

デカヒドロイソキノリン骨格を有する非ペプチド型

SARS 3CL プロテアーゼ阻害剤の設計と合成



## 論文目録

### 1 学位論文の基礎となる報文の著者名、題目、印刷・公表の方法、その時期および該当する章・節

- 1) Kenichi Akaji, Hiroyuki Konno, Hironori Mitsui, Kenta Teruya, Yasuhiro Shimamoto, Yasunao Hattori, Takeshi Ozaki, Masami Kusunoki, and Akira Sanjoh : Structure-based design, synthesis, and evaluation of peptide-mimetic SARS 3CL protease inhibitors. *J. Med. Chem.*, **54**, 7962—7973 (2011) . [1 章]
- 2) Yasuhiro Shimamoto, Yasunao Hattori, Kazuya Kobayashi, Kenta Teruya, Akira Sanjoh, Atsushi Nakagawa, Eiki Yamashita, and Kenichi Akaji : Fused-ring structure of decahydroisoquinolin as a novel scaffold for SARS 3CL protease inhibitors. *Bioorg. Med. Chem.*, **23**, 876—890 (2015) . [2 章] [3 章]

# 論文要旨

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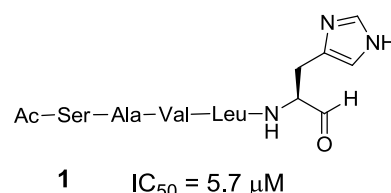
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## 1. はじめに

SARS (severe acute respiratory syndrome: 重症急性呼吸器症候群) は、2002 年 11 月中国広東省で発生し、8000 を超える症例と約 800 人の死者を出した呼吸器疾患である。発生と同時に発症原因の探索が精力的に行われ、新種のコロナウイルスである SARS-CoV (corona virus) が感染の原因であることが明らかにされた。しかし、未だ治療薬やワクチンはない。

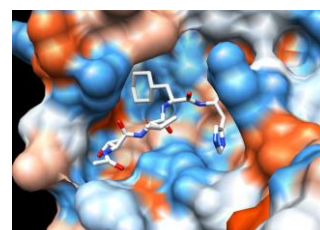
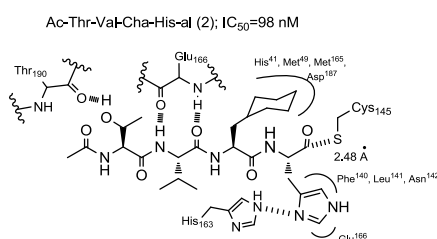
SARS-CoV は一本鎖(+)の RNA を持つコロナウイルスで、その複製に必須となるプロテアーゼ (SARS 3CL<sup>pro</sup>: SARS 3 chymotrypsin like protease) 阻害剤 が有望な抗 SARS 薬として期待されている。申請者の所属研究室ではこれまでに、①野生型 SARS 3CL<sup>pro</sup> が自己分解を起こしやすいこと、②プロテアーゼ 188 位のア르기ニンをイソロイシンに置換した変異プロテアーゼ R188I SARS 3CL<sup>pro</sup> がこの自己分解を起こさず、野生型プロテアーゼの約  $10^6$  倍の酵素活性を有すること、③このため、蛍光試薬などを用いない汎用型 HPLC を用いた再現性の高い阻害剤評価が可能であること、④自己分解抵抗性 R188I SARS 3CL<sup>pro</sup> の大量調製が可能なため、阻害剤との複合体結晶形成による分子レベルでの相互作用解析が容易になること、などを明らかにしてきた。さらに、これらの成果をもとに、プロテアーゼの基質配列に基づくペプチドアルデヒド型阻害剤 **1** (Ac-Ser-Ala-Val-Leu-His-al;  $IC_{50} = 5.7 \mu M$ ) とプロテアーゼ相互作用様式が解明された。

本研究ではまず、これまでの研究で得られた基質ペプチド型阻害剤 **1** とプロテアーゼとの相互作用解析に基づく構造最適化を進め、ナノモルレベルの  $IC_{50}$  値を持つ低分子ペプチドアルデヒド型阻害剤の開発を行った。ついで、 $S_2$  部位での疎水性相互作用に着目することによって、これまでに例のないデカヒドロイソキノリン骨格を有する非ペプチド型 SARS 3CL<sup>pro</sup> 阻害剤の設計と合成を行うとともに、そのプロテアーゼ相互作用解析を行った。



## 2. 低分子ペプチドアルデヒド型阻害剤の設計・合成と R188I SARS 3CL<sup>pro</sup> 相互作用解析

阻害剤 **1** と R188I SARS 3CL<sup>pro</sup> との X 線結晶構造解析の結果から、 $P_1$  ロイシンの側鎖部分は  $S_2$  部位に密に入っておらず、これを埋める構造を導入すれば阻害活性の向上が望めると考えた。また、 $S_4$  部位のアラニン側鎖構造の変換で新たな水素結合の付加が可能ではないかと推測した。さらに、 $S_5$  部位にあたるセリンはプロテアーゼ外側に位置していたので除去することができると考えた。これらの方針のもとに構造最適化を行い、阻害剤



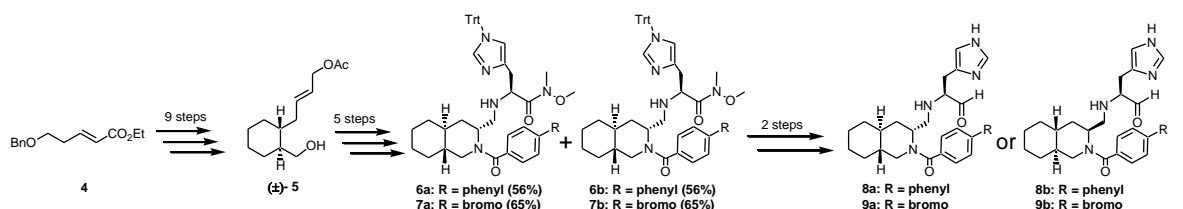


2 (Ac-Thr-Val-Cha-His-al; IC<sub>50</sub> = 98 nM) の創製に成功した (上図)。

### 3. 複合体結晶構造解析に基づく新規縮環型阻害剤の設計と合成

ペプチド型阻害剤の相互作用解析に基づき、デカヒドロイソキノリン骨格を有する非ペプチド型阻害剤の設計を行った。すなわち、P<sub>2</sub>シクロヘキサン環をメチレンリンカーでペプチド主鎖に連結したデカヒドロ

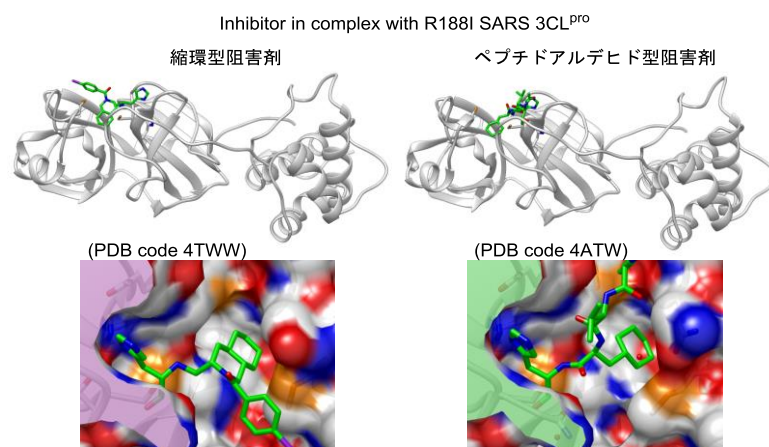
イソキノリン骨格を基本 scaffold とし、P<sub>1</sub>イミダゾール基と活性中心アルデヒド基の導入とともに新たにアシル置換基を付加した (上図)。まず、既知のエステルを出発原料として、4-フェニルベンゾイル基と 4-ブロモベンゾイル基を側鎖にもつジアステレオマー混合物を得た。ついで、HPLC により分離した各ジアステレオマーから単一の立体化学を持つ目的化合物を合成した (下図)。得られた化合物は中程度ながらも明らかな SARS 3CL<sup>pro</sup> 阻害活性を示したため、次に立体化学の決定と構造活性相関研究を行った。



### 4. 縮環型阻害剤の立体化学とプロテアーゼ相互作用解析

まず、文献法を参考にシクロヘキセンカルボン酸塩の光学分割を行い、得られた化合物の立体化学を既知化合物との比旋光度比較によって推定した。ついで、得られたキラルなシクロヘキセンカルボン酸誘導体を出発原料として、上記と同様の合成経路によって目的化合物を合成した。あわせて、異なるアシル置換基を有する化合物合成を行った。得られた化合物は数十  $\mu$  モルレベルの阻害能を示し、アシル基の構造は活性に大きな影響をおよぼさないことが分かった。一方、縮環構造の立体化学の差異により阻害活性が 1/2 から 1/3 に低下することが明らかになった。

最後に、これら阻害剤と R188I SARS 3CL<sup>pro</sup> との複合体構造解析を行い、①縮環型阻害剤とペプチドアルデヒド型阻害剤ではそのノンプライムサイトでの相互作用様式が大きく異なっていること、②縮環部位の立体化学の変化がアシル基とプロテアーゼとの相互作用様式を大きく左右すること、などを明らかにした (右図)。



以上、申請者は本研究によりプロテアーゼ基質配列に基づくペプチドアルデヒド型 SARS 3CL<sup>pro</sup> 阻害剤ならびに疎水性相互作用を核とする新規縮環型阻害剤の創製に成功した。

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## 理論の部

## 略号表

(注) 本論文で使用した略語を下記に示す。

### アミノ酸

Ala: alanine    Arg: arginine    Cha: cyclohexylalanine    Cys: cysteine    Gln: glutamine    Gly: glycine  
His: histidine    Ile: isoleucine    Leu: leucine    Lys: lysine    Phe: phenylalanine    Ser: serine  
Thr: threonine    Val: valine

### 1) その他の略号

CL<sup>pro</sup>: chymotrypsin-like protease  
CoV : coronavirus  
ESI : electrospray ionization  
EI : electron ionization  
FAB : fast atom bombardment  
HPLC : high-performance liquid chromatography  
MALDI : matrix-assisted laser desorption ionization  
MERS : middle east respiratory syndrome  
MS : mass spectrometry  
NMR : nuclear magnetic resonance  
ODS : octadecylsilyl  
PDB : protein data bank  
pp : polyprotein  
PL<sup>pro</sup> : papain-like protease  
RNA : ribonucleic acid  
TOF : time of flight  
SARS : severe acute respiratory syndrome  
WHO : world health organization

### 2) 試薬・溶媒・保護基など略号

Ac : acetyl  
Bn : benzyl  
DEAD : diethyl azodicarboxylate  
DIBALH : diisobutylaluminum hydride  
DIC : *N,N'*-diisopropylcarbodiimide  
DIPEA : diisopropylethylamine  
DMAP : *N,N*-4-dimethylaminopyridine  
DMF : *N,N*-dimethylformamide  
DPPA : diphenylphosphoryl azide

Et : ethyl

Fmoc : 9-fluorenylmethyloxycarbonyl

HBTU : *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate

NBS : *N*-bromosuccinimide

NMO : *N*-methylmorpholine *N*-oxide

HOBt : 1-hydroxybenzotriazole

IBCF : isobutyl chloroformate

LAH : lithium aluminum hydride

PCC : pyridinium chlorochromate

PPh<sub>3</sub> : triphenylphosphine

TBAF : tetra-*n*-butylammonium fluoride

TBDPS : *t*-butyldiphenylsilyl

TFA : trifluoroacetic acid

THF : tetrahydrofuran

TIS : triisopropylsilane

Trt : trityl

## 緒言

SARS は、2002 年 11 月中国広東省で発生し、短期間のうちに世界 30 カ国以上で 8000 を超える症例と約 800 人の死者を出した呼吸器疾患である。発生と同時に発症原因の探索が精力的に行われ、新種のコロナウイルスである SARS-CoV が感染の原因であることが明らかにされた。これにより感染の広がりを食い止めることができ、2003 年 7 月に WHO から終息宣言が出された。<sup>1-3</sup> しかし、未だ治療薬やワクチンはない。また 2005 年、コウモリから、SARS-CoV 類似のウイルスが発見され、新たなパンデミックの可能性が危惧されていた。<sup>4,5</sup> そして 2014 年には、新種の CoV を発症原因とする MERS が中東地域において確認された。<sup>6,7</sup>

SARS-CoV は、現在知られている中でもっとも大きな一本鎖(+)の RNA を持つ CoV である。ウイルス増殖機構は、後天性免疫不全症候群ウイルスなどのレトロウイルスとは違い、自身の RNA から直接複製に必要なプロテアーゼを転写、翻訳する。このとき複製に必要なプロテアーゼを産生するために、PP1a (~450 kDa) と PP1ab (~750 kDa) という 2 つの巨大な複合タンパク質を利用する。<sup>8-10</sup> 細胞に感染した SARS-CoV は自身の RNA を一度 PP1a、PP1ab に転写、翻訳する。この PP を切断し、複製に必要な RNA レプリカーゼなどを産生するためには、SARS 3CL<sup>pro</sup> が必須である (図 1)。このため、SARS 3CL<sup>pro</sup> 阻害剤は有望な抗 SARS 薬として期待されている。<sup>11</sup>

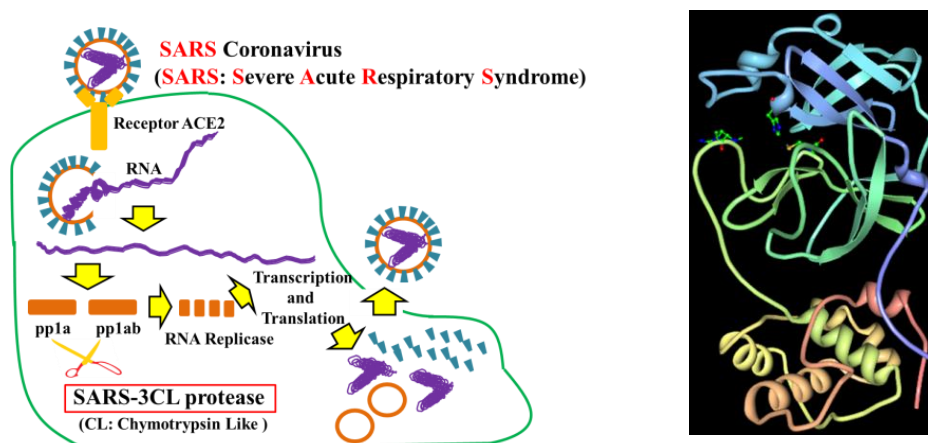


図 1 SARS-CoV の増殖機構と野生型 SARS 3CL<sup>pro</sup> (PDB code 2ZU4)

SARS 3CL<sup>pro</sup> は、3 つのドメインからなる 306 残基のアミノ酸配列を持つシステインプロテアーゼである。また本プロテアーゼはキモトリプシン様構造をとり、ドメイン 1,2 はβシート構造を多く含み、逆にドメイン 3 はαヘリックス構造を持つ特徴的なプロテアーゼである (図 1)。その触媒機構は His<sup>41</sup> により Cys<sup>145</sup> の水素を引き抜き活性化させ、標的蛋白質のペプチド結合の加水分解反応をおこなうものである (図 2)。

SARS 3CL<sup>pro</sup> は活性発現時に自身で二量体化しており、このとき直接活性発現に関与していないドメイン 3 がこの二量体化に関与していると考えられている。

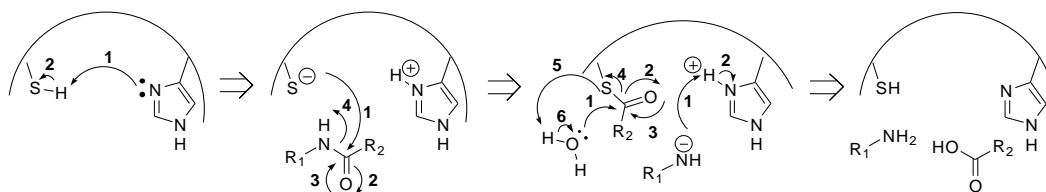


図2 システインプロテアーゼの蛋白質加水分解機構

SARS 3CL<sup>pro</sup> 阻害剤はペプチド型阻害剤と非ペプチド型阻害剤と既に多くの阻害剤が報告されている。これらの阻害剤には、ヒコナウイルス 3C プロテアーゼ阻害剤の構造模倣体や天然物から単離された低分子化合物やハイスループットスクリーニングから見つけれられた非ペプチド型阻害剤などがある (図 3)。<sup>12-26</sup>

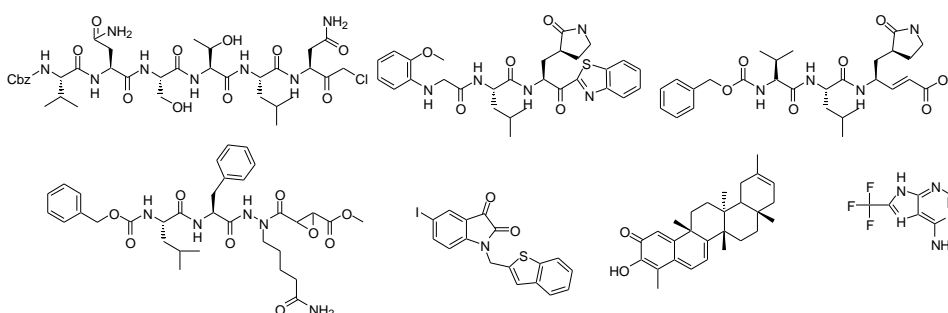
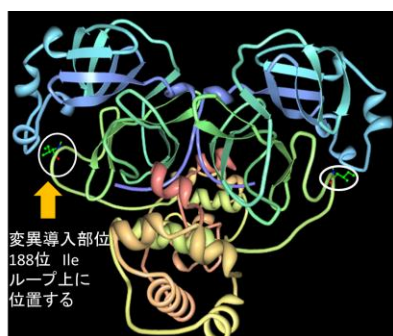


図3 SARS-3CL<sup>pro</sup> 阻害剤

著者の所属する研究室は、評価系構築の際に、野生型 SARS 3CL<sup>pro</sup> が、自己分解をおこしてしまい調整困難であることを確認している。原因の追求を行い、ドメイン 2 と 3 を繋ぐループ構造を形成するアミノ酸の 188 位 Arg を境に自己分解が起こっていることを突き止め、そのモチーフ構造として Ile に置換した分解抵抗性変異プロテアーゼ R188I SARS 3CL<sup>pro</sup> の開発に成功している (図 4)。本変異プロテアーゼを用いる利点は、①評価系内で自己分解による酵素活性の失活がないため、野生型プロテアーゼの約  $10^6$  倍の高い酵素活性を有すること、②本プロテアーゼを用いた評価系は蛍光試薬などを用いることなく、汎用型 HPLC を用いた再現性の高い評価試験が行えること、③プロテアーゼの大量調製が可能のため、阻害剤との共結晶も容易に作成でき迅速に X 線構造解析が行える点が挙げられる (図 4)。<sup>27</sup>



PDB code 3AW1

図4 野生型 SARS 3CL<sup>pro</sup> と R188I SARS 3CL<sup>pro</sup> との触媒効率比較

protease;		R188I mutant	R188I mutant of mature 3CL protease	
		R188I mutant	H	with C-terminal His-tag
protease	tag	K <sub>m</sub> (mM)	k <sub>cat</sub> (s <sup>-1</sup> )	k <sub>cat</sub> /K <sub>m</sub> (k <sub>cat</sub> /K <sub>m</sub> ) rel
wild	-	Not Reported		
	+	1150	0.20	1.7 x 10 <sup>-4</sup>
mutant	-	33.8	4753	156.9
	+	31.8	153	4.8

R188I SARS 3CL<sup>pro</sup>は野生型 SARS 3CL<sup>pro</sup>の188位 Arg を Ile に変換した変異体であるので野生型と比べ活性ポケットの構造に変化が出る可能性がある。その場合野生型とは違う酵素-基質親和性を示し、活性評価の結果の信頼性が危ぶまれる。このため R188I SARS 3CL<sup>pro</sup>の X 線結晶構造解析を行い、その構造がもとの野生型の構造と類似しているかの確認試験が行われた。その結果、導入した Ile の側鎖は、野生型の Arg の側鎖同様、活性ポケットとは反対側を向き活性ポケットに影響を与えていないことが明らかにされた (図 5-A, B)。また野生型と変異体の SARS 3CL<sup>pro</sup>を解析ソフト上で重ねあわせ電子密度の違いを比較した結果、Ileを除いた他のアミノ酸の電子密度は、野生型のプロテアーゼとほとんど同じであり、このことは先の結果を保障するものであった (図 5-C)。<sup>28</sup>

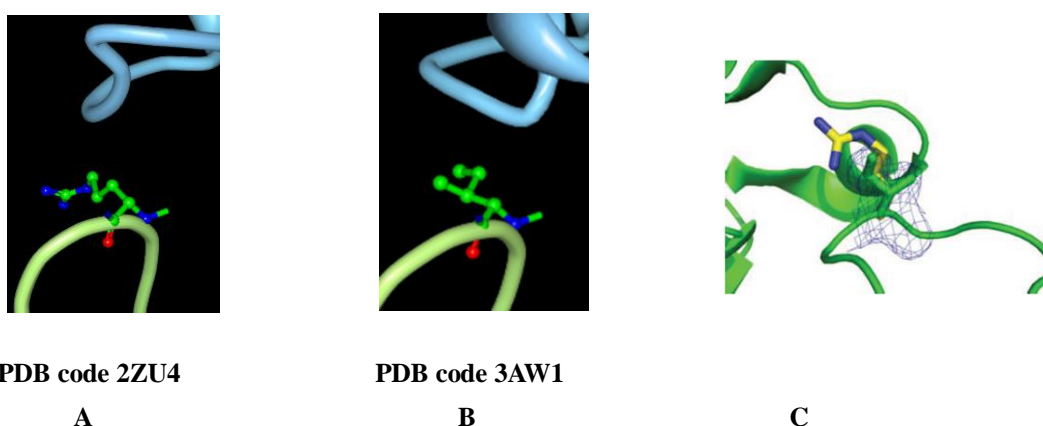


図 5 R188I SARS 3CL<sup>pro</sup> の変異部位

著者の所属する研究室ではこの新たに得られた評価系を用い SARS 3CL<sup>pro</sup>阻害剤開発研究が開始された。阻害剤開発にあたり、SARS 3CL<sup>pro</sup>のプロセシング部位のうち、効率よく認識されるプロテアーゼ自身の N 末部位にあたるアミノ酸配列 (...Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-...) をもとに、切断部位から N 末端側に 5 残基の基質アミノ酸に、プロテアーゼの活性中心の Cys 残基と相互作用するアルデヒド基を付加したペプチドアルデヒド型阻害剤 **1** (IC<sub>50</sub> = 37 μM) が開発された。<sup>27</sup> 得られた阻害剤 **1** を基に、S<sub>1</sub> site のアミノ酸の構造活性相関研究がなされ、His に置換した阻害剤 **2** が阻害活性を向上することが見出された。また、プロテアーゼとの X 線結晶構造解析から新たに導入した His の側鎖が活性ポケットに収まっていることも確認されている (図 6)。<sup>28</sup>



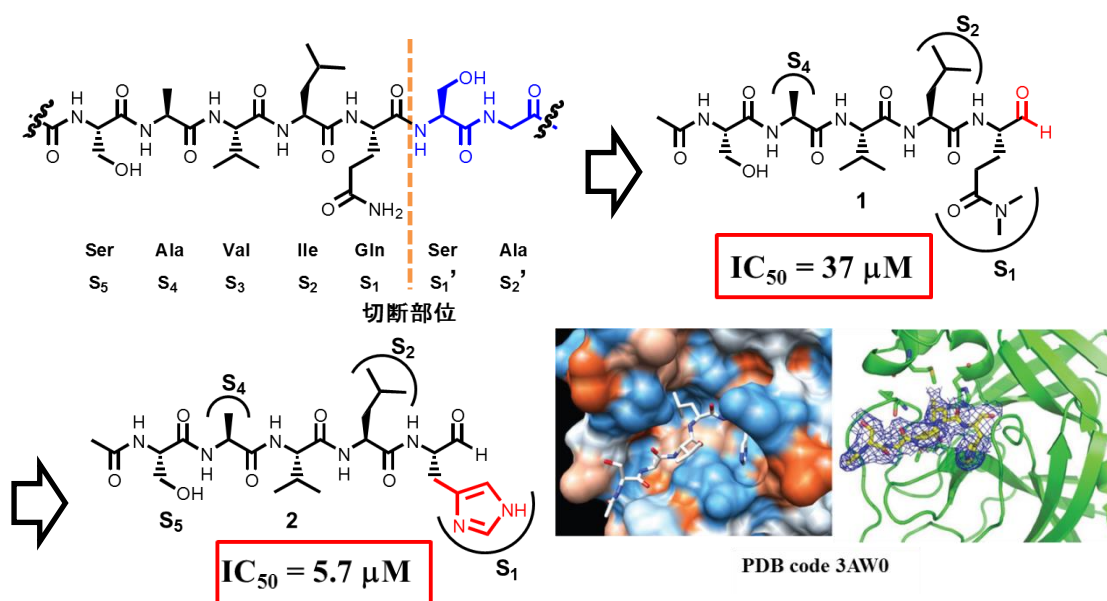


図 6 ペプチド型阻害剤の設計

以上の研究結果を踏まえ、著者は本論文において、第 1 章ではペプチド型阻害剤の阻害活性の向上のために、X 線結晶構造解析に基づく構造最適化について論じた。第 2 章では構造最適化したペプチド型阻害剤を非ペプチド型阻害剤に変換することを目的とし、デカヒドロイソキノリン骨格を有する非ペプチド型阻害剤の設計法と合成法について論じた。第 3 章では合成した阻害剤の立体化学を確認するために光学分割法を用い推定構造を提唱した。さらに共結晶 X 線構造解析を行ない阻害剤の推定構造とプロテアーゼとの相互作用の確認を行った。

## 第 1 章 ペプチドアルデヒド型阻害剤の構造最適化

### 1. ペプチドアルデヒド型阻害剤の最適化戦略

X 線結晶構造解析の結果を基に、構造変換が容易に行えるペプチド型阻害剤の利点を活かし、酵素と阻害剤の相互作用を最大限活かせる阻害剤デザインを行った。 $S_2$  site に位置する Leu の側鎖は密に詰まっておらず、空間的に余裕があることが分かった。そこでこの側鎖をより嵩高い構造に変換して、ポケットをより占有するようにすれば、阻害活性の向上に繋がると考えた。また  $S_3$  site の Val の側鎖は活性ポケットの外側に向いているのでこれ以上の構造変換はせず、 $S_4$  site の Ala の側鎖は逆に活性ポケットの内側に向いていたので水素結合可能な側鎖構造に変換を行うことにした。また  $S_5$  site の Ser の側鎖は活性ポケットの外側に出ていたので除去することを計画した (図 7)。

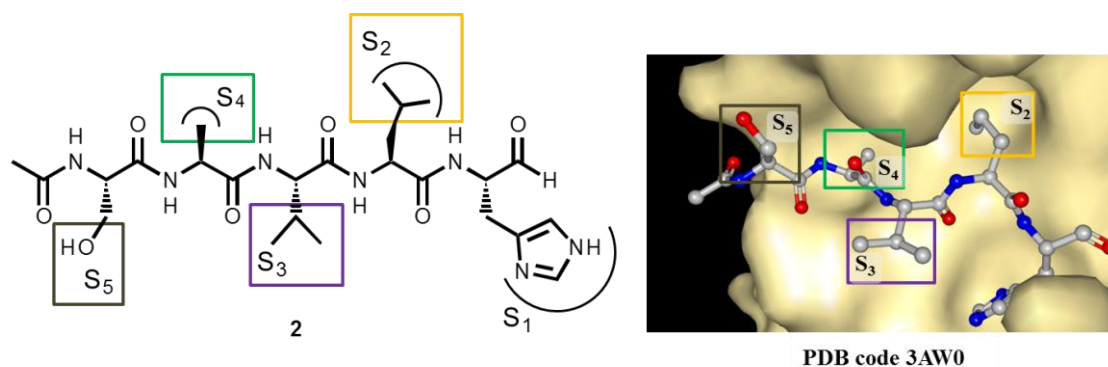


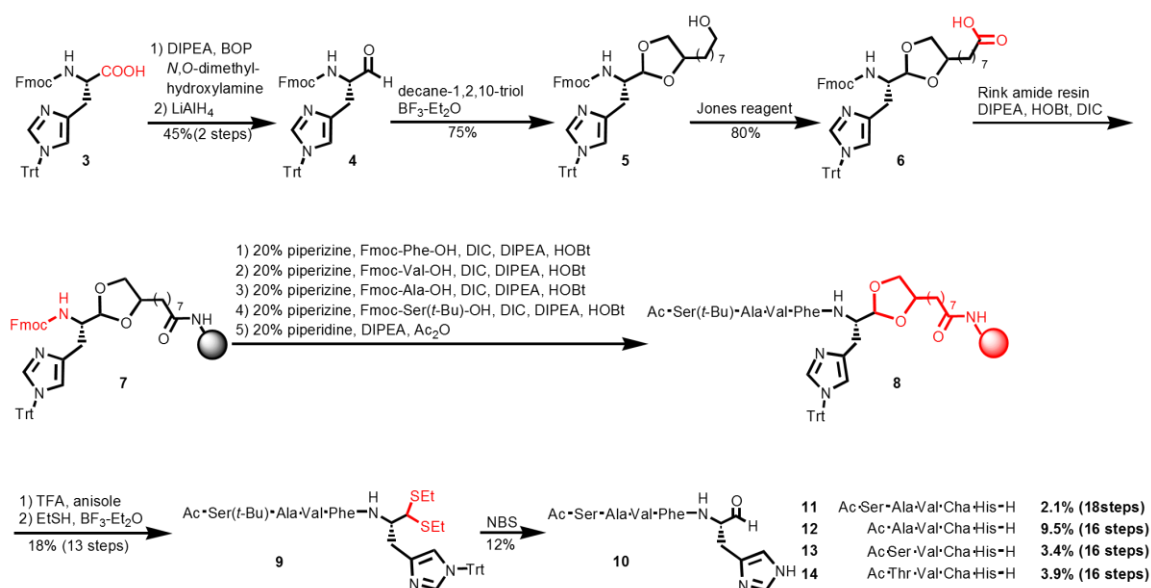
図 7 ペプチド型阻害剤の構造最適化戦略

## 2. ペプチドアルデヒド型阻害剤の合成

Fmoc-His(Trt)-OH (**3**)を Weinreb アミド化して、還元反応によりアルデヒド **4** を得た。次にアルデヒド **4** とリンカー部分となる 1,2,10-トリオールを連結しアセタール保護体 **5** とした。化合物 **5** の水酸基を Jones 試薬を用い酸化させカルボン酸 **6** とし、最後にリンクアミド樹脂に担持させた。この樹脂 **7** に一般的な固相合成法により必要なアミノ酸の伸長反応を行ない、末端を Ac 基で保護した。

一般的なペプチドアルデヒド合成法では次の反応段階で、この樹脂 **8** のすべての保護基の脱保護と脱樹脂を同時に行い、目的物を得る経路が採られる。しかしその方法を用いると収率が悪く、反応時間が長くなってしまうという欠点があった。そこで従来のペプチドアルデヒド合成法より収率良く目的物を得ることが可能な今野らが開発したアセタールリンカーを用いた合成法を採用した。<sup>29</sup> 本合成法は脱保護段階を 2 段階に分けることにより、収率と反応時間を改善した改良合成法である。

アミノ酸の伸長を終えた樹脂 **8** に TFA によりアミノ酸の保護基の脱保護と脱樹脂を同時に行い、その後三フッ化ホウ素とエタノールを反応させアセタール保護をチオアセタール保護に付替えを行い、化合物 **9** を得た。最後に NBS を用いチオアセタール保護の脱保護を行ない目的のペプチドアルデヒド **10** を収率良く得ることに成功した。構造最適化に用いたペプチドアルデヒドはこの合成法を用い合成した (Scheme 1)。



Scheme 1 アセタールリンカーを用いた改良ペプチドアルデヒド合成法

### 3. ペプチドアルデヒド型阻害剤の阻害活性

まず  $S_2$  site の構造最適化を行った。 $S_2$  site のポケットを密に埋める候補化合物は、平面的なベンゼン環を側鎖に持つ Phe に置換した化合物 **10** と嵩高いシクロヘキサン環を側鎖に持つ Cha に置換した化合物 **11** の 2 種類の化合物を比較した。阻害活性を調べてみると、どちらの化合物も構造変換前の阻害剤 **2** より高い阻害活性を示したが、ベンゼン環を側鎖に持つ化合物 **10** ( $IC_{50} = 380$  nM) よりもシクロヘキサン環を側鎖に持つ化合物 **11** ( $IC_{50} = 65$  nM) の方がより阻害活性を向上させる結果となった。そこで  $S_2$  site を阻害活性の高かった Cha に固定して  $S_5$  site の Ser を除去した阻害剤 **12** を合成した。低分子化を目的に Ser の除去を行った阻害剤 **12** は  $IC_{50} = 270$  nM の阻害活性を示し、阻害活性を大きく減弱することはなかった。従って  $S_5$  site の Ser の除去を行った状態で  $S_4$  site の構造変換を行うことにした。側鎖構造の水素結合を期待して水酸基をもつ Ser に置換した化合物 **13** と嵩高い側鎖を持つ Thr に置換した化合物 **14** を比べ、阻害活性評価を行うと、Ser に置換した化合物は同程度の阻害活性 ( $IC_{50} = 340$  nM) を示したのに対し、Thr に置換した化合物は阻害活性 ( $IC_{50} = 98$  nM) の向上が見られた。この結果、4 残基のアミノ酸で、5 残基のアミノ酸を持つ阻害剤と同程度の阻害能を示すナノモルオーダーの阻害活性を示す阻害剤 **14** の開発に成功した (図 8)。

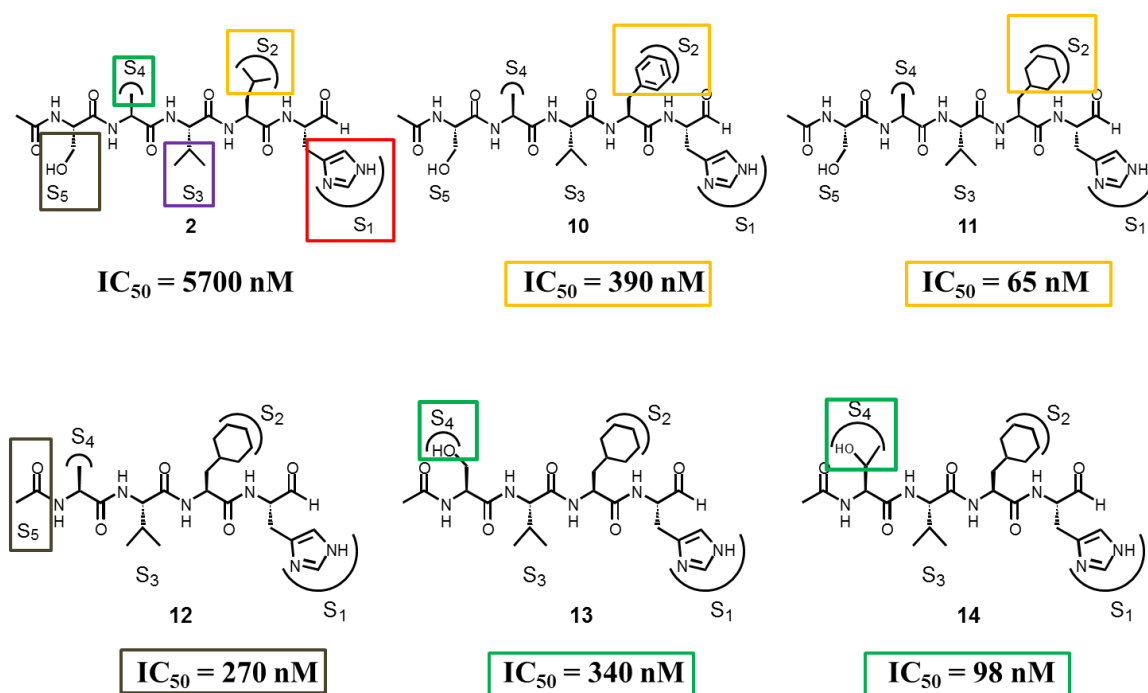


図 8 ペプチド型阻害剤の阻害活性

#### 4. R188I SARS 3CL<sup>pro</sup> との相互作用解析

阻害剤 **14** と R188I SARS 3CL<sup>pro</sup> との X 線結晶構造解析を行った。新たに導入した S<sub>2</sub> site のシクロヘキサン構造はポケット内に密に収まっていることが確認できた。また構造変換しなかった S<sub>3</sub> site の Val の側鎖は構造変換前の化合物同様ポケットの外側を向き、水素結合を期待して構造変換した S<sub>4</sub> site の Thr の側鎖は、プロテアーゼと水素結合していることが確認できた。これらの相互作用が合わさって 50 倍以上の阻害活性の増加に繋がったと考えられる (図 9)。

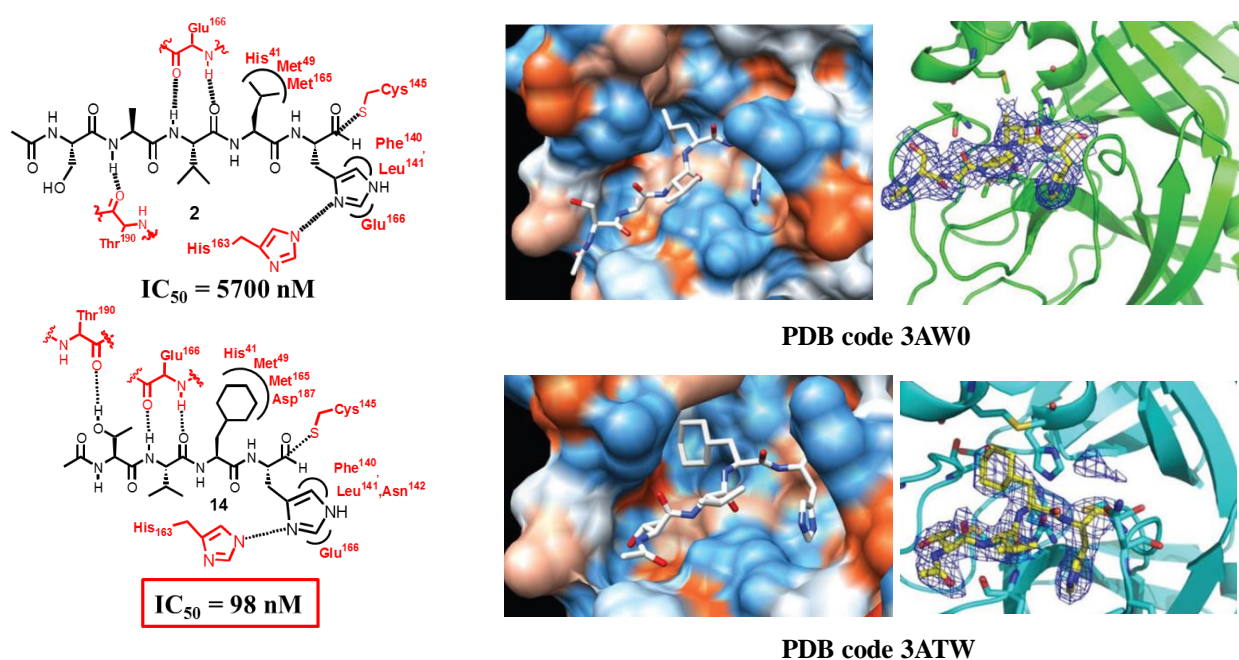


図 9 プロテアーゼとの相互作用解析

## 5. 阻害活性機構

X線結晶構造解析から阻害剤 **14** のアルデヒド基とプロテアーゼの活性中心の Cys は共有結合せず、アルデヒド基は  $sp^2$  構造を取っていることが強く示唆された。また活性中心のチオール基とアルデヒド基の炭素原子の距離は  $2.48 \text{ \AA}$  であり、これは C-S 結合が結合したときの距離の  $1.8 \text{ \AA}$  と比べると明らかに長いことが確認できた (図 10)。さらに阻害機構を調べるため、Lineweaver-Burk plot を取ると競争阻害機構を取っていることが明らかになった (図 11)。これらの結果から、アルデヒド基とチオール基は不可逆的な共有結合ではなく、可逆的な結合により相互作用していると考えられる。

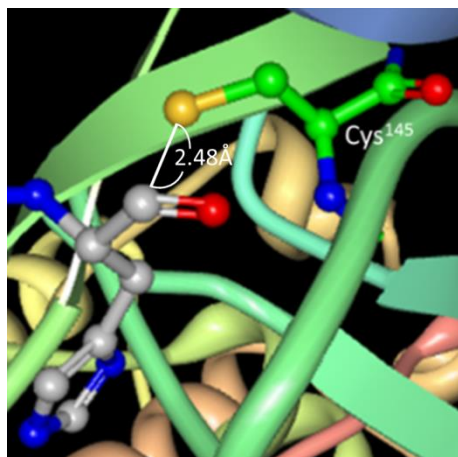


図 10 R188I SARS 3CLpro と阻害剤 **14** との触媒活性部位 (PDB code 3ATW)

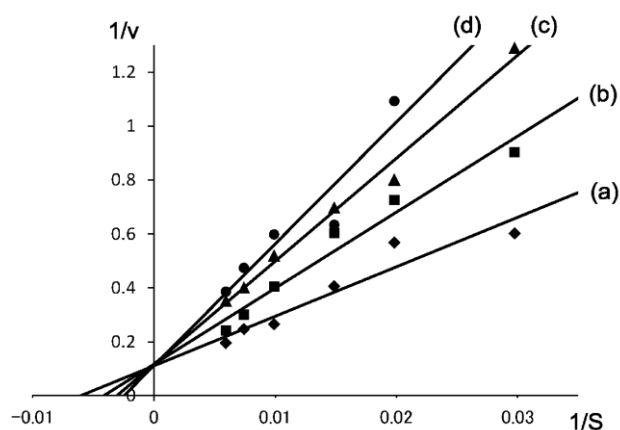


図 11 Lineweaver-Burk plot 図

(a) Incubated without inhibitor; (b) incubated with 25 nM of **14**; (c) incubated with 50 nM of **14**; (d) incubated with 100 nM of **14**.



## 第2章 複合体結晶構造解析に基づく新規縮環型阻害剤の設計と合成

### 1. ペプチド型阻害剤を基盤にした非ペプチド型阻害剤の設計

ペプチド型阻害剤は構造変換が容易であり多くの化合物評価が可能である反面、消化酵素により速やかに分解されるため吸収が低く持続性が短いという欠点を持つ。こういったバイオアベイラビリティの観点からも創薬を目指すにあたっては非ペプチド化の過程は避けられないものである。現在の創薬科学でも、ペプチド型阻害剤から非ペプチド型阻害剤への架け橋となる阻害剤デザイン法はまだまだ限りがあるのが実情である。事実、ペプチド型阻害剤を基にした阻害剤はペプチド結合を等価体構造に置き換えるペプチドミメティックな阻害剤として設計され、ペプチド型阻害剤を基に非ペプチド型阻害剤を設計する手法は過去にほとんど例を見ない。

非ペプチド型 SARS 3CL<sup>pro</sup> 阻害剤はいくつか知られているが、それらは天然物やハイスループットスクリーニングまたは他のプロテアーゼ阻害剤を基にデザインされている。<sup>16-26</sup> 本研究では、ペプチド型阻害剤から非ペプチド型阻害剤への変換を達成することを目的とし、デカヒドロイソキノリン型 SARS 3CL<sup>pro</sup> 阻害剤開発研究を行った。

ペプチド型阻害剤を非ペプチド型阻害剤に変換することにおいて最大の課題は、変換後も阻害剤とプロテアーゼが同様の相互作用を保てるかにある。これまでに得られた構造情報に基づく阻害剤設計を行うために、S<sub>2</sub> site の疎水性ポケットに着目することにした。阻害剤と変異プロテアーゼとの X 線結晶構造解析から S<sub>2</sub> site に位置するシクロヘキサン環とペプチド主鎖との間がかなり近接していることが確認できた。このことを利用し S<sub>2</sub> site の疎水性相互作用を核とした縮環骨格を基本構造として利用すればペプチド型阻害剤を模倣した分子設計が出来ると考えた。そこでペプチド主鎖とシクロヘキサン環をメチレンリンカーで連結したデカヒドロイソキノリン構造を母核 scaffold として設計考案した。この環状 scaffold 上にヒスチジン構造とアルデヒド基を付加し、さらにプロテアーゼとの相互作用を期待してアシル置換基を付加した 3 つの部位でプロテアーゼと相互作用が可能な非ペプチド型阻害剤 **15** を設計した。このとき、デカヒドロイソキノリン構造が S<sub>2</sub> 位に位置すると予測したときヒスチジン構造は S<sub>1</sub> 位に、アシル置換基は S<sub>4</sub> 位に位置すると期待し、配置した (図 12)。

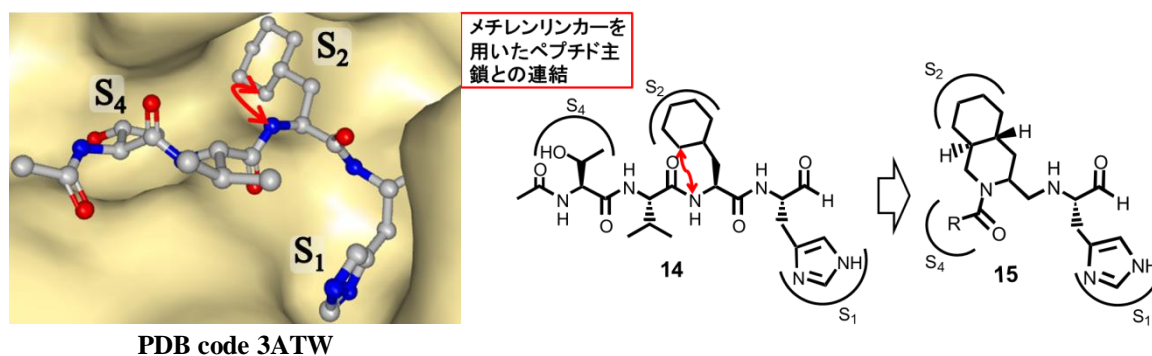
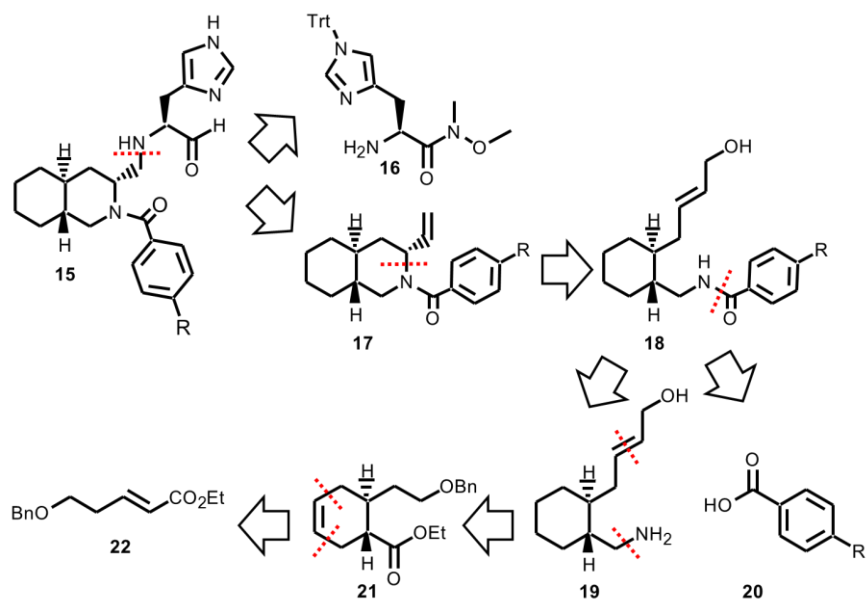


図 12 非ペプチド型阻害剤の設計

## 2. 逆合成解析

設計した非ペプチド型阻害剤の逆合成経路を示した。アシル置換基には活性に及ぼす影響を調べるに際し、合成工程数の短縮、および多様な誘導体が市販されていることから入手容易な構造を導入した。また置換基に剛直な構造のベンゼン環を持たせることで、分子運動を制御する動的制御の効果を狙った。目的の阻害剤 **15** は還元的アミノ化反応を用いたヒスチジン誘導体 **16** とデカヒドロイソキノリン **17** の連結反応後、還元反応を行うことにより得られると考えた。デカヒドロイソキノリン **17** は、環化前駆体 **18** にパラジウムを触媒として用いた環化反応により合成できると考えた。前駆体 **18** を得るためには、後の構造活性相関研究も見据え多種多様な分子設計ができる収束的経路を用いることとし、アミノアルコール **19** と種々のカルボン酸 **20** のカップリング反応により合成する経路を取ることにした。アミノアルコール **19** は環化体 **21** に官能基変換を行うことにより合成できると考えた。この環化体 **21** は既知のエステル **22** より合成できると考えた (Scheme 2)。



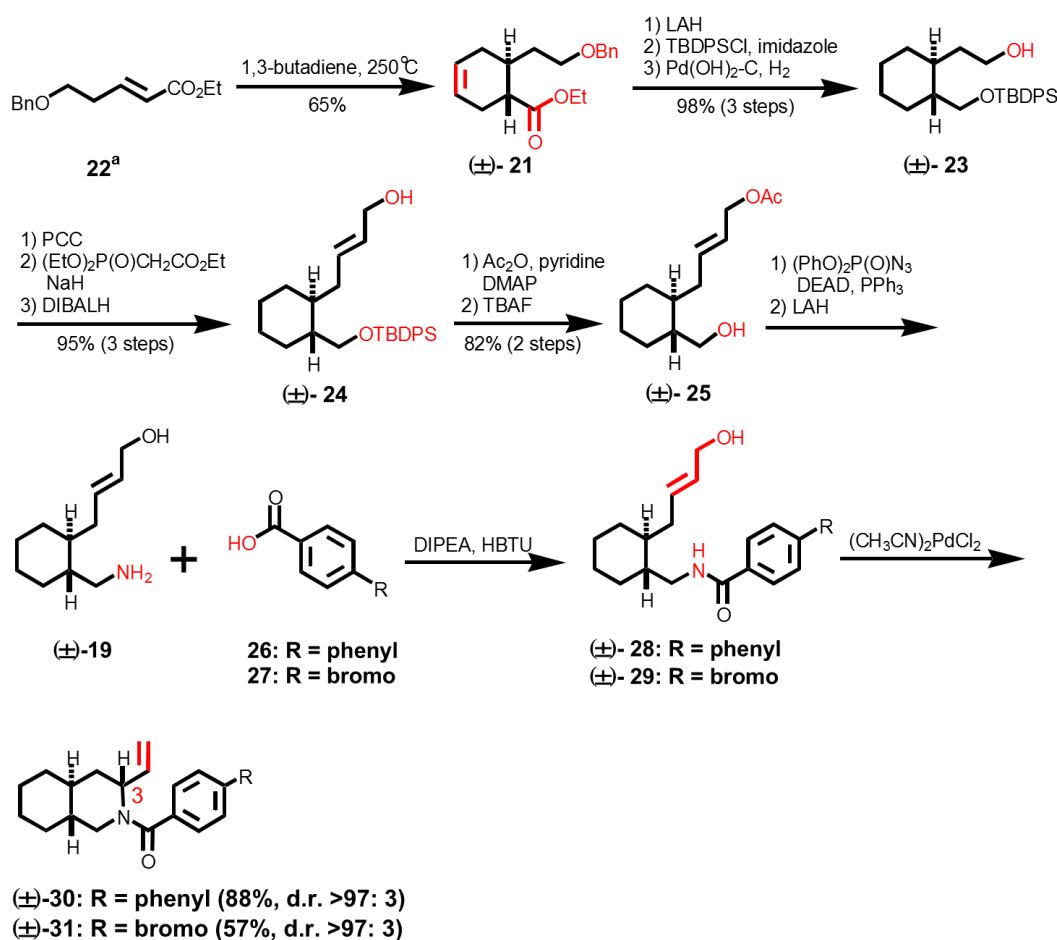
Scheme 2 逆合成経路



### 3. デカヒドロイソキノリン型阻害剤の合成

既知のエステル **22** と 1,3-ブタジエンとの Diels-Alder 反応により環化体 **21** をラセミ体として得た。<sup>30</sup> 環化体 **21** に還元反応を行い、得られた水酸基を TBDPS エーテルとして保護し、続く接触還元により二重結合の還元と Bn 基の脱保護を同時に行いアルコール **23** を得た。次にアルコール **23** の水酸基を PCC 酸化、Horner-Wadsworth-Emmons 反応により増炭し、増炭後のエチルエステルを DIBALH で還元しアリルアルコール **24** を得た。化合物 **24** の水酸基を Ac 基で保護し、TBDPS 基の脱保護を行いアルコール **25** を得た。この得られた水酸基をアジド化し、LAH を用いて Ac 基の脱保護とアジドの還元を同時に行い、アミノアルコール **19** を得た。

合成する阻害剤の側鎖構造に 4-フェニルベンゾイル基と 4-ブロモベンゾイル基を導入するため、アミノアルコール **19** と 4-フェニル安息香酸 **26** と 4-ブロモ安息香酸 **27** をカップリングさせ、環化前駆体 **28**, **29** を得た。環化反応は真壁らが報告している 2 価パラジウム触媒を用いたジアステレオ選択的な環化反応を参考に行った。<sup>31</sup> 環化前駆体 **28**, **29** に 2 価パラジウムである  $(\text{CH}_3\text{CN})_2\text{PdCl}_2$  を作用させると目的の環化反応が進行し、縮環化合物 **30**, **31** を得ることに成功した。得られた環化物が単一のジアステレオマーであることは  $^1\text{H}$  NMR を用い確認し、3 位の立体化学は文献を参考に推測した (Scheme 3)。

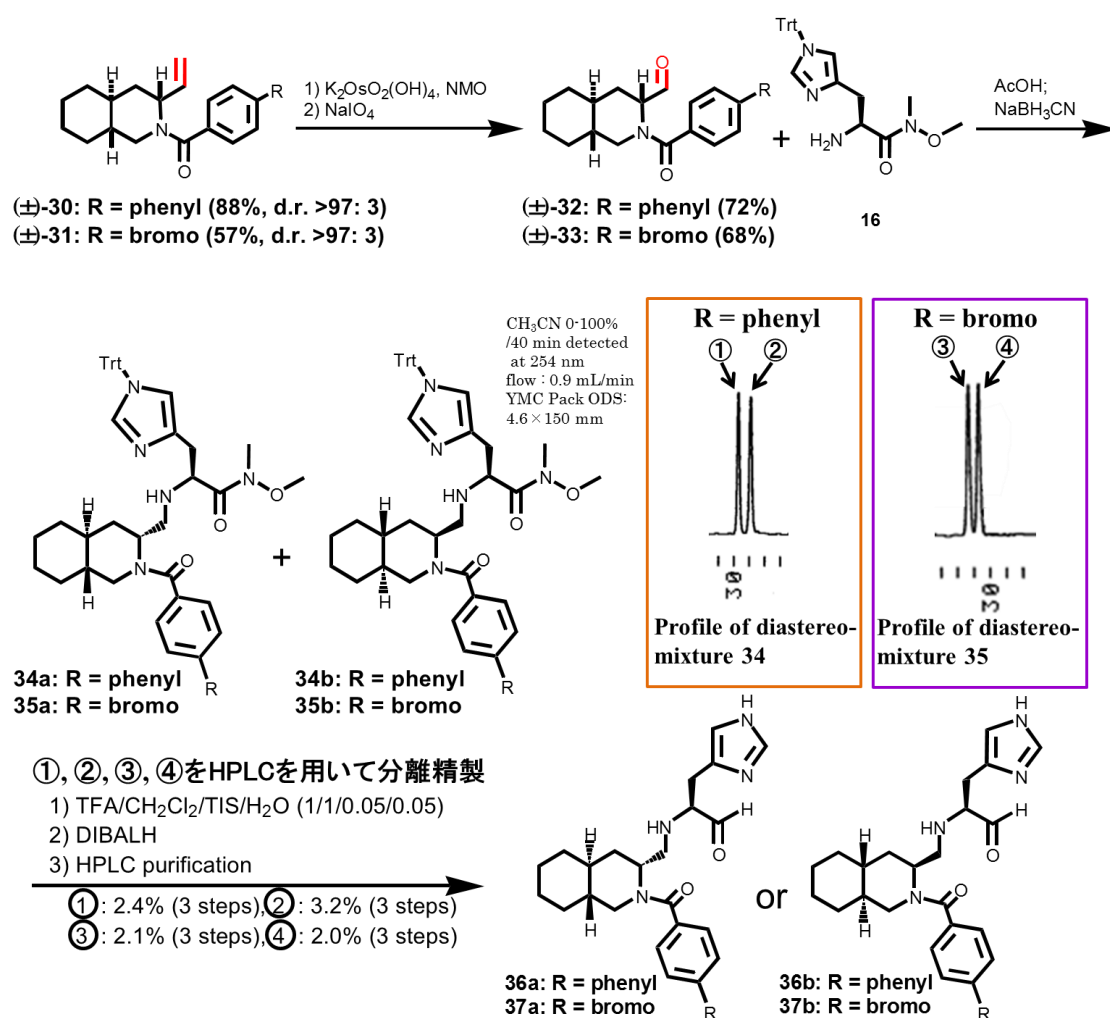


Scheme 3 環化体の合成

合成した環化体 **30, 31** を Lemieux-Johnson 酸化しアルデヒド **32, 33** とした後、別途合成したヒスチジン誘導体 **16** との還元的アミノ化反応を行いジアステレオマー混合物 **34, 35** を得た。

このジアステレオマー混合物 **34** 及び **35** は分離可能であり、側鎖に 4-フェニルベンゾイル基をもつジアステレオマー混合物 **34** の HPLC 分析を行うと、単一のジアステレオマーであるピーク①とピーク②に分離することが確認できた。側鎖に 4-ブロモベンゾイル基を持つジアステレオマー混合物 **35** も同様の結果を与えた。HPLC を用いピーク①-④の化合物を分離精製し、独立に TFA により Trt 基の脱保護を行い、DIBALH を用い還元反応を行った。最後に HPLC による精製を行ないそれぞれ単一のジアステレオマーであるデカヒドロイソキノリン骨格を有する阻害剤 **36a, 36b, 37a, 37b** の合成を達成した。合成した阻害剤はマイクロモルオーダーの R188I SARS 3CL<sup>pro</sup> 阻害活性を示し、デカヒドロイソキノリン骨格の有用性を確認できた (Scheme 4)。

しかし、この段階で合成した化合物の立体化学を決定することはできなかった。そのため立体化学の確認を行うことにした。



Scheme 4 デカヒドロイソキノリン骨格を有する阻害剤の合成

### 第3章 縮環型阻害剤の立体化学とプロテアーゼ相互作用解析

#### 1. 光学分割法を用いた立体化学の推定

立体化学の確認を行うためには幾つか方法が考えられた。原料の段階からキラルな化合物を用いるキラルプール法、または合成中間体をジアステレオマー塩化しキラルな化合物に誘導する光学分割法、不斉試薬を用い望みの立体化学を有する化合物を合成する不斉合成法などが挙げられる。

文献調査を行ったところ、合成中間体 **21** と類似の構造を有するカルボン酸 **38** が光学分割可能であるという報告例があった (図 13)。<sup>32</sup> そこでこの報告例を参考に合成中間体 **21** を光学分割により、キラルな化合物に誘導した後、この既知化合物(-)-**38**, (+)-**38** との比旋光度の符号を比較して立体化学の推定を行うことにした。

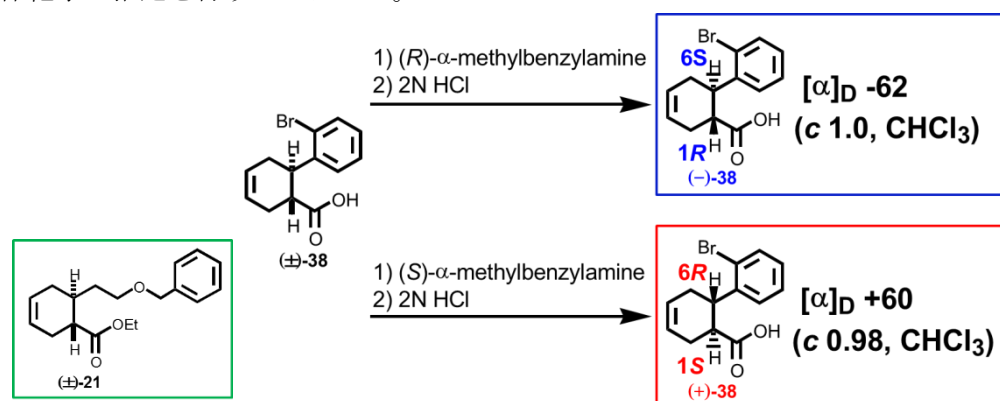


図 13 比旋光度の比較に用いた既知化合物

環化体 **21** を加水分解し、カルボン酸(±)-**39** とした。カルボン酸(±)-**39** に(R)-α-methylbenzylamine を作用させると結晶の析出が起り、塩解除後の比旋光度は -55 を示した。同様に(S)-α-methylbenzylamine を用いた場合、比旋光度は+52 を示した。このことから結晶として析出しなかったエナンチオマーは母液に存在している。得られた両光学分割体(-)-**39**, (+)-**39** の比旋光度の符号は既知化合物の比旋光度の符号(-)-**38**, (+)-**38** と一致しており、同様の立体化学を有する化合物が光学分割されたことが示唆された。

光学純度は、光学分割したカルボン酸をメチルエステル化し、キラルカラムを用いて HPLC 分析することにより確認した。エナンチオマー混合物の分析結果は、Peak area が 1 : 1 の均一な二本のピークに比べ、光学分割した化合物(-)-**40**, (+)-**40** の分析結果は Peak area が 10 : 1 となり、二本のピークが一本のピークに収束していることから純度の高い化合物が得られていると考えられる (図 14)。

純度の高い光学分割体(-)-**39**, (+)-**39** は得られたが、収率は 5% 以下と低く、この方法を使い今後の合成を続けることは困難と考えた。そこで収率の改善を目的に、基質の結晶性を良くするため、ベンゼン環の 4 位にブロモ基を導入した化合物を合成し、同様の方法を用いて光学分割することを計画した。

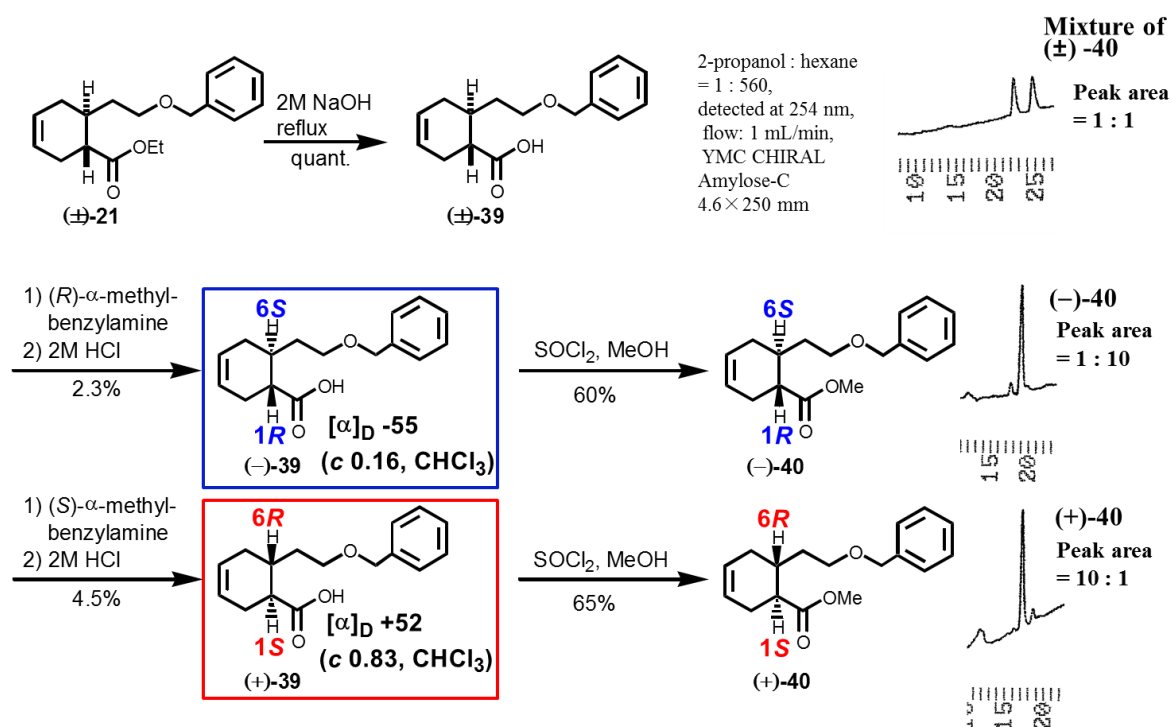


図 14 カルボン酸(±)-39 の光学分割

カルボン酸(±)-39 と同様に、カルボン酸(±)-42 を合成した。このカルボン酸(±)-42 に(*R*)- $\alpha$ -methylbenzylamine を作用させると比旋光度-33 を示す化合物が得られ、(*S*)- $\alpha$ -methylbenzylamine を作用させると比旋光度+22 を示す化合物が得られた。得られた結果はベンジル基の場合と同様、両光学分割体(-)-42, (+)-42 の比旋光度の符号は既知化合物(-)-38, (+)-38 の比旋光度の符号と一致しており、同じ立体化学を有する化合物が光学分割されたことが示唆された (図 15)。

光学純度の確認は先と同様の方法を用いた。ラセミ体(±)-42 と比べると両光学分割体の分析結果は明らかにピークの偏りが確認でき、純度の高い化合物が得られていると考えている。

ベンゼン環の4位にブロモ基を導入した結果、収率は5倍以上増加した。そこでこの化合物(-)-42, (+)-42 を使い以後の実験を行った。

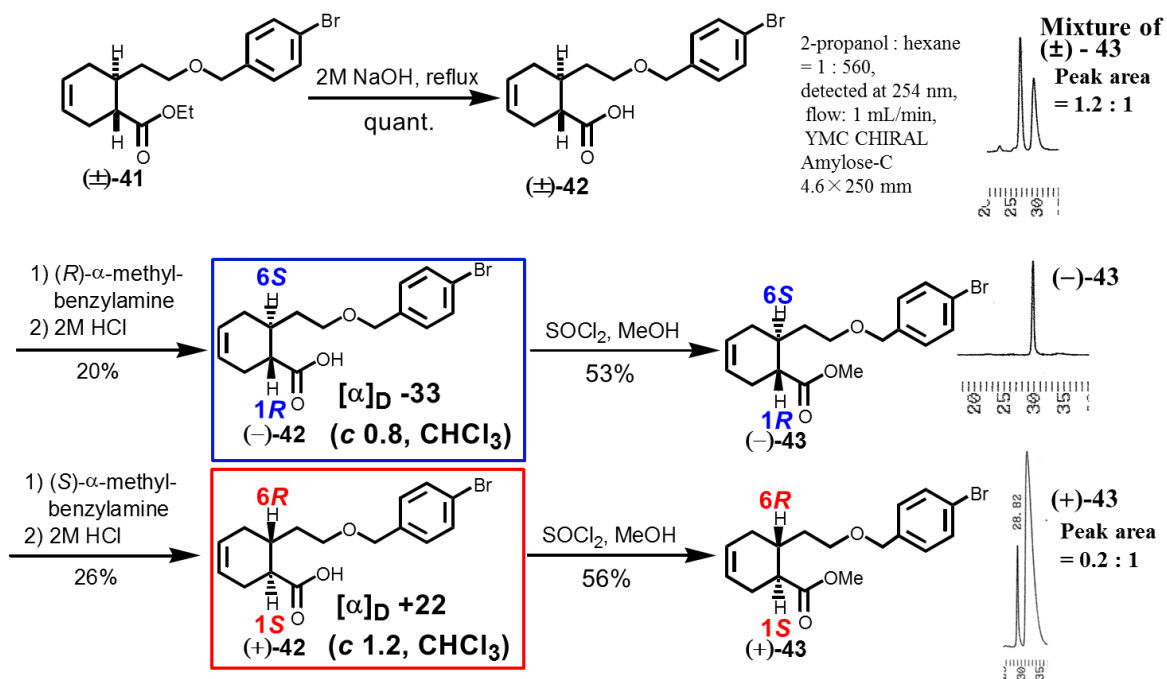
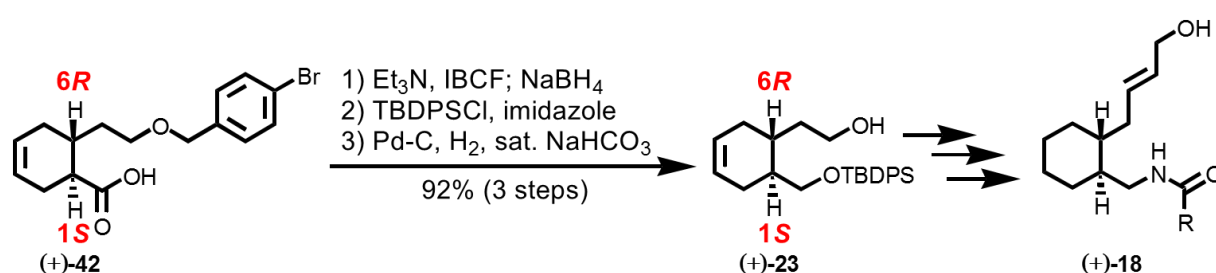


図 15 カルボン酸(±)-42 の光学分割

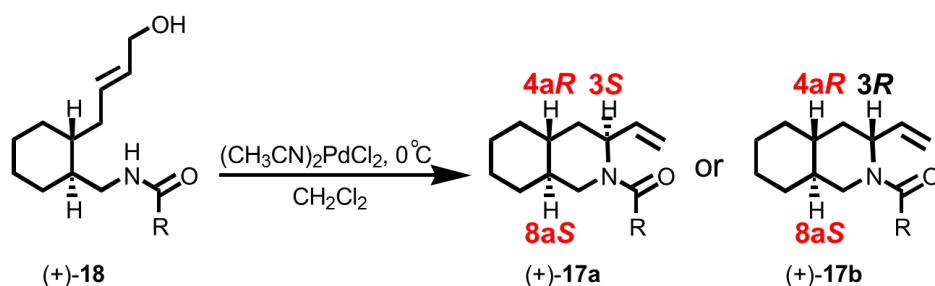
## 2. 環化反応と立体化学

光学分割体(+)-**42** に還元反応を行い、得られたアルコールを TBDPS エーテルとして保護し、続く接触還元によりアルコール(+)-**23** を得た (Scheme 5)。(–)-**42** に対しても同様の反応を行いアルコール(–)-**23** を得た。後の合成は前章と同様の方法を用いた。

環化反応の立体選択性は、化合物 **45** を除き単一のジアステレオマーを与えた (図 16)。単一のジアステレオマーを与えた置換基の場合、下側の遷移状態ではアミド部分とアリルアルコールとの立体反発が生じて上側の遷移状態を経由して反応が進行すると考えられる (図 17)。しかし 2 位にフェニル基がある場合は上側の遷移状態ではアリルアルコールとベンゼン環との立体反発が生じて、下側の遷移状態を経由することも可能となるためではないかと考察している (図 18)。



Scheme 5 アルコール(+)-23 の合成



compound	R	(3 <i>S</i> ,4 <i>aR</i> ,8 <i>aS</i> ) : (3 <i>R</i> ,4 <i>aR</i> ,8 <i>aS</i> )	compound	R	(3 <i>S</i> ,4 <i>aR</i> ,8 <i>aS</i> ) : (3 <i>R</i> ,4 <i>aR</i> ,8 <i>aS</i> )
(–)- <b>30</b>		>97 : 3 (–)- <b>30a</b> : (–)- <b>30b</b>	(–)- <b>31</b>		>97 : 3
<b>44</b>		>97 : 3	<b>46</b>		>97 : 3
<b>45</b>		68 : 32 <b>45a</b> : <b>45b</b>	<b>47</b>		>97 : 3

\* 選択比は<sup>1</sup>H NMR を用い決定した。

図 16 パラジウム環化の立体選択性

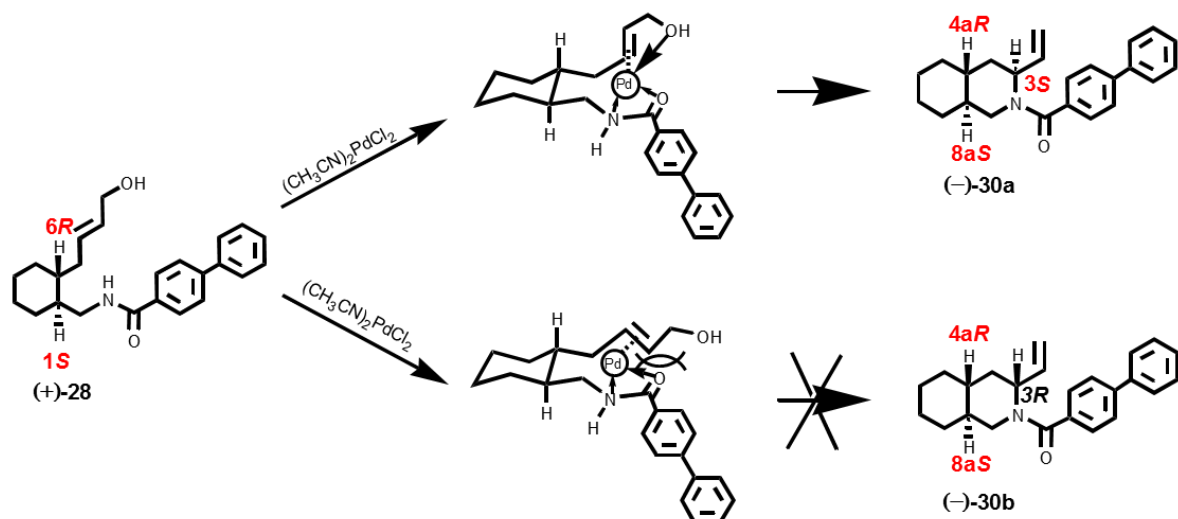


図 17 パラジウム環化時の推定遷移状態 (1)

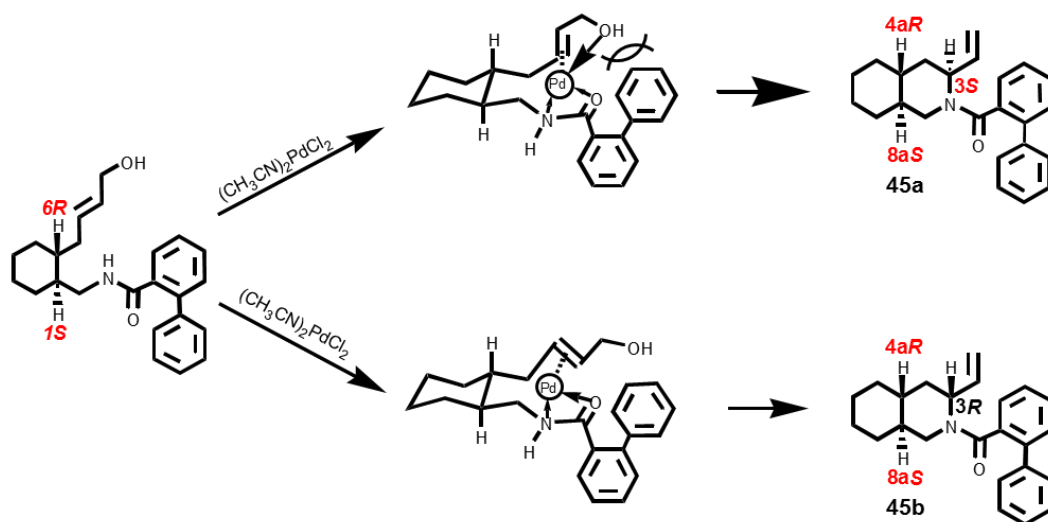
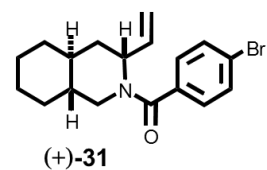


図 18 パラジウム環化時の推定遷移状態 (2)

得られた環化体 **30**, **31** は  $^1\text{H}$  NMR 上においてアミド結合による異性体由来するピークを確認できた。この異性体を配座異性体と推測し温度条件を変えて  $^1\text{H}$  NMR を測定することでその確認を行うことにした。

測定には環化体(+)-**31** を用いた。温度が上昇するに従ってピークの収束が確認できた (図 19)。これは加熱条件により配座間の交換スピードが早くなるためであると考えられ、23.7℃で二つの配座異性体を示すスペクトルが 80℃では一つの配座異性体を示すスペクトルに収束していることが確認できた。



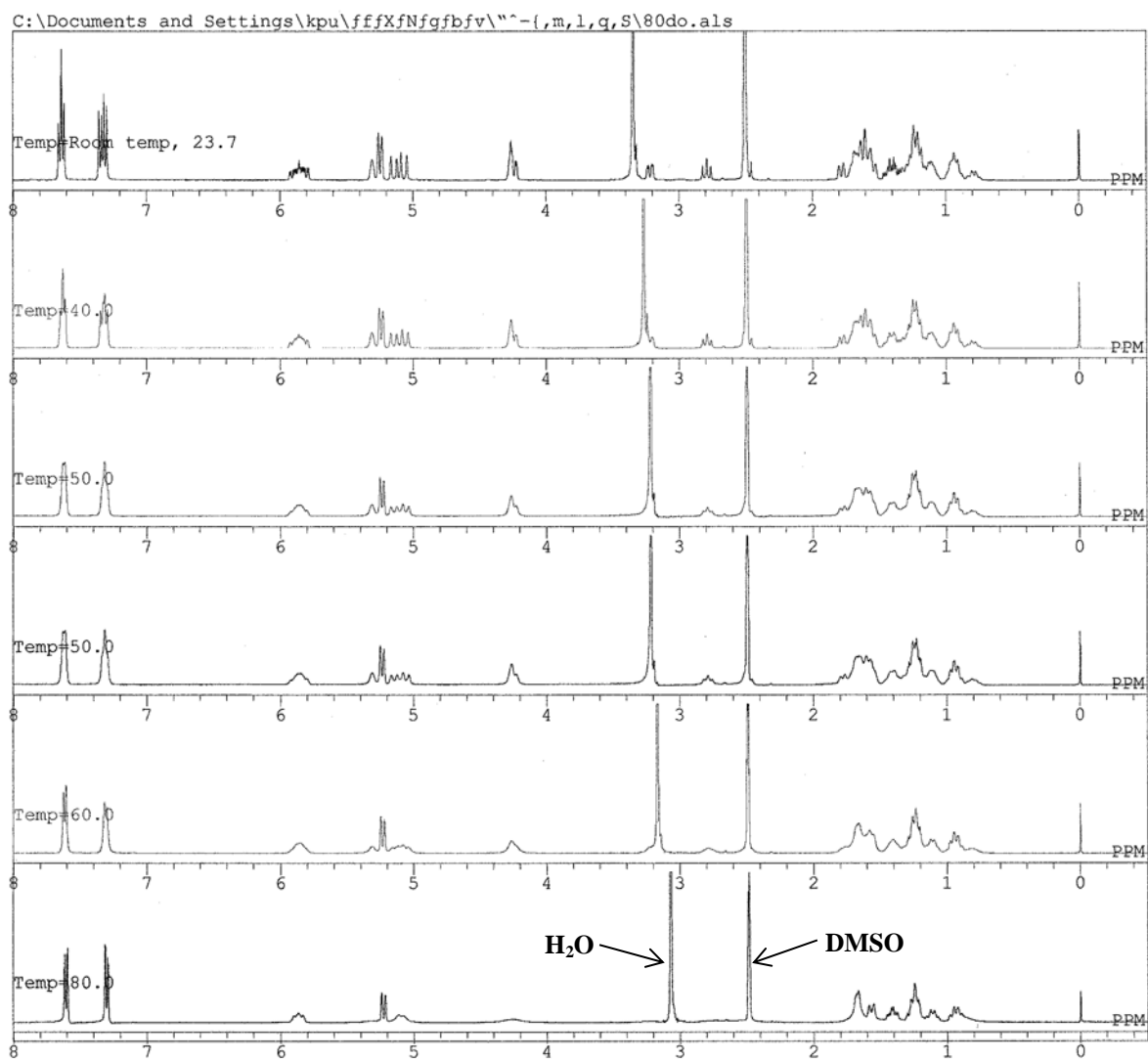


図 19 化合物(+)-31 の加温  $^1\text{H}$  NMR 結果 (400 MHz,  $\text{DMSO}-d_6$ )



### 3. デカヒドロイソキノリンの立体化学

環化体(-)-**30**, (+)-**30** をそれぞれ還元的アミノ化物 **48a**, **48b** に誘導した。この化合物 **48a** と **48b** の HPLC 分析の溶出時間を先ほど行ったジアステレオマー混合物との HPLC 分析の時間と比較すると化合物 **48a** は①のピークと一致し、化合物 **48b** は②のピークと一致することが分かった。このことからジアステレオマー混合物の①のピークの化合物のデカヒドロイソキノリン構造の立体化学は **3R,4aS,8aR** であり、②のピークの化合物のデカヒドロイソキノリン構造の立体化学は **3S,4aR,8aS** であることが強く示唆された (図 20)。

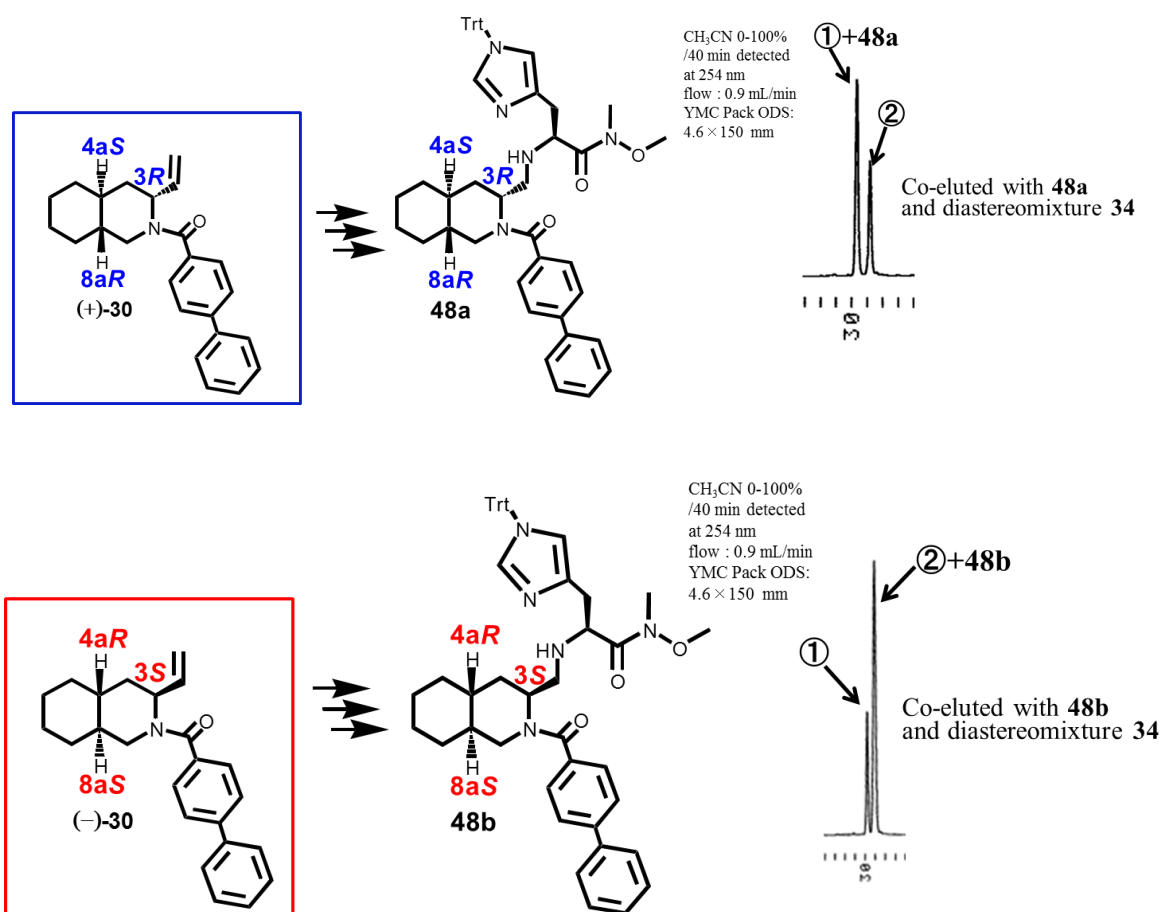


図 20 縮環骨格の立体化学の推定 (1)

次に側鎖に 4-ブロモベンゾイル基をもつ **49a**, **49b** に誘導した。化合物 **49a** と **49b** の HPLC 分析の溶出時間を比較すると、先の結果と相関して、化合物 **49a** は③のピークと一致し、化合物 **49b** は④のピークと一致することが分かった。このことからジアステレオマー混合物の③のピークの化合物のデカヒドロイソキノリン構造の立体化学は **3R,4aS,8aR** であり、④のピークの化合物のデカヒドロイソキノリン構造の立体化学は **3S,4aR,8aS** であることが強く示唆された (図 21)。

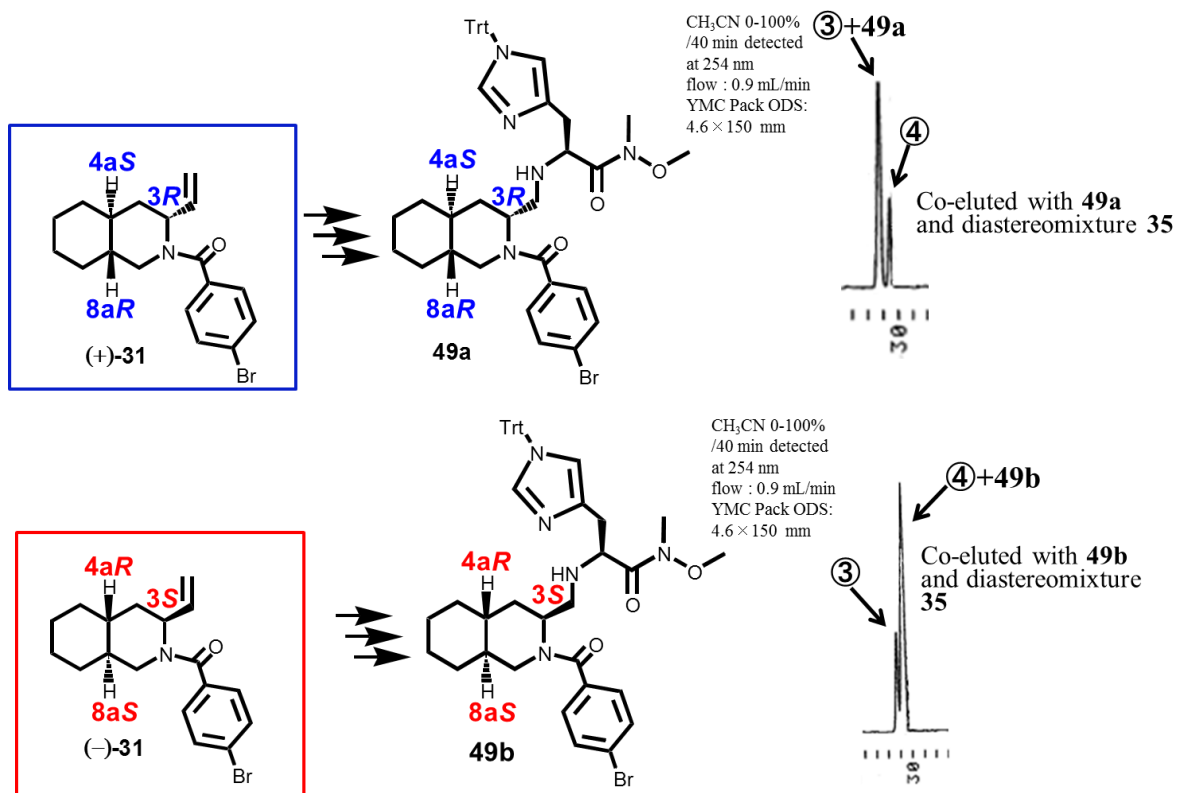
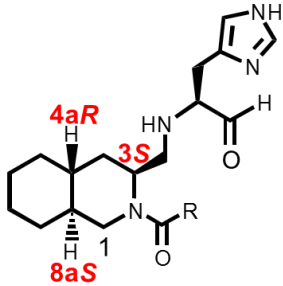
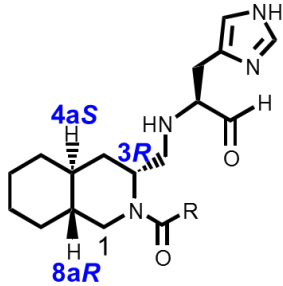


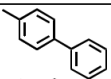

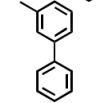

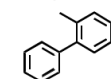

図 21 縮環骨格の立体化学の推定 (2)

#### 4. 側鎖構造の組み換えによる阻害活性評価

側鎖の組み換えを行い阻害活性評価を行った。4-フェニルベンゾイル基を側鎖にもつ阻害剤 **36** と 4-ブロモベンゾイル基を側鎖にもつ阻害剤 **37** では両化合物とも縮環骨格の立体化学が *3S,4aR,8aS* の立体化学をもつ化合物が *3R,4aS,8aR* の立体化学をもつ化合物より 2 倍以上阻害活性が高いことが明らかになった。構造の組み換えを行うにあたり、阻害活性の高かった *3S,4aR,8aS* の立体化学を持つ縮環構造を利用することにした。まず 4-フェニルベンゾイル基の位置異性体を合成し阻害活性を調べることにした。フェニル基の置換基を 3 位、2 位とした位置異性体 **50**, **51** は阻害活性に大きな変化はなかった。次に 4-ブロモベンゾイル基のブロモ基を水素、フルオロ基に置換した化合物 **52**, **53** もほとんど阻害活性の変化を示さなかった。以上の結果よりフェニル基の位置異性体、ハロゲンの有無は阻害活性に大きな変化を示さないことが明らかになった(図 22)。

今回得られた阻害剤は  $IC_{50}$  値がマイクロモルオーダーに留まっており、今後さらなる構造最適化が行われナノモルオーダーの阻害剤が開発されれば、他のシステインプロテアーゼに対する選択性の有無や細胞毒性試験などを行いたいと考えている。

compound	R	$IC_{50}$ ( $\mu$ M)		compound	R	$IC_{50}$ ( $\mu$ M)	
		( <i>3S,4aR,8aS</i> )	( <i>3R,4aS,8aR</i> )			( <i>3S,4aR,8aS</i> )	( <i>3R,4aS,8aR</i> )
<b>36</b>		108*	240*	<b>37</b>		63*	175*
<b>50</b>		135		<b>52</b>		68	
<b>51</b>		135 <sup>#</sup>		<b>53</b>		57	

\*ジアステレオマー分離体から誘導した阻害剤を阻害活性試験に供した

<sup>#</sup>ジアステレオマー混合物を阻害活性試験に供した

図 22 合成した阻害剤の阻害活性評価

## 5. プロテアーゼとの相互作用解析<sup>33-39</sup>

ジアステレオマー分離し、誘導した阻害剤 **37b** と R188I SARS 3CL<sup>pro</sup> との X 線結晶構造解析を行った。今までの推定実験により阻害剤 **37b** の内部渡環部分の立体化学は 4a*R*,8a*S* であることが示唆され、3 位の立体化学は *S* と予測していた。X 線結晶構造解析から得られた結果は、推定していた阻害剤の立体化学と同一であり、推定していた構造に間違いがないことが確認できた。また非ペプチド阻害剤 **37b** の末端アルデヒド基はプロテアーゼ活性中心の Cys<sup>145</sup> と相互作用し、His の側鎖は S<sub>1</sub> site に収まり、プロテアーゼと水素結合していることが明らかになった。さらに今回新たに設計したデカヒドロイソキノリン骨格は、設計当初予測していた通りに S<sub>2</sub> site に収まり、ペプチド型阻害剤 **14** と同様の相互作用をしていることが分かった。

またアルデヒドの炭素と活性中心のチオールとの結合距離を計算すると、2.42Å となり共有結合距離の 1.8Å よりも長いことが確認できた。このことから二つの置換基は共有結合せず相互作用していることが強く示唆された。これにより非ペプチド型阻害剤もペプチドアルデヒド型阻害剤同様、不可逆的な結合様式をとらず、競争阻害により阻害活性を発現していることが示唆される (図 23)。

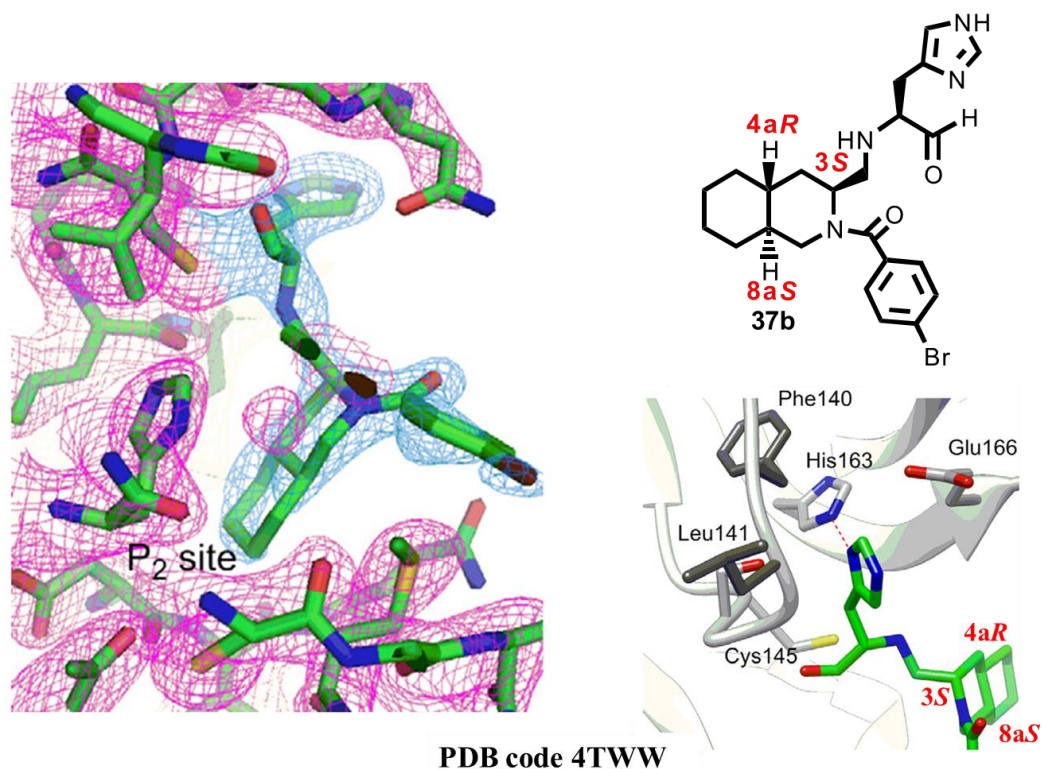


図 23 阻害剤 **37b** とプロテアーゼとの X 線結晶構造解析

ペプチド型阻害剤 **14** および非ペプチド型阻害剤 **37b** と変異プロテアーゼとの X 線結晶構造解析の結果を比較した。その結果、ペプチド型阻害剤は S<sub>3</sub>-S<sub>4</sub> site にアミノ酸残基が位置しているのに対し、非ペプチド型阻害剤はアシル置換基が S<sub>3</sub>-S<sub>4</sub> site の反対側に位置していることが確認できた。このことがペプチド型阻害剤ではナノモルオーダーの阻害能を示していたものが、非ペプチド型阻害剤では、マイクロモルオーダーの阻害能を示す結果になったと考察している (図 24)。

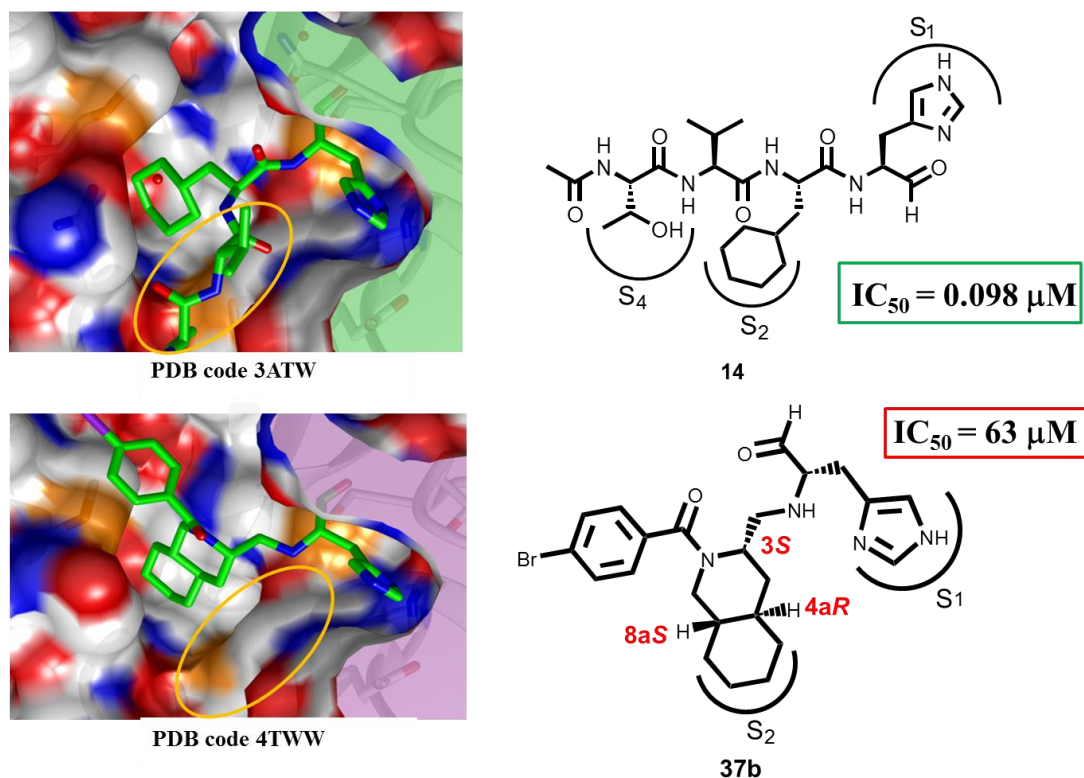


図 24 ペプチド型阻害剤と非ペプチド型阻害剤の相互作用比較

縮環骨格の立体化学の違いによる阻害活性の差が二倍以上あった。側鎖に 4-フェニルベンゾイル基をもつ阻害剤のジアステレオマー **36a** 或いは **36b** とプロテアーゼとの X 線結晶構造解析から二つのジアステレオマー **36a**, **36b** を比べると縮環構造の立体化学の違いにより側鎖構造の配置に差が生じることが明らかになった。阻害活性の高い **36b** の側鎖構造はプロテアーゼに沿うように位置し、プロテアーゼとの相互作用を受けており、逆に阻害活性の低い **36a** の側鎖構造はプロテアーゼから大きく外れる方向に位置しており相互作用を受けていないと考えられる。このことが縮環骨格の違いによる阻害活性の差に繋がったと考察している (図 25)。

また側鎖構造に 4-フェニルベンゾイル基を持つ化合物 **36b** と 4-ブロモベンゾイル基を持つ化合物 **37b** の違いによる阻害活性の差が 1.5 倍ほどあった。二つの化合物のプロテアーゼとの相互作用解析の結果を比べると阻害活性の低い **36b** は阻害活性の高い **37b** と比べると側鎖構造がプロテアーゼにかなり近接しており、空間的に余裕がなく、相互作用に影響することが考えられた。このことが阻害活性の差に繋がったのではないかと考察している (図 26)。



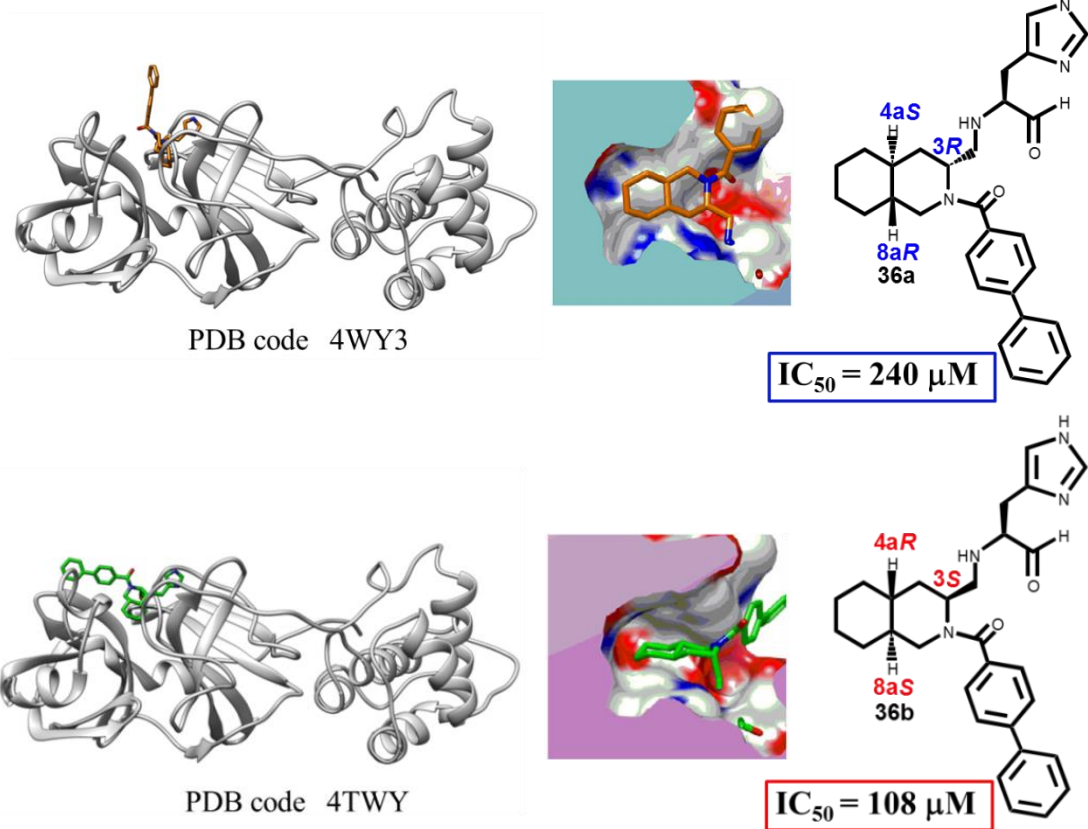


図 25 縮環骨格の立体化学による相互作用比較

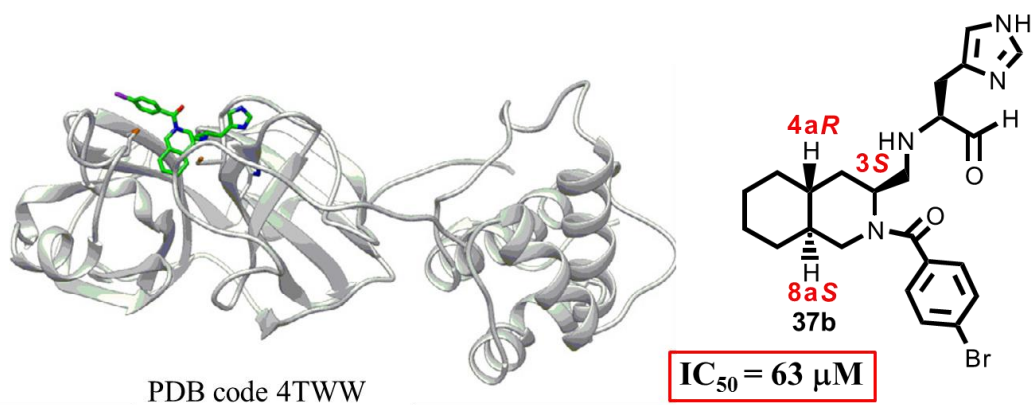


図 26 側鎖構造の相互作用比較

\* 図 25、図 26 は阻害剤を中心に作図した。

## 結語

ペプチド型阻害剤 **2** とプロテアーゼとの X 線結晶構造解析結果を基に阻害剤 **2** の構造最適化を行い、ナノモルオーダーの阻害能を示すペプチド型阻害剤 **14** を開発した。得られた阻害剤とプロテアーゼとの X 線結晶構造解析を行い、新たに導入したアミノ酸側鎖の相互作用様式を確認した。

X 線結晶構造解析を基に  $S_2$  site の疎水性相互作用に着目したデカヒドロイソキノリン骨格を母核 scaffold として考案し、非ペプチド型阻害剤の設計と合成を行った。合成した非ペプチド型阻害剤はマイクロモルオーダーの阻害能を有しており、デカヒドロイソキノリン骨格の有用性を証明できた。

立体化学の確認は光学分割法により行い、合成したデカヒドロイソキノリン構造の推定構造を示すことに成功した。この推定した構造はプロテアーゼとの X 線結晶構造解析から正しいことも確認できた。

デカヒドロイソキノリン型阻害剤と SARS 3CL プロテアーゼ との X 線結晶構造解析を行い、相互作用様式を確認した。活性中心の Cys 残基とアルデヒド基は相互作用し、His 側鎖は  $S_1$  site に収まり水素結合していることも確認できた。デカヒドロイソキノリン構造は予測した通り  $S_2$  site に密に収まっており、プロテアーゼと強く相互作用していることも確認できた。しかしアシル置換基はプロテアーゼの活性ポケットから外れており、阻害活性低下の原因と考察している。この点は今後の構造最適化の課題である。

## 実験の部



## 使用機器について

本実験において、反応に使用したジクロロメタンは水素化カルシウムを用いて蒸留したものを用いた。ジエチルエーテルは LAH を用い蒸留したものを使用した。THF は金属ナトリウムとベンゾフェノンを用いて蒸留したものを使用した。各反応の定性にはシリカゲル 70F254 Plate-Wako (厚さ 0.25 mm) を用い、紫外光の確認には 254 nm の波長を使用した。発色試薬は EtOH に溶いて 5 % に調整したリンモリブデン酸、ニンヒドリンと 10% に調整したアニスアルデヒドを反応に応じ使い分けた。カラムクロマトグラフィーに用いたシリカゲルはワコーゲル®C-200E (75~150 mm、破碎状)、ワコーゲル®C-300E (45~75  $\mu$ m、破碎状)、ワコーゲル®FC-40 (20~40  $\mu$ m) を使用した。分取薄層クロマトグラフィーは 70PF254 Plate- Wako (厚さ 0.75 mm) を用い行った。

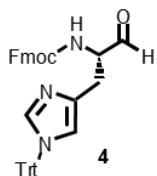
$^1\text{H}$ ,  $^{13}\text{C}$  NMR スペクトルは agilent UNITY INOVA 400 NB または JEOL JNM-ECS 400、Bruker AM-300、JEOL JNM-LA 500 のいずれかを用い測定を行った。溶媒に特に記載のないかぎり  $\text{CDCl}_3$  を用いた。ケミカルシフトには ppm を用い、特に記載がなければ  $^1\text{H}$  NMR では内部標準物質のテトラメチルシランの 0 ppm を、 $^{13}\text{C}$  NMR では 77.0 ppm を基準値として用いた。またカップリング定数には Hz を使用した。

比旋光度は、自動旋光計 HORIBA SEPA-300 を使用し測定を行った。

高分解能質量測定は JMS-HX-110A (FAB) または JEOL GCmateII (EI)、Shimadzu LCMS-IT-TOF (ESI) のいずれかを用い、低分解能質量測定は BRUKER autoflexII (TOF) または Shimadzu LCMS-2010EV (ESI) を用いた。

分析高速液体クロマトグラフィー (analytical HPLC) は HITACHI L7100 型 HPLC システム (ポンプ: HITACHI L-7100 型、紫外可視光検出器: HITACHI L-7400 型) を用い、分析用逆相カラム YMC-PACK ODS-AM (4.6 x 150 mm) または分析用キラルカラム (CHIRAL Amylose-C) を使用した。分取逆相高速液体クロマトグラフィー (preparative HPLC) は HITACHI L7100 型 HPLC システム (ポンプ: HITACHI L-7100 型、紫外可視光検出器: HITACHI L-7405 型) を用い、分取カラムは YMC-PACK ODS-AM (20 x 250 mm) を使用した。逆相分析 HPLC および分取 HPLC の溶出液は 0.1 % TFA を含む水溶液及びアセトニトリルを用い、2 液の濃度勾配による溶出を行った。光学純度の分析には 2-プロパノールとヘキサンを 1:540 の比率で事前に混合し脱気したものを用いアイソクラティック溶出法で分析を行った。

## 合成実験

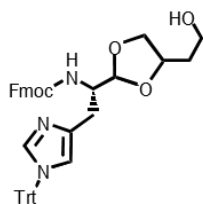


### Fmoc-His(Trt)-al (4).

室温下、Fmoc-His(Trt)-OH (1.67 g, 2.7 mmol) を溶かした DMF 溶液 (10 mL) に DIPEA (1.3 mL, 8.2 mmol)、BOP (1.2 g 2.7 mmol)、*N,O*-ジメチルヒドロキシアミン塩酸塩 (0.26 g, 2.7 mmol) の順に加え、90 分攪拌した。5%クエン酸を加え反応を停止し、5%炭酸水素ナトリウム水溶液、飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。溶媒を留去し、非晶質の白い粉を得た。

$^1\text{H}$  NMR (300 MHz):  $\delta$  = 7.75-7.10 (m, 24H), 6.58 (s, 1H), 6.14 (brd,  $J$  = 8.1 Hz, 1H), 4.96 (brdd,  $J$  = 8.1, 5.1 Hz 1H), 4.29 (d,  $J$  = 7.2 Hz, 2H), 4.19 (t,  $J$  = 7.2 Hz, 1H), 3.76 (s, 3H), 3.15 (s, 3H), 3.01 (brdd,  $J$  = 8.7, 5.1 Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz):  $\delta$  162.64, 156.43, 144.39, 142.29, 141.54, 138.98, 136.73, 130.15, 130.06, 128.32, 127.91, 127.37, 125.62, 120.18, 119.91, 75.52, 67.40, 61.98, 52.00, 47.48, 32.54, 31.00.

この未精製物を THF に溶かし、冷却攪拌下、LAH を加えた。10 分後水を加え反応を停止し、酢酸エチルを使い抽出した。飽和食塩水洗浄し、硫酸マグネシウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (hexane : AcOEt = 1 : 2) の流分から無色固体 (1.5 g, 64%) を得た。 $[\alpha]_D^{26} +18.2$  ( $c$  0.6,  $\text{CHCl}_3$ ),  $^1\text{H}$  NMR (300 MHz):  $\delta$  = 9.68 (brs), 7.77-7.08 (m, 24H), 6.62 (s, 1H), 6.45 (brd,  $J$  = 6.9 Hz, 1H), 4.97 (d,  $J$  = 7.5 Hz, 2H), 4.46 (dd,  $J$  = 6.9, 5.4 Hz 1H), 4.22 (t,  $J$  = 7.5 Hz, 1H), 3.16 (dd,  $J$  = 15.0, 5.4 Hz, 1H), 3.05 (dd,  $J$  = 15.0, 5.4 Hz, 1H);  $^{13}\text{C}$  NMR (75 MHz):  $\delta$  = 200.31, 156.39, 143.89, 142.47, 142.27, 141.29, 138.82, 135.88, 129.71, 128.13, 128.09, 128.02, 127.69, 127.54, 127.08, 125.24, 119.96, 119.85, 119.70, 75.37, 67.20, 59.89, 47.21, 27.24.



### Fmoc-His(Trt)-al 1-Octanol-ethylene Acetal [Fmoc-His(Trt)-acatal] (5).

室温下、Fmoc-His(Trt)-al **4** (0.59 g, 0.98mmol) と decane-1, 2, 10-triol (0.19 g, 0.98 mmol) の入ったジクロロメタン溶液 (10 mL) に三フッ化ホウ素ジエチルエーテル錯塩 (0.2 mL) を加え、30 分間攪拌した。水を加え反応を停止し、酢酸エチルを使い抽出した。飽和食塩水洗浄し、硫酸マグネシウムで乾燥させ、白い非晶質個体 (0.57 g, 75%) を得た。

$[\alpha]_D^{27} -6.3$  ( $c$  0.8,  $\text{CHCl}_3$ ),  $^1\text{H}$  NMR (300 MHz):  $\delta$  = 7.75-7.06 (m, 24H), 6.68 (s, 1H), 5.57-5.43 (m, 1H), 5.04-4.98 (m, 1H), 4.29-4.11 (m, 4H), 4.06-3.92 (m, 2H), 3.63-3.57 (m, 2H), 3.51-3.41 (m, 1H), 2.89-2.81 (m, 2H), 1.56-1.49 (m, 2H), 1.30-1.26 (m, 12H);  $^{13}\text{C}$  NMR (75 MHz):  $\delta$  = 162.49, 156.52, 144.20, 142.59,

141.38, 138.49, 137.77, 129.92, 128.87, 128.15, 128.00, 127.74, 127.19, 125.44, 120.02, 103.98, 75.38, 70.27, 67.09, 62.86, 53.42, 47.42, 33.25, 32.88, 29.52, 29.44, 29.35, 28.68, 25.84, 25.71.

#### Solid-Phase Synthesis of the Peptide Thioacetal [Ac-Ala-Val-Cha-His-(SEt)<sub>2</sub>]

氷冷下、アセトンに溶かした化合物 **4** (0.37 g, 0.48 mmol) に Jones 試薬 (0.3 mL; 2.67 M in acetone) を加え、その後室温下、40 分撹拌した。2-プロパノールを加え反応を停止させ、セライトショートパッドカラムに通し、2-プロパノールを使い溶出した。溶媒をエバポレーターで留去しクロロホルムを使い抽出した。有機層を飽和食塩水洗浄し、硫酸ナトリウムで乾燥させ、非晶質の粉末を得た。この化合物は精製せずに次の反応に用いた。

$[\alpha]_D^{27}$  -13.1 (c 0.8, CHCl<sub>3</sub>), <sup>1</sup>H NMR (300 MHz):  $\delta$  = 7.74-6.99 (m, 24H), 6.69 (s, 1H), 5.06 (d,  $J$  = 7.2 Hz, 1H), 4.38-4.06 (m, 6H), 3.95 (t,  $J$  = 6.9 Hz, 1H), 3.52-3.45 (m, 1H), 2.95-2.86 (m, 2H), 2.41-2.31 (m, 2H), 1.58-1.26 (m, 12H); <sup>13</sup>C NMR (75 MHz):  $\delta$  = 181.82, 155.60, 143.78, 141.20, 129.63, 128.47, 128.32, 128.23, 128.11, 127.66, 127.06, 125.29, 119.91, 66.85, 53.77, 47.15, 46.58, 34.32, 29.24, 28.93, 24.80.

DMF に十分膨潤させたリンクアミドレジンに 20% ピペリジン DMF 溶液を加え 25°C で 20 分間撹拌した。DMF を使いよく洗い、(この後の各反応の後処理はこの処理を行った、以後省略) その後、室温下、先のカルボン酸、HOBt (54 mg, 0.35 mmol), DIPEA (170  $\mu$ L, 1.1 mmol), DIC (62  $\mu$ L, 0.35 mmol), DMF (2 mL) を加え 10 時間撹拌した。次に 20% ピペリジン DMF 溶液を加え脱保護した。次に Fmoc-Cha-OH (170 mg, 0.44 mmol) を DIC/HOBt 条件を用い、同様の方法で縮合させた。同様に Fmoc-Val-OH (68 mg, 0.2 mmol), Fmoc-Ala-OH (62 mg, 0.2 mmol) と縮合させ、最後に DIPEA (130  $\mu$ L, 0.8 mmol) と無水酢酸 (76  $\mu$ L, 0.8 mmol) を加え Ac 化し、Ac-Ala-Val-Cha-His(Trt)-acetal resin の合成を達成した。その樹脂をよく乾燥させ、TFA (1.5 mL) とアニソール (87  $\mu$ L, 0.8 mmol) を加え、4 時間 25°C 撹拌した。反応液を濾過し、濃縮し反応を停止し、水を加えエーテルを用い洗った。最後に水槽を凍結乾燥した。

MALDI-TOF-MS. Calcd, 690.456 for C<sub>35</sub>H<sub>60</sub>N<sub>7</sub>O<sub>7</sub>, found, 690.302 for [M+H]<sup>+</sup>.

室温下、酢酸 (1 mL) に溶かしたその未精製物に、エタンチオール (0.13 mL, 1.8 mmol) と三フッ化ホウ素ジエチルエーテル錯塩 (100  $\mu$ L) を加え二時間撹拌した。水を加え反応を停止し、HPLC (CH<sub>3</sub>CN 10-60%, 60min, tR = 43.60 min) を使い精製し、目的物の白色粉末 (20 mg, 82%) を得た。

<sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O):  $\delta$  = 8.84 (s, 1H), 7.55 (s, 1H), 4.75-4.71 (m, 1H), 4.64-4.57 (m, 2H), 4.37 (d,  $J$  = 7.5 Hz, 1H), 4.32 (d,  $J$  = 4.8 Hz, 1H), 3.58 (dd,  $J$  = 15.3, 3.6 Hz, 1H), 3.32 (dd,  $J$  = 15.3, 10.8 Hz, 1H), 3.03 (q,  $J$  = 7.5 Hz, 2H), 3.01 (q,  $J$  = 7.5 Hz, 2H), 2.33-2.30 (m, 1H), 2.28 (s, 3H), 1.98-1.80 (m, 7H), 1.61 (d,  $J$  = 7.2 Hz, 3H), 1.56 (t,  $J$  = 7.5 Hz, 2H), 1.55 (t,  $J$  = 7.5 Hz, 3H), 1.56-1.47 (m, 4H), 1.23-1.20 (m, 2H), 1.15 (d,  $J$  = 6.6 Hz, 3H), 1.11 (d,  $J$  = 6.6 Hz, 3H); <sup>13</sup>C NMR (75 MHz):  $\delta$  = 174.88, 173.75, 173.72, 172.63, 133.66, 130.23, 117.30, 59.26, 54.88, 52.43, 51.67, 49.99, 39.33, 33.87, 33.36, 32.46, 30.69, 27.04, 26.44, 26.25, 26.15, 26.06, 25.98, 22.10, 18.79, 17.84, 16.99, 14.34, 14.18.

MALDI-TOF-MS. Calcd, 633.324 for C<sub>29</sub>H<sub>50</sub>N<sub>6</sub>O<sub>4</sub>S<sub>2</sub>Na, found, 633.299 for [M+Na]<sup>+</sup>.

#### Ac-Asn-Val-Cha-His-(SEt)<sub>2</sub>

上記の化合物と同様に合成した。Yield 74% <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O):  $\delta$  = 8.74 (s, 1H), 7.43 (s, 1H), 4.86 (brt,  $J$  = 6.9 Hz, 1H), 4.65-4.60 (m, 1H), 4.50-4.45 (m, 1H), 4.28 (d,  $J$  = 6.9 Hz, 1H), 4.21 (d,  $J$  = 5.1

Hz, 1H), 3.46 (dd,  $J = 15.3, 3.3$  Hz, 1H), 3.31 (dd,  $J = 15.6, 10.8$  Hz, 1H), 2.96-2.78 (m, 4H), 2.22-2.18 (m, 1H), 2.18 (s, 3H), 1.92-1.67 (m, 7H), 1.44 (t,  $J = 7.5$  Hz, 3H), 1.43 (t,  $J = 7.5$  Hz, 3H), 1.56-1.33 (m, 4H), 1.12-1.00 (m, 2H);  $^{13}\text{C}$  NMR (75 MHz):  $\delta = 174.36, 173.81, 173.80, 172.54, 172.53, 133.51, 130.00, 114.88, 59.17, 54.65, 52.25, 51.65, 50.67, 39.00, 36.44, 33.69, 33.18, 32.34, 30.60, 26.88, 26.32, 26.14, 26.02, 25.95, 25.85, 22.05, 18.65, 17.47, 14.23, 14.05$ .

MALDI-TOF-MS. Calcd, 654.347 for  $\text{C}_{30}\text{H}_{52}\text{N}_7\text{O}_2\text{S}_2$ , found, 654.442 for  $[\text{M}+\text{H}]^+$ .

#### Ac-Ser-Val-Cha-His-(SEt)<sub>2</sub>

上記の化合物と同様に合成した。Yield 21%  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 8.55$  (s, 1H), 7.26 (s, 1H), 4.53-4.43 (m, 2H), 4.34 (dd,  $J = 9.0, 6.3$  Hz, 1H), 4.13 (d,  $J = 7.2$  Hz, 1H), 4.08 (d,  $J = 4.8$  Hz, 1H), 3.81 (d,  $J = 6.3$  Hz, 1H), 3.31 (dd,  $J = 15.6, 3.0$  Hz, 1H), 3.07 (dd,  $J = 15.6, 11.1$  Hz, 1H), 2.753 (q,  $J = 7.5$  Hz, 2H), 2.746 (q,  $J = 7.5$  Hz, 2H), 2.06 (s, 3H), 2.10-2.03 (m, 1H), 1.69-1.54 (m, 7H), 1.29 (t,  $J = 7.5$  Hz, 3H), 1.27 (t,  $J = 7.5$  Hz, 3H), 1.36-1.15 (m, 4H), 1.00-0.9 (m, 2H), 0.89 (d,  $J = 6.9$  Hz, 3H), 0.84 (d,  $J = 6.9$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz):  $\delta = 174.35, 174.05, 172.61, 171.97, 133.43, 130.04, 116.93, 61.20, 59.23, 55.62, 54.34, 52.23, 51.52, 38.90, 33.51, 32.94, 32.05, 30.17, 26.86, 26.20, 26.06, 25.86, 25.80, 25.72, 21.84, 18.44, 17.49, 14.01, 13.82$ .

MALDI-TOF-MS. Calcd, 627.336 for  $\text{C}_{29}\text{H}_{51}\text{N}_6\text{O}_2\text{S}_2$  found, 627.332 for  $[\text{M}+\text{H}]^+$ .

#### Ac-Thr-Val-Cha-His-(SEt)<sub>2</sub>

上記の化合物と同様に合成した。Yield 31%  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 8.54$  (s, 1H), 7.22 (s, 1H), 4.50-4.43 (m, 1H), 4.32-4.28 (m, 1H), 4.22 (d,  $J = 5.7$  Hz, 1H), 4.08-4.01 (m, 3H), 3.26 (dd,  $J = 15.3, 3.0$  Hz, 1H), 3.03 (dd,  $J = 15.3, 11.1$  Hz, 1H), 2.70 (q,  $J = 7.5$  Hz, 2H), 2.69 (q,  $J = 7.5$  Hz, 2H), 2.02 (s, 3H), 2.02-1.98 (m, 1H), 1.60-1.48 (m, 7H), 1.23 (t,  $J = 7.5$  Hz, 3H), 1.22 (t,  $J = 7.5$  Hz, 3H), 1.13 (d,  $J = 6.6$  Hz, 3H), 1.30-1.11 (m, 4H), 0.95-0.84 (m, 2H), 0.85 (d,  $J = 6.6$  Hz, 3H), 0.75 (d,  $J = 6.6$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz):  $\delta = 174.38, 174.02, 172.45, 171.74, 133.23, 129.79, 119.88, 67.04, 59.49, 59.23, 54.27, 52.12, 51.35, 38.91, 26.74, 26.16, 26.00, 25.80, 25.76, 25.67, 21.76, 18.88, 18.37, 17.73, 13.94, 13.76$ .

MALDI-TOF-MS. Calcd, 641.352 for  $\text{C}_{30}\text{H}_{53}\text{N}_6\text{O}_5\text{S}_2$  found, 641.283 for  $[\text{M}+\text{H}]^+$ .

#### Ac-Ser-Ala-Val-Phe-His-(SEt)<sub>2</sub> (9)

上記の化合物と同様に合成した。Yield 18%  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 8.45$  (s, 1H), 7.33-7.12 (s, 1H), 4.53 (brt,  $J = 8.0$  Hz, 1H), 4.43-4.23 (m, 3H), 3.91 (d,  $J = 7.8$  Hz, 1H), 3.78 (d,  $J = 5.7$  Hz, 1H), 3.77 (d,  $J = 5.7$  Hz, 1H), 3.67 (d,  $J = 4.2$  Hz, 1H), 3.21 (dd,  $J = 15.3, 3.0$  Hz, 1H), 3.01 (dd,  $J = 15.3, 7.5$  Hz, 2H), 2.95-2.85 (m, 2H), 2.64 (q,  $J = 7.5$  Hz, 3H), 2.55 (q,  $J = 7.5$  Hz, 2H), 2.00 (s, 3H), 1.86-1.77 (m, 1H), 1.26 (d,  $J = 7.2$  Hz, 3H), 1.20 (t,  $J = 7.5$  Hz, 3H), 1.15 (t,  $J = 7.5$  Hz, 3H), 0.76 (d,  $J = 6.6$  Hz, 3H), 0.65 (d,  $J = 6.6$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz):  $\delta = 174.58, 174.53, 172.56, 172.36, 171.91, 136.15, 133.40, 130.02, 129.23, 128.84, 127.30, 116.86, 61.14, 59.32, 55.67, 54.62, 54.30, 52.33, 49.65, 37.41, 30.19, 26.32, 26.10, 21.78, 18.26, 17.71, 16.45, 13.94, 13.68$ .

MALDI-TOF-MS. Calcd, 692.327 for  $\text{C}_{32}\text{H}_{50}\text{N}_7\text{O}_6\text{S}_2$  found, 692.475 for  $[\text{M}+\text{H}]^+$ .

### Ac-Ser-Ala-Val-Cha-His-(SEt)<sub>2</sub>

Yield 17% <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ = 8.50 (s, 1H), 7.20 (s, 1H), 4.49-4.43 (m, 1H), 4.36-4.26 (m, 3H), 4.04 (d, *J* = 4.5 Hz, 1H), 3.96 (d, *J* = 8.1 Hz, 1H), 3.84-3.74 (m, 2H), 3.26 (d, *J* = 15.3, 3.3 Hz, 1H), 3.03 (dd, *J* = 15.3, 10.8 Hz, 1H), 2.69 (q, *J* = 7.5 Hz, 2H), 2.68 (q, *J* = 7.5 Hz, 2H), 2.01 (s, 3H), 2.01-1.88 (m, 1H), 1.58-1.42 (m, 7H), 1.32 (d, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 7.5 Hz, 3H), 1.20 (t, *J* = 7.5 Hz, 3H), 1.33-1.08 (m, 4H), 0.94-0.85 (m, 2H), 0.84 (d, *J* = 6.6 Hz, 3H), 0.75 (d, *J* = 6.6 Hz, 3H). <sup>13</sup>C NMR (75 MHz): δ = 174.66, 174.58, 174.22, 172.72, 171.98, 133.27, 129.87, 116.85, 61.11, 59.41, 55.72, 54.25, 52.22, 51.44, 49.75, 38.84, 33.41, 32.87, 31.88, 29.97, 26.79, 26.20, 25.97, 25.85, 25.73, 25.64, 21.75, 18.36, 17.73, 16.45, 13.88, 13.69.

MALDI-TOF-MS. Calcd, 698.374 for C<sub>32</sub>H<sub>56</sub>N<sub>7</sub>O<sub>6</sub>S<sub>2</sub> found, 698.389 for [M+H]<sup>+</sup>.

### Ac-Ala-Val-Cha-His-H

Ac-Ala-Val-Cha-His-(SEt)<sub>2</sub> (9.0 mg, 15 μmol)の溶けた混合溶媒 (THF : H<sub>2</sub>O = 2:1, 1.35 mL) に *N*-ブロモスクシンイミド [0.1 M in THF (159 μL, 15.9 μmol)] を加え、1 分間攪拌した。1 分後すぐに分取 HPLC を使い精製し、目的物 (3.2 mg, 43%) を得た。

Yield 21% <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ = 9.51 (brs), 8.53 (s, 1H), 7.21 (s, 1H), 4.31-4.20 (m, 2H), 4.11-4.06 (m, 1H), 4.00 (d, *J* = 8.1 Hz, 1H), 3.11 (dd, *J* = 15.3, 3.3 Hz, 1H), 2.84 (dd, *J* = 15.3, 10.8 Hz, 1H), 1.97 (s, 3H), 2.02-1.91 (m, 1H), 1.58-1.39 (m, 6H), 1.30 (d, *J* = 7.2 Hz, 3H), 1.25-1.05 (m, 5H), 0.86 (d, *J* = 6.9 Hz, 3H), 0.94-0.84 (m, 2H), 0.78 (d, *J* = 6.9 Hz, 3H).

MALDI-TOF-MS. Calcd, 505.314 for C<sub>25</sub>H<sub>41</sub>N<sub>6</sub>O<sub>5</sub> found, 505.353 for [M+H]<sup>+</sup>.

### Ac-Ser-Val-Cha-His-H

上記の化合物と同様に合成した。Yield 60% <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ = 9.42 (brs), 8.46 (s, 1H), 7.12 (s, 1H), 4.34-4.30 (m, 2H), 4.21-4.12 (m, 1H), 4.04-3.96 (m, 2H), 3.73-3.66 (m, 2H), 3.03 (dd, *J* = 15.3, 3.3 Hz, 1H), 2.76 (dd, *J* = 15.3, 10.8 Hz, 1H), 1.94 (s, 3H), 1.94-1.87 (m, 1H), 1.49-1.28 (m, 7H), 0.77 (d, *J* = 6.9 Hz, 3H), 0.72 (d, *J* = 6.9 Hz, 3H), 1.10-0.69 (m, 6H).

MALDI-TOF-MS. Calcd, 521.309 for C<sub>25</sub>H<sub>41</sub>N<sub>6</sub>O<sub>5</sub> found, 521.329 for [M+H]<sup>+</sup>.

### Ac-Thr-Val-Cha-His-H

上記の化合物と同様に合成した。Yield 40% <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ = 9.44 (brs), 8.44 (brs, 1H), 7.21 (brs, 1H), 4.41-4.37 (m, 1H), 4.25-4.13 (m, 3H), 4.05-4.02 (m, 1H), 3.31-3.24 (m, 1H), 3.08-3.01 (m, 1H), 2.18-2.11 (m, 1H), 2.03 (s, 3H), 1.63-1.41 (m, 7H), 1.24-1.11 (m, 7H), 0.93-0.89 (m, 8H).

MALDI-TOF-MS. Calcd, 535.324 for C<sub>26</sub>H<sub>43</sub>N<sub>6</sub>O<sub>6</sub> found, 535.383 for [M+H]<sup>+</sup>.

### Ac-Ser-Ala-Val-Phe-His-H (10)

上記の化合物と同様に合成した。Yield 12% <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ = 8.48 (brs, 1H), 7.31-7.15 (m, 6H), 4.51 (brt, *J* = 7.2 Hz, 1H), 4.34 (t, *J* = 5.7 Hz, 1H), 4.27 (q, *J* = 7.2 Hz, 1H), 4.04-3.99 (m, 1H),

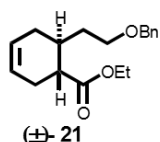
3.91 (d,  $J = 8.1$  Hz, 1H), 3.82-3.724 (m, 2H), 3.04 (dd,  $J = 15.6, 3.0$  Hz, 1H), 2.99-2.85 (m, 2H), 2.76 (dd,  $J = 15.6, 11.1$  Hz, 1H), 2.00 (s, 3H), 1.89-1.79 (m, 1H), 1.26 (d,  $J = 7.2$  Hz, 3H), 0.76 (d,  $J = 6.6$  Hz, 3H), 0.66 (d,  $J = 6.6$  Hz, 3H).

MALDI-TOF- MS. Calcd, 586.299 for  $C_{28}H_{40}N_7O_7$  found, 586.380 for  $[M+H]^+$ .

#### Ac-Ser-Ala-Val-Cha-His-H

上記の化合物と同様に合成した。Yield 46%  $^1H$  NMR (300 MHz,  $D_2O$ ):  $\delta = 9.47$  (brs), 8.28 (s, 1H), 7.10 (s, 1H), 4.37-4.25 (m, 3H), 4.09-4.03 (m, 1H), 3.96 (d,  $J = 8.1$  Hz, 1H), 3.81-3.74 (m, 2H), 3.06 (dd,  $J = 15.6, 3.6$  Hz, 1H), 2.79 (dd,  $J = 15.6, 10.8$  Hz, 1H), 2.01 (s, 3H), 2.01-1.89 (m, 1H), 1.59-1.42 (m, 6H), 1.33 (d,  $J = 7.2$  Hz, 3H), 1.24-1.00 (m, 5H), 0.85 (d,  $J = 6.6$  Hz, 3H), 0.93-0.79 (m, 2H), 0.78 (d,  $J = 6.6$  Hz, 3H).

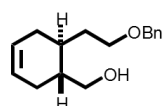
MALDI-TOF- MS. Calcd, 592.346 for  $C_{28}H_{46}N_7O_7$  found, 592.435 for  $[M+H]^+$ .



#### (1S/R, 6R/S)-ethyl 6-[2-(benzyloxy)ethyl]cyclohex-3-enecarboxylate (±)-21

耐圧反応装置 (TVS-N2 型 ポータブルリアクター、耐圧硝子工業製) に入れたエステル **22** (2.34 g, 10.0 mmol) に 1,3-ブタジエン [20% トルエン溶液 (17 mL, 40 mmol)] を加え、250 °C、60 時間加熱した。1M HCl を加え反応を停止させ、酢酸エチルで抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン : 酢酸エチル = 30:1) の流分から黄色の油状物 (1.87 g, 65%) を得た。

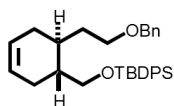
$^1H$  NMR (400 MHz):  $\delta = 7.36$ -7.31 (m, 4H), 7.29-7.26 (m, 1H), 5.64 (m, 2H), 4.51 (d,  $J = 11.6$  Hz, 1H), 4.46 (d,  $J = 12.0$  Hz, 1H), 4.14 (q,  $J = 7.2$  Hz, 2H), 3.54-3.50 (m, 2H), 2.41-2.35 (m, 1H), 2.31-2.20 (m, 3H), 2.09-2.04 (m, 1H), 1.84-1.73 (m, 2H), 1.54-1.45 (m, 1H), 1.25 (t,  $J = 7.2$  Hz, 3H);  $^{13}C$  NMR (100 MHz):  $\delta = 175.8, 138.5, 128.3, 127.6, 127.5, 125.7, 124.7, 72.8, 67.9, 60.2, 45.3, 33.7, 32.4, 29.9, 28.0, 14.2$ ; HRMS (EI) Calcd. For  $C_{18}H_{24}O_3$   $[M]^+$ : 288.1725. Found: 288.1722.



#### {(1S/R, 6R/S)-6-[2-(benzyloxy)ethyl]cyclohex-3-en-1-yl}methanol

氷冷攪拌下 LAH (387 mg, 10.2 mmol) の入ったエーテル溶液 (30 mL) にエステル(±)-**21** (1.47 g, 5.12 mmol) をゆっくり加えた。15 分後、水を加え反応を停止させ、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン : 酢酸エチル = 1:1) の流分から無色の油状物 (1.25 g, quant) を得た。

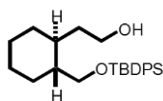
$^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.37-7.32 (m, 4H), 7.30-7.26 (m, 1H), 5.65-5.57 (m, 2H), 4.51 (s, 2H), 3.66 (dd,  $J$  = 10.8, 6.0 Hz, 1H), 3.62-3.48 (m, 3H), 2.14-2.09 (m, 2H), 2.01-1.75 (m, 5H), 1.66-1.59 (m, 1H), 1.55-1.48 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 138.3, 128.4, 127.7, 127.6, 125.8, 125.5, 73.1, 68.5, 65.0, 39.7, 32.9, 31.0, 29.5, 26.7; HRMS (EI) Calcd. For  $\text{C}_{16}\text{H}_{22}\text{O}_2$   $[\text{M}]^+$ : 246.1620. Found: 246.1618.



**{{(1*S*/R, 6*R*/S)-6-[2-(benzyloxy)ethyl]cyclohex-3-en-1-yl}methoxy)(*tert*-butyl)diphenylsilane**

室温下、イミダゾール (1.21 g, 10.3 mmol) と先のアルコール (2.92 g, 11.9 mmol) が入ったジクロロメタン溶液 (30 mL) に TBDPSCI (3.6 mL, 13.1 mmol) を入れ、16 時間攪拌した。飽和塩化アンモニウム水溶液を加え反応を停止させ、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン: 酢酸エチル = 20:1) の流分から無色の油状物 (5.76 g, quant) を得た。

$^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67-7.65 (m, 4H), 7.43-7.30 (m, 10H), 7.28 (m, 1H), 5.63-5.54 (m, 2H), 4.49 (d,  $J$  = 12.0 Hz, 1H), 4.45 (d,  $J$  = 12.0 Hz, 1H), 3.68 (dd,  $J$  = 10.0, 5.2 Hz, 1H), 3.62 (dd,  $J$  = 9.8, 7.0 Hz, 1H), 3.54-3.45 (m, 2H), 2.17-2.06 (m, 2H), 2.02-1.95 (m, 1H), 1.87-1.80 (m, 2H), 1.73-1.67 (m, 2H), 1.51-1.42 (m, 1H), 1.05 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 138.6, 135.62, 135.61, 133.98, 133.95, 129.5, 128.3, 127.58, 127.56, 127.4, 125.8, 125.4, 72.9, 68.6, 65.9, 39.6, 32.9, 30.9, 29.1, 26.9, 26.7, 19.3; HRMS (FAB) Calcd. For  $\text{C}_{32}\text{H}_{41}\text{O}_2\text{Si}$   $[\text{M}+\text{H}]^+$ : 485.2876. Found: 485.2870.

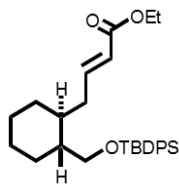


(±)-23

**2-[(1*R*/S, 6*S*/R)-6-[(*tert*-butyldiphenylsilyl)oxy]methyl]cyclohex-3-en-1-yl]ethanol (±)-23**

保護体 (3.40 g, 7.01 mmol) の入ったメタノール、酢酸エチル、ジクロロメタンの混合溶媒 (10:10:1, 21 mL) に活性炭に担持した水酸化パラジウム (610 mg) を加え、水素ガス雰囲気下室温で 12 時間攪拌した。水を加え、窒素ガス置換を十分行い、ドラフト内でジクロロメタンを使いろ紙濾過した。ろ液を濃縮し、シリカゲルカラムクロマトグラフィーに付し (ヘキサン: 酢酸エチル = 3:1) の流分から無色の油状物 (2.78 g, quant) を得た。

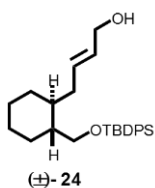
$^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.68-7.65 (m, 4H), 7.45-7.36 (m, 6H), 3.68-3.54 (m, 4H), 1.78-1.66 (m, 5H), 1.37-1.18 (m, 7H), 1.06 (s, 9H), 1.01-0.96 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 135.69, 135.66, 133.92, 133.90, 129.55, 129.54, 127.60, 127.57, 66.6, 61.1, 44.5, 36.5, 35.5, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3; HRMS (FAB) Calcd. For  $\text{C}_{25}\text{H}_{37}\text{O}_2\text{Si}$   $[\text{M}+\text{H}]^+$ : 397.2563. Found: 397.2569.



**(E)-ethyl 4-[(1R/S, 2S/R)-2-[(*tert*-butyldiphenylsilyl)oxy]methyl]cyclohex-1-yl]but-2-enoate**

氷冷撹拌下、アルコール(±)-**23** (2.50 g, 6.30 mmol) とセライト (3.5 g) の入ったジクロロメタン溶液 (20 mL) に、PCC (3.45 g, 16.0 mmol) を入れた。ゆっくりと室温までに昇温し、6 時間撹拌させた。反応液をシリカゲルショートパッドカラムに通し、エーテルを使い溶出させ黄色の油状物を得た。化合物が不安定なため精製作業を行わず、次の反応を行った。アルゴンガス雰囲気氷冷撹拌下、水素化ナトリウム [60% ミネラルオイル含有 (308 mg, 7.70 mmol)] の THF 溶液 (10 mL) にジエチルホスホ酢酸エチル (1.5 mL, 7.7 mmol) をゆっくり加えた。反応液が透明になるのを確認した後、-20 °C に冷やし、先のアルデヒドをゆっくり加え 90 分間撹拌した。飽和塩化アンモニウム水溶液を加え反応を停止させ、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン : 酢酸エチル = 20:1) の流分から無色の油状物 (2.70 g, 92%, 2 steps) を得た。

$^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67-7.64 (m, 4H), 7.45-7.36 (m, 6H), 6.91 (ddd,  $J$  = 15.4, 8.8, 6.4 Hz, 1H), 5.72 (d,  $J$  = 15.6 Hz, 1H), 4.18 (q,  $J$  = 7.1 Hz, 2H), 3.63-3.57 (m, 2H), 2.38-2.32 (m, 1H), 1.97 (td,  $J$  = 14.8, 8.1 Hz, 1H), 1.79-1.76 (m, 1H), 1.71-1.69 (m, 4H), 1.54-1.49 (m, 1H), 1.32-1.18 (m, 3H), 1.29 (t,  $J$  = 7.2 Hz, 3H), 1.05 (s, 9H), 1.03-0.97 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 166.6, 148.2, 135.62, 135.61, 133.82, 133.80, 129.59, 129.55, 127.62, 127.59, 122.4, 66.2, 60.1, 43.9, 37.8, 36.4, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3, 14.3; HRMS (FAB) Calcd. For  $\text{C}_{29}\text{H}_{40}\text{NaO}_3\text{Si}$   $[\text{M}+\text{Na}]^+$ : 487.2644. Found: 487.2651.



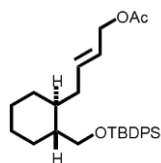
**(E)-4-[(1R/S, 2S/R)-2-[(*tert*-butyldiphenylsilyl)oxy]methyl]cyclohexyl]but-2-en-1-ol (±)-24**

-78 °C、アルゴンガス雰囲気下、エステル (1.92 g, 4.13 mmol) を入れたジクロロメタン溶液 (20 mL) に DIBALH [1.0 M ヘキサン溶液 (12.4 mL, 12.4 mmol)] をゆっくり加え、15 分間撹拌した。メタノールを加え反応を停止させ、徐々に室温まで昇温させ、シリカゲルショートパッドカラムに通し酢酸エチルを用い溶出した。ろ液を濃縮し、シリカゲルカラムクロマトグラフィーに付し (ヘキサン : 酢酸エチル = 1:1) の流分から無色の油状物 (1.74 g, quant) を得た。

$^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.68-7.65 (m, 4H), 7.44-7.36 (m, 6H), 5.64-5.48 (m, 2H), 4.04 (d,  $J$  = 6.0 Hz, 2H), 3.66 (dd,  $J$  = 10.0, 2.8 Hz, 1H), 3.58 (dd,  $J$  = 9.8, 5.4 Hz, 1H), 2.23-2.17 (m, 1H), 1.87-1.79 (m, 2H), 1.72-1.69 (m, 3H), 1.43-1.32 (m, 1H), 1.30-1.18 (m, 4H), 1.05 (s, 9H), 1.01-0.94 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 135.64, 135.63, 134.0, 131.6, 130.2, 129.52, 129.50, 127.58, 127.55, 66.3, 63.8, 43.9, 38.1, 36.2,



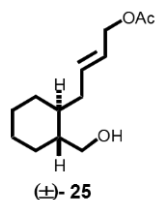
31.7, 30.0, 26.9, 26.2, 26.1, 19.4; HRMS (FAB) Calcd. For  $C_{27}H_{38}NaO_2Si$   $[M+Na]^+$ : 445.2539. Found: 445.2541.



**(E)-4-[(1R/S, 2S/R)-2-[(*tert*-butyldiphenylsilyl)oxy]methyl]cyclohexyl]but-2-en-1-yl acetate**

氷冷下、アルコール(±)-**24** (1.74 g, 4.11 mmol) とピリジン (0.50 mL, 6.2 mmol) の入ったジクロロメタン溶液 (20 mL) に、無水酢酸 (0.59 mL, 6.19 mmol) を加え 1 時間攪拌した。飽和塩化アンモニウム水溶液を加え反応を停止させ、酢酸エチルを使い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン: 酢酸エチル = 30:1) の流分から無色の油状物 (1.81 g, 95%) を得た。

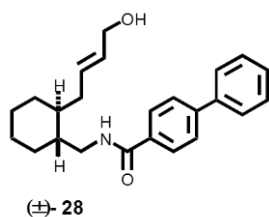
$^1H$  NMR (400 MHz):  $\delta$  = 7.67-7.64 (m, 4H), 7.44-7.36 (m, 6H), 5.71-5.64 (m, 1H), 5.49-5.42 (m, 1H), 4.47 (d,  $J$  = 6.4 Hz, 2H), 3.65 (dd,  $J$  = 9.8, 3.0 Hz, 1H), 3.57 (dd,  $J$  = 10.0, 4.8 Hz, 1H), 2.23-2.18 (m, 1H), 2.05 (s, 3H), 1.87-1.79 (m, 2H), 1.71-1.68 (m, 3H), 1.43-1.35 (m, 1H), 1.30-1.18 (m, 4H), 1.05 (s, 9H), 1.00-0.94 (m, 1H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 170.9, 135.6, 134.8, 133.94, 133.93, 129.5, 127.6, 125.0, 66.3, 65.3, 43.8, 38.0, 36.3, 31.7, 30.0, 26.9, 26.2, 26.1, 21.0, 19.3; HRMS (FAB) Calcd. For  $C_{29}H_{40}NaO_3Si$   $[M+Na]^+$ : 487.2644. Found: 487.2642.



**(E)-4-[(1R/S, 2S/R)-2-(hydroxymethyl)cyclohexyl]but-2-en-1-yl acetate (±)-25**

室温下、先の保護体 (1.81 g, 3.89 mmol) の入った THF 溶液 (20 mL) にフッ化テトラ-*n*-ブチルアンモニウム [1 M THF solution (7.8 mL, 7.8 mmol)] を加え、12 時間攪拌した。飽和塩化アンモニウム水溶液を加え反応を停止させ、酢酸エチルを使い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン: 酢酸エチル = 6:1) の流分から無色の油状物 (1.03 g, quant) を得た。

$^1H$  NMR (400 MHz):  $\delta$  = 5.80-5.72 (m, 1H), 5.60-5.53 (m, 1H), 4.51 (d,  $J$  = 6.4 Hz, 2H), 3.69 (dd,  $J$  = 10.8, 3.2 Hz, 1H), 3.59 (dd,  $J$  = 10.8, 5.6 Hz, 1H), 2.33-2.27 (m, 1H), 2.06 (s, 3H), 2.02-1.90 (m, 1H), 1.81-1.79 (m, 1H), 1.74-1.67 (m, 3H), 1.37-1.11 (m, 5H), 1.05-0.95 (m, 1H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 170.9, 134.5, 125.3, 65.7, 65.2, 43.8, 38.0, 36.4, 31.7, 29.5, 26.0, 25.8, 21.0; HRMS (FAB) Calcd. For  $C_{13}H_{22}NaO_3$   $[M+Na]^+$ : 249.1467. Found: 249.1460.



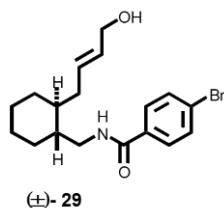
***N*-({(1*S*/*R*, 2*R*/*S*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl}-[1,1'-biphenyl]-4-carboxamide (±)-28**

アルゴンガス雰囲気氷冷撹拌下、アルコール(±)-25 (1.03 g, 4.56 mmol) を THF (10 mL) に溶かし、PPh<sub>3</sub> (2.80 g, 10.8 mmol)、DEAD [40% トルエン溶液 (4.2 mL, 10.8 mmol)]、DPPA (2.4 mL, 11 mmol) の順に加えた。温度を保ったまま、16 時間撹拌した後、反応停止のため濃縮した。シリカゲルショートパッドカラムに通し、混合溶液 (ヘキサン : 酢酸エチル = 30:1) を使い溶出した。得られた粗精製物は不安定なためこれ以上の精製を行わず次の反応に使用した。

<sup>1</sup>H NMR (400 MHz): δ = 5.77-5.69 (m, 1H), 5.62-5.54 (m, 1H), 4.52 (d, *J* = 6.0 Hz, 2H), 3.40 (dd, *J* = 12.0, 3.2 Hz, 1H), 3.25 (dd, *J* = 12.2, 6.2 Hz, 1H), 2.29-2.24 (m, 1H), 2.06 (s, 3H), 2.00-1.91 (m, 1H), 1.80-1.65 (m, 4H), 1.34-1.29 (m, 2H), 1.27-1.11 (m, 3H), 1.05-0.95 (m, 1H).

0°C、アルゴンガス雰囲気下 LAH (1.04 g, 27.4 mmol) の入ったエーテル溶液 (10 mL) に、先の粗精製物をゆっくりと加えた。12 時間加熱還流し、氷冷下メタノールを加え反応を停止した。反応液を濃縮し、残留溶媒が残らないように注意した。次にアルゴンガス雰囲気氷冷撹拌下、4-フェニル安息香酸 (903 mg, 4.56 mmol) と DIPEA (2.4 mL, 14 mmol) が入ったジクロロメタン溶液 (10 mL) に、HBTU (4.32 g, 11.4 mmol) を加えた。20 分撹拌後、先の濃縮物をジクロロメタン (10 mL) に溶かし加え、3 時間撹拌した。氷冷下飽和塩化アンモニウム水溶液を加え反応を停止させ、ジクロロメタンを使い抽出した。飽和食塩水洗浄し、硫酸ナトリウムを用い乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン : 酢酸エチル = 1:1) の流分から無色の油状物 (1.04 g, 63%, 3 steps) を得た。

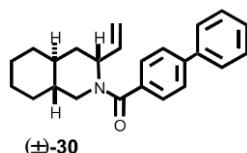
<sup>1</sup>H NMR (400 MHz): δ = 7.84-7.82 (m, 2H), 7.67-7.59 (m, 4H), 7.48-7.44 (m, 2H), 7.41-7.37 (m, 1H), 6.28 (brs, 1H), 5.79-5.67 (m, 2H), 4.10 (d, *J* = 4.4 Hz, 2H), 3.78 (ddd, *J* = 13.6, 6.0, 3.6 Hz, 1H), 3.20 (ddd, *J* = 13.7, 8.1, 5.9 Hz, 1H), 2.32-2.27 (m, 1H), 2.21-2.12 (m, 1H), 1.87-1.84 (m, 1H), 1.74-1.72 (m, 3H), 1.52-1.41 (m, 1H), 1.32-1.04 (m, 5H); <sup>13</sup>C NMR (100 MHz): δ = 167.2, 144.2, 140.0, 133.3, 131.2, 130.5, 128.9, 128.0, 127.3, 127.23, 127.17, 63.8, 43.3, 41.1, 39.6, 36.5, 31.9, 30.6, 26.0, 25.7; HRMS (EI) Calcd. For C<sub>24</sub>H<sub>29</sub>NO<sub>2</sub> [M]<sup>+</sup>: 363.2198. Found: 363.2207.



**4-bromo-*N*-({(1*S*/*R*, 2*R*/*S*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl}benzamide (±)-29**

化合物 28 と同様に合成した。Yield 50% (3 steps): <sup>1</sup>H NMR (400 MHz): δ = 7.64-7.61 (m, 2H),

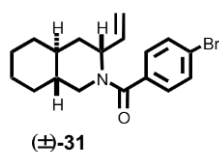
7.58-7.56 (m, 2H), 6.16 (m, 1H), 5.78-5.66 (m, 2H), 4.10 (d,  $J = 4.8$  Hz, 2H), 3.76 (ddd,  $J = 13.4, 5.8, 3.8$  Hz, 1H), 3.16 (ddd,  $J = 13.7, 8.1, 5.9$  Hz, 1H), 2.29-2.25 (m, 1H), 2.18-2.11 (m, 1H), 1.84-1.80 (m, 1H), 1.73-1.71 (m, 3H), 1.50-1.41 (m, 1H), 1.28-0.96 (m, 5H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 166.5, 133.5, 131.8, 131.2, 130.4, 128.5, 126.0, 63.7, 43.3, 41.0, 39.6, 36.4, 31.9, 30.5, 26.0, 25.7$ ; HRMS (EI) Calcd. For  $\text{C}_{18}\text{H}_{24}\text{BrNO}_2$   $[\text{M}]^+$  365.0990. Found 365.0996.



**(1,1'-biphenyl)-4-yl{(3S/R,4aR/S,8aS/R)-3-vinyloctahydroisoquinolin-2(1H)-yl}methanone (±)-30**

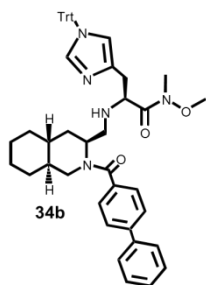
0°C、アルゴンガス雰囲気下、アルコール (120 mg, 0.331 mmol) を入れたジクロロメタン溶液 (1 mL) にビスアセトニトリルジクロロパラジウム (5 mg, 0.056 mmol) を加え、4 時間攪拌した。反応液を濃縮後、シリカゲルカラムクロマトグラフィーに付し (ヘキサン : 酢酸エチル = 10:1) の流分から無色の油状物 (100 mg, 88%) を得た。

$^1\text{H}$  NMR (400 MHz):  $\delta = 7.64$ -7.58 (m, 4H), 7.49-7.43 (m, 4H), 7.38-7.35 (m, 1H), 5.87 (ddd,  $J = 17.5, 10.7, 3.7$  Hz, 0.4H), 5.78 (ddd,  $J = 17.5, 10.7, 3.5$  Hz, 0.6H), 5.55 (brs, 0.4H), 5.31-5.28 (m, 1H), 5.23-5.16 (m, 1H), 4.54 (brs, 0.6H), 4.49 (dd,  $J = 13.2, 4.0$  Hz, 0.6H), 3.49 (dd,  $J = 13.0, 3.8$  Hz, 0.4H), 2.86 (dd,  $J = 13.2, 11.6$  Hz, 0.4H), 2.61 (dd,  $J = 12.8, 11.6$  Hz, 0.6H), 1.84-1.52 (m, 5H), 1.47-1.18 (m, 5H), 1.15-1.13 (m, 0.4H), 1.03-0.98 (m, 1H), 0.90-0.84 (m, 0.6H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 171.1, 170.4, 142.3, 142.2, 140.3, 137.1, 136.7, 135.4, 128.8, 127.69, 127.66, 127.4, 127.1, 126.8, 116.6, 116.1, 57.2, 50.8, 49.7, 43.5, 42.8, 41.9, 37.5, 36.8, 35.9, 32.9, 29.9, 29.7, 26.2, 26.1, 25.8, 25.7$ ; HRMS (EI) Calcd. For  $\text{C}_{24}\text{H}_{27}\text{BrNO}$   $[\text{M}]^+$ : 345.2093. Found: 345.2090.



**(4-bromophenyl)((3S,4aR/S,8aS/R)-3-vinyloctahydroisoquinolin-2(1H)-yl)methanone (±)-31**

化合物 **30** と同様に合成した。Yield 57%:  $^1\text{H}$  NMR (400 MHz):  $\delta = 7.56$ -7.50 (m, 2H), 7.29-7.27 (m, 2H), 5.84 (ddd,  $J = 17.4, 10.6, 3.8$  Hz, 0.4H), 5.74 (ddd,  $J = 17.5, 10.7, 3.5$  Hz, 0.6H), 5.49 (brs, 0.4H), 5.29-5.26 (m, 1H), 5.19-5.10 (m, 1H), 4.44 (dd,  $J = 13.4, 3.8$  Hz, 0.6H), 4.39 (s, 0.6H), 3.33 (dd,  $J = 13.2, 3.6$  Hz, 0.4H), 2.82 (dd,  $J = 13.0, 11.8$  Hz, 0.4H), 2.57 (dd,  $J = 13.0, 11.4$  Hz, 0.6H), 1.83-1.49 (m, 5H), 1.43-1.19 (m, 5H), 1.13-1.04 (m, 0.4H), 0.99-0.96 (m, 1H), 0.88-0.83 (m, 0.6H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 170.2, 169.6, 136.9, 136.5, 135.4, 131.7, 131.6, 128.6, 128.0, 123.7, 123.6, 116.7, 116.2, 57.2, 50.8, 49.6, 43.5, 42.8, 41.8, 37.5, 36.7, 35.9, 32.8, 29.9, 29.6, 26.1, 26.0, 25.7, 25.6$ ; HRMS (EI) Calcd. For  $\text{C}_{18}\text{H}_{22}\text{BrNO}$   $[\text{M}]^+$ : 347.0885. Found: 347.0879.



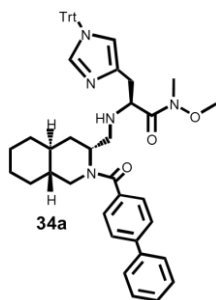
**(S)-2-([[(3S,4aR,8aS)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl]methyl]amino)-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide 34b**

室温下、環化体 **30** (286 mg, 0.829 mmol) の入った THF と水の混合溶液 (3:1, 10 mL) に、オスミウム(VI)酸カリウム二水和物 (3.1 mg, 0.0083 mmol) と NMO (389 mg, 3.32 mmol) を加えた。12 時間攪拌後、反応液に過ヨウ素酸ナトリウム (710 mg, 3.32 mmol) を加え 30 分攪拌させた。水を加え反応を停止させ、酢酸エチルを用い抽出した。飽和食塩水で洗い、硫酸ナトリウムを使い乾燥させた。シリカゲルショートパッドカラムに通し、混合溶媒 (ヘキサン : 酢酸エチル = 3:1) を使い溶出させ粗生成物 **32** を得た。

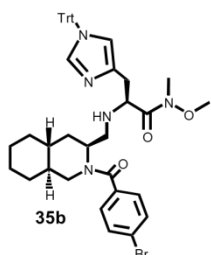
$^1\text{H}$  NMR (400 MHz):  $\delta$  = 9.69 (s, 0.75H), 9.65 (s, 0.25H), 7.69-7.54 (m, 5H), 7.49-7.37 (m, 4H), 5.50 (d,  $J$  = 6.4 Hz, 0.75H), 4.62-4.59 (m, 0.25H), 4.44 (d,  $J$  = 5.6 Hz, 0.25H), 3.69-3.65 (m, 0.75H), 2.81 (dd,  $J$  = 13.2, 11.6 Hz, 0.75H), 2.40 (t,  $J$  = 12.6 Hz, 0.25H), 2.33 (d,  $J$  = 13.6 Hz, 0.75H), 2.15 (dd,  $J$  = 13.6 Hz, 0.25H), 1.74-1.69 (m, 3H), 1.59-1.50 (m, 1H), 1.44-1.41 (m, 1H), 1.25-1.11 (m, 3H), 1.08-0.96 (m, 2H), 0.92-0.76 (m, 1H).

His 誘導体 (410 mg, 0.930 mmol) の入ったジクロロメタン溶液 (1 mL) に先の粗精製物 **32** と酢酸 (0.05 mL, 0.8 mmol) を加えた。2 時間攪拌後、氷冷下、水素化シアノホウ素ナトリウム (181 mg, 2.88 mmol) を加え、30 分攪拌した。1M 塩酸水溶液を加え反応を停止させ、酢酸エチルを用い抽出した。飽和炭酸水素ナトリウム水溶液、飽和食塩水で洗浄し、硫酸ナトリウムを使い乾燥させた。フラッシュカラムクロマトグラフィーに付し (クロロホルム : メタノール = 25:1) の流分から無色油状物 **34a** と **34b** を得た。

Compound **34b**: [80 mg, 13% (50% max.), 3 steps]  $[\alpha]_{\text{D}}^{28}$  -20 ( $c$  0.48,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.59-7.53 (m, 4H), 7.47-7.41 (m, 4H), 7.37-7.29 (m, 11H), 7.13-7.09 (m, 6H), 6.62 (m, 0.6H), 6.56 (m, 0.4H), 4.94 (brs, 0.6H), 4.41 (dd,  $J$  = 13.0, 3.0 Hz, 0.4H), 4.22-4.11 (m, 0.4H), 3.93 (m, 1H), 3.69 (s, 1.8H), 3.50 (s, 1.2H), 3.44-3.41 (m, 0.6H), 3.14 (s, 1.8H), 3.08 (s, 1.2H), 2.93-2.84 (m, 2.4H), 2.76-2.66 (m, 2H), 2.46 (t,  $J$  = 12.2 Hz, 0.6H), 1.80-1.70 (m, 3H), 1.61-1.54 (m, 1H), 1.43-1.17 (m, 6H), 1.08-0.85 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 175.4, 175.2, 171.3, 170.5, 142.44, 142.38, 141.88, 141.86, 140.42, 140.35, 138.2, 138.1, 137.6, 137.2, 135.9, 135.7, 129.72, 129.66, 129.3, 128.8, 128.7, 127.91, 127.87, 127.54, 127.46, 127.4, 127.2, 127.1, 127.04, 126.99, 119.3, 115.6, 77.2, 75.03, 75.02, 61.6, 61.5, 57.8, 57.4, 55.5, 49.5, 48.3, 47.1, 46.6, 43.1, 42.6, 42.1, 36.4, 36.2, 34.4, 33.0, 32.9, 32.6, 32.2, 32.0, 29.9, 29.7, 26.14, 26.05, 25.8, 25.7; HRMS (EI) Calcd. For  $\text{C}_{50}\text{H}_{53}\text{N}_5\text{O}_3$   $[\text{M}]^+$ : 771.4148. Found: 771.4141.

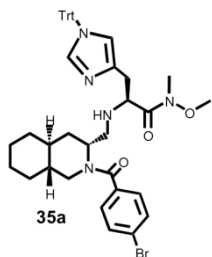


Compound **34a**: [75 mg, 12% (50% max.), 3 steps]  $[\alpha]_D^{28} +32$  (c 2.3,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.58-7.24 (m, 19H), 7.13-7.07 (m, 6H), 6.58 (m, 0.4H), 6.55 (m, 0.6H), 5.02-4.97 (m, 0.4H), 4.46 (dd,  $J$  = 13.2, 3.6 Hz, 0.6H), 4.13 (brs, 0.4H), 3.95 (m, 1H), 3.65 (s, 1.2H), 3.62-3.58 (m, 0.6H), 3.50 (s, 1.8H), 3.44 (dd,  $J$  = 13.4, 3.4 Hz, 0.4H), 3.14 (s, 1.2H), 3.11 (s, 1.8H), 3.01-2.94 (m, 1H), 2.89-2.81 (m, 2H), 2.65 (dd,  $J$  = 11.8, 6.6 Hz, 0.4H), 2.52 (dd,  $J$  = 12.0, 6.8 Hz, 0.6H), 2.50-2.44 (m, 0.6H), 2.26-2.24 (brs, 1H), 1.71-1.69 (m, 3H), 1.60-1.52 (m, 2H), 1.45-1.16 (m, 5H), 1.07-0.83 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 175.6, 175.3, 171.1, 170.8, 142.44, 142.37, 141.9, 141.8, 140.41, 140.39, 138.12, 138.08, 137.5, 137.3, 135.7, 129.72, 129.66, 128.73, 128.68, 127.9, 127.5, 127.4, 127.1, 127.05, 127.03, 126.95, 119.5, 119.3, 77.2, 75.0, 61.6, 61.5, 57.7, 57.5, 55.4, 49.3, 48.4, 47.4, 47.2, 43.0, 42.8, 42.0, 36.7, 36.5, 34.6, 33.5, 33.00, 32.96, 32.3, 32.1, 29.9, 29.7, 29.6, 26.2, 26.0, 25.8, 25.7; HRMS (EI) Calcd. For  $\text{C}_{50}\text{H}_{53}\text{N}_5\text{O}_3$   $[\text{M}]^+$ : 771.4148. Found: 771.4154.



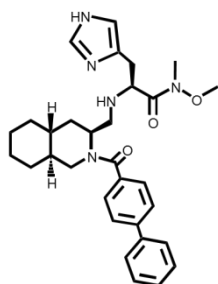
**(S)-2-([(3S,4aR,8aS)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl]methyl)amino)-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide 35b**

化合物 **34a** と **34b** と同様に合成した。Yield 11% (50% max., 3 steps):  $[\alpha]_D^{28} -31$  (c 0.83,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.47 (d,  $J$  = 8.4 Hz, 1.2H), 7.44 (d,  $J$  = 8.4 Hz, 0.8H), 7.34-7.31 (m, 10.8H), 7.22 (d,  $J$  = 8.4 Hz, 1.2H), 7.12-7.11 (m, 6H), 6.60 (brs, 0.6H), 6.55 (brs, 0.4H), 4.87 (m, 0.6H), 4.37 (dd,  $J$  = 13.2, 3.6 Hz, 0.4H), 4.10 (brs, 0.6H), 3.89 (brs, 0.4H), 3.78 (m, 0.6H), 3.64 (s, 1.8H), 3.51 (s, 1.2H), 3.24 (dd,  $J$  = 13.2, 3.6 Hz, 0.6H), 3.13 (s, 1.8H), 3.11 (s, 1.2H), 2.91-2.80 (m, 2.4H), 2.73-2.62 (m, 2H), 2.47-2.41 (m, 0.4H), 1.76-1.65 (m, 3.4H), 1.60-1.54 (m, 1.6H), 1.36-1.25 (m, 5H), 1.00-0.82 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 175.5, 175.1, 170.4, 169.7, 142.5, 142.4, 138.3, 138.1, 137.7, 137.2, 135.9, 135.8, 131.52, 131.49, 129.75, 129.72, 128.7, 128.4, 127.94, 127.91, 123.24, 123.21, 119.26, 119.25, 77.2, 75.1, 61.6, 61.5, 57.8, 57.5, 55.6, 49.3, 48.4, 47.1, 46.6, 43.1, 42.6, 42.0, 36.4, 36.2, 34.5, 33.0, 32.9, 32.7, 32.3, 32.0, 29.9, 29.7, 26.1, 26.0, 25.8, 25.7; HRMS (EI) Calcd. For  $\text{C}_{44}\text{H}_{48}\text{BrN}_5\text{O}_3$   $[\text{M}]^+$ : 773.2941. Found: 773.2948.



**(S)-2-([(3R,4aS,8aR)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl]methyl)amino)-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide 35a**

化合物 **34a** と **34b** と同様に合成した。Yield 11% (50% max., 3 steps):  $[\alpha]_D^{28} +4.5$  (c 0.42,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.47-7.43 (m, 2H), 7.37-7.29 (m, 12H), 7.12-7.10 (m, 6H), 6.55 (m, 1H), 4.98-4.95 (m, 0.4H), 4.40 (dd,  $J$  = 13.2, 3.6 Hz, 0.6H), 4.10 (brs, 0.4H), 3.90 (brs, 0.6H), 3.84-3.81 (m, 0.6H), 3.64-3.58 (m, 0.4H), 3.63 (s, 1.8H), 3.54 (s, 1.2H), 3.28 (dd,  $J$  = 13.2, 3.6 Hz, 0.4H), 3.13 (s, 1.8H), 3.11 (s, 1.2H), 2.98-2.91 (m, 1H), 2.86-2.74 (m, 2.6H), 2.60 (dd,  $J$  = 11.6, 6.0 Hz, 0.6H), 2.47-2.41 (m, 1.4H), 1.72-1.65 (m, 3H), 1.59-1.47 (m, 2H), 1.43-1.12 (m, 5H), 1.04-0.79 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 175.6, 175.2, 170.3, 170.0, 142.42, 142.37, 138.2, 138.1, 137.35, 137.25, 135.70, 135.66, 131.47, 131.45, 129.73, 129.70, 128.9, 128.7, 127.93, 127.91, 123.32, 123.26, 119.5, 119.3, 77.2, 75.1, 75.0, 61.5, 57.5, 57.4, 55.5, 49.2, 48.4, 47.4, 47.2, 42.9, 42.8, 42.0, 36.6, 36.5, 34.7, 33.6, 33.0, 32.9, 32.3, 32.0, 29.8, 29.6, 26.1, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For  $\text{C}_{44}\text{H}_{48}\text{BrN}_5\text{O}_3$   $[\text{M}]^+$ : 773.2941. Found: 773.2944.

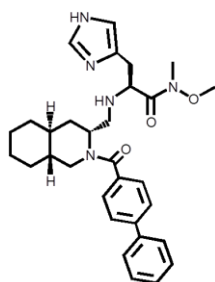


**(S)-2-([(3S,4aR,8aS)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl]methyl)amino]-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

還元的アミノ化物 **34b** (40 mg, 0.052 mmol) に TFA、ジクロロメタン、TIS、水の混合溶媒 (10:10:1.0:1.0, 5.5 mL) を加えた。反応停止のため濃縮し、その後飽和炭酸水素ナトリウム水溶液、飽和食塩水で洗い、酢酸エチル用い抽出した。硫酸ナトリウムを使い乾燥し、シリカゲルカラムクロマトグラフィーに付し (クロロホルム : メタノール = 10:1) の流分から黄色の油状物 (25 mg, 90%) を得た。

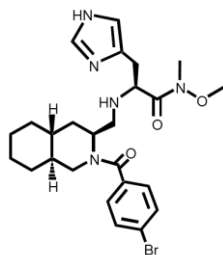
$[\alpha]_D^{28} -33$  (c 0.51,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.68-7.38 (m, 10H), 7.36 (s, 0.4H), 6.84 (s, 0.6H), 6.82 (s, 0.4H), 5.03-5.01 (m, 0.4H), 4.31-4.27 (m, 0.6H), 4.15 (brs, 0.6H), 3.86 (brs, 0.4H), 3.73 (s, 1.2H), 3.66 (s, 1.8H), 3.54-3.51 (m, 1H), 3.25 (s, 1.2H), 3.19 (s, 1.8H), 3.00-2.86 (m, 1H), 2.75-2.62 (m, 2H), 2.52-2.44 (m, 2H), 1.77-1.68 (m, 3.4H), 1.62-1.59 (m, 1.6H), 1.49-1.23 (m, 5H), 1.17-1.11 (m, 0.4H), 1.07-0.99 (m, 1H), 0.89-0.85 (m, 0.6H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 174.4, 171.0, 143.1, 142.4, 140.2, 140.1, 135.8, 135.3, 135.2, 134.8, 128.84, 128.83, 128.2, 127.8, 127.7, 127.5, 127.20, 127.18, 127.14, 127.08,

77.2, 61.7, 59.8, 58.4, 55.7, 49.5, 49.4, 48.6, 48.1, 43.5, 42.6, 42.0, 36.7, 34.3, 34.1, 33.0, 32.9, 32.2, 30.0, 29.6, 26.2, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For  $C_{31}H_{39}N_5O_3$   $[M]^+$ : 529.3053. Found: 529.3057.



**(S)-2-([(3R,4aS,8aR)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl]methyl)amino]-N-methoxy-N-methyl-3-(1H-imidazol-4-yl)propanamide**

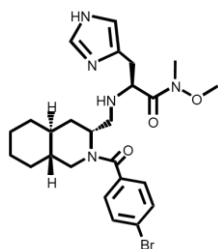
上記の化合物と同様に合成した。Yield quant:  $[\alpha]_D^{28}$  -41 (*c* 0.45,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.65-7.59 (m, 4H), 7.54 (s, 1H), 7.49-7.44 (m, 4H), 7.39-7.36 (m, 1H), 6.78 (m, 1H), 5.21-5.20 (m, 0.75H), 4.52-4.49 (m, 0.25H), 4.12 (m, 0.25H), 3.90-3.88 (m, 0.75H), 3.67 (s, 2.25H), 3.67-3.65 (m, 0.75H), 3.56 (s, 0.75H), 3.56-3.49 (m, 0.75H), 3.25 (s, 2.25H), 3.25-3.21 (m, 0.25H), 3.21 (s, 0.75H), 3.11-3.05 (m, 0.25H), 2.98-2.95 (m, 0.75H), 2.89-2.83 (m, 0.75H), 2.63-2.52 (m, 1.5H), 2.37 (dd, *J* = 12.0, 4.4 Hz, 1H), 2.29 (m, 1H), 1.72 (brs, 2H), 1.62-1.41 (m, 4H), 1.30-1.22 (m, 4H), 1.19-1.06 (m, 0.75H), 1.00-0.85 (m, 1.25H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 174.9, 171.6, 171.0, 142.4, 140.2, 135.6, 135.4, 135.2, 134.4, 128.9, 128.8, 127.7, 127.4, 127.2, 127.1, 127.0, 77.2, 61.7, 58.5, 55.5, 49.4, 49.1, 47.5, 42.8, 42.3, 36.9, 36.8, 35.2, 34.3, 33.1, 32.9, 32.3, 29.9, 29.65, 29.56, 29.2, 26.2, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For  $C_{31}H_{39}N_5O_3$   $[M]^+$ : 529.3053. Found: 529.3060.



**(S)-2-([(3S,4aR,8aS)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl]methyl)amino]-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

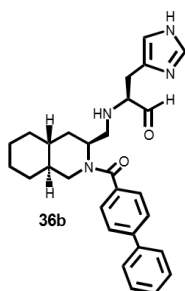
上記の化合物と同様に合成した。Yield 70%:  $[\alpha]_D^{28}$  -33.9 (*c* 0.42,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.66 (s, 0.6H), 7.56-7.53 (m, 2H), 7.38 (s, 0.4H), 7.32 (d, *J* = 8.4 Hz, 1.2H), 7.19 (d, *J* = 8.4 Hz, 0.8H), 6.83 (s, 0.6H), 6.81 (s, 0.4H), 4.97-4.95 (m, 0.4H), 4.26-4.22 (m, 0.6H), 4.00-3.98 (m, 0.6H), 3.85-3.84 (m, 0.4H), 3.72 (s, 1.2H), 3.66 (s, 1.8H), 3.56-3.53 (m, 0.6H), 3.35 (dd, *J* = 13.4, 3.8 Hz, 0.4H), 3.24 (s, 1.2H), 3.20 (s, 1.8H), 2.99-2.83 (m, 2H), 2.71-2.60 (m, 2H), 2.54-2.41 (m, 2H), 1.77-1.58 (m, 4H), 1.51-1.33 (m, 1H), 1.30-1.17 (m, 5H), 1.05-0.80 (m, 2H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 174.3, 173.1, 170.2, 135.8, 135.4, 135.3, 134.7, 131.9, 131.7, 129.3, 128.3, 124.2, 123.7, 77.2, 61.7, 59.7, 58.4, 55.8, 49.6, 49.4, 48.5, 48.0, 43.5, 42.6, 42.0, 36.6, 34.2, 34.0, 33.0, 32.8, 32.6, 29.9, 29.6, 26.1, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For

C<sub>25</sub>H<sub>34</sub>BrN<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 531.1845. Found: 531.1839.



**(S)-2-([(3R,4aS,8aR)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl]methyl)amino)-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide**

上記の化合物と同様に合成した。Yield 65%:  $[\alpha]_D^{28} -27.7$  (c 0.96, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.57-7.47 (m, 3H), 7.30-7.27 (m, 2H), 6.79 (s, 0.25H), 6.78 (s, 0.75H), 5.17-5.14 (m, 0.75H), 4.45 (dd,  $J$  = 13.4, 3.4 Hz, 0.25H), 3.94 (brs, 0.25H), 3.87-3.86 (m, 0.75H), 3.67 (s, 2.25H), 3.59 (s, 0.75H), 3.39 (dd,  $J$  = 13.6, 3.2 Hz, 0.75H), 3.25 (s, 2.25H), 3.21 (s, 0.75H), 3.19-3.16 (m, 0.75H), 3.07-3.01 (m, 0.25H), 2.98-2.89 (m, 1H), 2.82 (dd,  $J$  = 13.4, 11.8 Hz, 0.75H), 2.70-2.48 (m, 1.5H), 2.36 (dd,  $J$  = 12.2, 4.6 Hz, 0.75H), 2.30 (dd,  $J$  = 11.8, 5.8 Hz, 0.25H), 1.82-1.61 (m, 5H), 1.48-1.28 (m, 5H), 1.09-1.04 (m, 0.75H), 0.98-0.87 (m, 1.25H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 174.9, 170.7, 170.2, 135.6, 135.31, 135.26, 134.5, 131.8, 131.6, 128.7, 128.4, 123.8, 123.5, 77.2, 61.7, 58.4, 58.0, 55.4, 49.5, 49.1, 47.5, 47.4, 42.8, 42.7, 42.2, 36.8, 36.7, 35.1, 34.2, 33.0, 32.9, 32.3, 29.8, 29.5, 29.2, 26.1, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For C<sub>25</sub>H<sub>34</sub>BrN<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 531.1845. Found: 531.1839.



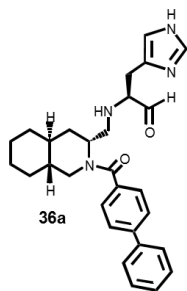
**(S)-2-([(3S,4aR,8aS)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl]methyl)amino]-3-(1H-imidazol-4-yl)propanal 36b**

-78°C、アルゴンガス雰囲気下、脱保護体 (33 mg, 0.070 mmol) を入れたジクロロメタン溶液 (1 mL) に、DIBALH [1.0 M in hexane (1.4 mL, 1.4 mmol)] を加え 5 分間攪拌した。反応停止のためメタノールを加え、直ちに中性シリカゲルを詰めたショートパッドカラムに通し、メタノールを用い溶出した。溶出液の濃縮後、分取 HPLC を使い精製しアルデヒド **36b** (10.5 mg, 28%) を得た。

$[\alpha]_D^{28} -3.2$  (c 0.48, CH<sub>3</sub>OH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.80 (brs, 1H), 7.75-7.73 (m, 2H), 7.67-7.65 (m, 2H), 7.58 (d,  $J$  = 8.4 Hz, 2H), 7.50 (brs, 1H), 7.48-7.45 (m, 2.5H), 7.40-7.36 (m, 1.5H), 5.13 (m, 1H), 4.82 (dd,  $J$  = 8.4, 3.2 Hz, 1H), 3.88-3.79 (m, 2H), 3.63-3.59 (m, 1H),

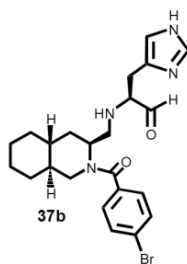


3.43-3.40 (m, 1H), 3.34 (s, 1H), 2.97 (t,  $J=12.6$  Hz, 1H), 1.77-1.68 (m, 5H), 1.45-1.34 (m, 5H), 1.06-0.98 (m, 2H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ , referenced to  $\text{CD}_3\text{OD}$ ):  $\delta = 175.1, 175.0, 163.0, 162.7, 144.70, 144.66, 141.1, 141.04, 135.5, 134.86, 134.80, 129.87, 129.86, 129.00, 128.97, 128.92, 128.0, 127.9, 118.6, 95.0, 94.9, 61.3, 61.0, 50.7, 50.5, 49.6, 47.2, 47.1, 43.2, 43.1, 37.59, 37.56, 35.2, 33.5, 30.2, 26.9, 26.5, 23.1, 22.9$ ; HRMS (ESI) Calcd. For  $\text{C}_{29}\text{H}_{35}\text{N}_4\text{O}_2$   $[\text{M}+\text{H}]^+$ : 471.2760. Found: 471.2756.



**(S)-2-[(*3R,4aS,8aR*)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methylamino]-3-(1H-imidazol-4-yl)propanal **36a****

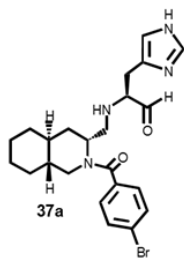
化合物 **36b** と同様に合成した。Yield 30%:  $[\alpha]_{\text{D}}^{29} -2.3$  ( $c$  0.61,  $\text{CH}_3\text{OH}$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , referenced to residual  $\text{CH}_3\text{OH}$ ):  $\delta = 8.76$  (s, 1H), 7.74 (d,  $J = 6.4$  Hz, 2H), 7.66 (d,  $J = 6.0$  Hz, 2H), 7.56 (d,  $J = 6.4$  Hz, 2H), 7.48-7.45 (m, 3.5H), 7.40-7.37 (m, 1.5H), 5.09 (brs, 1H), 3.86-3.75 (m, 2H), 3.64-3.59 (m, 1H), 3.55-3.48 (m, 1H), 3.35-3.32 (m, 1H), 3.28-3.26 (m, 1H), 2.93-2.91 (m, 1H), 1.79-1.66 (m, 5H), 1.47-1.28 (m, 5H), 1.07-0.97 (m, 2H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ , referenced to  $\text{CD}_3\text{OD}$ ):  $\delta = 175.5, 175.4, 163.1, 162.8, 145.0, 141.2, 135.7, 135.6, 134.9, 130.1, 129.2, 129.1, 128.2, 128.1, 119.0, 95.4, 95.1, 62.1, 61.6, 50.8, 43.2, 43.1, 37.7, 35.5, 35.4, 33.8, 33.7, 30.4, 27.0, 26.6, 24.5, 24.1$ ; LRMS (ESI) Calcd. For  $\text{C}_{29}\text{H}_{35}\text{N}_4\text{O}_2$   $[\text{M}+\text{H}]^+$ : 471.28. Found: 471.30.



**(S)-2-(((*3S,4aR,8aS*)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl)methylamino)-3-(1H-imidazol-4-yl)propanal **37b****

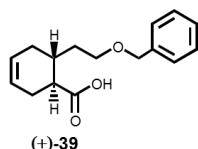
化合物 **36b** と同様に合成した。Yield 36%:  $[\alpha]_{\text{D}}^{28} -1.1$  ( $c$  0.40,  $\text{CH}_3\text{OH}$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ , referenced to residual  $\text{CH}_3\text{OH}$ ):  $\delta = 8.72$  (brs, 1H), 7.66-7.64 (m, 2H), 7.46 (brs, 1H), 7.41 (d,  $J = 8.4$  Hz, 2H), 5.11-5.03 (m, 1H), 4.78 (dd,  $J = 11.0, 3.0$  Hz, 1H), 3.85-3.75 (m, 2H), 3.47-3.39 (m, 2H), 3.26-3.24 (m, 1H), 2.96-2.84 (m, 1H), 1.78-1.54 (m, 5H), 1.43-1.22 (m, 5H), 1.07-0.93 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ , referenced to  $\text{CD}_3\text{OD}$ ):  $\delta = 174.1, 173.5, 163.1, 162.6, 135.7, 135.45, 135.39, 133.0, 130.39, 130.35, 130.2, 125.84, 125.78, 118.8, 118.7, 95.1, 94.9, 61.3, 61.0, 50.64, 50.58, 43.3, 43.2, 37.71, 37.70$ ,

37.68, 35.25, 35.22, 33.7, 30.4, 30.3, 27.0, 26.6, 23.2, 23.0; HRMS (ESI) Calcd. For  $C_{23}H_{30}BrN_4O_2$   $[M+H]^+$ : 473.1552. Found: 473.1543.



**(S)-2-((3R,4aS,8aR)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl)methylamino)-3-(1H-imidazol-4-yl)propanal 37a**

化合物 **36b** と同様に合成した。Yield 28%:  $[\alpha]_D^{29} -7.8$  (c 0.36,  $CH_3OH$ );  $^1H$  NMR (500 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.72 (brs, 1H), 7.66-7.62 (m, 2H), 7.45 (s, 1H), 7.43-7.36 (m, 2H), 5.06 (m, 1H), 3.83-3.75 (m, 2H), 3.49-3.46 (m, 2H), 3.34-3.33 (m, 1H), 3.28-3.23 (m, 1H), 2.92-2.85 (m, 1H), 1.76-1.58 (m, 5H), 1.44-1.26 (m, 5H), 1.06-0.93 (m, 2H);  $^{13}C$  NMR (125 MHz,  $CD_3OD$ , referenced to  $CD_3OD$ ):  $\delta$  = 174.43, 174.35, 163.1, 162.8, 135.7, 135.6, 135.3, 133.0, 130.3, 125.9, 118.8, 95.4, 95.1, 62.1, 61.6, 50.7, 43.1, 43.0, 37.7, 37.6, 35.4, 35.3, 33.7, 30.3, 27.0, 26.6, 24.6, 24.1; LRMS (ESI) Calcd. For  $C_{23}H_{30}BrN_4O_2$   $[M+H]^+$ : 473.16. Found: 473.25.

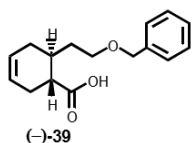


**(1S,6R)-6-[2-(benzyloxy)ethyl]cyclohex-3-enecarboxylic acid (+)-39**

環化体(±)-**21** (1.23 g, 4.27 mmol) を 2M 水酸化ナトリウムと THF 混合溶液 (1:1, 100 mL) に溶かし、15 時間加熱還流した。氷冷攪拌下、反応を停止させるため 2M 塩酸をゆっくり加えた。反応溶液が酸性になっているか pH 試験紙で確認し、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーを用い精製し (ヘキサン:酢酸エチル = 3:1) 黄色の油状物を得た。次に室温下、先のカルボン酸(±)-**39** (1.11 g, 4.27 mmol) をエーテルとトルエン混合溶媒 (5.5 mL, 10:1) に溶かし、(S)- $\alpha$ -methylbenzylamine (0.48 mL, 3.85 mmol) を入れた。よくかき混ぜ、セプタムの上から 0.9 x 38 mm の針を 1 本指して 3 日間室温で静置した。桐山ロートを使い、少量のエーテルを使い洗い込み白い結晶を得た。得られた結晶を 1M 塩酸を使い塩解除し、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン:酢酸エチル = 3:1) の流分から黄色の油状物(+)-**39** [50 mg, 4.5% (50% max.)] を得た。

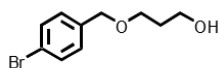
$[\alpha]_D^{25} +51.6$  (c 0.83,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.34-7.31 (m, 4H), 7.30-7.26 (m, 1H), 5.68-5.62 (m, 2H), 4.50 (dd,  $J$  = 18.0, 12.0 Hz, 2H), 3.59-3.51 (m, 2H), 2.47-2.21 (m, 4H), 2.12-2.08 (m, 1H), 1.92-1.86 (m, 1H), 1.81-1.75 (m, 1H), 1.60-1.52 (m, 1H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 181.4, 138.2, 128.4, 127.7, 127.6,

125.7, 124.5, 72.9, 67.8, 45.0, 33.6, 32.1, 29.5, 27.7; HRMS (EI) Calcd. For  $C_{16}H_{20}O_3$   $[M]^+$ : 260.1413. Found: 260.1417.



### (1R,6S)-6-[2-(benzyloxy)ethyl]cyclohex-3-enecarboxylic acid (-)-39

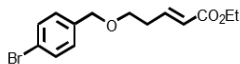
(*R*)- $\alpha$ -methylbenzylamine を用い (+)-39 と同様に合成した。Yield 2.3% (50% max.):  $[\alpha]_D^{28} -54.5$  (*c* 0.155,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.34-7.31 (m, 4H), 7.30-7.27 (m, 1H), 5.68-5.62 (m, 2H) 4.50 (dd,  $J$  = 18.0, 12.0 Hz, 2H), 3.59-3.51 (m, 2H), 2.47-2.21 (m, 4H), 2.12-2.08 (m, 1H), 1.92-1.86 (m, 1H), 1.81-1.75 (m, 1H), 1.60-1.52 (m, 1H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 181.4, 138.2, 128.4, 127.7, 127.6, 125.7, 124.5, 72.9, 67.8, 45.0, 33.7, 32.1, 29.5, 27.7; HRMS (EI) Calcd. For  $C_{16}H_{20}O_3$   $[M]^+$ : 260.1410. Found: 260.1417.



### 3-[(4-bromobenzyl)oxy]propan-1-ol

氷冷下、水素化ナトリウム [60% ミネラルオイル含有 (1.76 g, 44.0 mmol)] と 1,3-プロパンジオール (3.06 g, 40.2 mmol) を入れた DMF 溶液 (60 mL) に 4-ブロモベンジルブロミド (10.0 g, 40.0 mmol) を加え、16 時間攪拌した。氷冷下飽和塩化アンモニウム水溶液を加え反応を停止させ、酢酸エチルを使い抽出した。飽和食塩水洗浄し、硫酸ナトリウムを用い乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン: 酢酸エチル = 3:1) の流分から無色の油状物 (6.12 g, 62%) を得た。

$^1H$  NMR (400 MHz):  $\delta$  = 7.48-7.46 (m, 2H), 7.21-7.19 (m, 2H), 4.47 (s, 2H), 3.79 (t,  $J$  = 5.8 Hz, 2H), 3.65 (t,  $J$  = 5.8 Hz, 2H), 2.17 (brs, 1H), 1.87 (quint.,  $J$  = 5.8 Hz, 2H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 137.1, 131.6, 129.2, 121.6, 72.5, 69.4, 61.8, 32.1; HRMS (EI) Calcd. For  $C_{10}H_{13}BrO_2$   $[M]^+$ : 244.0099. Found: 244.0094.

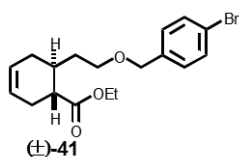


### (*E*)-ethyl 5-[(4-bromobenzyl)oxy]pent-2-enoate

氷冷攪拌下、アルコール (6.12 g, 24.9 mmol) とセライト (16.1 g) の入ったジクロロメタン溶液 (300 mL) に、PCC (16.1 g, 74.7 mmol) を入れた。ゆっくりと室温まで昇温し、5 時間攪拌させた。反応液をシリカゲルショートパッドカラムに通し、エーテルを使い溶出させ黄色の油状物を得た。化合物が不安定なため精製作業を行わず、次の反応を行った。アルゴンガス雰囲気氷冷攪拌下、水素化ナトリウム [60% ミネラルオイル含有 (1.20 g, 29.9 mmol)] の THF 溶液に (30 mL) ジエチルホスホ酢酸エチル (5.5 mL, 27.4 mmol) をゆっくり加えた。反応液が透明になるのを確認した後、 $-20^\circ C$  に冷やし、先の油状物をゆっくり加え 90 分間攪拌した。飽和塩化アンモニウ

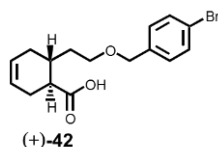
ム水溶液を加え反応を停止させ、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し（ヘキサン：酢酸エチル = 20:1）の流分から黄色の油状物（4.99 g, 64%, 2 steps）を得た。

$^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.47 (d,  $J$  = 8.0 Hz, 2H), 7.20 (d,  $J$  = 8.0 Hz, 2H), 6.97 (td,  $J$  = 15.6, 6.8 Hz, 1H), 5.89 (d,  $J$  = 15.6 Hz, 1H), 4.47 (s, 2H), 4.19 (q,  $J$  = 7.2 Hz, 2H), 3.58 (t,  $J$  = 6.6 Hz, 2H), 2.51 (ddd,  $J$  = 13.2, 6.4, 1.2 Hz, 2H), 1.29 (d,  $J$  = 7.2 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 166.4, 145.3, 137.1, 131.5, 129.2, 123.0, 121.5, 72.3, 68.4, 60.2, 32.6, 14.3; HRMS (EI) Calcd. For  $\text{C}_{14}\text{H}_{17}\text{BrO}_3$   $[\text{M}]^+$ : 312.0361. Found: 312.0365.



**(1*S*/R, 6*R*/S)-ethyl 6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-enecarboxylate (±)-41**

(±)-**21** と同様に合成した。Yield 50%:  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.47-7.45 (m, 2H), 7.22 (d,  $J$  = 8.4 Hz, 2H), 5.65 (m, 2H), 4.43 (dd,  $J$  = 18.8, 12.0 Hz, 2H), 4.14 (q,  $J$  = 7.2 Hz, 2H), 3.52-3.49 (m, 2H), 2.41-2.20 (m, 4H), 2.08-2.03 (m, 1H), 1.81-1.72 (m, 2H), 1.53-1.46 (m, 1H), 1.26 (t,  $J$  = 7.2 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 175.8, 137.5, 131.4, 129.2, 125.7, 124.8, 121.3, 72.1, 68.1, 60.3, 45.3, 33.7, 32.4, 29.9, 28.1, 14.3; HRMS (EI) Calcd. For  $\text{C}_{18}\text{H}_{23}\text{BrO}_3$   $[\text{M}]^+$ : 366.0831. Found: 366.0826.

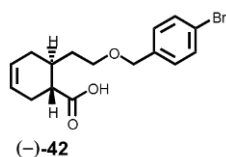


**(1*S*, 6*R*)-6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-enecarboxylic acid (+)-42**

環化体(±)-**41** (31.8 g, 86.6 mmol) を 2M 水酸化ナトリウムと THF 混合溶液 (1:1, 100 mL) に溶かし、15 時間加熱還流した。氷冷攪拌下、反応を停止させるため 2M 塩酸をゆっくり加えた。反応溶液が酸性になっているか pH 試験紙で確認し、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーを使い精製し（ヘキサン：酢酸エチル = 3:1）黄色の油状物 (29.3 g, quant.) を得た。次に室温下、先のカルボン酸 (29.3 g, 86.6 mmol) を酢酸エチル (300 mL) に溶かし、(*S*)- $\alpha$ -methylbenzylamine (11 mL, 87 mmol) を入れた。よくかき混ぜ、セプタムの上から 1.2 x 38 mm の針を 3 本指して 12 時間静置した。桐山ロートを使い、少量の酢酸エチルを使い洗い込み白い結晶を得た。得られた結晶を 1M 塩酸を使い塩解除し、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し（ヘキサン：酢酸エチル = 3:1）の流分から無色の油状物 [7.63 g, 26% (50% max.)] を得た。

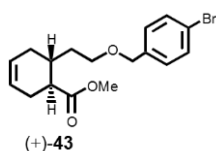
$[\alpha]_{\text{D}}^{25} +22$  (c 0.78,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.47-7.45 (m, 2H), 7.20 (d,  $J$  = 8.0 Hz, 2H), 5.69-5.66 (m, 2H), 4.44 (dd,  $J$  = 17.0, 12.2 Hz, 2H), 3.56-3.49 (m, 2H), 2.47-2.20 (m, 4H), 2.12-2.07 (m, 1H), 1.91-1.75 (m, 2H), 1.60-1.51 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 181.1, 137.3, 131.5, 129.3, 125.7,

124.5, 121.4, 72.2, 68.0, 44.9, 33.6, 32.1, 29.5, 27.7; HRMS (EI) Calcd. For  $C_{16}H_{19}BrO_3$   $[M]^+$ : 338.0518. Found: 338.0520.



**(1R,6S)-6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-enecarboxylic acid (-)-42**

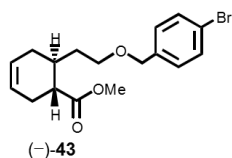
(*R*)- $\alpha$ -methylbenzylamineを用い、(+)-42と同様に合成した。Yield 20%;  $[\alpha]_D^{29}$  -33.2 (*c* 1.16,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.46 (d, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 5.66 (m, 2H), 4.44 (dd, *J* = 17.2, 12.0 Hz, 2H), 3.55-3.52 (m, 2H), 2.46-2.22 (m, 4H), 2.12-2.07 (m, 1H), 1.91-1.86 (m, 1H), 1.80-1.74 (m, 1H), 1.57-1.51 (m, 1H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 181.9, 137.3, 131.4, 129.2, 125.6, 124.4, 121.4, 77.2, 72.1, 67.9, 45.0, 33.5, 31.9, 29.5, 27.7; HRMS (EI) Calcd. For  $C_{16}H_{19}BrO_3$   $[M]^+$ : 338.0518. Found: 338.0511.



**(1S,6R)-methyl 6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-enecarboxylate (+)-43**

氷冷下、カルボン酸 (20 mg, 0.059 mmol) の入ったメタノール溶液 (2 mL) に塩化チオニル (18 mL, 0.24 mmol) を入れ 3 時間攪拌した。飽和塩化アンモニウム水溶液を加え反応を停止させ、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン: 酢酸エチル = 10:1) の流分から無色の油状物 (12 mg, 56%) を得た。

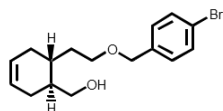
$[\alpha]_D^{27}$  +28.5 (*c* 1.18,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.46 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 5.68-5.65 (m, 2H), 4.45 (d, *J* = 12.0 Hz, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 3.68 (s, 3H), 3.52-3.48 (m, 2H), 2.43-2.35 (m, 1H), 2.32-2.24 (m, 3H), 2.10-2.01 (m, 1H), 1.80-1.72 (m, 2H), 1.53-1.44 (m, 1H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 176.2, 137.5, 131.4, 129.2, 125.7, 124.7, 121.3, 72.1, 68.1, 51.5, 45.2, 33.8, 32.4, 29.9, 28.1; HRMS (EI) Calcd. For  $C_{17}H_{21}BrO_3$   $[M]^+$ : 352.0674. Found: 352.0671.



**(1R,6S)-methyl 6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-enecarboxylate (-)-43**

(+)-43 と同様に合成した。Yield 52%;  $[\alpha]_D^{28}$  -40 (*c* 0.78,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.46 (d, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 5.67-5.61 (m, 2H), 4.43 (dd, *J* = 18.0, 12.0 Hz, 2H), 3.68 (s, 3H),

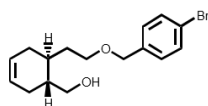
3.54-3.46 (m, 2H), 2.43-2.19 (m, 4H), 2.10-2.02 (m, 1H), 1.80-1.72 (m, 2H), 1.53-1.44 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 176.2, 137.5, 131.4, 129.2, 125.7, 124.7, 121.3, 72.1, 68.1, 51.5, 45.2, 33.8, 32.4, 29.9, 28.1; HRMS (EI) Calcd. For  $\text{C}_{17}\text{H}_{21}\text{BrO}_3$   $[\text{M}]^+$ : 352.0674. Found: 352.0681.



**[(1S,6R)-6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-en-1-yl]methanol**

アルゴンガス雰囲気下、 $-20^\circ\text{C}$ にてカルボン酸 (+)-**42** (7.70 g, 22.7 mmol)とトリエチルアミン (6.4 mL, 46 mmol) の入ったTHF溶液 (80 mL) にクロロギ酸イソブチル (4.5 mL, 34 mmol) を入れ、15分間攪拌した。温度を変えず、水素化ホウ素ナトリウム (3.47 g, 91.2 mmol) と水 (パスツールピペット10滴) をいれ、徐々に室温まで上げた。室温まで上がったら飽和塩化アンモニウム水溶液を加え反応を停止させ、酢酸エチルを用い抽出した。飽和食塩水洗浄し、硫酸ナトリウムで乾燥させた。シリカゲルカラムクロマトグラフィーに付し (ヘキサン : 酢酸エチル = 3:1) の流分から無色の油状物 (5.68 g, 77%) を得た。

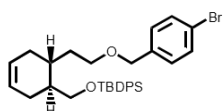
$[\alpha]_{\text{D}}^{29} +22$  (c 0.65,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.48-7.46 (m, 2H), 7.20 (d,  $J$  = 8.4 Hz, 2H), 5.65-5.58 (m, 2H), 4.45 (s, 2H), 3.68 (dd,  $J$  = 10.8, 6.4 Hz, 1H), 3.62 (dd,  $J$  = 10.8, 5.2 Hz, 1H), 3.58-3.47 (m, 2H), 2.15-2.09 (m, 2H), 2.00-1.76 (m, 4H), 1.66-1.49 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 137.4, 131.5, 129.3, 125.8, 125.5, 121.4, 72.3, 68.7, 65.0, 39.7, 32.9, 31.1, 29.5, 26.6; HRMS (EI) Calcd. For  $\text{C}_{16}\text{H}_{21}\text{BrO}_2$   $[\text{M}]^+$ : 324.0725. Found: 324.0732.



**[(1R,6S)-6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-en-1-yl]methanol**

上記の化合物と同様に合成した。

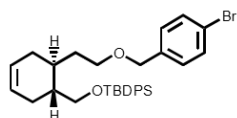
Yield 73%:  $[\alpha]_{\text{D}}^{29} -20$  (c 0.80,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.46 (d,  $J$  = 8.4 Hz, 2H), 7.20 (d,  $J$  = 8.4 Hz, 2H), 5.65-5.58 (m, 2H), 4.45 (s, 2H), 3.68 (dd,  $J$  = 10.8, 6.0 Hz, 1H), 3.61 (dd,  $J$  = 10.8, 4.8 Hz, 1H), 3.58-3.47 (m, 2H), 2.15-2.09 (m, 2H), 2.00-1.76 (m, 4H), 1.66-1.62 (m, 1H), 1.57-1.50 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 137.3, 131.5, 129.3, 125.8, 125.5, 121.4, 72.3, 68.7, 65.0, 39.7, 32.9, 31.1, 29.5, 26.6; HRMS (EI) Calcd. For  $\text{C}_{16}\text{H}_{21}\text{BrO}_2$   $[\text{M}]^+$ : 324.0725. Found: 324.0723.



**{[(1S, 6R)-6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-en-1-yl]methoxy}(tert-butyl)diphenylsilane**

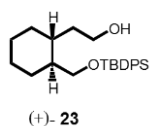
上記の化合物と同様に合成した。Yield quant:  $[\alpha]_{\text{D}}^{28} +18.6$  (c 1.74,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67-7.64 (m, 4H), 7.44-7.34 (m, 8H), 7.17 (d,  $J$  = 8.4 Hz, 2H), 5.63-5.54 (m, 2H), 4.41 (dd,  $J$  = 15.2, 12.0

Hz, 2H), 3.68 (dd,  $J = 9.8, 5.4$  Hz, 1H), 3.62 (dd,  $J = 10.0, 6.8$  Hz, 1H), 3.50-3.46 (m, 2H), 2.16-1.96 (m, 3H), 1.87-1.81 (m, 2H), 1.71-1.68 (m, 2H), 1.48-1.44 (m, 1H), 1.05 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 137.7, 135.61, 135.60, 133.94, 133.92, 131.4, 129.5, 129.1, 127.6, 125.8, 125.3, 121.2, 72.1, 68.7, 65.9, 39.6, 32.9, 30.8, 29.0, 26.9, 26.7, 19.3$ ; HRMS (FAB) Calcd. For  $\text{C}_{32}\text{H}_{40}\text{BrO}_5$   $[\text{M}+\text{H}]^+$ : 563.1981. Found: 563.1988.



**[(1R,6S)-6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-en-1-yl]methoxy)(*tert*-butyl)diphenylsilane**

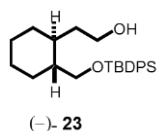
上記の化合物と同様に合成した。Yield 88%:  $[\alpha]_{\text{D}}^{28} -19.5$  ( $c$  1.15,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta = 7.67-7.64$  (m, 4H), 7.45-7.34 (m, 8H), 7.18 (d,  $J = 8.0$  Hz, 2H), 5.63-5.54 (m, 2H), 4.41 (dd,  $J = 15.2, 12.0$  Hz, 2H), 3.68 (dd,  $J = 9.8, 5.4$  Hz, 1H), 3.62 (dd,  $J = 10.0, 6.8$  Hz, 1H), 3.53-3.44 (m, 2H), 2.16-1.96 (m, 3H), 1.88-1.80 (m, 2H), 1.73-1.68 (m, 2H), 1.50-1.44 (m, 1H), 1.05 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 137.7, 135.61, 135.60, 133.94, 133.92, 131.4, 129.5, 129.1, 127.6, 125.8, 125.3, 121.2, 72.1, 68.7, 65.9, 39.6, 32.9, 30.8, 29.0, 26.9, 26.7, 19.3$ ; HRMS (FAB) Calcd. For  $\text{C}_{32}\text{H}_{40}\text{BrO}_5$   $[\text{M}+\text{H}]^+$ : 563.1981. Found: 563.1976.



**2-[(1R, 2S)-2-{[(*tert*-butyldiphenylsilyl)oxy]methyl}cyclohexyl]ethanol (+)-**23****

上記の保護体 (9.81 g, 17.4 mmol) の入ったメタノール、酢酸エチル、飽和炭酸水素ナトリウム水溶液の混合溶媒 (5:5:1, 110 mL) にパラジウム炭素 (3.8 g) を加え、水素ガス雰囲気下室温で 12 時間攪拌した。水を加え、窒素ガス置換を十分行い、ドラフト内でジクロロメタンを使いろ紙濾過した。ろ液を濃縮し、シリカゲルカラムクロマトグラフィーに付し (ヘキサン: 酢酸エチル = 6:1) の流分から無色の油状物 (6.90 g, quant.) を得た。

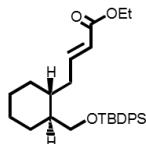
$[\alpha]_{\text{D}}^{28} +12$  ( $c$  0.65,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta = 7.68-7.65$  (m, 4H), 7.43-7.36 (m, 6H), 3.67-3.56 (m, 4H), 1.78-1.70 (m, 5H), 1.37-1.21 (m, 6H), 1.06 (s, 9H), 1.02-0.96 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 135.69, 135.66, 133.91, 133.89, 129.55, 129.54, 127.60, 127.57, 66.5, 61.0, 44.5, 36.5, 35.5, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3$ ; HRMS (FAB) Calcd. For  $\text{C}_{25}\text{H}_{37}\text{O}_2\text{Si}$   $[\text{M}+\text{H}]^+$ : 397.2563. Found: 397.2558.



**2-[(1R, 2S)-2-{[(*tert*-butyldiphenylsilyl)oxy]methyl}cyclohexyl]ethanol (-)-**23****

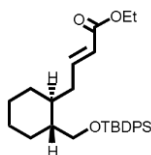
上記の化合物と同様に合成した。Yield 84%:  $[\alpha]_{\text{D}}^{28} -9.4$  ( $c$  1.1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta = 7.68-7.65$  (m, 4H), 7.45-7.36 (m, 6H), 3.68-3.54 (m, 4H), 1.78-1.66 (m, 5H), 1.37-1.18 (m, 7H), 1.06 (s,

9H), 1.02-0.97 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 135.68, 135.66, 133.91, 133.89, 129.55, 129.54, 127.60, 127.57, 66.6, 61.0, 44.5, 36.5, 35.5, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3; HRMS (FAB) Calcd. For  $\text{C}_{25}\text{H}_{37}\text{O}_2\text{Si}$   $[\text{M}+\text{H}]^+$ : 397.2563. Found: 397.2560.



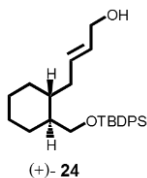
**(E)-ethyl 4-[(1R,2S)-2-[(*tert*-butyldiphenylsilyl)oxy]methyl]cyclohexyl]but-2-enoate**

上記の化合物と同様に合成した。Yield 98% (2 steps):  $[\alpha]_{\text{D}}^{28} +11$  (*c* 0.60,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67-7.64 (m, 4H), 7.44-7.36 (m, 6H), 6.91 (ddd,  $J$  = 15.4, 8.6, 6.6 Hz, 1H), 5.72 (d,  $J$  = 15.6 Hz, 1H), 4.18 (q,  $J$  = 7.1 Hz, 2H), 3.61-3.60 (m, 2H), 2.33 (m, 1H), 1.97 (td,  $J$  = 14.3, 8.6 Hz, 1H), 1.80-1.69 (m, 4H), 1.54-1.49 (m, 1H), 1.35-1.18 (m, 4H), 1.29 (t,  $J$  = 7.2 Hz, 3H), 1.05 (s, 9H), 1.00-0.97 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 166.6, 148.2, 135.62, 135.61, 133.82, 133.80, 129.59, 129.55, 127.62, 127.59, 122.4, 66.2, 60.1, 43.9, 37.8, 36.4, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3, 14.3; HRMS (FAB) Calcd. For  $\text{C}_{29}\text{H}_{40}\text{NaO}_3\text{Si}$   $[\text{M}+\text{Na}]^+$ : 487.2644. Found: 487.2649.



**(E)-ethyl 4-[(1S,2R)-2-[(*tert*-butyldiphenylsilyl)oxy]methyl]cyclohexyl]but-2-enoate**

上記の化合物と同様に合成した。Yield 98% (2 steps):  $[\alpha]_{\text{D}}^{28} -8.54$  (*c* 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67-7.64 (m, 4H), 7.44-7.36 (m, 6H), 6.91 (ddd,  $J$  = 15.5, 8.7, 6.7 Hz, 1H), 5.72 (d,  $J$  = 15.6 Hz, 1H), 4.18 (q,  $J$  = 7.2 Hz, 2H), 3.64-3.58 (m, 2H), 2.38-2.32 (m, 1H), 2.00-1.93 (m, 1H), 1.79-1.69 (m, 5H), 1.54-1.49 (m, 1H), 1.35-1.18 (m, 3H), 1.28 (t,  $J$  = 7.0 Hz, 3H), 1.05 (s, 9H), 1.03-0.97 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 166.6, 148.2, 135.62, 135.61, 133.83, 133.80, 129.6, 129.5, 127.62, 127.59, 122.4, 66.3, 60.1, 43.9, 37.8, 36.4, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3, 14.3; HRMS (FAB) Calcd. For  $\text{C}_{29}\text{H}_{40}\text{NaO}_3\text{Si}$   $[\text{M}+\text{Na}]^+$ : 487.2644. Found: 487.2640.

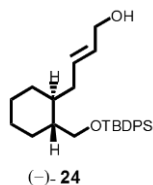


**(E)-4-[(1R,2S)-2-[(*tert*-butyldiphenylsilyl)oxy]methyl]cyclohexyl]but-2-en-1-ol (+)-24**

化合物(±)-24と同様に合成した。Yield 98%:  $[\alpha]_{\text{D}}^{28} +8.3$  (*c* 0.23,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.68-7.65 (m, 4H), 7.44-7.36 (m, 6H), 5.64-5.48 (m, 2H), 4.04 (d,  $J$  = 5.2 Hz, 2H), 3.66 (dd,  $J$  = 10.2, 3.2 Hz, 1H), 3.59 (dd,  $J$  = 10.2, 5.4 Hz, 1H), 2.22-2.18 (m, 1H), 1.87-1.79 (m, 2H), 1.72-1.69 (m, 3H), 1.37 (m,

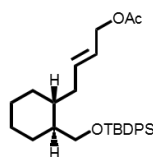


1H), 1.30-1.18 (m, 4H), 1.05 (s, 9H), 1.00-0.95 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 135.64, 135.62, 134.0, 131.6, 130.2, 129.52, 129.50, 127.58, 127.55, 66.3, 63.8, 43.8, 38.1, 36.2, 31.7, 30.0, 26.9, 26.2, 26.1, 19.4; HRMS (FAB) Calcd. For  $\text{C}_{27}\text{H}_{38}\text{NaO}_2\text{Si}$   $[\text{M}+\text{Na}]^+$ : 445.2539. Found: 445.2534.



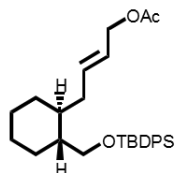
**(E)-4-[(1S,2R)-2-[[*tert*-butyldiphenylsilyl]oxy]methyl]cyclohexyl]but-2-en-1-ol (-)-24**

化合物(±)-**24**と同様に合成した。Yield 94%:  $[\alpha]_{\text{D}}^{28} -14$  (*c* 0.50,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.68-7.65 (m, 4H), 7.44-7.36 (m, 6H), 5.64-5.48 (m, 2H), 4.04 (d,  $J$  = 5.2 Hz, 2H), 3.66 (dd,  $J$  = 10.0, 2.8 Hz, 1H), 3.58 (dd,  $J$  = 9.8, 5.4 Hz, 1H), 2.21-2.18 (m, 1H), 1.87-1.79 (m, 2H), 1.72-1.69 (m, 3H), 1.42-1.33 (m, 1H), 1.30-1.18 (m, 4H), 1.05 (s, 9H), 1.00-0.95 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 135.66, 135.64, 134.0, 131.6, 130.2, 129.55, 129.52, 127.60, 127.58, 66.3, 63.8, 43.9, 38.1, 36.2, 31.7, 30.0, 26.9, 26.2, 26.1, 19.4; HRMS (FAB) Calcd. For  $\text{C}_{27}\text{H}_{38}\text{NaO}_2\text{Si}$   $[\text{M}+\text{Na}]^+$ : 445.2539. Found: 445.2534.



**(E)-4-((1R,2S)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)cyclohexyl)but-2-en-1-yl acetate**

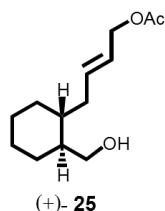
上記の化合物と同様に合成した。Yield 88%:  $[\alpha]_{\text{D}}^{27} +11$  (*c* 0.65,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67-7.64 (m, 4H), 7.44-7.36 (m, 6H), 5.71-5.64 (m, 1H), 5.48-5.42 (m, 1H), 4.47 (d,  $J$  = 6.4 Hz, 2H), 3.65 (dd,  $J$  = 10.0, 2.8 Hz, 1H), 3.57 (dd,  $J$  = 10.0, 5.2 Hz, 1H), 2.23-2.19 (m, 1H), 2.05 (s, 3H), 1.88-1.79 (m, 2H), 1.71-1.68 (m, 3H), 1.40-1.38 (m, 1H), 1.30-1.18 (m, 4H), 1.05 (s, 9H), 1.00-0.94 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 170.9, 135.63, 135.61, 134.8, 133.944, 133.935, 129.53, 129.51, 127.58, 127.56, 125.0, 66.3, 65.3, 43.8, 38.0, 36.3, 31.7, 30.0, 26.9, 26.2, 26.1, 21.0, 19.3; HRMS (FAB) Calcd. For  $\text{C}_{29}\text{H}_{40}\text{NaO}_3\text{Si}$   $[\text{M}+\text{Na}]^+$ : 487.2644. Found: 487.2641.



**(E)-4-[(1S,2R)-2-[[*tert*-butyldiphenylsilyl]oxy]methyl]cyclohexyl]but-2-en-1-yl acetate**

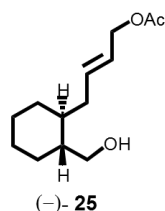
上記の化合物と同様に合成した。Yield 92%:  $[\alpha]_{\text{D}}^{28} -16.2$  (*c* 1.38,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67-7.64 (m, 4H), 7.44-7.36 (m, 6H), 5.71-5.64 (m, 1H), 5.49-5.42 (m, 1H), 4.47 (d,  $J$  = 6.4 Hz, 2H), 3.65 (dd,  $J$  = 10.0, 3.2 Hz, 1H), 3.57 (dd,  $J$  = 10.0, 5.2 Hz, 1H), 2.23-2.19 (m, 1H), 2.05 (s, 3H), 1.88-1.79 (m, 2H), 1.71-1.68 (m, 3H), 1.40-1.38 (m, 1H), 1.30-1.18 (m, 4H), 1.05 (s, 9H), 1.00-0.94 (m, 1H);  $^{13}\text{C}$  NMR

(100 MHz):  $\delta$  = 170.9, 135.63, 135.61, 134.8, 133.95, 133.94, 129.53, 129.51, 127.58, 127.56, 125.0, 66.3, 65.3, 43.8, 38.0, 36.3, 31.7, 30.0, 26.9, 26.2, 26.1, 21.0, 19.3; HRMS (FAB) Calcd. For  $C_{29}H_{40}NaO_3Si$   $[M+Na]^+$ : 487.2644. Found: 487.2644.



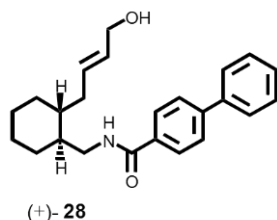
**(E)-4-[(1R,2S)-2-(hydroxymethyl)cyclohexyl]but-2-en-1-yl acetate (+)-25**

化合物(±)-**25**と同様に合成した。Yield 92%:  $[\alpha]_D^{28} +20.2$  (c 1.03,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 5.76 (ddd,  $J$  = 15.1, 8.1, 6.9 Hz, 1H), 5.60-5.53 (m, 1H), 4.51 (d,  $J$  = 6.4 Hz, 2H), 3.69 (dd,  $J$  = 10.6, 2.4 Hz, 1H), 3.57 (dd,  $J$  = 10.6, 5.8 Hz, 1H), 2.33-2.27 (m, 1H), 2.06 (s, 3H), 2.00-1.93 (m, 1H), 1.85-1.67 (m, 4H), 1.34-1.14 (m, 5H), 1.05-0.96 (m, 1H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 170.9, 134.5, 125.3, 65.5, 65.2, 43.8, 38.0, 36.3, 31.7, 29.5, 26.0, 25.8, 21.0; HRMS (FAB) Calcd. For  $C_{13}H_{22}NaO_3$   $[M+Na]^+$ : 249.1467. Found: 249.1469.



**(E)-4-[(1S,2R)-2-(hydroxymethyl)cyclohexyl]but-2-en-1-yl acetate (-)-25**

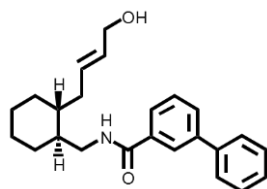
化合物(±)-**25**と同様に合成した。Yield 88%:  $[\alpha]_D^{28} -26.5$  (c 1.06,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 5.80-5.72 (m, 1H), 5.60-5.53 (m, 1H), 4.51 (d,  $J$  = 6.4 Hz, 2H), 3.69 (brd,  $J$  = 10.0 Hz, 1H), 3.57 (dd,  $J$  = 10.2, 5.0 Hz, 1H), 2.32-2.28 (m, 1H), 2.06 (s, 3H), 2.00-1.93 (m, 1H), 1.81-1.71 (m, 4H), 1.41 (brs, 1H), 1.33-1.29 (m, 1H), 1.25-1.14 (m, 4H), 1.05-0.96 (m, 1H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 170.9, 134.5, 125.3, 65.5, 65.2, 43.8, 38.0, 36.3, 31.7, 29.5, 26.0, 25.8, 21.0; HRMS (FAB) Calcd. For  $C_{13}H_{22}NaO_3$   $[M+Na]^+$ : 249.1467. Found: 249.1468.



**N-({(1S,2R)-2-[(E)-4-hydroxybut-2-en-1-yl]cyclohexyl}methyl)-[1,1'-biphenyl]-4-carboxamide (+)-28**

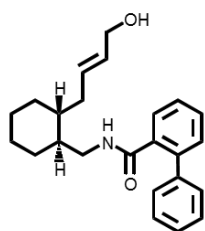
化合物(±)-**28**と同様に合成した。Yield 53% (3 steps):  $[\alpha]_D^{28} +13.4$  (c 0.41,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.84-7.82 (d,  $J$  = 8.4 Hz, 2H), 7.66-7.64 (d,  $J$  = 8.0 Hz, 2H), 7.62-7.60 (m, 2H), 7.48-7.45 (m, 2H), 7.41-7.37 (m, 1H), 6.24 (m, 1H), 5.79-5.64 (m, 2H), 4.11 (d,  $J$  = 4.4 Hz, 2H), 3.79 (ddd,  $J$  = 13.5, 5.9,

3.7 Hz, 1H), 3.21 (ddd,  $J = 13.6, 8.0, 5.8$  Hz, 1H), 2.31-2.27 (m, 1H), 2.19-2.12 (m, 1H), 1.88-1.84 (m, 1H), 1.75-1.72 (m, 3H), 1.52-1.44 (m, 1H), 1.32-1.05 (m, 5H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 167.2, 144.2, 140.0, 133.3, 131.2, 130.5, 128.9, 128.0, 127.3, 127.24, 127.19, 63.8, 43.3, 41.1, 39.6, 36.5, 31.9, 30.6, 26.1, 25.7$ ; HRMS (EI) Calcd. For  $\text{C}_{24}\text{H}_{29}\text{NO}_2$   $[\text{M}]^+$ : 363.2198. Found: 363.2191.



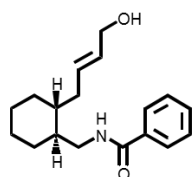
***N*-((1*S*,2*R*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl-(1,1'-biphenyl)-3-carboxamide**

化合物(±)-**28**と同様に合成した。Yield 45% (3 steps):  $[\alpha]_{\text{D}}^{28} +7.7$  ( $c$  0.60,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta = 7.98\text{--}7.97$  (m, 1H), 7.73-7.69 (m, 2H), 7.64-7.58 (m, 2H), 7.52-7.43 (m, 3H), 7.40-7.35 (m, 1H), 6.25 (m, 1H), 5.79-5.67 (m, 2H), 4.10 (d,  $J = 4.4$  Hz, 2H), 3.80 (ddd,  $J = 13.6, 6.0, 3.6$  Hz, 1H), 3.20 (ddd,  $J = 13.7, 8.1, 5.9$  Hz, 1H), 2.31-2.27 (m, 1H), 2.19-2.12 (m, 1H), 2.02 (brs, 1H), 1.87-1.83 (m, 1H), 1.71 (m, 3H), 1.58-1.43 (m, 1H), 1.30-1.01 (m, 5H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 167.5, 141.8, 140.2, 135.3, 131.2, 130.5, 130.1, 129.0, 128.9, 127.8, 127.2, 125.8, 125.4, 63.8, 43.3, 41.1, 39.6, 36.5, 31.9, 30.6, 26.0, 25.7$ ; HRMS (EI) Calcd. For  $\text{C}_{24}\text{H}_{29}\text{NO}_2$   $[\text{M}]^+$ : 363.2198. Found: 363.2193.



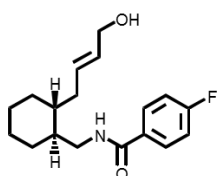
***N*-((1*S*,2*R*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl-(1,1'-biphenyl)-2-carboxamide**

化合物(±)-**28**と同様に合成した。Yield 55% (3 steps):  $[\alpha]_{\text{D}}^{28} +19.4$  ( $c$  1.58,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta = 7.72$  (dd,  $J = 7.4, 1.4$  Hz, 1H), 7.49-7.35 (m, 7H), 7.33 (dd,  $J = 7.4, 1.0$  Hz, 1H), 5.68-5.53 (m, 2H), 5.29 (m, 1H), 4.06 (d,  $J = 5.6$  Hz, 2H), 3.46 (ddd,  $J = 13.6, 6.0, 3.2$  Hz, 1H), 2.88 (ddd,  $J = 13.5, 7.6, 5.7$  Hz, 1H), 2.11-2.06 (m, 2H), 1.95-1.88 (m, 2H), 1.61-1.58 (m, 1H), 1.51-1.48 (m, 1H), 1.10-0.91 (m, 5H), 0.57-0.48 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 169.4, 140.4, 139.3, 135.7, 131.0, 130.4, 130.3, 130.0, 128.9, 128.74, 128.72, 127.7, 127.6, 63.7, 43.0, 40.6, 38.8, 36.3, 31.7, 30.0, 25.8, 25.7$ ; HRMS (EI) Calcd. For  $\text{C}_{24}\text{H}_{29}\text{NO}_2$   $[\text{M}]^+$ : 363.2198. Found: 363.2193.



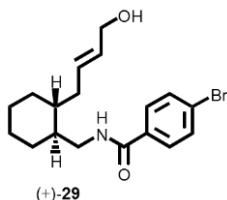
***N*-((1*S*,2*R*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methylbenzamide**

化合物(±)-**28**と同様に合成した。Yield 56% (3 steps):  $[\alpha]_D^{28} +17$  (*c* 0.60, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.76-7.74 (m, 2H), 7.52-7.48 (m, 1H), 7.45-7.41 (m, 2H), 6.19 (brs, 1H), 5.78-5.66 (m, 2H), 4.10 (d, *J* = 4.4 Hz, 2H), 3.77 (ddd, *J* = 13.5, 5.9, 3.7 Hz, 1H), 3.19 (ddd, *J* = 13.7, 8.1, 5.9 Hz, 1H), 2.32-2.26 (m, 1H), 2.18-2.11 (m, 1H), 1.85-1.82 (m, 1H), 1.73-1.71 (m, 3H), 1.51-1.41 (m, 1H), 1.32-1.03 (m, 6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 167.5, 134.7, 131.4, 131.2, 130.5, 128.6, 126.8, 63.8, 43.2, 41.1, 39.6, 36.5, 31.9, 30.6, 26.0, 25.7; HRMS (EI) Calcd. For C<sub>18</sub>H<sub>25</sub>NO<sub>2</sub> [M]<sup>+</sup>: 287.1885. Found: 287.1889.



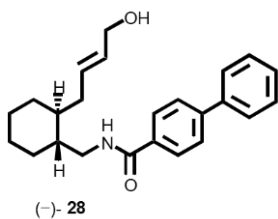
#### 4-fluoro-*N*-((1*S*,2*R*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl)benzamide

化合物(±)-**28**と同様に合成した。Yield 54% (3 steps) :  $[\alpha]_D^{28} +15.6$  (*c* 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.78-7.75 (m, 2H), 7.12-7.08 (m, 2H), 6.25 (brs, 1H), 5.77-5.65 (m, 2H), 4.09 (d, *J* = 4.4 Hz, 2H), 3.75 (ddd, *J* = 13.4, 5.8, 3.8 Hz, 1H), 3.16 (ddd, *J* = 13.7, 8.1, 5.9 Hz, 1H), 2.29-2.24 (m, 1H), 2.17-2.10 (m, 1H), 1.84-1.71 (m, 4H), 1.49-1.41 (m, 1H), 1.28-1.05 (m, 5H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 166.5, 164.6 (d, *J* = 250.4 Hz), 131.1, 130.8 (d, *J* = 3.2 Hz), 130.4, 129.2, 129.1, 115.7, 115.4, 63.7, 43.3, 41.0, 39.5, 36.4, 31.9, 30.5, 26.0, 25.7; HRMS (EI) Calcd. For C<sub>18</sub>H<sub>24</sub>FNO<sub>2</sub> [M]<sup>+</sup>: 305.1791. Found: 305.1787.



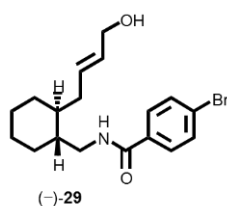
#### 4-bromo-*N*-((1*S*,2*R*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl)benzamide (+)-**29**

化合物(±)-**28**と同様に合成した。Yield 55% (3 steps):  $[\alpha]_D^{27} +17$  (*c* 0.51, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.63-7.61 (m, 2H), 7.57-7.55 (m, 2H), 6.16 (brs, 1H), 5.77-5.66 (m, 2H), 4.10 (d, *J* = 4.4 Hz, 2H), 3.76 (ddd, *J* = 13.6, 6.0, 3.6 Hz, 1H), 3.16 (ddd, *J* = 13.8, 8.3, 6.0 Hz, 1H), 2.29-2.25 (m, 1H), 2.17-2.11 (m, 1H), 2.02 (brs, 1H), 1.84-1.80 (m, 1H), 1.73-1.71 (m, 3H), 1.49-1.41 (m, 1H), 1.28-1.01 (m, 5H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 166.5, 133.5, 131.8, 131.2, 130.4, 128.5, 126.1, 63.8, 43.3, 41.0, 39.6, 36.4, 31.9, 30.5, 26.0, 25.7; HRMS (EI) Calcd. For C<sub>18</sub>H<sub>24</sub>BrNO<sub>2</sub> [M]<sup>+</sup>: 365.0990. Found 365.0997.



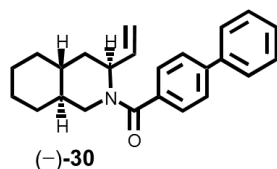
***N*-({(1*R*,2*S*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl}-(1,1'-biphenyl)-4-carboxamide (–)-28**

化合物(±)-28と同様に合成した。Yield 50% (3 steps):  $[\alpha]_D^{28}$  –9.1 (*c* 0.50, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.84-7.82 (m, 2H), 7.66-7.60 (m, 4H), 7.48-7.45 (m, 2H), 7.41-7.37 (m, 1H), 6.25 (m, 1H), 5.79-5.68 (m, 2H), 4.10 (d, *J* = 4.4 Hz, 2H), 3.79 (ddd, *J* = 13.5, 6.2, 3.7 Hz, 1H), 3.21 (ddd, *J* = 13.8, 8.2, 5.8 Hz, 1H), 2.31-2.27 (m, 1H), 2.19-2.12 (m, 2H), 2.08 (brs, 1H), 1.88-1.84 (m, 1H), 1.75-1.72 (m, 3H), 1.52-1.43 (m, 1H), 1.32-1.21 (m, 3H), 1.19-1.04 (m, 1H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 167.2, 144.2, 140.0, 133.3, 131.2, 130.5, 128.9, 128.0, 127.3, 127.24, 127.18, 63.8, 43.3, 41.1, 39.6, 36.5, 31.9, 30.6, 26.1, 25.7; HRMS (EI) Calcd. For C<sub>24</sub>H<sub>29</sub>NO<sub>2</sub> [M]<sup>+</sup>: 363.2198. Found: 363.2204.



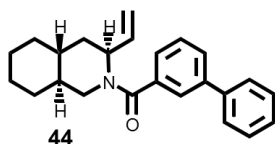
**4-bromo-*N*-({(1*R*,2*S*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl}benzamide (–)-29**

化合物(±)-28と同様に合成した。Yield 52% (3 steps):  $[\alpha]_D^{28}$  –6.9 (*c* 0.38, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.63-7.61 (m, 2H), 7.59-7.55 (m, 2H), 6.15 (m, 1H), 5.78-5.66 (m, 2H), 4.10 (d, *J* = 4.4 Hz, 2H), 3.76 (ddd, *J* = 13.5, 5.9, 3.9 Hz, 1H), 3.16 (ddd, *J* = 13.7, 8.1, 5.9 Hz, 1H), 2.29-2.25 (m, 1H), 2.17-2.11 (m, 2H), 1.84-1.80 (m, 1H), 1.73-1.71 (m, 3H), 1.60 (brs, 1H), 1.50-1.41 (m, 1H), 1.30-1.20 (m, 3H), 1.18-1.02 (m, 1H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 166.5, 133.5, 131.8, 131.2, 130.4, 128.5, 126.1, 63.8, 43.3, 41.0, 39.6, 36.4, 31.9, 30.5, 26.0, 25.7; HRMS (EI) Calcd. For C<sub>18</sub>H<sub>24</sub>BrNO<sub>2</sub> [M]<sup>+</sup>: 365.0990. Found: 365.0987.



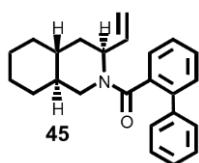
**(1,1'-biphenyl)-4-yl[(3*S*,4*aR*,8*aS*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl]methanone (–)-30**

化合物(±)-30と同様に合成した。Yield 64%:  $[\alpha]_D^{28}$  –19 (*c* 0.87, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.64-7.58 (m, 4H), 7.49-7.43 (m, 4H), 7.38-7.35 (m, 1H), 5.87 (ddd, *J* = 17.4, 10.6, 3.8 Hz, 0.4H), 5.78 (ddd, *J* = 17.4, 10.7, 3.6 Hz, 0.6H), 5.55 (brs, 0.4H), 5.31-5.28 (m, 1H), 5.23-5.16 (m, 1H), 4.53 (brs, 0.6H), 4.49 (dd, *J* = 13.2, 3.6 Hz, 0.6H), 3.49 (dd, *J* = 13.4, 3.8 Hz, 0.4H), 2.89-2.82 (m, 0.4H), 2.64-2.57 (m, 0.6H), 1.84-1.52 (m, 5H), 1.47-1.12 (m, 5.4H), 1.00-0.98 (m, 1H), 0.87-0.84 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 171.1, 170.4, 142.3, 142.2, 140.3, 137.1, 136.7, 135.4, 128.8, 127.68, 127.66, 127.4, 127.1, 126.8, 116.6, 116.1, 57.2, 50.8, 49.7, 43.5, 42.8, 41.9, 37.5, 36.8, 35.9, 32.9, 29.9, 29.7, 26.2, 26.1, 25.8, 25.7; HRMS (EI) Calcd. For C<sub>24</sub>H<sub>27</sub>NO [M]<sup>+</sup>: 345.2093. Found: 345.2091.



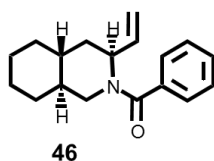
**(1,1'-biphenyl)-3-yl[(3*S*,4*aR*,8*aS*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl]methanone 44**

化合物(±)-**30**と同様に合成した。Yield 55%:  $[\alpha]_D^{28} -29$  (*c* 0.90, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.63-7.56 (m, 4H), 7.49-7.42 (m, 3H), 7.39-7.36 (m, 2H), 5.87 (ddd, *J* = 17.4, 10.6, 3.8 Hz, 0.4H), 5.77 (ddd, *J* = 17.6, 10.8 3.6 Hz, 0.6H), 5.56 (brs, 0.4H), 5.30-5.27 (m, 1H), 5.23-5.15 (m, 1H), 4.50 (d, *J* = 12.4, 3.6 Hz, 0.6H), 4.49 (brs, 0.6H), 3.44 (dd, *J* = 13.4, 3.8 Hz, 0.4H), 2.84 (dd, *J* = 13.2, 11.6 Hz, 0.4H), 2.61 (dd, *J* = 13.2, 11.2 Hz, 0.6H), 1.84-1.52 (m, 5H), 1.46-1.19 (m, 5.4H), 1.00-0.97 (m, 1H), 0.88-0.82 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 171.1, 170.5, 141.5, 141.4, 141.41, 140.36, 137.2, 137.15, 137.11, 136.7, 128.9, 128.11, 128.06, 127.62, 127.59, 127.2, 127.1, 125.54, 125.48, 125.0, 124.9, 116.6, 116.1, 77.2, 57.2, 50.7, 49.6, 43.4, 42.8, 41.9, 37.4, 36.8, 36.7, 35.9, 32.8, 29.9, 29.7, 26.1, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For C<sub>24</sub>H<sub>27</sub>NO<sub>2</sub> [M]<sup>+</sup>: 345.2093. Found: 345.2090.



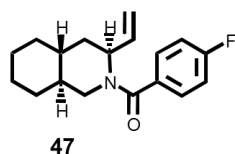
**(1,1'-biphenyl)-2-yl[(3*S*,4*aR*,8*aS*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl]methanone 45**

化合物(±)-**30**と同様に合成した。Yield 55%: <sup>1</sup>H NMR (400 MHz, 2.1:1, diastereomixture):  $\delta$  = 7.55-7.53 (m, 2H), 7.48-7.33 (m, 7H), 5.72 (ddd, *J* = 17.5, 10.7, 3.7 Hz, 0.38H), 5.49 (ddd, *J* = 17.3, 10.5 3.7 Hz, 0.38H), 5.43 (brs, 0.38H), 5.28 (ddd, *J* = 17.8, 10.6 3.7 Hz, 0.24H), 5.21-5.19 (m, 0.38H), 5.09 (s, 0.38H), 5.06-5.04 (m, 0.38H), 4.94-4.90 (m, 0.38H), 4.88-4.85 (m, 0.24H), 4.74 (m, 0.24H), 4.38 (dd, *J* = 12.6, 4.2 Hz, 0.38H), 4.34 (m, 0.38H), 4.01 (brs, 0.24H), 3.89 (brs, 0.38H), 2.90 (dd, *J* = 13.0, 3.8 Hz, 0.24H), 2.80 (dd, *J* = 13.4, 3.4 Hz, 0.38H), 2.44 (t, *J* = 12.4 Hz, 0.38H), 2.37 (t, *J* = 12.4 Hz, 0.38H), 2.21 (t, *J* = 12.4 Hz, 0.24H), 2.00 (m, 0.24H), 1.67-1.52 (m, 4.38H), 1.39-0.82 (m, 5H), 0.59-0.47 (m, 1.24H), -0.02--0.84 (m, 0.38H), -0.77--0.86 (m, 0.38H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 170.6, 170.1, 140.1, 140.0, 139.8, 138.8, 138.3, 138.1, 138.0, 136.8, 136.5, 136.3, 136.2, 136.0, 135.9, 129.6, 129.3, 129.2, 129.13, 129.06, 129.0, 128.94, 128.86, 128.6, 128.5, 128.4, 128.2, 128.0, 127.8, 127.7, 127.63, 127.59, 127.5, 127.4, 126.6, 115.9, 115.8, 115.7, 56.0, 50.4, 50.0, 48.6, 48.4, 42.9, 42.4, 40.7, 40.6, 36.4, 36.1, 35.9, 35.7, 35.6, 35.4, 32.8, 32.7, 32.6, 29.73, 29.68, 29.6, 29.3, 26.0, 25.9, 25.7, 25.6, 25.4; HRMS (EI) Calcd. For C<sub>24</sub>H<sub>27</sub>NO<sub>2</sub> [M]<sup>+</sup>: 345.2093. Found: 345.2096.

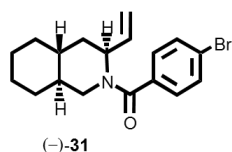


**Phenyl[(3*S*,4*aR*,8*aS*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl]methanone 46**

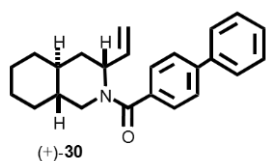
化合物(±)-**30**と同様に合成した。Yield 55%:  $[\alpha]_D^{28} -12$  (*c* 0.45, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.40-7.37 (m, 5H), 5.85 (ddd, *J* = 17.5, 10.7, 3.7 Hz, 0.4H), 5.75 (ddd, *J* = 17.4, 10.6 3.6 Hz, 0.6H), 5.53 (brs, 0.4H), 5.29-5.26 (m, 1H), 5.21-5.11 (m, 1H), 4.47 (dd, *J* = 13.2, 4.0 Hz, 0.6H), 4.43 (brs, 0.6H), 3.39 (dd, *J* = 13.4, 3.4 Hz, 0.4H), 2.81 (dd, *J* = 13.0, 11.8 Hz, 0.4H), 2.58 (dd, *J* = 12.8, 11.6 Hz, 0.6H), 1.83-1.50 (m, 5H), 1.44-1.18 (m, 5H), 1.14-1.06 (m, 0.4H), 0.99-0.97 (m, 1H), 0.88-0.82 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 171.2, 170.6, 137.1, 136.7, 136.6, 129.4, 129.3, 128.41, 128.38, 126.8, 126.2, 116.5, 116.0, 57.1, 50.7, 49.6, 43.4, 42.7, 41.9, 37.4, 36.7, 35.9, 32.9, 29.9, 29.6, 26.2, 26.1, 25.8, 25.7; HRMS (EI) Calcd. For C<sub>18</sub>H<sub>23</sub>NO [M]<sup>+</sup>: 296.1780. Found: 296.1784.

**(4-fluorophenyl)[(3*S*,4*aR*,8*aS*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl]methanone 47**

化合物(±)-**30**と同様に合成した。Yield 58%:  $[\alpha]_D^{28} -8.9$  (*c* 0.77, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.43-7.40 (m, 2H), 7.12-7.04 (m, 2H), 5.85 (ddd, *J* = 17.5, 10.7, 3.7 Hz, 0.4H), 5.76 (ddd, *J* = 17.4, 10.6 3.6 Hz, 0.6H), 5.50 (brs, 0.4H), 5.30-5.27 (m, 1H), 5.20-5.12 (m, 1H), 4.44 (dd, *J* = 13.2, 4.0 Hz, 0.6H), 4.43 (brs, 0.6H), 3.37 (dd, *J* = 13.4, 3.4 Hz, 0.4H), 2.83 (dd, *J* = 13.0, 11.8 Hz, 0.4H), 2.58 (dd, *J* = 12.8, 11.6 Hz, 0.6H), 1.83-1.49 (m, 5H), 1.44-1.17 (m, 5H), 1.14-1.05 (m, 0.4H), 0.99-0.95 (m, 1H), 0.89-0.80 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 170.3, 169.7, 163.3 (d, *J* = 247.3 Hz), 163.2 (d, *J* = 247.8 Hz), 137.0, 136.6, 132.6 (d, *J* = 3.4 Hz), 129.1, 129.0, 128.6, 128.5, 116.6, 116.1, 115.6, 115.5, 115.4, 115.3, 57.3, 50.9, 49.7, 43.6, 42.8, 41.8, 37.5, 36.7, 35.9, 32.8, 29.9, 29.6, 26.1, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For C<sub>18</sub>H<sub>22</sub>FNO [M]<sup>+</sup>: 287.1685. Found: 287.1689.

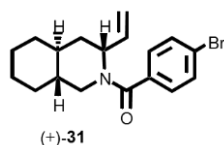
**(4-bromophenyl)[(3*S*,4*aR*,8*aS*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl]methanone (-)-31**

化合物(±)-**30**と同様に合成した。Yield 63%:  $[\alpha]_D^{28} -10$  (*c* 0.25, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.55 (d, *J* = 8.4 Hz, 0.8H), 7.51 (d, *J* = 8.4 Hz, 1.2H), 7.29-7.27 (m, 2H), 5.84 (ddd, *J* = 17.4, 10.6, 4.0 Hz, 0.4H), 5.75 (ddd, *J* = 17.5, 10.9, 3.7 Hz, 0.6H), 5.49 (brs, 0.4H), 5.29-5.26 (m, 1H), 5.19-5.11 (m, 1H), 4.44 (dd, *J* = 13.2, 4.0 Hz, 0.6H), 4.38 (brs, 0.6H), 3.33 (dd, *J* = 13.2, 4.0 Hz, 0.4H), 2.85-2.79 (m, 0.4H), 2.60-2.54 (m, 0.6H), 1.82-1.49 (m, 5H), 1.43-1.19 (m, 5H), 1.10-1.07 (m, 0.4H), 0.99-0.96 (m, 1H), 0.88-0.83 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 170.2, 169.6, 136.9, 136.5, 135.4, 131.7, 131.6, 128.6, 128.0, 123.7, 123.6, 116.7, 116.2, 57.2, 50.8, 49.6, 43.5, 42.7, 41.8, 37.5, 36.7, 35.9, 32.8, 29.9, 29.6, 26.1, 26.0, 25.7, 25.6; HRMS (EI) Calcd. For C<sub>18</sub>H<sub>22</sub>BrNO [M]<sup>+</sup>: 347.0885. Found: 347.0891.



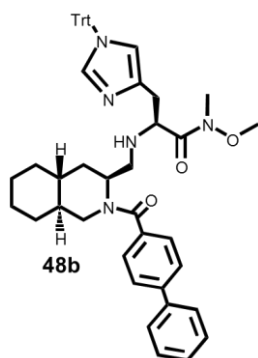
**(1,1'-biphenyl)-4-yl[(3*R*,4*aS*,8*aR*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl]methanone (+)-30**

化合物(±)-**30**と同様に合成した。Yield 58%:  $[\alpha]_D^{29} +29$  (*c* 0.80, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.64-7.58 (m, 4H), 7.49-7.43 (m, 4H), 7.38-7.35 (m, 1H), 5.87 (ddd, *J* = 17.4, 10.6, 3.6 Hz, 0.4H), 5.78 (ddd, *J* = 17.5, 10.7, 3.5 Hz, 0.6H), 5.55 (brs, 0.4H), 5.31-5.28 (m, 1H), 5.23-5.16 (m, 1H), 4.54 (brs, 0.6H), 4.49 (dd, *J* = 13.2, 3.6 Hz, 0.6H), 3.49 (dd, *J* = 12.8, 3.6 Hz, 0.4H), 2.86 (dd, *J* = 13.2, 11.6 Hz, 0.4H), 2.61 (dd, *J* = 13.2, 11.6 Hz, 0.6H), 1.84-1.52 (m, 5H), 1.47-1.18 (m, 5H), 1.15-0.98 (m, 1.4H), 0.89-0.81 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 171.1, 170.4, 142.3, 142.2, 140.4, 137.1, 136.7, 135.4, 128.8, 127.7, 127.4, 127.1, 126.8, 116.6, 116.1, 57.2, 50.8, 49.7, 43.5, 42.8, 41.9, 37.5, 36.8, 35.9, 32.9, 29.9, 29.7, 26.2, 26.1, 25.8, 25.7; HRMS (EI) Calcd. For C<sub>24</sub>H<sub>27</sub>NO [M]<sup>+</sup>: 345.2093. Found: 345.2091.



**(4-bromophenyl)[(3*R*,4*aS*,8*aR*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl]methanone (+)-31**

化合物(±)-**30**と同様に合成した。Yield 52%:  $[\alpha]_D^{28} +22$  (*c* 0.59, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.56-7.50 (m, 2H), 7.29-7.27 (m, 2H), 5.84 (ddd, *J* = 17.3, 10.7, 3.9 Hz, 0.4H), 5.75 (ddd, *J* = 17.2, 10.8, 3.6 Hz, 0.6H), 5.49 (brs, 0.4H), 5.29-5.26 (m, 1H), 5.19-5.10 (m, 1H), 4.44 (dd, *J* = 13.2, 4.0 Hz, 0.6H), 4.38 (brs, 0.6H), 3.33 (dd, *J* = 13.2, 3.6 Hz, 0.4H), 2.82 (dd, *J* = 13.0, 11.4 Hz, 0.4H), 2.57 (dd, *J* = 13.0, 11.4 Hz, 0.6H), 1.82-1.49 (m, 5H), 1.43-1.15 (m, 5H), 1.13-1.04 (m, 0.4H), 1.02-0.90 (m, 1H), 0.89-0.79 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 170.2, 169.6, 136.9, 136.5, 135.4, 131.7, 131.6, 128.6, 128.0, 123.7, 123.6, 116.6, 116.2, 57.2, 50.8, 49.6, 43.5, 42.7, 41.8, 37.4, 36.7, 35.9, 32.8, 29.9, 29.7, 26.1, 26.0, 25.7, 25.6; HRMS (EI) Calcd. For C<sub>18</sub>H<sub>22</sub>BrNO [M]<sup>+</sup>: 347.0885. Found: 347.0891.

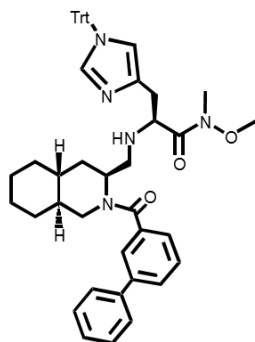


**(*S*)-2-({[(3*S*,4*aR*,8*aS*)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl]methyl}amino)-*N*-met**

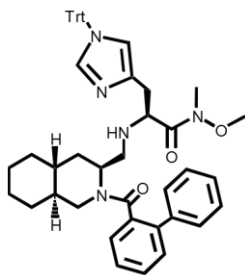


**hoxy-*N*-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide 48b**

化合物 **34b** と同様に合成した。Yield 50% (3 steps):  $[\alpha]_D^{29} -21$  (*c* 0.49, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.59-7.53 (m, 4H), 7.48-7.40 (m, 4H), 7.36-7.28 (m, 11H), 7.14-7.09 (m, 6H), 6.62 (brs, 0.6H), 6.56 (brs, 0.4H), 4.91 (m, 0.6H), 4.42 (dd, *J* = 13.6, 3.2 Hz, 0.4H), 4.13 (m, 0.4H), 3.93 (brs, 1H), 3.67 (s, 1.8H), 3.49 (s, 1.2H), 3.40 (dd, *J* = 13.0, 3.4 Hz, 0.6H), 3.14 (s, 1.8H), 3.08 (s, 1.2H), 2.87-2.83 (m, 2.4H), 2.72-2.66 (m, 2H), 2.49-2.43 (m, 0.6H), 1.80-1.70 (m, 3H), 1.61-1.55 (m, 1H), 1.39-1.26 (m, 6H), 1.05-0.83 (m, 2H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.5, 175.2, 171.3, 170.5, 142.5, 142.4, 141.9, 141.88, 141.86, 140.44, 140.37, 138.2, 138.1, 137.7, 137.2, 135.9, 135.8, 129.73, 129.67, 129.3, 128.8, 128.7, 127.91, 127.87, 127.55, 127.47, 127.4, 127.2, 127.1, 127.05, 127.00, 119.2, 115.6, 77.2, 75.02, 75.01, 61.6, 61.5, 57.8, 57.5, 55.5, 49.5, 48.3, 47.1, 46.6, 43.1, 42.6, 42.1, 36.4, 36.2, 34.5, 33.0, 32.9, 32.6, 32.3, 32.0, 29.9, 29.7, 26.2, 26.1, 25.8, 25.7; HRMS (EI) Calcd. For C<sub>50</sub>H<sub>53</sub>N<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 771.4148. Found: 771.4154.

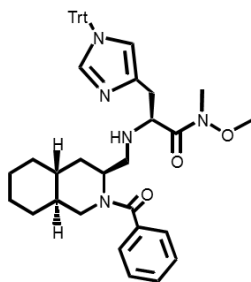
**(*S*)-2-[(1,1'-biphenyl)-3-carbonyl]decahydroisoquinolin-3-ylmethylamino]-*N*-methoxy-*N*-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide**

化合物 **34b** と同様に合成した。Yield 65% (3 steps):  $[\alpha]_D^{28} -18$  (*c* 0.67, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.60-7.53 (m, 4H), 7.45-7.29 (m, 15H), 7.14-7.08 (m, 6H), 6.61 (brs, 0.55H), 6.53 (brs, 0.45H), 4.91 (m, 0.55H), 4.43 (dd, *J* = 13.4, 3.8 Hz, 0.45H), 4.13-4.11 (m, 0.45H), 3.90 (m, 1H), 3.67 (s, 1.65H), 3.45 (s, 1.35H), 3.37 (dd, *J* = 13.4, 3.8 Hz, 0.55H), 3.13 (s, 1.65H), 3.04 (s, 1.35H), 2.90-2.76 (m, 2.55H), 2.73-2.64 (m, 2H), 2.48-2.42 (m, 0.45H), 1.82-1.52 (m, 4.55H), 1.35-1.19 (m, 5.45H), 1.04-0.78 (m, 2H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.4, 175.1, 171.3, 170.6, 142.52, 142.45, 141.34, 141.31, 140.5, 140.4, 138.2, 138.1, 137.69, 137.65, 137.58, 137.3, 129.8, 129.7, 127.93, 127.89, 127.8, 127.7, 127.5, 127.4, 127.2, 127.1, 125.6, 125.45, 125.37, 125.3, 119.2, 77.2, 75.04, 75.02, 61.7, 61.4, 57.9, 57.4, 55.5, 49.6, 48.2, 47.1, 46.5, 43.0, 42.6, 42.3, 42.1, 36.4, 36.2, 34.5, 33.0, 32.9, 32.5, 32.3, 32.0, 30.0, 29.7, 26.2, 26.1, 25.9, 25.7; HRMS (EI) Calcd. For C<sub>50</sub>H<sub>53</sub>N<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 771.4148. Found: 771.4141.



**(S)-2-[(3S,4aR,8aS)-2-[(1,1'-biphenyl)-2-carbonyl]decahydroisoquinolin-3-yl]methylamino]-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide**

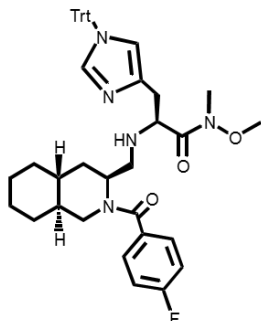
化合物 **34b** と同様に合成した。Yield 62% (3 steps):  $^1\text{H}$  NMR (400 MHz, 2.1:1 diastereomixture):  $\delta$  = 7.52-7.48 (m, 1.4H), 7.45-7.29 (m, 17.6H), 7.14-7.12 (m, 6H), 6.60 (brs, 0.6H), 6.55 (brs, 0.3H), 6.51 (brs, 0.1H), 4.93-4.91 (m, 0.3H), 4.87-4.84 (m, 0.1H), 4.78 (dd,  $J$  = 13.2, 7.2 Hz, 0.6H), 4.72-4.70 (m, 0.3H), 4.46-4.43 (m, 0.1H), 4.39 (dd,  $J$  = 13.6, 4.0 Hz, 0.6H), 4.07 (m, 0.6H), 4.01 (m, 0.4H), 3.82-3.76 (m, 0.6H), 3.69 (s, 1.8H), 3.64 (s, 0.9H), 3.61 (s, 0.6H), 3.33-3.30 (m, 0.1H), 3.27-3.22 (m, 0.3H), 3.18 (s, 0.9H), 3.14 (s, 1.8H), 2.97-2.95 (m, 0.3H), 2.88-2.67 (m, 2H), 2.55-2.44 (m, 1H), 2.38-1.83 (m, 2.7H), 1.66-1.50 (m, 2H), 1.35-1.03 (m, 3H), 0.89-0.81 (m, 2H), 0.69 (td,  $J$  = 12.8, 5.5 Hz, 0.6H), 0.59-0.36 (m, 1.4H), -0.23 (td,  $J$  = 13.1, 5.7 Hz, 0.1H), -0.35 (td,  $J$  = 12.7, 5.5 Hz, 0.3H), -0.73--0.89 (0.6H, m);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 175.5, 170.3, 169.9, 142.54, 142.52, 142.48, 140.1, 139.93, 139.85, 138.3, 138.2, 138.1, 137.81, 137.76, 137.3, 136.4, 136.2, 129.8, 129.7, 129.1, 128.9, 128.85, 128.76, 128.73, 128.66, 128.52, 128.47, 128.4, 128.3, 128.03, 127.96, 127.93, 127.90, 127.87, 127.7, 127.5, 127.4, 119.2, 119.1, 77.2, 75.0, 62.6, 61.7, 61.5, 61.4, 61.0, 57.8, 57.5, 57.4, 54.5, 51.1, 48.8, 48.6, 48.5, 47.8, 47.5, 47.4, 46.4, 42.4, 41.9, 40.59, 40.56, 40.2, 35.96, 35.94, 35.5, 35.4, 33.0, 32.80, 32.78, 32.7, 32.32, 32.26, 32.1, 32.0, 31.6, 29.8, 29.6, 29.4, 29.3, 25.99, 25.97, 25.84, 25.78, 25.6, 25.46, 25.38; HRMS (EI) Calcd. For  $\text{C}_{50}\text{H}_{53}\text{N}_5\text{O}_3$   $[\text{M}]^+$ : 771.4148. Found: 771.4154.



**{(3S,4aR,8aS)-3-[(R)-1-[methoxy(methyl)amino]-2-(1-trityl-1H-imidazol-4-yl)ethyl]amino)methyl]octahydroisoquinolin-2(1H)-yl}(phenyl)methanone**

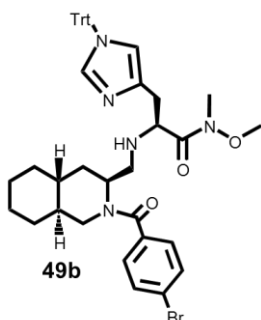
化合物 **34b** と同様に合成した。Yield 55% (3 steps):  $[\alpha]_{\text{D}}^{28}$  -23 (c 0.12,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.31 (brs, 15H), 7.11 (brs, 6H), 6.61 (brs, 0.6H), 6.54 (brs, 0.4H), 4.89 (brs, 0.6H), 4.41 (d,  $J$  = 10.8 Hz, 0.4H), 4.12 (brs, 0.6H), 3.90 (brs, 0.4H), 3.82 (brs, 0.4H), 3.66 (s, 1.8H), 3.50 (s, 1.2H), 3.30 (d,  $J$  = 10.8 Hz, 0.6H), 3.13 (s, 1.8H), 3.08 (s, 1.2H), 2.87-2.80 (m, 2.6H), 2.66-2.61 (m, 2H), 2.43 (t,  $J$  = 12.0 Hz, 0.4H), 1.79-1.69 (m, 3H), 1.60-1.53 (m, 2H), 1.35-1.26 (m, 5H), 1.03-0.80 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 175.5, 175.3, 171.4, 170.7, 142.5, 142.4, 138.2, 138.1, 137.7, 137.3, 137.1, 137.0, 129.75, 129.70,

129.01, 128.96, 128.33, 128.29, 127.93, 127.88, 126.7, 126.6, 119.2, 77.2, 75.0, 61.7, 61.5, 57.9, 57.4, 55.4, 49.4, 48.2, 47.1, 46.5, 43.0, 42.5, 42.1, 36.4, 36.2, 34.4, 33.0, 32.9, 32.6, 32.3, 32.1, 29.9, 29.7, 26.2, 26.1, 25.8, 25.7; HRMS (EI) Calcd. For  $C_{44}H_{49}N_5O_3$   $[M]^+$ : 695.3835. Found: 695.3829.



**(S)-2-({[(3S,4aR,8aS)-2-(4-fluorobenzoyl)decahydroisoquinolin-3-yl]methyl}amino)-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide**

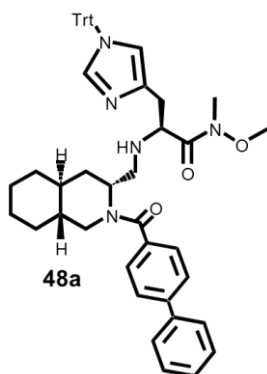
化合物 **34b** と同様に合成した。Yield 55% (3 steps):  $[\alpha]_D^{28} -17$  (c 0.65,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.42-7.39 (m, 1.2H), 7.36-7.31 (m, 10.8H), 7.12-7.10 (m, 6H), 7.04-6.97 (m, 2H), 6.60 (brs, 0.6H), 6.55 (brs, 0.4H), 4.89-4.87 (m, 0.6H), 4.37 (dd,  $J$  = 13.2, 3.6 Hz, 0.4H), 4.11 (brs, 0.4H), 3.91 (m, 0.4H), 3.82 (m, 0.6H), 3.65 (s, 1.8H), 3.52 (s, 1.2H), 3.28 (d,  $J$  = 13.2, 3.6 Hz, 0.6H), 3.13 (s, 1.8H), 3.09 (s, 1.2H), 2.91-2.80 (m, 2.6H), 2.73-2.63 (m, 2H), 2.46-2.40 (m, 0.4H), 1.77-1.66 (m, 3H), 1.60-1.52 (m, 2H), 1.36-1.24 (m, 5H), 1.03-0.82 (m, 2H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 175.5, 175.2, 170.6, 169.8, 163.0 (d,  $J$  = 247.2 Hz), 162.9 (d,  $J$  = 246.9 Hz), 142.5, 142.4, 138.2, 138.1, 137.6, 137.2, 133.1 (d,  $J$  = 3.4 Hz), 133.0 (d,  $J$  = 3.5 Hz), 129.73, 129.70, 129.2, 129.1, 128.9, 128.8, 127.92, 127.89, 119.24, 119.22, 115.4, 115.2, 77.2, 75.0, 61.6, 61.5, 57.9, 57.5, 55.6, 49.5, 48.4, 47.2, 46.6, 43.2, 42.6, 42.0, 36.4, 36.2, 34.5, 33.0, 32.9, 32.7, 32.3, 32.0, 29.9, 29.7, 26.1, 26.0, 25.8, 25.7; HRMS (EI) Calcd. For  $C_{44}H_{48}FN_5O_3$   $[M]^+$ : 713.3741. Found: 771.3748.



**(S)-2-({[(3S,4aR,8aS)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl]methyl}amino)-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide **49b****

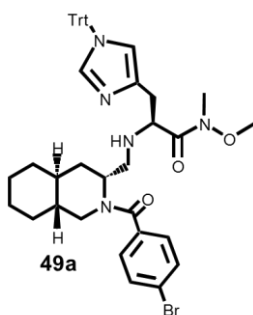
化合物 **34b** と同様に合成した。Yield 58% (3 steps):  $[\alpha]_D^{28} -17$  (c 0.67,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.47 (d,  $J$  = 8.4 Hz, 1.2H), 7.44 (d,  $J$  = 8.4 Hz, 0.8H), 7.35-7.29 (m, 10.8H), 7.22 (d,  $J$  = 8.4 Hz, 1.2H), 7.14-7.11 (m, 6H), 6.60 (s, 0.6H), 6.55 (s, 0.4H), 4.87 (m, 0.6H), 4.37 (dd,  $J$  = 13.4, 3.8 Hz, 0.4H), 4.10

(brs, 0.6H), 3.90 (brs, 0.4H), 3.78 (brs, 0.6H), 3.64 (s, 1.8H), 3.51 (s, 1.2H), 3.24 (dd,  $J = 13.2, 3.6$  Hz, 0.6H), 3.13 (s, 1.8H), 3.09 (s, 1.2H), 2.91-2.79 (m, 2.4H), 2.73-2.63 (m, 2H), 2.46-2.40 (m, 0.4H), 1.76-1.71 (m, 3.4H), 1.60-1.54 (m, 1.6H), 1.37-1.26 (m 5H), 1.00-0.82 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 175.4, 175.1, 170.4, 169.6, 142.46, 142.41, 138.2, 138.1, 137.6, 137.2, 135.9, 135.8, 131.50, 131.46, 129.73, 129.70, 128.7, 128.4, 127.92, 127.89, 123.21, 123.18, 119.23, 119.22, 77.2, 75.0, 61.6, 61.5, 57.8, 57.5, 55.5, 49.4, 48.3, 47.1, 46.6, 43.1, 42.5, 42.0, 36.4, 36.2, 34.5, 33.0, 32.9, 32.7, 32.3, 32.0, 29.9, 29.7, 26.1, 26.0, 25.8, 25.6$ ; HRMS (EI) Calcd. For  $\text{C}_{44}\text{H}_{48}\text{BrN}_5\text{O}_3$   $[\text{M}]^+$ : 773.2941. Found: 773.2949.



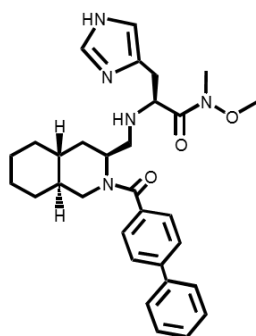
**(S)-2-([(3R,4aS,8aR)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methyl]amino)-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide 48a**

化合物 **34b** と同様に合成した。Yield 66% (3 steps):  $[\alpha]_{\text{D}}^{28} +58$  ( $c$  1.2,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta = 7.58\text{--}7.24$  (m, 19H), 7.13-7.07 (m, 6H), 6.57 (brs, 0.4H), 6.55 (m, 0.6H), 5.00-4.98 (m, 0.4H), 4.46 (dd,  $J = 13.2, 3.6$  Hz, 0.6H), 4.13-4.11 (m, 0.4H), 3.94 (m, 1H), 3.65 (s, 1.2H), 3.62-3.58 (m, 0.6H), 3.50 (s, 1.8H), 3.44 (dd,  $J = 13.6, 3.2$  Hz, 0.4H), 3.14 (s, 1.2H), 3.11 (s, 1.8H), 3.01-2.93 (m, 1H), 2.88-2.77 (m, 2H), 2.65 (dd,  $J = 12.0, 6.4$  Hz, 0.4H), 2.55-2.43 (m, 1.2H), 1.71-1.69 (m, 3H), 1.60-1.52 (m, 2H), 1.45-1.19 (m, 5H), 1.05-0.83 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 175.7, 175.4, 171.1, 170.8, 142.5, 142.4, 142.0, 141.9, 140.5, 140.3, 138.2, 138.1, 137.5, 137.4, 135.7, 129.8, 129.7, 128.8, 128.7, 127.9, 127.54, 127.47, 127.12, 127.11, 127.08, 127.00, 119.5, 119.3, 77.2, 75.1, 62.0, 61.6, 57.7, 57.6, 55.5, 49.3, 48.4, 47.5, 47.3, 43.0, 42.9, 42.1, 36.7, 36.6, 34.6, 33.5, 33.1, 33.0, 32.3, 32.1, 29.9, 29.73, 29.67, 26.2, 26.1, 25.9, 25.7$ ; HRMS (EI) Calcd. For  $\text{C}_{50}\text{H}_{53}\text{N}_5\text{O}_3$   $[\text{M}]^+$ : 771.4148. Found: 771.4154.



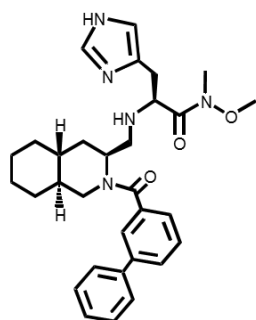
**(S)-2-([(3R,4aS,8aR)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl)methyl]amino)-N-methoxy-N-methyl-3-(1-trityl-1H-imidazol-4-yl)propanamide 49a**

化合物 **34b** と同様に合成した。Yield 66% (3 steps):  $[\alpha]_D^{28} +8.1$  (*c* 0.25, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.47-7.31 (m, 14H), 7.12-7.11 (m, 6H), 6.55 (brs, 1H), 4.94 (m, 0.4H), 4.40 (dd, *J* = 13.4, 3.4 Hz, 0.6H), 4.10 (brs, 0.4H), 3.91 (brs, 0.6H), 3.80 (m, 0.6H), 3.63 (s, 1.8H), 3.54 (s, 1.6H), 3.27 (dd, *J* = 13.0, 3.4 Hz, 0.4H), 3.12 (s, 1.8H), 3.11 (s, 1.2H), 2.98-2.91 (m, 1H), 2.86-2.73 (m, 2.6H), 2.61 (dd, *J* = 11.6, 6.0 Hz, 0.6H), 2.47-2.41 (m, 1.4H), 1.68-1.66 (m, 3H), 1.59-1.47 (m, 2H), 1.38-1.18 (m, 5H), 0.99-0.88 (m, 2H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.6, 175.3, 170.3, 170.0, 142.5, 142.4, 138.2, 138.1, 137.4, 137.3, 135.8, 135.7, 131.50, 131.49, 129.8, 129.7, 128.9, 128.7, 127.9, 123.33, 123.28, 119.5, 119.3, 77.2, 75.1, 75.0, 61.6, 57.8, 57.5, 55.5, 49.2, 48.5, 47.4, 47.2, 42.9, 42.8, 42.0, 36.7, 36.5, 34.7, 33.5, 33.0, 32.9, 32.3, 32.1, 29.9, 29.7, 26.2, 26.0, 25.8, 25.7; HRMS (EI) Calcd. For C<sub>44</sub>H<sub>48</sub>BrN<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 773.2941. Found: 771.2935.



**(S)-2-([(3S,4aR,8aS)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methyl]amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

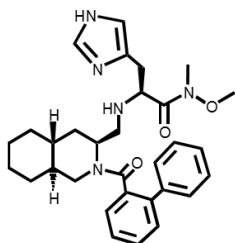
上記の化合物と同様に合成した。Yield 73%:  $[\alpha]_D^{28} -62$  (*c* 0.085, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.68-7.36 (m, 10H), 6.84 (m, 0.6H), 6.82 (m, 0.4H), 5.03 (brs, 0.4H), 4.30-4.27 (m, 0.6H), 4.15-4.11 (m, 0.6H), 3.87-3.85 (m, 0.4H), 3.73 (s, 1.2H), 3.66 (s, 1.8H), 3.54-3.51 (m, 1H), 3.25 (s, 1.2H), 3.19 (s, 1.8H), 2.99-2.86 (m, 1H), 2.75-2.62 (m, 2H), 2.56-2.44 (m, 2H), 1.77-1.56 (m, 5H), 1.48-1.39 (m, 2H), 1.31-1.19 (m, 3H), 1.14-1.12 (m, 0.4H), 1.03-1.02 (m, 1H), 0.88-0.84 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 174.4, 171.0, 143.0, 142.4, 140.2, 140.1, 135.7, 135.3, 135.2, 134.8, 128.84, 128.83, 128.2, 127.8, 127.7, 127.5, 127.19, 127.17, 127.14, 127.08, 77.2, 61.7, 59.8, 58.4, 55.7, 49.6, 49.4, 48.6, 48.1, 43.4, 42.6, 42.0, 36.6, 34.2, 34.0, 33.0, 32.9, 32.2, 30.0, 29.6, 26.2, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For C<sub>31</sub>H<sub>39</sub>N<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 529.3053. Found: 529.3051.



**(S)-2-([(3S,4aR,8aS)-2-[(1,1'-biphenyl)-3-carbonyl]decahydroisoquinolin-3-yl)methyl]amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

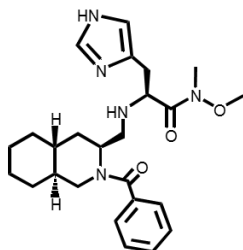
**midazol-4-yl)-N-methoxy-N-methylpropanamide**

上記の化合物と同様に合成した。Yield 78%:  $[\alpha]_D^{28} -35$  (c 0.27,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.66-7.63 (d,  $J$  = 9.6 Hz, 2H), 7.58-7.55 (m, 2H), 7.50-7.41 (m, 3.6H), 7.39-7.35 (m, 1.4H), 7.26 (s, 1H), 6.84 (s, 0.6H), 6.83 (s, 0.4H), 5.03-5.01 (m, 0.4H), 4.32 (dd,  $J$  = 13.2, 3.2 Hz, 0.6H), 4.13-4.11 (m, 0.6H), 3.88-3.86 (m, 0.4H), 3.72 (s, 1.2H), 3.64 (s, 1.8H), 3.57-3.55 (m, 0.6H), 3.48 (dd,  $J$  = 13.0, 3.4 Hz, 0.4H), 3.24 (s, 1.2H), 3.18 (s, 1.8H), 3.00-2.86 (m, 1H), 2.73-2.62 (m, 2H), 2.55-2.44 (m, 2H), 1.79-1.68 (m, 3H), 1.64-1.44 (m, 2H), 1.41-1.19 (m, 5H), 1.14-0.82 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 174.3, 171.1, 141.7, 141.6, 140.3, 140.2, 137.2, 137.0, 135.6, 134.8, 129.3, 128.89, 128.87, 128.2, 127.69, 127.65, 127.2, 127.1, 126.5, 126.1, 125.29, 125.27, 77.2, 61.7, 59.8, 58.4, 55.7, 49.5, 49.4, 48.5, 48.0, 43.4, 42.6, 42.0, 36.65, 36.58, 34.3, 34.0, 33.0, 32.9, 32.2, 30.0, 29.6, 26.2, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For  $\text{C}_{31}\text{H}_{39}\text{N}_5\text{O}_3$   $[\text{M}]^+$ : 529.3053. Found: 529.3057.



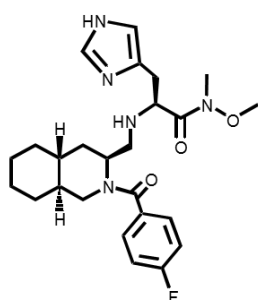
**(S)-2-([(3S,4aR,8aS)-2-[(1,1'-biphenyl)-2-carbonyl]decahydroisoquinolin-3-yl)methylamino]-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

上記の化合物と同様に合成した。2.7:1 のジアステレオマー混合物。Yield 84%:  $^1\text{H}$  NMR (400 MHz, 2.7:1):  $\delta$  = 7.65 (brs, 1H), 7.52-7.30 (m, 9H), 6.83 (m, 0.9H), 6.75 (brs, 0.1H), 5.14-5.12 (m, 1H), 4.92 (brs, 0.6H), 4.78-4.76 (m, 0.3H), 4.46-4.43 (m, 0.1H), 4.37 (dd,  $J$  = 13.2, 3.6 Hz, 0.6H), 3.80 (m, 1H), 3.71 (s, 0.9H), 3.67 (s, 0.3H), 3.62 (s, 1.8H), 3.50 (m, 0.4H), 3.38 (m, 0.6H), 3.25 (m, 0.3H), 3.23 (s, 0.9H), 3.18 (s, 1.8H), 2.99-2.86 (m, 2.3H), 2.74-2.54 (m, 2H), 2.47-1.97 (m, 2H), 1.66-1.53 (2H, m), 1.43-1.02 (4H, m), 0.86 (2H, m), 0.59-0.38 (m, 1.4H), -0.21 (brs, 0.3H), -0.78--0.86 (m, 0.3H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 174.3, 172.0, 170.6, 170.1, 140.2, 139.9, 139.8, 138.8, 138.4, 138.1, 137.9, 137.8, 136.3, 136.0, 135.9, 135.5, 134.7, 134.4, 129.6, 126.5, 129.4, 129.12, 129.08, 129.0, 128.9, 128.7, 128.6, 128.5, 128.2, 127.8, 127.7, 127.64, 127.58, 127.42, 127.38, 126.5, 77.2, 61.7, 59.6, 58.4, 57.7, 55.3, 54.3, 48.9, 48.8, 48.4, 48.3, 48.1, 47.8, 45.7, 42.5, 41.8, 40.5, 40.4, 36.0, 35.7, 33.3, 32.8, 32.7, 32.6, 32.2, 29.7, 29.6, 29.5, 29.3, 26.0, 25.9, 25.7, 25.5, 25.3; HRMS (EI) Calcd. For  $\text{C}_{31}\text{H}_{39}\text{N}_5\text{O}_3$   $[\text{M}]^+$ : 529.3053. Found: 529.3046.



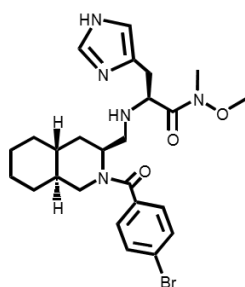
**(S)-2-([(3S,4aR,8aS)-2-benzoyldecahydroisoquinolin-3-yl)methyl]amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

上記の化合物と同様に合成した。Yield 74%:  $[\alpha]_D^{28}$  -45 (c 0.29,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67 (brs, 0.6H), 7.43-7.40 (m, 4.4H), 7.33-7.31 (m, 1H), 6.83 (s, 0.6H), 6.81 (s, 0.4H), 5.00 (m, 0.4H), 4.28 (brd,  $J$  = 12.0 Hz, 0.6H), 4.07 (brs, 0.6H), 3.84-3.81 (m, 0.4H), 3.72 (s, 1.2H), 3.66 (s, 1.8H), 3.51 (brs, 0.6H), 3.43 (d,  $J$  = 11.2 Hz, 0.4H), 3.24 (s, 1.2H), 3.19 (s, 1.8H), 2.99-2.84 (m, 1.4H), 2.72-2.59 (m, 2H), 2.48-2.42 (m, 1.6H), 1.77-1.58 (m, 4H), 1.46-1.21 (m, 6H), 1.13-0.94 (m, 1.4H), 0.90-0.81 (m, 0.6H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 174.3, 171.2, 136.6, 136.5, 135.8, 134.8, 130.1, 129.5, 128.7, 128.5, 127.6, 126.6, 125.7, 77.2, 61.7, 59.9, 58.5, 55.6, 49.3, 48.6, 43.4, 42.6, 42.0, 36.6, 34.2, 33.0, 32.9, 32.2, 30.0, 29.6, 26.2, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For  $\text{C}_{25}\text{H}_{35}\text{N}_5\text{O}_3$   $[\text{M}]^+$ : 453.2740. Found: 453.2744.



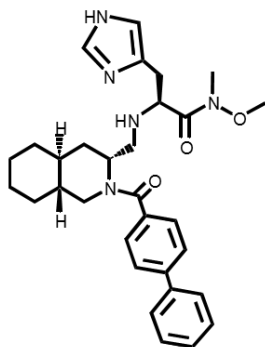
**(S)-2-([(3S,4aR,8aS)-2-(4-fluorobenzoyl)decahydroisoquinolin-3-yl)methyl]amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

上記の化合物と同様に合成した。Yield 78%:  $[\alpha]_D^{28}$  -35 (c 0.41,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.75-7.69 (m, 0.4H), 7.66 (s, 0.6H), 7.48-7.44 (m, 1.2H), 7.37 (s, 0.4H), 7.34-7.30 (m, 1H), 7.12-7.06 (m, 1.4H), 6.83 (s, 0.6H), 6.81 (s, 0.4H), 4.98-4.96 (m, 0.4H), 4.24 (dd,  $J$  = 13.2, 3.2 Hz, 0.6H), 4.05-4.02 (m, 0.6H), 3.86-3.84 (m, 0.4H), 3.72 (s, 1.2H), 3.66 (s, 1.8H), 3.56-3.54 (m, 0.4H), 3.39 (dd,  $J$  = 13.4, 3.4 Hz, 0.4H), 3.24 (s, 1.2H), 3.20 (s, 1.8H), 2.95-2.83 (m, 1.2H), 2.72-2.64 (m, 2H), 2.50-2.43 (m, 2H), 1.76-1.36 (m, 5H), 1.33-1.25 (m, 5H), 1.06-0.97 (m, 1.4H), 0.89-0.83 (m, 0.6H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 174.3, 173.3, 170.2, 163.6 (d,  $J$  = 249.1 Hz), 163.2 (d,  $J$  = 248.1 Hz), 135.6, 134.7, 132.6 (d,  $J$  = 3.4 Hz), 132.5 (d,  $J$  = 3.4 Hz), 130.0, 129.9, 128.9, 128.82, 128.78, 127.5, 127.3, 127.0, 115.9, 115.71, 115.65, 115.4, 77.2, 61.7, 59.7, 58.3, 55.8, 49.6, 49.4, 48.6, 48.0, 43.6, 42.6, 42.0, 36.6, 34.2, 34.0, 33.0, 32.8, 32.2, 29.9, 29.6, 26.1, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For  $\text{C}_{25}\text{H}_{34}\text{FN}_5\text{O}_3$   $[\text{M}]^+$ : 471.2646. Found: 471.2644.



**(S)-2-([(3S,4aR,8aS)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl)methyl]amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

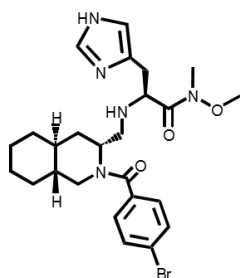
上記の化合物と同様に合成した。Yield 74%:  $[\alpha]_D^{28} -36$  (c 0.28, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.65 (brs, 0.6H), 7.55-7.52 (m, 2H), 7.39 (m, 0.4H), 7.32 (d,  $J$  = 7.6 Hz, 1.2H), 7.18 (d,  $J$  = 8.4 Hz, 0.8H), 6.82 (s, 0.6H), 6.80 (s, 0.4H), 4.95 (brs, 0.4H), 4.25 (d,  $J$  = 12.0 Hz, 0.6H), 3.98 (brs, 0.6H), 3.86-3.80 (m, 0.4H), 3.71 (s, 1.2H), 3.65 (s, 1.8H), 3.54-3.53 (m, 0.6H), 3.35-3.27 (m, 0.4H), 3.23 (s, 1.2H), 3.20 (s, 1.8H), 2.99-2.82 (m, 2H), 2.71-2.60 (m, 2H), 2.51-2.42 (m, 2H), 1.76-1.58 (m, 4H), 1.47-1.24 (m, 5H), 1.05-0.83 (m, 2H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 174.2, 173.4, 170.1, 135.6, 135.4, 135.3, 134.7, 131.9, 131.7, 129.3, 128.3, 124.4, 123.6, 77.2, 61.7, 59.7, 58.2, 55.7, 49.5, 49.3, 48.5, 47.9, 43.4, 42.5, 41.9, 36.5, 34.2, 33.9, 32.9, 32.8, 32.2, 29.9, 29.6, 26.1, 25.9, 25.7, 25.5; HRMS (EI) Calcd. For C<sub>25</sub>H<sub>34</sub>BrN<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 531.1845. Found: 531.1849.



**(S)-2-([(3R,4aS,8aR)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methyl]amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

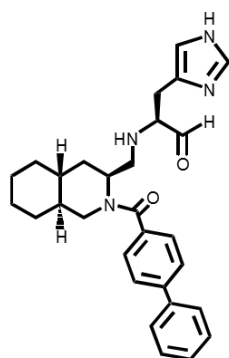
上記の化合物と同様に合成した。Yield 84%:  $[\alpha]_D^{28} -30.7$  (c 1.25, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.65-7.60 (m, 4H), 7.55 (s, 1H), 7.49-7.44 (m, 4H), 7.40-7.36 (m, 1H), 6.79 (s, 1H), 5.23-5.21 (m, 0.75H), 4.53-4.51 (m, 0.25H), 4.13 (m, 0.25H), 3.90-3.88 (m, 0.75H), 3.67 (s, 2.25H), 3.67-3.65 (m, 0.75H), 3.57 (s, 0.75H), 3.57-3.53 (m, 0.75H), 3.28-3.26 (m, 0.25H), 3.26 (s, 2.25H), 3.22 (s, 0.75H), 3.12-3.06 (m, 0.25H), 2.97 (dd,  $J$  = 15.4, 3.4 Hz, 0.75H), 2.86 (dd,  $J$  = 13.4, 11.8 Hz, 0.75H), 2.62-2.53 (m, 1.5H), 2.36 (dd,  $J$  = 12.4, 4.4 Hz, 1H), 1.73 (m, 2H), 1.62-1.41 (m, 4H), 1.30-1.22 (m, 4H), 1.15-1.12 (m, 0.75H), 0.97-0.88 (m, 1.25H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.0, 171.6, 171.0, 142.5, 140.2, 135.6, 135.4, 135.2, 134.4, 129.0, 128.9, 127.7, 127.4, 127.3, 127.2, 127.1, 127.0, 77.2, 61.7, 58.6, 55.5, 49.5, 49.1, 47.5, 42.8, 42.3, 37.0, 36.8, 35.2, 34.3, 33.1, 33.0, 32.3, 29.9, 29.7, 29.6, 29.2, 26.2, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For C<sub>31</sub>H<sub>39</sub>N<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 529.3053. Found: 529.3061.





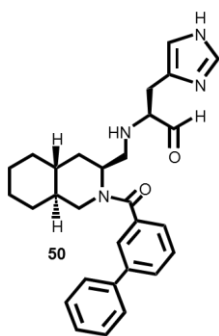
**(S)-2-([(3R,4aS,8aR)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl]methyl)amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

上記の化合物と同様に合成した。Yield 85%:  $[\alpha]_D^{28}$  -26 (*c* 0.36,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.56-7.47 (m, 3H), 7.31-7.28 (m, 2H), 6.78 (s, 0.25H), 6.77 (s, 0.75H), 5.16-5.13 (m, 0.75H), 4.44 (dd, *J* = 13.4, 3.8 Hz, 0.25H), 3.89-3.87 (m, 1H), 3.66 (s, 2.25H), 3.58 (s, 0.75H), 3.38 (dd, *J* = 13.6, 3.6 Hz, 0.75H), 3.24 (s, 2.25H), 3.20 (s, 0.75H), 3.18-3.15 (m, 0.75H), 3.03 (dd, *J* = 11.6, 9.6 Hz, 0.25H), 2.98-2.88 (m, 1H), 2.82 (dd, *J* = 13.4, 11.8 Hz, 0.75H), 2.70-2.48 (m, 1.5H), 2.38 (dd, *J* = 12.2, 4.6 Hz, 0.75H), 2.31 (dd, *J* = 11.8, 5.4 Hz, 0.25H), 1.72-1.67 (m, 2H), 1.60-1.37 (m, 5H), 1.30-1.21 (m, 5H), 1.11-1.05 (m, 0.75H), 0.97-0.83 (m, 1.25H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 174.8, 170.6, 170.2, 135.6, 135.3, 135.2, 134.6, 131.7, 131.6, 128.8, 128.4, 123.7, 123.4, 77.2, 61.7, 58.4, 57.9, 55.4, 49.4, 49.1, 47.5, 47.3, 42.8, 42.7, 42.1, 36.8, 36.7, 35.0, 34.2, 33.0, 32.9, 32.3, 29.8, 29.5, 29.3, 26.1, 25.9, 25.8, 25.5; HRMS (EI) Calcd. For  $\text{C}_{25}\text{H}_{34}\text{BrN}_5\text{O}_3$   $[\text{M}]^+$ : 531.1845. Found: 531.1837.



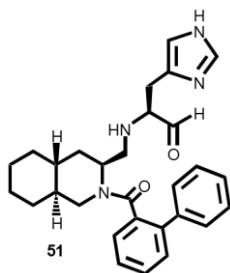
**(S)-2-([(3S,4aR,8aS)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl]methyl)amino)-3-(1H-imidazol-4-yl)propanal**

化合物 **36b** と同様に合成した。Yield 30%:  $[\alpha]_D^{28}$  -4.3 (*c* 0.83,  $\text{CH}_3\text{OH}$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , referenced to residual  $\text{CH}_3\text{OH}$ ):  $\delta$  = 8.81 (brs, 1H), 7.75-7.73 (m, 2H), 7.67-7.65 (m, 2H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.51 (brs, 1H), 7.48-7.45 (m, 2.5H), 7.40-7.36 (m, 1.5H), 5.14-5.13 (m, 1H), 4.81 (dd, *J* = 9.8, 2.6 Hz, 1H), 3.89-3.80 (m, 2H), 3.63-3.59 (m, 1H), 3.44-3.39 (m, 1H), 3.34 (s, 1H), 2.97 (t, *J* = 12.6 Hz, 1H), 1.82-1.62 (m, 5H), 1.45-1.28 (m, 5H), 1.09-0.89 (m, 2H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ , referenced to  $\text{CD}_3\text{OD}$ ):  $\delta$  = 175.24, 175.17, 163.2, 162.8, 144.9, 144.8, 141.21, 141.20, 135.6, 135.03, 134.97, 130.1, 129.22, 129.18, 129.12, 128.2, 128.1, 118.98, 118.95, 95.0, 94.9, 61.3, 60.9, 50.73, 50.69, 49.8, 47.1, 47.0, 43.33, 43.30, 37.8, 37.7, 35.4, 33.7, 30.4, 27.0, 26.6, 23.1, 22.9; HRMS (ESI) Calcd. For  $\text{C}_{29}\text{H}_{35}\text{N}_4\text{O}_2$   $[\text{M}+\text{H}]^+$ : 471.2760. Found: 471.2765.



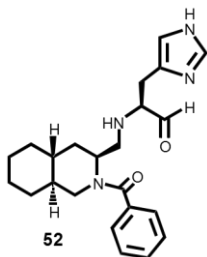
**(S)-2-[(3S,4aR,8aS)-2-[(1,1'-biphenyl)-3-carbonyl]decahydroisoquinolin-3-yl)methyl]amino]-3-(1H-imidazol-4-yl)propanal 50**

化合物 **36b** と同様に合成した。Yield 31%:  $[\alpha]_D^{29} -6.7$  (*c* 0.10, CH<sub>3</sub>OH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.66 (brs, 1H), 7.79-7.73 (m, 2H), 7.64-7.55 (m, 4H), 7.48-7.45 (m, 3.5H), 7.41-7.37 (m, 1.5H), 5.19-5.18 (m, 1H), 4.77 (dd, *J* = 12.6, 3.2 Hz, 1H), 3.87-3.79 (m, 2H), 3.63-3.58 (m, 1H), 3.46-3.40 (m, 1H), 3.36-3.34 (m, 0.5H), 3.25-3.23 (m, 1.5H), 2.99-2.93 (m, 1H), 1.79-1.62 (m, 5H), 1.42-1.21 (m, 5H), 1.10-0.89 (m, 2H); LRMS (ESI) Calcd. For C<sub>29</sub>H<sub>35</sub>N<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 471.28. Found: 471.35.



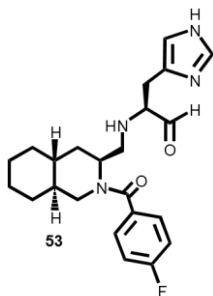
**(S)-2-[(3S,4aR,8aS)-2-[(1,1'-biphenyl)-2-carbonyl]decahydroisoquinolin-3-yl)methyl]amino]-3-(1H-imidazol-4-yl)propanal 51**

化合物 **36b** と同様に合成した。ジアステレオマー混合物。Yield 27%: <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH, diastereomixture):  $\delta$  = 8.84 (brs, 1H), 7.59-7.38 (m, 11H), 5.03 (m, 1H), 4.81 (dd, *J* = 10.0, 2.8 Hz, 1H), 3.90 (m, 1H), 3.69-3.59 (m, 1H), 3.41 (m, 1H), 3.34 (s, 1H), 3.27-3.24 (m, 1H), 2.93-2.87 (m, 1H), 2.61-2.54 (m, 1H), 1.59-1.56 (m, 2H), 1.50 (d, *J* = 10.4 Hz, 1H), 1.42 (d, *J* = 12.4 Hz, 1H), 1.18-1.08 (m, 2H), 0.99-0.88 (m, 3H), 0.70-0.61 (m, 1H), 0.46-0.44 (m, 1H); LRMS (ESI) Calcd. For C<sub>29</sub>H<sub>35</sub>N<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 471.28. Found: 471.35.



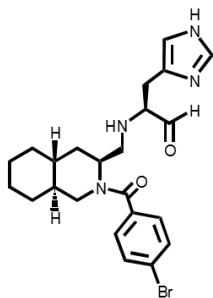
**(S)-2-[(3S,4aR,8aS)-2-benzoyldecahydroisoquinolin-3-yl]methylamino]-3-(1H-imidazol-4-yl)propanal 52**

化合物 **36b** と同様に合成した。Yield 25%:  $[\alpha]_D^{29} -3.7$  (*c* 0.15, CH<sub>3</sub>OH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.61 (brs, 1H), 7.52-7.46 (m, 6H), 7.45-7.41 (m, 1H), 5.13-5.11 (m, 2H), 4.77 (dd, *J* = 11.8, 3.4 Hz, 1H), 3.79-3.66 (m, 1H), 3.56-3.50 (m, 1H), 3.42-3.32 (m, 1H), 3.26-3.20 (m, 1H), 2.97-2.91 (m, 1H), 1.78-1.59 (m, 5H), 1.40-1.20 (m, 5H), 1.08-0.86 (m, 2H); LRMS (ESI) Calcd. For C<sub>23</sub>H<sub>31</sub>N<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 395.24. Found: 395.30.



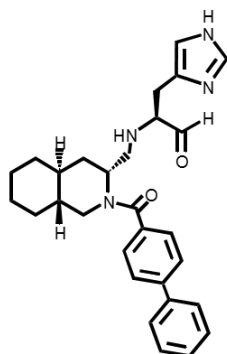
**(S)-2-[(3S,4aR,8aS)-2-(4-fluorobenzoyl)decahydroisoquinolin-3-yl]methylamino]-3-(1H-imidazol-4-yl)propanal 53**

化合物 **36b** と同様に合成した。Yield 18%:  $[\alpha]_D^{29} -3.6$  (*c* 0.18, CH<sub>3</sub>OH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.68 (brs, 1H), 7.56-7.53 (m, 2H), 7.44 (brs, 1H), 7.25-7.19 (m, 3H), 5.10 (m, 1H), 4.77 (dd, *J* = 11.4, 3.2 Hz, 1H), 3.84-3.75 (m, 2H), 3.52-3.47 (m, 1H), 3.40-3.34 (m, 1H), 3.25-3.23 (m, 1H), 2.96-2.89 (m, 1H), 1.78-1.65 (m, 5H), 1.43-1.23 (m, 5H), 1.05-0.93 (m, 2H); LRMS (ESI) Calcd. For C<sub>23</sub>H<sub>30</sub>FN<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 413.24. Found: 413.35.



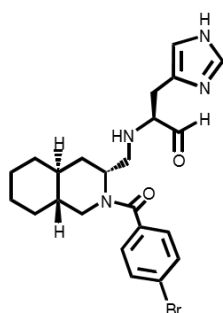
**(S)-2-[(3S,4aR,8aS)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl]methylamino]-3-(1H-imidazol-4-yl)propanal**

化合物 **36b** と同様に合成した。Yield 23%:  $[\alpha]_D^{28} -0.64$  ( $c$  0.88, CH<sub>3</sub>OH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.80 (brs, 1H), 7.65-7.63 (m, 2H), 7.49 (brs, 1H), 7.42 (d,  $J$  = 8.4 Hz, 2H), 5.11 (m, 1H), 4.81-4.78 (m, 1H), 3.86-3.78 (m, 2H), 3.47-3.34 (m, 2H), 2.97-2.91 (m, 1H), 1.75-1.60 (m, 5H), 1.42-1.24 (m, 5H), 1.04-0.96 (m, 2H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD, referenced to CD<sub>3</sub>OD):  $\delta$  = 174.2, 174.1, 163.2, 162.8, 135.6, 135.44, 135.39, 132.9, 130.40, 130.36, 130.0, 125.8, 125.7, 119.0, 118.9, 95.0, 94.9, 61.2, 60.8, 50.64, 50.60, 43.24, 43.22, 37.69, 37.66, 35.25, 35.23, 33.7, 30.4, 30.3, 27.0, 26.6, 23.1, 22.9; HRMS (ESI) Calcd. For C<sub>23</sub>H<sub>30</sub>BrN<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 473.1552. Found: 473.1546.



**(S)-2-([(3R,4aS,8aR)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methyl]amino)-3-(1H-imidazol-4-yl)propanal**

化合物 **36b** と同様に合成した。Yield 31%:  $[\alpha]_D^{28} -1.62$  ( $c$  1.23, CH<sub>3</sub>OH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.75 (s, 1H), 7.75 (d,  $J$  = 8.0 Hz, 2H), 7.66 (d,  $J$  = 7.2 Hz, 2H), 7.57 (d,  $J$  = 8.0 Hz, 2H), 7.47-7.45 (m, 3.5H), 7.40-7.37 (m, 1.5H), 5.09 (brs, 1H), 3.80 (m, 2H), 3.66-3.63 (m, 1H), 3.51 (m, 1H), 3.26 (m, 1H), 2.93-2.91 (m, 1H), 1.77-1.68 (m, 5H), 1.45-1.35 (m, 5H), 1.07-0.97 (m, 2H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD, referenced to CD<sub>3</sub>OD):  $\delta$  = 175.5, 175.4, 163.2, 162.8, 144.9, 141.2, 135.6, 135.5, 134.9, 130.1, 129.8, 129.7, 129.2, 129.1, 128.2, 128.1, 119.1, 95.2, 95.0, 62.0, 61.4, 50.8, 43.12, 43.10, 37.73, 37.69, 35.5, 35.4, 33.7, 30.4, 27.0, 26.6, 24.4, 23.9; HRMS (ESI) Calcd. For C<sub>29</sub>H<sub>35</sub>N<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 471.2760. Found: 471.2756.



**(S)-2-([(3R,4aS,8aR)-2-(4-bromobenzoyl)decahydroisoquinolin-3-yl)methyl]amino)-3-(1H-imidazol-4-yl)propanal**

化合物 **36b** と同様に合成した。Yield 30%:  $[\alpha]_D^{28} -6.1$  ( $c$  1.0, CH<sub>3</sub>OH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.82 (brs, 1H), 7.65 (d,  $J$  = 8.4 Hz, 2H), 7.49 (s, 1H), 7.40 (d,  $J$  = 8.0

Hz, 2H), 5.06 (m, 1H), 4.83 (m, 1H), 3.86-3.76 (m, 2H), 3.51-3.43 (m, 2H), 3.27-3.25 (m, 1H), 2.93-2.90 (m, 1H), 1.76-1.66 (m, 5H), 1.44-1.30 (m, 5H), 1.04-0.96 (m, 2H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ , referenced to  $\text{CD}_3\text{OD}$ ):  $\delta$  = 174.43, 174.35, 163.1, 162.8, 135.6, 135.5, 135.3, 133.0, 130.4, 125.9, 119.1, 95.3, 95.0, 61.8, 61.3, 50.7, 43.03, 43.01, 37.7, 37.6, 35.4, 35.3, 33.7, 30.33, 30.31, 27.0, 26.6, 24.4, 23.9; HRMS (ESI) Calcd. For  $\text{C}_{23}\text{H}_{30}\text{BrN}_4\text{O}_2$   $[\text{M}+\text{H}]^+$ : 473.1552. Found: 4731537. Found: 4731537.

## 評価試験・X線結晶構造解析

### 1) 阻害活性評価

基質ペプチド (H-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-NH<sub>2</sub>) (111  $\mu$ M) 溶液 [DTT (7 mM)を含む緩衝液 (25  $\mu$ L of 20 mM Tris-HCl buffer pH 7.5)に溶かした] に R188I SARS 3CL<sup>pro</sup> (56 nM) と様々な濃度に調整した阻害剤を加え 37°C、60 分温置した。阻害率を求めるためには HPLC を用い、基質ペプチドのピーク面積の減少率を酵素の切断率として測定した。すべての阻害剤の IC<sub>50</sub> はシグモイド容量反応曲線を用い求めた。またこの実験は三回繰り返し行なった。

### 2) Lineweaver-Burk plot

加水分解反応の初速度は阻害活性評価に用いた方法と同様の方法を用いて算出した。R188I SARS 3CL<sup>pro</sup> (56 nM) 存在下、阻害剤 **14** を添加しない系と阻害剤 **14** の濃度 (25 nM, 50 nM, 100 nM) の幅で変化させた系に、基質 (濃度 34-168  $\mu$ M) を加え、反応を行いデータの収集をした。基質の消化時間は 10-15 分と濃度の差により変化した。HPLC を用い初速度は基質のピーク面積の減少率を基に算出し、また [S] は基質濃度を基にした。得られたデータは縦軸を 1/v、横軸を 1/[S] とした図にプロットした。

### 3) X線結晶構造解析

#### a) ペプチド型阻害剤

ペプチド型阻害剤 **14** と R188I SARS 3CL<sup>pro</sup> の共結晶作成には、シッティングドロップ蒸気拡散法を用いた。プロテアーゼと阻害剤 **14** の混合溶液 (8 mg/mL、プロテアーゼ : 阻害剤 = 1 : 4 の比率に調製した DMSO 溶液) を 1 時間 4°C 条件下インキュベートした後、混合溶液 (pH 6.0 に調整した 100 mM の MES 溶液、9-11% の PEG20000 溶液と 5 mM DTT の混合溶液) を加えた。次に沈降溶液 (pH 6.2 に調整した 100 mM の MES 溶液と 5-10% の PEG20000 溶液と 5 mM の DTT 溶液の混合溶液) を先の混合溶液と同量加え、3 日のうちに 4°C で結晶を析出させた。次に析出した結晶を沈降溶液 (pH 6.0 に調整した 100 mM MES、11% PEG20000、5 mM DTT、15% エチレングリコールの混合溶液) 中に移し、窒素ガス雰囲気下保存した。X 線回折は -178°C 下、Photon Factory の ADSC Quantum 315r CCD detector, BL-6A を用い 0.9780 Å の波長で測定し、回折データは HKL-2000 software system により処理した。

得られた回折データの構造解析は、初めに PDB に登録済みの R188I SARS-3CL<sup>pro</sup> (PDB code 2ZU4) を指標とし、Molrep の分子置換法により位相解析を行った。次に CCP package の Refmac 5 プログラムを使いプロテアーゼの細部の解析 (Rigid body refinement 解析と restrained refinement 解析) を行った。再解析は Coot プログラムを使い行った。水分子は蛋白質のすべての構造解析が済んだ後、Coot プログラムを使い配置した。最後に、電子密度に合わせ阻害剤を設置し、全体の再解析をもう一度行った。構造解析したデータの描画ソフトには Pymol または chimera software を用いた。

## b) 非ペプチド型阻害剤

精製済みの R188I SARS 3CL<sup>pro</sup> に混合溶液 (pH 5.5 20 mM の Bis-Tris 溶液、10 mM の NaCl 溶液、1 mM DTT 溶液の混合溶液) を加え 8 mg/mL の濃度に調整した。結晶化にはシッティングドロップ蒸気拡散法を用いることとし、沈降溶液 (pH 6.2 に調整した 100 mM の MES 溶液と 5-10% の PEG20000 溶液と 5 mM の DTT 溶液の混合溶液) を先の混合溶液と同量加え、4°C で結晶を析出させた。3 日以内に 6 面体 (0.3 mm x 0.3 mm x 0.3 mm) の結晶に成長した。次にその結晶に 3 mM の阻害剤 **36a** もしくは **36b** を含んだ混合溶液 (pH 6.2 に調整した 100 mM の MES 溶液、5-8% の PEG20000 溶液と 5 mM DTT の混合溶液) を加え、24 時間静置した。得られた結晶は、窒素ガス雰囲気下 -173°C、3 mM の阻害剤 **36a** もしくは **36b** を含む混合溶液 (pH 6.2 に調整した 100 mM の MES 溶液、10% の PEG20000 溶液、5 mM の DTT 溶液と 15% のエチレングリコールの混合溶液) 中で保存した。阻害剤 **36a**, **36b** と R188I SARS 3CL<sup>pro</sup> の X 線結晶構造の回折データ収集は SPring-8 の Rayonix MX300HE CCD detector を用い波長 0.900 Å のビームライン BL44XU を用い行った。

阻害剤 **37b** と R188I SARS-3CL<sup>pro</sup> の共結晶作成には、シッティングドロップ蒸気拡散法を用いた。プロテアーゼと阻害剤 **37b** (阻害剤最大濃度 3 mM) の混合溶液に沈降溶液 (pH 6.0 の 100 mM の MES 溶液、5-6% PEG20000 の溶液、5 mM の DTT の混合溶液) をプロテアーゼ・阻害剤の混合溶液と同量加え、4°C で結晶を析出させた。3 日間のうちに 6 面体 (0.2 mm x 0.2 mm x 0.2 mm) の結晶に成長した。次に析出した結晶を 3 mM の阻害剤を含む混合溶液 (pH 6.0 の 100 mM MES, 6% PEG20000, 5 mM DTT, 15% エチレングリコール) 中に移し、-173°C 窒素ガス雰囲気下保存した。X 線結晶構造の回折データ収集は Rigaku RAXIS VII imaging-plate detector を用い 1.5418 Å の波長の in-house rotating anode FR-E/Super Bright X-ray generator で測定し、回折データは Confocal VariMax (VariMax HF) optics system を使い処理した。

得られた回折データの構造解析は、初めに PDB に登録済みの R188I SARS 3CL<sup>pro</sup> (PDB code 3AW1) を指標とし、Molrep の分子置換法により位相解析を行った。次に CCP package の Refmac 5 プログラムを使いプロテアーゼの細部の解析 (Rigid body refinement 解析と restrained refinement 解析) を行った。再解析は Coot program を使い行った。水分子は蛋白質のすべての構造解析が済んだ後、Coot プログラムを使い配置した。最後に、JLigand ソフトを使い阻害剤をプロテアーゼ内の電子密度に合わせ設置し、全体の再解析をもう一度行った。構造解析したデータの描画ソフトには Pymol または chimera software を用いた。

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## Structure-Based Design, Synthesis, and Evaluation of Peptide-Mimetic SARS 3CL Protease Inhibitors

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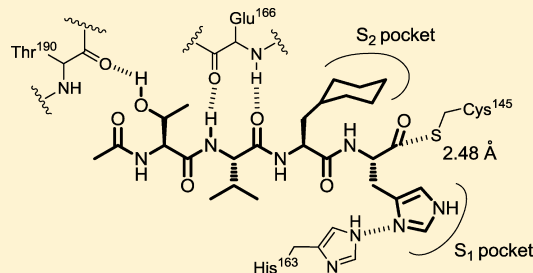
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### **S** Supporting Information

**ABSTRACT:** The design and evaluation of low molecular weight peptide-based severe acute respiratory syndrome (SARS) chymotrypsin-like protease (3CL) protease inhibitors are described. A substrate-based peptide aldehyde was selected as a starting compound, and optimum side-chain structures were determined, based on a comparison of inhibitory activities with Michael type inhibitors. For the efficient screening of peptide aldehydes containing a specific C-terminal residue, a new approach employing thioacetal to aldehyde conversion mediated by *N*-bromosuccinimide was devised. Structural optimization was carried out based on X-ray crystallographic analyses of the R188I SARS 3CL protease in a complex with each inhibitor to provide a tetrapeptide aldehyde with an IC<sub>50</sub> value of 98 nM. The resulting compound carried no substrate sequence, except for a P<sub>3</sub> site directed toward the outside of the protease. X-ray crystallography provided insights into the protein–ligand interactions.



## ■ INTRODUCTION

Severe acute respiratory syndrome (SARS), a life-threatening form of pneumonia, is caused by a new coronavirus (SARS CoV).<sup>1–3</sup> Although the primary SARS epidemic that affected about 8500 patients and left 800 dead was eventually brought under control, no effective therapy exists for this viral infection. In addition, the recent identification of a SARS CoV-like virus in Chinese bats raises the possibility of a re-emergence of SARS or related diseases.<sup>4,5</sup> Thus, developing anti-SARS agents against future outbreaks remains a formidable challenge.

SARS is a positive-sense, single-stranded RNA virus featuring the largest viral RNA genome known to date.<sup>6,7</sup> The genomic RNA produces two large proteins with overlapping sequences, polyproteins 1a (~450 kDa) and 1ab (~750 kDa), which are autocatalytically cleaved by two or three viral proteases to yield functional polypeptides.<sup>8</sup> The key enzyme in this processing is a 33 kDa protease, which is called the chymotrypsin-like protease (3CL).<sup>9,10</sup> The SARS 3CL protease is a cysteine protease with a chymotrypsin fold and cleaves precursor proteins at as many as 11 conserved sites involving a conserved Gln at the P<sub>1</sub> position and a small amino acid (Ser, Ala, or Gly) at the P<sub>1</sub>' position with varying efficiency.<sup>11,12</sup> The 3CL protease exists as a homodimer, and each 33 kDa protomer has its own active site containing a Cys-His catalytic dyad. Because of its functional

importance in the viral life cycle, the 3CL protease is considered an attractive target for the structure-based design of drugs against SARS.<sup>13–20</sup> Several crystal forms of the SARS 3CL protease with or without inhibitors have also been used to evaluate various types of inhibitors for this protease.<sup>10,21–25</sup> Most inhibitors contain a functional group such as a chloromethyl ketone, a Michael acceptor, or an epoxide that can react irreversibly with the active-site cysteine residue. Few studies dealing with inhibitors containing an aldehyde group as a functional group have been reported.<sup>26</sup> In the course of our own studies on the SARS 3CL protease and its inhibitors,<sup>27</sup> we found that the mature SARS 3CL protease is sensitive to degradation at the 188Arg/189Gln site, which causes a loss of catalytic activity. The stability of the 3CL protease is dramatically increased by mutating the Arg at the 188 position to Ile. The enzymatic efficiency of the R188I mutant was increased by a factor of more than 1 × 10<sup>6</sup>. The potency of the mutant protease makes it possible to quantitatively evaluate substrate-based peptide-aldehyde inhibitors using conventional high-performance liquid chromatography (HPLC). The evaluations revealed that a peptide aldehyde containing the substrate

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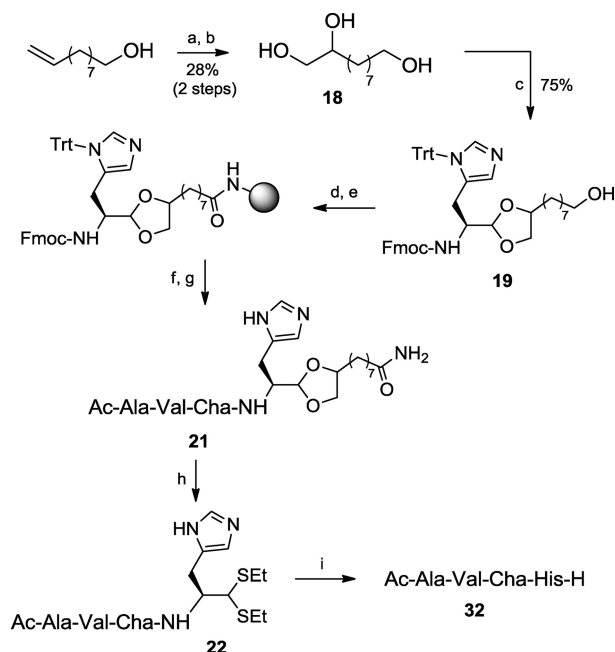




oxazolidine linker, however, a thioacetal compound was produced, more or less as a side product. In the present study, we found that the conversion of an acetal to an aldehyde is quite slow but that the thioacetal can be efficiently converted into the desired aldehyde by treatment with *N*-bromosuccinimide (NBS).

On the basis of this finding, the Fmoc-His(Trt)-acetal **19** containing a linker<sup>32</sup> was selected as a C-terminal residue (Scheme 3).

**Scheme 3. Solid-Phase Synthesis of Peptide Aldehyde Inhibitors 30–35<sup>a</sup>**



compounds	peptide sequence
22, 32;	Ac-Ala-Val-Cha-
23, 33;	Ac-Asn-Val-Cha-
24, 34;	Ac-Ser-Val-Cha-
25, 35;	Ac-Thr-Val-Cha-
26, 30;	Ac-Ser-Ala-Val-Phe-
27, 31;	Ac-Ser-Ala-Val-Cha-

<sup>a</sup>(a) *m*CPBA, 25 °C, 30 min. (b) 1M NaOH, reflux, 2 h. (c) Fmoc-His(Trt)-al, BF<sub>3</sub>·Et<sub>2</sub>O, 25 °C, 30 min. (d) Jones reagent, 25 °C, 40 min. (e) Rink amide resin, DIPCDI, HOBT, DIEA, 25 °C, 10 h. (f) Fmoc-based peptide synthesis. (g) TFA-anisole, 25 °C, 4 h. (h) EtSH, BF<sub>3</sub>·Et<sub>2</sub>O, 25 °C, 2 h. (i) NBS, 25 °C, 1 min.

Epoxidation of the commercially available 9,10-decadien-1-ol with *m*CPBA and hydrolysis gave a triol linker **18**. Fmoc-His(Trt)-al, prepared from the corresponding Weinreb amide, was then reacted with **18** in the presence of BF<sub>3</sub> etherate (BF<sub>3</sub>·Et<sub>2</sub>O) to yield the corresponding acetal **19**. The acetal alcohol was oxidized by treatment with the Jones reagent, and

the resulting carboxylic acid, without any further purification, was anchored to a Rink amide resin<sup>33</sup> [4-(2,4-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin] using diisopropylcarbodiimide (DIPCDI)/1-hydroxybenzotriazole (HOBT) as the coupling reagents. The coupling of the corresponding amino acid residues was conducted by conventional Fmoc-based peptide synthesis using DIPCDI/HOBT coupling and Fmoc deprotection by treatment with piperidine. The product resin was treated with TFA to afford the desired peptide acetal amide **21**. The crude product was dissolved in AcOH and treated with ethanethiol in the presence of BF<sub>3</sub>·Et<sub>2</sub>O as above. After that was quenched with H<sub>2</sub>O, the desired thioacetal peptide **22** was easily purified by HPLC, although a prolonged reaction (more than 4 h) of the Ser-containing acetal-peptide may lower the yield due to acetylation of the Ser side-chain hydroxyl group. The thioacetal thus obtained was treated with NBS; the conversion required less than 1 min. The reaction mixture was immediately subjected to HPLC to give the desired peptide aldehyde **32** showing a single peak.

## RESULTS AND DISCUSSION

The inhibitory activities of Michael acceptor type derivatives were first evaluated based on IC<sub>50</sub> values (Table 1) according to a published procedure.<sup>27</sup> While the IC<sub>50</sub> of a previously reported peptide aldehyde **1** was reported to be 37 μM,<sup>27</sup> the value for **5**, containing the same peptide sequence as **1**, was 330 μM. The Michael type analogues **9** and **10** that had been elongated toward the prime site showed no inhibitory activities. These results suggest that the aldehyde type inhibitor containing the substrate sequence would be more active toward the R188I 3CL protease than the irreversible Michael type inhibitor. The results regarding analogues **9** and **10** also suggest that the recognition of the prime-site main-chain structure by the 3CL protease is strict and that no linker structure inserted between the P<sub>1</sub> site and the reactive functional group would be tolerated.

For optimization of the side-chain structures of **1**, optimization at the P<sub>1</sub> site was first necessary. Conversion of the side-chain structure at the P<sub>1</sub> site would be expected to have a dramatic effect on inhibitory activity, since the P<sub>1</sub> site is conserved in all cleavage sequences of the mature 3CL protease. Six different side-chain structures were examined (Table 2): aliphatic isobutyl and *tert*-butyl groups, aromatic and aliphatic ring structures, and heteroatom-containing ring structures. Each peptide aldehyde was prepared using a conventional Weinreb amide resin and purified by HPLC, but the overall yields were not impressive.

The inhibitory activities of the synthesized aldehydes were evaluated based on IC<sub>50</sub> values calculated from a decrease in the substrate by protease digestion in the presence of different inhibitor concentrations. Incubation of the protease with an inhibitor prior to the addition of the substrate gave an IC<sub>50</sub>

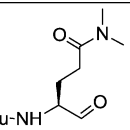
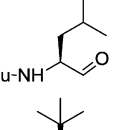
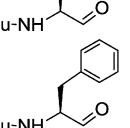
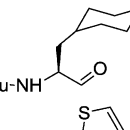
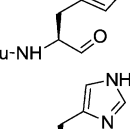
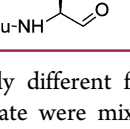
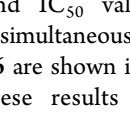
**Table 1. Inhibitory Activities of Michael Type Inhibitors**

compd	structure	IC <sub>50</sub> (μM)
<b>1</b>	Ac-Ser-Ala-Val-Leu-NHCH(CH <sub>2</sub> CH <sub>2</sub> CON(CH <sub>3</sub> ) <sub>2</sub> )-CHO	37
<b>5</b>	Ac-Ser-Ala-Val-Leu-NHCH(CH <sub>2</sub> CH <sub>2</sub> CON(CH <sub>3</sub> ) <sub>2</sub> )-CH=CHCOOEt	330
<b>9</b>	Ac-Ser-Ala-Val-Leu-NHCH(CH <sub>2</sub> CH <sub>2</sub> CON(CH <sub>3</sub> ) <sub>2</sub> )-CH <sub>2</sub> -CH=CHCOOEt	NI <sup>a</sup>
<b>10</b>	Ac-Ser-Ala-Val-Leu-NHCH(CH <sub>2</sub> CH <sub>2</sub> CON(CH <sub>3</sub> ) <sub>2</sub> )-(CH <sub>2</sub> ) <sub>2</sub> -CH=CHCOOEt	NI

<sup>a</sup>NI, no inhibition.



**Table 2. Inhibitory Activities of P<sub>1</sub> Site-Substituted Peptide Aldehydes**

compound	structure	IC <sub>50</sub> (μM)
1		37
11		~3000
12		~3000
13		~2000
14		62
15		48
16		5.7

value that was not significantly different from that obtained when the inhibitor and substrate were mixed simultaneously. Typical sigmoidal curves and IC<sub>50</sub> values (4.0 μM by preincubation vs 5.7 μM by simultaneous mixing) obtained by these two procedures for **16** are shown in Figure S-1 in the Supporting Information. These results suggest that the

inhibitory mode of the aldehyde inhibitor does not involve a suicide mechanism, since the presence of a large excess of inhibitor (μM scale inhibitor vs nM scale protease) had no effect on the cleavage of the substrate when it was added afterward. Thus, the IC<sub>50</sub> values summarized in Table 2 were obtained using a simple simultaneous mixing procedure. Replacement with an aliphatic group or an aromatic ring abolished the inhibitory activity (compounds **11**–**13**), but replacement with a cyclohexyl or a thiophen structure had little effect on the inhibitory activity (**14** and **15**). In contrast, replacement with an imidazole ring (**16**) increased the inhibitory activity by more than 6-fold as compared to **1**. From these results, the pentapeptide aldehyde **16** containing a His unit at the P<sub>1</sub> site was selected as a lead compound for further optimization.

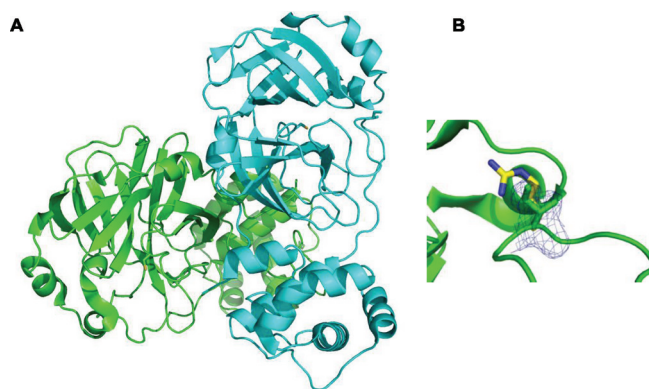
To achieve further optimization using a structure-based design, crystallization and X-ray crystallographic analyses of the R188I mutant protease and its inhibitor complex were conducted. The structure of the mutant protease containing no inhibitor (PDB code: 3AW1) was first refined to a resolution of 2.00 Å (data collection and refinement statistics are summarized in Table 3).

As compared with the reported mature 3CL protease (PDB code: 2ZU4), X-ray crystallography revealed that the R188I mutation did not cause any major change in the overall tertiary organization of the SARS 3CL protease (Figure 2A). The electron density at the active site Cys145 and the dimer's interface, such as residues Ser139-Leu141,<sup>34</sup> was clearly detected, and the expected dimeric structure was folded similarly to the mature protease. The side chain of the mutated Ile was directed toward the outside of the protease, and the electron density at the mutated site was nearly the same as that of structures lacking the guanidine group of Arg in the mature protease (Figure 2B).

**Table 3. Data Collection and Refinement Statistics for the R188I SARS 3CL Protease and Its Inhibitor in Complexes with Compounds 16, 31, and 35**

PDB ID	3AW1 without inhibitor	3AW0 complexed with 16	3AVZ complexed with 31	3ATW complexed with 35
space group	P1	C121	C121	P1
unit cell parameters				
length <i>a</i>	52.13	108.384	109.185	54.517
length <i>b</i>	67.454	81.606	80.533	58.546
length <i>c</i>	67.384	53.33	52.987	67.745
angle $\alpha$	71.23	90	90	94.29
angle $\beta$	77.46	104.24	105.05	104.03
angle $\gamma$	81.74	90	90	106.64
resolution	30.0–2.00	50.0–2.30	50.0–2.50	46.4–2.30
observations	212842	145804	113847	61338
unique observations	51940	19005	14594	29416
redundancy	3.8	7.3	7.4	2
completeness	97.2	99.9	97.4	96.7
mean <i>I</i> /σ( <i>I</i> )	24.2	21.8	16.7	11.8
<i>R</i> merge	0.038	0.082	0.122	0.079
resolution range	30.0–2.00	50.0–2.30	50.0–2.50	46.4–2.30
<i>R</i> <sub>cryst</sub>	0.22	0.23	0.22	0.23
<i>R</i> <sub>free</sub>	0.26	0.29	0.26	0.3
rmsd from ideal				
bond length (Å)	0.023	0.022	0.018	0.01
bond angle (deg)	2.007	1.943	1.781	1.308

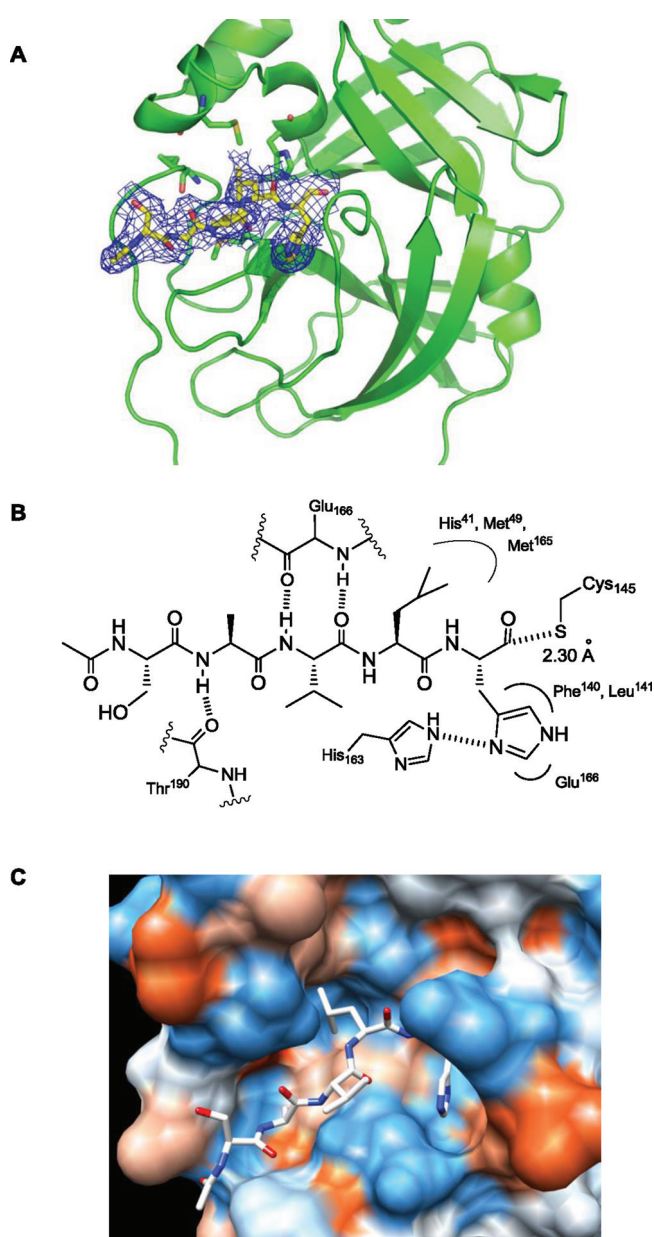




**Figure 2.** (A) Overall dimeric organization of the R188I mutant protease; side chain structures of the mutated Ile and active center Cys are shown as a stick model. (B) Structures near the mutated Ile residue superimposed on the mature 3CL protease (PDB code 2ZU4). The Match Maker Program in the UCSF Chimera package was utilized for superimposing 2ZU4 on 3AW1.

The structure of the mutant protease, in a complex with the lead compound **16**, was refined to a resolution of 2.30 Å (Table 3). The X-ray crystallography revealed that the overall structure was folded similar to the R188I mutant protease solved above. Each monomer of the dimer contains the inhibitor **16** at its active center (PDB code: 3AW0). The binding did not cause changes in the overall organization of the dimer, and **16** was located in the active site cleft. The carbonyl carbon of the aldehyde in **16** was detected at a sufficiently close distance (2.30 Å) from the thiol of the active center Cys-145, and its electron density could be fitted to an  $sp^2$  carbonyl carbon (Figure 3A). The side-chain imidazole nitrogen of the  $P_1$  site His formed a hydrogen bond with His-163 of the mutant protease, which placed the other side of the  $P_1$  site imidazole into a  $S_1$  pocket formed from the side chains of Phe-140, Leu-141, and Glu-166 of the protease (Figure 3B). The isobutyl group of the  $P_2$  site Leu was located at the  $S_2$  pocket made by Met-45, Met-165, and His-41, but some space still remained from Met-45 and -165 (Figure 3C). The amide-bound nitrogen and carbonyl oxygen atoms of the  $P_3$ -Val of **16** formed hydrogen bonds with the protease main chain at Glu-166, and the amide-bound nitrogen of the  $P_4$ -Ala of **16** also formed a hydrogen bond with the protease main chain at Thr-190. Although those hydrogen bonds hold the main chain of the inhibitor into the active-site cleft of the protease, side chains of the  $P_3$ -Val and the  $P_5$ -Ser were directed to the outside of the protease, and no interaction with the protease was detected at these sites (Figure 3).

On the basis of these structural analyses, small molecular inhibitors containing an aldehyde functional group were designed as follows. First, the side chain of the  $P_2$  site was replaced with a bulky group to permit the side chain to fit into the  $S_2$  pocket more tightly than **16**. Next, the outwardly directed  $P_5$  site was removed to lower the molecular weight of the inhibitors, since no interactions at the corresponding side chains with the protease were detected. Third, to more closely attach the  $P_5$  site-deleted inhibitor to the active-site cleft, a heteroatom was introduced into the side chain of the  $P_4$  site to create the possibility of additional hydrogen bonds with the protease. Thus, six additional inhibitors (**30–35** in Table 4) were designed and synthesized using the newly developed acetal-thioacetal conversion method (Scheme 3).



**Figure 3.** (A) X-ray structure of the inhibitor **16** bound to the R188I SARS 3CL protease (PDB code 3AW0); oxygen (red), nitrogen (blue), and sulfur (yellow); the main chain of **16** is also shown in yellow. (B) Mode of the interaction. (C) Molecular graphics image produced using the UCSF Chimera package from the Resource for Biocomputing Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).

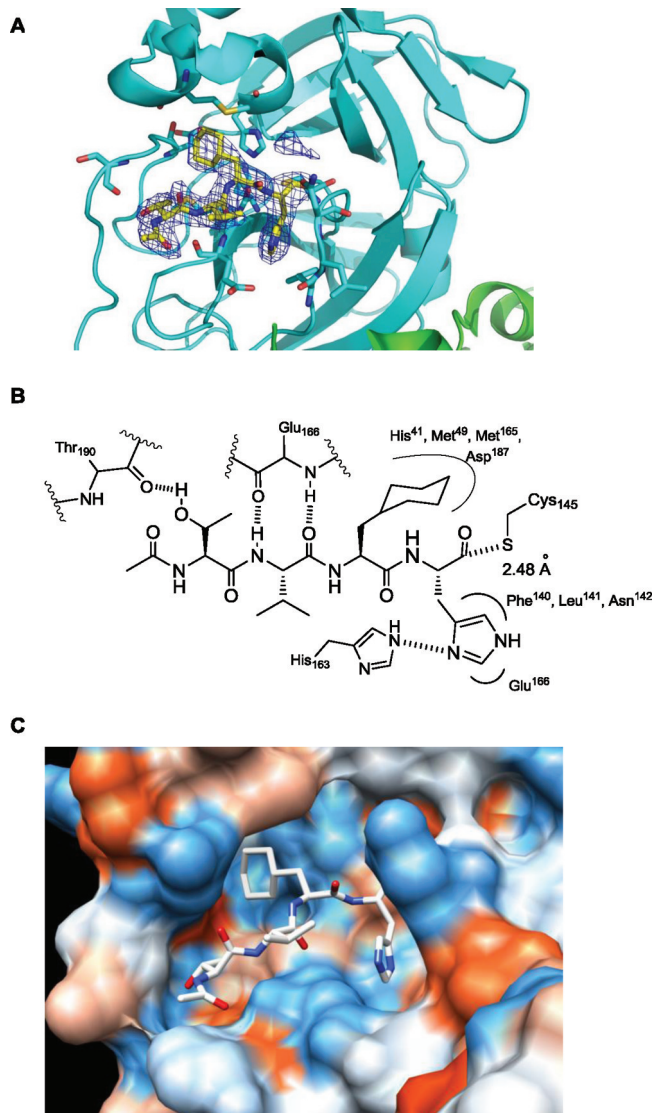
The inhibitory activities of the newly synthesized inhibitors were evaluated using  $IC_{50}$  values obtained by the simultaneous mixing procedure, as detailed above (Table 4). The peptide aldehydes containing a more bulky phenyl or cyclohexyl group at the  $P_2$  site showed dramatically increased inhibitory activity (compounds **30** and **31**) as expected. Substitution with a cyclohexyl group was more effective than substitution with a planar aromatic group, and the inhibitory activity of **31** was increased by more than 80 times as compared with **16** (a reduction in the  $IC_{50}$  value from 5.7  $\mu$ M to 65 nM).<sup>35</sup> Reducing the molecular weight of **31** caused by the simple deletion of the  $P_5$  site lowered the inhibitory activity to 1/6, but the resulting compound **32** was still 20 times more potent than the lead

**Table 4. Inhibitory Activities of P<sub>2</sub> and P<sub>4</sub> Site-Substituted Peptide Aldehydes**

compound	structure	IC <sub>50</sub> (nM)
16		5700
30		390
31		65
32		270
33		10000
34		340
35		98

compound **16** (5.7  $\mu$ M vs 270 nM in IC<sub>50</sub>). The inhibitory activity of **32** was nearly the same as that of the P<sub>2</sub>-replaced pentapeptide inhibitor **30**. Replacement of the P<sub>4</sub> site of **32** with an oxygen-containing side chain to create an additional hydrogen bond was then examined. Replacement of the P<sub>4</sub> site Ala with Ser to introduce an alcohol group at the side chain produced no significant increase in the inhibitory effect of **32**, although introducing an amide group by replacement with Asn resulted in a remarkable lowering in the inhibitory activity of **32**. In contrast, the introduction of a more hindered secondary alcohol at the P<sub>4</sub> site, replacement with Thr instead of Ser, gave a tetrapeptide aldehyde **35** having nearly the same inhibitory activity (IC<sub>50</sub> = 98 nM) as the pentapeptide aldehyde **31** (IC<sub>50</sub> = 65 nM). Thus, the molecular weight of the inhibitor was reduced to 534 for **35** from 591 for **31**, while the inhibitory activity remained the same.

The mode of binding of the small molecular inhibitor **35** was confirmed by X-ray crystallography as above (PDB code: 3ATW). The structure of the mutant protease in a complex with **35** was refined to a resolution of 2.30 Å (Table 3). The overall fitting of the inhibitor **35** was similar to that of **16**, but



**Figure 4.** (A) X-ray structure of the inhibitor **35** bound to the R188I SARS 3CL protease (PDB code 3ATW); oxygen (red), nitrogen (blue), and sulfur (yellow); main chain of **35** is also shown in yellow. (B) Mode of the interaction. (C) Molecular graphics image produced using the UCSF Chimera package from the Resource for Biocomputing Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).

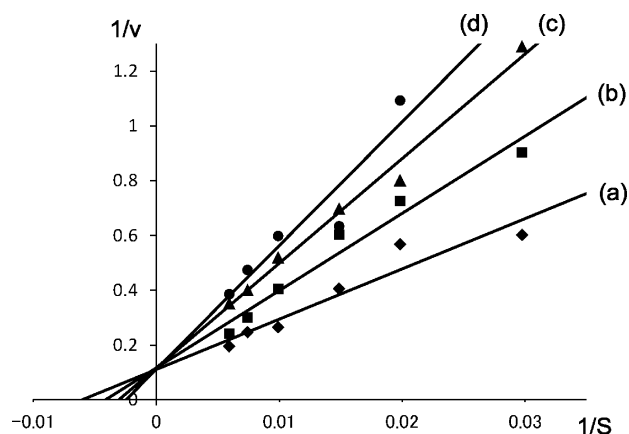
tight interactions were observed, especially at the P<sub>2</sub> and P<sub>4</sub> sites (Figure 4A). The carbonyl carbon of the aldehyde group in **35** was detected at a distance of 2.48 Å from the active center thiol of Cys-145, and its electron density could be fitted to an sp<sup>2</sup> carbonyl carbon as in **16**. The nitrogen atom of the P<sub>1</sub> site imidazole of **35** formed a hydrogen bond with the imidazole nitrogen of His-163, and the inhibitor imidazole was slightly twisted as compared to **16** (Figure 3), resulting in close fitting at the other side of the S<sub>1</sub> pocket formed from the Phe-140, Leu-141, and Glu-166 side chains of the protease. The cyclohexyl group at the P<sub>2</sub> site of **35** was inserted into a large S<sub>2</sub> pocket created by His-41, Met-49, Met-165, and Asp-187. Most of the S<sub>2</sub> pocket was occupied by a cyclohexyl group, as shown in Figure 4C. The carbonyl oxygen and amide nitrogen atom of the P<sub>3</sub> site Val of **35** formed hydrogen bonds with Glu-166 of the protease, as observed in **16**. The side-chain oxygen of Thr at the P<sub>4</sub> site formed an additional hydrogen bond with



Thr-190 of the protease, which was not observed in the interaction with **16**. Those interactions, especially at the P<sub>1</sub>, P<sub>2</sub>, and P<sub>4</sub> sites, function to hold the main chain of the truncated inhibitor tightly into the active site cleft, which resulted in the compact fitting of the tetra-peptide inhibitor **35** to the mutant protease.

In these X-ray structural analyses of inhibitors bound to the mutant protease, the electron density of the aldehyde group in the inhibitors could be fitted to an expected sp<sup>2</sup> carbon. In the inhibitory assays, no significant difference was observed between the IC<sub>50</sub> value obtained after preincubation of the inhibitor with the protease and that obtained by simultaneous mixing (Figure S-1 in the Supporting Information). These results strongly suggest that the aldehyde inhibitor functions as a competitive inhibitor and that no stable covalent bonds are formed with the protease. To estimate the inhibitory mechanism, an inhibitory kinetics experiment with **35** was performed by collecting a Lineweaver–Burk plot.

The rate of cleavage for different amounts of substrate [S] by the R188I mutant protease in the absence or presence of **35** (25, 50, or 100 nM) was monitored during the initial 10–15 min reaction period using HPLC. The enzymatic reaction rate ( $v$ ,  $\mu\text{M}/\text{min}$ ) was obtained by monitoring the decrease in the area corresponding to the substrate, and the resulting  $1/v$  was plotted against  $1/S$ . The plots resulted in four straight lines with the same  $y$ -axis intercept reflecting competitive inhibition toward the protease [Figure 5, each plot obtained at each



**Figure 5.** (a) Incubated without inhibitor; (b) incubated with 25 nM of **35**; (c) incubated with 50 nM of **35**; (d) incubated with 100 nM of **35**.

inhibitor concentration (0, 25, 50, or 100 nM) was shown in Figures S-2–S-5 in the Supporting Information].

## CONCLUSION

SARS 3CL protease inhibitors containing an aldehyde at the C terminus were found to be more effective than an irreversible inhibitor containing a Michael acceptor at the same site. The initial lead sequence, Ac-Ser-Ala-Val-Leu-His-H **16** (IC<sub>50</sub> = 5.7  $\mu\text{M}$ ), was selected by the screening of P<sub>1</sub> site residues of a previously reported peptide aldehyde inhibitor, **1**. Systematic modification guided by the X-ray crystal structure of the lead compound-bound R188I SARS 3CL protease resulted in the production of a small molecular weight inhibitor **35** with an IC<sub>50</sub> value of 98 nM. All of the side-chain structures of **35** differed from the substrate sequence except at the P<sub>3</sub> site, where the side-chain was directed outward and no evidence was found for interactions with the protease. Kinetic inhibition data for **35**

obtained from Lineweaver–Burk plots suggested that inhibitors containing an aldehyde at the C terminus can be expected to function as a competitive inhibitor. The interactions of the inhibitor **35** at the P<sub>1</sub> and P<sub>2</sub> sites with the protease seemed remarkably effective, and further modification of **35** into a nonpeptide inhibitor focusing on P<sub>1</sub> and P<sub>2</sub> site interactions is currently underway in our laboratory.

## EXPERIMENTAL SECTION

**General.** All solvents were of reagent grade. THF was distilled from sodium and benzophenone ketyl. CH<sub>2</sub>Cl<sub>2</sub> was distilled from CaH<sub>2</sub>. All commercial reagents were of the highest purity available. Analytical TLC was performed on silica gel (60 F-254, 0.25 mm plates). Column chromatography was carried out on Wakogel 60 (particle size, 63–200  $\mu\text{m}$ ) or Wakogel FC-40 (particle size, 20–40  $\mu\text{m}$ ). NMR spectra were recorded on a Bruker AM-300 or Bruker-DMX 500. Chemical shifts are expressed in ppm relative to TMS (0 ppm) or CHCl<sub>3</sub> (7.28 ppm). High-resolution mass spectra (HRMS) were obtained on a JMS-HX-110A (FAB), Bruker Autoflex-II (MALDI-TOF), or Bruker Daltonics HCTplus (ESI). The purity of the test compounds was determined by HRMS and HPLC. All test compounds showed  $\geq 95\%$  purity.

**Boc-NHCH[CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>]-OBn (**2**).** To a solution of Boc-Glu-OBn (1.0 g, 3.0 mmol) and BOP (2.0 g, 4.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) were added dimethylamine hydrochloride (0.49 g, 6.0 mmol) and DIEA (1.6 mL, 9.0 mmol), and the mixture was stirred for 15 h at room temperature. The reaction was quenched with saturated aqueous NH<sub>4</sub>Cl, and the entire solution was extracted with AcOEt. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography using CHCl<sub>3</sub>:MeOH (50:1, v/v) to give 1.1 g (98%) of **2** as a colorless solid, mp 97–99 °C.  $[\alpha]_D^{24}$  –10.4° (c 1.00, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.43 (s, 9H), 1.98–2.07 (m, 1H), 2.14–2.27 (m, 1H), 2.30–2.40 (m, 2H), 2.89 (s, 3H), 2.92 (s, 3H), 4.30 (dd,  $J$  = 12.0 Hz, 7.5 Hz, 1H), 5.13 (d,  $J$  = 12.3 Hz, 1H), 5.21 (d,  $J$  = 12.3 Hz, 1H), 5.43 (d,  $J$  = 7.2 Hz, 1H), 7.30–7.37 (m, 5H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  27.5, 28.3, 29.3, 35.5, 37.0, 53.5, 67.0, 79.8, 128.3, 128.3, 128.5, 135.5, 155.6, 171.8, 172.3. MS (ESI)  $m/z$ : 365.2  $[M + H]^+$ .

**(4S)-4-[N-(tert-Butoxycarbonyl)amino]-5-hydroxypentanoic Acid (N,N-Dimethyl)amide, Boc-Gln(Me)<sub>2</sub>-ol (**3**).** To a suspension of LiAlH<sub>4</sub> (0.11 g, 2.8 mmol) in THF (2.0 mL) was added benzyl ester **2** (0.50 g, 1.4 mmol) in THF (2.0 mL) at 0 °C, and the mixture was stirred for an additional 15 min at the same temperature. The reaction was quenched with water, and the resulting solution was extracted with AcOEt. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography using CHCl<sub>3</sub>:MeOH (20:1, v/v) to give 0.26 g (72%) of **3** as colorless oil.  $[\alpha]_D^{25}$  –13.4° (c 2.05, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.44 (s, 9H), 1.82–1.97 (m, 2H), 2.37–2.43 (m, 2H), 2.96 (s, 3H), 3.02 (s, 3H), 3.57 (brs, 2H), 3.74 (brs, 1H), 5.24 (brs, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):  $\delta$  25.7, 28.3, 29.6, 35.6, 37.2, 52.7, 64.4, 79.2, 156.2, 173.1. MS (ESI)  $m/z$ : 261.3  $[M + H]^+$ .

**Ethyl {(2E,4S)-4-[N-(tert-Butoxycarbonyl)amino]-7-one-7-dimethylamino]-2-heptenoate (Boc-NHCH[CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>]-CH=CHCOOC<sub>2</sub>H<sub>5</sub>) (**4**).** To a stirred solution of oxaly chloride (4 mL, 47 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) were added DMSO (6.6 mL, 93 mmol) and Boc-Gln(Me)<sub>2</sub>-ol **3** (5.5 g, 21 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL), and the mixture was stirred at –40 °C for 2 h. After Et<sub>3</sub>N (30 mL, 0.21 mol) was added, the mixture was washed with H<sub>2</sub>O and brine. The organic phase was dried with MgSO<sub>4</sub>, and the solvent was evaporated. The residue was partially purified by silica gel column chromatography using CHCl<sub>3</sub>:MeOH (95:5, v/v) to afford 2.3 g (41%) of Boc-Gln(Me)<sub>2</sub>-al. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.20 (s, 9H), 1.56 (m, 1H), 1.72 (m, 1H), 2.19 (m, 2H), 2.71 (s, 3H), 2.79 (s, 3H), 4.29 (brt,  $J$  = 8.7 Hz, 1H), 5.04 (brd,  $J$  = 8.7 Hz, 1H), 9.34 (brs). The product was used without further purification. To a stirred solution of NaH (0.37 g, 15 mmol) in THF (15 mL) was added

triethyl phosphonoacetate (3.1 mL, 15 mmol), and the mixture was stirred at 4 °C for 30 min. Boc-Gln(Me)<sub>2</sub>-al (2.0 g, 7.6 mmol) was added, and the mixture was stirred at 25 °C for 90 min. Ether (100 mL) was added, and the organic layer was washed with H<sub>2</sub>O and then brine, dried, and evaporated. The residue was purified by silica gel column chromatography using CHCl<sub>3</sub> to yield 1.2 g (50%) of **4**. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.28 (t, *J* = 7.2 Hz, 3H), 1.45 (s, 9H), 1.89–2.06 (m, 2H), 2.41 (t, *J* = 7.1 Hz, 2H), 2.97 (s, 3H), 3.01 (s, 3H), 4.21 (q, *J* = 7.2 Hz, 2H), 4.31 (brs, 1H), 5.08 (brs, 1H), 5.94 (d, *J* = 15.9 Hz, 1H), 6.87 (dd, *J* = 5.3 Hz, 15.9 Hz, 1H).

**Ac-Ser-Ala-Val-Leu-NHCH[CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>]CH=CHCOOC<sub>2</sub>H<sub>5</sub> (**5**).** To a stirred solution of Boc-NHCH[CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>]CH=CHCOOC<sub>2</sub>H<sub>5</sub> **4** (17 mg, 52 μmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.4 mL) was added TFA (0.12 mL), and the mixture was stirred at 25 °C for 20 min. The solvent was removed by evaporation. The residue, after it was washed with hexane, was dissolved in DMF (1 mL). To the solution were added Ac-Ser(<sup>t</sup>Bu)-Ala-Val-Leu-OH (prepared by conventional Fmoc-based SPPS; 25 mg, 52 μmol), WSC (water-soluble carbodiimide) (16 mg, 104 μmol), 3-hydroxy-1,2,3-benzotriazine-4-one (HOObt) (10 mg, 68 μmol), and DIEA (14 μL, 78 μmol), and the mixture was stirred at 25 °C for 12 h. The solvent was removed by evaporation, and the residue was dissolved in AcOEt (10 mL). The organic phase was washed with 5% citric acid, 5% NaHCO<sub>3</sub>, and brine and dried. The solvent was removed, and the residue was dissolved in TFA (30 μL). The solution was allowed to stir at 25 °C for 1 h and was then evaporated. After it was washed with ether, the residue was dissolved in MeOH and purified by semipreparative HPLC using a Cosmosil 5C18 (10 mm × 250 mm) column to yield 7 mg (20%) of **5** showing a single peak on analytical HPLC: *R*<sub>t</sub> 12.99 min (CH<sub>3</sub>CN gradient; 20–45% in 30 min). MALDI TOF-MS. Calcd, 663.370 for C<sub>30</sub>H<sub>52</sub>N<sub>6</sub>O<sub>9</sub>Na; found, 663.653 for [M + Na]<sup>+</sup>.

**Ac-Ser-Ala-Val-Leu-NHCH[CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>]CH<sub>2</sub>CH=CHCOOC<sub>2</sub>H<sub>5</sub> (**9**).** To a stirred solution of methoxymethyltriphenylphosphonium chloride (11.5 g, 34 mmol) in THF (150 mL) was added <sup>t</sup>BuOK (3.7 g, 34 mmol). The mixture was stirred at 25 °C for 30 min, and Boc-Gln(Me)<sub>2</sub>-al (1.75 g, 6.7 mmol) in THF (30 mL) was added. The resulting solution was further stirred at 25 °C for 1 h and quenched with saturated aqueous NH<sub>4</sub>Cl. The crude product was partially purified by silica gel column chromatography using CHCl<sub>3</sub>, and the product, containing a small amount of methoxymethyltriphenylphosphonium chloride, was dissolved in THF (31 mL). To the solution was added 1 N HCl (6.2 mL), and the mixture was stirred at 25 °C for 30 min and quenched with a saturated aqueous solution of NH<sub>4</sub>Cl. The crude product was extracted with CH<sub>2</sub>Cl<sub>2</sub>, and the organic phase was washed with H<sub>2</sub>O and then brine, dried over MgSO<sub>4</sub>, and evaporated. The residue was purified by silica gel column chromatography using CHCl<sub>3</sub> to afford 0.95 g (52%) of Boc-NHCH[CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>]CH<sub>2</sub>CH=O **6**. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.42 (s, 9H), 1.86–1.93 (m, 1H), 2.40 (brt, *J* = 7.4 Hz, 2H), 2.65 (brd, *J* = 6.0 Hz, 2H), 2.95 (s, 3H), 3.00 (s, 3H), 3.97–4.09 (m, 1H), 5.08 (brd, *J* = 7.5 Hz, 1H), 9.76 (brs). The aldehyde was reacted with triethyl phosphonoacetate, and the resulting crude product **7** was treated with TFA followed by hexane washing as above to afford NH<sub>2</sub>CH[CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>]CH<sub>2</sub>CH=CHCOOC<sub>2</sub>H<sub>5</sub>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.27 (t, *J* = 7.2 Hz, 3H), 1.89–1.96 (m, 2H), 2.52–2.72 (m, 4H), 2.96 (s, 3H), 3.07 (s, 3H), 3.44 (tt, *J* = 6.3 Hz, 6.3 Hz, 1H), 4.20 (q, *J* = 7.2 Hz, 2H), 6.07 (d, *J* = 15.6 Hz, 1H), 6.93 (dt, *J* = 15.6 Hz, 7.2 Hz, 1H). The N<sup>α</sup>-deblocked product was then coupled with Ac-Ser(<sup>t</sup>Bu)-Ala-Val-Leu-OH, and the product was treated with TFA as above to yield **9** showing a single peak on analytical HPLC: *R*<sub>t</sub> 13.84 min (CH<sub>3</sub>CN gradient; 20–45% in 30 min). MALDI TOF-MS. Calcd, 677.385 for C<sub>31</sub>H<sub>54</sub>N<sub>6</sub>O<sub>9</sub>Na; found, 677.149 for [M + Na]<sup>+</sup>.

**Ac-Ser-Ala-Val-Leu-NHCH[CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>]CH<sub>2</sub>CH<sub>2</sub>CH=CHCOOC<sub>2</sub>H<sub>5</sub> (**11**).** This compound was prepared similarly through the homologation of **6** by a Wittig reaction using methoxymethyltriphenylphosphonium chloride followed by a reaction with triethyl phosphonoacetate to yield **8** and coupling with the tetrapeptide: *R*<sub>t</sub>

17.64 min (CH<sub>3</sub>CN gradient; 20–40% in 30 min). MALDI TOF-MS. Calcd, 691.401 for C<sub>32</sub>H<sub>56</sub>N<sub>6</sub>O<sub>9</sub>Na; found, 691.344 for [M + Na]<sup>+</sup>.

**Solid-Phase Synthesis of the P<sub>1</sub> Site-Substituted Peptide Aldehyde Ac-Ser-Ala-Val-Leu-P<sub>1</sub>-H.** The peptide aldehyde was synthesized using the Weinreb AM resin (Novabiochem, Merck) according to published procedures,<sup>29</sup> and the product was purified by semipreparative HPLC using Cosmosil 5C18 (10 mm × 250 mm) to afford a white powder with a single chromatographic peak. Ac-Ser-Ala-Val-Leu-Leu-H **11**: yield, 0.8%; *R*<sub>t</sub> 14.76 min (CH<sub>3</sub>CN gradient; 20–50% in 30 min). MALDI TOF-MS. Calcd, 550.322 for C<sub>25</sub>H<sub>45</sub>N<sub>5</sub>O<sub>7</sub>Na; found, 550.249 for [M + Na]<sup>+</sup>. Ac-Ser-Ala-Val-Leu-<sup>t</sup>Bu-Gly-H **12**: yield, 0.5%; *R*<sub>t</sub> 17.46 min (CH<sub>3</sub>CN gradient; 20–40% in 30 min). MALDI TOF-MS. Calcd, 550.322 for C<sub>25</sub>H<sub>45</sub>N<sub>5</sub>O<sub>7</sub>Na; found, 550.254 for [M + Na]<sup>+</sup>. Ac-Ser-Ala-Val-Leu-Phe-H **13**: yield, 1.0%; *R*<sub>t</sub> 16.42 min (CH<sub>3</sub>CN gradient; 20–50% in 30 min). MALDI TOF-MS. Calcd, 584.306 for C<sub>28</sub>H<sub>43</sub>N<sub>5</sub>O<sub>7</sub>Na; found, 584.230 for [M + Na]<sup>+</sup>. Ac-Ser-Ala-Val-Leu-Cha-H **14**: yield, 0.5%; *R*<sub>t</sub> 19.55 min (CH<sub>3</sub>CN gradient; 20–50% in 30 min). MALDI TOF-MS. Calcd, 590.353 for C<sub>28</sub>H<sub>49</sub>N<sub>5</sub>O<sub>7</sub>Na; found, 590.310 for [M + Na]<sup>+</sup>. Ac-Ser-Ala-Val-Leu-Thi-H **15**: yield, 0.5%; *R*<sub>t</sub> 12.30 min (CH<sub>3</sub>CN gradient; 20–50% in 30 min). MALDI TOF-MS. Calcd, 590.262 for C<sub>26</sub>H<sub>41</sub>N<sub>5</sub>O<sub>7</sub> S<sub>1</sub>Na; found, 590.263 for [M + Na]<sup>+</sup>. Ac-Ser-Ala-Val-Leu-His-H **16**: yield, 4.4%; *R*<sub>t</sub> 12.04 min (CH<sub>3</sub>CN gradient; 15–30% in 30 min). MALDI TOF-MS. Calcd, 574.297 for C<sub>25</sub>H<sub>41</sub>N<sub>7</sub>O<sub>7</sub>Na; found, 574.258 for [M + Na]<sup>+</sup>.

**9,10-Epoxydecan-1-ol (**17**).** To a solution of 9-decen-1-ol (2.0 g, 12 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) was added *m*CPBA (2.4 g, 14 mmol) at 25 °C. After 30 min of stirring, saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (20 mL) and saturated NaHCO<sub>3</sub>(aq) (20 mL) were added. The organic layer was washed with H<sub>2</sub>O and brine, dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography using hexane:AcOEt (2:1, v/v) to give 1.5 g (64%) of **17** as a colorless oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.33 (brs, 8H), 1.46–1.56 (m, 6H), 2.46 (dd, *J* = 5.0 Hz, 3.0 Hz, 1H), 2.74 (t, *J* = 5.0 Hz, 1H), 2.90 (m, 1H), 3.56 (t, *J* = 6.5 Hz, 2H), 3.89 (brs, 1H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 25.3, 25.4, 28.8, 29.0, 31.9, 32.1, 46.6, 51.9, 61.7. IR (film) ν<sub>max</sub> cm<sup>−1</sup>: 3408, 3046, 1465, 1056, 833. ESIHRMS [M + Na]<sup>+</sup> calcd for C<sub>10</sub>H<sub>20</sub>O<sub>2</sub>Na, 195.1361; found, 195.1308.

**Decan-1,2,10-triol (**18**).** 9,10-Epoxydecan-1-ol **17** (1.5 g, 7.5 mmol) in THF:1 M NaOH (1:1, 20 mL) was refluxed for 2 h. After it was cooled to room temperature, the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography using AcOEt:MeOH (4:1, v/v) to give 0.7 g (43%) of decan-1,2,10-triol **18** as a white solid; mp 58–64 °C. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 1.32 (brs, 10H), 1.46–1.57 (m, 4H), 3.39 (dd, *J* = 11.5 Hz, 6.5 Hz, 1H), 3.44 (dd, *J* = 11.5 Hz, 4.5 Hz, 1H), 3.52 (t, *J* = 6.5 Hz, 2H), 3.55 (m, 1H). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ 25.4, 25.6, 29.3, 29.4, 29.5, 32.3, 33.1, 61.7, 66.1, 71.9. IR (KBr) ν<sub>max</sub> cm<sup>−1</sup>: 3285, 2900, 1468, 1327, 1057, 1020, 965, 878. ESIHRMS [M + Na]<sup>+</sup> calcd for C<sub>10</sub>H<sub>22</sub>O<sub>3</sub>Na, 213.1467; found, 213.1455.

**Fmoc-His(Trt)-al.** HN(OMe)Me HCl salt (0.26 g, 2.7 mmol), BOP (1.2 g, 2.7 mmol), and DIEA (1.3 mL, 8.2 mmol) were added to Fmoc-His(Trt)-OH in DMF (10 mL), and the mixture was stirred at 25 °C for 90 min. The solvent was evaporated, and the residue was dissolved in AcOEt. The organic phase was washed with 5% citric acid, 5% NaHCO<sub>3</sub>, and brine and then dried over MgSO<sub>4</sub>. The solvent was evaporated to afford 1.6 g (98%) of the corresponding Weinreb amide as a white amorphous powder. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 3.01 (brdd, *J* = 8.7 Hz, 5.1 Hz, 2H), 3.15 (s, 3H), 3.76 (s, 3H), 4.19 (t, *J* = 7.2 Hz, 1H), 4.29 (d, *J* = 7.2 Hz, 2H), 4.96 (brdd, *J* = 8.1 Hz, 5.1 Hz, 1H), 6.14 (brd, *J* = 8.1 Hz, 1H), 6.58 (s, 1H), 7.10–7.75 (m, 24H). <sup>13</sup>C NMR: δ 31.00, 32.54, 47.48, 52.00, 61.98, 67.40, 75.52, 119.91, 120.18, 125.62, 127.37, 127.91, 128.32, 130.06, 130.15, 136.73, 138.98, 141.54, 142.29, 144.39, 156.43, 162.64. The product (0.66 g, 10 mmol), without further purification, was dissolved in THF (10 mL), and LiAlH<sub>4</sub> (50 mg) was added. The mixture was stirred at 25 °C for 10 min, filtered, and evaporated. The residue was dissolved in CHCl<sub>3</sub>, and the organic phase was washed with H<sub>2</sub>O and dried over MgSO<sub>4</sub>.



The solvent was removed by evaporation, and the residue was purified by flash chromatography using hexane:AcOEt (1:2 v/v) to yield the desired product as an oil. Yield, 0.50 g (42%);  $[\alpha]_D^{26} +18.2$  (c 0.6, CHCl<sub>3</sub>). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 3.05 (dd, *J* = 15.0 Hz, 5.4 Hz, 1H), 3.16 (dd, *J* = 15.0 Hz, 5.4 Hz, 1H), 4.22 (t, *J* = 7.5 Hz, 1H), 4.97 (d, *J* = 7.5 Hz, 2H), 4.46 (dd, *J* = 6.9 Hz, 5.4 Hz, 1H), 6.45 (brd, *J* = 6.9 Hz, 1H), 6.62 (s, 1H), 7.08–7.77 (m, 24H), 9.68 (brs). <sup>13</sup>C NMR: δ 27.24, 47.21, 59.89, 67.20, 75.37, 119.70, 119.85, 119.96, 125.24, 127.08, 127.54, 127.69, 128.02, 128.09, 128.13, 129.71, 135.88, 138.82, 141.29, 142.27, 142.47, 143.89, 156.39, 200.31.

**Fmoc-His(Trt)-al 1-Octanol-ethylene Acetal [Fmoc-His(Trt)-acetal] (19).** To a stirred solution of Fmoc-His(Trt)-al (0.59 g, 0.98 mmol) and decane-1, 2, 10-triol **18** (0.19 g, 0.98 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added BF<sub>3</sub>·Et<sub>2</sub>O (0.2 mL), and the mixture was stirred at 25 °C for 30 min. H<sub>2</sub>O (10 mL) was added, and the organic phase was washed with H<sub>2</sub>O and dried on MgSO<sub>4</sub>. The solvent was evaporated to yield 0.57 g (75%) of a white amorphous powder, and the product was used for the next reaction without further purification:  $[\alpha]_D^{27} -6.3$  (c 0.8, CHCl<sub>3</sub>). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.26–1.30 (m, 12H), 1.49–1.56 (m, 2H), 2.81–2.89 (m, 2H), 3.41–3.51 (m, 1H), 3.57–3.63 (m, 2H), 3.92–4.06 (m, 2H), 4.11–4.29 (m, 4H), 4.98–5.04 (m, 1H), 5.43–5.57 (m, 1H), 6.68 (s, 1H), 7.06–7.75 (m, 24H). <sup>13</sup>C NMR: δ 25.71, 25.84, 28.68, 29.35, 29.44, 29.52, 32.88, 33.25, 47.42, 53.42, 62.86, 67.09, 70.27, 75.38, 103.98, 120.02, 125.44, 127.19, 127.74, 128.00, 128.15, 128.87, 129.92, 137.77, 138.49, 141.38, 142.59, 144.20, 156.52, 162.49.

**Solid-Phase Synthesis of the Peptide Thioacetal [Ac-Ala-Val-Cha-His-(SEt)<sub>2</sub>] (22).** To a stirred solution of **19** (0.37 g, 0.48 mmol) in acetone (5 mL) was added Jones reagent (0.3 mL; 2.67 M solution) at 0 °C, and the mixture was stirred for 40 min at 25 °C. 2-Propanol (0.1 mL) was then added, and the mixture was filtered through a Celite pad. The solvent was removed by evaporation, and the residue was extracted with CHCl<sub>3</sub>. The organic layer was washed with H<sub>2</sub>O, dried over MgSO<sub>4</sub>, and evaporated to yield 0.30 g (80%) of the corresponding carboxylic acid **20** as an amorphous powder:  $[\alpha]_D^{27} -13.1$  (c 0.8, CHCl<sub>3</sub>). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.26–1.58 (m, 12H), 2.31–2.41 (m, 2H), 2.86–2.95 (m, 2H), 3.45–3.52 (m, 1H), 3.95 (t, *J* = 6.9 Hz, 1H), 4.06–4.38 (m, 6H), 5.06 (d, *J* = 7.2 Hz, 1H), 6.69 (s, 1H), 6.99–7.74 (m, 24H). <sup>13</sup>C NMR: δ 24.80, 28.93, 29.24, 34.32, 46.58, 47.15, 53.77, 66.85, 119.91, 125.29, 127.06, 127.66, 128.11, 128.23, 128.32, 128.47, 129.63, 141.20, 143.78, 155.60, 181.82. The product was used without further purification.

Piperidine (20%) in DMF (3 mL) was added to a DMF-swelled Rink amide resin [4-(2,4-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin] (140 mg, 89 μmol), and the mixture was agitated at 25 °C for 20 min. The resin was washed with DMF, the above carboxylic acid **20** (0.28 g, 0.35 mmol), HOBt (54 mg, 0.35 mmol), DIEA (170 μL, 1.1 mmol), and DIPCDI (62 μL, 0.35 mmol) in DMF (2 mL) were added, and the mixture was agitated at 25 °C for 10 h. Deprotection of the *N*<sup>α</sup>-Fmoc group by treatment with 20% piperidine in DMF and coupling with *N*<sup>α</sup>-Fmoc-β-cyclohexyl-L-alanine (Fmoc-Cha-OH) (170 mg, 0.44 mmol) using DIPCDI/HOBt were carried out as above. An aliquot of the resulting resin [Fmoc-Cha-His(Trt)-acetal resin] (0.10 g, 40 μmol) was then used for coupling with Fmoc-Val-OH (68 mg, 0.20 mmol) and Fmoc-Ala-OH (62 mg, 0.20 mmol) and acetylation using Ac<sub>2</sub>O (76 μL, 0.80 mmol) and DIEA (130 μL, 0.80 mmol) to afford the Ac-Ala-Val-Cha-His(Trt)-acetal resin. To the dried resin were added anisole (87 μL, 0.80 mmol) and TFA (1.5 mL), and the mixture was agitated at 25 °C for 4 h. The mixture was filtered, and the solvent was removed by evaporation. Ether and H<sub>2</sub>O were added to the residue, and the aqueous phase was washed with ether. The solvent was removed by lyophilization to afford **21** as a powder: MALDI-TOF MS. Calcd, 690.456 for C<sub>35</sub>H<sub>60</sub>N<sub>7</sub>O<sub>7</sub>; found, 690.302 for [M + H]<sup>+</sup>.

To the crude product in AcOH (1 mL) were added ethanethiol (0.13 mL, 1.8 mmol) and BF<sub>3</sub>·Et<sub>2</sub>O (100 μL). The mixture was stirred at 25 °C for 2 h, and H<sub>2</sub>O (400 μL) was added. Then, 150 μL of the solution was applied to a semipreparative HPLC column (Cosmosil 5C18, 10 mm × 250 mm) and eluted with a gradient of CH<sub>3</sub>CN (10–60%, 60 min) in 0.1% aqueous TFA at 3 mL/min. The desired thioacetal **22** eluted at 43.60 min. The rest of the solution was similarly

purified and lyophilized to yield 20 mg (82%) of **22** as a white powder. <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 1.11 (d, *J* = 6.6 Hz, 3H), 1.15 (d, *J* = 6.6 Hz, 3H), 1.20–1.23 (m, 2H), 1.47–1.66 (m, 4H), 1.55 (t, *J* = 7.5 Hz, 3H), 1.56 (t, *J* = 7.5 Hz, 3H), 1.61 (d, *J* = 7.2 Hz, 3H), 1.80–1.98 (m, 7H), 2.28 (s, 3H), 2.30–2.33 (m, 1H), 3.01 (q, *J* = 7.5 Hz, 2H), 3.03 (q, *J* = 7.5 Hz, 2H), 3.32 (dd, *J* = 15.3 Hz, 10.8 Hz, 1H), 3.58 (dd, *J* = 15.3 Hz, 3.6 Hz, 1H), 4.32 (d, *J* = 4.8 Hz, 1H), 4.37 (d, *J* = 7.5 Hz, 1H), 4.57–4.64 (m, 2H), 4.71–4.75 (m, 1H), 7.55 (s, 1H), 8.84 (s, 1H). <sup>13</sup>C NMR: δ 14.18, 14.34, 16.99, 17.84, 18.79, 22.10, 25.98, 26.06, 26.15, 26.25, 26.44, 27.04, 30.69, 32.46, 33.36, 33.87, 39.33, 49.99, 51.67, 52.43, 54.88, 59.26, 117.30, 130.23, 133.66, 172.63, 173.72, 173.75, 174.88. MALDI-TOF MS. Calcd, 633.324 for C<sub>29</sub>H<sub>50</sub>N<sub>6</sub>O<sub>4</sub>S<sub>2</sub>Na; found, 633.299 for [M + Na]<sup>+</sup>. Peptide thioacetals **23–27** were similarly prepared as above.

**Ac-Asn-Val-Cha-His-(SEt)<sub>2</sub> (23).** Yield, 74%. <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 0.99 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 6.9 Hz, 3H), 1.00–1.12 (m, 2H), 1.33–1.56 (m, 4H), 1.43 (t, *J* = 7.5 Hz, 3H), 1.44 (t, *J* = 7.5 Hz, 3H), 1.67–1.92 (m, 7H), 2.18 (s, 3H), 2.18–2.22 (m, 1H), 2.78–2.96 (m, 4H), 3.31 (dd, *J* = 15.6 Hz, 10.8 Hz, 1H), 3.46 (dd, *J* = 15.3 Hz, 3.3 Hz, 1H), 4.21 (d, *J* = 5.1 Hz, 1H), 4.28 (d, *J* = 6.9 Hz, 1H), 4.45–4.50 (m, 1H), 4.60–4.65 (m, 1H), 4.86 (brt, *J* = 6.9 Hz, 1H), 7.43 (s, 1H), 8.74 (s, 1H). <sup>13</sup>C NMR: δ 14.05, 14.23, 17.47, 18.65, 22.05, 25.85, 25.95, 26.02, 26.14, 26.32, 26.88, 30.60, 32.34, 33.18, 33.69, 36.44, 39.00, 50.67, 51.65, 52.25, 54.65, 59.17, 114.88, 130.00, 133.51, 172.53, 172.54, 173.80, 173.81, 174.36. MALDI-TOF MS. Calcd, 654.347 for C<sub>30</sub>H<sub>52</sub>N<sub>7</sub>O<sub>5</sub>S<sub>2</sub>; found, 654.442 for [M + H]<sup>+</sup>.

**Ac-Ser-Val-Cha-His-(SEt)<sub>2</sub> (24).** Yield, 21%. <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 0.84 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.90–1.00 (m, 2H), 1.15–1.36 (m, 4H), 1.27 (t, *J* = 7.5 Hz, 3H), 1.29 (t, *J* = 7.5 Hz, 3H), 1.54–1.69 (m, 7H), 2.03–2.10 (m, 1H), 2.06 (s, 3H), 2.746 (q, *J* = 7.5 Hz, 2H), 2.753 (q, *J* = 7.5 Hz, 2H), 3.07 (dd, *J* = 15.6 Hz, 11.1 Hz, 1H), 3.31 (dd, *J* = 15.6 Hz, 3.0 Hz, 1H), 3.81 (d, *J* = 6.3 Hz, 1H), 4.08 (d, *J* = 4.8 Hz, 1H), 4.13 (d, *J* = 7.2 Hz, 1H), 4.34 (dd, *J* = 9.0 Hz, 6.3 Hz, 1H), 4.43–4.53 (m, 2H), 7.26 (s, 1H), 8.55 (s, 1H). <sup>13</sup>C NMR: δ 13.82, 14.01, 17.49, 18.44, 21.84, 25.72, 25.80, 25.86, 26.06, 26.20, 26.86, 30.17, 32.05, 32.94, 33.51, 38.90, 51.52, 52.23, 54.34, 55.62, 59.23, 61.20, 116.93, 130.04, 133.43, 171.97, 172.61, 174.05, 174.35. MALDI-TOF MS. Calcd, 627.336 for C<sub>29</sub>H<sub>51</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>; found, 627.332 for [M + H]<sup>+</sup>.

**Ac-Ser(Ac)-Val-Cha-His-(SEt)<sub>2</sub> (28).** <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 0.82 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H), 0.91–0.96 (m, 2H), 1.18–1.35 (m, 4H), 1.27 (t, *J* = 7.5 Hz, 3H), 1.29 (t, *J* = 7.5 Hz, 3H), 1.55–1.69 (m, 7H), 2.04–2.10 (m, 1H), 2.07 (s, 3H), 2.10 (s, 3H), 2.746 (q, *J* = 7.5 Hz, 2H), 2.755 (q, *J* = 7.5 Hz, 2H), 3.09 (dd, *J* = 15.3 Hz, 10.8 Hz, 1H), 3.32 (dd, *J* = 15.3 Hz, 3.3 Hz, 1H), 4.09 (d, *J* = 4.5 Hz, 1H), 4.11 (d, *J* = 7.5 Hz, 1H), 4.27–4.42 (m, 3H), 4.50–4.54 (m, 1H), 7.28 (s, 1H), 8.60 (s, 1H). <sup>13</sup>C NMR: δ 13.82, 14.02, 17.73, 18.46, 20.28, 21.81, 25.74, 25.80, 25.85, 26.06, 26.20, 26.80, 30.36, 32.10, 32.95, 33.53, 38.99, 51.51, 52.18, 52.73, 54.37, 59.28, 63.44, 116.94, 129.89, 130.05, 133.33, 170.68, 172.31, 173.47, 173.99, 174.27. MALDI-TOF MS. Calcd, 669.347 for C<sub>31</sub>H<sub>53</sub>N<sub>6</sub>O<sub>6</sub>S<sub>2</sub>; found, 669.348 for [M + H]<sup>+</sup>.

**Ac-Thr-Val-Cha-His-(SEt)<sub>2</sub> (25).** Yield, 31%. <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 0.75 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.84–0.95 (m, 2H), 1.11–1.30 (m, 4H), 1.13 (d, *J* = 6.6 Hz, 3H), 1.22 (t, *J* = 7.5 Hz, 3H), 1.23 (t, *J* = 7.5 Hz, 3H), 1.48–1.60 (m, 7H), 1.98–2.02 (m, 1H), 2.02 (s, 3H), 2.69 (q, *J* = 7.5 Hz, 2H), 2.70 (q, *J* = 7.5 Hz, 2H), 3.03 (dd, *J* = 15.3 Hz, 11.1 Hz, 1H), 3.26 (dd, *J* = 15.3 Hz, 3.0 Hz, 1H), 4.01–4.08 (m, 3H), 4.22 (d, *J* = 5.7 Hz, 1H), 4.28–4.32 (m, 1H), 4.43–4.50 (m, 1H), 7.22 (s, 1H), 8.54 (s, 1H). <sup>13</sup>C NMR: δ 13.76, 13.94, 17.73, 18.37, 18.88, 21.76, 25.67, 25.76, 25.80, 26.00, 26.16, 26.74, 38.91, 51.35, 52.12, 54.27, 59.23, 59.49, 67.04, 119.88, 129.79, 133.23, 171.74, 172.45, 174.02, 174.38. MALDI-TOF MS. Calcd, 641.352 for C<sub>30</sub>H<sub>53</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>; found, 641.283 for [M + H]<sup>+</sup>.

**Ac-Ser-Ala-Val-Phe-His-(SEt)<sub>2</sub> (26).** Yield, 18%. <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 0.65 (d, *J* = 6.6 Hz, 3H), 0.76 (d, *J* = 6.6 Hz, 3H), 1.15 (t, *J* = 7.5 Hz, 3H), 1.20 (t, *J* = 7.5 Hz, 3H), 1.26 (d, *J* = 7.2 Hz, 3H), 1.77–1.86 (m, 1H), 2.00 (s, 3H), 2.55 (q, *J* = 7.5 Hz, 2H), 2.64 (q, *J* = 7.5 Hz, 2H), 2.85–2.95 (m, 2H), 3.01 (dd, *J* = 15.3 Hz, 7.5 Hz, 1H), 3.21 (dd, *J* = 15.3 Hz, 3.0 Hz, 1H), 3.67 (d, *J* = 4.2 Hz, 1H),

3.770 (d,  $J = 5.7$  Hz, 1H), 3.775 (d,  $J = 5.7$  Hz, 1H), 3.91 (d,  $J = 7.8$  Hz, 1H), 4.23–4.43 (m, 3H), 4.53 (brt,  $J = 8.0$  Hz, 1H), 7.12–7.33 (m, 6H), 8.45 (s, 1H).  $^{13}\text{C}$  NMR:  $\delta$  13.68, 13.94, 16.45, 17.71, 18.26, 21.78, 26.10, 26.32, 30.19, 37.41, 49.65, 52.33, 54.30, 54.62, 55.67, 59.32, 61.14, 116.86, 127.30, 128.84, 129.23, 130.02, 133.40, 136.15, 171.91, 172.36, 172.56, 174.53, 174.58. MALDI-TOF MS. Calcd, 692.327 for  $\text{C}_{32}\text{H}_{50}\text{N}_7\text{O}_6\text{S}_2$ ; found, 692.475 for  $[\text{M} + \text{H}]^+$ .

**Ac-Ser-Ala-Val-Cha-His-(SEt)<sub>2</sub> (27).** Yield, 17%.  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.75 (d,  $J = 6.6$  Hz, 3H), 0.84 (d,  $J = 6.6$  Hz, 3H), 0.85–0.94 (m, 2H), 1.08–1.33 (m, 4H), 1.20 (t,  $J = 7.5$  Hz, 3H), 1.22 (t,  $J = 7.5$  Hz, 3H), 1.32 (d,  $J = 7.2$  Hz, 3H), 1.42–1.58 (m, 7H), 1.88–2.01 (m, 1H), 2.01 (s, 3H), 2.68 (q,  $J = 7.5$  Hz, 2H), 2.69 (q,  $J = 7.5$  Hz, 2H), 3.03 (dd,  $J = 15.3$  Hz, 10.8 Hz, 1H), 3.26 (dd,  $J = 15.3$  Hz, 3.3 Hz, 1H), 3.74–3.84 (m, 2H), 3.96 (d,  $J = 8.1$  Hz, 1H), 4.04 (d,  $J = 4.5$  Hz, 1H), 4.26–4.36 (m, 3H), 4.43–4.49 (m, 1H), 7.20 (s, 1H), 8.50 (s, 1H).  $^{13}\text{C}$  NMR:  $\delta$  13.69, 13.88, 16.45, 17.73, 18.36, 21.75, 25.64, 25.73, 25.85, 25.97, 26.20, 26.79, 29.97, 31.88, 32.87, 33.41, 38.84, 49.75, 51.44, 52.22, 54.25, 55.72, 59.41, 61.11, 116.85, 129.87, 133.27, 171.98, 172.72, 174.22, 174.58, 174.66. MALDI-TOF MS. Calcd, 698.374 for  $\text{C}_{32}\text{H}_{56}\text{N}_7\text{O}_6\text{S}_2$ ; found, 698.389 for  $[\text{M} + \text{H}]^+$ .

**Ac-Ser(Ac)-Ala-Val-Cha-His-(SEt)<sub>2</sub> (29).**  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.91 (d,  $J = 6.6$  Hz, 3H), 0.97 (d,  $J = 6.6$  Hz, 3H), 0.98–1.09 (m, 2H), 1.24–1.51 (m, 4H), 1.36 (t,  $J = 7.5$  Hz, 3H), 1.37 (t,  $J = 7.5$  Hz, 3H), 1.45 (d,  $J = 7.2$  Hz, 3H), 1.59–1.75 (m, 7H), 2.11–2.16 (m, 1H), 2.15 (s, 3H), 2.19 (s, 3H), 2.83 (q,  $J = 7.5$  Hz, 2H), 2.84 (q,  $J = 7.5$  Hz, 2H), 3.15 (dd,  $J = 15.6$  Hz, 10.8 Hz, 1H), 3.40 (dd,  $J = 15.3$  Hz, 3.3 Hz, 1H), 4.13–4.18 (m, 2H), 4.40–4.51 (m, 4H), 4.54–4.61 (m, 1H), 7.36 (s, 1H), 8.66 (s, 1H).  $^{13}\text{C}$  NMR:  $\delta$  13.95, 14.13, 16.72, 17.78, 18.58, 20.34, 21.98, 25.84, 25.93, 26.14, 26.82, 30.37, 32.15, 33.12, 33.59, 39.08, 49.86, 51.53, 52.23, 53.04, 54.49, 59.26, 63.52, 117.02, 129.95, 132.42, 170.72, 172.58, 173.47, 173.89, 174.13, 174.29. MALDI-TOF MS. Calcd, 740.384 for  $\text{C}_{34}\text{H}_{58}\text{N}_7\text{O}_7\text{S}_2$ ; found, 740.190 for  $[\text{M} + \text{H}]^+$ .

**Ac-Ala-Val-Cha-His-H (32).** Ac-Ala-Val-Cha-His-(SEt)<sub>2</sub> **22** (9.0 mg, 15  $\mu\text{mol}$ ) was dissolved in  $\text{H}_2\text{O}$ :THF (2:1, 1.35 mL). A 150  $\mu\text{L}$  amount of the solution was mixed with 53  $\mu\text{L}$  of a 0.1 mmol/L THF solution of NBS, and the mixture was applied to a semipreparative HPLC column (Cosmosil SC18, 10 mm  $\times$  250 mm). The desired product was eluted with a gradient of  $\text{CH}_3\text{CN}$  (10–60%, 60 min) in 0.1% aqueous TFA at 3 mL/min, appearing at 23.26 min. The rest of the solution was similarly purified and lyophilized to yield 3.2 mg (43%) of **32** as a white powder:  $R_f$  on analytical HPLC, 12.87 min (single peak).  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.78 (d,  $J = 6.9$  Hz, 3H), 0.84–0.94 (m, 2H), 0.86 (d,  $J = 6.9$  Hz, 3H), 1.05–1.25 (m, 5H), 1.30 (d,  $J = 7.2$  Hz, 3H), 1.39–1.58 (m, 6H), 1.91–2.02 (m, 1H), 1.97 (s, 3H), 2.84 (dd,  $J = 15.3$  Hz, 10.8 Hz, 1H), 3.11 (dd,  $J = 15.3$  Hz, 3.3 Hz, 1H), 4.00 (d,  $J = 8.1$  Hz, 1H), 4.06–4.11 (m, 1H), 4.20–4.31 (m, 2H), 7.21 (s, 1H), 8.53 (s, 1H), 9.51 (brs). MALDI-TOF MS. Calcd, 505.314 for  $\text{C}_{25}\text{H}_{41}\text{N}_6\text{O}_5$ ; found, 505.353 for  $[\text{M} + \text{H}]^+$ . The following peptidealdehydes were similarly prepared as above.

**Ac-Asn-Val-Cha-His-H (33).** Yield, 51%;  $R_f$  on analytical HPLC, 12.35 min (single peak).  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.89–0.96 (m, 8H), 1.10–1.26 (m, 4H), 1.44–1.64 (m, 7H), 1.98 (s, 3H), 2.16–2.20 (m, 1H), 2.71–2.81 (m, 3H), 2.93–2.98 (m, 1H), 3.98–4.02 (m, 2H), 4.15–4.20 (m, 1H), 4.61–4.64 (m, 1H), 7.18 (s, 1H), 8.46 (s, 1H), 9.44 (brs). MALDI-TOF MS. Calcd, 548.320 for  $\text{C}_{26}\text{H}_{42}\text{N}_7\text{O}_6$ ; found, 548.375 for  $[\text{M} + \text{H}]^+$ .

**Ac-Ser-Val-Cha-His-H (34).** Yield, 60%;  $R_f$  on analytical HPLC, 13.11 min (single peak).  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.69–1.10 (m, 6H), 0.72 (d,  $J = 6.9$  Hz, 3H), 0.77 (d,  $J = 6.9$  Hz, 3H), 1.28–1.49 (m, 7H), 1.87–1.94 (m, 1H), 1.94 (s, 3H), 2.76 (dd,  $J = 15.3$  Hz, 10.8 Hz, 1H), 3.03 (dd,  $J = 15.3$  Hz, 3.3 Hz, 1H), 3.66–3.73 (m, 2H), 3.96–4.04 (m, 2H), 4.12–4.21 (m, 1H), 4.30–4.34 (m, 1H), 7.12 (brs, 1H), 8.46 (brs, 1H), 9.42 (brs). MALDI-TOF MS. Calcd, 521.309 for  $\text{C}_{25}\text{H}_{41}\text{N}_6\text{O}_6$ ; found, 521.329 for  $[\text{M} + \text{H}]^+$ .

**Ac-Thr-Val-Cha-His-H (35).** Yield, 40%;  $R_f$  on analytical HPLC, 12.27 min (single peak).  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.89–0.93 (m, 8H), 1.11–1.24 (m, 7H), 1.41–1.63 (m, 7H), 2.03 (s, 3H), 2.11–2.18 (m, 1H), 3.01–3.08 (m, 1H), 3.24–3.31 (m, 1H), 4.02–4.05 (m, 1H), 4.13–4.25 (m, 3H), 4.37–4.41 (m, 1H), 7.21 (brs, 1H), 8.44 (brs,

1H), 9.44 (brs). MALDI-TOF MS. Calcd, 535.324 for  $\text{C}_{26}\text{H}_{43}\text{N}_6\text{O}_6$ ; found, 535.383 for  $[\text{M} + \text{H}]^+$ .

**Ac-Ser-Ala-Val-Phe-His-H (30).** Yield, 12%;  $R_f$  on analytical HPLC, 10.27 min (single peak).  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.66 (d,  $J = 6.6$  Hz, 3H), 0.76 (d,  $J = 6.6$  Hz, 3H), 1.26 (d,  $J = 7.2$  Hz, 3H), 1.79–1.89 (m, 1H), 2.00 (s, 3H), 2.76 (dd,  $J = 15.6$  Hz, 11.1 Hz, 1H), 2.85–2.99 (m, 2H), 3.04 (dd,  $J = 15.6$  Hz, 3.0 Hz, 1H), 3.724–3.82 (m, 2H), 3.91 (d,  $J = 8.1$  Hz, 1H), 3.99–4.04 (m, 1H), 4.27 (q,  $J = 7.2$  Hz, 1H), 4.34 (t,  $J = 5.7$  Hz, 1H), 4.51 (brt,  $J = 7.2$  Hz, 1H), 7.15–7.31 (m, 6H), 8.48 (brs, 1H). MALDI-TOF MS. Calcd, 586.299 for  $\text{C}_{28}\text{H}_{40}\text{N}_7\text{O}_7$ ; found, 586.380 for  $[\text{M} + \text{H}]^+$ .

**Ac-Ser-Ala-Val-Cha-His-H (31).** Yield, 46%;  $R_f$  on analytical HPLC, 12.66 min (single peak).  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.78 (d,  $J = 6.6$  Hz, 3H), 0.79–0.93 (m, 2H), 0.85 (d,  $J = 6.6$  Hz, 3H), 1.00–1.24 (m, 5H), 1.33 (d,  $J = 7.2$  Hz, 3H), 1.42–1.59 (m, 6H), 1.89–2.01 (m, 1H), 2.01 (s, 3H), 2.79 (dd,  $J = 15.6$  Hz, 10.8 Hz, 1H), 3.06 (dd,  $J = 15.6$  Hz, 3.6 Hz, 1H), 3.74–3.81 (m, 2H), 3.96 (d,  $J = 8.1$  Hz, 1H), 4.03–4.09 (m, 1H), 4.25–4.37 (m, 3H), 7.10 (s, 1H), 8.28 (s, 1H), 9.47 (brs). MALDI-TOF MS. Calcd, 592.346 for  $\text{C}_{28}\text{H}_{46}\text{N}_7\text{O}_7$ ; found, 592.435 for  $[\text{M} + \text{H}]^+$ .

**X-ray Crystallography.** Prior to the crystallization of the inhibitor complex, the purified R188I mutant SARS 3CL protease in 20 mM Bis-Tris, pH 5.5/100 mM NaCl/5 mM DTT (8 mg/mL) was crystallized at 4 °C using the sitting drop vapor diffusion method by mixing it with an equal volume of a precipitant solution (9–11% (w/v) of PEG20000, 100 mM MES, pH 6.0, and 5 mM DTT). Diffraction-quality crystals were formed within 3 days and reached a typical size of 0.3 mm  $\times$  0.3 mm  $\times$  0.2 mm.

For crystallization of the inhibitor complex, the R188I SARS 3CL protease solution (8 mg/mL) was mixed with the inhibitor dissolved in DMSO at a molar ratio of 1:4 and then incubated for 1 h at 4 °C before being combined with an equal volume of the precipitant solution described above. The crystal form of the inhibitor complex was then transferred to a cryoprotectant solution of 11% PEG20000, 100 mM MES, pH 6.0, 5 mM DTT, and 15% (v/v) ethylene glycol and flash-frozen in a liquid nitrogen stream prior to the collection of X-ray diffraction data.

X-ray data were collected from frozen crystals at 95 K at the Photon Factory (Tsukuba, Japan). Diffraction data were collected from a crystal of the R188I SARS 3CL protease on a beamline BL-5A with an ADSC Quantum 315r CCD detector at a wavelength of 1.0000 Å. A data set from a protease crystal in a complex with the inhibitor was collected on a BL-6A on an ADSC Quantum 4r CCD detector at a wavelength of 0.9780 Å. The diffraction data were processed using the HKL-2000 software program.

The structures of the R188I SARS 3CL protease alone and complexed with inhibitors were determined by molecular replacement using the Molrep program with a wild-type SARS 3CL protease structure (PDB code: 2ZU4) as the search model. Rigid body refinement and subsequent restrained refinement protocols were performed with the program Refmac 5 of the CCP package. The Coot program was used for manual model rebuilding. Water molecules were added using Coot only after the refinement of protein structures had converged. Ligands were directly built into the corresponding difference electron density, and the model was then subjected to an additional round of refinement.

**Estimation of IC<sub>50</sub> Values.** Peptide substrate SO1 [H-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-NH<sub>2</sub>]<sup>27</sup> (111  $\mu\text{M}$ ) in a reaction solution (25  $\mu\text{L}$  of 20 mM Tris-HCl buffer, pH 7.5, containing 7 mM DTT) was incubated with the R188I SARS protease<sup>27</sup> (56 nM) at 37 °C for 60 min in the presence of various inhibitor concentrations. In a preincubation procedure, the mutant protease was incubated with the inhibitor at 37 °C for 20–30 min before addition of the substrate. The substrate was then added to the mixture, and the cleavage reaction was continued for a further 60 min. In a simultaneous mixing procedure, the protease, inhibitor, and substrate were simultaneously mixed, and the mixture was incubated at 37 °C for 60 min. The cleavage reaction was monitored by analytical HPLC [Cosmosil SC18 column (4.6 mm  $\times$  150 mm), a linear gradient of  $\text{CH}_3\text{CN}$  (10–20%) in an aqueous 0.1% TFA over 30 min], and the cleavage rates were calculated from

the decrease of the substrate peak area. Each  $IC_{50}$  value was obtained from the sigmoidal dose–response curve (see Figure S-1 in the Supporting Information for a typical sigmoidal curve). Each experiment was repeated three times, and the results were averaged.

**Lineweaver–Burk Plot.** Initial rate measurements for the hydrolysis were carried out using basically the same procedure as above. Each reaction was initiated by adding the protease (56 nM) to various solutions containing different final concentrations of the substrate (34–168  $\mu$ M) in the absence or presence of **35** (25, 50, or 100 nM). The digestion time (10–15 min) varied depending on the amount of substrate used. After the reaction, each mixture was analyzed by HPLC, as described above. The initial digestion rate ( $v$ ,  $\mu$ M/min) was calculated from the decrease in the peak area of the substrate, and  $1/v$  was plotted vs  $1/[S]$ , where  $[S]$  is the concentration of the substrate ( $\mu$ M).

## ■ ASSOCIATED CONTENT

### ● Supporting Information

HPLC data for inhibitors, typical sigmoidal curve used to obtain  $IC_{50}$  values, and Lineweaver–Burk plots obtained at different concentrations (0, 25, 50, and 100 nM) of **35**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

### Accession Codes

PDB codes: 3AW1, 3AW0, 3AVZ, and 3ATW.

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## ■ ABBREVIATIONS USED

SARS, severe acute respiratory syndrome; CoV, coronavirus; 3CL, chymotrypsin-like protease; DIPCDI, diisopropylcarbodiimide; HOBt, 1-hydroxybenzotriazole; BOP, benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; HOOBt, 3-hydroxy-1,2,3-benzotriazine-4-one; WSC, water-soluble carbodiimide

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# Fused-ring structure of decahydroisoquinolin as a novel scaffold for SARS 3CL protease inhibitors

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## ABSTRACT

The design and evaluation of a novel decahydroisoquinolin scaffold as an inhibitor for severe acute respiratory syndrome (SARS) chymotrypsin-like protease (3CL<sup>pro</sup>) are described. Focusing on hydrophobic interactions at the S<sub>2</sub> site, the decahydroisoquinolin scaffold was designed by connecting the P<sub>2</sub> site cyclohexyl group of the substrate-based inhibitor to the main-chain at the α-nitrogen atom of the P<sub>2</sub> position via a methylene linker. Starting from a cyclohexene enantiomer obtained by salt resolution, *trans*-decahydroisoquinolin derivatives were synthesized. All decahydroisoquinolin inhibitors synthesized showed moderate but clear inhibitory activities for SARS 3CL<sup>pro</sup>, which confirmed the fused ring structure of the decahydroisoquinolin functions as a novel scaffold for SARS 3CL<sup>pro</sup> inhibitor. X-ray crystallographic analyses of the SARS 3CL<sup>pro</sup> in a complex with the decahydroisoquinolin inhibitor revealed the expected interactions at the S<sub>1</sub> and S<sub>2</sub> sites, as well as additional interactions at the *N*-substituent of the inhibitor.

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## 1. Introduction

Although the primary epidemic of SARS (Severe Acute Respiratory Syndrome)<sup>1–3</sup> affecting about 8500 patients and 800 dead was eventually brought under control, the recent identification of a SARS CoV (coronavirus)-like virus in Chinese bats<sup>4,5</sup> and of a novel coronavirus MERS-CoV (Middle East Respiratory Syndrome Corona Virus, previously known as human CoV-EMC) raise the possibility of a reemergence of SARS or related diseases.<sup>6,7</sup> Since no effective therapy exists for these viral infections, developing anti-SARS agents against future outbreaks remains a formidable challenge.

SARS is a positive-sense, single-stranded RNA virus featuring the largest known viral RNA which produces two large proteins with overlapping sequences, polyproteins 1a (~450 kDa) and 1ab (~750 kDa).<sup>8–10</sup> SARS 3CL (chymotrypsin like) protease (3CL<sup>pro</sup>) is a key enzyme to cleave the polyproteins to yield functional polypeptides.<sup>11,12</sup> The 3CL<sup>pro</sup> is a cysteine protease containing a Cys-His catalytic dyad and it exists as a homodimer; each monomer contains the catalytic dyad at each active site. Due to its functional importance in the viral life cycle, 3CL<sup>pro</sup> is considered an attractive target for the structure-based design of drugs against SARS. Thus,

numerous inhibitors of 3CL<sup>pro</sup> have been reported including peptide-mimics<sup>13–17</sup> and small molecules derived from natural products,<sup>18–20</sup> anti-viral agents,<sup>21,22</sup> anti-malaria agents,<sup>23</sup> or high throughput screening.<sup>24–27</sup>

In the course of our own studies on the SARS 3CL<sup>pro</sup> and its inhibitors,<sup>28</sup> we found that the addition of an extra sequence to the N- or C-terminus of the mature SARS 3CL<sup>pro</sup> lowered the catalytic activity and that the mature SARS 3CL<sup>pro</sup> is sensitive to degradation at the 188Arg/189Gln site, which causes a loss of catalytic activity. The stability of 3CL<sup>pro</sup> is dramatically increased by mutating the Arg at the 188 position to Ile. The enzymatic efficiency of the R188I mutant was increased by a factor of more than  $1 \times 10^6$ . The potency of the mutant protease makes it possible to quantitatively evaluate substrate-based peptide-mimetic inhibitors easily by conventional HPLC using a substrate peptide containing no fluorescence derivatives. The evaluations revealed that a peptide aldehyde covering the P-site sequence of substrate, Ac-Ser-Ala-Val-Leu-NHCH(CH<sub>2</sub>CH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>)-CHO, inhibits the SARS 3CL<sup>pro</sup> with an IC<sub>50</sub> value of 37 μM. Systematic modification guided by the X-ray crystal structure of a series of peptide-mimics in a complex with R188I SARS 3CL<sup>pro</sup> resulted in **1** with an IC<sub>50</sub> value of 98 nM (Fig. 1).<sup>13</sup> All of the side-chain structures of **1** differed from the substrate sequence except at the P<sub>3</sub> site, where the side-chain was directed outward. Kinetic inhibition data for **1**

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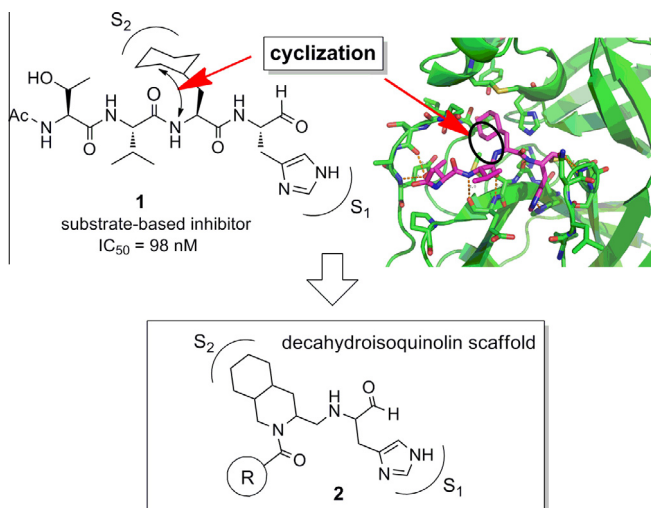


Figure 1. Design of a decahydroisoquinolin scaffold.

obtained from Lineweaver–Burk plots suggested that inhibitors containing an aldehyde at the C-terminus can be expected to function as competitive inhibitors.

In the present study, we designed a novel non-peptide inhibitor focusing on the interactions at the  $S_1$  and  $S_2$  sites of the 3CL<sup>pro</sup>. Confirmed to be critical to make the **1** potent competitive inhibitor. Among the key interactions clarified by X-ray crystallographic study, we focused on hydrophobic interactions at the cyclohexyl side-chain to design a novel inhibitor scaffold. Thus, the cyclohexyl ring is connected to the main-chain at an  $\alpha$ -nitrogen atom of the  $P_2$  position Cha (cyclohexylalanine) via a methylene linker to yield compound **2** (Fig. 1). The resulting decahydroisoquinolin scaffold of **2** is expected to keep the hydrophobic interactions at the cyclohexyl ring of the substrate-based inhibitor at the  $S_2$  pocket. In addition, the resulting decahydroisoquinolin scaffold arranges the  $P_1$  site imidazole and active site functional aldehyde at each required position, giving the fused-ring structure of decahydroisoquinolin as a scaffold for a novel inhibitor. The acyl substituent on the nitrogen in the decahydroisoquinolin scaffold may add an extra position for the interactions with the 3CL<sup>pro</sup>.

## 2. Results and discussion

### 2.1. Chemistry

The retro synthetic route for the desired decahydroisoquinolin derivative **2** is shown in Scheme 1. The  $P_1$  site His derivative could be introduced by a reductive amination reaction using an aldehyde derivative prepared by oxidative cleavage of the olefin bond of **3**. The *trans*-decahydroisoquinolin scaffold of **3** could be constructed via Pd-mediated stereoselective intra-molecular cyclization<sup>29</sup> by nucleophilic attack of a nitrogen atom to the Pd-activated olefin moiety of an allyl alcohol of **4**. The olefin structure of **4** could be constructed by a Horner–Emmons reaction utilizing an aldehyde of precursor **5**, and the amino group of **4** could be introduced by a Mitsunobu reaction to the alcohol of **5**. The six-membered ring structure of **5** could be constructed by a Diels–Alder reaction of known ester **6**<sup>30</sup> with butadiene.

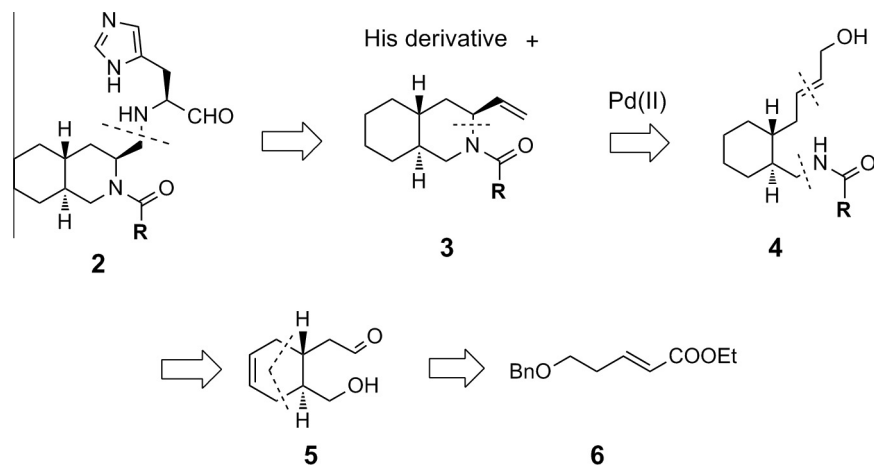
Thus, the key intermediates **12** and **13**, a precursor of the Pd-mediated cyclization, were prepared according to the route shown in Scheme 2. The known ester **6** was first reacted with butadiene to construct the six-membered ring structure to yield **7** as an enantiomer mixture of 1,6-*trans*-substituted cyclohexene. The product was reduced with LAH and the resulting alcohol was then

protected as *tert*-butyldiphenylsilyl ether to give **8**. The benzyl group was removed by catalytic hydrogenation, which reduced the cyclohexene to cyclohexane at the same time. The resulting hydroxyl group was then oxidized with PCC and the resulting aldehyde was then reacted with  $(EtO)_2P(O)CH_2COOEt$  to yield **9**. The ethyl ester of **9** was reduced with DIBALH and the resulting alcohol was protected as acetyl ester to give **10**. After treatment with TBAF, the resulting alcohol was converted to the azide derivative **11** by a Mitsunobu reaction. Since the product **11** was rather unstable, **11** was immediately reduced to the corresponding amine. Without further purification, the amine derivative was coupled with *p*-phenylbenzoic acid using HBTU to yield **12** as an enantiomer mixture. Coupling with *p*-bromobenzoic acid was similarly conducted to yield a related derivative **13**.

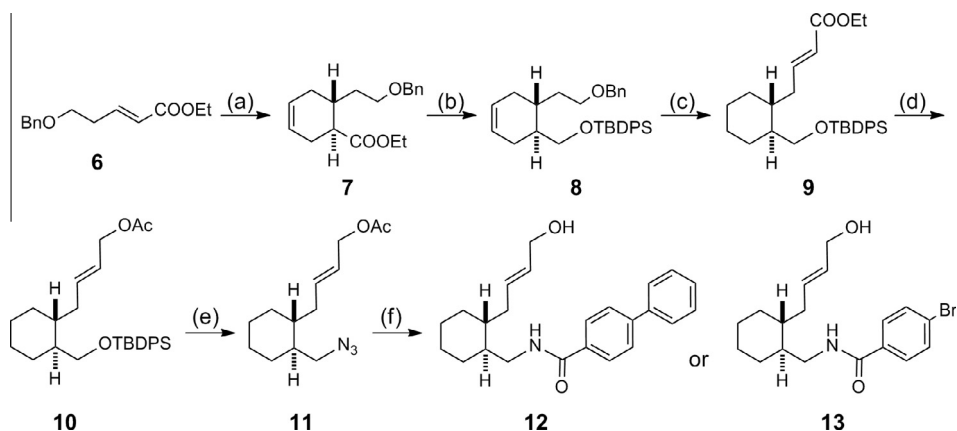
Construction of the decahydroisoquinolin scaffold was achieved as shown in Scheme 3.  $(CH_3CN)_2PdCl_2$ -mediated cyclization of **12/13** gave the desired *trans*-decahydroisoquinolin derivative **14/15** as a major product. The product was an enantiomer mixture which was thought to have the relative configuration of **14/15** due to the cyclization through a less hindered Pd-chelated intermediate. Thus, the vinyl substituent of the product **14/15** was thought to be axial, which was clearly confirmed by X-ray crystallographic studies of the inhibitor in a complex with the R188I mutant SARS 3CL<sup>pro</sup> as discussed below. The olefin bond of **14/15** was oxidatively cleaved by the treatment with  $K_2O_8(OH)_4$  followed by  $NaIO_4$  to yield aldehyde **16/17**. Reductive amination by H-His(Trt)-N( $OCH_3$ )- $CH_3$  gave the coupling products **18** and **20** or **19** and **21** as a 1:1 diastereomer mixture which was separable on a reversed-phase column (YMC Pack ODS) by analytical HPLC (Fig. S1). The diastereomers could also be separated by conventional silica-gel column chromatography to yield diastereomers **18** and **20** or **19** and **21**, each having single peak on the above reversed-phase column. Each separated diastereomer was then treated with TFA to cleave the Trt group at the imidazole ring, and the product was reduced with DIBALH to yield the desired aldehyde **22/23** or **24/25**. Although the absolute configuration of each product was not determined at this stage, the purity of each product was confirmed by analytical HPLC. Since moderate but clear inhibitory activities were observed in a preliminary evaluation on the inhibitory potency of **22** and **24**, the identification of the stereo-structure was then conducted.

To separately prepare the above diastereomers and estimate the absolute configurations, cyclohexene carboxylic acid obtained by a Diels–Alder reaction was converted to a salt with (*R*)- or (*S*)- $\alpha$ -methylbenzylamine and resolved according to the literature procedure for (1*R*/6*S*,1*S*/6*R*)-6-(2-bromophenyl)cyclohex-3-ene-1-carboxylic acid **26**<sup>31</sup> (Scheme 4). Resolution of a carboxylic acid derived from compound **7** and compound **29** having the corresponding *p*-bromobenzyl group gave compounds showing the same polarimetric characters as the literature compounds.<sup>31</sup> (–) Carboxylic acid **27** or **30** was obtained by salt formation with (*R*)- $\alpha$ -methylbenzylamine and following salt-liberation with HCl, whereas the salt with (*S*)- $\alpha$ -methylbenzylamine gave (+) carboxylic acid **28** or **31**. Compared with the literature values, these results strongly suggest that **27** and **30** would have (1*R*,6*S*) and **28** and **31** would have (1*S*,6*R*) absolute configurations. Optical purity of each enantiomer was further confirmed using a chiral column (YMC CHIRAL Amylose-C) by HPLC (Fig. S2). Since the chemical yield from the *p*-bromobenzyl derivative **29** was superior to the benzyl derivative **7**, enantiomer **30** or **31** was used as the starting compound for the separate synthesis of decahydroisoquinolin diastereomers.

The separated (1*S*,6*R*) enantiomer **31** was then used to synthesize the corresponding decahydroisoquinolin diastereomer **40** or **41** using basically the same route as above (Scheme 5i). (1*R*,6*S*) Enantiomer **30** was also employed for the syntheses of diastereo-



**Scheme 1.** Retro synthetic route for the decahydroisoquinolin derivative.



**Scheme 2.** Synthesis of intermediate **12** or **13**. Configurations in the racemic compounds **7**–**13** indicate the relative 1,6-*trans* configurations. Reagents: (a) 1,3-butadiene; (b) (1) LAH, (2) TBDPS-Cl/imidazole; (c) (1)  $\text{H}_2/\text{Pd}(\text{OH})_2\text{-C}$  (2) PCC (3)  $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{COOEt}/\text{NaH}$ ; (d) (1) DIBALH (2)  $\text{Ac}_2\text{O}/\text{pyridine}/\text{DMAP}$ ; (e) (1) TBAF, (2)  $(\text{EtO})_2\text{P}(\text{O})\text{N}_3/\text{DIAD}/\text{PPh}_3$ ; (f) (1) LAH, (2) 4-phenylbenzoic acid or 4-bromobenzoic acid/HBTU/DIPEA.

mer **44** or **45** (Scheme 5ii). The protected intermediate **38** ( $\text{R} = p$ -phenylphenyl) from **31** and the diastereomer **42** ( $\text{R} = p$ -phenylphenyl) from (1*R*,6*S*) enantiomer **30** were co-eluted with a previously synthesized diastereomixture of **18** and **20** on a reversed-phase column (YMC Pack ODS). Intermediate **38** had the same retention time as **18**, whereas intermediate **42** had the same retention time as **20** (Fig. S3). The comparison was also conducted on **39** and **43** having a *p*-bromophenyl *N*-substituent with the corresponding diastereomers **19** and **21**, and the same results as above were obtained (Fig. S4). These results clearly demonstrated that the two diastereomers **18** and **20** were derived from the *trans*-decahydroisoquinolin structure constructed from enantiomer **7**. Each protected diastereomer **38/39** and **42/43** thus synthesized was converted to the desired derivatives **40/41** and **44/45** without difficulty. Several analogs shown in Table 1 containing different *N*-acyl substituents of the decahydroisoquinolin scaffold were also prepared using the same synthetic route (Fig. S5).

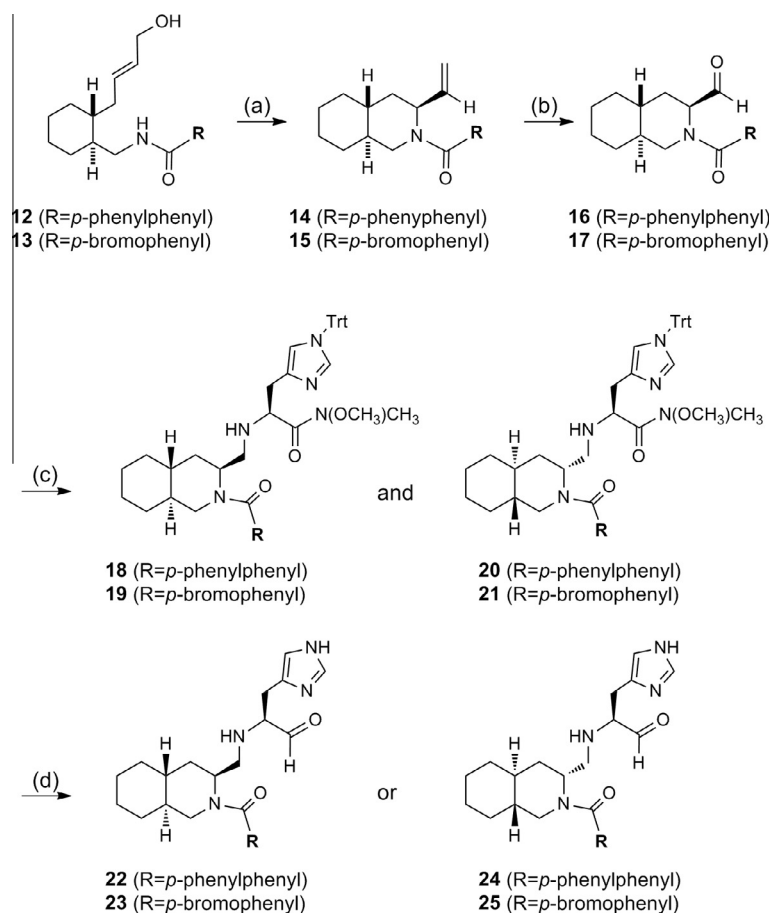
## 2.2. Inhibitory activity

Digestion of the substrate peptide with R188I SARS 3CL<sup>Pro</sup> in the presence of decahydroisoquinolin derivatives of different concentrations was conducted according to the published procedure.<sup>13</sup> The inhibitory activities were evaluated based on IC<sub>50</sub> values calculated from the decrease in the substrate digested by R188I SARS

3CL<sup>Pro</sup>; a typical sigmoidal curve used for estimation of the IC<sub>50</sub> value is shown in Figure S6. As summarized in Table 1, synthesized decahydroisoquinolin derivatives all showed inhibitory activities for the mutant 3CL<sup>Pro</sup>. The results strongly suggest that the decahydroisoquinolin fused-ring can function as an inhibitor scaffold. Comparison of IC<sub>50</sub> values of *trans*-decahydroisoquinolin diastereomers in *N*-4-phenylbenzoyl derivatives (**40** vs **44**) or *N*-4-bromobenzoyl derivative (**41** vs **45**) clearly showed that the (4*aR*,8*aS*) isomer is more potent than (4*aS*,8*aR*) isomer. The results suggest the importance of the interaction at the S<sub>2</sub> pocket of the mutant 3CL<sup>Pro</sup>. It was also demonstrated that a series of the *N*-benzoyl derivative was more potent than *N*-4-phenylbenzoyl derivatives. Substitution at the 4-position of the benzoyl substituent in **48** with halogen showed no significant effect on the inhibitory activity (**41** and **49**), whereas substitution at the 4-position of the phenyl group in the *N*-biphenylacetyl derivative **40** gave a slightly more potent inhibitor than 2- or 3-substituted biphenyl derivatives (**46** and **47**). The results suggest that the substituent on the nitrogen atom of the decahydroisoquinolin scaffold may have some interactions with R188I SARS 3CL<sup>Pro</sup>.

## 2.3. Evaluation of the interactions

To clarify the interactions of a newly synthesized decahydroisoquinolin inhibitor with R188I SARS 3CL<sup>Pro</sup>, the structure of



**Scheme 3.** Construction of the decahydroisoquinolin scaffold. Reagents: (a)  $(\text{CH}_3\text{CN})_2\text{PdCl}_2$ ; (b)  $\text{K}_2\text{OsO}_2(\text{OH})_4/\text{NaIO}_4$ ; (c) (1)  $\text{H-His}(\text{Trt})\text{-N}(\text{OCH}_3)\text{CH}_3/\text{NaBH}_3\text{CN}$ ; (d) (1) TFA, (2) DIBALH.

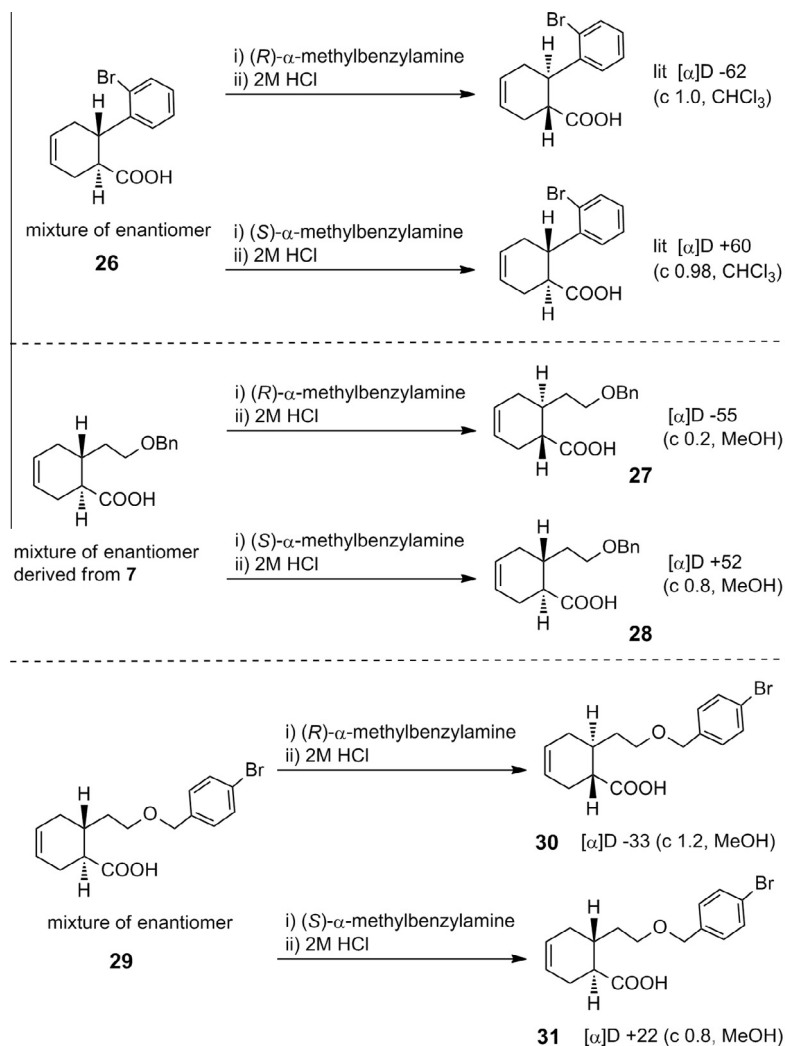
the protease in a complex with the inhibitor was revealed by X-ray crystallography. Subsequently, a co-crystal of the inhibitor with 3CL<sup>pro</sup> was prepared and analyzed. Structures of the 3CL<sup>pro</sup> in a complex with inhibitors **40**, **41**, and **44** were refined to resolutions of 1.60 Å, 2.42 Å, and 1.89 Å, respectively (PDB code 4TWY, 4TWW, and 4WY3). The data obtained are summarized in Table 2.

The overall structure of the 3CL<sup>pro</sup> in complex with inhibitor **41** ( $\text{IC}_{50} = 63 \mu\text{M}$ ) was first compared with the substrate-based inhibitor **1** (PDB code 3ATW) (Fig. 2). Basically, the decahydroisoquinolin inhibitor **41** was at the active site cleft of the 3CL<sup>pro</sup> as observed in the highly potent inhibitor **1**. The aldehyde group and imidazole ring of His-al, as well as the decahydroisoquinolin structure of **41**, had an almost identical conformation with **1** and similarly interacted with 3CL<sup>pro</sup>. In contrast, the direction of the *p*-bromobenzoyl group was outward from 3CL<sup>pro</sup> and opposite to the P<sub>3</sub> to P<sub>4</sub> sites of **1**. The *N*-*p*-bromobenzoyl group, however, was at the surface of 3CL<sup>pro</sup>, where additional hydrophobic interaction with Met of the 3CL<sup>pro</sup> may be possible (Fig. S7).

The carbonyl carbon of the aldehyde group in **41** was detected at a distance of 2.43 Å from the active center thiol of Cys-145, and its electron density could be fitted to an  $\text{sp}^2$  carbonyl carbon as in **1** (Fig. 3i). The results suggest that the decahydroisoquinolin inhibitor would function as a competitive inhibitor as do the peptide-aldehyde inhibitor **1**.<sup>13</sup> It was clearly confirmed that the decahydroisoquinolin scaffold of **41** took a *trans*-fused (4*aR*,8*aS*) configuration, as expected from the salt-resolution of enantiomixture **29**. It was also confirmed that the P<sub>1</sub> His-al substituent on the decahydroisoquinolin scaffold took an axial-configuration, as expected from the Pd(II)-mediated cyclization. The decahydroiso-

quinolin scaffold of **41** was inserted into a large S<sub>2</sub> pocket created by His-41, Met-49, Met-165, and Asp-187, as in the case of a parent peptide aldehyde inhibitor, and most of the S<sub>2</sub> pocket was occupied by the fused-ring structure of decahydroisoquinolin (Fig. 3i). The nitrogen atom of the P<sub>1</sub> site imidazole of **41** formed a hydrogen bond with the imidazole nitrogen of His-163, resulting in close fitting at the other side of the S<sub>1</sub> pocket formed from the Phe-140, Leu-141, and Glu-166 side chains of the protease (Fig. 3ii). These interactions, especially of the decahydroisoquinolin scaffold in the S<sub>2</sub> pocket, function to hold the P<sub>1</sub> site imidazole and terminal aldehyde tightly inside the active site cleft, which resulted in the compact fitting of the novel scaffold to the 3CL<sup>pro</sup>.

To evaluate the effects of absolute configuration of the decahydroisoquinolin scaffold, structures of the 3CL<sup>pro</sup> in complex with (4*aR*,8*aS*)-*N*-4-phenylbenzoyl decahydroisoquinolin inhibitor **40** and (4*aS*,8*aR*)-*N*-4-phenylbenzoyl decahydroisoquinolin inhibitor **44** were compared (Fig. 4i). In both inhibitors, the P<sub>1</sub> site imidazole ring and the terminal aldehyde group had nearly the same interactions as in the (4*aR*,8*aS*)-*N*-bromobenzoyl decahydroisoquinolin inhibitor **41** described above. Due to the configuration change at the decahydroisoquinolin moiety, however, the (4*aS*,8*aR*) decahydroisoquinolin scaffold was clearly twisted compared to the (4*aR*,8*aS*) decahydroisoquinolin in the S<sub>2</sub> pocket (Fig. 4ii). This conformation change of the decahydroisoquinolin scaffold transferred to the direction of the *N*-substituent. Thus, the substituent of (4*aR*,8*aS*) decahydroisoquinolin **40** took nearly the same conformation as the *N*-*p*-bromobenzoyl inhibitor **41** located on the surface of the 3CL<sup>pro</sup>, whereas the substituent of (4*aS*,8*aR*) decahydroisoquinolin directed outside from the protease surface. These



**Scheme 4.** Resolution by salt formation.

conformational differences at the *N*-substituent, as well as the interactions at the S<sub>2</sub> pocket, explain the discrepancy in the inhibitory activity between (4aR,8aS) and (4aS,8aR) decahydroisoquinolin inhibitors (**41** vs **44**).

### 3. Conclusion

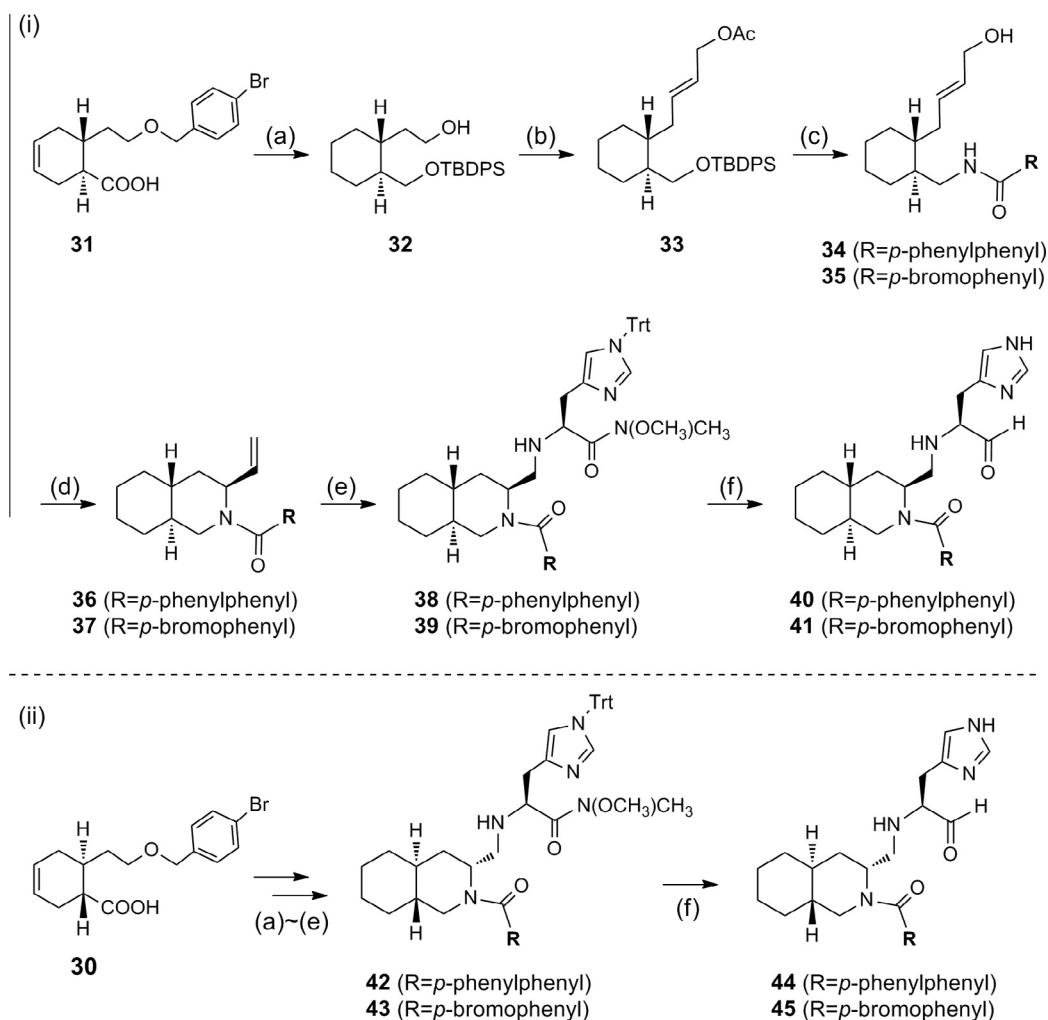
A novel non-peptide inhibitor based on the interactions at the  $S_1$  and  $S_2$  sites of SARS 3CL<sup>Pro</sup> was designed and synthesized. Focusing on cleavage site interaction at the  $S_1$  site and hydrophobic interaction at the  $S_2$  site, a decahydroisoquinolin scaffold was designed. Using a cyclohexene enantiomer obtained by salt resolution using chiral amine, the *trans*-decahydroisoquinolin derivative was synthesized as an enantiomer. Several analogs containing different *N*-substituents were also prepared similarly. All decahydroisoquinolin inhibitors showed moderate but clear inhibitory activities for SARS 3CL<sup>Pro</sup>, which confirmed that the fused ring structure of the decahydroisoquinolin scaffold functions as an inhibitor for SARS 3CL<sup>Pro</sup>. By X-ray crystallographic studies, it was confirmed that the decahydroisoquinolin inhibitors were at the active site cleft of 3CL<sup>Pro</sup>, as observed in the highly potent peptide-aldehyde inhibitor. The decahydroisoquinolin scaffold was inserted into a large  $S_2$  pocket and occupied most of the pocket. The  $P_1$  site imidazole was inserted into the  $S_1$  pocket as expected. These interactions were effective to hold the terminal aldehyde

tightly inside the active site cleft, which resulted in the compact fitting of the novel scaffold to 3CL<sup>pro</sup>. The acyl substituent on the nitrogen in the decahydroisoquinolin scaffold was at the surface of the 3CL<sup>pro</sup>, where additional interactions with the 3CL<sup>pro</sup> may be possible. Evaluations on the analogs focusing on the interactions at the *N*-substituent are now underway.

## 4. Experimental

#### 4.1. General

All solvents were of reagent grade. THF was distilled from sodium and benzophenone ketyl.  $\text{CH}_2\text{Cl}_2$  was distilled from  $\text{CaH}_2$ . All commercial reagents were of the highest purity available. Analytical TLC was performed on silica gel (60 F-254, 0.25 mm Plates). Column chromatography was carried out on Wakogel C-200E (particle size, 75–150  $\mu\text{m}$ ) or Wakogel FC-40 (particle size, 20–40  $\mu\text{m}$ ).  $^1\text{H}$  NMR spectra were recorded in  $\text{CDCl}_3$  (unless otherwise stated) on agilent UNITY INOVA 400 NB, JEOL JNM-ECS 400, Bruker AM-300, or JEOL JNM-LA 500 spectrometers. Chemical shifts are expressed in ppm relative to tetramethylsilane (0 ppm) or  $\text{CHCl}_3$  (7.28 ppm). The coupling constants are given in Hz.  $^{13}\text{C}$  NMR spectra were recorded on the same spectrometers at 100 or 125 MHz, using the central resonance of  $\text{CDCl}_3$  ( $\delta$  77.0 ppm) as the internal reference unless otherwise stated. High-resolution mass spectra



**Scheme 5.** Construction of the decahydroisoquinolin scaffold starting from the separated enantiomer. Reagents: (a) (1) IBCF/NaBH<sub>4</sub>, (2) TBDPS-Cl/imidazole, (3) H<sub>2</sub>/Pd-C/sat. NaHCO<sub>3</sub> aq.; (b) (1) PCC, (2) (EtO)<sub>2</sub>P(O)CH<sub>2</sub>COOEt/NaH, (3) DIBALH, (4) Ac<sub>2</sub>O/pyridine/DMAP; (c) (1) TBAF, (2) (EtO)<sub>2</sub>P(O)N<sub>3</sub>/DIAD/PPh<sub>3</sub>, (3) LAH, (4) 4-phenylbenzoic acid or 4-bromobenzoic acid/HBTU/DIPEA; (d) (CH<sub>3</sub>CN)<sub>2</sub>PdCl<sub>2</sub>; (e) (1) K<sub>2</sub>OsO<sub>2</sub>(OH)<sub>4</sub>/NaIO<sub>4</sub>, (2) H-His(Trt)-N(OCH<sub>3</sub>)CH<sub>3</sub>/NaBH<sub>3</sub>CN; (f) (1) TFA, (2) DIBALH.

(HRMS) were obtained on a JMS-HX-110A (FAB), and Shimadzu LCMS-IT-TOF (ESI). Low-resolution mass spectra (LRMS) were obtained on a Shimadzu LCMS-2010EV (ESI). Optical rotations were determined with a HORIBA SEPA-300 polarimeter. Preparative HPLC was performed using a COSMOSIL 5C18-ARII column (20 × 250 mm) with a linear gradient of CH<sub>3</sub>CN in 0.1% aqueous TFA at a flow rate of 5.0 mL/min on a HITACHI LaChrom system (OD, 254 nm). For analytical HPLC, unless otherwise noted, a COSMOSIL 5C18-ARII column (4.6 × 150 mm) was employed with a linear gradient of CH<sub>3</sub>CN in 0.1% aqueous TFA at a flow rate of 0.9 mL/min on a HITACHI LaChrom system (OD, 254 nm). The purity of the test compounds was determined by analytical HPLC. All test compounds showed ≥95% purity.

#### 4.1.1. (1*S*,6*R*/*S*)-Ethyl 6-[2-(benzyloxy)ethyl]cyclohex-3-enecarboxylate **7**

To a solution of 1,3-butadiene (20 wt% solution in hexane, 17 mL, 40 mmol) was added ester **6** (2.34 g, 10.0 mmol), heated at 250 °C for 60 h. After the reaction mixture was cooled to room temperature, water was added and the whole was extracted with AcOEt. The organic layer was washed with 1 M HCl and brine, dried over MgSO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 30:1) to give **7** (1.87 g, 65%) as a yellow pale oil. <sup>1</sup>H NMR (400 MHz): δ = 7.36–7.31

(m, 4H), 7.29–7.26 (m, 1H), 5.64 (m, 2H), 4.51 (d, *J* = 11.6 Hz, 1H), 4.46 (d, *J* = 12.0 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 3.54–3.50 (m, 2H), 2.41–2.35 (m, 1H), 2.31–2.20 (m, 3H), 2.09–2.04 (m, 1H), 1.84–1.73 (m, 2H), 1.54–1.45 (m, 1H), 1.25 (t, *J* = 7.2 Hz, 3H); <sup>13</sup>C NMR (100 MHz): δ = 175.8, 138.5, 128.3, 127.6, 127.5, 125.7, 124.7, 72.8, 67.9, 60.2, 45.3, 33.7, 32.4, 29.9, 28.0, 14.2; HRMS (EI) calcd for C<sub>18</sub>H<sub>24</sub>O<sub>3</sub> [M]<sup>+</sup>: 288.1725. Found: 288.1722.

#### 4.1.2. {(1*S*,6*R*/*S*)-6-[2-(Benzyloxy)ethyl]cyclohex-3-en-1-yl}methanol

To a suspension of LiAlH<sub>4</sub> (387 mg, 10.2 mmol) in ether (30 mL) was added **7** (1.47 g, 5.12 mmol) at 0 °C. After being stirred for 15 min at 0 °C, the reaction was quenched with H<sub>2</sub>O. The mixture was warmed to room temperature and filtered through Celite and a silica gel layer, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to give a title alcohol (1.25 g, quant.) as a colorless oil. <sup>1</sup>H NMR (400 MHz): δ = 7.37–7.32 (m, 4H), 7.30–7.26 (m, 1H), 5.65–5.57 (m, 2H), 4.51 (s, 2H), 3.66 (dd, *J* = 10.8, 6.0 Hz, 1H), 3.62–3.48 (m, 3H), 2.14–2.09 (m, 2H), 2.01–1.75 (m, 5H), 1.66–1.59 (m, 1H), 1.55–1.48 (m, 1H); <sup>13</sup>C NMR (100 MHz): δ = 138.3, 128.4, 127.7, 127.6, 125.8, 125.5, 73.1, 68.5, 65.0, 39.7, 32.9, 31.0, 29.5, 26.7; HRMS (EI) calcd for C<sub>16</sub>H<sub>22</sub>O<sub>2</sub> [M]<sup>+</sup>: 246.1620. Found: 246.1618.



**Table 1**  
Inhibitory activities of the decahydroisoquinolin derivatives

R	IC <sub>50</sub>	
	(3S,4aR,8aS)	(3R,4aS,8aR)
	<b>40</b> 108 μM	<b>44</b> 240 μM
	<b>46</b> 135 μM	
	<b>47</b> 135 μM	
	<b>48</b> 68 μM	
	<b>41</b> 63 μM	45 175 μM
	<b>49</b> 57 μM	

#### 4.1.3. ((1S/R,6R/S)-6-[2-(Benzyloxy)ethyl]cyclohex-3-en-1-yl)methoxy)(*tert*-butyl)diphenylsilane **8**

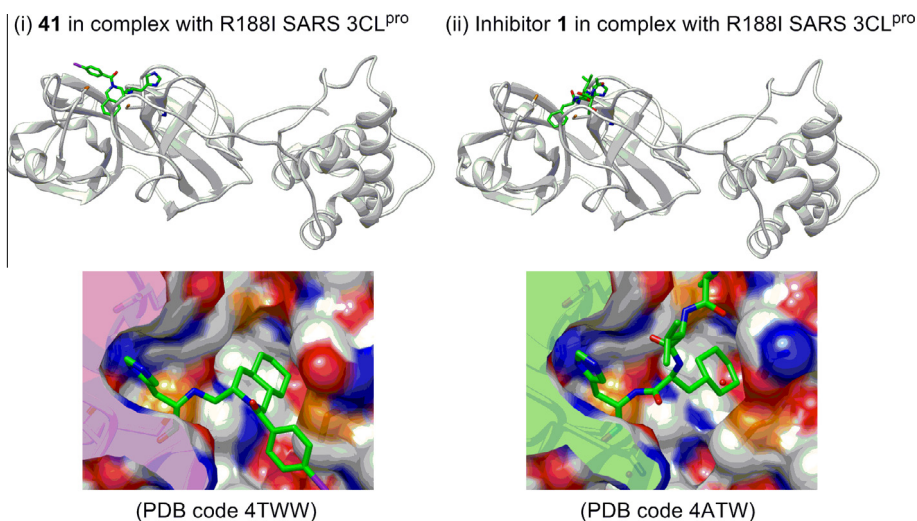
TBDPS-Cl (3.6 mL, 13.1 mmol) was added to a solution of the above alcohol (2.92 g, 11.9 mmol) and imidazole (1.21 g, 17.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and the mixture was stirred for 16 h. The reaction was quenched with saturated aqueous NH<sub>4</sub>Cl, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 20:1) to give **8** (5.76 g, quant.) as a colorless oil. <sup>1</sup>H NMR (400 MHz): δ = 7.67–7.65 (m, 4H), 7.43–7.30 (m, 10H), 7.28 (m, 1H), 5.63–5.54 (m, 2H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.45 (d, *J* = 12.0 Hz, 1H), 3.68 (dd, *J* = 10.0, 5.2 Hz, 1H), 3.62 (dd, *J* = 9.8, 7.0 Hz, 1H), 3.54–3.45 (m, 2H), 2.17–2.06 (m, 2H), 2.02–1.95 (m, 1H), 1.87–1.80 (m, 2H), 1.73–1.67 (m, 2H), 1.51–1.42 (m, 1H), 1.05 (s, 9H); <sup>13</sup>C NMR (100 MHz): δ = 138.6, 135.62, 135.61, 133.98, 133.95, 129.5, 128.3, 127.58, 127.56, 127.4, 125.8, 125.4, 72.9, 68.6, 65.9, 39.6, 32.9, 30.9, 29.1, 26.9, 26.7, 19.3; HRMS (FAB) calcd for C<sub>32</sub>H<sub>41</sub>O<sub>2</sub>Si [M+H]<sup>+</sup>: 485.2876. Found: 485.2870.

#### 4.1.4. 2-[(1R/S,6S/R)-6-[[*tert*-Butyldiphenylsilyl]oxy]methyl]-cyclohexyl]ethanol

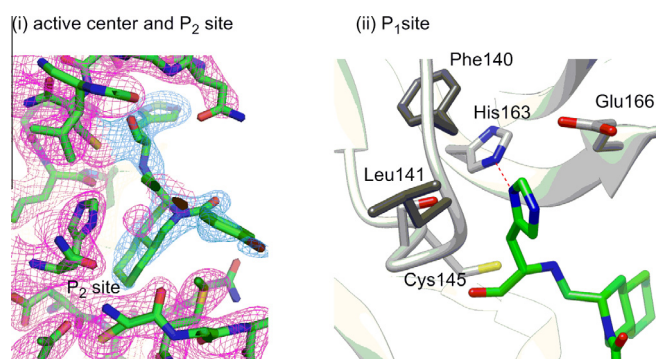
To a solution of **8** (3.40 g, 7.01 mmol) in CH<sub>3</sub>OH/AcOEt/CH<sub>2</sub>Cl<sub>2</sub> (10:10:1, 21 mL) Pd(OH)<sub>2</sub>-C (610 mg) was added and stirred under a hydrogen gas atmosphere at room temperature for 12 h. The mixture was filtered through Celite and a silica gel layer, and the filtrate was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3:1) to give a title alcohol (2.78 g, quant.) as a colorless oil. <sup>1</sup>H NMR (400 MHz): δ = 7.68–7.65 (m, 4H), 7.45–7.36 (m, 6H), 3.68–3.54 (m, 4H), 1.78–1.66 (m, 5H), 1.37–1.18 (m, 7H), 1.06 (s, 9H), 1.01–0.96 (m, 1H); <sup>13</sup>C NMR (100 MHz): δ = 135.69, 135.66, 133.92, 133.90, 129.55, 129.54, 127.60, 127.57, 66.6, 61.1, 44.5, 36.5, 35.5, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3; HRMS (FAB) calcd for C<sub>25</sub>H<sub>37</sub>O<sub>2</sub>Si [M+H]<sup>+</sup>: 397.2563. Found: 397.2569.

**Table 2**  
Data collection and refinement statistics for the R188I SARS 3CL protease in complexes with compounds **40**, **41**, and **44**

PDB ID	4TWY In complex with <b>40</b>	4TWW In complex with <b>41</b>	4WY3 In complex with <b>44</b>
Space group	C121	P1	C121
Unit cell parameters			
Length <i>a</i>	107.83	54.89	108.11
Length <i>b</i>	82.128	59.52	81.82
Length <i>c</i>	53.271	68.40	53.24
Angle α	90	93.11	90
Angle β	104.98	102.82	104.69
Angle γ	90	107.30	90
Resolution	1.60	2.42	1.89
Observations			
Unique observations	57,490	31,213	49,270
Redundancy	4.2	1.75	4.1
Completeness	88.6	94.3	93.2
Mean <i>I</i> /σ( <i>I</i> )	2.18 (at 1.60 Å)	9.96 (at 2.42 Å)	2.49 (at 1.89 Å)
<i>R</i> merge	0.08	0.05	0.07
Refinement			
Resolution range	25.3–1.60	66.1–2.42	30.6–1.89
<i>R</i> <sub>cryst</sub>	0.29	0.23	0.27
<i>R</i> <sub>free</sub>	0.32	0.26	0.30
RMSZ from ideal			
Bond length (Å)	0.93	0.73	0.86
Bond angle (°)	0.96	0.86	0.90



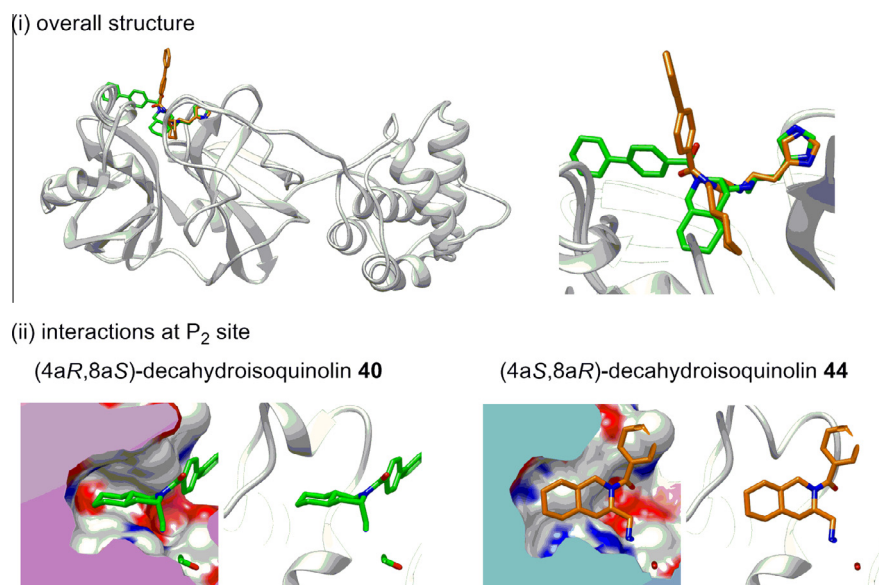
**Figure 2.** (i) X-ray structure of the inhibitor **41** in complex with R188I SARS 3CL<sup>pro</sup> (PDB code 4TWW) and molecular graphics image around the P<sub>1</sub> and P<sub>2</sub> sites. (ii) X-ray structure of the inhibitor **1** in complex with R188I SARS 3CL<sup>pro</sup> (Ref. 13; PDB code 4ATW) and molecular graphics image around the P<sub>1</sub> and P<sub>2</sub> sites.



**Figure 3.** (i) Interactions of the inhibitor **41** with R188I SARS 3CL<sup>pro</sup> at the active center and P<sub>2</sub> site. (ii) Interactions at the P<sub>1</sub> site.

#### 4.1.5. (*E*)-Ethyl 4-[(1*R*/5,2*S*/*R*)-2-[[*tert*-butyldiphenylsilyl]oxy]methyl]cyclohex-1-yl]but-2-enoate **9**

To a solution of PCC (3.45 g, 16.0 mmol) and Celite (3.5 g) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL), above alcohol (2.50 g, 6.30 mmol) was added at 0 °C. The temperature was gradually raised to room temperature. After being stirred for 6 h, the reaction mixture was filtered through a silica gel layer and the filtrate was concentrated. This compound was immediately used for the next step without purification. Triethylphosphonoacetate (1.5 mL, 7.7 mmol) was added to a suspension of NaH [60% in mineral oil (308 mg, 7.70 mmol)] in THF (10 mL) at –20 °C under an argon gas atmosphere and the mixture was stirred for 0.5 h. The oxidized product was added drop-wise to the reaction mixture and stirred for 1.5 h at –20 °C. The reaction was quenched with saturated aqueous NH<sub>4</sub>Cl, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 20:1) to give **9** (2.70 g, 92%, 2 steps) as a colorless oil. <sup>1</sup>H



**Figure 4.** (i) X-ray structures of R188I SARS 3CL<sup>pro</sup> in complex with (4a*R*/8a*S*)-*N*-4-phenylbenzoyl decahydroisoquinolin inhibitor **40** (PDB code 4TWY) and (4a*S*/8a*R*)-*N*-4-phenylbenzoyl decahydroisoquinolin inhibitor **44** (PDB code 4WY3). (ii) Interactions of **40** and **44** at the P<sub>2</sub> site.



NMR (400 MHz):  $\delta$  = 7.67–7.64 (m, 4H), 7.45–7.36 (m, 6H), 6.91 (ddd,  $J$  = 15.4, 8.8, 6.4 Hz, 1H), 5.72 (d,  $J$  = 15.6 Hz, 1H), 4.18 (q,  $J$  = 7.1 Hz, 2H), 3.63–3.57 (m, 2H), 2.38–2.32 (m, 1H), 1.97 (td,  $J$  = 14.8, 8.1 Hz, 1H), 1.79–1.76 (m, 1H), 1.71–1.69 (m, 4H), 1.54–1.49 (m, 1H), 1.32–1.18 (m, 4H), 1.29 (t,  $J$  = 7.2 Hz, 3H), 1.05 (s, 9H), 1.03–0.97 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 166.6, 148.2, 135.62, 135.61, 133.82, 133.80, 129.59, 129.55, 127.62, 127.59, 122.4, 66.2, 60.1, 43.9, 37.8, 36.4, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3, 14.3; HRMS (FAB) calcd for  $\text{C}_{29}\text{H}_{40}\text{NaO}_3\text{Si}$   $[\text{M}+\text{Na}]^+$ : 487.2644. Found: 487.2651.

#### 4.1.6. (E)-4-[(1R,2S,2R)-2-[(*tert*-Butyldiphenylsilyl)oxy]-methyl]cyclohexyl]but-2-en-1-ol

To a solution of **9** (1.92 g, 4.13 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL), DIBALH (1.0 mol/L solution in hexane, 12.4 mL, 12.4 mmol) was added at  $-78^\circ\text{C}$ . After being stirred for 15 min at the same temperature, the reaction was quenched with  $\text{CH}_3\text{OH}$  (5.0 mL). The mixture was warmed to room temperature, and filtered through Celite and a silica gel layer. The filtrate was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to give a title alcohol (1.74 g, quant.) as a colorless oil.  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.68–7.65 (m, 4H), 7.44–7.36 (m, 6H), 5.64–5.48 (m, 2H), 4.04 (d,  $J$  = 6.0 Hz, 2H), 3.66 (dd,  $J$  = 10.0, 2.8 Hz, 1H), 3.58 (dd,  $J$  = 9.8, 5.4 Hz, 1H), 2.23–2.17 (m, 1H), 1.87–1.79 (m, 2H), 1.72–1.69 (m, 3H), 1.43–1.32 (m, 1H), 1.30–1.18 (m, 4H), 1.05 (s, 9H), 1.01–0.94 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 135.64, 135.63, 134.0, 131.6, 130.2, 129.52, 129.50, 127.58, 127.55, 66.3, 63.8, 43.9, 38.1, 36.2, 31.7, 30.0, 26.9, 26.2, 26.1, 19.4; HRMS (FAB) calcd for  $\text{C}_{27}\text{H}_{38}\text{NaO}_2\text{Si}$   $[\text{M}+\text{Na}]^+$ : 445.2539. Found: 445.2541.

#### 4.1.7. (E)-4-[(1R,2S,2R)-2-[(*tert*-Butyldiphenylsilyl)oxy]-methyl]cyclohexyl]but-2-en-1-yl acetate **10**

To a solution of above alcohol (1.74 g, 4.11 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL), pyridine (0.50 mL, 6.2 mmol), acetic anhydride (0.59 mL, 6.19 mmol), and DMAP (50 mg, 0.41 mmol) were added at  $0^\circ\text{C}$ . The mixture was stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$ . The mixture was extracted with AcOEt. The organic layer was washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 30:1) to give **10** (1.81 g, 95%) as a colorless oil.  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.67–7.64 (m, 4H), 7.44–7.36 (m, 6H), 5.71–5.64 (m, 1H), 5.49–5.42 (m, 1H), 4.47 (d,  $J$  = 6.4 Hz, 2H), 3.65 (dd,  $J$  = 9.8, 3.0 Hz, 1H), 3.57 (dd,  $J$  = 10.0, 4.8 Hz, 1H), 2.23–2.18 (m, 1H), 2.05 (s, 3H), 1.87–1.79 (m, 2H), 1.71–1.68 (m, 3H), 1.43–1.35 (m, 1H), 1.30–1.18 (m, 4H), 1.05 (s, 9H), 1.00–0.94 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 170.9, 135.6, 134.8, 133.94, 133.93, 129.5, 127.6, 125.0, 66.3, 65.3, 43.8, 38.0, 36.3, 31.7, 30.0, 26.9, 26.2, 26.1, 21.0, 19.3; HRMS (FAB) calcd for  $\text{C}_{29}\text{H}_{40}\text{NaO}_3\text{Si}$   $[\text{M}+\text{Na}]^+$ : 487.2644. Found: 487.2642.

#### 4.1.8. (E)-4-[(1R,2S,2R)-2-(Hydroxymethyl)cyclohexyl]but-2-en-1-yl acetate

To a solution of **10** (1.81 g, 3.89 mmol) in THF (20 mL), TBAF [1.0 M solution in THF (7.8 mL, 7.8 mmol)] was added at room temperature. After the mixture was stirred for 12 h, the reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 6:1) to give a title alcohol (1.03 g, quant.) as a colorless oil.  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 5.80–5.72 (m, 1H), 5.60–5.53 (m, 1H), 4.51 (d,  $J$  = 6.4 Hz, 2H), 3.69 (dd,  $J$  = 10.8, 3.2 Hz, 1H), 3.59 (dd,  $J$  = 10.8, 5.6 Hz, 1H), 2.33–2.27 (m, 1H), 2.06 (s, 3H), 2.02–1.90 (m, 1H), 1.81–1.79 (m, 1H), 1.74–1.67 (m, 3H), 1.37–1.11 (m,

5H), 1.05–0.95 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 170.9, 134.5, 125.3, 65.7, 65.2, 43.8, 38.0, 36.4, 31.7, 29.5, 26.0, 25.8, 21.0; HRMS (FAB) calcd for  $\text{C}_{13}\text{H}_{22}\text{NaO}_3$   $[\text{M}+\text{Na}]^+$ : 249.1467. Found: 249.1460.

#### 4.1.9. N-((1S,2R,2S)-2-[(E)-4-Hydroxybut-2-en-1-yl]cyclohexyl)methyl)-[1,1'-biphenyl]-4-carboxamide **12**

DPPA (2.4 mL, 11 mmol) was added drop-wise to a solution of above alcohol (1.03 g, 4.56 mmol), triphenylphosphine (2.80 g, 10.8 mmol), and DEAD (40% solution in toluene, 4.2 mL, 10.8 mmol) in THF (10 mL) at  $0^\circ\text{C}$ . The mixture was stirred for 16 h at the same temperature, and then the reaction mixture was concentrated. The residue was roughly purified by silica gel column chromatography (hexane/AcOEt = 30:1) to give **11**.  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 5.77–5.69 (m, 1H), 5.62–5.54 (m, 1H), 4.52 (d,  $J$  = 6.0 Hz, 2H), 3.40 (dd,  $J$  = 12.0, 3.2 Hz, 1H), 3.25 (dd,  $J$  = 12.2, 6.2 Hz, 1H), 2.29–2.24 (m, 1H), 2.06 (s, 3H), 2.00–1.91 (m, 1H), 1.80–1.65 (m, 4H), 1.34–1.29 (m, 2H), 1.27–1.11 (m, 3H), 1.05–0.95 (m, 1H).

The crude **11** was dissolved in ether (10 mL) and added to a suspension of  $\text{LiAlH}_4$  (1.04 g, 27.4 mmol) in ether (10 mL) at  $0^\circ\text{C}$ . The reaction was quenched with  $\text{CH}_3\text{OH}$  and concentrated. The mixture was stirred for 6 h under reflux. The reaction mixture cooled to room temperature and then quenched with  $\text{CH}_3\text{OH}$  and concentrated to give a corresponding amine derivative.  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 5.63–5.48 (m, 2H), 3.94–3.92 (m, 2H), 2.81 (dd,  $J$  = 12.6, 3.0 Hz, 1H), 2.43 (dd,  $J$  = 12.8, 7.6 Hz, 1H), 2.20–2.15 (m, 1H), 1.97–1.86 (m, 1H), 1.79–1.75 (m, 1H), 1.69–1.60 (m, 3H), 1.24–1.09 (m, 5H), 1.05–0.96 (m, 1H).

The residue was used in the next step without purification. The crude product in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added to a solution of HBTU (4.32 g, 11.4 mmol), DIPEA (2.4 mL, 14 mmol), and 4-biphenyl carboxylic acid (903 mg, 4.56 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) at  $0^\circ\text{C}$ . The mixture was stirred for 3 h at room temperature. The reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  and extracted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was washed with brine and dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **12** (1.04 g, 63%, 3 steps) as a colorless oil.  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.84–7.82 (m, 2H), 7.67–7.59 (m, 4H), 7.48–7.44 (m, 2H), 7.41–7.37 (m, 1H), 6.28 (br s, 1H), 5.79–5.67 (m, 2H), 4.10 (d,  $J$  = 4.4 Hz, 2H), 3.78 (ddd,  $J$  = 13.6, 6.0, 3.6 Hz, 1H), 3.20 (ddd,  $J$  = 13.7, 8.1, 5.9 Hz, 1H), 2.32–2.27 (m, 1H), 2.21–2.12 (m, 1H), 1.87–1.84 (m, 1H), 1.74–1.72 (m, 3H), 1.52–1.41 (m, 1H), 1.32–1.04 (m, 5H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 167.2, 144.2, 140.0, 133.3, 131.2, 130.5, 128.9, 128.0, 127.3, 127.23, 127.17, 63.8, 43.3, 41.1, 39.6, 36.5, 31.9, 30.6, 26.0, 25.7; HRMS (EI) calcd for  $\text{C}_{24}\text{H}_{29}\text{NO}_2$   $[\text{M}]^+$ : 363.2198. Found: 363.2207.

#### 4.1.10. 4-Bromo-N-((1S,2R,2S)-2-[(E)-4-hydroxybut-2-en-1-yl]cyclohexyl)methyl)benzamide **13**

A title compound was similarly prepared from **10** as above. Colorless oil; yield 50% (3 steps):  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 7.64–7.61 (m, 2H), 7.58–7.56 (m, 2H), 6.16 (m, 1H), 5.78–5.66 (m, 2H), 4.10 (d,  $J$  = 4.8 Hz, 2H), 3.76 (ddd,  $J$  = 13.4, 5.8, 3.8 Hz, 1H), 3.16 (ddd,  $J$  = 13.7, 8.1, 5.9 Hz, 1H), 2.29–2.25 (m, 1H), 2.18–2.11 (m, 1H), 1.84–1.80 (m, 1H), 1.73–1.71 (m, 3H), 1.50–1.41 (m, 1H), 1.28–0.96 (m, 5H);  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 166.5, 133.5, 131.8, 131.2, 130.4, 128.5, 126.0, 63.7, 43.3, 41.0, 39.6, 36.4, 31.9, 30.5, 26.0, 25.7; HRMS (EI) calcd for  $\text{C}_{18}\text{H}_{24}\text{BrNO}_2$   $[\text{M}]^+$ : 365.0990. Found: 365.0996.

#### 4.1.11. (1,1'-Biphenyl)-4-yl((3S,4aR,5S,8aR)-3-vinyloctahydroisoquinolin-2(1H)-yl)methanone **14**

To a solution of **12** (120 mg, 0.331 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (1 mL),  $(\text{CH}_3\text{CN})_2\text{PdCl}_2$  (15 mg, 0.056 mmol) was added at  $0^\circ\text{C}$  under an argon gas atmosphere, and the mixture was stirred at the same

temperature for 4 h. The reaction mixture was filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 10:1) to give **14** (100 mg, 88%) as a colorless oil. <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.64–7.58 (m, 4H), 7.49–7.43 (m, 4H), 7.38–7.35 (m, 1H), 5.87 (ddd,  $J$  = 17.5, 10.7, 3.7 Hz, 0.4H), 5.78 (ddd,  $J$  = 17.5, 10.7, 3.5 Hz, 0.6H), 5.55 (br s, 0.4H), 5.31–5.28 (m, 1H), 5.23–5.16 (m, 1H), 4.54 (br s, 0.6H), 4.49 (dd,  $J$  = 13.2, 4.0 Hz, 0.6H), 3.49 (dd,  $J$  = 13.0, 3.8 Hz, 0.4H), 2.86 (dd,  $J$  = 13.2, 11.6 Hz, 0.4H), 2.61 (dd,  $J$  = 12.8, 11.6 Hz, 0.6H), 1.84–1.52 (m, 5H), 1.47–1.18 (m, 5H), 1.15–1.13 (m, 0.4H), 1.03–0.98 (m, 1H), 0.90–0.84 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 171.1, 170.4, 142.3, 142.2, 140.3, 137.1, 136.7, 135.4, 128.8, 127.69, 127.66, 127.4, 127.1, 126.8, 116.6, 116.1, 57.2, 50.8, 49.7, 43.5, 42.8, 41.9, 37.5, 36.8, 35.9, 32.9, 29.9, 29.7, 26.2, 26.1, 25.8, 25.7; HRMS (EI) calcd for C<sub>24</sub>H<sub>27</sub>NO [M]<sup>+</sup>: 345.2093. Found: 345.2090.

#### 4.1.12. (4-Bromophenyl)((3*S*,4*aR*,5*aS*/*R*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl)methanone **15**

A title compound was similarly prepared as above. Colorless oil; yield 57%: <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.56–7.50 (m, 2H), 7.29–7.27 (m, 2H), 5.84 (ddd,  $J$  = 17.4, 10.6, 3.8 Hz, 0.4H), 5.74 (ddd,  $J$  = 17.5, 10.7, 3.5 Hz, 0.6H), 5.49 (br s, 0.4H), 5.29–5.26 (m, 1H), 5.19–5.10 (m, 1H), 4.44 (dd,  $J$  = 13.4, 3.8 Hz, 0.6H), 4.39 (s, 0.6H), 3.33 (dd,  $J$  = 13.2, 3.6 Hz, 0.4H), 2.82 (dd,  $J$  = 13.0, 11.8 Hz, 0.4H), 2.57 (dd,  $J$  = 13.0, 11.4 Hz, 0.6H), 1.83–1.49 (m, 5H), 1.43–1.19 (m, 5H), 1.13–1.04 (m, 0.4H), 0.99–0.96 (m, 1H), 0.88–0.83 (m, 0.6H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 170.2, 169.6, 136.9, 136.5, 135.4, 131.7, 131.6, 128.6, 128.0, 123.7, 123.6, 116.7, 116.2, 57.2, 50.8, 49.6, 43.5, 42.8, 41.8, 37.5, 36.7, 35.9, 32.8, 29.9, 29.6, 26.1, 26.0, 25.7, 25.6; HRMS (EI) Calcd for C<sub>18</sub>H<sub>22</sub>BrNO [M]<sup>+</sup>: 347.0885. Found: 347.0879.

#### 4.1.13. (S)-2-(((3*S*,4*aR*,5*aS*)-2-[(1,1'-Biphenyl)-4-carbonyl]-decahydroisoquinolin-3-yl)methyl)amino)-*N*-methoxy-*N*-methyl-3-(1-trityl-1*H*-imidazol-4-yl)propanamide **18**

To a solution of K<sub>2</sub>OsO<sub>2</sub>(OH)<sub>4</sub> (3.1 mg, 0.0083 mmol) and *N*-methylmorpholine *N*-oxide (389 mg, 3.32 mmol), **14** (286 mg, 0.829 mmol) was added in THF/H<sub>2</sub>O (3:1, 10 mL). After being stirred for 12 h, NaIO<sub>4</sub> (710 mg, 3.32 mmol) was added to the mixture. The resultant mixture was stirred for 30 min. The reaction was quenched with H<sub>2</sub>O, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/AcOEt = 3:1) to give **16**. <sup>1</sup>H NMR (400 MHz):  $\delta$  = 9.69 (s, 0.75H), 9.65 (s, 0.25H), 7.69–7.54 (m, 5H), 7.49–7.37 (m, 4H), 5.50 (d,  $J$  = 6.4 Hz, 0.75H), 4.62–4.59 (m, 0.25H), 4.44 (d,  $J$  = 5.6 Hz, 0.25H), 3.69–3.65 (m, 0.75H), 2.81 (dd,  $J$  = 13.2, 11.6 Hz, 0.75H), 2.40 (t,  $J$  = 12.6 Hz, 0.25H), 2.33 (d,  $J$  = 13.6 Hz, 0.75H), 2.15 (dd,  $J$  = 13.6 Hz, 0.25H), 1.74–1.69 (m, 3H), 1.59–1.50 (m, 1H), 1.44–1.41 (m, 1H), 1.25–1.11 (m, 3H), 1.08–0.96 (m, 2H), 0.92–0.76 (m, 1H).

The product was used without further purification. To a solution of **16** and H-His(Trt)-N(OCH<sub>3</sub>)CH<sub>3</sub> (410 mg, 0.930 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), AcOH (0.05 mL, 0.8 mmol) was added. The mixture was stirred at room temperature for 2 h and then NaBH<sub>3</sub>CN (181 mg, 2.88 mmol) was added. The resultant mixture was stirred for 30 min. The reaction was quenched with 1 M HCl and the whole was extracted with AcOEt. The organic layer was washed with saturated aqueous NaHCO<sub>3</sub> and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by flash column chromatography (CHCl<sub>3</sub>/CH<sub>3</sub>OH = 25:1) to give **18** and **20**.

**Compound 18**: [80 mg, 13% (50% max.), 3 steps] as a colorless oil. [ $\alpha$ ]<sub>D</sub><sup>28</sup> –20 (c 0.48, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.59–7.53 (m, 4H), 7.47–7.41 (m, 4H), 7.37–7.29 (m, 11H), 7.13–7.09 (m, 6H), 6.62 (m, 0.6H), 6.56 (m, 0.4H), 4.94 (br s, 0.6H), 4.41 (dd,  $J$  = 13.0, 3.0 Hz, 0.4H), 4.12–4.11 (m, 0.4H), 3.93 (m, 1H), 3.69 (s, 1.8H), 3.50

(s, 1.2H), 3.44–3.41 (m, 0.6H), 3.14 (s, 1.8H), 3.08 (s, 1.2H), 2.93–2.84 (m, 2.4H), 2.76–2.66 (m, 2H), 2.46 (t,  $J$  = 12.2 Hz, 0.6H), 1.80–1.70 (m, 3H), 1.61–1.54 (m, 1H), 1.43–1.17 (m, 6H), 1.08–0.85 (m, 2H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.4, 175.2, 171.3, 170.5, 142.44, 142.38, 141.88, 141.86, 140.42, 140.35, 138.2, 138.1, 137.6, 137.2, 135.9, 135.7, 129.72, 129.66, 129.3, 128.8, 128.7, 127.91, 127.87, 127.54, 127.46, 127.4, 127.2, 127.1, 127.04, 126.99, 119.3, 115.6, 77.2, 75.03, 75.02, 61.6, 61.5, 57.8, 57.4, 55.5, 49.5, 48.3, 47.1, 46.6, 43.1, 42.6, 42.1, 36.4, 36.2, 34.4, 33.0, 32.9, 32.6, 32.2, 32.0, 29.9, 29.7, 26.14, 26.05, 25.8, 25.7; HRMS (EI) calcd for C<sub>50</sub>H<sub>53</sub>N<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 771.4148. Found: 771.4141.

**Compound 20**: [75 mg, 12% (50% max.), 3 steps] as a colorless oil. [ $\alpha$ ]<sub>D</sub><sup>28</sup> +32 (c 2.3, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.58–7.24 (m, 19H), 7.13–7.07 (m, 6H), 6.58 (m, 0.4H), 6.55 (m, 0.6H), 5.02–4.97 (m, 0.4H), 4.46 (dd,  $J$  = 13.2, 3.6 Hz, 0.6H), 4.13 (br s, 0.4H), 3.95 (m, 1H), 3.65 (s, 1.2H), 3.62–3.58 (m, 0.6H), 3.50 (s, 1.8H), 3.44 (dd,  $J$  = 13.4, 3.4 Hz, 0.4H), 3.14 (s, 1.2H), 3.11 (s, 1.8H), 3.01–2.94 (m, 1H), 2.89–2.81 (m, 2H), 2.65 (dd,  $J$  = 11.8, 6.6 Hz, 0.4H), 2.52 (dd,  $J$  = 12.0, 6.8 Hz, 0.6H), 2.50–2.44 (m, 0.6H), 2.26–2.24 (br s, 1H), 1.71–1.69 (m, 3H), 1.60–1.52 (m, 2H), 1.45–1.16 (m, 5H), 1.07–0.83 (m, 2H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.6, 175.3, 171.1, 170.8, 142.44, 142.37, 141.9, 141.8, 140.41, 140.39, 138.12, 138.08, 137.5, 137.3, 135.7, 129.72, 129.66, 128.73, 128.68, 127.9, 127.5, 127.4, 127.1, 127.05, 127.03, 126.95, 119.5, 119.3, 77.2, 75.0, 61.6, 61.5, 57.7, 57.5, 55.4, 49.3, 48.4, 47.4, 47.2, 43.0, 42.8, 42.0, 36.7, 36.5, 34.6, 33.5, 33.00, 32.96, 32.3, 32.1, 29.9, 29.7, 29.6, 26.2, 26.0, 25.8, 25.7; HRMS (EI) calcd for C<sub>50</sub>H<sub>53</sub>N<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 771.4148. Found: 771.4154.

#### 4.1.14. (S)-2-(((3*S*,4*aR*,5*aS*)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl)methyl)amino)-*N*-methoxy-*N*-methyl-3-(1-trityl-1*H*-imidazol-4-yl)propanamide **19**

**Compound 19** was similarly synthesized as **18**. Colorless oil; yield 11% (50% max., 3 steps): [ $\alpha$ ]<sub>D</sub><sup>28</sup> –31 (c 0.83, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.47 (d,  $J$  = 8.4 Hz, 1.2H), 7.44 (d,  $J$  = 8.4 Hz, 0.8H), 7.34–7.31 (m, 10.8H), 7.22 (d,  $J$  = 8.4 Hz, 1.2H), 7.12–7.11 (m, 6H), 6.60 (br s, 0.6H), 6.55 (br s, 0.4H), 4.87 (m, 0.6H), 4.37 (dd,  $J$  = 13.2, 3.6 Hz, 0.4H), 4.10 (br s, 0.6H), 3.89 (br s, 0.4H), 3.78 (m, 0.6H), 3.64 (s, 1.8H), 3.51 (s, 1.2H), 3.24 (dd,  $J$  = 13.2, 3.6 Hz, 0.6H), 3.13 (s, 1.8H), 3.11 (s, 1.2H), 2.91–2.80 (m, 2.4H), 2.73–2.62 (m, 2H), 2.47–2.41 (m, 0.4H), 1.76–1.65 (m, 3.4H), 1.60–1.54 (m, 1.6H), 1.36–1.25 (m, 5H), 1.00–0.82 (m, 2H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.5, 175.1, 170.4, 169.7, 142.5, 142.4, 138.3, 138.1, 137.7, 137.2, 135.9, 135.8, 131.52, 131.49, 129.75, 129.72, 128.7, 128.4, 127.94, 127.91, 123.24, 123.21, 119.26, 119.25, 77.2, 75.1, 61.6, 61.5, 57.8, 57.5, 55.6, 49.3, 48.4, 47.1, 46.6, 43.1, 42.6, 42.0, 36.4, 36.2, 34.5, 33.0, 32.9, 32.7, 32.3, 32.0, 29.9, 29.7, 26.1, 26.0, 25.8, 25.7; HRMS (EI) calcd for C<sub>44</sub>H<sub>48</sub>BrN<sub>5</sub>O<sub>3</sub> [M]<sup>+</sup>: 773.2941. Found: 773.2948.

#### 4.1.15. (S)-2-(((3*R*,4*aS*,5*aR*)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl)methyl)amino)-*N*-methoxy-*N*-methyl-3-(1-trityl-1*H*-imidazol-4-yl)propanamide **21**

**Compound 21** was similarly synthesized as **20**. Colorless oil; yield 11% (50% max., 3 steps): [ $\alpha$ ]<sub>D</sub><sup>28</sup> +4.5 (c 0.42, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.47–7.43 (m, 2H), 7.37–7.29 (m, 12H), 7.12–7.10 (m, 6H), 6.55 (m, 1H), 4.98–4.95 (m, 0.4H), 4.40 (dd,  $J$  = 13.2, 3.6 Hz, 0.6H), 4.10 (br s, 0.4H), 3.90 (br s, 0.6H), 3.84–3.81 (m, 0.6H), 3.64–3.58 (m, 0.4H), 3.63 (s, 1.8H), 3.54 (s, 1.2H), 3.28 (dd,  $J$  = 13.2, 3.6 Hz, 0.4H), 3.13 (s, 1.8H), 3.11 (s, 1.2H), 2.98–2.91 (m, 1H), 2.86–2.74 (m, 2.6H), 2.60 (dd,  $J$  = 11.6, 6.0 Hz, 0.6H), 2.47–2.41 (m, 1.4H), 1.72–1.65 (m, 3H), 1.59–1.47 (m, 2H), 1.43–1.12 (m, 5H), 1.04–0.79 (m, 2H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.6, 175.2, 170.3, 170.0, 142.42, 142.37, 138.2, 138.1, 137.35, 137.25, 135.70, 135.66, 131.47, 131.45, 129.73, 129.70, 128.9, 128.7, 127.93, 127.91, 123.32, 123.26, 119.5, 119.3, 77.2, 75.1, 75.0, 61.5, 57.5,

57.4, 55.5, 49.2, 48.4, 47.4, 47.2, 42.9, 42.8, 42.0, 36.6, 36.5, 34.7, 33.6, 33.0, 32.9, 32.3, 32.0, 29.8, 29.6, 26.1, 26.0, 25.8, 25.6; HRMS (EI) calcd for  $C_{44}H_{48}BrN_5O_3$   $[M]^+$ : 773.2941. Found: 773.2944.

**4.1.16. (S)-2-(((3S,4aR,8aS)-2-[(1,1'-Biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methyl)amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

TFA/ $CH_2Cl_2$ /TIS/ $H_2O$  (10:10:1.0:1.0, 5.5 mL) was added to **18** (40 mg, 0.052 mmol). The mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure. The residue was diluted with AcOEt and basified by saturated aqueous  $NaHCO_3$ . The whole was extracted with AcOEt and the organic layer was washed with brine, dried over  $Na_2SO_4$ , filtered, and concentrated. The residue was purified by silica gel column chromatography ( $CHCl_3/CH_3OH$  = 10:1) to give the de-tritylated product (25 mg, 90%) as a yellowish oil.  $[\alpha]_D^{28}$  –33 (c 0.51,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.68–7.36 (m, 10H), 6.84 (s, 0.6H), 6.82 (s, 0.4H), 5.03–5.01 (m, 0.4H), 4.31–4.27 (m, 0.6H), 4.15 (br s, 0.6H), 3.86 (br s, 0.4H), 3.73 (s, 1.2H), 3.66 (s, 1.8H), 3.54–3.51 (m, 1H), 3.25 (s, 1.2H), 3.19 (s, 1.8H), 3.00–2.86 (m, 1H), 2.75–2.62 (m, 2H), 2.52–2.44 (m, 2H), 1.77–1.68 (m, 3.4H), 1.62–1.59 (m, 1.6H), 1.49–1.23 (m, 5H), 1.17–1.11 (m, 0.4H), 1.07–0.99 (m, 1H), 0.89–0.85 (m, 0.6H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 174.4, 171.0, 143.1, 142.4, 140.2, 140.1, 135.8, 135.3, 135.2, 134.8, 128.84, 128.83, 128.2, 127.8, 127.7, 127.5, 127.20, 127.18, 127.14, 127.08, 77.2, 61.7, 59.8, 58.4, 55.7, 49.5, 49.4, 48.6, 48.1, 43.5, 42.6, 42.0, 36.7, 34.3, 34.1, 33.0, 32.9, 32.2, 30.0, 29.6, 26.2, 26.0, 25.8, 25.6; HRMS (EI) calcd for  $C_{31}H_{39}N_5O_3$   $[M]^+$ : 529.3053. Found: 529.3057.

Compounds **19**, **20**, and **21** were similarly treated with TFA/ $CH_2Cl_2$ /TIS/ $H_2O$  (10:10:1.0:1.0, 5.5 mL) as above to yield the corresponding de-tritylated products.

**4.1.17. From 19: (S)-2-(((3S,4aR,8aS)-2-(4-bromobenzoyl)-decahydroisoquinolin-3-yl)methyl)amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide**

Yellowish oil; yield, 70%;  $[\alpha]_D^{28}$  –33.9 (c 0.415,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.66 (s, 0.6H), 7.56–7.53 (m, 2H), 7.38 (s, 0.4H), 7.32 (d,  $J$  = 8.4 Hz, 1.2H), 7.19 (d,  $J$  = 8.4 Hz, 0.8H), 6.83 (s, 0.6H), 6.81 (s, 0.4H), 4.97–4.95 (m, 0.4H), 4.26–4.22 (m, 0.6H), 4.00–3.98 (m, 0.6H), 3.85–3.84 (m, 0.4H), 3.72 (s, 1.2H), 3.66 (s, 1.8H), 3.56–3.53 (m, 0.6H), 3.35 (dd,  $J$  = 13.4, 3.8 Hz, 0.4H), 3.24 (s, 1.2H), 3.20 (s, 1.8H), 2.99–2.83 (m, 2H), 2.71–2.60 (m, 2H), 2.54–2.41 (m, 2H), 1.77–1.58 (m, 4H), 1.51–1.33 (m, 1H), 1.30–1.17 (m, 5H), 1.05–0.80 (m, 2H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 174.3, 173.1, 170.2, 135.8, 135.4, 135.3, 134.7, 131.9, 131.7, 129.3, 128.3, 124.2, 123.7, 77.2, 61.7, 59.7, 58.4, 55.8, 49.6, 49.4, 48.5, 48.0, 43.5, 42.6, 42.0, 36.6, 34.2, 34.0, 33.0, 32.8, 32.6, 29.9, 29.6, 26.1, 26.0, 25.8, 25.6; HRMS (EI) calcd for  $C_{25}H_{34}BrN_5O_3$   $[M]^+$ : 531.1845. Found: 531.1839.

**4.1.18. From 20: (S)-2-(((3R,4aS,8aR)-2-[(1,1'-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methyl)amino)-N-methoxy-N-methyl-3-(1H-imidazol-4-yl)propanamide**

Yellowish oil; yield, quantitative;  $[\alpha]_D^{28}$  –41 (c 0.45,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.65–7.59 (m, 4H), 7.54 (s, 1H), 7.49–7.44 (m, 4H), 7.39–7.36 (m, 1H), 6.78 (m, 1H), 5.21–5.20 (m, 0.75H), 4.52–4.49 (m, 0.25H), 4.12 (m, 0.25H), 3.90–3.88 (m, 0.75H), 3.67 (s, 2.25H), 3.67–3.65 (m, 0.75H), 3.56 (s, 0.75H), 3.56–3.49 (m, 0.75H), 3.25 (s, 2.25H), 3.25–3.21 (m, 0.25H), 3.21 (s, 0.75H), 3.11–3.05 (m, 0.25H), 2.98–2.95 (m, 0.75H), 2.89–2.83 (m, 0.75H), 2.63–2.52 (m, 1.5H), 2.37 (dd,  $J$  = 12.0, 4.4 Hz, 1H), 2.29 (m, 1H), 1.72 (br s, 2H), 1.62–1.41 (m, 4H), 1.30–1.22 (m, 2H), 1.19–1.06 (m, 0.75H), 1.00–0.85 (m, 1.25H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 174.9, 171.6, 171.0, 142.4, 140.2, 135.6, 135.4, 135.2, 134.4, 128.9, 128.8, 127.7, 127.4, 127.23, 127.16, 127.1, 127.0, 77.2,

61.7, 58.5, 55.5, 49.4, 49.1, 47.5, 42.8, 42.3, 36.9, 36.8, 35.2, 34.3, 33.1, 32.9, 32.3, 29.9, 29.65, 29.56, 29.2, 26.2, 26.0, 25.8, 25.6; HRMS (EI) calcd for  $C_{31}H_{39}N_5O_3$   $[M]^+$ : 529.3053. Found: 529.3060.

**4.1.19. From 21: (S)-2-(((3R,4aS,8aR)-2-(4-bromobenzoyl)-decahydroisoquinolin-3-yl)methyl)amino)-N-methoxy-N-methyl-3-(1H-imidazol-4-yl)propanamide**

Yellowish oil; yield, 65%;  $[\alpha]_D^{28}$  –27.7 (c 0.96,  $CHCl_3$ );  $^1H$  NMR (400 MHz):  $\delta$  = 7.57–7.47 (m, 3H), 7.30–7.27 (m, 2H), 6.79 (s, 0.25H), 6.78 (s, 0.75H), 5.17–5.14 (m, 0.75H), 4.45 (dd,  $J$  = 13.4, 3.4 Hz, 0.25H), 3.94 (br s, 0.25H), 3.87–3.86 (m, 0.75H), 3.67 (s, 2.25H), 3.59 (s, 0.75H), 3.39 (dd,  $J$  = 13.6, 3.2 Hz, 0.75H), 3.25 (s, 2.25H), 3.21 (s, 0.75H), 3.19–3.16 (m, 0.75H), 3.07–3.01 (m, 0.25H), 2.98–2.89 (m, 1H), 2.82 (dd,  $J$  = 13.4, 11.8 Hz, 0.75H), 2.70–2.48 (m, 1.5H), 2.36 (dd,  $J$  = 12.2, 4.6 Hz, 0.75H), 2.30 (dd,  $J$  = 11.8, 5.8 Hz, 0.25H), 1.82–1.61 (m, 5H), 1.48–1.28 (m, 5H), 1.09–1.04 (m, 0.75H), 0.98–0.87 (m, 1.25H);  $^{13}C$  NMR (100 MHz):  $\delta$  = 174.9, 170.7, 170.2, 135.6, 135.31, 135.26, 134.5, 131.8, 131.6, 128.7, 128.4, 123.8, 123.5, 77.2, 61.7, 58.4, 58.0, 55.4, 49.5, 49.1, 47.5, 47.4, 42.8, 42.7, 42.2, 36.8, 36.7, 35.1, 34.2, 33.0, 32.9, 32.3, 29.8, 29.5, 29.2, 26.1, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For  $C_{25}H_{34}BrN_5O_3$   $[M]^+$ : 531.1845. Found: 531.1839.

**4.1.20. (S)-2-(((3S,4aR,8aS)-2-[(1,1'-Biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methyl)amino)-3-(1H-imidazol-4-yl)-propanal 22**

To a solution of above de-tritylated product of **18** (33 mg, 0.61 mmol) in  $CH_2Cl_2$  (1 mL), DIBALH (1.0 mol/L solution in hexane, 1.2 mL, 1.2 mmol) was added drop-wise at –78 °C. The reaction mixture was stirred for 5 min. The reaction was quenched with  $CH_3OH$  and concentrated. The residue was dissolved in  $CH_3OH$  and filtered through a silica gel layer. The filtrate was concentrated. The residue was purified by HPLC to give **22** (10.5 mg, 28%) as a colorless oil.  $[\alpha]_D^{28}$  –3.2 (c 0.48,  $CH_3OH$ );  $^1H$  NMR (500 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.80 (br s, 1H), 7.75–7.73 (m, 2H), 7.67–7.65 (m, 2H), 7.58 (d,  $J$  = 8.4 Hz, 2H), 7.50 (br s, 1H), 7.48–7.45 (m, 2.5H), 7.40–7.36 (m, 1.5H), 5.13 (m, 1H), 4.82 (dd,  $J$  = 8.4, 3.2 Hz, 1H), 3.88–3.79 (m, 2H), 3.63–3.59 (m, 1H), 3.43–3.40 (m, 1H), 3.34 (s, 1H), 2.97 (t,  $J$  = 12.6 Hz, 1H), 1.77–1.68 (m, 5H), 1.45–1.34 (m, 5H), 1.06–0.98 (m, 2H);  $^{13}C$  NMR (125 MHz,  $CD_3OD$ , referenced to  $CD_3OD$ ):  $\delta$  = 175.1, 175.0, 163.0, 162.7, 144.70, 144.66, 141.1, 141.04, 135.5, 134.86, 134.80, 129.87, 129.86, 129.00, 128.97, 128.92, 128.0, 127.9, 118.6, 95.0, 94.9, 61.3, 61.0, 50.7, 50.5, 49.6, 47.2, 47.1, 43.2, 43.1, 37.59, 37.56, 35.2, 33.5, 30.2, 26.9, 26.5, 23.1, 22.9; HRMS (ESI) calcd for  $C_{29}H_{35}N_4O_2$   $[M+H]^+$ : 471.2760. Found: 471.2760.

**4.1.21. (S)-2-(((3S,4aR,8aS)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl)methyl)amino)-3-(1H-imidazol-4-yl)propanal 23**

A title compound **23** was synthesized from the de-tritylated product of **19** as above. Colorless oil; yield, 36%;  $[\alpha]_D^{28}$  –1.1 (c 0.40,  $CH_3OH$ );  $^1H$  NMR (400 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.72 (br s, 1H), 7.66–7.64 (m, 2H), 7.46 (br s, 1H), 7.41 (d,  $J$  = 8.4 Hz, 2H), 5.11–5.03 (m, 1H), 4.78 (dd,  $J$  = 11.0, 3.0 Hz, 1H), 3.85–3.75 (m, 2H), 3.47–3.39 (m, 2H), 3.26–3.24 (m, 1H), 2.96–2.84 (m, 1H), 1.78–1.54 (m, 5H), 1.43–1.22 (m, 5H), 1.07–0.93 (m, 2H);  $^{13}C$  NMR (100 MHz,  $CD_3OD$ , referenced to  $CD_3OD$ ):  $\delta$  = 174.1, 173.5, 163.1, 162.6, 135.7, 135.45, 135.39, 133.0, 130.39, 130.35, 130.2, 125.84, 125.78, 118.8, 118.7, 95.1, 94.9, 61.3, 61.0, 50.64, 50.58, 43.3, 43.2, 37.71, 37.70, 37.68, 35.25, 35.22, 33.7, 30.4, 30.3, 27.0, 26.6, 23.2, 23.0; HRMS (ESI) calcd for  $C_{23}H_{30}BrN_4O_2$   $[M+H]^+$ : 473.1552. Found: 473.1543.

#### 4.1.22. (S)-2-[(*[(3R,4aS,8aR)-2-[(1,1'-Biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl)methyl*amino]-3-(1*H*-imidazol-4-yl)propanal 24

A title compound **24** was synthesized from the de-tritylated product of **20** as above. Colorless oil; yield, 30%;  $[\alpha]_D^{29} -2.3$  (c 0.61, CH<sub>3</sub>OH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.76 (s, 1H), 7.74 (d,  $J$  = 6.4 Hz, 2H), 7.66 (d,  $J$  = 6.0 Hz, 2H), 7.56 (d,  $J$  = 6.4 Hz, 2H), 7.48–7.45 (m, 3.5H), 7.40–7.37 (m, 1.5H), 5.09 (br s, 1H), 3.86–3.75 (m, 2H), 3.64–3.59 (m, 1H), 3.55–3.48 (m, 1H), 3.35–3.32 (m, 1H), 3.28–3.26 (m, 1H), 2.93–2.91 (m, 1H), 1.79–1.66 (m, 5H), 1.47–1.28 (m, 5H), 1.07–0.97 (m, 2H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD, referenced to CD<sub>3</sub>OD):  $\delta$  = 175.5, 175.4, 163.1, 162.8, 145.0, 141.2, 135.7, 135.6, 134.9, 130.1, 129.2, 129.1, 128.2, 128.1, 119.0, 95.4, 95.1, 62.1, 61.6, 50.8, 43.2, 43.1, 37.7, 35.5, 35.4, 33.8, 33.7, 30.4, 27.0, 26.6, 24.5, 24.1; LRMS (ESI) calcd for C<sub>29</sub>H<sub>35</sub>N<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 471.28. Found: 471.30.

#### 4.1.23. (S)-2-[(*[(3R,4aS,8aR)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl)methyl*amino]-3-(1*H*-imidazol-4-yl)propanal 25

A title compound **25** was synthesized from the de-tritylated product of **21** as above. Colorless oil; yield, 28%;  $[\alpha]_D^{29} -7.8$  (c 0.36, CH<sub>3</sub>OH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, referenced to residual CH<sub>3</sub>OH):  $\delta$  = 8.72 (br s, 1H), 7.66–7.62 (m, 2H), 7.45 (s, 1H), 7.43–7.36 (m, 2H), 5.06 (m, 1H), 3.83–3.75 (m, 2H), 3.49–3.46 (m, 2H), 3.34–3.33 (m, 1H), 3.28–3.23 (m, 1H), 2.92–2.85 (m, 1H), 1.76–1.58 (m, 5H), 1.44–1.26 (m, 5H), 1.06–0.93 (m, 2H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD, referenced to CD<sub>3</sub>OD):  $\delta$  = 174.43, 174.35, 163.1, 162.8, 135.7, 135.6, 135.3, 133.0, 130.3, 125.9, 118.8, 95.4, 95.1, 62.1, 61.6, 50.7, 43.1, 43.0, 37.7, 37.6, 35.4, 35.3, 33.7, 30.3, 27.0, 26.6, 24.6, 24.1; LRMS (ESI) calcd for C<sub>23</sub>H<sub>30</sub>BrN<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 473.16. Found: 473.25.

#### 4.1.24. (1*S*,6*R*)-6-{2-[(4-Bromobenzyl)oxy]ethyl}cyclohex-3-enecarboxylic acid 31

To a solution of 1,3-butadiene (20 wt% solution in toluene, 108 mL, 255 mmol) was added (*E*)-ethyl 5-[(4-bromobenzyl)oxy]pent-2-enoate<sup>32</sup> (20.0 g, 63.9 mmol), and the mixture was heated at 225 °C for 60 h. After the reaction mixture was cooled to room temperature, water was added and the whole was extracted with AcOEt. The organic layer was washed with 1 M HCl and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 35:1) to give an ethyl ester of **29**, (1*S*/*R*, 6*R*/*S*)-ethyl 6-{2-[(4-bromobenzyl)oxy]ethyl}cyclohex-3-enecarboxylate, (11.7 g, 50%) as a yellow pale oil. <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.47–7.45 (m, 2H), 7.22 (d,  $J$  = 8.4 Hz, 2H), 5.65 (m, 2H), 4.43 (dd,  $J$  = 18.8, 12.0 Hz, 2H) 4.14 (q,  $J$  = 7.2 Hz, 2H), 3.52–3.49 (m, 2H), 2.41–2.20 (m, 4H), 2.08–2.03 (m, 1H), 1.81–1.72 (m, 2H), 1.53–1.46 (m, 1H), 1.26 (t,  $J$  = 7.2 Hz, 3H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 175.8, 137.5, 131.4, 129.2, 125.7, 124.8, 121.3, 72.1, 68.1, 60.3, 45.3, 33.7, 32.4, 29.9, 28.1, 14.3; HRMS (EI) Calcd for C<sub>18</sub>H<sub>23</sub>BrO<sub>3</sub> [M]<sup>+</sup>: 366.0831. Found: 366.0826.

The above ester (31.8 g, 86.6 mmol) was dissolved in 2 M NaOH/THF (1:1, 100 mL). After being stirred for 15 h under reflux, the reaction mixture was cooled to room temperature. The mixture was acidified with 2 M HCl, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue purified by silica gel column chromatography (hexane/AcOEt = 3:1). The product was dissolved in AcOEt (300 mL) and then (*S*)-(-)-phenylethylamine (11 mL, 87 mmol) was added. After 12 h, the solid was collected by suction filtration. The free acid was liberated from the salt by treatment with 2 M HCl and extraction with AcOEt. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by silica gel column chroma-

tography (hexane/AcOEt = 3:1) to give **31** [7.63 g, 26% (50% max.)] as a colorless oil.  $[\alpha]_D^{28} +22$  (c 0.78, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.47–7.45 (m, 2H), 7.20 (d,  $J$  = 8.0 Hz, 2H), 5.69–5.66 (m, 2H), 4.44 (dd,  $J$  = 17.0, 12.2 Hz, 2H) 3.56–3.49 (m, 2H), 2.47–2.20 (m, 4H), 2.12–2.07 (m, 1H), 1.91–1.75 (m, 2H), 1.60–1.51 (m, 1H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 181.1, 137.3, 131.5, 129.3, 125.7, 124.5, 121.4, 72.2, 68.0, 44.9, 33.6, 32.1, 29.5, 27.7; HRMS (EI) calcd for C<sub>16</sub>H<sub>19</sub>BrO<sub>3</sub> [M]<sup>+</sup>: 338.0518. Found: 338.0520.

#### 4.1.25. [(1*S*,6*R*)-6-{2-[(4-Bromobenzyl)oxy]ethyl}cyclohex-3-en-1-yl]methanol

To a solution of **31** (7.70 g, 22.7 mmol) in THF (80 mL), Et<sub>3</sub>N (6.4 mL, 46 mmol) and IBCF (4.5 mL, 34 mmol) were added at –20 °C. After being stirred for 15 min at the same temperature, NaBH<sub>4</sub> (3.47 g, 91.2 mmol) and H<sub>2</sub>O (10 drops from a pipette) was added. The mixture was warmed up to room temperature and then the reaction was quenched with saturated aqueous NH<sub>4</sub>Cl. The whole was extracted with AcOEt and the organic layer was washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3:1) to give a title alcohol (5.68 g, 77%) as a colorless oil.  $[\alpha]_D^{29} +22$  (c 0.65, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.48–7.46 (m, 2H), 7.20 (d,  $J$  = 8.4 Hz, 2H), 5.65–5.58 (m, 2H), 4.45 (s, 2H), 3.68 (dd,  $J$  = 10.8, 6.4 Hz, 1H), 3.62 (dd,  $J$  = 10.8, 5.2 Hz, 1H), 3.58–3.47 (m, 2H), 2.15–2.09 (m, 2H), 2.00–1.76 (m, 4H), 1.66–1.49 (m, 3H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 137.4, 131.5, 129.3, 125.8, 125.5, 121.4, 72.3, 68.7, 65.0, 39.7, 32.9, 31.1, 29.5, 26.6; HRMS (EI) calcd for C<sub>16</sub>H<sub>21</sub>BrO<sub>2</sub> [M]<sup>+</sup>: 324.0725. Found: 324.0732.

#### 4.1.26. [(1*S*,6*R*)-6-{2-[(4-Bromobenzyl)oxy]ethyl}cyclohex-3-en-1-yl]methoxy(tert-butyl)diphenylsilane

TBDPS-Cl (5.0 mL, 19 mmol) was added to a solution of above alcohol (5.66 g, 17.4 mmol) and imidazole (1.43 g, 21.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL), and the mixture was stirred for 8 h at room temperature. The reaction was quenched with saturated aqueous NH<sub>4</sub>Cl, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 30:1) to give a di-protected alcohol compound (9.81 g, quant.) as a colorless oil.  $[\alpha]_D^{28} +18.6$  (c 1.74, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.67–7.64 (m, 4H), 7.44–7.34 (m, 8H), 7.17 (d,  $J$  = 8.4 Hz, 2H), 5.63–5.54 (m, 2H), 4.41 (dd,  $J$  = 15.2, 12.0 Hz, 2H), 3.68 (dd,  $J$  = 9.8, 5.4 Hz, 1H), 3.62 (dd,  $J$  = 10.0, 6.8 Hz, 1H), 3.50–3.46 (m, 2H), 2.16–1.96 (m, 3H), 1.87–1.81 (m, 2H), 1.71–1.68 (m, 2H), 1.48–1.44 (m, 1H), 1.05 (s, 9H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 137.7, 135.61, 135.60, 133.94, 133.92, 131.4, 129.5, 129.1, 127.6, 125.8, 125.3, 121.2, 72.1, 68.7, 65.9, 39.6, 32.9, 30.8, 29.0, 26.9, 26.7, 19.3; HRMS (FAB) Calcd. For C<sub>32</sub>H<sub>40</sub>BrO<sub>5</sub> [M+H]<sup>+</sup>: 563.1981. Found: 563.1988.

#### 4.1.27. 2-[(1*R*,2*S*)-2-[(tert-Butyldiphenylsilyl)oxy]methyl]cyclohexyl]ethanol 32

To a solution of above di-protected alcohol (9.81 g, 17.4 mmol) in CH<sub>3</sub>OH/EtOAc/saturated aqueous NaHCO<sub>3</sub> (5:5:1, 110 mL), Pd-C (3.8 g) was added, and the mixture was stirred under a hydrogen gas atmosphere at room temperature for 6 h. The mixture was filtered through Celite and a silica gel layer, and the filtrate was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 6:1) to give **32** (6.90 g, quant.) as a colorless oil.  $[\alpha]_D^{28} +12$  (c 0.65, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 7.68–7.65 (m, 4H), 7.43–7.36 (m, 6H), 3.67–3.56 (m, 4H), 1.78–1.70 (m, 5H), 1.37–1.21 (m, 6H), 1.06 (s, 9H), 1.02–0.96 (m, 1H); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 135.69, 135.66, 133.91, 133.89, 129.55, 129.54, 127.60, 127.57, 66.5, 61.0, 44.5,

36.5, 35.5, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3; HRMS (FAB) calcd for  $C_{25}H_{37}O_2Si$   $[M+H]^+$ : 397.2563. Found: 397.2558.

**4.1.28. (S)-2-(((3S,4aR,8aS)-2-((1,1'-Biphenyl)-4-carbonyl)decahydroisoquinolin-3-yl)methyl)amino]-3-(1H-imidazol-4-yl)propanal 40**

Title compound was prepared from **32** according to the same procedure<sup>33</sup> employed for the synthesis of **22** starting from enantiomer mixture **7**. Colorless solid; yield, 30%:  $[\alpha]_D^{28}$  –4.3 (c 0.83,  $CH_3OH$ );  $^1H$  NMR (500 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.81 (br s, 1H), 7.75–7.73 (m, 2H), 7.67–7.65 (m, 2H), 7.58 (d,  $J$  = 8.0 Hz, 2H), 7.51 (br s, 1H), 7.48–7.45 (m, 2.5H), 7.40–7.36 (m, 1.5H), 5.14–5.13 (m, 1H), 4.81 (dd,  $J$  = 9.8, 2.6 Hz, 1H), 3.89–3.80 (m, 2H), 3.63–3.59 (m, 1H), 3.44–3.39 (m, 1H), 3.34 (s, 1H), 2.97 (t,  $J$  = 12.6 Hz, 1H), 1.82–1.62 (m, 5H), 1.45–1.28 (m, 5H), 1.09–0.89 (m, 2H);  $^{13}C$  NMR (125 MHz,  $CD_3OD$ , referenced to  $CD_3OD$ ):  $\delta$  = 175.24, 175.17, 163.2, 162.8, 144.9, 144.8, 141.21, 141.20, 135.6, 135.03, 134.97, 130.1, 129.22, 129.18, 129.12, 128.2, 128.1, 118.98, 118.95, 95.0, 94.9, 61.3, 60.9, 50.73, 50.69, 49.8, 47.1, 47.0, 43.33, 43.30, 37.8, 37.7, 35.4, 33.7, 30.4, 27.0, 26.6, 23.1, 22.9; HRMS (ESI) Calcd. For  $C_{29}H_{35}N_4O_2$   $[M+H]^+$ : 471.2760. Found: 471.2765.

Compounds **41**, **44**, and **45–49** listed in Table 1 were similarly prepared as above.

**4.1.29. Compound 41**

**4.1.29.1. (S)-2-(((3S,4aR,8aS)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl)methyl)amino]-3-(1H-imidazol-4-yl)propanal 41.** Colorless solid; yield, 23%:  $[\alpha]_D^{28}$  –0.64 (c 0.88,  $CH_3OH$ );  $^1H$  NMR (400 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.80 (br s, 1H), 7.65–7.63 (m, 2H), 7.49 (br s, 1H), 7.42 (d,  $J$  = 8.4 Hz, 2H), 5.11 (m, 1H), 4.81–4.78 (m, 1H), 3.86–3.78 (m, 2H), 3.47–3.34 (m, 2H), 2.97–2.91 (m, 1H), 1.75–1.60 (m, 5H), 1.42–1.24 (m, 5H), 1.04–0.96 (m, 2H);  $^{13}C$  NMR (100 MHz,  $CD_3OD$ , referenced to  $CD_3OD$ ):  $\delta$  = 174.2, 174.1, 163.2, 162.8, 135.6, 135.44, 135.39, 132.9, 130.40, 130.36, 130.0, 125.8, 125.7, 119.0, 118.9, 95.0, 94.9, 61.2, 60.8, 50.64, 50.60, 43.24, 43.22, 37.69, 37.66, 35.25, 35.23, 33.7, 30.4, 30.3, 27.0, 26.6, 23.1, 22.9; HRMS (ESI) calcd for  $C_{23}H_{30}BrN_4O_2$   $[M+H]^+$ : 473.1552. Found: 473.1546.

**4.1.30. Compound 44**

Colorless solid; yield, 31%:  $[\alpha]_D^{28}$  –1.62 (c 1.23,  $CH_3OH$ );  $^1H$  NMR (500 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.75 (s, 1H), 7.75 (d,  $J$  = 8.0 Hz, 2H), 7.66 (d,  $J$  = 7.2 Hz, 2H), 7.57 (d,  $J$  = 8.0 Hz, 2H), 7.47–7.45 (m, 3.5H), 7.40–7.37 (m, 1.5H), 5.09 (br s, 1H), 3.80 (m, 2H), 3.66–3.63 (m, 1H), 3.51 (m, 1H), 3.26 (m, 1H), 2.93–2.91 (m, 1H), 1.77–1.68 (m, 5H), 1.45–1.35 (m, 5H), 1.07–0.97 (m, 2H);  $^{13}C$  NMR (125 MHz,  $CD_3OD$ , referenced to  $CD_3OD$ ):  $\delta$  = 175.5, 175.4, 163.2, 162.8, 144.9, 141.2, 135.6, 135.5, 134.9, 130.1, 129.2, 129.1, 128.2, 128.1, 119.1, 95.2, 95.0, 62.0, 61.4, 50.8, 43.12, 43.10, 37.73, 37.69, 35.5, 35.4, 33.7, 30.4, 27.0, 26.6, 24.4, 23.9; HRMS (ESI) calcd for  $C_{29}H_{35}N_4O_2$   $[M+H]^+$ : 471.2760. Found: 471.2756.

**4.1.31. Compound 45**

Colorless solid; yield, 30%:  $[\alpha]_D^{28}$  –6.1 (c 1.0,  $CH_3OH$ );  $^1H$  NMR (500 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.82 (br s, 1H), 7.65 (d,  $J$  = 8.4 Hz, 2H), 7.49 (s, 1H), 7.40 (d,  $J$  = 8.0 Hz, 2H), 5.06 (m, 1H), 4.83 (m, 1H), 3.86–3.76 (m, 2H), 3.51–3.43 (m, 2H), 3.27–3.25 (m, 1H), 2.93–2.90 (m, 1H), 1.76–1.66 (m, 5H), 1.44–1.30 (m, 5H), 1.04–0.96 (m, 2H);  $^{13}C$  NMR (125 MHz,  $CD_3OD$ , referenced to  $CD_3OD$ ):  $\delta$  = 174.43, 174.35, 163.1, 162.8, 135.6, 135.5, 135.3, 133.0, 130.4, 125.9, 119.1, 95.3, 95.0, 61.8, 61.3, 50.7, 43.03, 43.01, 37.7, 37.6, 35.4, 35.3, 33.7, 30.33, 30.31, 27.0, 26.6, 24.4, 23.9; HRMS (ESI) calcd for  $C_{23}H_{30}BrN_4O_2$   $[M+H]^+$ : 473.1552. Found: 4731537. Found: 4731537.

**4.1.32. Compound 46**

Colorless solid; yield, 31%:  $[\alpha]_D^{29}$  –6.7 (c 0.10,  $CH_3OH$ );  $^1H$  NMR (400 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.66 (br s, 1H), 7.79–7.73 (m, 2H), 7.64–7.55 (m, 4H), 7.48–7.45 (m, 3.5H), 7.41–7.37 (m, 1.5H), 5.19–5.18 (m, 1H), 4.77 (dd,  $J$  = 12.6, 3.2 Hz, 1H), 3.87–3.79 (m, 2H), 3.63–3.58 (m, 1H), 3.46–3.40 (m, 1H), 3.36–3.34 (m, 0.5H), 3.25–3.23 (m, 1.5H), 2.99–2.93 (m, 1H), 1.79–1.62 (m, 5H), 1.42–1.21 (m, 5H), 1.10–0.89 (m, 2H); LRMS (ESI) calcd for  $C_{29}H_{35}N_4O_2$   $[M+H]^+$ : 471.28. Found: 471.35.

**4.1.33. Compound 47**

Colorless solid; yield, 27% (obtained as the mixture of a diastereomer derived from Pd-mediated cyclization):  $^1H$  NMR (400 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.84 (br s, 1H), 7.59–7.38 (m, 11H), 5.03 (m, 1H), 4.81 (dd,  $J$  = 10.0, 2.8 Hz, 1H), 3.90 (m, 1H), 3.69–3.59 (m, 1H), 3.41 (m, 1H), 3.34 (s, 1H), 3.27–3.24 (m, 1H), 2.93–2.87 (m, 1H), 2.61–2.54 (m, 1H), 1.59–1.56 (m, 2H), 1.50 (d,  $J$  = 10.4 Hz, 1H), 1.42 (d,  $J$  = 12.4 Hz, 1H), 1.18–1.08 (m, 2H), 0.99–0.88 (m, 3H), 0.70–0.61 (m, 1H), 0.46–0.44 (m, 1H); LRMS (ESI) calcd for  $C_{29}H_{35}N_4O_2$   $[M+H]^+$ : 471.28. Found: 471.35.

**4.1.34. Compound 48**

Colorless solid; yield, 25%:  $[\alpha]_D^{29}$  –3.7 (c 0.15,  $CH_3OH$ );  $^1H$  NMR (400 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.61 (br s, 1H), 7.52–7.46 (m, 6H), 7.45–7.41 (m, 1H), 5.13–5.11 (m, 2H), 4.77 (dd,  $J$  = 11.8, 3.4 Hz, 1H), 3.79–3.66 (m, 1H), 3.56–3.50 (m, 1H), 3.42–3.32 (m, 1H), 3.26–3.20 (m, 1H), 2.97–2.91 (m, 1H), 1.78–1.59 (m, 5H), 1.40–1.20 (m, 5H), 1.08–0.86 (m, 2H); LRMS (ESI) calcd for  $C_{23}H_{31}N_4O_2$   $[M+H]^+$ : 395.24. Found: 395.30.

**4.1.35. Compound 49**

Colorless solid; yield, 18%:  $[\alpha]_D^{29}$  –3.6 (c 0.18,  $CH_3OH$ );  $^1H$  NMR (400 MHz,  $CD_3OD$ , referenced to residual  $CH_3OH$ ):  $\delta$  = 8.68 (br s, 1H), 7.56–7.53 (m, 2H), 7.44 (br s, 1H), 7.25–7.19 (m, 3H), 5.10 (m, 1H), 4.77 (dd,  $J$  = 11.4, 3.2 Hz, 1H), 3.84–3.75 (m, 2H), 3.52–3.47 (m, 1H), 3.40–3.34 (m, 1H), 3.25–3.23 (m, 1H), 2.96–2.89 (m, 1H), 1.78–1.65 (m, 5H), 1.43–1.23 (m, 5H), 1.05–0.93 (m, 2H); LRMS (ESI) calcd for  $C_{23}H_{30}FN_4O_2$   $[M+H]^+$ : 413.24. Found: 413.35.

**4.2. Estimation of  $IC_{50}$  values**

Peptide substrate [H-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-NH<sub>2</sub>]<sup>28</sup> (111  $\mu$ M) in a reaction solution (25  $\mu$ L of 20 mM Tris-HCl buffer pH 7.5 containing 7 mM DTT) was incubated with the R1881 SARS 3CL<sup>pro</sup> (56 nM) at 37 °C for 60 min in the presence of various inhibitor concentrations at 37 °C for 60 min. The cleavage reaction was monitored by analytical HPLC [Cosmosil 5C18 column (4.6  $\times$  150 mm), a linear gradient of  $CH_3CN$  (10–20%) in an aq0.1% TFA over 30 min], and the cleavage rates were calculated from the reduction in the substrate peak area. Each  $IC_{50}$  value was obtained from the sigmoidal dose-response curve (see Fig. S1 for a typical sigmoidal curve). Each experiment was repeated 3 times and the results were averaged.

**4.3. X-ray crystallography**

The purified SARS 3CL<sup>pro</sup> in 20 mM Bis-Tris pH 5.5, 10 mM NaCl, and 1 mM DTT was concentrated to 8 mg/mL.<sup>13</sup> Crystals of SARS 3CL<sup>pro</sup> were grown at 4 °C using a sitting-drop vapor diffusion method by mixing it with an equal volume of reservoir solution containing 100 mM MES pH 6.2, 5–10% PEG20000, and 5 mM DTT. Cubic-shaped crystals with dimensions of 0.3 mm  $\times$  0.3 mm  $\times$  0.3 mm grew within 3 days. The crystals were then soaked for 24 h with reservoir-based solution of 100 mM MES



pH 6.2, 5–8% PEG20000, and 5 mM DTT containing 3 mM of **40** or **44**. Crystals were then transferred into a cryobuffer of 100 mM MES pH 6.2, 10% PEG20000, 5 mM DTT, 15% ethylene glycol containing 3 mM of **40** or **44**, and flash-frozen in a nitrogen stream at 100 K. X-ray diffraction data of SARS 3CL<sup>pro</sup> in complexes with inhibitor **40** or **44** were collected at the Spring-8, beamline BL44XU with a Rayonix MX300HE CCD detector at a wavelength of 0.900 Å.

Crystals of SARS 3CL<sup>pro</sup> in a complex with **41** were obtained by co-crystallization using sitting-drop vapor diffusion at 4 °C and mixing an equal volume of protein-inhibitor complex (final inhibitor concentration of 3 mM) and a reservoir solution containing 100 mM MES pH 6.0, 5–6% PEG20000, and 5 mM DTT. Cubic-shaped crystals with dimensions of 0.2 mm × 0.2 mm × 0.2 mm were obtained within 3 days. Crystals were transferred into cryobuffer with 100 mM MES pH 6.0, 6% PEG20000, 5 mM DTT, 15% ethylene glycol, and 3 mM of **41** and then flash-frozen in a nitrogen stream at 100 K. X-ray diffraction data were collected on a Rigaku RAXIS VII imaging-plate detector at a wavelength of 1.5418 Å equipped with an in-house rotating anode FR-E/Super Bright X-ray generator and Confocal VariMax (VariMax HF) optics system.

The structures of SARS 3CL<sup>pro</sup> in a complex with inhibitors were determined by molecular replacement using the Molrep<sup>34</sup> program with a R1881 SARS 3CL<sup>pro</sup> structure (PDB code 3AW1<sup>13</sup>) as the search model. Rigid body refinement and subsequent restrained refinement protocols were performed with the program Refmac 5<sup>35</sup> of the CCP package.<sup>36</sup> The Coot program<sup>37</sup> was used for manual model rebuilding. Water molecules were added using Coot only after the refinement of protein structures had converged. Ligands generated on JLigand<sup>38</sup> software were directly built into the corresponding difference in electron density, and the model was then subjected to an additional round of refinement. The figures for structural representation were generated on Pymol<sup>39</sup> or chimera<sup>40</sup> software.

## 5. PDB ID codes

4TWY, 4TWW, and 4WY3.

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## Supplementary data

Supplementary data (the HPLC data for the evaluation of purities using a reversed-phase or chiral column, typical sigmoidal curves used to obtain IC<sub>50</sub> values, and NMR data of synthesized compounds) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2014.12.028>.

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