetylaminopropyl)-3-ethylcarbodiimide hydrochloride (243 mg, 1.27 mmol) at 0 ºC. After being stirred for 20 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was used for the next reaction without further purification. To a solution of the residue in THF (3 mL) and CH_2Cl_2 (4 mL) were added pyridine (0.23 mL, 2.85 mmol) and trifluoroacetic anhydride (0.12 mL, 0.86 mmol) at 0 ºC. After being stirred for 15 min, the reaction mixture was quenched with water and extracted with CH_2Cl_2. The organic layer was successively washed with 1M HCl, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting crystalline solid was washed with ^{t}BuOMe and collected by filtration and dried under reduced pressure to yield **81** (214 mg, 69%). TLC R_f = 0.44 (CH_2Cl_2/MeOH/AcOH, 10/1/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21−1.45 (m, 9H), 1.61−2.57 (m, 6H), 2.82 (s, 3H), 3.02 (s, 3H), 3.16−3.76 (m, 5H), 4.28−4.47 (m, 1H), 4.75−4.85 (m, 1H), 4.92−5.05 (m, 1H).

(3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*-dimethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (72)

Compound **72** was obtained as a white powder in 92% yield from **81** according to the same procedures as described for the preparation of **10** from **29a**. TLC R_f = 0.13 (CH_2Cl_2/MeOH, 5/1); MS (MALDI, Pos.) *m/z* 265 $(M+H)^+$; IR (KBr) 2243, 1647, 1452, 1219, 1171, 1123, 1034, 1010, 683, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.02−2.17 (m, 6H), 2.36 (s, 3H), 2.64−2.77 (m, 1H), 2.89 (s, 3H), 2.93 (s, 3H), 3.33−3.45 (m, 1H), 3.45−3.67 (m, 2H), 3.68−3.79 (m, 1H), 4.79−4.86 (m, 1H), 5.02 (s, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.0 Hz, 2H), 8.28 (s, 1H), 9.59 (s, 1H); HRMS (FAB) calcd for $C_{13}H_{21}N_4O_2$: 265.1665. Found: 265.1668.

Methyl (2*S*)-1-(*tert*-Butoxycarbonyl)-4-(3-hydroxy-1-propyn-1-yl)-2,5-dihydro-1*H*-pyrrole-2-carboxylate (82)

To a mixture of **51** (750 mg, 2.0 mmol), diisopropylamine (1.6 mL, mmol), copper(I) iodide (57 mg, 0.6 mol), bis(triphenylphosphine)palladium(II) dichloride (70 mg, 0.20 mol) in THF (5 mL) was added 2-propyn-1-ol (0.12 mL, 4.0 mmol). The reaction mixture was stirred for 30 min under argon atmosphere. The reaction mixture was quenched with aqueous NH_4Cl and extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/3) as an eluent to yield **82** (579 mg, 100%). TLC R_f = 0.40 (EtOAc/hexane, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.38−1.51 (m, 9H), 1.61−1.76 (m, 1H), 3.72−3.77 (m, 3H), 4.17−4.36 (m, 2H), 4.41 (dd, *J* = 6.3, 2.8 Hz, 2H), 4.98−5.13 (m, 1H), 5.84−6.05 (m, 1H).

Methyl (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(3-hydroxypropyl)-2-pyrrolidinecarboxylate (83)

To a solution of **82** (809 mg, 2.88 mmol) in MeOH (30 mL) was added 10 % palladium on carbon (162 mg). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 3 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/1) as an eluant to yield **83** (455 mg, 55%). TLC R_f = 0.50 (EtOAc/hexane, 1/2); ^1H NMR (300 MHz, $CDCl_3$) δ 1.37−1.49 (m, 9H), 1.50−1.67 (m, 6H), 2.06−2.27 (m, 1H), 2.32−2.50 (m, 1H), 3.02 (t, *J* = 10.1 Hz, 1H), 3.53−3.86 (m, 6H), 4.13−4.33 (m, 1H).

3-[(3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-(methoxycarbonyl)-3-pyrrolidinyl]propanoic acid (84)

To a solution of **83** (497 mg, 1.73 mmol) in CH_3CN (9 mL) were added 2,2,6,6-tetramethylpiperidine 1-oxyl (19 mg, 0.12 mmol) and sodium phosphate buffer (pH 6.86, 6.5 mL). To the reaction mixture were added $NaClO_2$ (313 mg, 3.4 mmol) and 12% NaClO (0.3 mL) at 35 ºC. The reaction mixture was vigorously stirred at 35 ºC for 3 h, diluted

with ^{t}BuOMe and extracted with aqueous $NaHCO_3$. The aqueous layer was acidified with 1M HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to yield **84**(451 mg, 85%). TLC R_f = 0.43 (EtOAc/hexane, 3/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 and 1.36 (s, 9H), 1.40−1.60 (m, 3H), 1.99−2.27 (m, 3H), 2.28−2.43 (m, 1H), 2.80−2.87 (m, 1H), 3.50−3.60 (m, 1H), 3.60 and 3.63 (s, 3H), 4.07−4.26 (m, 1H), 12.10 (s, 1H).

Methyl (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-[3-(dimethylamino)-3-oxopropyl]-2-pyrrolidinecarboxylate (85)

Compound **85** was obtained as a colorless oil in 75% yield from **84** according to the same procedures as described for the preparation of **37** from **36**. TLC R_f = 0.63 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37−1.50 (m, 9H), 1.52−1.94 (m, 3H), 2.11−2.51 (m, 4H), 2.94 (s, 3H), 2.97−3.08 (m, 4H), 3.71−3.75 (m, 3H), 3.79 (dd, *J* = 10.2, 7.1 Hz, 1H), 4.14 −4.31 (m, 1H).

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-[3-(dimethylamino)-3-oxopropyl]-2-pyrrolidinecarboxylic acid (86)

Compound **86** was obtained from **85** according to the same procedures as described for the preparation of **34** from **33**, which was used for the next reaction without further purification.

***tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[3-(dimethylamino)-3-oxopropyl]-1-pyrrolidinecarboxylate (87)**

To a stirred solution of **86** (285 mg, 0.91 mmol) in CH_2Cl_2 (2 mL) were added L-prolineamide (156 mg, 1.37 mmol), 1-hydroxybenzotriazole (209 mg, 1.37 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (262 mg, 1.37 mmol) at 0 ºC. After being stirred for 4 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated. To a solution of the residue in THF (5 mL) were added pyridine (0.37 mL, 4.5 mmol) and trifluoroacetic anhydride (0.19 mL, 1.5 mmol) at 0 ºC. After being stirred for 1 h at room temperature, the reaction mixture was quenched with water and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (40/1) as an eluant to yield **87** (214 mg, 60%) as a white powder. TLC R_f = 0.44 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.34 (s, 9H), 1.37−1.43 (m, 1H), 1.54−1.71 (m, 2H), 2.00−2.23 (m, 5H), 2.29 (t, *J* = 7.1 Hz, 2H), 2.39−2.46 (m, 1H), 2.70−2.93 (m, 7H), 3.46−3.73 (m, 3H), 4.36 (t, *J* = 8.1 Hz, 1H), 4.62−4.91 (m, 1H).

3-((3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinyl)-*N*,*N*-dimethylprop

anamide 4-methylbenzenesulfonate (73)

Compound **73** was obtained as a white powder in 88% yield from **87** according to the same procedures as described for the preparation of **10** from **29a**. TLC R_f = 0.28 ($CHCl_3$/MeOH/AcOH, 3/1/1); MS (APCI, pos. 20 V) *m/z* 293 $(M+H)^+$; IR (KBr) 2924, 1658, 1650, 1643, 1634, 1454, 1186, 1122, 1034, 1010, 683 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.20−1.44 (m, 1H), 1.46−1.76 (m, 2H), 1.94−2.09 (m, 2H), 2.08−2.44 (m, 5H), 2.27 (s, 3H), 2.56−2.75 (m, 1H), 2.79 (s, 3H), 2.80−2.91 (m, 1H), 2.93 (s, 3H), 3.26−3.43 (m, 1H), 3.55 (t, *J* = 6.6 Hz, 2H), 4.32−4.55 (m, 1H), 4.82 (dd, *J* = 7.9, 4.6 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 8.69 (s, 1H), 9.28 (s, 1H); HRMS (FAB) calcd for $C_{15}H_{25}N_4O_2$: 293.1978. Found: 293.1975..

Benzyl (3*S*,5*S*)-5-{[(2*S*)-2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)- 3-pyrrolidinecarboxylate (100)

To a stirred solution of **80** (4.16 g, 11.7 mmol) in DMF (12 mL) were added K_2CO_3 (1.78 g, 12.9 mmol) and benzylbromide (1.4 mL, 12 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with water and brine, dried over $MgSO_4$, and evaporated to yield **100** (4.97 g, 96%). TLC R_f = 0.48 ($CHCl_3$/MeOH, 9/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.39 and 1.45 (s, 9H), 1.75−2.63 (m, 4H), 3.05−3.25 (m, 1H), 3.40−4.77 (m, 7H), 5.03−5.29 (m, 3H), 6.98 (s, 1H), 7.29−7.50 (m, 5H), 7.91 and 8.02 (s, 1H).

Benzyl (3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-3-pyrrolidinecarboxylate (101a)

To a stirred solution of **100** (4.97 g, 11.2 mmol) in THF (75 mL) were added pyridine (4.5 mL, 56 mmol) and trifluoroacetic anhydride (2.4 mL, 17 mmol) at 0 ºC. After being stirred for 1 h at room temperature, the reaction mixture was quenched with water and extracted with hexane/EtOAc (1/1). The organic layer was successively washed with 0.5 M HCl, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated to yield **101a** (4.63 g, 97%). TLC R_f = 0.67 ($CHCl_3$/MeOH, 9/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.35−1.50 (m, 9H), 2.07−2.68 (m, 6H), 2.98−3.26 (m, 1H), 3.51−3.64 (m, 1H), 3.66−4.04 (m, 3H), 4.30−4.56 (m, 1H), 4.75−4.95 (m, 1H), 5.03−5.30 (m, 2H), 7.29−7.54 (m, 5H).

(3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidine carboxylic acid (101b)

To a solution of **101a** (4.63 g, 10.8 mmol) in EtOAc (43 mL) was added 20 % palladium hydroxide on carbon (460 mg). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 1 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield **101b** (3.68 g, 100%). TLC R_f = 0.43

($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24−1.45 (m, 9H), 1.77−1.92 (m, 1H), 1.99−2.22 (m, 4H), 2.51−2.65 (m, 1H), 2.96−3.23 (m, 1H), 3.37−3.82 (m, 4H), 4.29−4.58 (m, 1H), 4.66−5.15 (m, 1H), 12.58 (s, 1H).

***tert*-Butyl (2*S*,4*S*)-4-(aminocarbonyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (102a)**

To a stirred solution of **101b** (300 mg, 0.89 mmol) in THF (5 mL) were added triethylamine (0.14 mL, 1.0 mmol) and ethyl chloroformate (0.10 mL, 1.0 mmol) at 0 ºC. After being stirred for 2 h at room temperature, the reaction mixture was filtered and the filtrate was concentrated in vacuo. To a stirred solution of the residue in THF (5 mL) was added 28% aqueous NH_3 (2 mL) at 0 ºC. After being stirred for 2 h at room temperature, the reactcion mixure was diluted with CH_2Cl_2. The organic layer was successively washed with water, 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (20/1) as an eluant to yield **102a** (117 mg, 39%). TLC R_f = 0.54 ($CHCl_3$/MeOH, 4/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24−1.40 (m, 9H), 1.73−1.93 (m, 1H), 1.97−2.31 (m, 4H), 2.37−2.46 (m, 1H), 2.80−2.99 (m, 1H), 3.08−3.23 (m, 1H), 3.36−3.64 (m, 2H), 3.64−3.78 (m, 1H), 4.28−4.46 (m, 1H), 4.72−5.00 (m, 1H), 7.01 (s, 1H), 7.44 (s, 1H).

According to the same procedures as described above, **102b−c** were prepared from **101b**.

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(methylamino)carbonyl]-1-pyrrolidinecarboxylate (102b)**

Yield 41%. A white powder. R_f =0.41 (EtOAc/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.36 (s, 9 H) 1.87−2.00 (m, 1H) 2.02−2.11 (m, 2H) 2.12−2.22 (m, 2H), 2.44−2.48 (m, 1H), 2.61 (d, J = 4.5 Hz, 3H), 2.87−2.93 (m, 1H), 3.28 (t, J = 10.0 Hz, 1H), 3.50−3.64 (m, 2H), 3.73 (dd, J = 10.0, 8.2 Hz, 1H), 4.41 (dd, J = 8.7, 7.9 Hz, 1H), 4.73−4.83 (m, 1H), 7.10−7.99 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(ethylamino)carbonyl]-1-pyrrolidinecarboxylate (102c)**

Yield 59%. A white powder. R_f = 0.64 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.01 (t, J = 7.5 Hz, 3H), 1.30 and 1.38 (s, 9H), 1.75−1.93 (m, 1H), 1.97−2.31 (m, 4H), 2.37−2.46 (m, 1H), 2.80−2.99 (m, 1H), 3.00−3.75 (m, 6H), 4.32−4.43 (m, 1H), 4.75−5.00 (m, 1H), 7.92−8.01 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(propylamino)carbonyl]-1-pyrrolidinecarboxylate (102d)**

To a stirred solution of **101b** (300 mg, 0.89 mmol) in CH_2Cl_2 (2 mL) were added propyl

amine (0.11 mL, 1.3 mmol), triethylamine (0.19 mL, 1.3 mmol), 1-hydroxybenzotriazole (142 mg, 1.05 mmol) and 1-(3-dimetylaminopropyl)-3-ethylcarbodiimide hydrochloride (243 mg, 1.3 mmol) at 0 ºC. After being stirred for 16 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (50/1) as an eluant to yield **102d** (333 mg, 99%) as a white powder. TLC R_f = 0.61 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos.) *m/z* 379 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, *J* = 7.5 Hz, 3H), 1.25−1.44 (m, 2H), 1.28 and 1.36 (s, 9H), 1.88−2.20 (m, 5H), 2.40−2.50 (m, 1H), 2.84−3.06 (m, 3H), 3.10−3.24 (m, 1H), 3.40−3.76 (m, 3H), 4.33−4.43 (m, 1H), 4.77−4.82 (m, 1H), 7.93−8.00 (m, 1H).

According to the same procedures as described for the preparation of **81** from **80**, **102e**−**i** were prepared from **80**.

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-{[ethyl(methyl)amino]carbonyl}-1-pyrrolidinecarboxylate (102e)**

Yield 66%. A white powder. R_f = 0.42 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos.) *m/z* 379 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.07 (t, *J* = 7.5 Hz, 3H), 1.37 (s, 9H), 1.84−2.22 (m, 5H), 2.46−2.57 (m, 1H), 2.97 (s, 3H), 3.08−3.42 (m, 4H), 3.52−3.75 (m, 3H), 4.40−4.47 (m, 1H), 4.75−4.82 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(diethylamino)carbonyl]-1-pyrrolidinecarboxylate (102f)**

Yield 58%. A white powder. R_f = 0.61 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos.) *m/z* 393 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 0.99−1.18 (m, 6H), 1.36 (s, 9H), 1.85−2.12 (m, 3H), 2.12−2.23 (m, 2H), 2.49−2.55 (m, 1H), 3.22−3.43 (m, 6H), 3.49−3.64 (m, 2H), 3.66−3.76 (m, 1H), 4.43 (dd, *J* = 9.2, 7.7 Hz, 1H), 4.73−4.85 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-4-(1-azetidinylcarbonyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (102g)**

Yield 42%. A white powder. R_f = 0.58 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos.) *m/z* 393 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.36 (s, 9H), 1.78−1.94 (m, 1H), 2.00−2.11 (m, 2H), 2.12−2.28 (m, 4H), 2.50−2.53 (m, 1H), 2.95−3.08 (m, 1H), 3.27 (t, *J* = 10.4 Hz, 1H), 3.49−3.61 (m, 2H), 3.64−3.77 (m, 1H), 3.83−4.24 (m, 4H), 4.40 (dd, *J* = 9.0, 7.7 Hz, 1H), 4.69−4.88 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (102h)**

Yield 77%. A white powder. R_f = 0.46 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23−1.40 (m, 9H), 1.66−1.96 (m, 5H), 1.96−2.18 (m, 4H), 2.49−2.60 (m, 1H), 3.08−3.31 (m, 5H), 3.48 (t, J = 6.6 Hz, 2 H), 3.53−3.76 (m, 2H), 4.25−4.46 (m, 1H), 4.65−5.06 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(1-piperidinylcarbonyl)-1-pyrrolidinecarboxylate (102i)**

Yield 41%. A white powder. R_f = 0.55 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24−1.38 (m, 9H), 1.39−1.64 (m, 7H), 1.78-1.90 (m, 1H), 1.93−2.31 (m, 4H), 3.29−3.53 (m, 7H), 3.53−3.74 (m, 2H), 4.27−4.46 (m, 1H), 4.65−5.08 (m, 1H).

According to the same procedures as described for the preparation of **102d** from **101b**, **102j−k** were prepared from **101b**.

***tert*-Butyl (2*S*,4*S*)-4-(1-azepanylcarbonyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (102j)**

Yield 78%. A white powder. R_f =0.56 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21−1.40 (m, 9H), 1.40−1.71 (m, 8H), 1.72−1.93 (m, 1H), 1.97−2.31 (m, 4H), 2.36−2.62 (m, 1H), 3.15−3.78 (m, 9H), 4.21−4.48 (m, 1H), 4.57−5.17 (m, 1H).

***tert*-Butyl (2*S*,4*S*)-4-(1-azocanylcarbonyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (102k)**

Yield 83%. A white powder. R_f = 0.49 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.36 (s, 9H), 1.40−1.80 (m, 10H), 1.90−2.30 (m, 5H), 2.45−2.55 (m, 1H), 3.30−3.80 (m, 9H), 4.43 (dd, J = 9.3, 7.8 Hz, 1H), 4.74−4.84 (m, 1H).

According to the same procedures as described for the preparation of **10** from **29a**, **88−98** were prepared from **102a−k**, respectively.

(3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (88)

Yield 76%. A white powder. TLC R_f = 0.11 ($CHCl_3$/MeOH/AcOH, 3/1/1); MS (APCI, pos. 20 V) *m/z* 237 $(M+H)^+$; IR (KBr) 2239, 1693, 1661, 1379, 1230, 1171, 1126, 1038, 1014, 685, 569 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78−1.95 (m, 1H), 1.94−2.07 (m, 2H), 2.08−2.33 (m, 2H), 2.27 (s, 3H), 2.62−2.79 (m, 1H), 2.98−3.20 (m, 1H), 3.23−3.46 (m, 2H), 3.46−3.64 (m, 2H), 4.40−4.57 (m, 1H), 4.81 (dd, J = 7.8, 4.9 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 7.20 (s, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.61 (s, 1H), 9.09 (s, 2H); HRMS (FAB) calcd for $C_{11}H_{17}N_4O_2$: 237.1352. Found: 237.1356.

(3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-methyl-3-pyrrolidinecarboxamide

4-methylbenzenesulfonate (89)

Yield 77%. A white powder. TLC R_f = 0.13 (EtOAc/AcOH/H_2O, 3/1/1); MS (APCI, pos. 20 V) *m/z* 251 $(M+H)^+$; IR (KBr) 3105, 2964, 2783, 2246, 1663, 1567, 1455, 1186, 1124, 1035, 1010, 685, 569 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.80−2.25 (m, 5H), 2.28 (s, 3H), 2.59 (d, *J* = 4.2 Hz, 3H), 2.64−2.75 (m, 1H), 2.98−3.17 (m, 1H), 3.24−3.36 (m, 1H), 3.38−3.62 (m, 3H), 4.45−4.59 (m, 1H), 4.82 (dd, *J* = 7.8, 4.9 Hz, 1H), 7.10 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 8.12 (d, *J* = 4.2 Hz, 1H), 8.73−8.94 (m, 1H), 9.32−9.53 (m, 1H); HRMS (FAB) calcd for $C_{12}H_{19}N_4O_2$: 251.1508. Found: 251.1510.

(3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-ethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (90)

Yield 83%. A white powder. TLC R_f = 0.31 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 265 $(M+H)^+$; IR (KBr) 3423, 2976, 2245, 1662, 1560, 1452, 1376, 1186, 1123, 1034, 1010, 684, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.01 (t, *J* = 7.2 Hz, 3H), 1.78−2.26 (m, 5H), 2.28 (s, 3H), 2.63−2.79 (m, 1H), 2.99−3.18 (m, 3H), 3.19−3.49 (m, 2H), 3.54−3.62 (m, 2H), 4.42−4.60 (m, 1H), 4.82 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.47 (d, *J* = 7.8 Hz, 2H), 8.08−8.24 (m, 1H), 8.68−8.97 (m, 1H), 9.33−9.57 (m, 1H); HRMS (FAB) calcd for $C_{13}H_{21}N_4O_2$: 265.1665. Found: 265.1668.

(3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-propyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (91)

Yield 86%. A beige powder. TLC R_f = 0.38 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 279 $(M+H)^+$; IR (KBr) 3434, 2247, 1661, 1189, 1124, 1035, 1011, 684 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.83 (t, *J* = 7.4 Hz, 3H), 1.31−1.49 (m, 2H), 1.75−1.94 (m, 1H), 1.95−2.09 (m, 2H), 2.09−2.26 (m, 2H), 2.28 (s, 3H), 2.36−2.47 (m, 1H), 2.64−2.84 (m, 1H), 2.94−3.18 (m, 3H), 3.20−3.38 (m, 1H), 3.55 (t, *J* = 6.6 Hz, 2H), 4.43−4.61 (m, 1H), 4.82 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.41−7.54 (m, 2H), 8.11−8.19 (m, 1H), 8.81 (s, 1H), 9.44 (s, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 279.1821. Found: 279.1820.

(3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-ethyl-*N*-methyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (92)

Yield 98%. A beige powder. TLC R_f = 0.30 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 279 $(M+H)^+$; IR (KBr) 3450, 2978, 2243, 1637, 1454, 1187, 1123, 1035, 1011, 684, 569 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.92−1.20 (m, 3H), 1.64−2.26 (m, 5H), 2.28 (s, 3H), 2.71−3.04 (m, 4H), 3.13−3.71 (m, 7H), 4.43−4.61 (m, 1H), 4.82 (dd, *J* = 7.6, 4.6 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.47 (d, *J* = 7.8 Hz, 2H), 8.69−8.98 (m, 1H), 9.18−9.59 (m, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 279.1821. Found: 279.1818.

(3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*-diethyl-3-pyrrolidinecarboxami

de 4-methylbenzenesulfonate (93)

Yield 97%. A white powder. TLC R_f = 0.18 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 293 $(M+H)^+$; IR (KBr) 3568, 3449, 1663, 1655, 1646, 1638, 1451, 1214, 1190, 684, 569 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.95−1.06 (m, 3H), 1.07−1.18 (m, 3H), 1.69−1.84 (m, 1H), 1.97−2.07 (m, 2H), 2.09−2.25 (m, 2H), 2.28 (s, 3H), 2.73−2.88 (m, 1H), 3.17−3.50 (m, 7H), 3.51−3.64 (m, 2H), 4.43−4.60 (m, 1H), 4.83 (dd, *J* = 7.78, 4.8 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.40−7.54 (m, 2H), 8.84 (s, 1H), 9.37 (s, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 293.1978. Found: 293.1981.

(2*S*)-1-{[(2*S*,4*S*)-4-(1-Azetidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (94)

Yield 98%. A white powder. TLC R_f = 0.19 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 277 $(M+H)^+$; IR (KBr) 3439, 2242, 1654, 1648, 1446, 1188, 1123, 1034, 1010, 683, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.67−1.86 (m, 1H), 1.95−2.08 (m, 2H), 2.11−2.26 (m, 4H), 2.28 (s, 3H), 2.66−2.87 (m, 1H), 3.07−3.22 (m, 1H), 3.22−3.36 (m, 2H), 3.49−3.65 (m, 2H), 3.86 (t, *J* = 7.6 Hz, 2H), 4.18 (t, *J* = 7.6 Hz, 2H), 4.38−4.59 (m, 1H), 4.83 (dd, *J* = 7.6, 4.7 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.42−7.50 (m, 2H), 8.88 (s, 1H), 9.40 (s, 1H); HRMS (FAB) calcd for $C_{14}H_{21}N_4O_2$: 277.1665. Found: 277.1667.

(2S)-1-{[(2S,4S)-4-(1-Pyrrolidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (95)

Yield 96%. A white powder. TLC R_f = 0.67 ($CHCl_3$/MeOH/AcOH, 3/1/1); MS (APCI, pos. 20 V) *m/z* 291 $(M+H)^+$; IR (KBr) 3434, 3061, 2974, 2881, 1660, 1633, 1450, 1187, 1123, 1034, 1010, 683 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.68−2.08 (m, 7H), 2.09−2.26 (m, 2H), 2.27 (s, 3H), 2.50−2.61 (m, 1H), 2.77−2.93 (m, 1H), 3.28 (t, *J* = 6.7 Hz, 2H), 3.42−3.79 (m, 6H), 4.44−4.61 (m, 1H), 4.82 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 8.86 (s, 1H), 9.41 (s, 1H); HRMS (FAB) calcd for $C_{15}H_{23}N_4O_2$: 291.1821. Found: 291.1821.

(2*S*)-1-{[(2*S*,4*S*)-4-(1-Piperidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (96)

Yield 98%. A white powder. TLC R_f = 0.61 ($CHCl_3$/MeOH/AcOH, 3/1/1); MS (APCI, pos. 20 V) *m/z* 305 $(M+H)^+$; IR (KBr) 2938, 2241, 1662, 1636, 1448, 1218, 1186, 1123, 1033, 1010, 682 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.37−1.69 (m, 7H), 1.94−2.23 (m, 4H), 2.36 (s, 3H), 2.61−2.80 (m, 1H), 3.22−3.82 (m, 9H), 4.76−4.88 (m, 1H), 4.93−5.10 (m, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.0 Hz, 2H), 8.24 (s, 1H), 9.59 (s, 1H); HRMS (FAB) calcd for $C_{16}H_{25}N_4O_2$: 305.1978. Found: 305.1977.

(2*S*)-1-{[(2*S*,4*S*)-4-(1-Azepanylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonit

rile hydrochloride (97)

Yield 97%. A white powder. TLC R_f = 0.22 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 319 $(M+H)^+$; IR (KBr) 3411, 2927, 2722, 1634, 1448, 1369, 1267, 1191, 1101, 1046, 730 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.39−1.72 (m, 8H), 1.72−1.88 (m, 1H), 1.91−2.06 (m, 2H), 2.08−2.32 (m, 2H), 2.36−2.60 (m, 1H), 2.67−2.86 (m, 1H), 3.33−3.82 (m, 8H), 4.43−4.58 (m, 1H), 4.81 (dd, *J* = 7.8, 4.7 Hz, 1H), 9.59 (s, 2H); HRMS (FAB) calcd for $C_{17}H_{27}N_4O_2$: 319.2134. Found: 319.2135.

(2*S*)-1-{[(2*S*,4*S*)-4-(1-Azocanylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (98)

Yield 87%. A beige powder. TLC R_f = 0.22 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 333 $(M+H)^+$; IR (KBr) 2927, 2242, 1662, 1636, 1450, 1214, 1174, 1123, 1033, 1010, 682, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.34−1.72 (m, 10H), 1.74−1.90 (m, 1H), 1.93−2.09 (m, 2H), 2.09−2.25 (m, 2H), 2.27 (s, 3H), 2.38−2.62 (m, 1H), 2.67−2.92 (m, 1H), 3.27−3.66 (m, 8H), 4.44−4.57 (m, 1H), 4.82 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 8.85 (s, 1H), 9.38 (s, 1H); HRMS (FAB) calcd for $C_{18}H_{29}N_4O_2$: 333.2291. Found: 333.2288.

1-*tert*-Butyl 3-ethyl (3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-1,3-pyrrolidinedicarboxylate (103)

To a stirred solution of **78** (23.4 g, 81 mmol) in CH_2Cl_2 (160 mL) were added pyrrolidine (7.14 mL, 86 mmol), 1-hydroxybenzotriazole (13.7 g, 90 mmol), *N*-methylmorpholine (10.7 mL, 98 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (18.7 g, 98 mmol) at room temperature. After being stirred for 19 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated to give **103** (27 g, 97%) as a pale orange powder. TLC R_f = 0.38 (EtOAc/hexane, 4/1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.17 (t, *J* = 7.0 Hz, 3H), 1.24−1.43 (m, 9H), 1.61−1.98 (m, 6H), 3.03−3.53 (m, 6H), 3.56−3.76 (m, 1H), 3.98−4.15 (m, 2H), 4.26−4.56 (m, 1H).

***tert*-butyl (2*S*,4*S*)-4-(dimethylcarbamoyl)-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (104)**

To a stirred solution of **103** (1.56 g, 3.4 mmol) in MeOH (6.8 mL) was added 2 M NaOH (3.4 mL) at 0 ºC. After being stirred at 0 ºC for 1 h, the reaction was quenched with 2 M HCl (3.4 mL). The organic solvent was removed by evaporation. The resulting residue was diluted with EtOH. Insoluble substance was removed by filtration and the filtrate was evaporated to yield crude carboxylic acid (709 mg, 66%) as a white powder. To a stirred solution of crude carboxylic acid (100 mg, 0.32 mmol) in CH_2Cl_2 (1 mL) were added dimethylamine hydrochloride (39 mg, 0.48 mmol), 1-hydroxybenzotriazole (43 mg, 0.32 mmol),

N-methylmorpholine (0.10 mL, 0.96 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (92 mg, 0.48 mmol) at room temperature. After being stirred for 19 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated to give **104** (98 mg, 90%) as a white powder. TLC R_f = 0.36 (CH_2Cl_2/MeOH, 10/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.75−2.07 (m, 4H), 2.25−2.54 (m, 2H), 2.96 (s, 3H), 3.07 (s, 3H), 3.10−3.30 (m, 1H), 3.28−4.03 (m, 6H), 4.28−4.60 (m, 1H).

(3*S*,5*S*)-*N*,*N*-Dimethyl-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (99)

A solution of **104** (93 mg, 0.27 mmol) and *p*-toluenesulfonic acid (78 mg, 0.41 mmol) in EtOH (2 mL) was stirred at 90 ºC for 5 h. After cooling to room temperature, the reaction mixture was evaporated to give **99** (113 mg, 100%) as a colorless oil. TLC R_f = 0.21 (CH_2Cl_2/MeOH, 9/1); MS (APCI, pos.) *m/z* 240 $(M+H)^+$; IR (KBr) 3418, 2979, 1645, 1496, 1455, 1172, 1123, 1035, 1011, 685 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.68−1.97 (m, 5H), 2.28 (s, 3H), 2.67−2.80 (m, 1H), 2.82 (s, 3H), 3.00 (s, 3H), 3.27−3.60 (m, 7H), 4.38−4.54 (m, 1H), 7.11 (d, *J*=8.1 Hz, 2H), 7.47 (d, *J*=8.1 Hz, 2H), 8.56−8.75 (m, 1H), 9.23−9.44 (m, 1H); HRMS (FAB) calcd for $C_{12}H_{22}N_3O_2$: 240.1712. Found: 240.1715.

2-*tert*-Butyl 4-methyl (2*S*)-1-benzyloxycarbonyl-5-methyl-2,3-dihydro-1*H*-pyrrole-2,4-dicarboxylate (114)

To a stirred solution of lithium bis(trimethylsilyl)amide in THF (400 mL ,1.0 M) was added dropwise a solution of **113** (51.9 g, 142 mmol) in THF (500 mL) at −78 ºC. After being stirred for 30 min, acetyl chloride (31.4 mL, 441 mmol) was added and the reaction mixture was stirred at −78 ºC for additional 1 h. The reaction mixture was quenched with AcOH (280 mL), warmed up to 50 ºC, and stirred for 2 h. The reaction mixture was cooled to room temperature, diluted with EtOAc/hexane (1/1, 400 mL), and filtered. The filtrate was concentrated in vacuo and the resulting residue was diluted with EtOAc/hexane (1/1, 1000 mL). The organic layer was successively washed with 1M HCl, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/10) as an eluant to yield **114** (33.5 g, 60%) as a white powder. TLC R_f = 0.70 (EtOAc/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 376 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9H), 2.65 (s, 3H), 2.66−2.75 (m, 1H), 3.03−3.17 (m, 1H), 3.71 (s, 3H), 4.59−4.66 (m, 1H), 5.17 (s, 2H), 7.30−7.38 (m, 5H).

2-*tert*-Butyl 4-methyl (2*S*,4*S*,5*S*)-5-methyl-2,4-pyrrolidinedicarboxylate (115a) and 2-*tert*-Butyl 4-methyl (2*S*,4*R*,5*S*)-5-methyl-2,4-pyrrolidinedicarboxylate (115b)

To a solution of **114** (6.24 g, 16.6 mmol) in AcOH (80 mL) was added 10 % palladium on

carbon (1.2 g). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 9 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (3/1) as an eluant to yield **115a** (1.91 g, 47%) and **115b** (1.15 g, 28%) as a colorless oil. **115a** : TLC R_f = 0.36 (EtOAc/hexane, 2/1); MS (APCI, pos. 20 V) *m/z* 244 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, *J* = 6.6 Hz, 3H), 1.48 (s, 9H), 2.14−2.37 (m, 2H), 2.90−2.99 (m, 1H), 3.32−3.43 (m, 1H), 3.66−3.70 (m, 1H), 3.67 (s, 3H). **115b** : TLC R_f = 0.17 (EtOAc/hexane, 1/1); MS (APCI, pos. 20 V) *m/z* 244 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, *J* = 6.3 Hz, 3H), 1.47 (s, 9H), 2.10−2.19 (m, 1H), 2.35−2.50 (m, 2H), 3.20−3.30 (m, 1H), 3.68−3.78 (m, 1H), 3.70 (s, 3H).

2-*tert*-Butyl 4-methyl (2*S*,4*S*,5*S*)-5-methyl-2,4-pyrrolidinedicarboxylate (115a)

To a solution of **114** (14.7 g, 39.2 mmol) in AcOH (80 mL) was added platinum(IV) oxide (890 mg, 3.92 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 8 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (20/1) as an eluant to yield **115a** (8.50 g, 89%). TLC R_f = 0.36 (EtOAc/hexane, 2/1); MS (APCI, pos. 20 V) *m/z* 244 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, *J* = 6.6 Hz, 3H), 1.48 (s, 9H), 2.14−2.37 (m, 2H), 2.90−2.99 (m, 1H), 3.32−3.43 (m, 1H), 3.66−3.70 (m, 1H), 3.67 (s, 3H).

(2*S*,4*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-4-(methoxycarbonyl)-5-methyl-2-pyrrolidinecarboxylic acid (118)

A solution of **115a** (9.68 g, 39.8 mmol) in trifluoroacetic acid (31 mL) and water (3 mL) was stirred for 19 h at room temperature. The reaction mixture was evaporated. To a stirred solution of the residue in THF (5 mL) and water (20 mL) were added $NaHCO_3$ (15 g, 178 mmol) and a solution of di-*tert*-butyl-dicarbonate (10.4 g, 47.8 mmol) in THF (15 mL) at room temperature. After being stirred for 3 h, the reaction mixture was quenched with 2 M HCl and extracted with EtOAc. The organic layer was dried over $MgSO_4$, and evaporated to yield **118** (1.16 g, 100%). TLC R_f = 0.44 (EtOAc/MeOH, 20/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, *J* = 6.6 Hz, 3H), 1.48 (s, 9H), 2.38−2.78 (m, 2H), 3.11−3.21 (m, 1H), 3.73 (s, 3H), 4.20−4.40 (m, 2H).

Methyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylate (119a)

Compound **119a** was obtained as a white powder in 85% yield from **118** according to the

same procedures as described for the preparation of **79** from **78**. TLC R_f = 0.44 (EtOAc/MeOH, 9:1); MS (APCI, pos. 20 V) *m/z* 384 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18−1.27 (m, 3H), 1.39−1.46 (m, 9H), 1.60−2.60 (m, 7H), 3.10−3.70 (m, 3H), 3.72 (s, 3H), 4.20−4.72 (m, 2H), 5.28 and 5.51 (s, 1H), 7.02 and 7.98 (s, 1H).

(2*S*,3*S*,5*S*)-5-{[(2*S*)-2-(Aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylic acid (119b)

To a stirred solution of **119a** (12.5 g, 32.5 mmol) in MeOH (65 mL) was added 1 M LiOH (36 mL) at 0 ºC. After being stirred for 3 h at room temperature, the reaction mixture was quenched with 2 M HCl (18 mL). The organic solvent was removed in vacuo. The resulting residue was diluted with EtOH and filtered. The filtrate was evaporated to yield **119b** (12.0 g), which was used for the next reaction without further purification.

Benzyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylate (119c)

To a stirred solution of **119b** (12.0 g, 32.5 mmol) in DMF (33 mL) were added K_2CO_3 (4.94 g, 35.8 mmol) and benzylbromide (4.3 mL, 36 mmol) at room temperature. After being stirred for 15 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, and evaporated to yield **119c** (14.9 g), which was used for the next reaction without further purification.

Benzyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylate (120)

To a stirred solution of **119c** (14.9 g, 32.5 mmol) in THF (100 mL) were added pyridine (7.9 mL, 98 mmol) and trifluoroacetic anhydride (5.5 mL, 39 mmol) at 0 ºC. After being stirred for 30 min, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was successively washed with 1 M HCl, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (3/1) as an eluant to yield **120** (6.09 g, 42% from **119a**). TLC R_f = 0.29 (EtOAc/hexane, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (d, *J* = 7.5 Hz, 3H), 1.34 and 1.42 (s, 9H), 2.05−2.50 (m, 6H), 3.10−3.22 (m, 1H), 3.53−3.83 (m, 2H), 4.22−4.41 (m, 2H), 4.77−4.84 (m, 1H), 5.12−5.22 (m, 2H), 7.30−7.42 (m, 5H).

(2*S*,3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-pyrrolidinecarboxylic acid (121)

To a solution of **120** (6.0 g, 13.6 mmol) in EtOAc (45 mL) was added 20 % palladium hydroxide on carbon (1.2 g). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 20 min. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield **121** (4.45 g, 93%). TLC R_f = 0.20

(EtOAc/hexane, 2/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, J = 7.5 Hz, 3H), 1.27 and 1.37 (s, 9H), 1.80−2.43 (m, 6H), 3.12−3.24 (m, 1H), 3.40−3.68 (m, 2H), 4.03−4.13 (m, 1H), 4.30−4.39 (m, 1H), 4.76−4.82 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-[(dimethylamino)carbonyl]-2-methyl-1-pyrrolidinecarboxylate (122a)**

To a stirred solution of **121** (200 mg, 0.57 mmol) in CH_2Cl_2 (2 mL) were added dimethyl amine hydrochloride (93 mg, 1.14 mmol), triethylamine (0.28 mL, 2.0 mmol), 1-hydroxybenzotriazole (87 mg, 0.57 mmol) and 1-(3-dimetylaminopropyl)-3-ethylcarbodiimide hydrochloride (164 mg, 0.85 mmol) at room temperature. After being stirred for 4 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was recrystalized from EtOAc and hexane to yield **122a** (164 mg, 76%) as a white powder. TLC R_f = 0.40 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (d, J = 7.5 Hz, 3H), 1.27 and 1.36 (s, 9H), 1.98−2.28 (m, 6H), 2.82 (s, 3H), 3.01 (s, 3H), 3.38−3.68 (m, 3H), 4.08−4.16 (m, 2H), 4.77−4.83 (m, 1H).

According to the same procedures as described above, **122b−h** were prepared from **121**.

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-{[ethyl(methyl)amino]carbonyl}-2-methyl-1-pyrrolidinecarboxylate (122b)**

Yield 41%. A white powder. R_f = 0.37 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87−1.13 (m, 6H), 1.21−1.46 (m, 9H), 2.00−2.31 (m, 6H), 2.73−3.05 (m, 3H), 3.05−3.20 (m, 1H), 3.33−3.54 (m, 3H), 3.54−3.68 (m, 1H), 4.05−4.22 (m, 1H), 4.22−4.38 (m, 1H), 4.70−5.03 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-3-(1-azetidinylcarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-1-pyrrolidinecarboxylate (122c)**

Yield 42%. A white powder. R_f = 0.42 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91−1.06 (m, 3H), 1.22−1.44 (m, 9H), 1.99−2.31 (m, 8H), 2.93−3.19 (m, 1H), 3.37−3.70 (m, 2H), 3.83 (t, J = 7.7 Hz, 2H), 3.95−4.23 (m, 3H), 4.28 (dd, J=10.1, 7.6 Hz, 1H), 4.66−5.01 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (122d)**

Yield 72%. A white powder. R_f = 0.32 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88−1.02 (m, 3H), 1.23−1.42 (m, 9H), 1.67−1.95 (m, 4H), 2.00−2.31 (m, 6H), 3.21−3.70 (m,

7H), 4.12−4.22 (m, 1H), 4.23−4.37 (m, 1H), 4.64−5.07 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-(1-piperidinylcarbonyl)-1-pyrrolidinecarboxylate (122e)**

Yield 96%. A white powder. R_f = 0.53 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.89−1.00 (m, 3H), 1.23−1.38 (m, 9H), 1.37−1.68 (m, 6H), 1.99−2.32 (m, 6H), 3.24−3.73 (m, 7H), 4.02−4.16 (m, 1H), 4.20−4.36 (m, 1H), 4.71−5.01 (m, 1 H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-(4-morpholinylcarbonyl)-1-pyrrolidinecarboxylate (122f)**

Yield 84%. A white powder. R_f = 0.36 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.94−1.04 (m, 3H), 1.24−1.39 (m, 9H), 1.99−2.30 (m, 6H), 3.33−3.72 (m, 11H), 4.05−4.20 (m, 1H), 4.20−4.37 (m, 1H), 4.71−5.06 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-(4-thiomorpholinylcarbonyl)-1-pyrrolidinecarboxylate (122g)**

Yield 97%. A white powder. R_f = 0.59 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.03 (d, J = 6.4 Hz, 3H), 1.35 (s, 9H), 1.97−2.39 (m, 6H), 2.50−2.53 (m, 1H), 2.51−2.65 (m, 4H), 3.36−3.47 (m, 1H), 3.52−3.64 (m, 2H), 3.78 (t, J = 5.2 Hz, 3H), 4.11−4.19 (m, 1H), 4.33 (t, J = 8.7 Hz, 1H), 4.70−4.85 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-(1,3-dihydro-2*H*-isoindol-2-ylcarbonyl)-2-methyl-1-pyrrolidinecarboxylate (122h)**

Yield 50%. A white powder. R_f =0.29 (EtOAc/hexane, 9/1); MS (APCI, pos. 20 V) *m/z* 453 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.08 (d, J = 6.4 Hz, 3H), 1.38 (s, 9H), 2.00−2.39 (m, 6H), 3.40−3.50 (m, 1H), 3.56−3.64 (m, 2H), 4.17−4.23 (m, 2H), 4.60−5.03 (m, 5H), 7.28−7.38 (m, 4H).

(2*S*,3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*,2-trimethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (105)

A solution of **122a** (2.0 g, 5.29 mmol) and *p*-toluenesulfonic acid (1.21 g, 6.36 mmol) in EtOH (20 mL) and ^{i}PrOH (10 mL) was stirred at 90 ºC for 2 h. After cooling to room temperature, the resulting precipitates were collected by filtration and dried under reduced pressure to yield **105** (1.91 g, 80%) as a white powder. TLC R_f = 0.24 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 279$(M+H)^+$; IR (KBr) 3449, 2243, 1654, 1647, 1639, 1450, 1219, 1188, 1122, 1010, 682, 568 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, J = 6.7 Hz, 3H), 1.94−2.26 (m, 5H), 2.28 (s, 3H), 2.57−2.71 (m, 1H), 2.85 (s, 3H), 3.01 (s, 3H), 3.47−3.67 (m, 3H), 3.92 (s, 1H), 4.51 (s, 1H), 4.81 (dd, J = 7.8, 5.1 Hz, 1H), 7.10 (d, J = 8.2 Hz, 2H), 7.46

(d, *J* = 7.8 Hz, 2H), 8.10−8.44 (m, 1H), 9.53−9.80 (m, 1H); Anal. Calcd for $C_{21}H_{30}N_4O_5S$: C, 55.98; H, 6.71; N, 12.43. Found: C, 55.72; H, 6.76; N, 12.31.

According to the same procedures as described above, **106-112** were prepared from **122b-h**, respectively.

(2*S*,3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-ethyl-*N*,2-dimethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (106)

Yield 95%. A white powder. TLC R_f = 0.50 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 293 $(M+H)^+$; IR (KBr) 2980, 2244, 1663, 1560, 1496, 1452, 1222, 1173, 1122, 1010, 682, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.94−1.13 (m, 3H), 1.12−1.19 (m, 3H), 1.92−2.25 (m, 5H), 2.27 (s, 3H), 2.53−2.75 (m, 1H), 2.79−2.99 (m, 3H), 3.10−3.34 (m, 1H), 3.36−3.66 (m, 4H), 3.81−3.97 (m, 1H), 4.44−4.60 (m, 1H), 4.80 (dd, *J* = 7.7, 5.2 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 8.21 (s, 1H), 9.64 (s, 1H); HRMS (FAB) calcd for $C_{15}H_{25}N_4O_2S$: 293.1978. Found: 293.1975.

(2*S*)-1-{[(2*S*,4*S*,5*S*)-4-(1-Azetidinylcarbonyl)-5-methyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (107)

Yield 68%. A white powder. TLC R_f = 0.41 (CH_2Cl_2/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 291 $(M+H)^+$; IR (KBr) 2951, 1671, 1644, 1465, 1456, 1441, 1379, 1222, 1155, 1121, 1031, 681, 574 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.20 (d, *J* = 6.7 Hz, 3H), 1.91−2.25 (m, 7H), 2.27 (s, 3H), 2.52−2.73 (m, 1H), 3.14 (q, *J* = 7.6 Hz, 1H), 3.49−3.60 (m, 2H), 3.75−3.94 (m, 3H), 4.16 (t, *J* = 7.6 Hz, 2H), 4.43−4.57 (m, 1H), 4.79 (dd, *J* = 7.7, 5.2 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 8.21 (s, 1H), 9.67 (s, 1H).

(2*S*)-1-{[(2*S*,4*S*,5*S*)-5-Methyl-4-(1-pyrrolidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (108)

Yield 93%. A white powder. TLC R_f = 0.51 ($CHCl_3$/MeOH, 5/1); MS (FAB, pos.) *m/z* 305$(M+H)^+$; IR (KBr) 2975, 2880, 2242, 1666, 1637, 1449, 1226, 1169, 1121, 1009, 681 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.17 (d, *J* = 6.7 Hz, 3H), 1.70−2.25 (m, 9H), 2.27 (s, 3H), 2.54−2.73 (m, 1H), 3.21−3.37 (m, 2H), 3.36−3.60 (m, 5H), 3.84−4.01 (m, 1H), 4.40−4.61 (m, 1H), 4.80 (dd, *J* = 7.8, 5.3 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 8.20 (s, 1H), 9.68 (s, 1H); Anal. Calcd for $C_{23}H_{32}N_4O_5S$: C, 57.96; H, 6.77; N, 11.76. Found: C, 57.96; H, 6.75; N, 11.55.

(2*S*)-1-{[(2*S*,4*S*,5*S*)-5-Methyl-4-(1-piperidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (109)

Yield 87%. A pale yellow powder. TLC R_f = 0.44 ($CHCl_3$/MeOH, 4/1); MS (APCI, pos. 20 V) *m/z* 319 $(M+H)^+$; IR (KBr) 2939, 2242, 1665, 1644, 1561, 1447, 1370, 1248, 1227, 1167,

1122, 1032, 1009, 681, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, J = 6.7 Hz, 3H), 1.22−1.66 (m, 6H), 1.92−2.26 (m, 5H), 2.27 (s, 3H), 2.52−2.66 (m, 1H), 3.28−3.73 (m, 7H), 3.76−3.93 (m, 1H), 4.39−4.61 (m, 1H), 4.80 (dd, J = 7.8, 5.3 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 8.24 (s, 1H), 9.57 (s, 1H); HRMS (FAB) calcd for $C_{17}H_{27}N_4O_2$: 319.2134. Found: 319.2130.

(2*S*)-1-{[(2*S*,4*S*,5*S*)-5-Methyl-4-(4-morpholinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (110)

Yield 93%. A white powder. TLC R_f = 0.43 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 321 $(M+H)^+$; IR (KBr) 2242, 1654, 1448, 1230, 1169, 1119, 1032, 1009, 681, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.09−1.30 (m, 3H), 1.88−2.26 (m, 5H), 2.27 (s, 3H), 2.55−2.74 (m, 1H), 3.33−3.69 (m, 11H), 3.80−3.96 (m, 1H), 4.46−4.59 (m, 1H), 4.72−4.87 (m, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 8.27 (s, 1H), 9.63 (s, 1H).

(2*S*)-1-{[(2*S*,4*S*,5*S*)-5-Methyl-4-(4-thiomorpholinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (111)

Yield 99%. A white powder. TLC R_f = 0.60 ($CHCl_3$/MeOH, 4/1); MS (APCI, pos. 20 V) *m/z* 337 $(M+H)^+$; IR (KBr) 2242, 1652, 1450, 1221, 1198, 1168, 1122, 1032, 1009, 682 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.17 (d, J = 6.7 Hz, 3H), 1.91−2.10 (m, 2H), 2.11−2.26 (m, 2H), 2.28 (s, 3H), 2.52−2.75 (m, 3H), 3.47−3.87 (m, 11H), 4.40−4.61 (m, 1H), 4.81 (dd, J = 7.6, 5.1 Hz, 1H), 7.10 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 8.2 Hz, 2H), 8.17−8.38 (m, 1H), 9.51−9.71 (m, 1H); HRMS (FAB) calcd for $C_{16}H_{25}N_4O_2S$: 337.1698. Found: 337.1689.

(2*S*)-1-{[(2*S*,4*S*,5*S*)-4-(1,3-Dihydro-2*H*-isoindol-2-ylcarbonyl)-5-methyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (112)

Yield 98%. A white powder. TLC R_f = 0.29 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 353 $(M+H)^+$; IR (KBr) 2980, 2243, 1656, 1449, 1370, 1227, 1166, 1121, 1032, 1008, 681, 566 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.23 (d, J = 6.7 Hz, 3H), 1.95−2.33 (m, 5H), 2.28 (s, 3H), 2.65−2.80 (m, 1H), 3.46−3.65 (m, 3H), 3.94−4.08 (m, 1H), 4.50−4.64 (m, 1H), 4.64−4.74 (m, 2H), 4.82 (dd, J = 7.8, 5.3 Hz, 1H), 4.86−5.01 (m, 2H), 7.10 (d, J = 8.2 Hz, 2H), 7.23−7.41 (m, 4H), 7.47 (d, J = 8.2 Hz, 2H), 8.13−8.50 (m, 1H), 9.57−9.96 (m, 1H); HRMS (FAB) calcd for $C_{20}H_{25}N_4O_2$: 353.1978. Found: 353.1975.

1-Benzyl 2-*tert*-butyl 4-methyl (2*S*)-5-ethyl-2,3-dihydro-1*H*-pyrrole-1,2,4-tricarboxylate (135)

To a stirred solution of lithium bis(trimethylsilyl)amide in THF (100 mL, 1.0 M) was added dropwise a solution of **113** (14.0 g, 40 mmol) in THF (40 mL) at −78 ºC. After being stirred for 30 min, the reaction mixture was treated with propionyl chloride (5.2 mL, 60 mmol) and

stirred at −78 ºC for additional 2 h. The reaction was quenched with 5% aqueous $KHSO_4$ and extracted with EtOAc. The organic layer was successively washed with aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated. To a stirred solution of the resulting residue in CH_2Cl_2 (40 mL) was added trifluoroacetic acid (3.1 mL, 40 mmol) at 0 ºC. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc and treated with 1M NaOH. The organic layer was successively washed with aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/4) as an eluant to yield **135** (10.1 g, 65%) as a white powder. TLC R_f = 0.78 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, J = 7.5 Hz, 3H), 1.36 (s, 9H), 2.67 (dd, J = 15.9, 3.9 Hz, 1H), 3.02−3.29 (m, 3H), 3.71 (s, 3H), 4.63 (dd, J = 12.0, 4.2 Hz, 1H), 5.16 (s, 2H), 7.30−7.38 (m, 5H).

(2*S*,4*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-ethyl-4-(methoxycarbonyl)-2-pyrrolidinecarboxylic acid (137)

To a solution of **135** (10.0 g, 25.7 mmol) in AcOH (50 mL) was added platinum(IV) oxide (1.0 g, 4.4 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 8 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. A solution of the resulting residue in trifluoroacetic acid (18 mL) and water (2 mL) was stirred at room temperature for 19 h. The reaction mixture was evaporated. To a stirred solution of the resulting residue in THF (3 mL) and water (13 mL) were added $NaHCO_3$ (10.8 g, 129 mmol) and a solution of di-*tert*-butyl-dicarbonate (6.72 g, 31 mmol) in THF (10 mL) at room temperature. After being stirred for 15 h, the reaction was quenched with 2 M HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to yield **137** (3.60 g, 47%). TLC R_f = 0.33 (EtOAc); MS (APCI, pos. 20 V) *m/z* 302 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.2 Hz, 3H), 1.30−1.50 (m, 2H), 1.48 (s, 9H), 2.30−2.50 (m, 1H), 2.60−2.85 (m, 1H), 3.08−3.18 (m, 1H), 3.72 (s, 3H), 4.20-4.26 (m, 1H), 4.30−4.43 (m, 1H).

1-*tert*-Butyl 3-methyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-2-ethyl-1,3-pyrrolidinedicarboxylate (138)

To a stirred solution of **137** (3.58 g, 11.9 mmol) in CH_2Cl_2 (12 mL) were added L-prolinamide (1.49 g, 13.1 mmol), 1-hydroxybenzotriazole (1.84 g, 11.9 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.73 g, 14.3 mmol) at room temperature. After being stirred for 3 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated to give **138** (4.77 g) , which was used for the next reaction without further purification.

(2*S*,3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-2-eth

yl-3-pyrrolidinecarboxylic acid (139)

To a stirred solution of **138** (4.77 g, 11.9 mmol) in MeOH (24 mL) was added 1 M LiOH (13 mL) at 0 ºC. After being stirred at room temperature for 4 h, the reaction was quenched with 2 M HCl (18 mL). The organic solvent was removed by evaporation. The resulting residue was diluted with EtOH. Insoluble substance was removed by filtration. The filtrate was evaporated to yield **139** (4.56 g), which was used for the next reaction without further purification.

3-Benzyl 1-*tert*-butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-2-ethyl-1,3-pyrrolidinedicarboxylate (140)

To a stirred solution of **139** (4.56 g, 11.9 mmol) in DMF (24 mL) were added K_2CO_3 (1.86 g, 13.5 mmol) and benzylbromide (4.3 mL, 36 mmol) at room temperature. After being stirred for 15 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, and evaporated to yield **140** (5.63 g), which was used for the next reaction without further purification.

3-Benzyl 1-*tert*-butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-ethyl-1,3-pyrrolidinedicarboxylate (141)

To a stirred solution of **140** (5.63 g, 11.9 mmol) in THF (40 mL) were added pyridine (2.4 mL, 30 mmol) and trifluoroacetic anhydride (1.85 mL, 13 mmol) at 0 ºC. After being stirred for 30 min, the reaction was quenched with water and extracted with EtOAc. The organic layer was successively washed with 1 M HCl, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/1) as an eluant to yield **141** (2.34 g, 43% from **138**). TLC R_f = 0.29 (EtOAc/hexane, 1/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.78−0.91 (m, 3H), 1.20−1.50 (m, 2H), 1.29 and 1.38 (s, 9H), 1.90−2.20 (m, 6H), 3.30−3.68 (m, 3H), 3.97−4.05 (m, 1H), 4.35−4.43 (m, 1H), 4.78−4.83 (m, 1H), 5.10−5.18 (m, 2H), 7.30−7.42 (m, 5H).

(2*S*,3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-ethyl-3-pyrrolidinecarboxylic acid (142)

To a solution of **141** (1.27 g, 2.79 mmol) in EtOAc (5.5 mL) and THF (3 mL) was added 20 % palladium hydroxide on carbon (127 mg). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 20 min. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield **142** (1.01 g, 99%). TLC R_f = 0.20 (EtOAc/hexane, 2/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86−0.98 (m, 3H), 1.20−1.50 (m, 2H), 1.29 and 1.38 (s, 9H), 1.85−2.45 (m, 6H), 3.09−3.20 (m, 1H), 3.40−3.65 (m, 2H), 3.95−4.05 (m, 1H), 4.35−4.42 (m, 1H), 4.78−4.83 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-(dimethylcarbamoyl)-2-ethyl-1-pyrrolidinecarboxylate (143)**

To a stirred solution of **142** (300 mg, 0.82 mmol) in CH_2Cl_2 (2 mL) were added dimethylamine hydrochloride (134 mg, 1.64 mmol), triethylamine (0.40 mL, 2.9 mmol), 1-hydroxybenzotriazole (125 mg, 0.82 mmol) and 1-(3-dimetylaminopropyl)-3-ethylcarbodiimide hydrochloride (235 mg, 1.23 mmol) at room temperature. After being stirred for 4 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was recrystallized from EtOAc and hexane to yield **143** (162 mg, 50%) as a white powder. TLC R_f = 0.59 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 0.84 (t, J = 7.5 Hz, 3H), 1.25−1.40 (m, 1H), 1.37 (s, 9H), 1.44−1.60 (m, 1H), 2.00−2.33 (m, 6H), 2.83 (brs, 3H), 3.04 (brs, 3H), 3.33−3.42 (m, 1H), 3.55−3.63 (m ,1H), 4.01−4.09 (m, 1H), 4.37 (t, J = 6.9 Hz, 1H), 4.78−4.83 (m, 1H).

(2*S*,3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-2-ethyl-*N*,*N*-dimethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (123)

A solution of **143** (162 mg, 0.41 mmol) and *p*-toluenesulfonic acid (86 mg, 0.45 mmol) in EtOH (2 mL) was stirred at 90 ºC for 2 h. After cooling to room temperature, the resulting precipitates were collected by filtration and dried under reduced pressure to yield **123** (188 mg, 98%) as a white powder. TLC R_f = 0.44 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 293 $(M+H)^+$; IR (KBr) 3459, 2972, 1661, 1556, 1496, 1454, 1223, 1167, 1121, 1033, 1009, 682, 567 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (t, J = 7.4 Hz, 3H), 1.43−1.79 (m, 2H), 1.90−2.31 (m, 5H), 2.28 (s, 3H), 2.58−2.78 (m, 1H), 2.84 (s, 3H), 3.03 (s, 3H), 3.43− 3.62 (m, 3H), 3.62−3.77 (m, 1H), 4.46−4.63 (m, 1H), 4.81 (dd, J = 7.8, 5.3 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.97−8.28 (m, 1H), 9.60−9.89 (m, 1H); HRMS (FAB) calcd for $C_{15}H_{25}N_4O_2$: 293.1978. Found: 293.1976.

(2*S*,4*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-4-(methoxycarbonyl)-5-methyl-2-pyrrolidinecarboxylic acid (144)

A solution of **115b** (1.14 g, 4.69 mmol) in trifluoroacetic acid (9 mL) and water (1 mL) was stirred at room temperature for 3 h. The reaction mixture was evaporated. To a stirred solution of the resulting residue in THF (10 mL) and water (10 mL) were added $NaHCO_3$ to adjust to pH 9 and then di-*tert*-butyl-dicarbonate (1.54 g, 7.04 mmol) at room temperature. After being stirred for 17 h, the reaction mixture was acidified with 10% aqueous citric acid and extracted with EtOAc. The organic layer was dried over $MgSO_4$, and evaporated to yield **144** (1.45 g), which was used for the next reaction without further purification.

1-*tert*-Butyl 3-methyl (2*S*,3*R*,5*S*)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-2-methyl-1,3-pyrrolidinedicarboxylate (145)

Compound **145** was prepared as a white powder in 65% yield from **144** according to the same

procedures as described for the preparation of **138** from **137**. TLC R_f = 0.23 (EtOAc/MeOH, 20/1); MS (APCI, pos. 20 V) *m/z* 384 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35−1.50 (m, 12H), 1.70−2.20 (m, 4H), 2.30−2.50 (m, 2H), 2.80−3.05 (m, 1H), 3.50−3.90 (m, 2H), 3.72 (s, 3H), 4.07−4.48 (m, 2H), 4.54−4.73 (m, 2H), 5.30 and 5.74 (brs, 1H), 6.83 and 6.98 (s, 1H).

(2*S*,3*R*,5*S*)-5-{[(2*S*)-2-(Aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylic acid (146)

To a stirred solution of **145** (430 mg, 1.12 mmol) in MeOH (3 mL) was added 2 M NaOH (0.67 mL) at 0 ºC. After being stirred at room temperature for 3 h, the reaction was quenched with 2 M HCl (0.67 mL). The organic solvent was removed by evaporation. The resulting residue was diluted with EtOH. Insoluble substance was removed by filtration and the filtrate was evaporated to yield **146** (412 mg), which was used for the next reaction without further purification.

***tert*-Butyl (2*S*,3*R*,5*S*)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (147)**

Compound **147** was prepared as a white powder in 90% yield from **146** according to the same procedures as described for the preparation of **143** from **142**. TLC R_f = 0.48 (EtOAc/MeOH/H_2O, 3/1/1); MS (APCI, pos. 20 V) *m/z* 397 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35−1.50 (m, 12H), 1.70−2.20 (m, 5H), 2.25−2.60 (m, 2H), 2.95−2.97 (m, 3H), 3.01−3.05 (m, 3H), 3.50−3.90 (m, 2H), 4.00−4.17 (m, 1H), 4.57−4.80 (m, 2H), 5.30 and 5.50 (s, 1H), 6.76 and 7.04 (s, 1H).

***tert*-Butyl (2*S*,3*R*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-[(dimethylamino)carbonyl]-2-methyl-1-pyrrolidinecarboxylate (148)**

Compound **148** was prepared as a white powder in 40% yield from **147** according to the same procedures as described for the preparation of **141** from **140**. TLC R_f = 0.31 (EtOAc/MeOH, 10/1); MS (APCI, pos. 20 V) *m/z* 379 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.31 (d, *J* = 6.3 Hz, 3H), 1.18 (s, 9H), 1.93−2.35 (m, 6H), 3.09−3.17 (m, 1H), 3.55−3.64 (m, 2H), 3.97−4.04 (m, 1H), 4.51−4.58 (m, 1H), 4.76−4.81 (m, 1H).

(2*S*,3*R*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*,2-trimethyl-3-pyrrolidinecarboxamide hydrochrolide (124)

To a stirred solution of **148** (153 mg, 0.40 mmol) in EtOAc (1 mL) was added 4M HCl in EtOAc (1 mL). After being stirred for 4 h, the reaction mixture was concentrated in vacuo. The resulting solid was washed with EtOAc to yield **124** (137 mg, 100%) as a white powder. TLC R_f = 0.58 (CH_2Cl_2/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 279 $(M+H)^+$; IR (KBr) 3423, 2944, 2244, 1639, 1508, 1452, 1403, 1256, 1191, 1156, 637 cm^{-1}; ^{1}H NMR (300 MHz,

DMSO-d_6) δ 1.32 (d, J = 6.5 Hz, 3H), 1.93−2.44 (m, 6H), 2.85 (s, 3H), 2.98 (s, 3H), 3.04−3.22 (m, 1H), 3.29−3.69 (m, 2H), 3.71−3.88 (m, 1H), 4.52−4.69 (m, 1H), 4.77−4.87 (m, 1H), 8.64 (s, 1H), 10.41 (s, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 279.1821. Found: 279.1819.

1-*tert*-Butyl 2-ethyl (2*S*,4*R*)-4-[(benzyloxy)methyl]-5-oxo-1,2-pyrrolidinedicarboxylate (149a) and 1-*tert*-Butyl 2-ethyl (2*S*,4*S*)-4-[(benzyloxy)methyl]-5-oxo-1,2-pyrrolidinedic arboxylate (149b)

To a stirred solution of lithium bis(trimethylsilyl)amide in THF (22 mL ,1.0 M) was added dropwise a solution of **24** (5.15 g, 20.0 mmol) in THF (20 mL) and HMPA (5 mL) at −78 ºC. After being stirred for 1 h, the reaction mixture was added to a stirred solution of benzyloxymethyl chloride (5.5 mL, 40 mmol) in THF (10 mL) at −78 ºC and stirred for additional 1 h. The reaction was quenched with 1 M NH_4Cl and extracted with ^{t}BuOMe. The organic layer was successively washed with aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1/2) as an eluant to yield **149a** (2.20 g, 27%) and **149b** (1.37 g, 18%) as a colorless oil. **149a** : TLC R_f = 0.35 (EtOAc/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 378 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, J = 7.1 Hz, 3H), 1.49 (s, 9H), 1.99−2.13 (m, 1H), 2.45−2.60 (m, 1H), 2.80−2.94 (m, 1H), 3.66 (dd, J = 9.3, 7.3 Hz, 1H), 3.76 (dd, J = 9.3, 4.2 Hz, 1H), 4.04−4.24 (m, 2H), 4.44−4.59 (m, 3H), 7.17−7.45 (m, 5H). **149b** : TLC R_f = 0.42 (EtOAc/hexane, 1/2); MS (APCI, pos. 20 V) *m/z* 378 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (q, J = 7.1 Hz, 3H), 1.50 (s, 9H), 2.10−2.24 (m, 1H), 2.29−2.47 (m, 1H), 2.77−2.99 (m, 1H), 3.60−3.84 (m, 2H), 4.22 (q, J = 7.1 Hz, 2H), 4.42−4.66 (m, 3H), 7.21−7.41 (m, 5H).

1-*tert*-Butyl 2-ethyl (2*S*,4*R*)-4-[(benzyloxy)methyl]-5-methoxy-1,2-pyrrolidinedicarboxy late (150)

To a stirred solution of **149a** (1.41 g, 3.74 mmol) in THF (20 mL) was added a solution of lithium triethylborohydride in THF (4.5 mL, 1.0 M) at −78 ºC. After being stirred for 30 min, the reaction was quenched with aqueous $NaHCO_3$ and warmed up to 0 ºC. After the addition of 30% H_2O_2 (2 mL), the reaction mixture was stirred at 0 ºC. After being stirred for 30 min, the reaction mixture was evaporated to remove organic solvent and extracted with ^{t}BuOMe. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. To a stirred solution of the resulting residue in MeOH (20 mL) was added *p*-toluenesulfonic acid (142 mg, 0.74 mmol) at room temperature. After being stirred for 18 h, the reaction was quenched with aqueous $NaHCO_3$. The reaction mixture was evaporated to remove organic solvent and extracted with ^{t}BuOMe. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to yield **150** (1.71 g), which was used for the next reaction without further purification.

1-*tert*-Butyl 2-ethyl (2*S*,4*S*,5*R*)-4-[(benzyloxy)methyl]-5-methyl-1,2-pyrrolidinedicarbox

ylate (151)

To a stirred suspension of copper (I) bromide-dimethyl sulfide complex (3.58 g, 17.4 mmol) in Et_2O (34 mL) was added MeMgBr in Et_2O (5.8 mL, 3.0 M) at −40 ºC. After being stirred for 1 h, the reaction mixture was cooled to −78 ºC and treated with boron trifluoride etherate (2.2 mL, 17 mmol). After being stirred for 30 min, to the above described reaction mixture was added a solution of **150** (1.71 g, 3.74 mmol) in Et_2O (6 mL). After being stirred for 15 min, the reaction mixture was warmed up to room temperature. After 1 h, the reaction was quenched with a mixture of saturated NH_4Cl aq (10 mL) and 28% NH_3 aq (10 mL). After being stirred for 30 min, the reaction mixture was extracted with ^{t}BuOMe. The organic layer was successively washed with H_2O, brine, then dried over $MgSO_4$, and concentrated in vacuo. To a stirred solution of the resulting residue in THF (10 mL) were added aqueous $NaHCO_3$ and di-*tert*-butyl-dicarbonate (816 mg, 3.74 mmol) at room temperature. After being stirred for 3 h, the reaction mixture was extracted with EtOAc. The organic layer was washed with brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (3/7) as an eluant to yield **151** (732 mg, 45%) as a colorless oil. TLC R_f = 0.57 (EtOAc/hexane, 7/3); MS (APCI, pos. 20 V) *m/z* 378 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.18 (t, *J* = 7.0 Hz, 3H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.37 (s, 9H), 1.67−1.78 (m, 1H), 2.06−2.20 (m, 1H), 2.37−2.47 (m, 1H), 3.37 (dd, *J* = 9.7, 7.3 Hz, 1H), 3.51 (dd, *J* = 9.7, 7.5 Hz, 1H), 3.65−3.77 (m, 1H), 4.01−4.13 (m, 2H), 4.19 (dd, *J* = 9.8, 4.1 Hz, 1H), 4.39−4.53 (m, 2H), 7.21−7.39 (m, 5H).

1-*tert*-Butyl 2-ethyl (2*S*,4*S*,5*R*)-4-(hydroxymethyl)-5-methyl-1,2-pyrrolidinedicarboxylate (152)

To a solution of **151** (732 mg, 1.94 mmol) in EtOH (10 mL) and AcOH (1 mL) was added 10 % palladium on carbon (200 mg). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 3 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield **152** (566 mg, 100%) as a colorless oil. TLC R_f = 0.53 (CH_2Cl_2/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 378 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19−1.35 (m, 6H), 1.37−1.50 (m, 9H), 1.74−1.95 (m, 1H), 1.97−2.18 (m, 1H), 2.39−2.58 (m, 1H), 3.48−3.64 (m, 1H), 3.65−3.80 (m, 1H), 3.83−4.03 (m, 1H), 4.05−4.40 (m, 3H)

(2*R*,3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-(ethoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylic acid (153)

To a stirred solution of **152** (566 mg, 1.94 mmol) in acetone (5 mL) was added Jones reagent (1 mL) at 0 ºC. After being stirred for 2 h at room temperature, the reaction was quenched with ice-water. The reaction mixture was extracted with EtOAc. The organic layer was successively washed with H_2O, brine, then dried over $MgSO_4$, and concentrated in vacuo to yield **153** (544 mg, 93%) as a colorless oil. R_f = 0.47 (CH_2Cl_2/MeOH, 9/1); ^{1}H NMR (300

MHz, $CDCl_3$) δ 1.21−1.33 (m, 6H), 1.37−1.50 (m, 9H), 2.44−2.52 (m, 2H), 2.64−2.76 (m, 1H), 4.00−4.64 (m, 4H)

1-*tert*-Butyl 2-ethyl (2*S*,4*S*,5*R*)-4-(dimethylcarbamoyl)-5-methyl-1,2-pyrrolidinedicarboxylate (154)

Compound **154** was prepared as a colorless oil in 70% yield from **153** according to the same procedures as described for the preparation of **37** from **36**. TLC R_f = 0.35 (acetone/hexane, 1/2); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09−1.28 (m, 6H), 1.28−1.42 (m, 9H), 1.83−1.97 (m, 1H), 2.36−2.46 (m, 1H), 2.80 (s, 3H), 2.98 (s, 3H), 3.00−3.12 (m, 1H), 3.83−3.97 (m, 1H), 3.96−4.14 (m, 2H), 4.21 (dd, J = 8.6, 5.9 Hz, 1H)

(2*S*,4*S*,5*R*)-1-(*tert*-Butoxycarbonyl)-4-(dimethylcarbamoyl)-5-methyl-2-pyrrolidinecarboxylic acid (155)

To a stirred solution of **154** (200 mg, 0.61 mmol) in MeOH (2 mL) was added 1 M NaOH (1.2 mL) at room temperature. After being stirred at 60 ºC for 3 h, the reaction mixture was cooled to 0 ºC and the reaction was quenched with 1 M HCl (1.2 mL). The organic solvent was removed by evaporation. The resulting residue was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to yield **155** (159 mg, 87%) as a colorless oil. TLC R_f = 0.46 (EtOAc/AcOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (d, J = 6.0 Hz, 3H), 1.30−1.41 (m, 9H), 1.80−1.92 (m, 1H), 2.35−2.46 (m, 1H), 2.81 (s, 3H), 2.99 (s, 3H), 3.01−3.10 (m, 1H), 3.82−3.95 (m, 1H), 4.12 (t, J = 7.8 Hz, 1H), 12.33−12.54 (m, 1H).

***tert*-Butyl (2*R*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (156)**

To a stirred solution of **155** (159 g, 0.53 mmol) in CH_2Cl_2 (2 mL) were added (2*S*)-2-cyanopyrrolidine 4-methylbenzenesulfonate (116 mg, 0.61 mmol), 1-hydroxybenzotriazole (74 mg, 0.61 mmol), triethylamine (0.085 mL, 0.61 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (116 mg, 0.61 mmol) at room temperature. After being stirred for 15 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 1M HCl, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (3/7) as an eluant to yield **156** (130 mg, 75%) as a colorless oil. TLC R_f = 0.38 (EtOAc/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (d, J = 6.0 Hz, 3H), 1.35 (s, 9H), 1.55−1.80 (m, 1H), 1.98−2.25 (m, 4H), 2.40−2.56 (m, 1H), 2.91−2.97 (m, 6H), 2.96−3.07 (m, 1H), 3.45−3.67 (m, 2H), 3.90−4.12 (m, 1H), 4.49 (dd, J = 8.8, 7.7 Hz, 1H), 4.71−4.81 (m, 1H).

(2*R*,3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*,2-trimethyl-3-pyrrolidinecar

boxamide 4-methylbenzenesulfonate (125)

Compound **125** was prepared as a white powder in 57% yield from **156** according to the same procedures as described for the preparation of **10** from **29a**. TLC R_f = 0.35 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 279$(M+H)^+$; IR (KBr) 3057, 2239, 1663, 1646, 1619, 1455, 1369, 1225, 1167, 682 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.29 (d, *J* = 6.5 Hz, 3H), 1.61−1.80 (m, 1H), 1.94−2.08 (m, 2H), 2.08−2.25 (m, 2H), 2.28 (s, 3H), 2.84 (s, 3H), 2.87−2.99 (m, 1H), 3.02 (s, 3H), 3.07−3.26 (m, 1H), 3.55 (t, *J* = 6.5 Hz, 2H), 3.73−3.85 (m, 1H), 4.49−4.66 (m, 1H), 4.82 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 8.86−9.41 (m, 2H); Anal. Calcd for $C_{21}H_{30}N_4O_5S$: C, 55.98; H, 6.71; N, 12.43. Found: C, 55.83; H, 6.80; N, 12.27.

1-*tert*-Butyl 2-ethyl (2*S*,4*S*)-4-[(benzyloxy)methyl]-5-methoxy-1,2-pyrrolidinedicarboxylate (157)

Compound **157** was prepared from **149b** according to the same procedures as described for the preparation of **150** from **149a**, which was used for the next reaction without further purification.

1-*tert*-Butyl 2-ethyl (2*S*,4*R*)-4-[(benzyloxy)methyl]-5-methyl-1,2-pyrrolidinedicarboxylate (158)

Compound **158** was prepared as a colorless oil in 41% from **157** according to the same procedures as described for the preparation of **151** from **150**. TLC R_f = 0.65 (acetone/hexane, 1/2); ^1H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 0.95−1.32 (m, 6H), 1.38 (s, 9H), 1.73−2.69 (m, 3H), 3.31−3.55 (m, 2H), 3.61−4.28 (m, 4H), 4.42−4.55 (m, 2H), 7.14−7.43 (m, 5H)

1-*tert*-Butyl 2-ethyl (2*S*,4*R*)-4-(hydroxymethyl)-5-methyl-1,2-pyrrolidinedicarboxylate (159)

Compound **159** was prepared as a colorless oil in 83% from **158** according to the same procedures as described for the preparation of **152** from **151**. TLC R_f = 0.42 (acetone/hexane, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.03−1.39 (m, 3H), 1.27 (q, *J* = 7.1 Hz, 3H), 1.39−1.50 (m, 9H), 1.84−2.79 (m, 3H), 3.51−3.76 (m, 2H), 4.04−4.39 (m, 4H).

(3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-(ethoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylic acid (160)

Compound **160** was prepared as a colorless oil in 90% from **159** according to the same procedures as described for the preparation of **153** from **152**. TLC R_f = 0.44 (CH_2Cl_2/MeOH, 9/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.02−1.21 (m, 3H), 1.22−1.33 (m, 3H), 1.38−1.50 (m, 9H), 2.00−2.29 (m, 1H), 2.42−2.70 (m, 1H), 3.26−3.57 (m, 1H), 4.05−4.55 (m, 4H).

1-*tert*-Butyl 2-ethyl

(2*S*,4*R*,5*R*)-4-(dimethylcarbamoyl)-5-methyl-1,2-pyrrolidinedicarboxylate (161)

Compound **161** was prepared as a colorless oil in 70% yield from **160** according to the same procedures as described for the preparation of **37** from **36**. TLC R_f = 0.40 (acetone/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96−1.10 (m, 3H), 1.22−1.33 (m, 3H), 1.37−1.51 (m, 9H), 1.85−2.00 (m, 1H), 2.75−2.94 (m, 1H), 2.93−2.99 (m, 3H), 3.04−3.11 (m, 3H), 3.34−3.54 (m, 1H), 4.05−4.50 (m, 4H).

(2*S*,4*R*,5*R*)-1-(*tert*-Butoxycarbonyl)-4-(dimethylcarbamoyl)-5-methyl-2-pyrrolidinecarboxylic acid (162)

Compound **162** was prepared as a white powder in 81% yield from **161** according to the same procedures as described for the preparation of **155** from **154**. TLC R_f = 0.50 (EtOAC/AcOH, 10/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.79−0.94 (m, 3H), 1.29−1.43 (m, 9H), 1.68−1.87 (m, 1H), 2.53−2.75 (m, 1H), 2.81 (s, 3H), 2.98 (s, 3H), 3.32−3.44 (m, 1H), 4.08−4.30 (m, 2H).

***tert*-Butyl (2*R*,3*R*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (163)**

Compound **163** was prepared as a white powder in 75% yield from **162** according to the same procedures as described for the preparation of **156** from **155**. TLC R_f = 0.38 (acetone/hexane, 1/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95 (d, J = 6.6 Hz, 3H), 1.35 (s, 9H), 1.72 (dd, J = 12.7, 6.1 Hz, 1H), 2.00−2.11 (m, 2H), 2.13−2.23 (m, 2H), 2.63−2.77 (m, 1H), 2.80−3.08 (m, 6H), 3.42−3.64 (m, 3H), 4.19−4.34 (m, 1H), 4.49 (d, J = 9.2 Hz, 1H), 4.70−4.81 (m, 1H).

(2*R*,3*R*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*,2-trimethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (126)

Compound **126** was prepared as a white powder in 75% yield from **163** according to the same procedures as described for the preparation of **10** from **29a**. TLC R_f = 0.45 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 279$(M+H)^+$; IR (KBr) 3449, 2957, 2243, 1667, 1644, 1217, 1204, 1186, 1169, 567 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (d, J = 6.8 Hz, 3H), 1.91−2.08 (m, 3H), 2.08−2.26 (m, 2H), 2.28 (s, 3H), 2.65−2.80 (m, 1H), 2.87 (s, 3H), 3.02 (s, 3H), 3.49−3.64 (m, 2H), 3.64−3.75 (m, 1H), 3.83−4.01 (m, 1H), 4.60−4.74 (m, 1H), 4.83 (dd, J = 8.0, 4.9 Hz, 1H), 7.10 (d, J = 8.1 Hz, 2H), 7.46 (d, J = 8.1 Hz, 2H), 8.99 (s, 1H), 9.20 (s, 1H); Anal. Calcd for $C_{21}H_{30}N_4O_5S$: C, 55.98; H, 6.71; N, 12.43. Found: C, 55.27; H, 6.71; N, 12.03.

***tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-3-(diazoacetyl)-2-methyl-1-pyrrolidinecarboxylate (164)**

To a stirred solution of **119b** (100 mg, 0.23 mmol) in THF (2 mL) were added triethylamine (0.038 mL, 0.27 mmol) and ethyl chloroformate (0.026 mL, 0.27 mmol) at 0 ºC. After being stirred at room temperature for 2 h, the reaction mixture was treated with a solution of

diazomethane in Et_2O and stirred for 2 h. The reaction was quenched with H_2O and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (6/1) as an eluant to yield **164** (38 mg, 42%) as a colorless oil. TLC R_f = 0.32 (EtOAc/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 394 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18−1.30 (m, 3H), 1.35−1.50 (m, 9H), 1.70−2.60 (m, 6H), 3.04−3.17 (m, 1H), 3.50−3.80 (m, 2H), 4.10−4.50 (m, 2H), 4.55−4.72 (m, 1H), 5.23−5.54 (m, 2H), 6.97−7.12 (m, 1H).

***tert*-Butyl (2*S*,3*R*,5*S*)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-3-(2-methoxy-2-oxoethyl)-2-methyl-1-pyrrolidinecarboxylate (165)**

To a stirred solution of **164** (279 mg, 0.71 mmol) in MeOH (2 mL) were added triethylamine (0.10 mL, 0.72 mmol) and silver benzoate (8 mg, 0.03 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (6/1) as an eluant to yield **165** (219 mg, 78%) as a colorless oil. TLC R_f = 0.48 (EtOAc/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 398 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.03 (d, J = 6.6 Hz, 3H), 1.35 (s, 9H), 1.42−1.63 (m, 1H), 1.83−2.06 (m, 5H), 2.26−2.58 (m, 3H), 3.46−3.58 (m, 2H), 3.63 (s, 3H), 3.92−4.04 (m, 1H), 4.26−4.52 (m, 2H), 6.68 (s, 2H).

[(2*S*,3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-2-methyl-3-pyrrolidinyl]acetic acid (166)

Compound **166** was prepared from **165** according to the same procedures as described for the preparation of **119b** from **119a**. This compound was used for the next reaction without further purification.

***tert*-Butyl (2*S*,3*R*,5*S*)-5-{[(2*S*)-2-carbamoyl-1-pyrrolidinyl]carbonyl}-3-[2-(dimethylamino)-2-oxoethyl]-2-methyl-1-pyrrolidinecarboxylate (167)**

Compound **167** was prepared as a white powder in 85% yield from **166** according to the same procedures as described for the preparation of **37** from **36**. TLC R_f = 0.50 (EtOAc/MeOH/AcOH, 5/1/0.1); MS (APCI, pos. 20 V) *m/z* 398 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11−1.23 (m, 3H), 1.36−1.49 (m, 9H), 1.52−1.73 (m, 1H), 1.81−2.83 (m, 8H), 2.92−2.98 (m, 3H), 2.98−3.03 (m, 3H), 3.43−3.84 (m, 2H), 4.07−4.73 (m, 3H), 5.22−5.57 (m, 1H), 6.86−7.09 (m, 1H).

***tert*-Butyl (2*S*,3*R*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-[2-(dimethylamino)-2-oxoethyl]-2-methyl-1-pyrrolidinecarboxylate (168)**

Compound **168** was prepared as a white powder in 40% yield from **167** according to the same procedures as described for the preparation of **120** from **119c**. TLC R_f = 0.30 (hexane/acetone, 1/1); MS (APCI, pos. 20 V) *m/z* 393 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 ºC) δ 1.03

(d, *J* = 6.6 Hz, 3H), 1.35 (s, 9H), 1.42−1.57 (m, 1H), 2.00−2.20 (m, 4H), 2.24−2.60 (m, 3H), 3.50−3.70 (m, 2H), 3.98−4.08 (m, 1H), 4.55 (dd, *J* = 7.2, 6.6 Hz, 1H), 4.70−4.80 (m, 1H).

2-((2*S*,3*R*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-pyrrolidinyl)-*N*,*N*-dimethylacetamide 4-methylbenzenesulfonate (127)

Compound **127** was prepared as a white powder in 100% yield from **168** according to the same procedures as described for the preparation of **10** from **29a**. TLC R_f = 0.30 ($CHCl_3$/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 293$(M+H)^+$; IR (KBr) 2949, 2242, 1645, 1452, 1221, 1122, 1033, 1009, 682, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.13 (d, *J* = 7.0 Hz, 3H), 1.52−1.69 (m, 1H), 1.91−2.25 (m, 6H), 2.28 (s, 3H), 2.59−2.74 (m, 2H), 2.80 (s, 3H), 2.93 (s, 3H), 3.43−3.67 (m, 2H), 3.80−3.95 (m, 1H), 4.40−4.55 (m, 1H), 4.80 (dd, *J* = 7.9, 4.9 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 8.25 (s, 1H), 9.58 (s, 1H).

4-Benzyl 1-*tert*-butyl 2-methyl (2*S*)-5-methyl-2,3-dihydro-1*H*-pyrrole-1,2,4-tricarboxylate (170)

Compound **170** was prepared as a white powder in 82% yield from **169** according to the same procedures as described for the preparation of **135** from **113**. TLC R_f = 0.55 (EtOAc/hexane, 1/3); ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.64 (s, 3H), 2.69−2.80 (m, 1H), 2.97−3.30 (m, 1H), 3.75 (s, 3H), 4.67 (dd, *J* = 12.4, 5.2 Hz, 1H), 5.16 (s, 2H), 7.16−7.48 (m, 5H).

(5*S*)-1-(*tert*-Butoxycarbonyl)-5-(methoxycarbonyl)-2-methyl-4,5-dihydro-1*H*-pyrrole-3-carboxylic acid (171)

To a solution of **170** (230 g, 613 mmol) in MeOH (900 mL) was added 10 % palladium on carbon (23 g). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was recrystallized from $^iPr_2O$−hexane to yield **171** (161 g, 92%) as a white powder. TLC R_f = 0.25 (EtOAc/hexane, 1/3); ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.64 (s, 3H), 2.67−2.77 (m, 1H), 2.93−3.19 (m, 1H), 3.77 (s, 3H), 4.69 (dd, *J* = 12.5, 5.1 Hz, 1H).

1-*tert*-Butyl 2-methyl (2*S*)-4-(dimethylcarbamoyl)-5-methyl-2,3-dihydro-1*H*-pyrrole-1,2-dicarboxylate (172)

Compound **172** was prepared as a white powder in 100% yield from **171** according to the same procedures as described for the preparation of **143** from **142** TLC R_f = 0.57 (CH_2Cl_2/MeOH, 10/1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.17 (s, 3H), 2.57−2.69 (m, 1H), 2.99 (s, 6H), 3.09−3.29 (m, 1H), 3.76 (s, 3H), 4.69 (dd, *J* = 12.0, 4.9 Hz, 1H).

1-*tert*-Butyl 2-methyl (2*S*,4*S*,5*S*)-4-(dimethylcarbamoyl)-5-methyl-1,2-pyrrolidinedicarboxylate (173a)

To a solution of **172** (11 g, 35.1 mmol) in AcOH (120 mL) was added platinum(IV) oxide (3.0 g, 13.2 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 5 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was dissolved in EtOAc. The solution was successively washed with water, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and concentrated in vacuo to yield **173a** (10.3 g, 93%) as a colorless oil. TLC R_f = 0.55 (CH_2Cl_2/MeOH, 10/1) ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07−1.19 (m, 3H), 1.38−1.52 (m, 9H), 2.21−2.37 (m, 1H), 2.53−2.77 (m, 1H), 2.93−3.01 (m, 3H), 3.03−3.13 (m, 3H), 3.17−3.38 (m, 1H), 3.69−3.80 (m, 3H), 4.06−4.45 (m, 2H)

(2*S*,4*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-4-(dimethylcarbamoyl)-5-methyl-2-pyrrolidinecarboxylic acid (174)

To a stirred solution of **173a** (10.3 g, 32.8 mmol) in THF (100 mL) was added 2 M NaOH (21.3 mL) at room temperature. After being stirred for 23 h, the reaction was quenched with 2 M HCl (21.3 mL). The reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, concentrated in vacuo, and recrystallized from EtOAc to yield **174** (5.62 g, 57%) as a white powder. TLC R_f = 0.36 (CH_2Cl_2/MeOH, 10/1) ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (d, J = 6.6 Hz, 3H), 1.44 (s, 9H), 2.25−2.44 (m, 1H), 2.58−2.83 (m, 1H), 2.98 (s, 3H), 3.08 (s, 3H), 3.17−3.43 (m, 1H), 4.12−4.56 (m, 2H), 8.11 (s, 1H).

(2*S*,4*S*,5*S*)-1-[(Benzyloxy)carbonyl]-4-(dimethylcarbamoyl)-5-methyl-2-pyrrolidinecarboxylic acid (175)

To a stirred solution of **174** (5.62 g, 18.7 mmol) in CH_2Cl_2 (20 mL) was added 4 N HCl in dioxane (45 mL) at room temperature. After being stirred for 1 h, the reaction mixture was concentrated in vacuo. To a stirred solution of the resulting residue in H_2O (20 mL) − ^{i}Pr_2O (5 mL) were added $NaHCO_3$ (6.28 g, 74.8 mmol) and a solution of benzyloxycarbonyl chloride (3.0 mL, 21 mmol) in ^{i}Pr_2O (15 mL). After being stirred for 15 h, the reaction was quenched with 2 M HCl and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over MgSO4, and concentrated in vacuo to yield **175** (5.44 g, 87%) as a white powder. TLC R_f = 0.22 (CH_2Cl_2/MeOH, 10/1) ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, J = 6.2 Hz, 3H), 2.32−2.51 (m, 1H), 2.64−2.81 (m, 1H), 2.98 (s, 3H), 3.07 (s, 3H), 3.21−3.36 (m, 1H), 4.20−4.55 (m, 2H), 5.02−5.26 (m, 2H), 7.20−7.44 (m, 5H).

Benzyl (2*S*,3*S*,5*S*)-5-[(2-amino-2-oxoethyl)(methyl)carbamoyl]-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (176)

To a stirred solution of **175** (340 mg, 1.02 mmol) in CH_2Cl_2 (7 mL) were added oxaly chloride (0.10 mL, 1.12 mmol) and DMF (one drop) at room temperature. After being stirred for 20 min, the reaction mixture was concentrated in vacuo. To a stirred solution of the resulting residue in CH_2Cl_2 (5 mL) were added *N*-methylglycinamide hydrochloride (152 mg, 1.22

mmol) and triethylamine (0.36 mmol, 2.54 mmol). After being stirred for 1 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated to give **176** (270 mg, 66%). TLC R_f = 0.36 (EtOAc/MeOH, 10/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01−1.48 (m, 6H), 2.09−2.39 (m, 1H), 2.53−2.80 (m, 1H), 2.78−3.13 (m, 9H), 3.24−3.40 (m, 1H), 4.19−5.51 (m, 5H), 6.20 and 6.40 (s, 1H), 7.24−7.41 (m, 5H), 7.68 (s, 1H)

tert-Butyl (2*S*,3*S*,5*S*)-5-[(cyanomethyl)carbamoyl]-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (178a)

To a stirred solution of **174** (300 mg, 1.0 mmol) in CH_2Cl_2 (4 mL) were added pyridine (0.25 mL, 3.0 mmol) and cyanuric fluoride (0.085 mL, 1.0 mmol) at 0 ºC. After being stirred for 1 h, the reaction was quenched with ice-water. Insoluble substance was removed by filtration. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. To a stirred solution of the resulting residue in 1,2-dichloroethane (4 mL) were added aminoacetonitrile (84 mg, 1.5 mmol) and pyridine (0.16 mL, 2 mmol) at room temperature. After being stirred for 30 min at 60 ºC, the reaction was quenched with 10% aqueous citric acid and extracted with CH_2Cl_2. The organic layer was successively washed with aqueous $NaHCO_3$, brine, then dried over Na_2SO_4, and concentrated in vacuo. The resulting solid was washed with ^{t}BuOMe to yield **178a** (250 mg, 74%) as a white powder. TLC R_f = 0.41 (CH_2Cl_2/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (d, J = 6.6 Hz, 3H), 1.47 (s, 9H), 2.25−2.42 (m, 1H), 2.54−2.79 (m, 1H), 2.97 (s, 3H), 3.08 (s, 3H), 3.22−3.34 (m, 1H), 3.97−4.12 (m, 1H), 4.17 (dd, J = 10.4, 7.7 Hz, 1H), 4.26−4.47 (m, 2H), 6.38−6.82 (m, 1H).

tert-Butyl (2*S*,3*S*,5*S*)-5-[(cyanomethyl)(methyl)carbamoyl]-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (178b)

To a solution of **176** (261 mg, 0.645 mmol) in THF (6 mL) was added 10 % palladium on carbon (55 mg). After being stirred at room temperature under an atmospheric pressure of hydrogen for 2 h, the catalyst was removed by filtration. To the filtrate was added a solution of di-*tert*-butyl-dicarbonate (169 mg, 0.774 mmol) in THF (6 mL). After being stirred for 1 h, the reaction mixture was treated with pyridine (0.26 mL, 3.23 mmol) and trifluoroacetic anhydride (0.14 mL, 0.97 mmol) and stirred for additional 30 min. The reaction was quenched with water and extracted with EtOAc. The organic layer was successively washed with 1 M HCl, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (40/1) as an eluant to yield **178b** (170 mg, 75%) as a white powder. TLC R_f = 0.31 (CH_2Cl_2/MeOH, 10/1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (d, J = 6.6 Hz, 3H), 1.26−1.39 (m, 9H), 2.11−2.23 (m, 2H), 2.81 (s, 3H), 3.01 (s, 3H), 3.11 (s, 3H), 4.06−4.31 (m, 2H), 4.36−4.67 (m, 3H).

According to the same procedures as described for the preparation of **178a** from **174**,

compounds **178c−g** were prepared from **174**.

***tert*-Butyl (2*S*,3*S*,5*S*)-5-[(cyanomethyl)(ethyl)carbamoyl]-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (178c)**

Yield 75%. A white powder. R_f = 0.21 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, *J* = 6.6 Hz, 3H), 1.34 (t, *J* = 7.1 Hz, 3H), 1.39−1.49 (m, 9H), 2.17−2.37 (m, 1H), 2.53−2.71 (m, 1H), 2.96 (s, 3H), 3.05−3.13 (m, 3H), 3.23−3.39 (m, 1H), 3.47−3.71 (m, 2H), 3.98−4.70 (m, 4H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-[(cyanomethyl)(propyl)carbamoyl]-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (178d)**

Yield 82%. A white powder. R_f = 0.28 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91−1.05 (m, 3H), 1.16 (d, *J* = 6.6 Hz, 3H), 1.40−1.48 (m, 9H), 1.63−1.83 (m, 2H), 2.13−2.37 (m, 1H), 2.56−2.77 (m, 1H), 2.96−2.99 (m, 3H), 3.05−3.11 (m, 3H), 3.19−3.66 (m, 3H), 3.91−4.73 (m, 4H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-[allyl(cyanomethyl)carbamoyl]-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (178e)**

Yield 80%. A white powder. R_f = 0.29 (EtOAc); MS (APCI, pos.) *m/z* 379 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (d, *J* = 6.6 Hz, 3H), 1.40−1.51 (m, 9H), 2.15−2.29 (m, 1H), 2.56−2.77 (m, 1H), 2.96 (s, 3H), 3.03−3.13 (m, 3H), 3.18−3.35 (m, 1H), 3.90−4.78 (m, 6H), 5.24−5.44 (m, 2H), 5.70−6.07 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-[(cyanomethyl)(cyclopropyl)carbamoyl]-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (178f)**

Yield 73%. A white powder. R_f = 0.24 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81−1.10 (m, 4H), 1.12−1.22 (m, 3H), 1.41−1.48 (m, 9H), 2.20−2.41 (m, 1H), 2.50−2.68 (m, 1H), 2.80−3.02 (m, 4H), 3.05−3.14 (m, 3H), 3.23−3.40 (m, 1H), 3.99−4.16 (m, 1H), 4.17−4.46 (m, 1H), 4.49−4.62 (m, 1H), 4.88−5.05 (m, 1H).

***tert*-Butyl (2*S*,3*S*,5*S*)-5-[(cyanomethyl)(2-propyn-1-yl)carbamoyl]-3-(dimethylcarbamoyl)-2-methyl-1-pyrrolidinecarboxylate (178g)**

Yield 61%. A white powder. R_f = 0.32 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, *J* = 6.6 Hz, 3H), 1.34−1.51 (m, 9H), 2.25−2.51 (m, 2H), 2.55−2.76 (m, 1H), 2.97 (s, 3H), 3.05−3.14 (m, 3H), 3.19−3.42 (m, 1H), 4.06−4.83 (m, 6H)

According to the same procedures as described for the preparation of **10** from **29a**, compounds **128-134** were prepared from **178a-g**, respectively.

(2*S*,4*S*,5*S*)-*N*-(Cyanomethyl)-4-(dimethylaminocarbonyl)-5-methyl-2-pyrrolidinecarboxa

mide 4-methylbenzenesulfonate (128)

Yield 100%. A white powder. TLC R_f = 0.33 (CH_2Cl_2/MeOH, 4/1); MS (APCI, pos. 20 V) *m/z* 239(M+H)$^+$; IR (KBr) 1632, 1551, 1495, 1214, 1160, 1120, 1031, 1007, 680, 566 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11 (d, *J* = 6.8 Hz, 3H), 2.13−2.27 (m, 1H), 2.28 (s, 3H), 2.39 −2.48 (m, 1H), 2.83 (s, 3H), 2.99 (s, 3H), 3.57−3.68 (m, 1H), 3.86−4.00 (m, 1H), 4.18−4.35 (m, 3H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 8.23−8.39 (m, 1H), 9.16 (t, *J* = 5.6 Hz, 1H), 9.59 (s, 1H); HRMS (FAB) calcd for $C_{11}H_{19}N_4O_2$: 239.1508. Found: 239.1515.

(2*S*,4*S*,5*S*)-*N*-(Cyanomethyl)-*N*,5-dimethyl-4-(dimethylcarbamoyl)-2-pyrrolidinecarboxa mide 4-methylbenzenesulfonate (129)

Yield 100%. A white powder. TLC R_f = 0.50 (CH_2Cl_2/MeOH, 5/1); MS (APCI, pos. 20 V) *m/z* 253(M+H)$^+$; IR (KBr) 3426, 2940, 2250, 1665, 1635, 1183, 1124, 1034, 1010, 685 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, *J* = 6.8 Hz, 3H), 2.02−2.17 (m, 1H), 2.28 (s, 3H), 2.56 −2.71 (m, 1H), 2.83 (s, 3H), 3.00 (s, 3H), 3.06 (s, 3H), 3.56−3.68 (m, 1H), 3.81−3.95 (m, 1H), 4.39 (d, *J* = 18.0 Hz, 1H), 4.56 (d, *J* = 18.0 Hz, 1H), 4.63−4.76 (m, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 8.04−8.22 (m, 1H), 9.57−9.71 (m, 1H).

(2*S*,4*S*,5*S*)-*N*-(Cyanomethyl)-*N*-ethyl-4-(dimethylcarbamoyl)-5-methyl-2-pyrrolidinecar boxamide 4-methylbenzenesulfonate (130)

Yield 100%. A white powder. TLC R_f = 0.29 (CH_2Cl_2/MeOH, 4/1); MS (APCI, pos. 20 V) *m/z* 267(M+H)$^+$; IR (KBr) 2975, 2939, 2245, 1654, 1637, 1217, 1162, 1120, 1008, 566 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05−1.27 (m, 6H), 1.99−2.19 (m, 1H), 2.28 (s, 3H), 2.52−2.70 (m, 1H), 2.83 (s, 3H), 3.00 (s, 3H), 3.32−3.52 (m, 2H), 3.63 (q, *J* = 7.4 Hz, 1H), 3.82−4.01 (m, 1H), 4.31−4.58 (m, 2H), 4.57−4.84 (m, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 7.99−8.38 (m, 1H), 9.44−9.84 (m, 1H); HRMS (FAB) calcd for $C_{13}H_{23}N_4O_2$: 267.1821. Found: 267.1818.

(2*S*,4*S*,5*S*)-*N*-(Cyanomethyl)-4-(dimethylcarbamoyl)-5-methyl-*N*-propyl-2-pyrrolidineca rboxamide 4-methylbenzenesulfonate (131)

Yield 89%. A white powder. TLC R_f = 0.53 ($CHCl_3$/MeOH, 4/1); MS (APCI, pos. 20 V) *m/z* 281(M+H)$^+$; IR (KBr) 3464, 3112, 2246, 1655, 1458, 1223, 1163, 1120, 1008, 680 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83−0.93 (m, 3H), 1.16 (d, *J* = 6.8 Hz, 3H), 1.46−1.77 (m, 2H), 2.01−2.17 (m, 1H), 2.28 (s, 3H), 2.55−2.72 (m, 1H), 2.83 (s, 3H), 3.00 (s, 3H), 3.32 (t, *J* = 7.5 Hz, 2H), 3.59−3.72 (m, 1H), 3.85−3.97 (m, 1H), 4.31−4.63 (m, 2H), 4.59−4.78 (m, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 8.08−8.23 (m, 1H), 9.57−9.74 (m, 1H); HRMS (FAB) calcd for $C_{14}H_{25}N_4O_2$: 281.1978. Found: 281.1988.

(2*S*,4*S*,5*S*)-*N*-Allyl-*N*-(Cyanomethyl)-4-(dimethylcarbamoyl)-5-methyl-2-pyrrolidinecarb oxamide 4-methylbenzenesulfonate (132)

Yield 90%. A white powder. TLC R_f = 0.57 ($CHCl_3$/MeOH, 4/1); MS (APCI, pos. 20 V) *m/z* 279(M+H)$^+$; IR (KBr) 3448, 2246, 1661, 1636, 1560, 1496, 1224, 1120, 1008, 681 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.16 (d, *J* = 6.8 Hz, 3H), 1.99−2.19 (m, 1H), 2.28 (s, 3H), 2.53 −2.69 (m, 1H), 2.83 (s, 3H), 3.00 (s, 3H), 3.56−3.70 (m, 1H), 3.84−3.95 (m, 1H), 3.96−4.17 (m, 2H), 4.21−4.53 (m, 2H), 4.59−4.76 (m, 1H), 5.22−5.39 (m, 2H), 5.73−5.96 (m, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 8.12−8.26 (m, 1H), 9.58−9.76 (m, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 279.1821. Found: 279.1819.

(2*S*,4*S*,5*S*)-*N*-(Cyanomethyl)-*N*-cyclopropyl-4-(dimethylcarbamoyl)-5-methyl-2-pyrrolidinecarboxamide 4-methylbenzenesulfonate (133)

Yield 100%. A white powder. TLC R_f = 0.42 (CH_2Cl_2/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 279(M+H)$^+$; IR (KBr) 1639, 1442, 1364, 1226, 1161, 1119, 1031, 1008, 680, 565 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83−1.06 (m, 4H), 1.15 (d, *J* = 6.8 Hz, 3H), 2.18−2.34 (m, 1H), 2.28 (s, 3H), 2.58−2.73 (m, 1H), 2.81−2.94 (m, 1H), 2.84 (s, 3H), 3.01 (s, 3H), 3.59−3.74 (m, 1H), 3.90−4.05 (m, 1H), 4.35−4.54 (m, 2H), 4.72−4.84 (m, 1H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 8.07−8.31 (m, 1H), 9.33−9.64 (m, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 279.1821. Found: 279.1820.

(2*S*,4*S*,5*S*)-*N*-(Cyanomethyl)-4-(dimethylcarbamoyl)-5-methyl-*N*-propargyl-2-pyrrolidinecarboxamide 4-methylbenzenesulfonate (134)

Yield 100%. A white powder. TLC R_f = 0.44 (CH_2Cl_2/MeOH, 9/1); MS (APCI, pos. 20 V) *m/z* 277(M+H)$^+$; IR (KBr) 2120, 1667, 1634, 1466, 1161, 1120, 1032, 1008, 680, 566 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, *J* = 6.8 Hz, 3H), 2.04−2.26 (m, 1H), 2.28 (s, 3H), 2.57 −2.77 (m, 1H), 2.84 (s, 3H), 3.01 (s, 3H), 3.36−3.71 (m, 2H), 3.83−3.97 (m, 1H), 4.21−4.84 (m, 5H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 8.10−8.35 (m, 1H), 9.58−9.79 (m, 1H); HRMS (FAB) calcd for $C_{14}H_{21}N_4O_2$: 277.1665. Found: 277.1663.

4-16-2. Biological methods

Multiple oral glucose tolerance tests in rats

The effect of inhibitor **72** and **105** on the outcome of multiple oral glucose tolerance tests was assessed in male SD rats (364-473 g). The rats were fasted for at least 20 h before the study, and then were dosed orally with the vehicle (0.5% methyl cellulose) or with compound **72** or **105** (1 mg/kg) at -0.5 h. Blood samples (75 μl) were collected from the tail vein into heparinized tubes at -0.08 h. Glucose (1 g/kg) was administered orally at 0, 6, and 12 h. Additional blood samples (75 μl) were collected at 0.17, 0.5, 1, 2, 6, 6.17, 6.5, 7, 8, 12, 12.17, 12.5, 13, and 14 h after the first glucose load. Plasma was obtained from each sample by centrifugation and was stored at -80 °C until measurement of the glucose level, with a glucose oxidase peroxidase dye system (Diacolor GC, Toyobo, Japan).

Enzyme assays

Enzymatic activity was determined at 37 °C by the cleavage rate of a substrate, Gly-Pro-AMC (30 μM) (Sigma-Aldrich, USA).[15] Briefly, 10 μL of DPP-IV solution was added to each well of a 96-well flat-bottomed microtiter plate, followed by the addition of 50 μL of 60 μM Gly-Pro-AMC, 10 μL of 500 mM Tris-HCl (pH 7.4), 20 μL of distilled water, and 10 μL of a test compound. Then the change of fluorescence was monitored at 37 °C using a spectrofluorometer (excitation at 355 nm/ emission at 460 nm) (fmax, Molecular Devices, USA). The initial rate of DPP-IV activity was calculated over the first 15 min of the reaction, and was defined as the rate of increase in the fluorescence intensity (arbitrary units 1 mL) under these conditions. The percent inhibition was calculated relative to the addition of the solvent alone and IC_{50} values were determined by logistic regression analysis. To study slow binding, the apparent inhibitory potency of compound **17**, **72** or **105** was determined as a function of the preincubation time in a standard IC_{50} experiment. Test compounds were preincubated with the enzyme for 0, 10, 30, 60, or 120 min, and then the reaction was initiated by adding the substrate.

To study dissociation rate,

Dissociation rate was determined at 37 °C by the cleavage rate of a substrate, Gly-Pro-AMC (30 μM) (Sigma-Aldrich, USA).[15] Briefly, 10 μL of DPP-IV solution was added to each well of a 96-well flat-bottomed microtiter plate, followed by the addition of 10 μL of a test compound (1 μM for **17** and **105**, 0.3 μM for **72**), 10 μL of 500 mM Tris-HCl (pH 7.4), and 70 μL of distilled water. After the preincubation for 90 min, 10 μL of reaction mixture was diluted with 90 μL of 33 μM Gly-Pro-AMC (30 μM). Then the change of fluorescence was monitored for 120 min at 37 °C using a spectrofluorometer (excitation at 355 nm/ emission at 460 nm) (fmax, Molecular Devices, USA)

DPP-VIII Enzyme assays

Reaction solutions containing 10 μL of a test compound, 10 μL of DPP-VIII enzyme preparations, 5 μL of 1 mM Tris-HCl (pH 7.4), 10 μL of 100 mM EDTA, 15 μL of distilled water and 50 μL of 200 μM Gly-Pro-AMC were incubated at 37 °C using a 96-well flat-bottomed microtiter plate. The change of fluorescence was monitored at 37 °C using a spectrofluorometer (excitation at 355 nm/ emission at 460 nm) (fmax, Molecular Devices, USA). The initial rate of DPP-VIII enzyme activity was calculated over the first 15 min of the reaction, with units/mL being defined as the rate of increase in the fluorescence intensity (arbitrary units) under these conditions. The percent inhibition relative to addition of the solvent alone was calculated and IC_{50} values were determined by logistic analysis.

DPP-IX Enzyme assays

Reaction solutions containing 10 μL of a test compound, 10 μL of DPP-IX enzyme preparations, 10 μL of 500 mM Tris-HCl (pH 7.4), 20 μL of distilled water and 50 μL of 60 μM Gly-Pro-AMC were incubated at 37 °C using a 96-well flat-bottomed microtiter plate. The change of fluorescence was monitored at 37 °C using a spectrofluorometer (excitation at 355 nm/ emission at 460 nm) (fmax, Molecular Devices, USA). The initial rate of DPP-IX enzyme activity was calculated over the first 15 min of the reaction, with units/mL being defined as the rate of increase in the fluorescence intensity (arbitrary units) under these conditions. The percent inhibition relative to addition of the solvent alone was calculated and IC_{50} values were determined by logistic analysis.

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第五章　結論

近年、Glucagon-like peptide-1（GLP-1）の作用増強をターゲットとした創薬は次世代の2型糖尿病治療薬として注目されている。数多くの製薬会社がGLP-1の代謝酵素であるDipeptidyl peptidase IV（DPP-IV）の阻害剤の開発に取り組んでいる。本研究は、患者の生活の質（QOL）の改善を目的とし、長時間作用の持続するDPP-IV阻害剤の開発を目指し行われた。

本研究においては、あまり研究のされていなかったプロリル-2-シアノピロリジン**7**からリード化合物が創製できないか検討した。化合物**7**からリード化合物を創製するためには、①低い投与量を可能にする高い酵素阻害活性、②一日一回投与を可能にする持続性、③分子内環化反応に対する安定性という3つの課題を解決する必要があった。これら3つの課題を解決するためには、P2位のピロリジン環の4位に置換基を導入するのが妥当と考察し、様々な置換基の導入を行った。その結果、2位のカルボニル基と*cis*配置でフェニル基またはジメチルアミノカルボニルメチル基を導入した化合物**8**, **17**において、これら3つの性質すべてを満たす可能性が示唆され、2種の異なる骨格のリード化合物を見出すことができた。

4-フェニルピロリジン体**8**はラットにおいて持続性を示したが、化合物**8**の血中濃度からは持続性が説明できず、活性代謝物の存在が示唆された。化合物**8**の代謝物は水酸化、さらにグルクロン酸抱合を受けた構造であることを明らかにした。水酸化の位置をベンゼン環上と推定し、フェノール誘導体を合成し、代謝物のグルクロニダーゼ処理により、パラ位のフェノール体**45**が化合物**8**の代謝の中間体であることが明らかとなった。化合物**45**はラットにおいて持続性を示した。そこで、フェノール体の分子内環化反応に対する安定性を高め、阻害剤としての最適化を目指した。オルト位のフェノール体**43**においてフェノールの酸性プロトンが分子内環化反応を促進していることを見出し、オルト位の置換基が分子内環化反応の際にニトリル基の近傍に存在しうると考察した。その考察を基に、２ヶ所のオルト位に置換基を導入したフェノール誘導体を設計し合成した。その結果、分子内環化反応に対する安定性、作用持続性の高まった化合物**64**を見出すことに成功した。化合物**64**はグルクロン酸抱合を受けやすい化合物であることがわかり、グルクロン酸抱合体の生成、胆汁排泄、再吸収を繰り返すことにより、持続性が発現していると推定している。

4位アミド誘導体**17**の最適化として、まずアミド部分のメチレン鎖の長さの検討を行った。その結果、ピロリジン環にジメチルアミノカルボニル基が直接結合した化合物**72**においてラットにおける持続性が見られた。化合物**72**のアミノ基部分の最適化を行った結果、比較的小さい二級アミンがラットにおける持続性の面で優れており、4位のアミド部分を嵩高くして分子内環化反応に対する安定性を高める方針を取る事は困難と考えられた。そこで、分子内環化反応に対する安定性を高める目的で5位にメチル基を導入した。その結果、化合物**105**においてラットにおける持続性が飛躍的に向上し、分子内環化反応に対する安定性も高まった。化合物**105**は経口糖負荷試験

においても1 mg/kgという低投与量で、投与12時間後においても抑制傾向が見られ、持続性が確認された。化合物**105**はDPP-IVとの複合体からの解離速度が遅いことが、酵素阻害活性の測定により明らかとなった。この解離速度の遅さが、持続性の発現に寄与していると考えられる。また、解離速度が遅い原因は、DPP-IVの酵素三次元構造より、DPP-IVのチロシン547残基との水を介した水素結合に基づくと推察している。

以上、化合物**7**からの合成展開により、低投与量で作用持続性を示すDPP-IV阻害剤として、化合物**64**、化合物**105**が見出された。また、分子内環化反応に対する安定性を大幅に改善することに成功した。これら化合物は、患者の生活の質（QOL）を改善する糖尿病治療薬の候補化合物、GLP-1に関する研究用のツールとして期待される。

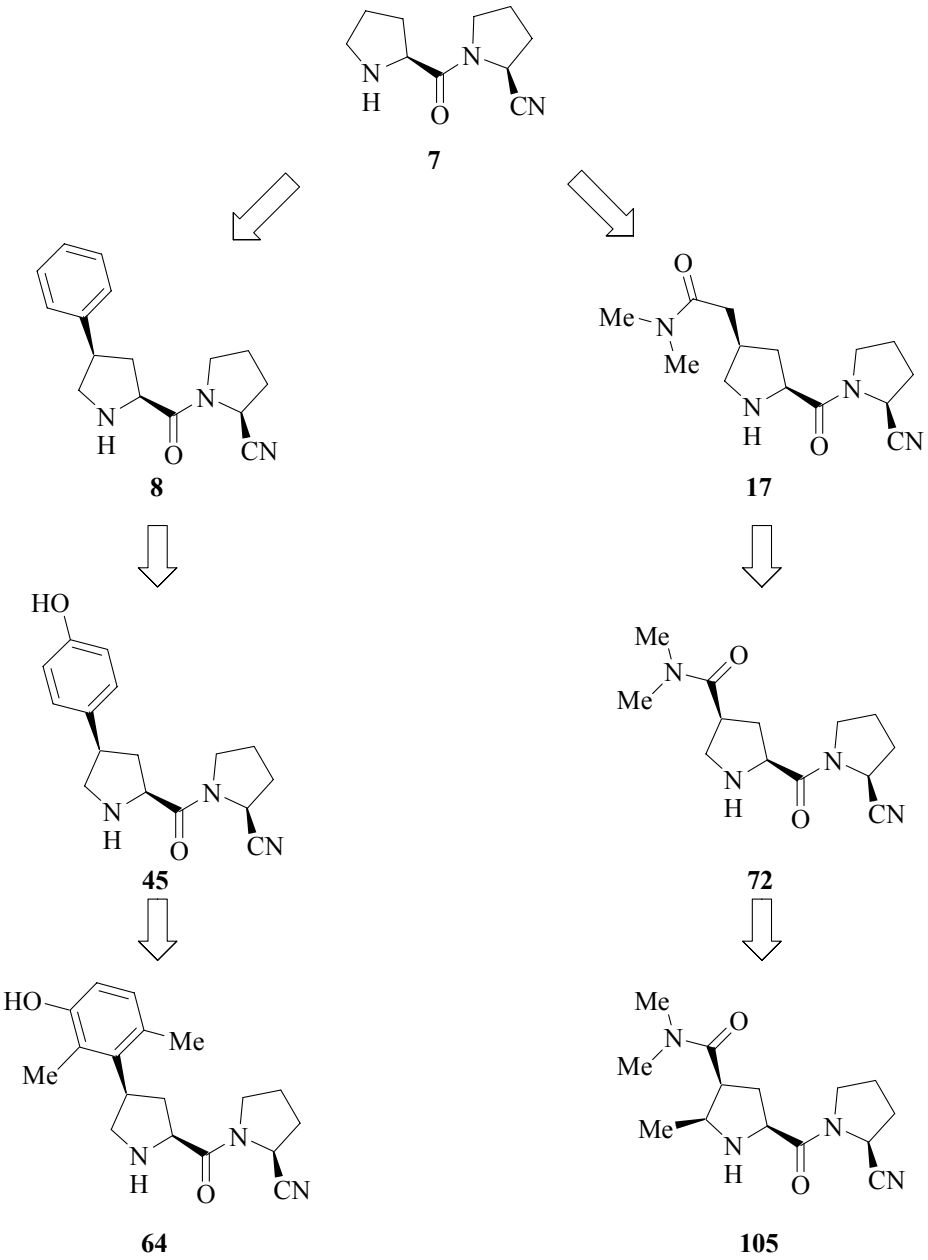

Figure 5-1. プロリル-2-シアノピロリジン**7**の最適化

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