博士学位論文

アトピー性皮膚炎治療薬を志向した、キマーゼを阻害する化合物の分子設計と合成に関する研究

第一三共・創薬化学研究所

田中 太作

目次

序章　アトピー性皮膚炎とキマーゼの阻害剤

第 1 節 キマーゼの役割とアトピー性皮膚炎治療への可能性

アトピー性皮膚炎の薬物治療は外用療法が中心であり、ステロイドや免疫抑制剤などが用いられているが、長期間の使用では副作用が懸念され、痒みの抑制を目的に経口の抗ヒスタミン薬も投与されることが多く、治療に対する満足度は必ずしも高くない。キマーゼ（chymase：EC 3.4.21.39）はマスト細胞の顆粒内に含まれるキモトリプシン様セリンプロテアーゼであり[1)]、フェニルアラニン、チロシンなど芳香族アミノ酸を認識し、その C 末端側を切断する。本酵素の生理的基質候補としてはアンジオテンシン I[2)]、膜結合型 stem cell factor (SCF)[3)]、transforming growth factor (TGF)－β前駆体[4)]などが挙げられ、本酵素がさまざまな疾患に関与すると示唆されている。

キマーゼ陽性を示すマスト細胞は主として皮膚や小腸に、特に皮膚に多く分布している[5)]。皮膚が何らかのアレルゲン刺激を受けると、脱顆粒によりキマーゼが細胞外に放出され、膜結合型 SCF などの基質を切断、遊離型 SCF を産生する(Figure 1)。この遊離型 SCF がマスト細胞の c-kit 受容体に結合、細胞内シグナル伝達を経て IgE 受容体が活性化、アトピー性皮膚等を引き起こす[6)]。

一連の研究から、マウスへのキマーゼ皮内投与が浮腫や炎症性細胞の集積を引き起こすこと[7)]、慢性皮膚炎モデルにおけるマスト細胞の増加およびキマーゼ活性の上昇[3)]が見いだされている。また、キマーゼ遺伝子の single nucleotide polymorphism とアトピー性皮膚炎の発症率との相関[8-10)]、アトピー性皮膚炎患者の皮膚で、キマーゼ陽性マスト細胞数の増加[11)]も報告されており、キマーゼのアトピー性皮膚炎への関与が注目されている。

一方、アンジオテンシン I が基質の候補化合物であることから、キマーゼを阻害した際、循環動態に影響を与える可能性が副作用として懸念されてきた。しかし、血管組織にはキマーゼ陽性を示すマスト細胞が存在するものの、正常時にはキマーゼはその顆

粒内に貯蔵され、強力な傷害を受けた場合にのみ、細胞外に放出され作用を示す。これらのことから、心不全を誘発する血管の肥厚や心筋の線維化など、心臓の組織に重篤な疾患がある場合を除き、キマーゼは循環動態に対する直接的な役割は少ない[12]。

その上皮膚においても、キマーゼはアレルゲン刺激を受けた部位でのみ局所的に作用する[10]。本酵素を阻害しても特定部位のみで作用し、全身的な影響は少なく、それに伴う副作用が起こる可能性が少ないと期待される。

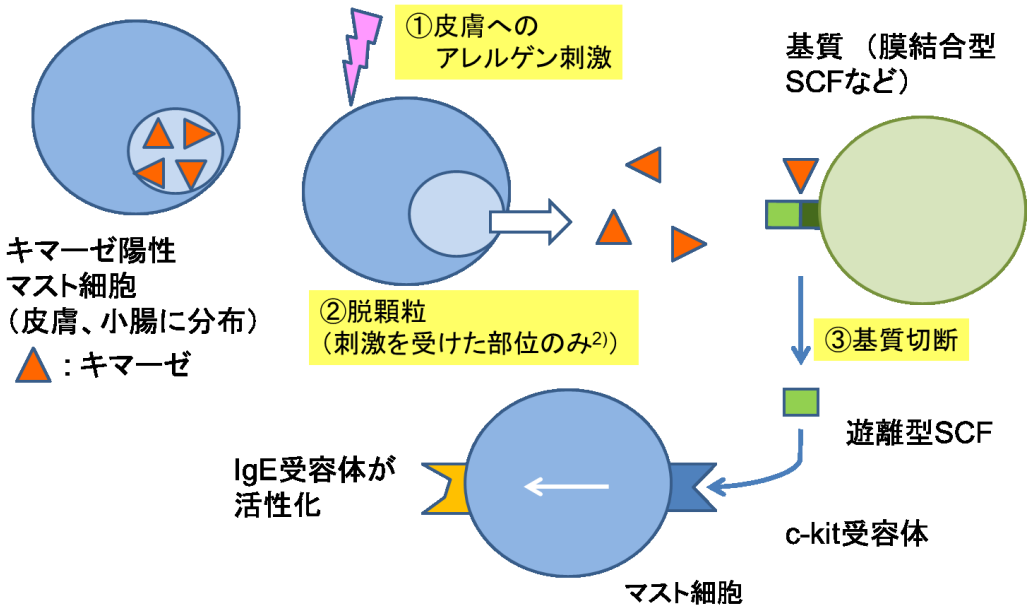


Figure 1 キマーゼによる IgE 受容体活性化および阻害剤が、アトピー性皮膚炎治療薬になりうる可能性

キマーゼを低分子化合物で阻害する場合、同じく生体内に存在する、他のセリンプロテアーゼの阻害に起因する副作用が懸念される。そこで、同様のさまざまな加水分解酵素の活性部位を比較し、副作用の回避に不可欠な、キマーゼに対してのみ強い阻害を示す低分子化合物を探索する手掛りとした。

ここで、基質および酵素の活性部位に関し、本論文で用いる用語について説明する。基質の各アミノ酸残基は、加水分解される部位から N 末端側に P_1 site、C 末端側に P_1', P_2'

site (以後、両者を合わせたものを prime site と定義する)と数える。一方、酵素の活性中心には、基質の P_1 と相互作用する S_1 site、P_1', P_2'と作用する S_1', S_2' site が存在する。さらに、S_1 site を形成する領域の中に、加水分解酵素に特徴的な S_1 hole と呼ばれる、奥行きが深いポケット構造が存在する(Figure 2)

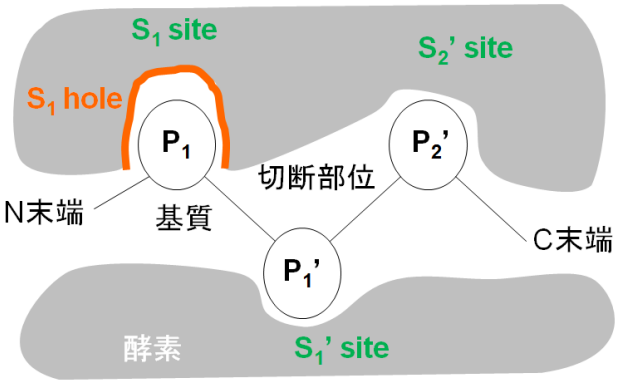


Figure 2　基質および酵素の活性部位の名称

この S_1 hole は、基質の認識に重要であり、その形状により、セリンプロテアーゼは、キモトリプシン様、トリプシン様、エラスターゼ様の 3 種類に分類される(Figure 3)。S_1 hole の大きさや性質は、側面に存在する 216, 226 番目のアミノ酸残基によって主に決められる。キマーゼを含むキモトリプシン様セリンプロテアーゼでは、ともにグリシンである。このため、S_1 hole は広く、疎水的な環境である。

トリプシン様セリンプロテアーゼの両アミノ酸残基も同様にグリシンだが、ポケットの奥に位置するアミノ酸残基は、キモトリプシン様酵素のセリン 189 と異なり、アスパラギン酸 189 である。これにより、トリプシン様酵素は、塩基性を示す官能基と強く結合する可能性が高い。

一方、エラスターゼ様セリンプロテアーゼは、キモトリプシン様酵素と 216, 226 番目のアミノ酸残基が異なる。それぞれバリン 216, スレオニン 226 であり、ポケットは狭く、脂肪族官能基を好む。このように 3 種類のセリンプロテアーゼは、ポケットの特

性により、それぞれ異なった基質認識能を持つ。このことから、トリプシン様、エラスターゼ様セリンプロテアーゼに対し、高い特異性を示す化合物の分子設計は可能と考えた。

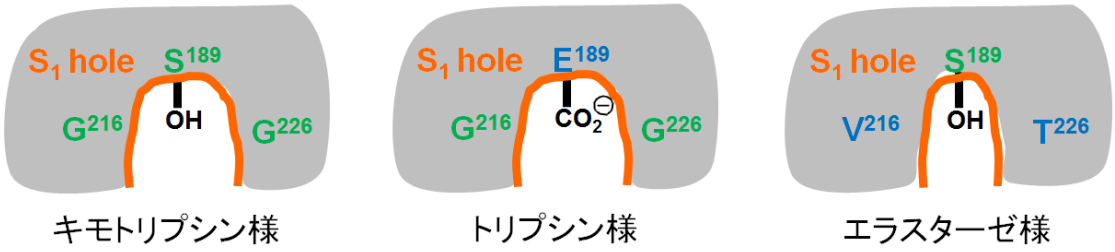


Figure 3　各種セリンプロテアーゼ間における S_1 hole の違い

さらに、この中でキモトリプシン様セリンプロテアーゼ間の特異性に関しては、さまざまな酵素をとりあげてアミノ酸配列の相同性を検討した。相同性はキマーゼに最も近いとされるカテプシン G でも 52%程度と低く [13]、分子構造の工夫により、キマーゼに高い特異性を示す化合物が得られると期待した。

第 2 節　キマーゼ阻害剤に関する先行研究

ヒトキマーゼの X 線結晶構造解析が 1999 年に報告されており、その構造を Figure 4 に示す [14]。活性中心であるセリン 195 の近傍に S_1 hole が確認できる。低分子化合物が阻害剤として、高い親和性を示すには、酵素が 500 $Å^3$ を超えるポケット容積を酵素の活性部位に持つことが重要であるとされている [15]。キマーゼの活性部位に注目すると、その容積の大部分を S_1 hole で充当することができる。このため、Figure 4 の右上に示に示した例のように、分子量が小さく、表面積が限られる阻害剤でも、S_1 hole にその近傍領域を加えたごく限られた酵素の内部と効率良く接触することによって、高い親和性が期待できる。また、これまで、キマーゼと同じように大きな容積の S_1 hole を持つ酵素に対し、その部位に強く結合、薬理作用を発現する低分子量の阻害剤が数多く報告され

ている[16]。これらの前例と同様に、低分子量のキマーゼ阻害剤を創製できると考えた。一方、分子量 500 以下の化合物は、一般に消化管における吸収性が良い[17]。このように「経口投与可能なアトピー性皮膚炎治療薬」を目標として、キマーゼを阻害する化合物を探索する意義は深い。

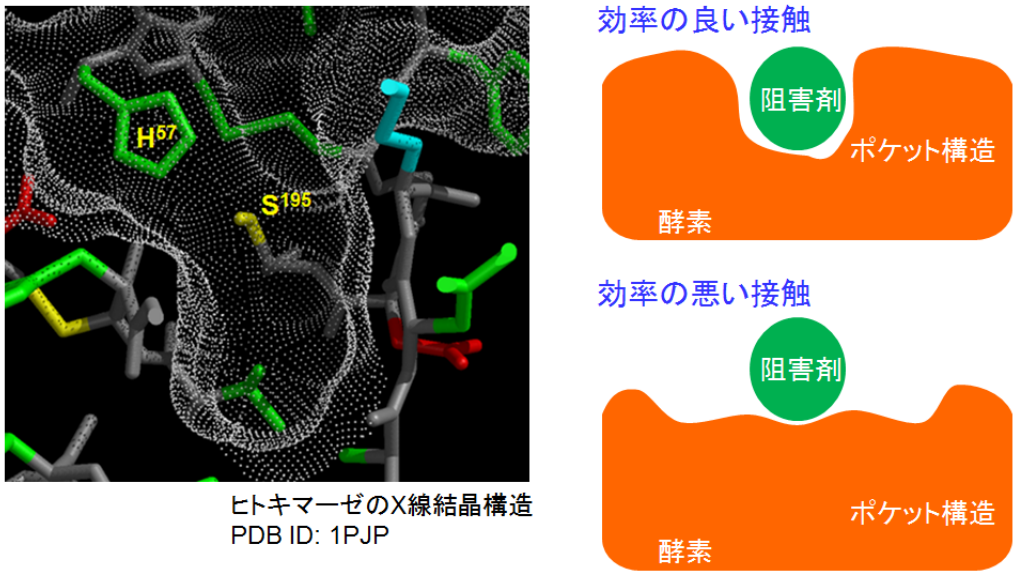


Figure 4 低分子化合物と高い親和性を示す S_1 hole の構造、および、阻害剤－酵素間の接触効率

本研究開始以前に、すでにキマーゼを阻害する物質が広く探索されており、基質に類似したペプチド構造を持つ阻害剤[A[18], B[19], C[20]]が見出されていた(Figure 5)。しかし、これらのいずれも、極性が高いアミド結合を分子内に数多く持ち、消化管を経由する良好な吸収は期待できない。さらに、血中等生体内において、プロテアーゼによりすみやかに分解され、失活すると予想される。そこで、消化管からの吸収性と生体内における安定性の向上を目指し、ペプチド構造を模倣する分子設計が進められた[21]。三菱化学から報告された D[22]を Figure 5 に示す。

一方、多様な構造を持つ低分子化合物を集めたライブラリーのスクリーニング、ホモロジーモデルを用いた in silico screening 等により探索研究が進められた[21]。モデル構

薬には、その時点までに解明されていた、ヒト以外の動物に由来するキマーゼを手本としている。帝人から報告されたE[23]，三菱東京製薬のF[24]，トーアエイヨーのG[25]をFigure 5に示す。いずれも、疎水性のS_1 holeと相互作用しうる、赤で示す部分構造を同じく持っている。ところが、このうちFとGは、消化管を経由する吸収が期待できない。なぜなら、これらの化合物は、カルボキシル基およびスルホンアミドという、生体中の条件下イオン化しうる構造を分子内に2ヶ所持ち、極性が高いからである。

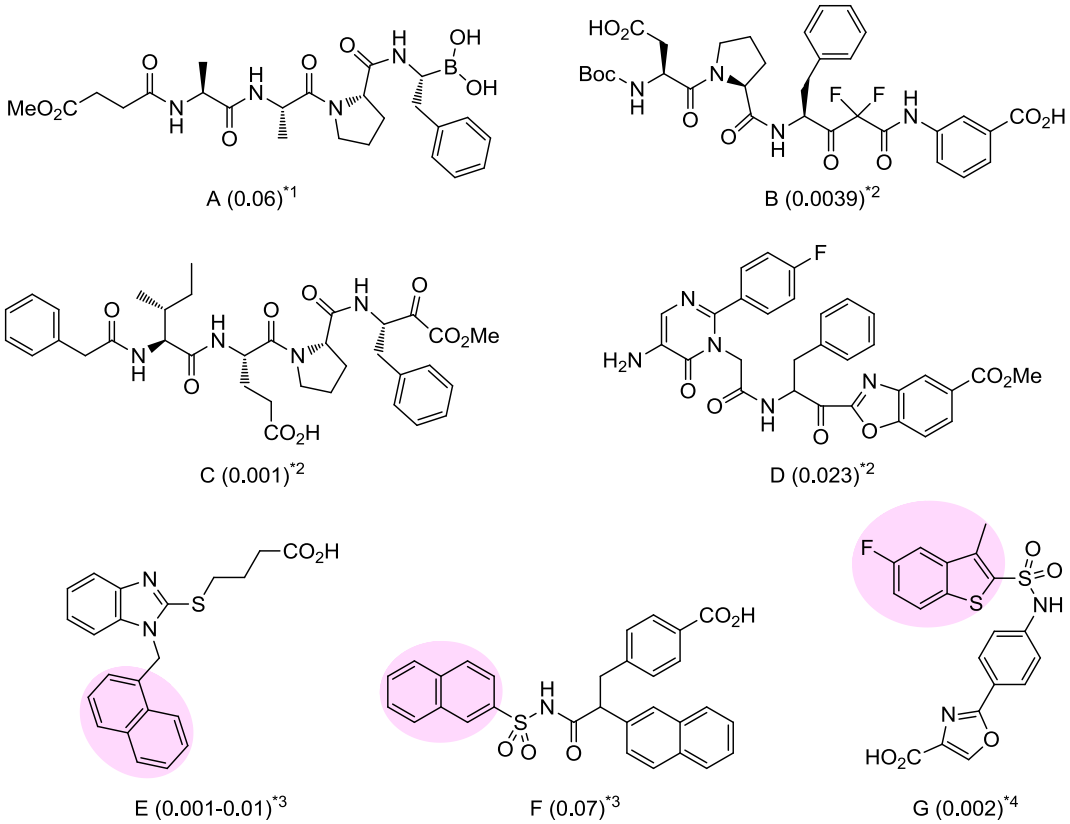
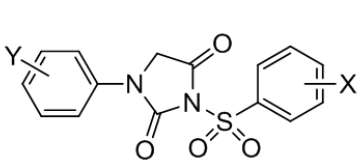


Figure 5 研究開始時期に報告されていた、ペプチド性および低分子のキマーゼ阻害剤

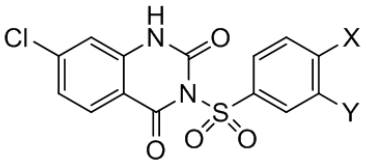
著者が所属したサントリー生物医学研究所においても、経口吸収性を示し、生体内で安定な化合物を志向して、化合物が探索された。その中からイミダゾリジン環を持つ

キマーゼ阻害剤 **1**[26]が 1997 年に見出された。それをベースにさまざまな誘導体が合成され、キナゾリン環を持つ阻害剤 **2**[27]まで到達していた(Table 1)。この化合物は、ヒトキマーゼに対し強い阻害を示した。

Table 1 サントリー生物医学研究所における先行研究

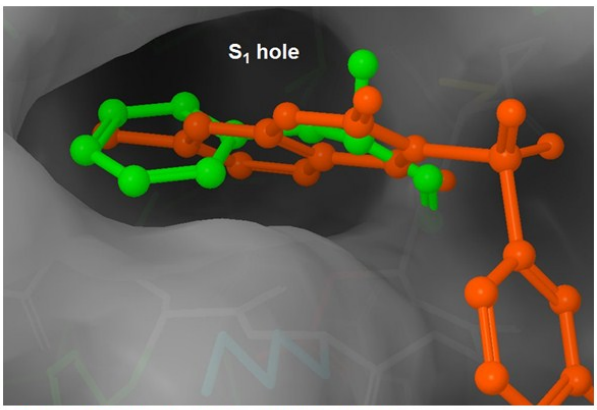


イミダゾリジン型阻害剤 **1**

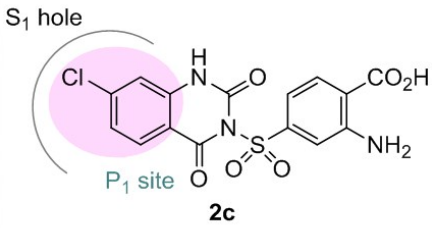


キナゾリン型阻害剤 **2a-c**

化合物	X	Y	ヒトキマーゼに対する IC_{50} (μM)
2a	Cl	H	0.018
2b	H	NH_2	0.36
2c	COOH	NH_2	0.13



オレンジ:キナゾリン型阻害剤 **2c**
緑:アンジオテンシン I （フェニルアラニン部位）



2c

Figure 6 複合体モデルにおいて、酵素の S_1 hole への化合物の P_1 site の収容

複合体モデル作成：アスビオファーマ（株）創薬技術ファンクション、ドッキングプログラム：GOLD

キナゾリン型化合物は、複数の *in vivo* 皮膚炎モデルにおいて薬理効果を示していた。

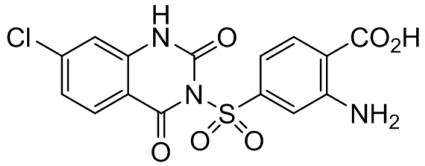
具体的には **2b** や **2c** で、1) アスカリス抽出物によって誘発される、マウス 2 相性皮膚炎モデルを用い、「即時相」および「遅発相」の両者ともに浮腫を抑制する作用 [7]、2) マウスに 2,4-ジニトロフルオロベンゼン（ハプテン）を反復的に塗布して誘発した皮膚炎のモデルで、皮膚の肥厚を抑制する作用 [3, 28]、3) NC / Nga マウスにおける clinical / histological score が低減する作用 [29]が確認されている。しかし、ハプテンを反復塗布する皮膚炎モデルでは、薬効発現には 50 mg / kg 程度 [30]が必要とされ(Table 2)、炎症を抑制する効果は不十分で、アトピー性皮膚炎治療薬として十分とは言えなかった。

著者は、*in vivo* 皮膚炎モデルにおいて、より低い用量の経口投与においても有意な抑制効果を示し、アトピー性皮膚炎治療薬として機能する化合物の創製を目標に、キナゾリン型阻害剤 **2** を出発点として、2001 年に分子設計・合成研究を開始した。

著者は、キナゾリン型化合物 **2c** がキマーゼの S_1 hole と相互作用しうるかドッキングシミュレーションを試みた。探索医薬研究所（現 : アスビオファーマ創薬技術ファンクション）に化合物 **2c** とヒトキマーゼの複合体モデル作成を依頼したところ、本酵素の基質である緑で示したアンジオテンシン I 同様に、P_1 site 芳香環が親和性に重要な S_1 hole に入っていると示唆された(Figure 6)。

しかしキナゾリン **2** は血漿中、半減期が 7 分 [31]と短い(Table 2)。このように生体内で不安定であったことが、*in vivo* 活性の弱い大きな原因と考え、この克服を最大のキーワードとし、まず、S_1 hole との相互作用を維持しつつ、キナゾリン骨格をさまざまに改変することにした。構造改変に伴い、IC_{50} として表わされる活性はさまざまに変化する。その情報を常にフィードバックし、酵素の S_1 site, S_1' site, S_2' site 等への作用を考慮しながら、阻害作用の一層の向上を心がけることとした。

Table 2 キナゾリン型化合物 **2c** の *in vivo* 活性、および血漿中半減期



化合物	抑制傾向を示す 腹腔内投与量 (mg/kg)*1	血漿中半減期 $T_{1/2}$ (min)*2
2c	50	7

*1 ハプテン反復塗布マウスにおける効果

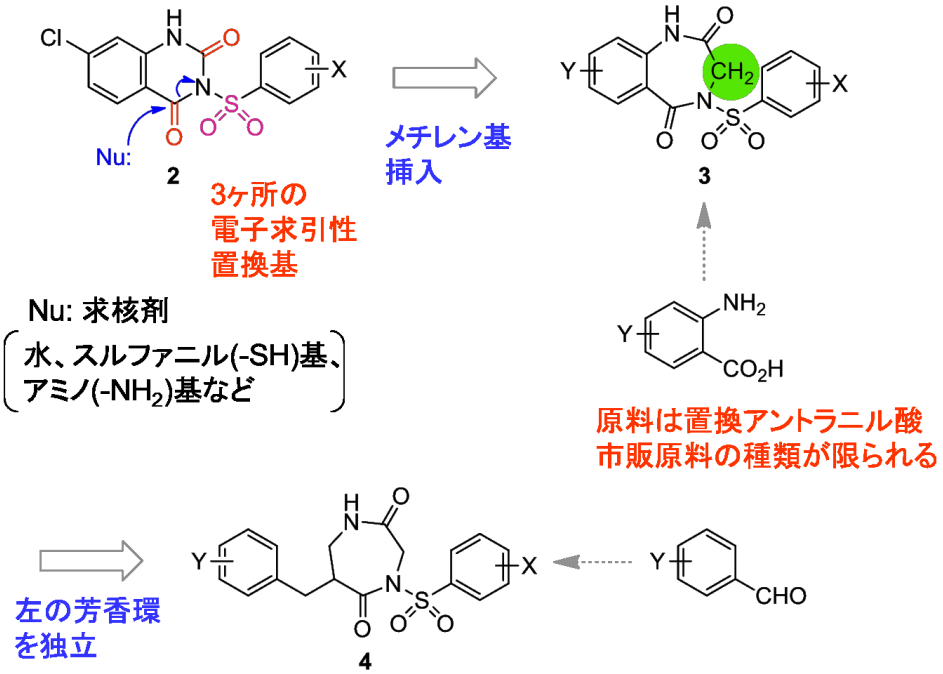
*2 マウス静脈内投与後の血漿中半減期

第 1 章　生体内安定性の向上を意図したジアゼパン骨格への置き換え [32)]

第 1 節 分子設計

既存の阻害剤には、3 ヶ所の電子求引基が隣接する不安定なスルホニルイミド構造が存在する。このカルボニル基は、水や、タンパク質中に含まれるシステイン残基のスルファニル基、リジン残基のアミノ基など求核剤の攻撃を受ける可能性が高く、その結果生体内における安定性が低下したと考えた。

この不安定な構造から脱却しようと、一方の電子求引基との間にメチレン基を挿入した、化合物 **3** を考えた。しかし、この場合、市販原料の種類が限られる置換アントラニル酸を原料として用いる必要があり、結果として合成可能な化合物の種類も限られてしまう。そこで、左端の芳香環を切り離し、ヘテロ環部分をジアゼパン構造とした化合物 **4** を合成することにした。こうすると多彩な構造を持ち、市販化合物として入手容易な置換ベンズアルデヒドから合成できるようになる(Scheme 1)。



Scheme 1　不安定な部分構造から脱却を目指した分子設計

新しく設計したジアゼパン環を持つ化合物は、序章第 2 節で述べた酵素の活性部位を構成する各サイト、とりわけ S_1 hole に、キナゾリン型化合物 **2c** と同様、効果的に収容される必要がある(Figure 7)。この検証を目的とし、**4a** についてドッキングスタディを実施した。この化合物は一か所のみ不斉中心を有し、鏡像関係にある 2 種類の異性体が存在する。異性体が存在しうる化合物については、可能な全てについてドッキングシミュレーションを依頼した。それらの中から、最も高いドッキングスコアを示した異性体を用いて、以下の議論を進めた。

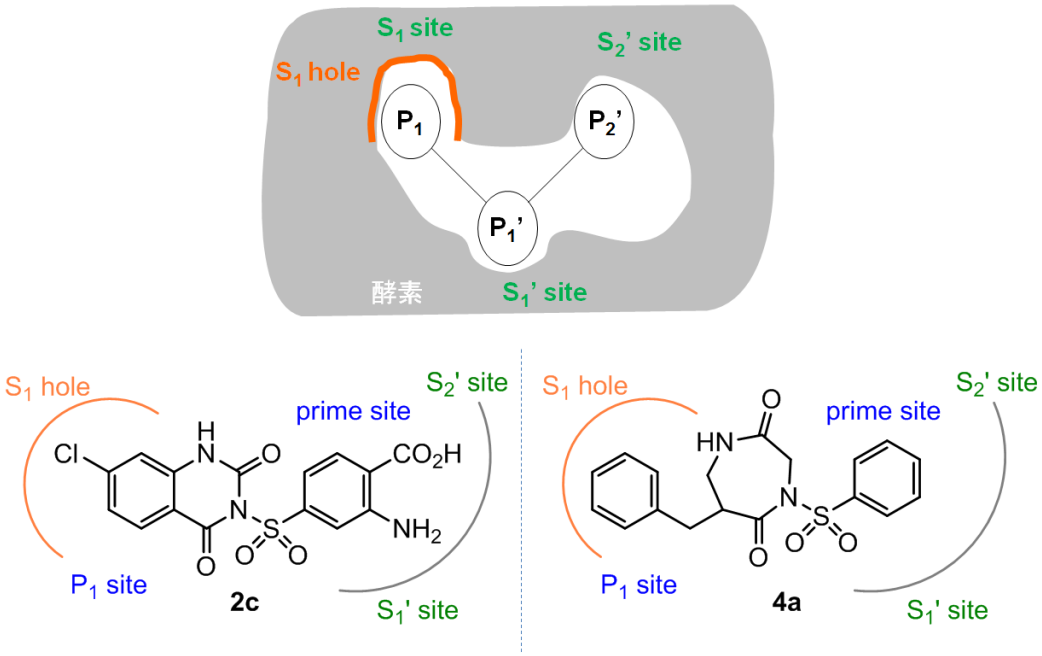


Figure 7 基質の結合様式から推定される、低分子化合物と酵素の相互作用

ジアゼパン環を持つ **4a** がキマーゼの触媒部位と結合する複合体モデルを Figure 8 の右に示した。これを先ほどの **2c** と比較した。青で示した **4a** も、左に示す **2c** と同じように P_1 サイトが S_1 hole の奥深く入り込み、新たに分子設計した化合物は十分に機能すると期待した。さらに、活性の一層の向上を目指し、先行研究により得られていた、キナゾリン型化合物の構造活性相関情報 [27]を参考に、疎水的な S_1 hole により適合するよ

う疎水性の官能基を P_1 site 芳香環に導入することにした。

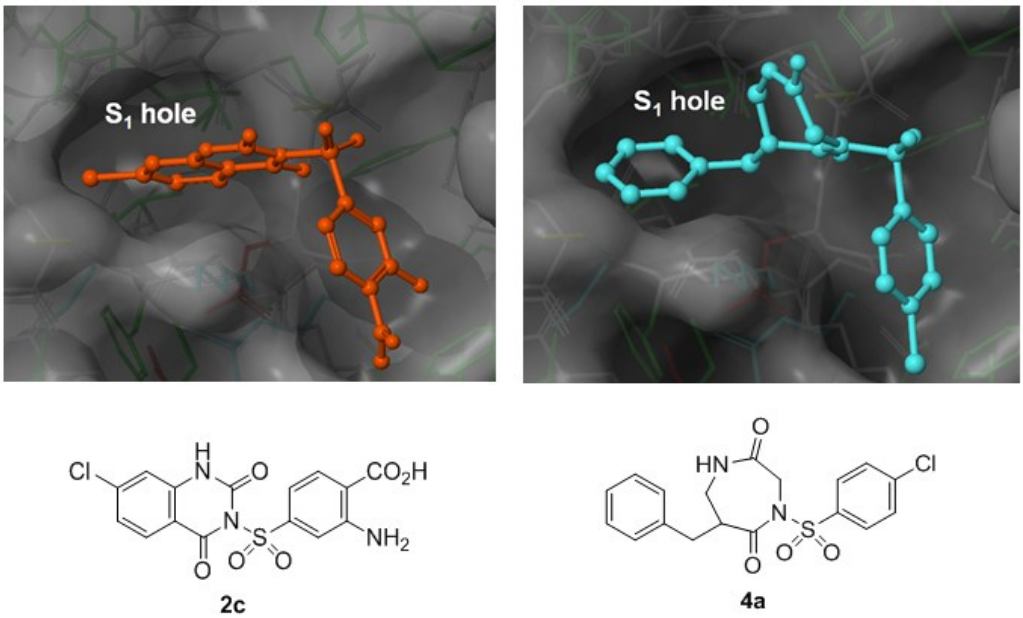


Figure 8 化合物 **2c** および **4a** とヒトキマーゼの複合体モデル

複合体モデル作成：アスビオファーマ（株）創薬技術ファンクション、ドッキングプログラム：GOLD 可能な立体異性体のうち、高いドッキングスコアを示した異性体を示す

一方、Figure 9 に示す複合体モデルの prime site （P_1', P_2' sites を合わせたもの）を解析したところ、化合物の prime site 芳香環が対応する S_2' site に、オレンジで示したリジン 40 の側鎖アミノ基、アルギニン 143 の側鎖グアニジル基という塩基性官能基、青で示したフェニルアラニン 41 の主鎖アミドカルボニル基が存在することが判った。前者は阻害剤に含まれるカルボキシル基と、後者は水素結合しうる官能基との相互作用が期待できる。そこで、化合物の prime site 芳香環にアミノ基、カルボキシル基を導入することにした。

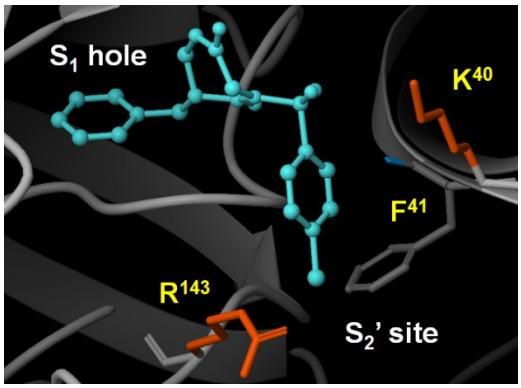


Figure 9 化合物 **4a** とヒトキマーゼの複合体モデル

複合体モデル作成：アスビオファーマ（株）創薬技術ファンクション、ドッキングプログラム：GOLD 可能な立体異性体のうち、高いドッキングスコアを示した異性体を示す

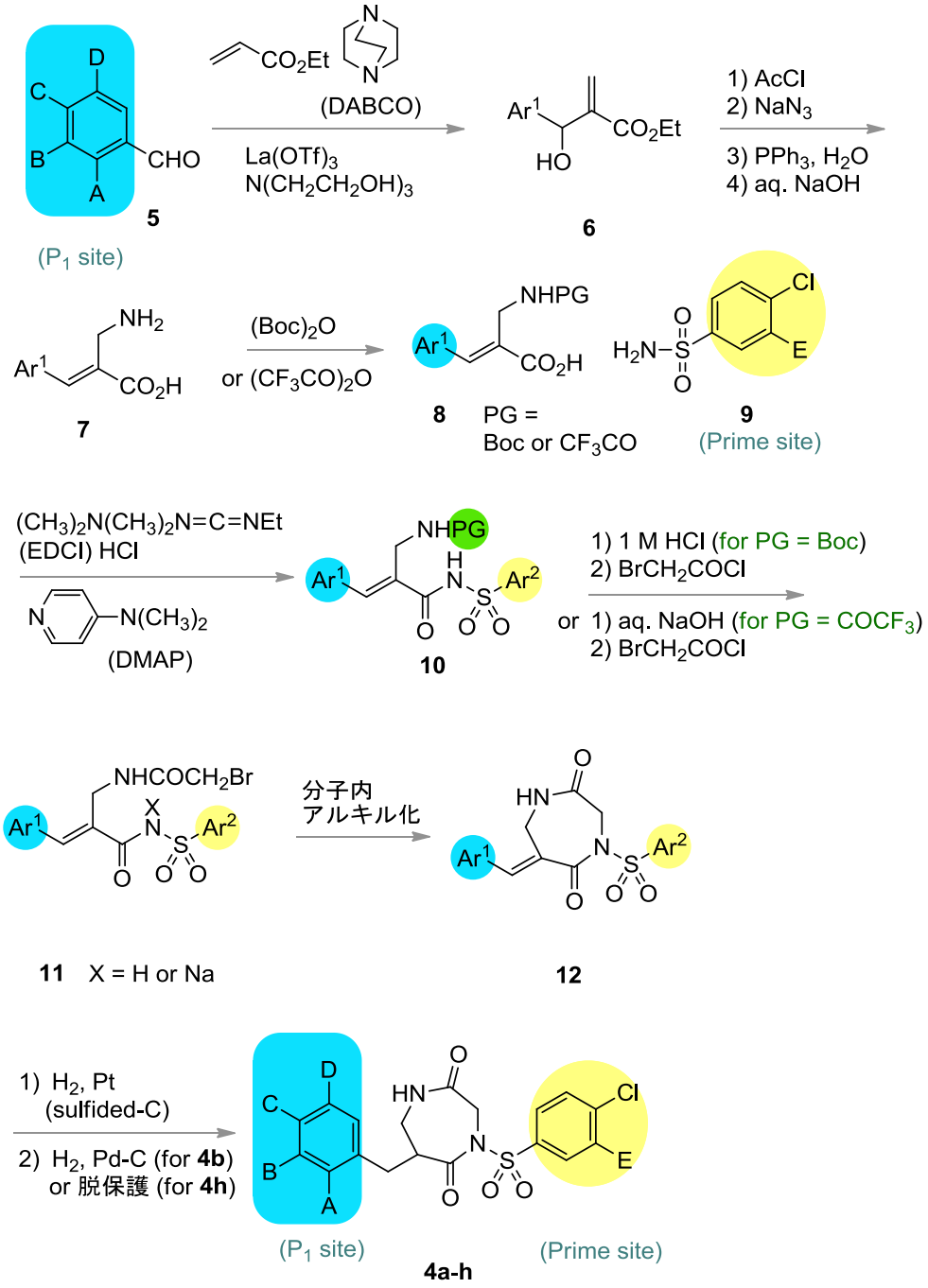
第 2 節 化合物の合成

以上、ジアゼパン環を持つさまざまな化合物を、阻害剤の候補として設定した。合成を Scheme 2 に示す。置換ベンズアルデヒド **5** とアクリル酸エチルから、1,4-ジアザビシクロ[2.2.2]オクタン（DABCO）を用いた Baylis-Hillman 反応により、アリルアルコール **6** を合成した。生じた第二級アルコールをアセチル化、アセトキシ基を脱離基とし、アジ化ナトリウムを求核剤とする S_N2'反応でアジド基を導入し、次いでアジド基の還元とエステルの加水分解によりカルボン酸 **7** を得た。その後、アミノ基を *t*-ブトキシカルボニル(Boc)基またはトリフルオロアセチル基で保護し **8** とした。続いて、**8** と **9** を縮合し、スルホンアミド **10** を合成した。得られた **10** のうち、Boc 基を持つ化合物は酸性条件下脱保護し、ブロモアセチル基を導入後、塩基として水素化ナトリウムを用いた分子内アルキル化により閉環しジアゼパン **12** とした。

一方、トリフルオロアセチル基を持つ **10** は塩基性条件で脱保護し、ブロモアセチル化の工程で遊離型またはナトリウム塩として **11** を調製した。遊離型の **11** は上述の方法と同様に水素化ナトリウムを用い、一方、ナトリウム塩として得た **11** は塩基を用いず加熱するのみで閉環し、**12** を得た。最後に二重結合を還元、さらに、**4b** については **4c**

を接触水素還元することにより塩素原子を除き、また、**4h** は保護基を有する前駆体を脱保護し、**4a-h** とした。

本合成法は、青で示した P_1 site に対応する芳香環、黄色で示す prime site に対応する芳香環を含む前駆体をそれぞれ合成し、合成経路半ばで連結するので、さまざまな置換基が導入可能である。保護基については、導入した置換基の性質にあわせ、酸性条件下除去できる Boc 基、塩基性で除去できるトリフルオロアセチル基の二種類を使い分けている。



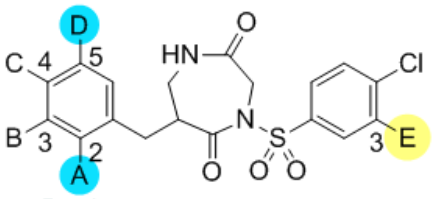
Scheme 2 ジアゼパン型化合物の合成

第 3 節 活性評価と解析

合成した化合物は、ヒトキマーゼの阻害に関しアスビオファーマ免疫・炎症疾患フィールドに測定を依頼した。いずれの化合物も、もとのキナゾリン型化合物と遜色ない活性を示した(Table 3)。さらに、活性の維持には、P_1 site 芳香環上置換基が、以下のように貢献していることが判った。S_1 hole への一層の適合を目指し、P_1 site 芳香環に疎水性官能基として塩素原子を導入した化合物(**4e**, **f**, **g**)の中で、A 位に塩素原子を有する **4e** が最も強い活性を示した。メトキシ基とした **4b** の活性も強く、A 位への置換基導入はいずれも活性向上に寄与していた。さらに、A 位に加え、D 位に塩素原子を持つ **4c** の活性は一層強く、A, D 位への置換基の導入は、相乗的効果を示していた。また、P_1 site 芳香環に置換基を持たない **4a** と比べ、**4c** の活性は 10 倍以上強く、このような大幅な活性の向上は期待通り、親和性発現に重要な部位、つまり、S_1 hole と効果的に相互作用した結果と考えた。複合体モデルが示したように、P_1 site 芳香環は、S_1 hole に入り込み、ジアゼパン骨格への変換は分子設計として妥当であったといえる。

一方、prime site に極性基を加えた化合物(**4d**, **h**)の活性はそれ以上に向上しなかった。導入した極性基が、先述の S_2’ site に存在するアミノ酸残基と相互作用し、活性が一層向上すると期待したが、そのような効果は示されていない。ジアゼパン化合物 **4** のこれらの官能基は、分子設計の段階で懸念された通り、相互作用にはほとんど関わっていなかった。

Table 3 ジアゼパン型化合物の阻害活性

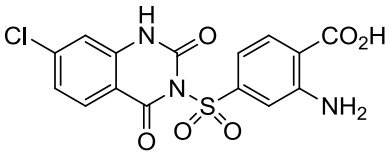


4	A	B	C	D	E	ヒトキマーゼに対するIC50 (μM)*
2c	—	—	—	—	—	0.13
a	H	H	H	H	H	0.42
b	OMe	H	H	H	H	0.14
c	OMe	H	H	Cl	H	0.034
d	OMe	H	H	Cl	NH_2	0.062
e	Cl	H	H	H	H	0.11
f	H	Cl	H	H	H	0.31
g	H	H	Cl	H	H	0.46
h	OMe	H	H	Cl	CO_2H	0.16

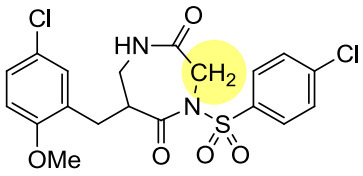
* アスビオファーマ（株）免疫・炎症疾患フィールド

一番強い活性を示した **4c** について、血漿中半減期の測定[33]を依頼したところ、キナゾリン型の **2c** と比べ、その値は 18 分にまで延長していた(Table 4)。しかしその程度では依然として、生体内における安定性は不十分である。皮膚炎モデルを用いた *in vivo* 薬効試験において、阻害剤の効果を正確に判断するには、短くとも 60 分程度の血漿中半減期が必要と考えられ、ジアゼパン型化合物 **4c** は、この目標値に到底至っていない。

Table 4 化合物 **2c** および **4c** の血漿中半減期



2c



4c

化合物	血漿中半減期$T_{1/2}$ (マウス, min)*
2c	7
4c	18
(目標値)	60

* アスビオファーマ（株）創薬技術ファンクション

このように生体内安定性が低い原因を究明するには、ジアゼパン型化合物の挙動を詳細に知る必要がある。そこで、以下の実験を依頼した。キマーゼと阻害剤 **4c** を 30 分間混合し、その後低分子化合物をゲル濾過により取り除き、酵素活性を測定した。ところが、低分子化合物を分離後、2 時間経過した後でも、酵素活性は 58%と依然として低く、回復しなかった。(Figure 10)。

一般的に、酵素と阻害剤が、水素結合やファンデルワールス力など可逆的な相互作用を介して結合する場合、2 時間後には大部分が解離し、酵素の活性は回復する [34]。このような持続的な阻害が残った原因として、今回のケースでは阻害剤とキマーゼが非可逆的に共有結合したと考えた。

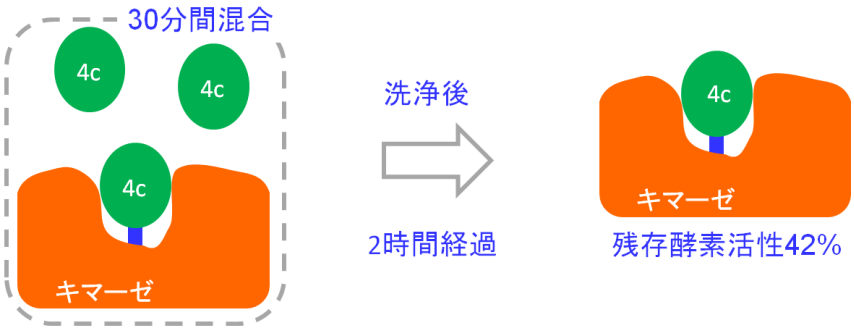
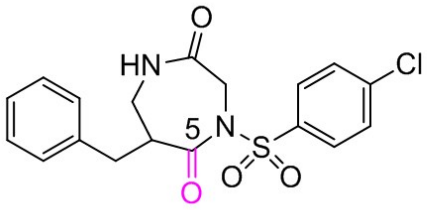


Figure 10 化合物 **4c** とキマーゼの非可逆的相互作用

非可逆的結合が形成する機構を、以下のように考察した。Figure 8 の右に示したドッキングシミュレーションの図を回転し、見る角度を変えたものを改めて Figure 11 として示す。ここに示す **4a** とヒトキマーゼの複合体モデルでは、ジアゼパン環 5 位のカルボニル基を赤紫色で示した。電子求引性のスルホニル基が隣接する、このカルボニル基のすぐ近傍に、キマーゼの活性残基であるセリン 195 に含まれる、黄色で示すヒドロキシ基が位置している。



4a

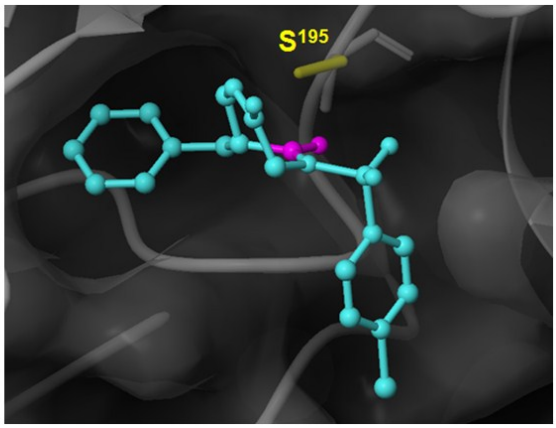


Figure 11 化合物 **4a** とヒトキマーゼの複合体モデル
（Figure 8 の右に示す複合体モデルを回転）

セリン 195 は、ヒスチジン 57, アスパラギン酸 102 とともに catalytic triad と呼ばれ、セリンプロテアーゼが基質を加水分解する機構を担っている(Figure 12)。セリン 195、ヒスチジン 57、アスパラギン酸 102 とプロトンが受け渡され、活性残基であるセリン 195 が活性化、S_1 hole によって認識された基質のアミノ酸残基の C 末端側アミド結合を求核攻撃する。

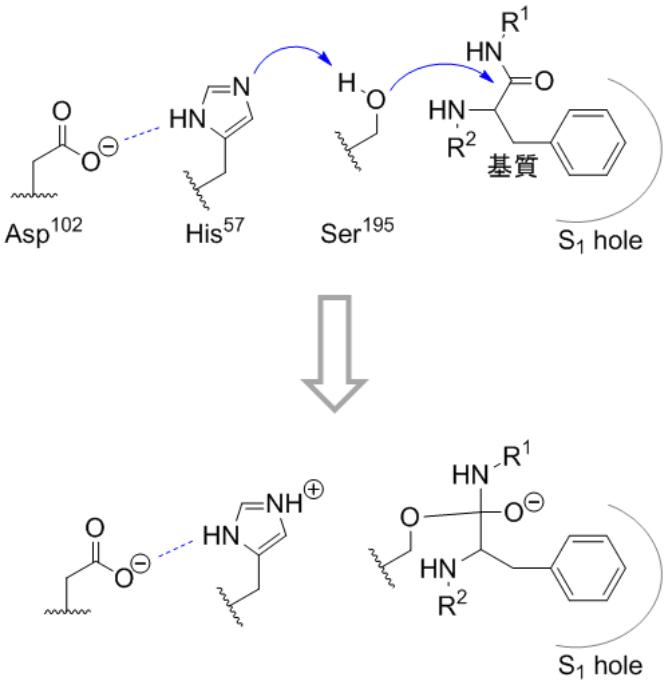


Figure 12 セリンプロテアーゼにおいて、加水分解が触媒される反応機構

ジアゼパン **4c** のカルボニル基に、まずキマーゼの活性残基であるセリン 195 のヒドロキシ基が求核攻撃し、アシル酵素複合体を形成する(Figure 13)。

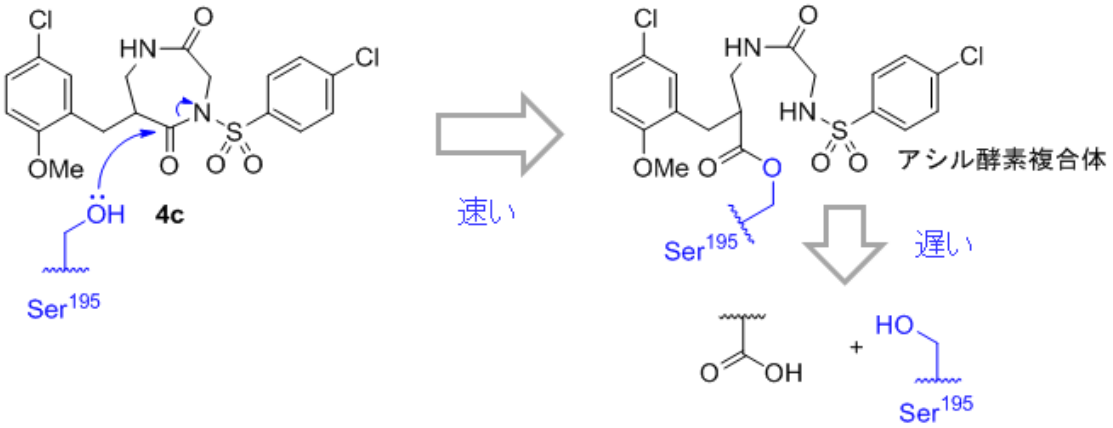


Figure 13 化合物 **4c** とキマーゼが共有結合を形成する機構

次に、この複合体そのものについて、アスビオファーマ創薬技術ファンクションに

X 線回折測定を依頼し、測定結果を解析した。阻害剤にキマーゼの活性部位、すなわちセリン 195 のヒドロキシ基が共有結合したアシル酵素複合体がはっきりと確認できた (Figure 14)。このことから複合体の加水分解が遅いことが確かめられた。

さらに、この X 線結晶構造を見ると、ドッキングシミュレーションで予測した通り、化合物の P_1 site 芳香環が酵素の S_1 hole に入り込んでいる。なお、Prime site については、ジアゼパン環が開環し、キマーゼの活性部位から離脱しているため、複合体モデルとの比較は困難である。

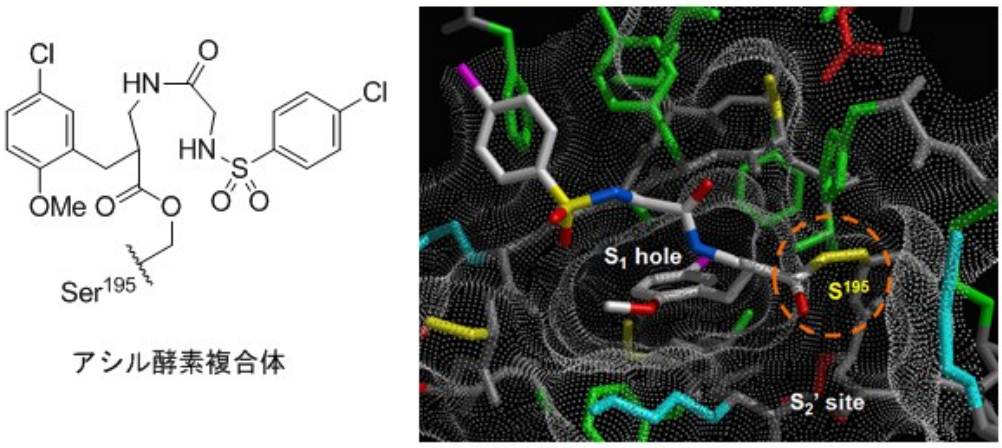


Figure 14 化合物 **4c** とヒトキマーゼのアシル酵素複合体（X 線結晶構造解析）
共結晶作成、単結晶 X 線測定：アスビオファーマ（株）創薬技術ファンクション（分解能：1.90 Å）

以上、キマーゼに対する阻害の持続性および X 線結晶構造解析から、ジアゼパンとした化合物は求核攻撃を受けやすく、生体内においても各種低分子と反応し、安定性が低いと予想した。さらに、このような反応は生体内で非特異的に起こると懸念される。セリンプロテアーゼ間の特異性が低下することに加え、さまざまなタンパク質に結合、機能に悪い影響を与え、毒性発現を誘発する可能性がある。アトピー性皮膚炎のような慢性疾患には、治療薬の長期投与が必要と想定され、薬剤の安全性は極めて重要である。これらのことから、生体内における安定性および安全性の改善を目指し、化合物への求核攻撃が抑制されるよう一層の構造改変が必要と考えた。

第 2 章　生体内安定性の向上を意図したカルバモイル基への置き換え

第 1 節　分子設計

第 1 章第 3 節で示したように、ジアゼパン骨格とした化合物は求核攻撃を受けやすく、生体内における安定性が低い。この考察に基づき、電子求引性の強いスルホニル基をカルバモイル基に置き換えることとした。ジアゼパン化合物 **4** では、環内窒素原子に強い電子求引性を示すスルホニル基が結合している。このことがジアゼパン環 5 位のカルボニル基が求核剤の攻撃を受けやすくする原因である。もし、スルホニル基をカルバモイル基に置き換えた **13** を想定すれば、カルバモイル基は窒素原子から電子を押し込む、Figure 15 に示すような共鳴の極限構造式が描ける。環内窒素原子の電子密度を下げず、結果として環内のカルボニル基への求核攻撃も起こりにくくなると考えた。

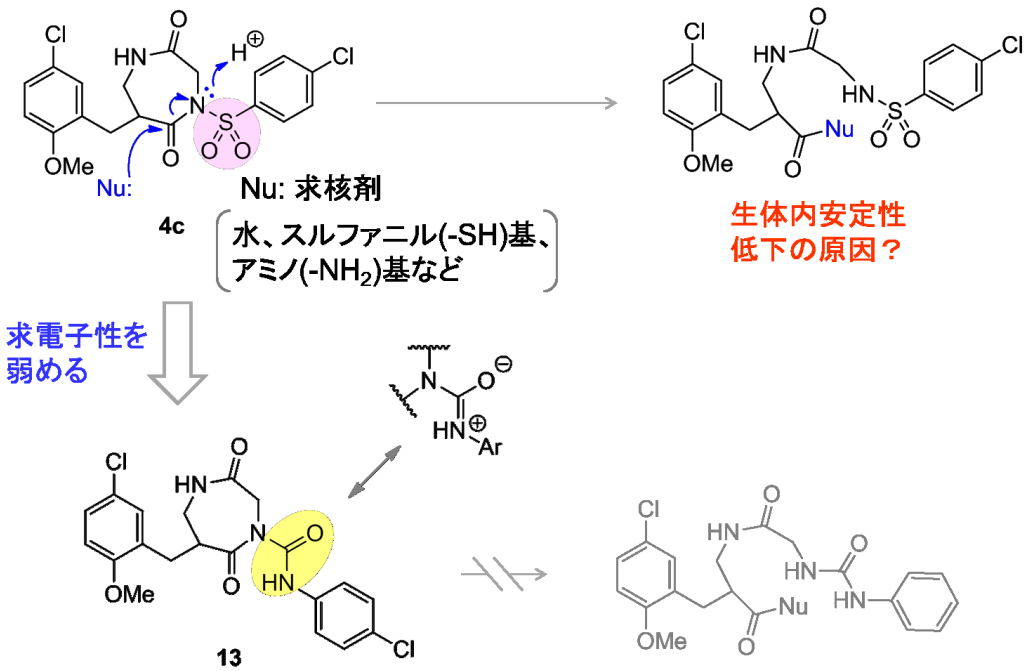
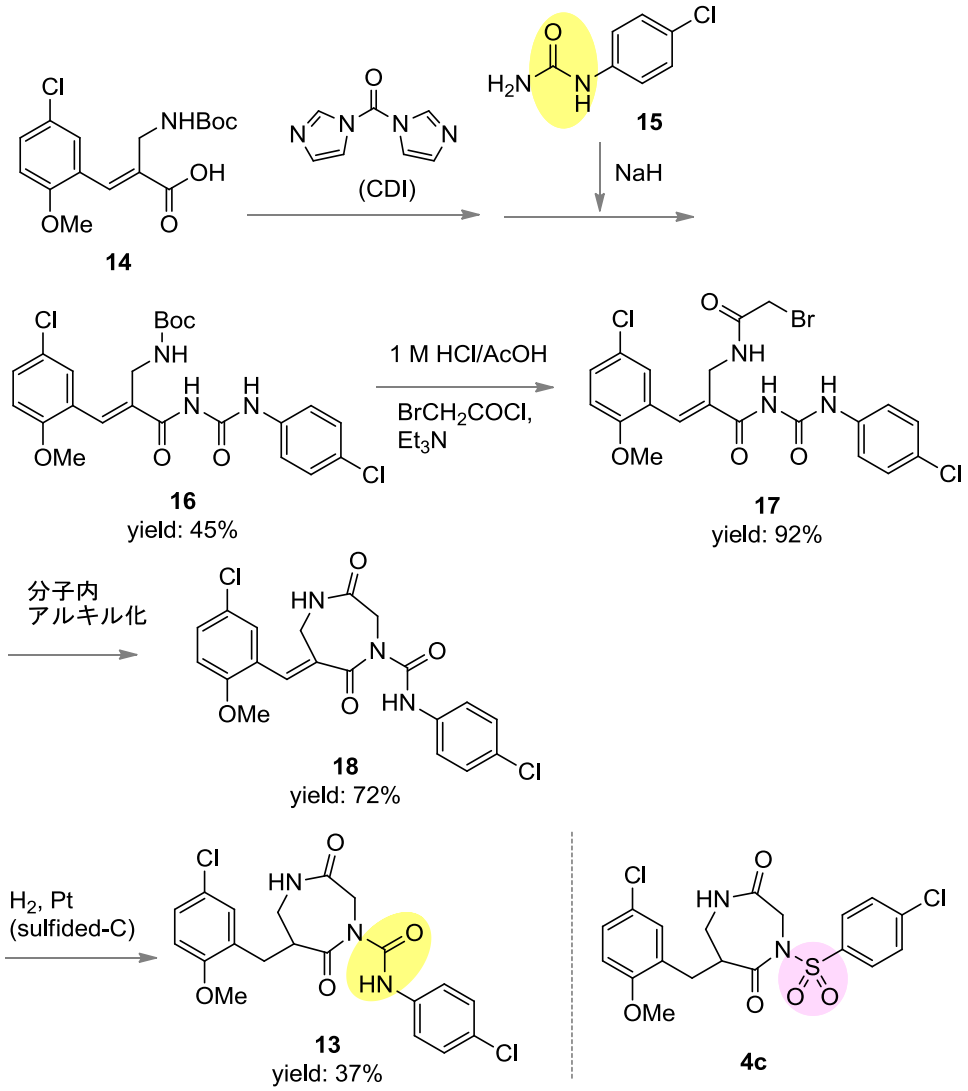


Figure 15 電子求引性が弱いカルバモイル基に変換する分子設計

第 2 節 化合物の合成

カルバモイル基に置き換えた化合物 **13** は Scheme 3 に示す方法で合成した。第 1 章第 2 節の Scheme 2 に従い合成したアミノカルボン酸 **14** (Scheme 2 における、化合物 **8** に相当）と尿素誘導体 **15** を、カルボニルジイミダゾール(CDI)を用いて縮合し、**16** を得た。続いて **16** の Boc 基を酸性条件で除去し、ブロモアセチル基を導入後、分子内アルキル化により閉環、最後に二重結合を還元し **13** とした。この方法は、最初の段階がスルホニル型化合物 **4** の合成と異なるのみで、それ以外は、同様な条件、共通の中間体を利用できる効率の良いルートである。

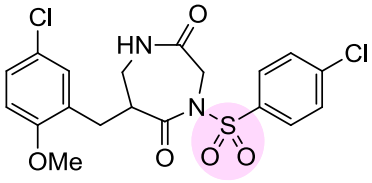


Scheme 3 カルバモイル型化合物の合成

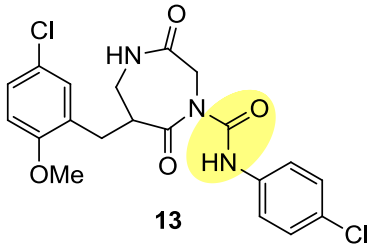
第 3 節 活性評価と解析

合成した化合物の血漿中半減期 [33]および阻害の測定をアスビオファーマ創薬技術ファンクションおよび免疫・炎症疾患フィールドにそれぞれ依頼した。カルバモイル基に置き換えた **13** は、血漿中半減期が 60 分以上と、もとのスルホニル型化合物と比べ、生体内における安定性は大幅な改善を示した(Table 5)。長時間経過した後も化合物 **13** がそのまま確認できたことから、狙い通り、求核攻撃が抑制されたためと考えた。しかしその一方で、活性は大きく下がってしまった。そこで化合物 **13** をベースとした上で、再度分子設計し直すことにした。

Table 5 化合物 **4c** および **13** の血漿中半減期・阻害活性



4c



13

化合物	血漿中半減期 $T_{1/2}$ (マウス，min)[1)]	ヒトキマーゼに対する IC_{50} (μM)[2)]
4c	18	0.034
13	>60	5.4

1) 静脈内投与後の血漿中半減期　アスビオファーマ（株）創薬技術ファンクション

2) 同社免疫・炎症疾患フィールド

第 3 章　阻害活性の回復を目指したベンジル型への置き換え [35)]

第 1 節 分子設計

第 2 章第 3 節の考察に基づいて分子を再設計するにあたり、**13** とヒトキマーゼの複合体モデルの作成を依頼した。第 1 章第 1 節で示した **4a** と同様、1 か所の不斉中心のため、**13** には鏡像関係にある 2 種類の異性体が存在する。両者のうち高いドッキングスコアを示した異性体を用いて議論を進めている。Figure 16 の左にその絵を示す。カルバモイル基に置き換えた化合物は、分子が伸長した型で、prime site 芳香環と S_1' site の相互作用が期待できる。しかし、S_1' site は、prime site 芳香環を無理なく収容できるほど奥行きが深くない。そのため **13** は実際には入りにくく、活性が大幅に低下したと考えた。

これに対し、右に示す元のスルホンアミドでは、広い S_2' site と効果的に相互作用している。この解析から、活性向上を目指すには、立体的な反発を避けつつ狭い S_1' site に対応するより、広い S_2' site と良好に相互作用するように構造を改変すべきと考えた。

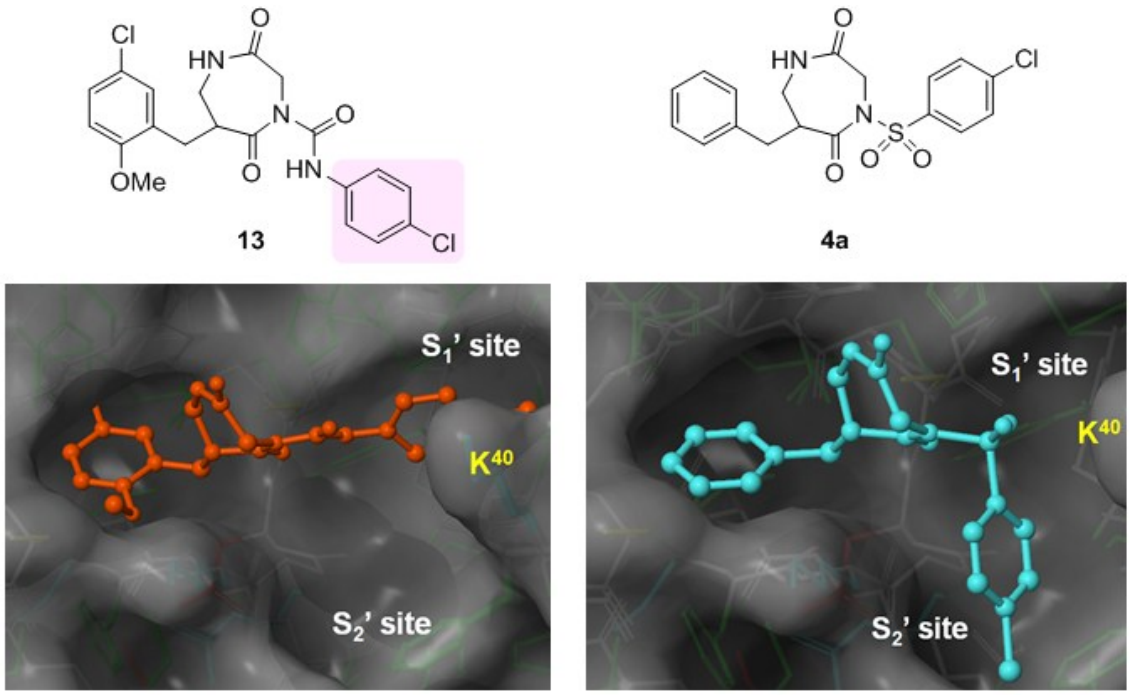


Figure 16 化合物 **13** および **4a** とヒトキマーゼの複合体モデル

複合体モデル作成：アスビオファーマ（株）創薬技術ファンクション、ドッキングプログラム：GOLD
可能な立体異性体のうち、高いドッキングスコアを示した異性体を示す

そこで、カルバモイル型化合物 **13** をもとに、prime site の芳香環と窒素原子の間に一炭素を挿入したベンジル型化合物 **19a** を考えた(Figure 17)。構造が屈曲し、prime site 芳香環が S_2' site に配置されうると期待した。さらに、挿入した **19a** のメチレン基は、S_1' site の入口に位置すると予想した。上述のように、S_1' site は狭く、カルバモイル型化合物 **13** が持つ芳香環では、立体障害により効果的に相互作用できない。一方、サイズがより小さい置換基であれば無理なく収容されるであろう。そこで、S_1' site に効果的に収容されるよう、アルキル基をベンジル位に置換した **19b**, **c** も想定した。

これらの候補化合物の中から、エチル基を持つ **19b** とキマーゼの複合体モデル作成を依頼した。この化合物には 2 か所の不斉中心が存在し、結果として合計 4 種類の異性体が可能である。全立体異性体についてドッキングシミュレーションし、最も高いドッキングスコアを示した異性体の結果を Figure 17 の右に示す。芳香環が S_2' site に、エチ

ル基が S_1' site に無理なく収容されると考えられる。

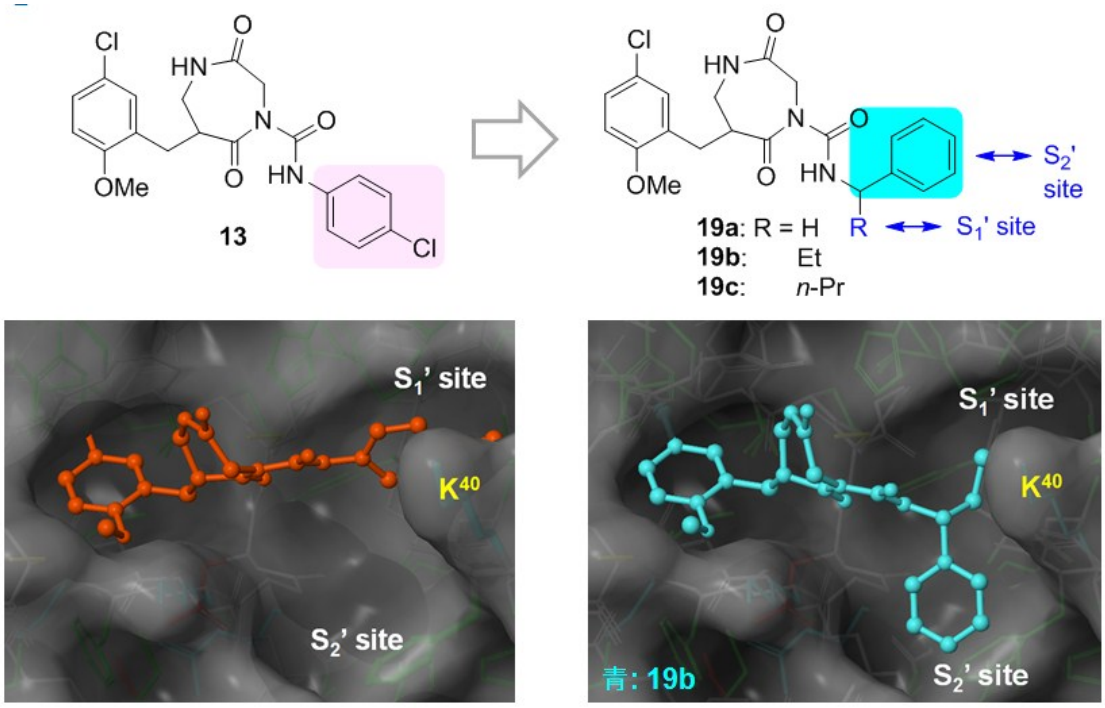


Figure 17 構造が屈曲するベンジル型化合物の分子設計

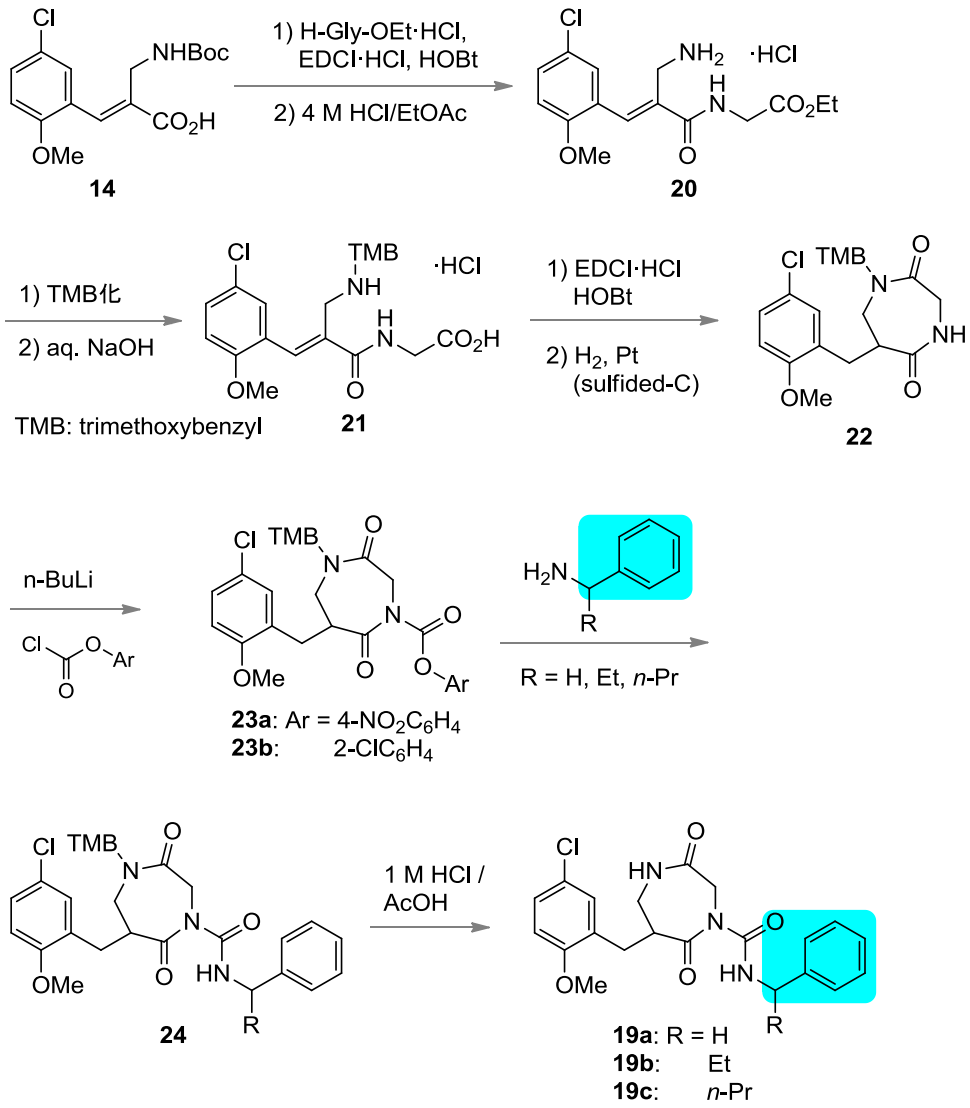
複合体モデル作成：アスビオファーマ（株）創薬技術ファンクション、ドッキングプログラム：GOLD
可能な立体異性体のうち、高いドッキングスコアを示した異性体を示す

第 2 節 化合物の合成

そこで、ベンジル型とした化合物を、Scheme 4 に示す方法に従って合成した。アミノカルボン酸 **14** とグリシンエチルエステルを縮合、酸性条件下 Boc 基を除去し、**20** とした。遊離になったアミノ基をトリメトキシベンジル(TMB)化、塩基性条件でエステルを加水分解し、**21** を得た。続いてアミド結合を形成しラクタム環を構築、二重結合を還元し、クロロギ酸アリールとの縮合により **23a, b** とした。最後に種々のベンジルアミンと結合、酸性条件下 TMB 基を除去し、目的とする **19a-c** を合成した。

これらの化合物の合成法については、これまでのカルバモイル型化合物のそれとは大幅に変更し、ジアゼパン環を先に構築、prime site にあたるベンジルアミンを合成経路の後半で導入している。これにより、同一の骨格に対し prime site をさまざまに変え

た化合物を効率良く合成できる。

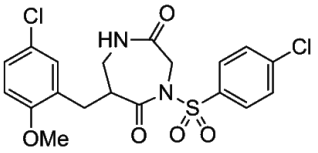


Scheme 4 ベンジル型化合物の合成

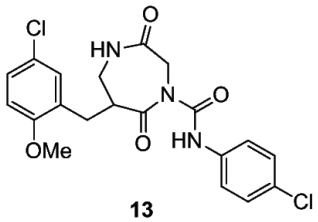
第 3 節 活性評価と解析

合成した化合物のヒトキマーゼに対する阻害の測定を依頼した。ところが、構造改変前のカルバモイル型化合物 **13** と比べ、ほとんど活性が向上しないばかりか、*n*-プロピル基を持つ **19c** では大幅に低下してしまった(Table 6)。一方、ベンジル型とした **19a**, **b** が **13** と同程度の活性を示したことから、相互作用は不十分とはいえ、複合体モデル通り、ベンジル型化合物は、キマーゼの活性部位に収容されていると考えた。prime site 芳香環を S_2' site に、一方、ベンジル位に導入したアルキル基を S_1' site に配置させる分子設計は妥当であった。**19c** については、**19b** のエチル基と比べ *n*-プロピル基は一炭素長く、S_1' site の奥行き部分との立体的な反発が大きくなり、そのため活性が低下したと思われる。ベンジル型化合物をもとにした上で、何らかの打開策を見出す必要がある。

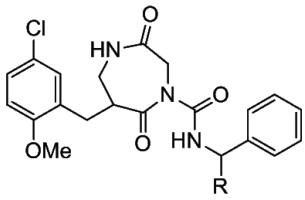
Table 6 化合物 **4c**, **13** および **19a-c** の阻害活性



4c



13



19a-c

化合物	R	ヒトキマーゼ に対するIC_{50} (μM)*
4c	—	0.034
13	—	5.4
19a	H	4.9
19b	Et	2.8
19c	*n*-Pr	69.3

* アスビオファーマ（株）免疫・炎症疾患フィールド

第 4 章　阻害活性の回復を目指した prime site 芳香環の修飾および変換

第 1 節 S_2' site と相互作用しうる置換基の導入 [35)]

第 3 章第 3 節で示したように、酵素の S_1'および S_2' site との相互作用を期待し、ベンジル型としたのみでは、活性は回復しなかった。そこで、prime site 芳香環自体に、酵素の S_2' site と効果的に相互作用しうる官能基を導入することとした。第 1 章第 1 節で示したように、S_2' site にはオレンジで示したリジン 40 の側鎖アミノ基、アルギニン 143 の側鎖グアニジル基、青で示したフェニルアラニン 41 の主鎖アミドカルボニル基が存在する。そこで、エチル基を導入した **19b** とヒトキマーゼの複合体モデル(Figure 18)を見直した。化合物 prime site 芳香環の 3-または 4-位に、それらと相互作用しうるカルボキシル基やアミノ基を挿入すれば活性が向上すると考え、化合物 **25** を設計した。

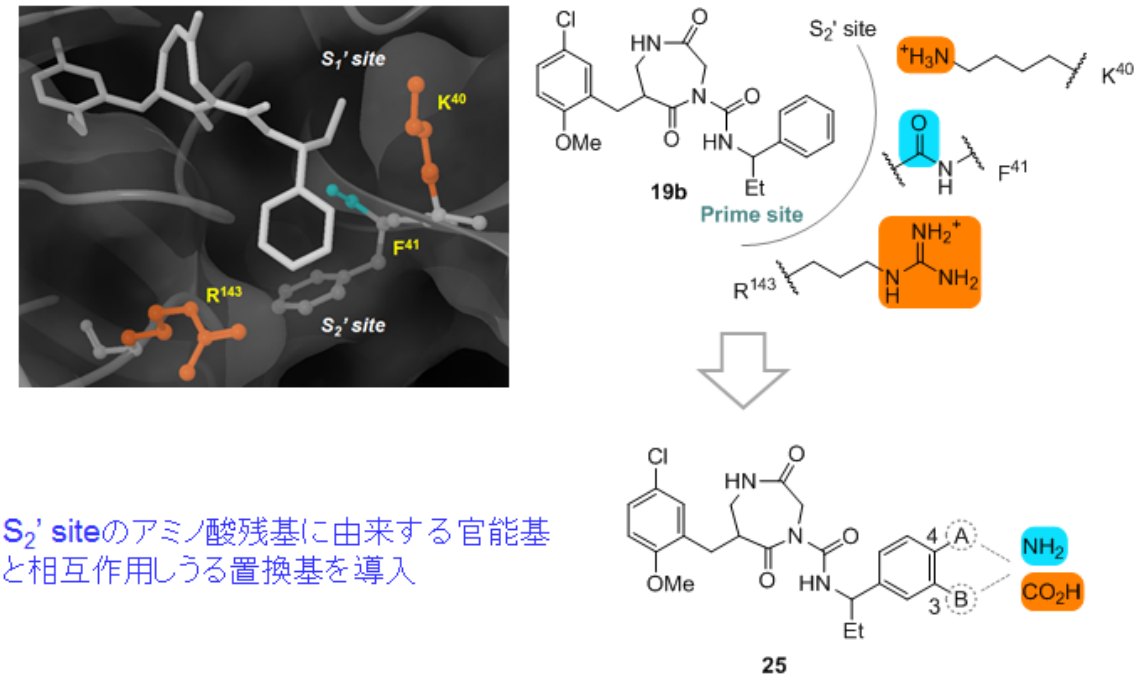
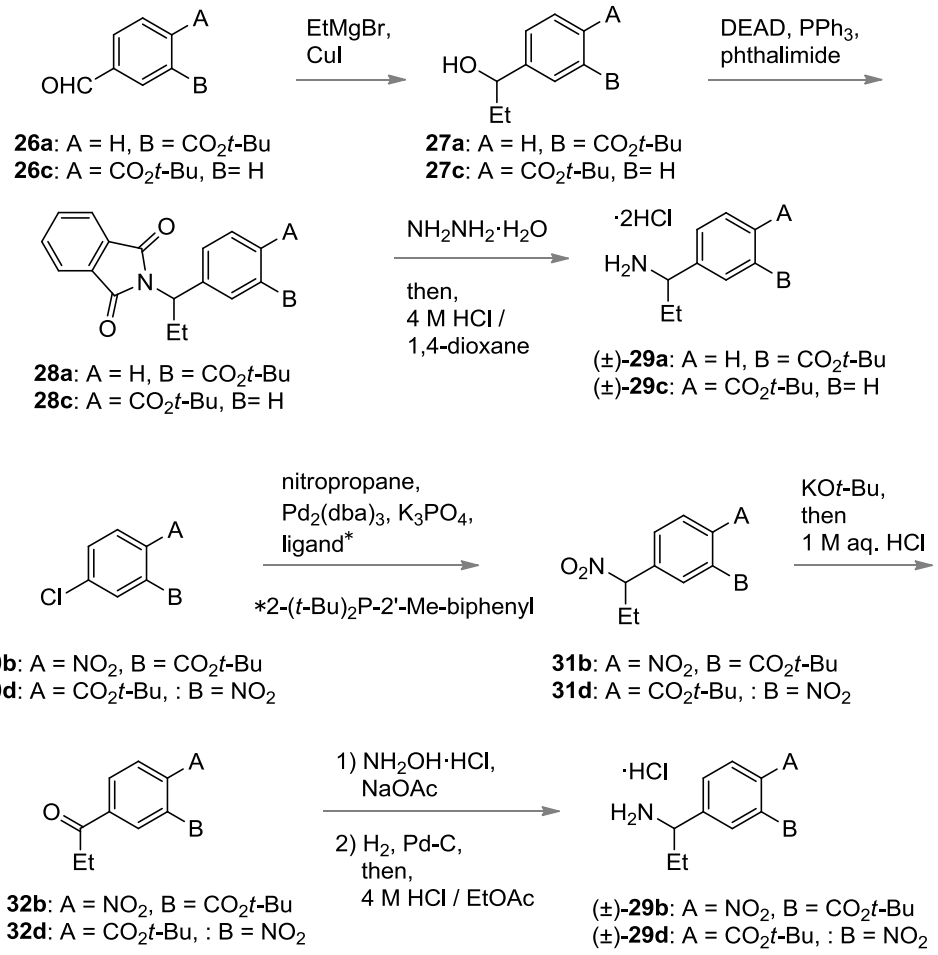


Figure 18 K^{40}, F^{41}, R^{141} との相互作用を意図した分子設計

複合体モデル作成：アスビオファーマ（株）創薬技術ファンクション、ドッキングプログラム：GOLD
可能な立体異性体のうち、高いドッキングスコアを示した異性体を示す

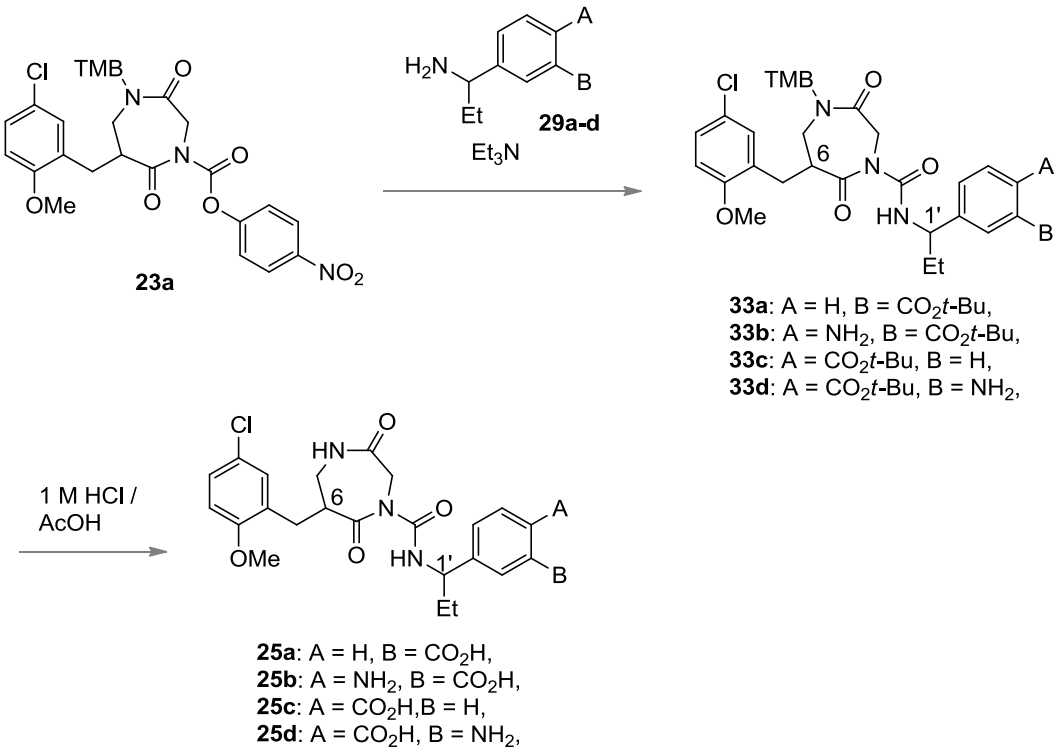
第 2 節 化合物の合成

化合物 **25** の合成には、3-または 4-位に官能基を導入したベンジルアミン(**29a-d**)が不可欠である。それらはベンズアルデヒド **26** またはハロゲン化アリール **30** から合成した(Scheme 5)。ベンズアルデヒド **26** からは Grignard 反応でエチル基を導入し **27** とし、生じた第二級アルコールを光延反応でフタルイミドに置換(**28**)、最後に脱保護によりアミノ基を持つ **29a**, **c** としている。一方、ハロゲン化アリール **30** からは、パラジウム触媒を用いたニトロプロパンとのカップリングにより **31** を合成し、ニトロ基は Nef 反応によって、いったんカルボニル基とした。ケトン **32** に対し改めてアミノ化し、**29b**, **d** を合成した。合成中間体 **31b**, **d** の分子内 2 カ所に含まれるニトロ基を直接還元する方法も試みたが、**32b**, **d** を経るルートが総収率で勝っていた。



Scheme 5 Prime site 芳香環部分に官能基を持つベンジルアミンの合成

合成したベンジルアミン **29a-d** を用い、第 3 章第 2 節の Scheme 4 に示した方法と同様に **25a-d** を合成した(Scheme 6)。まず、先に示した **23a** にベンジルアミン **29a-d** を結合、続いてカルボキシル基上の *t*-ブチルエステルを酸性条件で除去した。

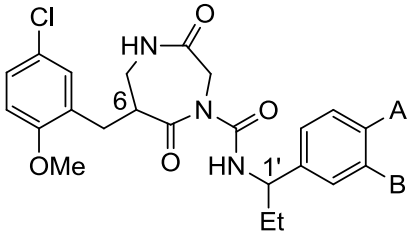


Scheme 6 Prime site 芳香環部分に官能基を導入したベンジル型化合物の合成

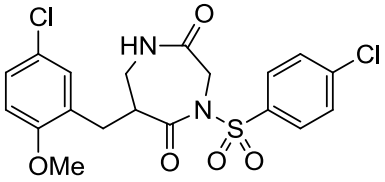
第 3 節 活性評価と解析

新たに合成した化合物の阻害測定を依頼した。その結果を Table 7 に示す。スルホニル型化合物 **4c** にはまだ 1 桁及ばないが、無置換の **19b** と比べいずれも活性は大幅に向上し、複合体モデルで予想した通り、S_2' site と強く相互作用しうる置換基導入が活性回復に有効なことがわかった。

Table 7 Prime site 芳香環上へ置換基を導入した化合物の阻害活性



19b, 25a-d



4c

化合物	置換基		ヒトキマーゼに対する IC_{50} (μM)*
	A	B	
4c	—	—	0.034
19b	H	H	2.8
25a	H	CO_2H	0.54
b	NH_2	CO_2H	0.63
c	CO_2H	H	0.47
d	CO_2H	NH_2	0.50

* アスビオファーマ（株）免疫・炎症疾患フィールド

第 4 節 S_2' site との相互作用を期待したヘテロ原子の導入 [36)]

以上のように prime site 芳香環への極性基導入は活性の回復に有効だったので、次にベンゼン環をヘテロ環に変えてみた(Figure 19)。キマーゼと相互作用する部位を増やすことを念頭に、窒素、酸素、硫黄原子などを導入する。ヘテロ環とした化合物 34 には、特に、S_2' site に存在するリジン 40 との水素結合を期待した。

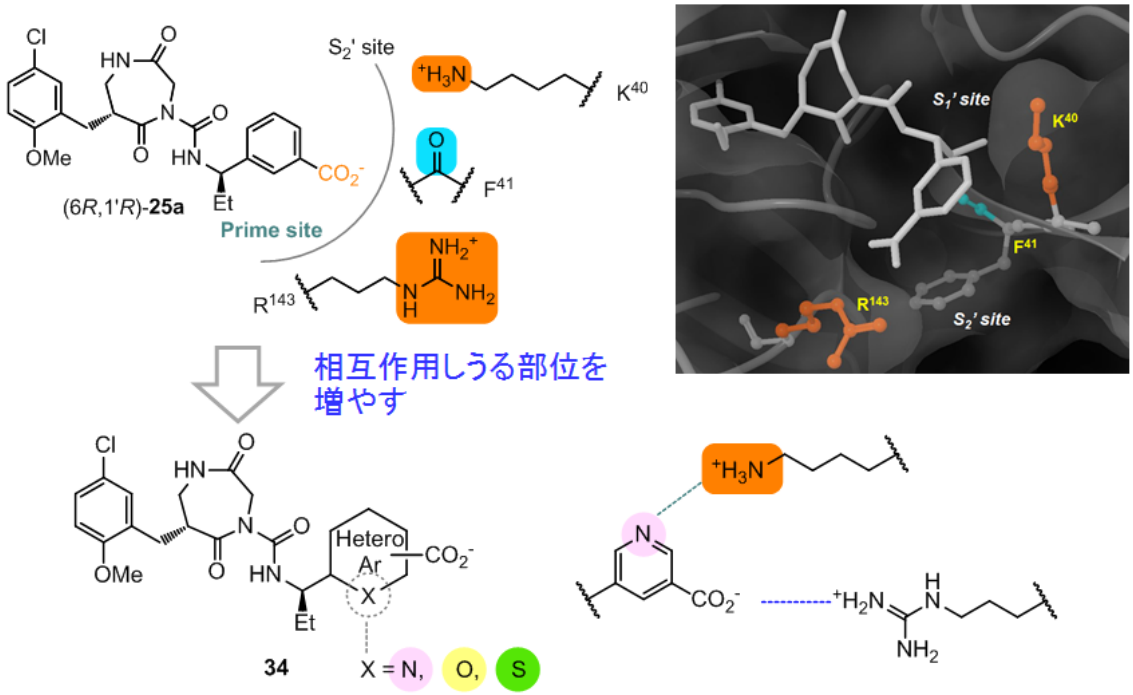
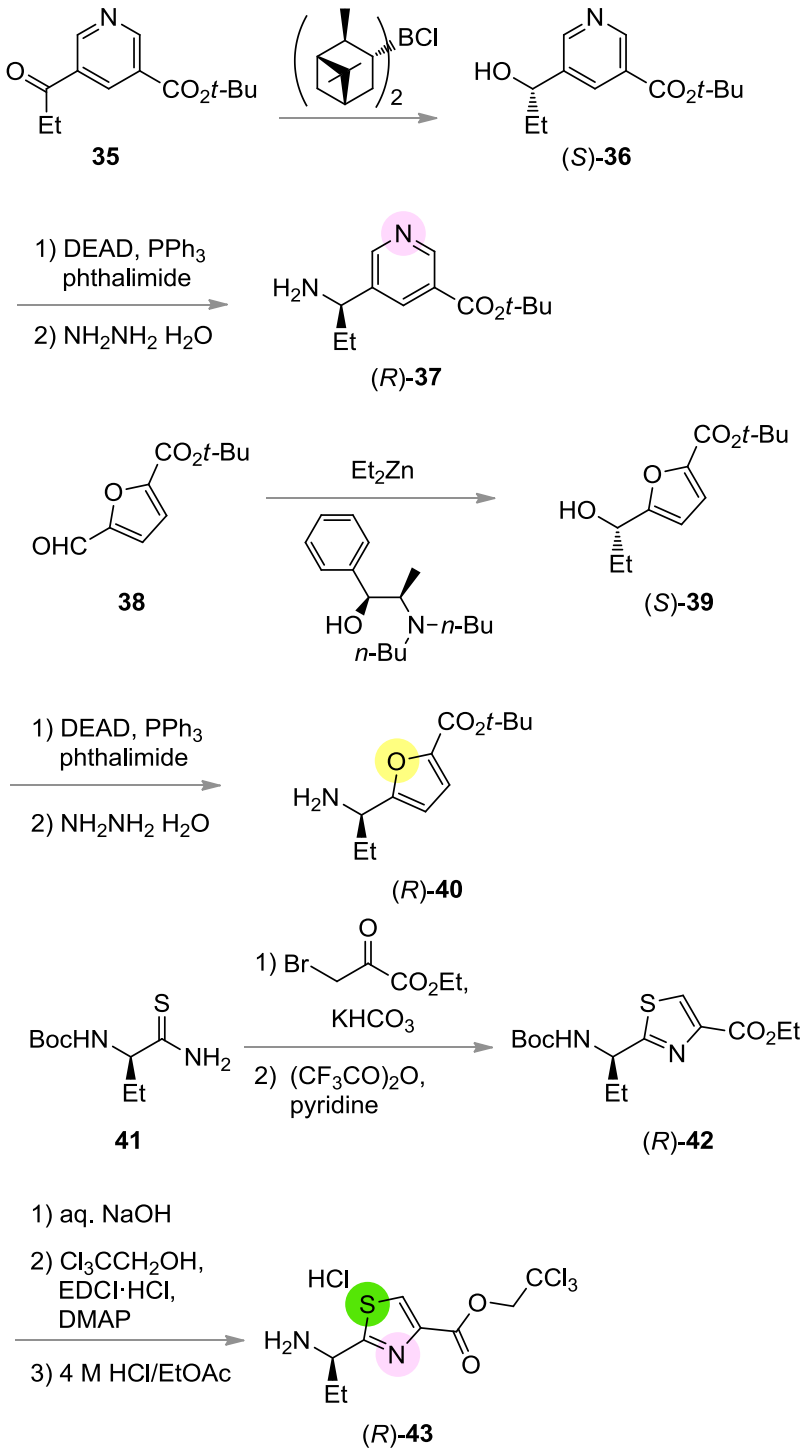


Figure 19 S_2' site に対する親和性を一層高める分子設計

複合体モデル作成：アスビオファーマ（株）創薬技術ファンクション、ドッキングプログラム：GOLD
化合物 **25a** とヒトキマーゼの複合体モデルの作成を依頼、可能な立体異性体のうち、高いドッキングスコアを示した異性体を示す

第 5 節 化合物の合成

化合物 **34** の合成に不可欠なヘテロ環を含むアミンは、Scheme 7 に示す方法で合成した。まずピリジン環を持つ(*R*)-**37** は、ケトンを Brown らの方法 [37]で不斉還元し、第二級アルコールに対し光延反転で窒素原子を導入することにより合成した。フラン(*R*)-**40** の合成においては、アルデヒド **38** を基質とし、砂合らの方法 [38]で不斉アルキル化している。一方、チアゾール(*R*)-**43** については、市販の(*R*)-2-アミノ酪酸から誘導されるチオアミドを原料として、ブロモピルビン酸エチルを用いた Hantzsch 合成法により、目的とするヘテロ環を構築した(Scheme 7)。得られたアミンを用い、第 4 章第 2 節の Scheme 6 と同様の方法で化合物 **34** を合成した。

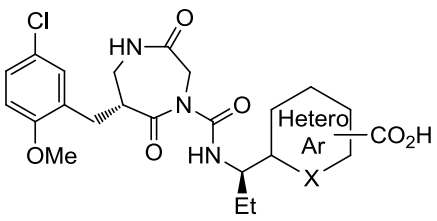


Scheme 7 ヘテロ環を含むアミン類の合成

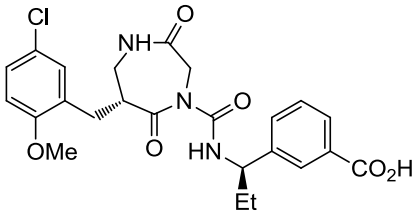
第 6 節 活性評価と解析

ところが、合成したヘテロ環化合物 **34** の活性は、ベンゼン環のそれと比べ、同様か、あるいは大幅に低下し、期待したヘテロ原子の効果は観察されなかった(Table 8)。ヘテロ原子は、アミノ基と水素結合するなどの積極的な相互作用は示さず、活性が低下した化合物では、キマーゼのアミドカルボニル基と何らかの反発を招いたと考えられる(Figure 20)。

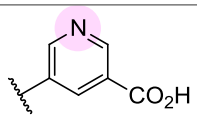
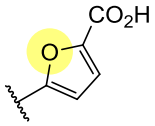
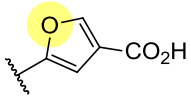
Table 8 ヘテロ環とした化合物の阻害活性

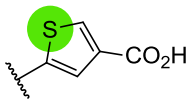
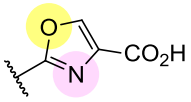
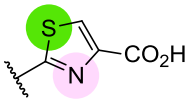


(6*R*,1'*R*)-**34a-f**



(6*R**,1'*R**)-**25a**
IC_{50} = 0.54 μM*

34	Ar	ヒトキマーゼに対する IC_{50} (μM)*
a		0.38
b		0.41
c		1.2

34	Ar	ヒトキマーゼに対する IC_{50} (μM)*
d		0.52
e		2.77
f		1.64

* アスビオファーマ（株）免疫・炎症疾患フィールド

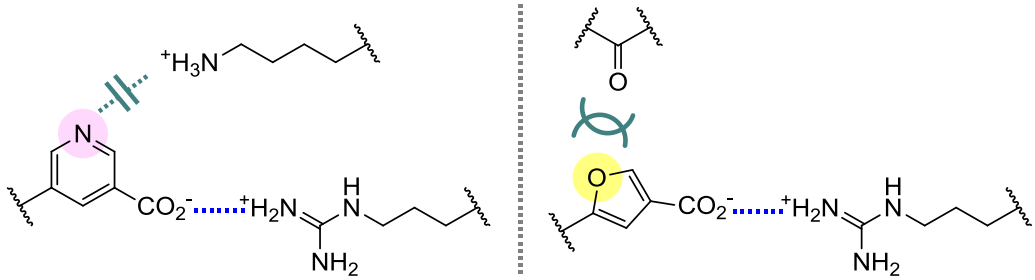


Figure 20 ヘテロ環部分と S_2' site の相互作用に関する考察

第 7 節 カルバモイル基の挿入 [36)]

そこで、再びベンゼン環に戻り、活性を示した化合物のベンジル位と芳香環の間にカルバモイル基を挿入することにした(Figure 21)。二原子の挿入により、prime site 上の極性基の位置が変わることに異論はない。しかも、ペプチド結合でよく知られているように、アミド結合は回転障壁が高いことから、S_2' site に対し、適切な位置に官能基を固定できるのではないかと期待した。

剛直な構造をとると、思惑とは逆に、酵素の特定アミノ酸残基と相互作用できない位置に、化合物の官能基を固定してしまう懸念もある。しかし、構造が柔軟なエチレン基等では、酵素に収容され固定される際に配座の自由度が減り、エントロピー的に不利になると考え、上述の剛直なアミド結合を優先的に採用した。

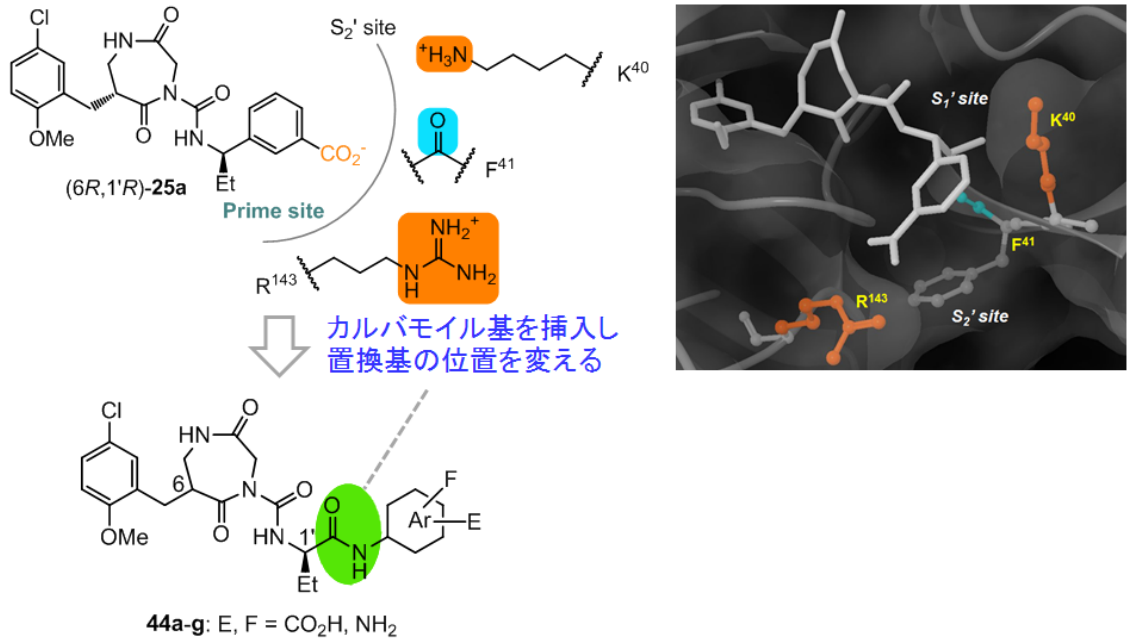
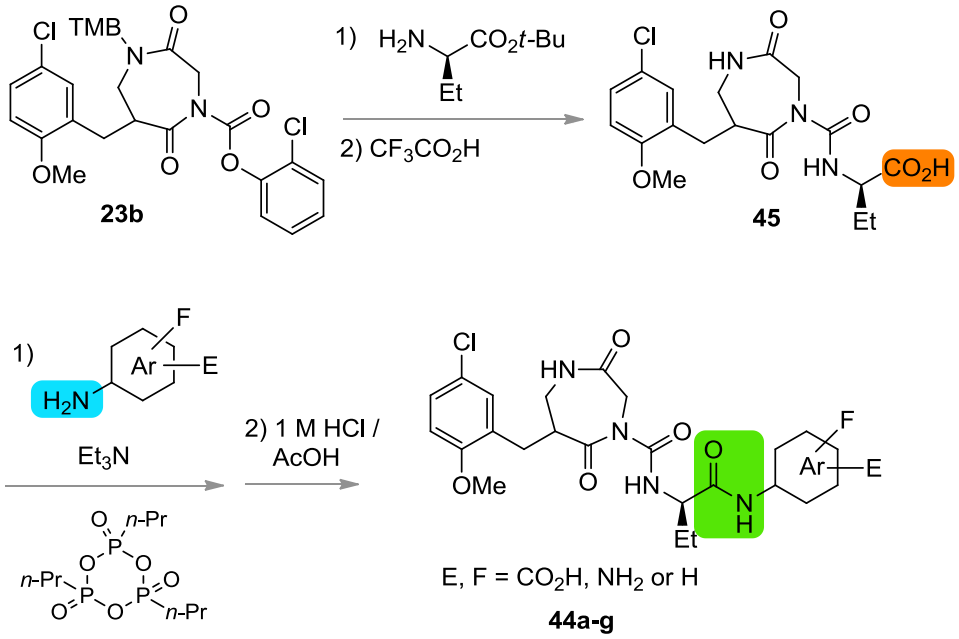


Figure 21 カルバモイル基挿入による、相互作用様式の変化

複合体モデル作成：アスビオファーマ（株）創薬技術ファンクション、ドッキングプログラム：GOLD
可能な立体異性体のうち、高いドッキングスコアを示した異性体を示す

第 8 節 化合物の合成

カルバモイル基は Scheme 8 に示す方法で構築した。まず、第 3 章第 2 節の Scheme 4 に示したアリールエステル **23b** に対し、市販の(*R*)-2-アミノ酪酸 *t*-ブチルを結合させ、脱保護を経て、カルボン酸 **45** とした。このものに prime site を構成する種々のアミンを縮合させ、当初の目的を達成している。

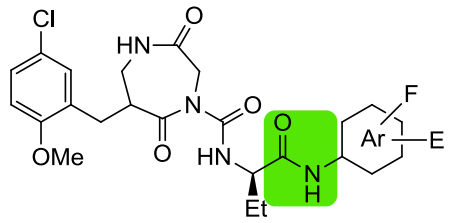


Scheme 8 カルバモイル基を挿入した化合物の合成

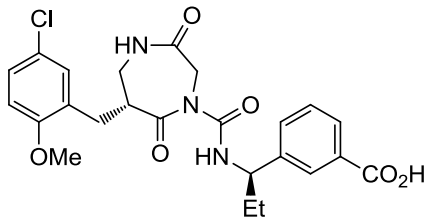
第 9 節 活性評価と解析

合成した化合物の阻害の測定を依頼したところ、芳香環上にカルボキシル基、アミノ基を持つ化合物は、いずれも IC_{50} 値が 1 μM 以下の活性を示した。両者を併せ持つ **44f** の活性は 0.38 μM と、カルバモイル基を挿入する以前の **25a** のそれを凌いでいる(Table 9)。この結果から、キマーゼと相互作用しうる prime site 芳香環のカルボキシル基およびアミノ基を適切な位置に配置すれば、相乗的に作用することがわかった(Figure 22)。しかし残念ながら、活性の飛躍的上昇は認められていない。

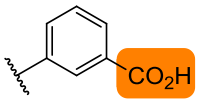
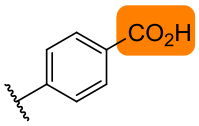
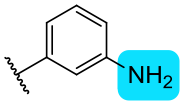
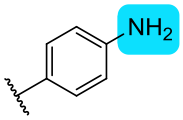
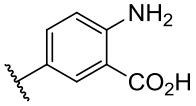
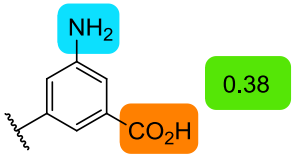
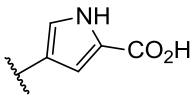
Table 9 カルバモイル基を挿入した化合物の阻害活性



44a-g



(6*R**,1'*R**)-**25a**
IC_{50} = 0.54 μM*

44	Ar	ヒトキマーゼに対する IC_{50} (μM)*
a		0.57
b		0.77
c		0.66
d		0.79
e		0.73
f		0.38
g		0.85

* アスビオファーマ（株）免疫・炎症疾患フィールド

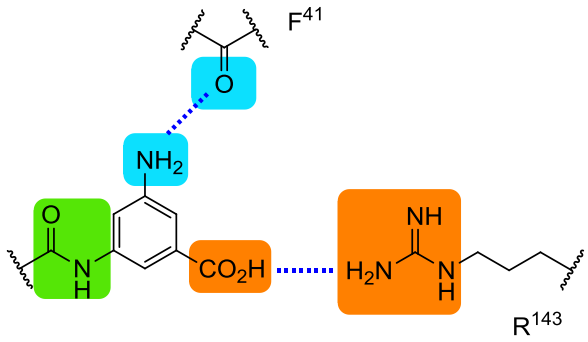


Figure 22 二種類の官能基導入による相互作用様式の変化

第 10 節 テトラゾール環が S_2' site と相互作用する可能性

さらに、これまでの知見を結集し、カルボキシル基、ヘテロ原子、カルバモイル基を組み合わせ、カルボキシル基の等価体のひとつ、テトラゾール環を持つ **44h** を阻害剤の候補として設定した(Figure 23)。

まず、テトラゾール環は、カルボキシル基と同程度の酸解離定数を示すので、先に示した prime site 芳香環にカルボキシル基を持つ化合物と同様、リジン 40, アルギニン 143 との相互作用が期待できる。次に、4 箇所の窒素原子のいずれかを経る水素結合も可能である。さらに、**44h** のようにカルバモイル基を介しテトラゾール環を持つ構造とすれば、これまでに合成した化合物とは異なる位置に官能基を配置する可能性もある。これらのことから、S_2' site に対し、一層強い相互作用を期待した。

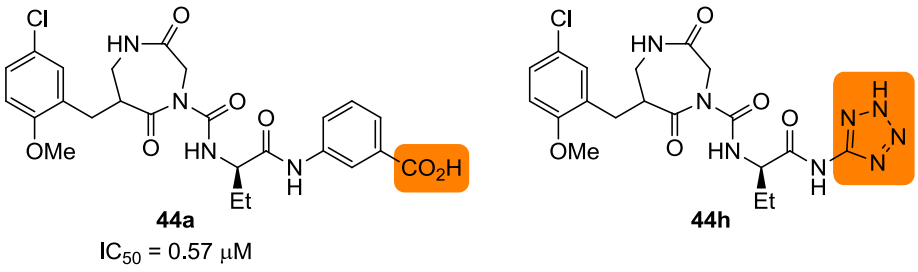
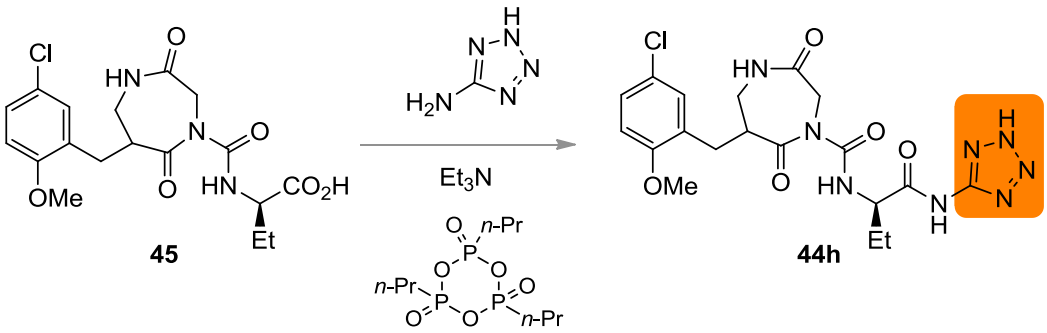


Figure 23 さまざまな官能基の比較統合により設計した、テトラゾール環を持つ化合物

テトラゾール環を持つ化合物 **44h** は、第 4 章第 8 節(Scheme 8)に述べた中間体の一つ、カルボン酸 **45** に 5-アミノテトラゾールを縮合することによって合成した(Scheme 9)。



Scheme 9 テトラゾール環を持つ化合物の合成

活性測定を依頼したところ、テトラゾール環を持つ **44h** は、ヒトキマーゼに対する IC_{50} が 0.26 μM という強い阻害を示した。S_2' site に非常に好ましい形で収容され、官能基も相互作用していると期待し、そこで、キマーゼとの共結晶の作成と単結晶 X 線回折測定を依頼した。しかし、その結果を解析したところ、テトラゾール環は、タンパク質から突出し、S_2' site の内部ではなく酵素の分子表面でリジン 40 の側鎖アミノ基と直接相互作用していた(Figure 24)。残念ながら、目論んだ分子設計とは異なる結果であった。

分子が単独で生体内に存在する場合、周囲の水分子と水素結合して安定化する。分子同士が直接相互作用する場合は、それぞれの作用部位に水素結合していた水分子を排除する必要があり、その損失を脱溶媒和ペナルティと呼ぶ。イオン間の直接的な相互作用が起きる際、脱溶媒和ペナルティは、特に多くの水分子が結合しているタンパク質表面で非常に大きい。

以上の理由から、分子を設計する際、結合水を排除してまで、タンパク質表面においてイオン間相互作用が起こると予想していなかった。今回見出した **44h** のテトラゾール環とリジン 40 の相互作用は、X 線結晶構造解析の結果から初めて判った。この相互作用には、イオン間の静電的引力に加え、分子に含まれる原子間の距離から判断して、置換基同士のファンデルワールス力も寄与していると考えられ、これらが十分に脱溶媒和ペナルティを補ったと推察している。

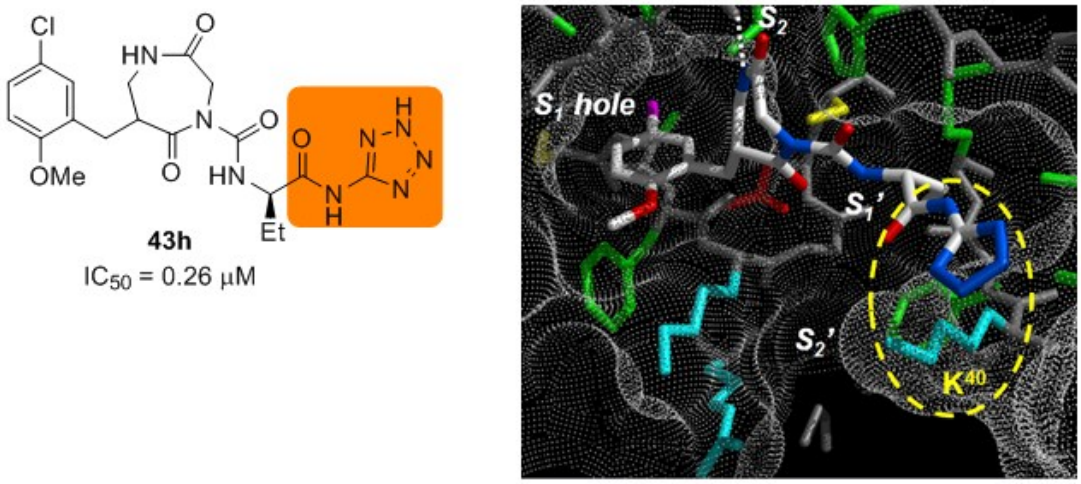


Figure 24　ヒトキマーゼに対する化合物 **44h** の阻害活性と共結晶 X 線構造解析

* 共結晶作成、単結晶 X 線測定：アスビオファーマ（株）創薬技術ファンクション（分解能：2.00　Å）

第 11 節　構造改変を通じた阻害様式の変化

以上のように prime site の構造改変を種々試み、活性が回復した化合物を見出した。それらの中から、構造がシンプルな **25a** を選び、生体内安定性を確認した。研究初期に、血漿中における半減期が 60 分以上と、安定性が高いカルバモイル型化合物 **13** を見出していたが（第 2 章第 3 節）、そのものと比較しても、**25a** は、半減期が 54 分と、ほぼ同じ程度であった。さらに、別途依頼したキマーゼの阻害実験で、**25a** とインキュベーションし、阻害剤を洗浄した直後 77%に低下した酵素活性が、2 時間経過した後では 97%にまで回復することが判った(Figure 25)。この結果は、時間経過に伴い阻害剤がキマーゼの触媒部位から脱落することを意味している。これにより、第 1 章第 3 節で示したスルホニル型化合物と異なり、毒性を引き起こす可能性が高い、酵素に対する非可逆的な結合形成は起こっていないと判断した。

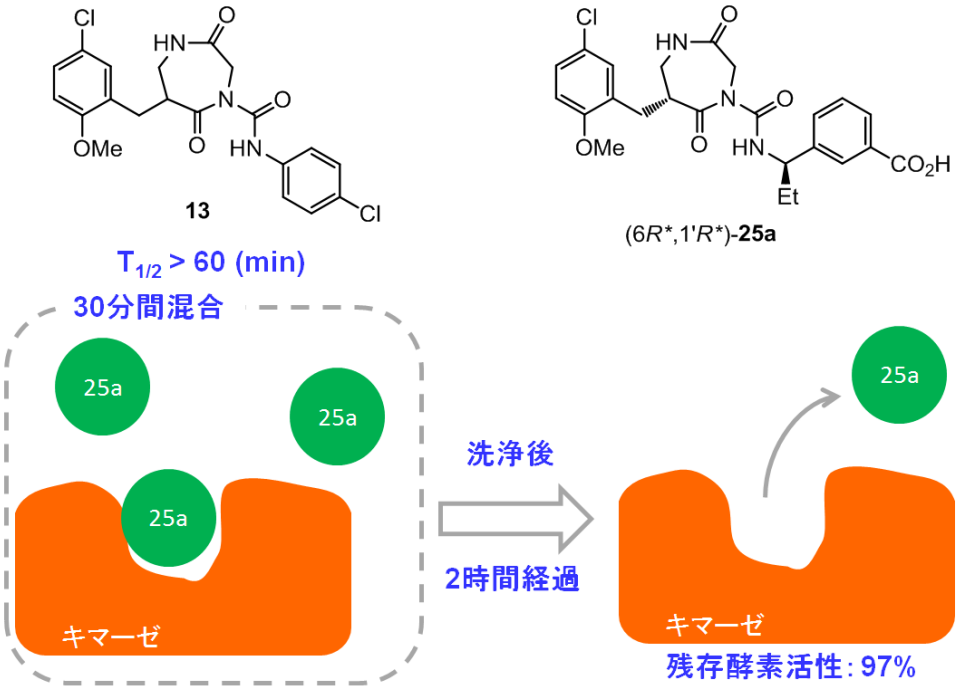


Figure 25 化合物 **25a** の生体内安定性と酵素阻害の持続性

* アスビオファーマ（株）免疫・炎症疾患フィールド

第 5 章　立体化学の解明と活性に与える影響

第 1 節 X 線結晶構造解析と不斉合成を組み合わせる、絶対立体配置解明

第 4 章第 11 節に述べたように、毒性が低く、かつ活性が回復した化合物 **25a** を見出したので、立体化学が活性に与える影響を調べることとした。これまで、ドッキングシミュレーションを頼りに分子構造を設計し、合成を進めてきた。しかし実際には、合成品は立体異性体の混合物で、2 ヶ所の不斉中心を持つ場合、合計 4 種類の異性体が存在し(Figure 26)、1 種類のみが有効で、残りは全く効いていない可能性もある。

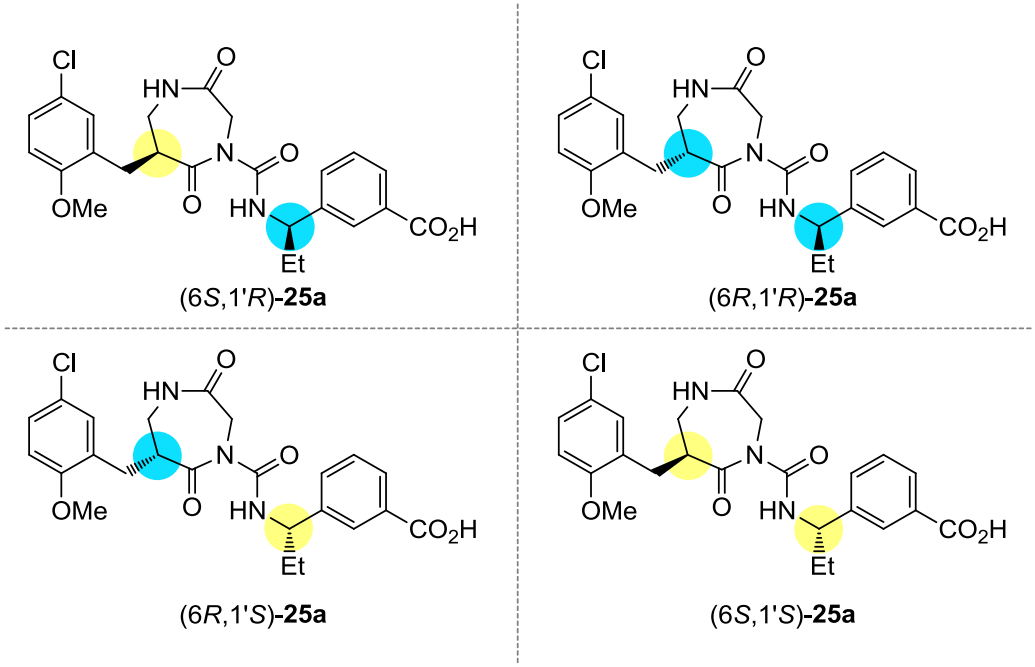
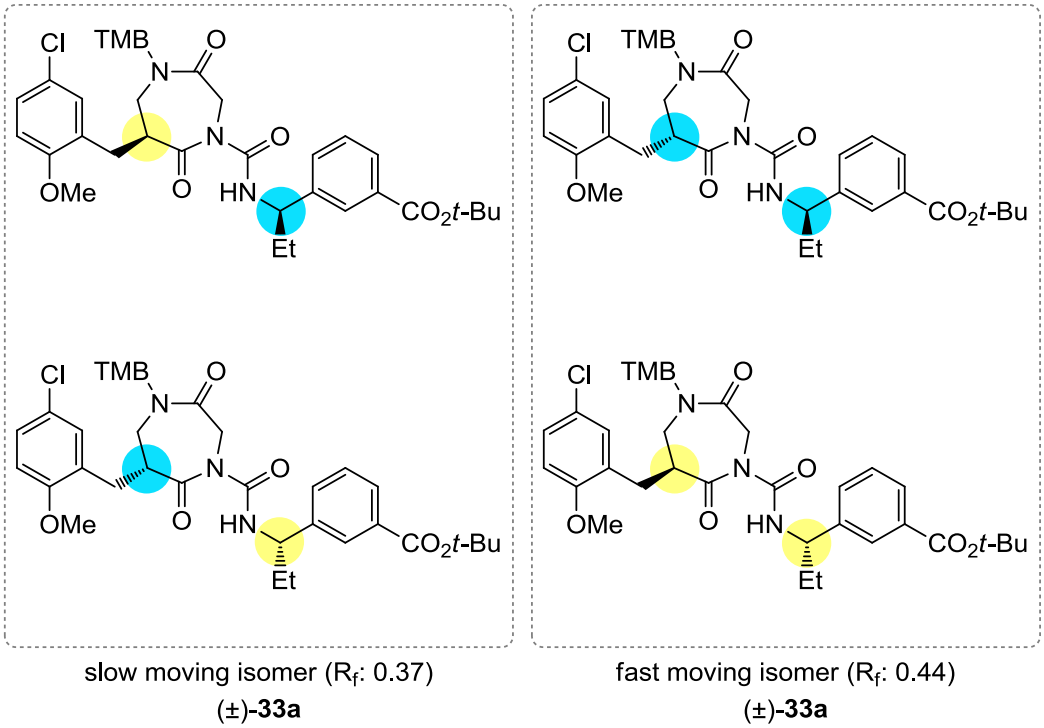


Figure 26　化合物 **25a** の全立体異性体

そこで、強い活性を示した **25a** について、全立体異性体の絶対立体配置を決定することにした。2 ヶ所の不斉中心のうち、右側のエチル基の付け根の部分については、第 4 章第 5 節に示した、「ヘテロ環を持つ化合物の合成」で用いた不斉合成や絶対立体配置既知化合物からの誘導によって、多くの情報を得ることが可能である。しかし、左側のジアゼパン部分の不斉中心については、不斉合成も誘導も困難で、絶対立体配置の決

定は挑戦的課題である。そこで、以下に示す間接的、演繹的手法で解明を試みた。

まず前駆体 **33a** を選び、4 種類の異性体のうちジアステレオマーをカラムクロマトグラフィーにより分離した。この先、薄層クロマトグラフィー分析で R_f 値が 0.37 と移動が遅い異性体を slow moving isomer、R_f 値が 0.44 と移動が速い異性体を fast moving isomer と呼んで説明する(Figure 27)。Slow moving isomer, fast moving isomer とも、それぞれ鏡像異性体の等量混合物、即ちラセミ体である。

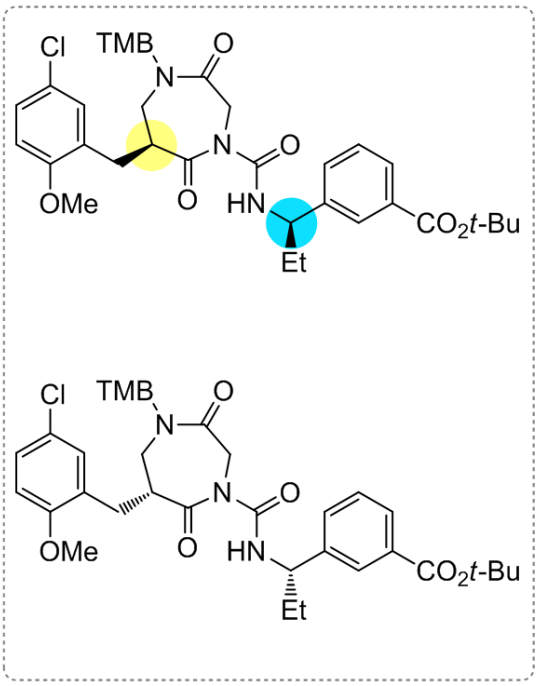


slow moving isomer (R_f: 0.37)
(±)-33a

fast moving isomer (R_f: 0.44)
(±)-33a

Figure 27 カラムクロマトグラフィーによる、前駆体 **33a** のジアステレオマー分離

このうち slow moving isomer は結晶化したので、単結晶 X 線測定を依頼し、その結果、相対立体配置は(6*R**,1'*S**)であることがわかった(Figure 28)。即ち(6*R*,1'*S*)-体と(6*S*,1'*R*)-体の等量混合物である。



slow moving isomer (R_f: 0.37)
(6*R**,1'*S**)-**33a**

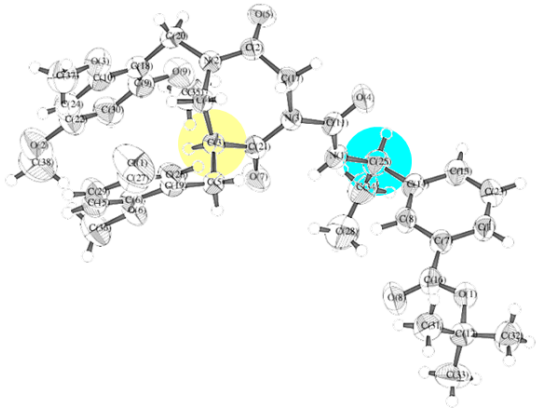


Figure 28 X 線結晶構造解析で確定した、一方のジアステレオマーの相対立体配置

単結晶 X 線測定：アスビオファーマ（株）創薬技術ファンクション

2 ヶ所の不斉中心それぞれを持つ部分構造をラセミ体同士で合成すれば、生成する異性体は 4 種類である。一方、Figure 29 において青で示すように、ベンジルアミン部分を純粋な鏡像異性体としてから合成すれば、赤枠で示す(6*S*,1'*R*)-体と(6*R*,1'*R*)-体の 2 種類しか生じない。

赤枠で囲った(6*S*,1'*R*)-体と(6*R*,1'*R*)-体は、ジアステレオマーの関係にあるので、カラムクロマトグラフィーにより分離可能である。両者を、先ほど X 線結晶構造解析により先に相対立体配置を決定した、青枠で示すサンプルと、薄層クロマトグラフィー上の挙動や NMR スペクトルを比較する。一致した立体異性体が(6*S*,1'*R*)-体であり、残り 3 種類の立体異性体も、この情報をもとに立体化学が確定したサンプルを調製できる。

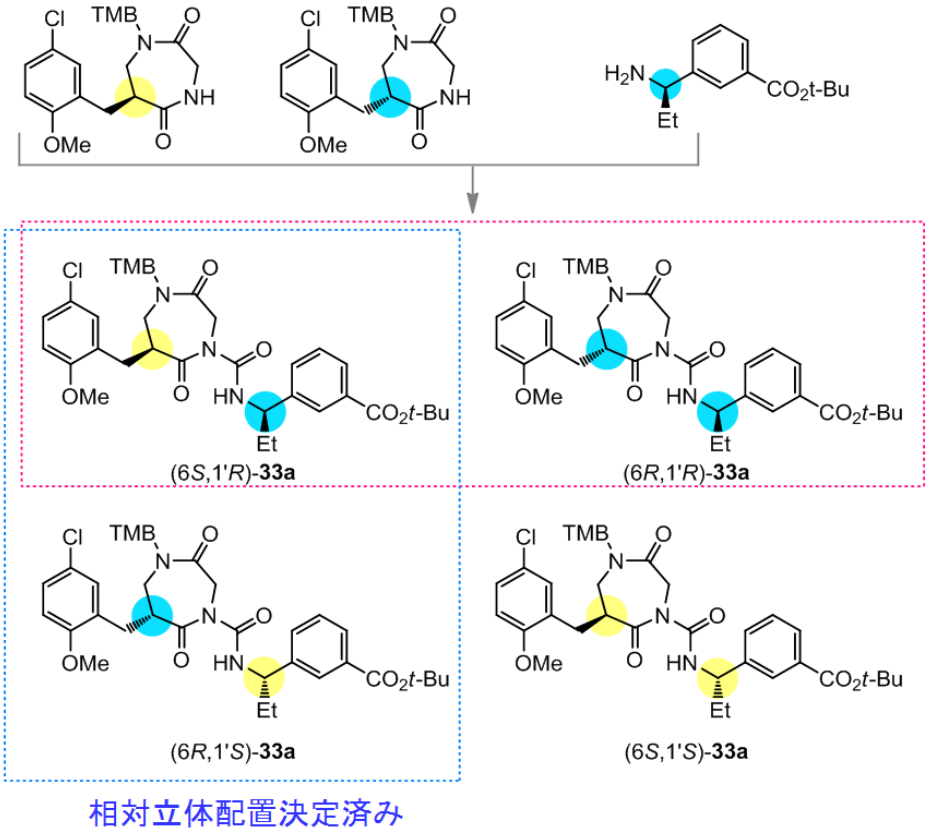
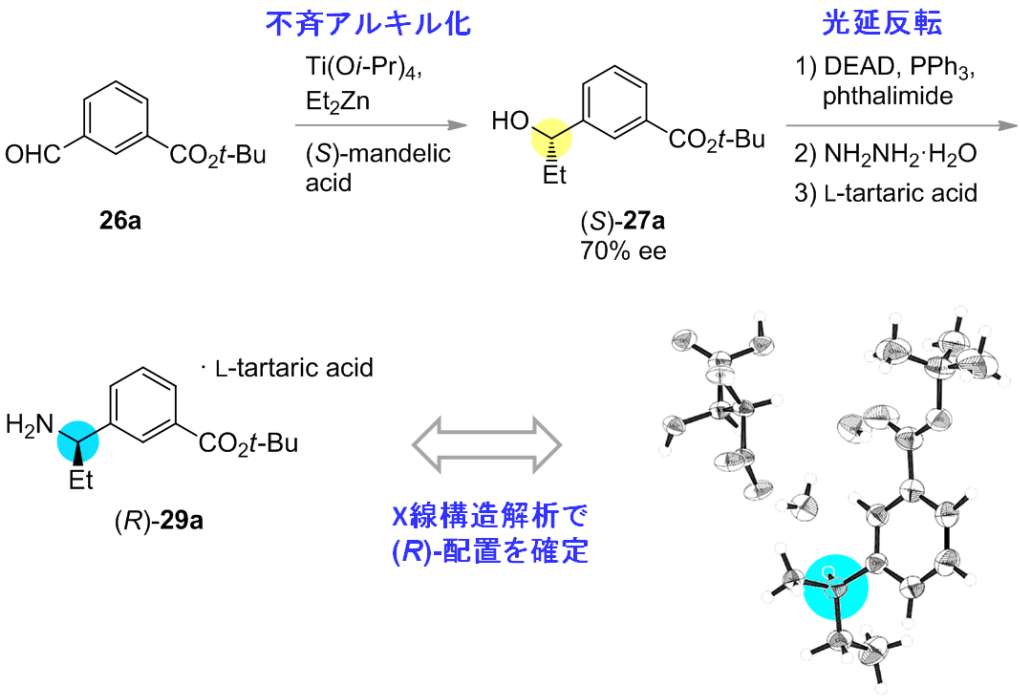


Figure 29 不斉合成と結晶構造解析を組み合わせることにより絶対立体配置を決定

この目的に向け、(*R*)-体の絶対立体配置を持つベンジルアミンを不斉合成した(Scheme 10)。まず、Bauer の方法 [39]によってアルデヒドから第二級アルコール(*S*)-**27a** を 70% ee、即ち(*S*) : (*R*) = 85 : 15 で調製した。このものに対しフタルイミドを窒素求核剤として光延反応に付した。求核置換反応は立体化学の反転を伴って進行するので、(*R*)-**29a** が生じる。

この不斉アルキル化は多数の例が報告されており、経験則から(*S*)-**27a** であることに疑いの余地はないが、(*R*)-**29a** は新規化合物なので、念のため酒石酸塩としたのち単結晶 X 線測定を依頼、立体化学が(*R*)-であることを確認した。



Scheme 10 ベンジルアミン：不斉合成で単一の立体異性体を調製

単結晶 X 線測定：アスビオファーマ（株）創薬技術ファンクション

第 2 節 立体化学が阻害活性に与える影響

以上のように、まずジアステレオマーのうち一方の相対立体配置を確定した。次いで不斉合成を活用して得た標品と物性を比較することにより、 (6*R*,1'*R*)-**25a** と (6*S*,1'*R*)-**25a** が確定し、残りも絶対立体配置が定まった。それらの 4 種の立体異性体について、それぞれ阻害測定を依頼した。結果を Figure 30 に示す。4 種のうち 1 種類、(6*R*,1'*R*)-体が強い活性を示している。第 4 章第 4 節の Figure 18 に示したように、ドッキングシミュレーションの段階で、化合物の P_1 site 芳香環が S_1 hole に深く入り込み、prime site が S_1'および S_2' site に良好に収容されると予測し、分子設計した立体化学とよく一致している。

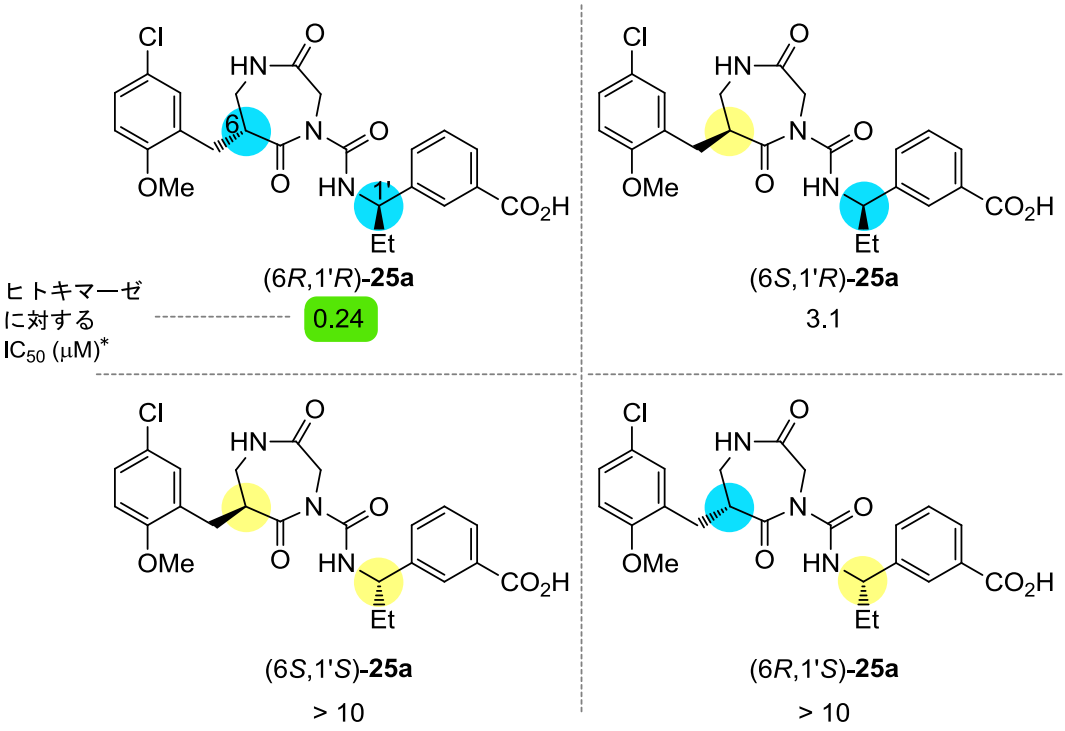


Figure 30 化合物 **25a** 全立体異性体の阻害活性

* アスビオファーマ（株）免疫・炎症疾患フィールド

第 3 節 前駆体であるジアゼパンの段階で立体化学の確定

前節で述べた手法では、もし Figure 31 の右上に示すアミン部分の構造を変えた場合、すべてのケースで同じ手順を繰り返さないと、化合物の立体化学は確定しない。これに対し、部分構造であるジアゼパン **22** のどちらか一方の鏡像体を用い、同じ縮合によって **33a** を合成すれば、左の(6*S*,1'*R*)-体か右の(6*R*,1'*R*)-体のどちらか一方のみが必ず一致する。その結果から遡及して、**22** の段階で絶対立体配置が決まるので、結果として、絶対立体配置を確定した **22** の両鏡像体の調製が可能になる。このことから、Figure 31 に示す、**22** のうち一方の鏡像体を用いた検証は、以後 **22** を出発原料として用いる合成で、正しい配置を持つ化合物群への到達に有益な情報を与える。

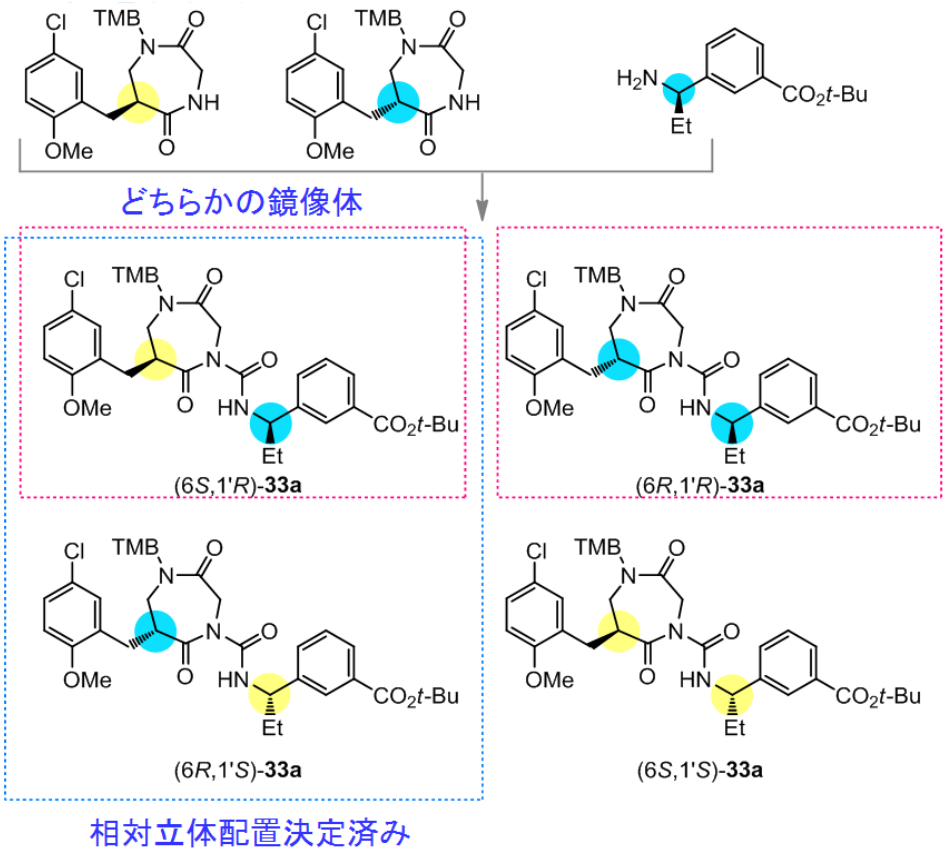


Figure 31　ジアゼパン **22** のうち一方の鏡像体を用いる、立体配置決定の試み

そこで、ラセミ体の **22** に対し、キラル固定相を持つカラムクロマトグラフィーによる鏡像異性体の分離を試みた。固定相や溶媒・流速など条件をさまざまに検討した結果、両鏡像異性体に分離することができた(Figure 32)。

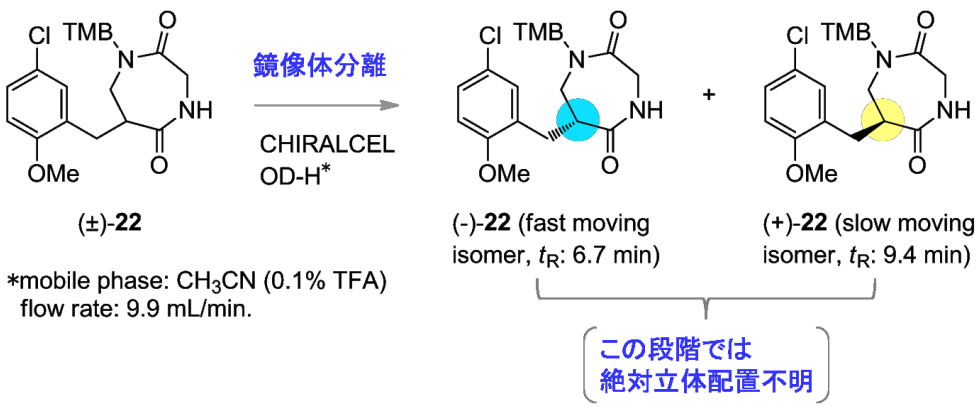


Figure 32　ジアゼパン部分：HPLC を用いた両鏡像体の分離

このうち、保持時間 6.7 分を示し、速く溶出した **22** を(*R*)-配置のベンジルアミン **29a** と縮合し、**33a** を合成した。この化合物の薄層クロマトグラフィーにおける R_f 値および NMR スペクトルは、 ラセミ体の(6*R**,1'*R**)-体と一致した。遡って、**22** の fast moving isomer は(*R*)-体、slow moving isomer は(*S*)-体と結論された(Figure 33)。

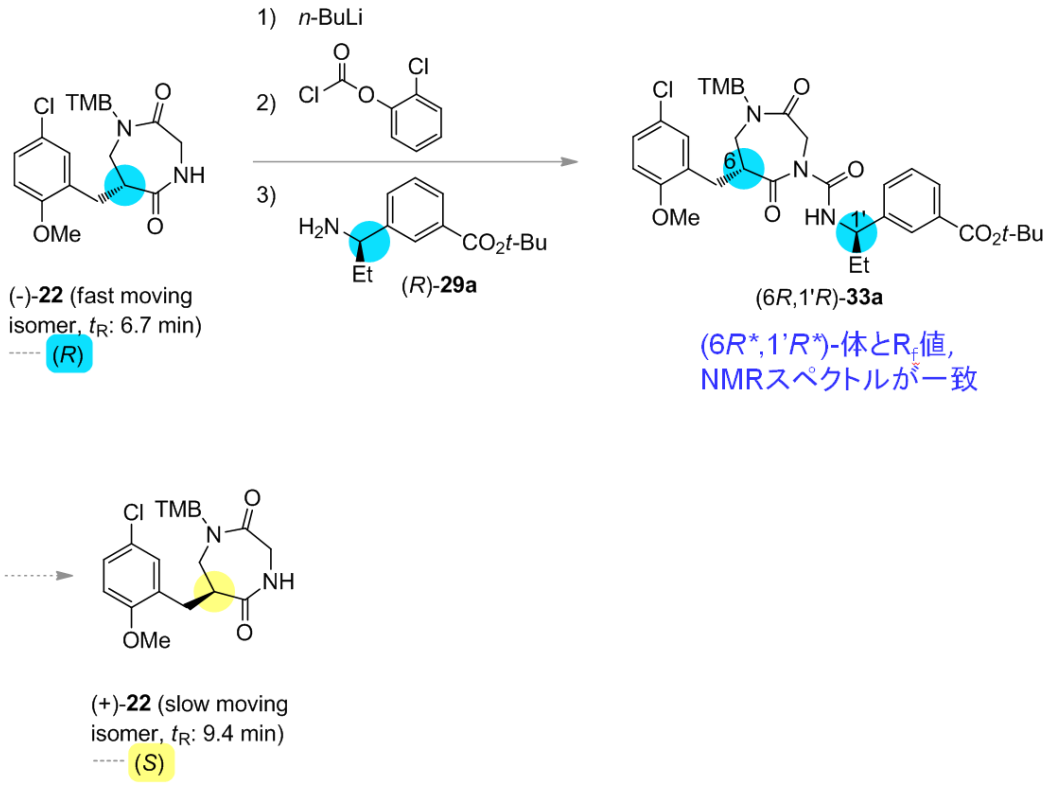
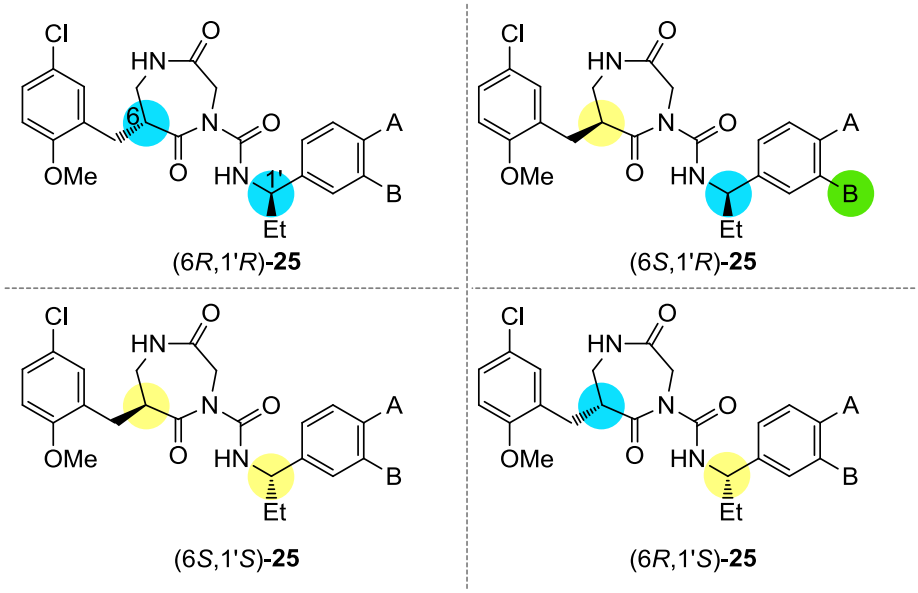


Figure 33 ジアゼパン部分：純粋な **33a** の立体化学から遡及した絶対立体配置決定

第 4 節 芳香環上の置換基に影響を受ける、活性と立体化学との関係

このようにしてジアゼパン部分 **22** は(*R*)-体、(*S*)-体を独立して調製できるようになったので、このものから出発し、prime site 芳香環にアミノ基を導入した **25d** の全立体異性体を合成した。それぞれについて阻害の測定を依頼、その結果を **25a** のそれとあわせて Table 10 に示した。アミノ基を持たない **25a** と同様に(6*R*, 1'*R*)-体は強い活性を示したが、**25d** では、ジアステレオマーである(6*S*,1'*R*)-体が、(6*R*,1'*R*)-体のそれをわずかながら上回った。

Table 10 化合物 **25a** および **25d** に含まれる、4 種の立体異性体の阻害活性



(6*R*,1'*R*)-**25**　(6*S*,1'*R*)-**25**

(6*S*,1'*S*)-**25**　(6*R*,1'*S*)-**25**

	絶対立体配置		置換基		ヒトキマーゼに対する IC_{50} (μM)*
25	6	1'	A	B	
a	*R*	*R*	H	CO_2H	0.24
	S	*S*	H	CO_2H	>10
	S	*R*	H	CO_2H	3.1
	R	*S*	H	CO_2H	>10
d	*R*	*R*	CO_2H	NH_2	0.30
	S	*S*	CO_2H	NH_2	>10
	S	*R*	CO_2H	NH_2	0.17
	R	*S*	CO_2H	NH_2	>10

* アスビオファーマ（株）免疫・炎症疾患フィールド

第 6 章 阻害剤複合体の X 線結晶構造解析から判明した、相互作用様式の違い

第 1 節 酵素の構造変化が阻害活性に与えた影響

前章の最後に示した(6*R*,1'*R*)-**25d** と(6*R*,1'*R*)-**25d** に着目し、キマーゼとの共結晶作成と単結晶 X 線回折の測定を依頼した。その結果を解析したところ、まず Figure 34 に示すように(6*R*,1'*R*)-**25d** は、P_1 site、prime site ともに、分子設計通り S_1 hole、S_1'および S_2' site に収容されていた。ジアゼパン環の 1 位アミドプロトンに関しては、複合体モデルで予想した通り、セリン 214 の主鎖に含まれるカルボニル基と水素結合していた。これに対し、ジアステレオマーで、しかもその活性が(6*R*,1'*R*)-体を上回った(6*S*,1'*R*)-**25d** については、P_1 site、prime site は(6*R*,1'*R*)-体と同様に収容されていたものの、上述のアミドプロトンは水素結合していなかった。

ここで、(6*S*,1'*R*)-**25d** および(6*R*,1'*R*)-**25d** の結晶構造において、ジアゼパン部位が酵素に収容される向きに着目した。両者では全く逆である。複合体を形成すると、酵素の構造自体も、リジン 40 およびリジン 192 の位置が大きく異なっている。

本研究と並行して Johnson &Johnson 社はキマーゼ阻害剤の創製を検討しており、その過程で化合物 H を見出していた(Figure 35)[40]。これらの化合物に着目して、韓国・慶尚大学校の Lee らを中心とするグループは、キマーゼとの共結晶の X 線結晶構造解析を 2013 年に報告[41]、本論文の研究と同様の構造変化を明らかにしている。

本研究では、(6*S*,1'*R*)-**25d** を収容した場合、S_2' site が狭く変化した。化合物の prime site 芳香環と一層接近し、より強く相互作用するようになったことが、(6*S*,1'*R*)-**25d** の活性が上回った理由の一つと思われる。

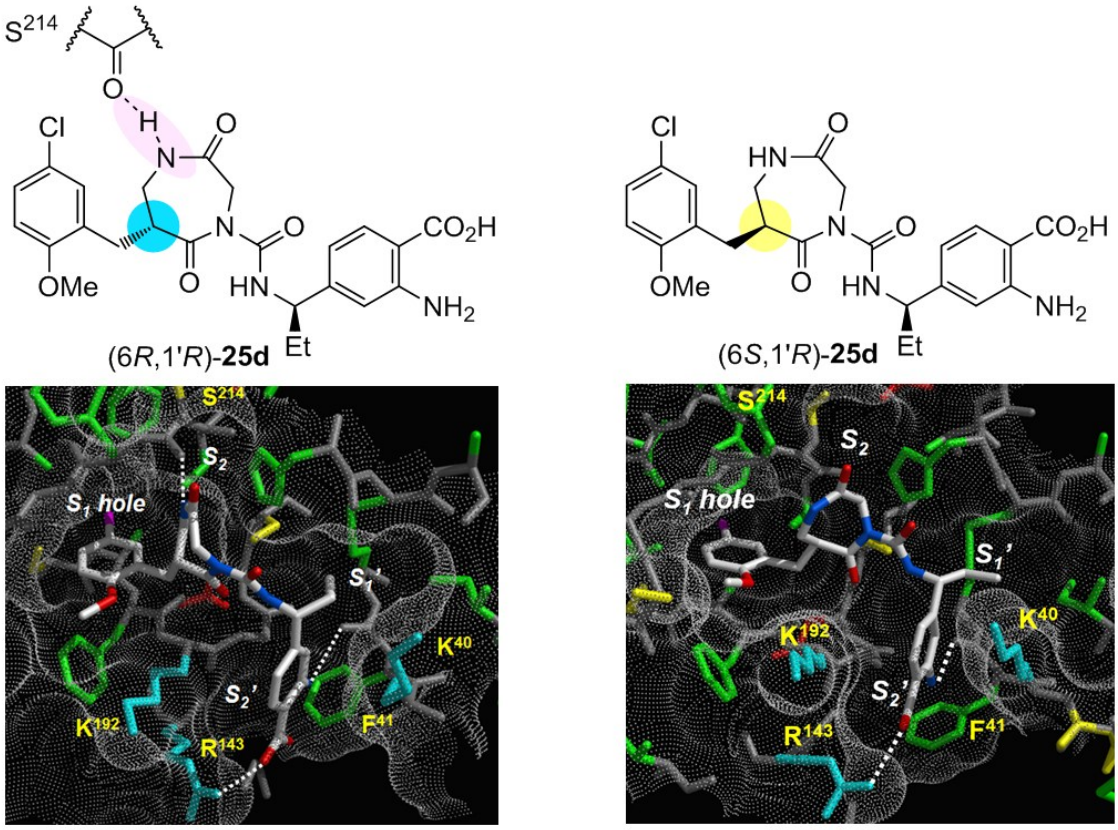
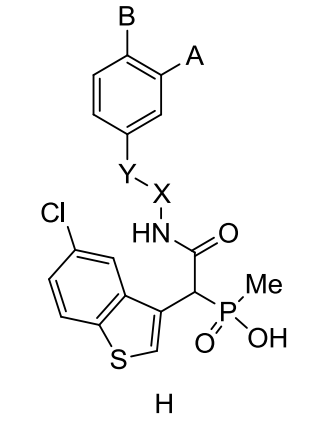


Figure 34 (6*R*,1'*R*)-**25d** および(6*S*,1'*R*)-**25d** とヒトキマーゼの共結晶 X 線構造解析

共結晶作成、単結晶 X 線測定：アスビオファーマ（株）創薬技術ファンクション

[分解能 (6*R*,1'*R*)-**25d**: 1.78 Å, (6*S*,1'*R*)-**25d**: 1.73 Å]



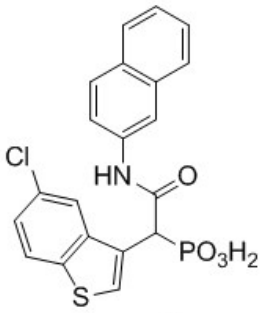
A = F, Cl　　X, Y = CH=CH

B = F, H　　CH_2

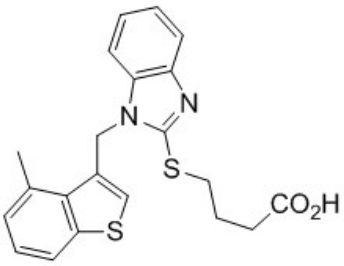
Figure 35 Johnson & Johnson 社が見出した、キマーゼ阻害剤 H

第 2 節 本研究対象化合物が相互作用するサイトと、他社により創製された化合物のそれらとの比較

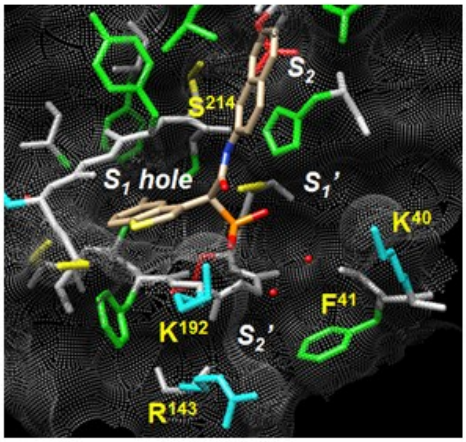
本研究において、キマーゼ - 阻害剤複合体の共結晶構造が明らかにされた前後に、他社で創製された化合物についても同様の情報が徐々に公刊されてきた。Johnson & Johnson の I[40], 帝人の J[42], Boehringer Ingelheim の K[43]を Figure 36 に示す。なお、I は H と同様の経緯で見出され、J は序章第 2 節で示した E を構造改変した化合物[44]である。これらの阻害剤と **25d** のキマーゼとの共結晶構造を比較した。S_1 hole の奥深くに置換基が入り込む結合様式は、**25d**, I-K いずれも同じだが、I-K は、S_2 site を含めた non-prime site のみに置換基のほとんどの部分が収容されている。これまでに見出されたプロテアーゼ阻害剤の多くは、I-K と同様に、non-prime site を主に利用している[45]。

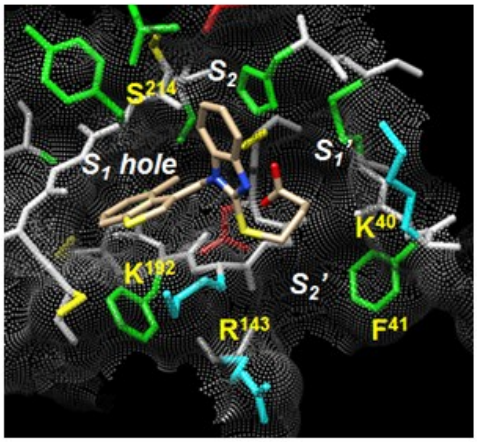


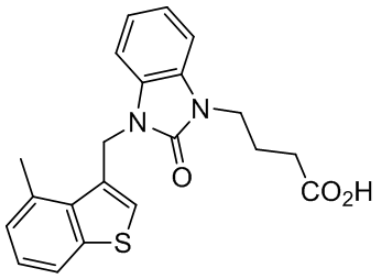
I (0.029)*1



J (0.002)*2







K (0.180)*1

*1 カッコ内はIC50 (μM, ヒトキマーゼ),
*2 カッコ内はKi (μM, ヒトキマーゼ)

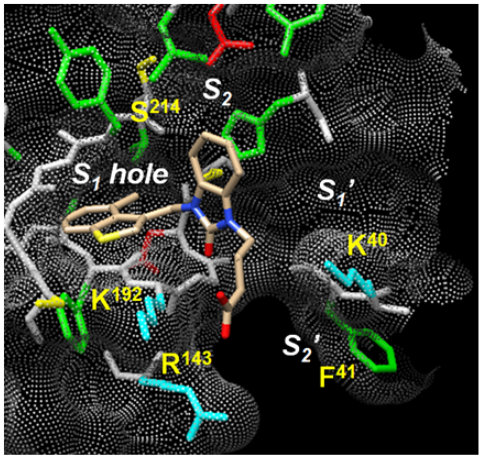


Figure 36 I, J および K とヒトキマーゼの共結晶 X 線構造解析
(H 分解能: 2.6 Å, PDBID: 2HVX; I 分解能: 2.8 Å, PDBID: 4kp0; J 分解能: 1.95 Å, PDBID: 3S0N)

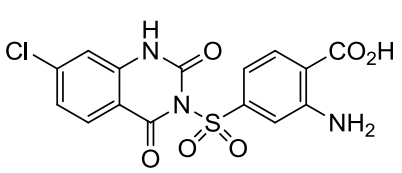
本研究を通じ到達した **25d** は、prime site との相互作用が強いという対照的な結果であった。測定条件等が同一ではなく、厳密な意味で直接比較はできないが、単純に阻害の強さを比較すると、I-K のように prime site を用いない創薬の方向性が勝っていたのでは、という印象を与えるかもしれない。

しかし、本研究の成果は、キマーゼ阻害剤の分子設計に、これまでにない視点を加えている。Novartis のグループでは、レニン阻害剤の創薬研究において、prime site の重要性を見出しており [46, 47]、著者の追及した方向性は、新しい展開の礎の 1 つになりうると考えている。

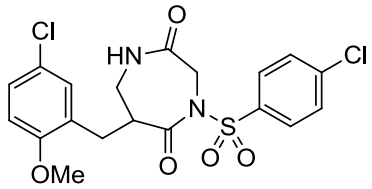
第 7 章　*in vivo* 皮膚炎モデルにおける有効性向上の確認

第 4 章第 11 節に示した、強い活性を示す化合物、具体的には(6*R*,1'*R*)-**25d** および(6*S*,1'*R*)-**25d** について、生体内安定性の確認と *in vivo* 皮膚炎モデルにおける有効性の検証を依頼した(Table 11)。その結果、血漿中の半減期は、スルホニル基を持つ阻害剤 **4c** が 18 分と不十分だったのに対し、60 分程度と十分な生体内安定性を示した。さらに動物モデルにおける評価では、先行研究のキナゾリン型阻害剤 **2c** が 50 mg / kg を腹腔内投与、カルバモイル型とした阻害剤 **13** が 10 mg / kg を経口投与することにより、ようやく抑制効果を示したのに対し、それぞれ 2.0, 0.4 mg / kg という低い用量で経口投与するという条件でさえ、皮膚炎を有意に抑制した [48]。*in vivo* 活性の向上という本研究の目的を達成した。

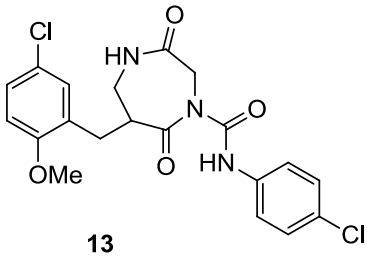
Table 11 生体内における安定性が改善、その結果 *in vivo* 活性が向上した



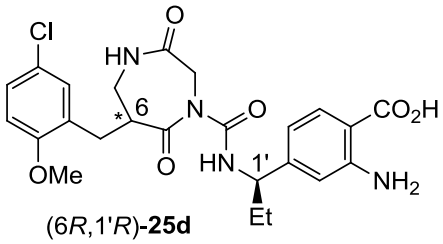
2c



4c



13



(6*R*,1'*R*)-**25d**
(6*S*,1'*R*)-**25d**

化合物	ヒトキマーゼ に対するIC$_{50}$ (μM)[1)]	マウス血中半減期 $T_{1/2}$ (min)[2)]	有意な抑制を示す 投与量(mg / kg)[3)]
2c	0.13	7	50 （腹腔内）
4c	0.034	18	2.0 (経口）
13	5.4	> 60	10 （経口）
(6*R*,1'*R*)-**25d**	0.3	60	2.0 (経口）
(6*S*,1'*R*)-**25d**	0.17	54	0.4 (経口）

1) アスビオファーマ（株）免疫・炎症疾患フィールド

2) 静脈内投与後の血漿中半減期　同社創薬技術ファンクション

3) ハプテン反復塗布マウスにおける効果　同社免疫・炎症疾患フィールド

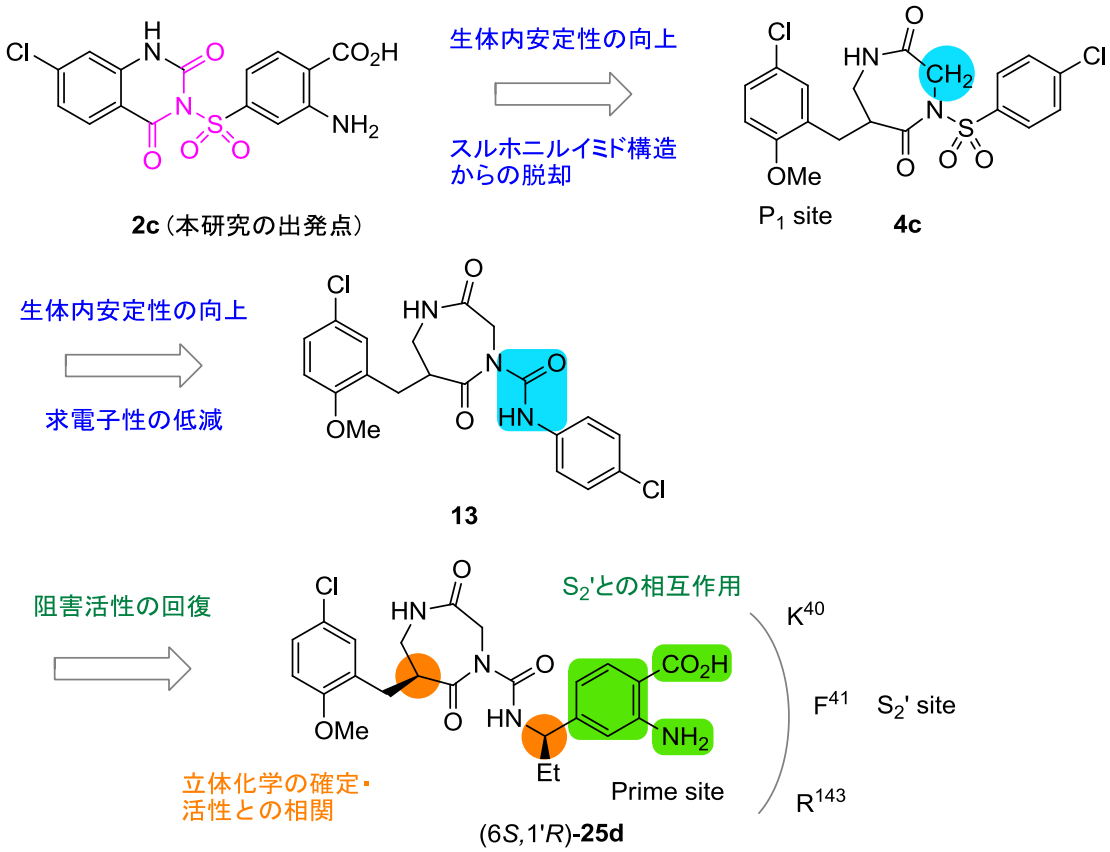
第 8 章　総括

本研究の成果を Table 12 にまとめた。アトピー性皮膚炎治療に有効なキマーゼ阻害剤の創製をめざし、活性を示した **2c** を出発点として最適化研究を試みた。まず、生体内における安定性の向上を目指し、3 つの電子求引基が隣接する、不安定なスルホニルイミド構造をメチレン鎖の導入により解消、P_1 site 芳香環を独立させ、ジアゼパン骨格とした。IC_{50} が 0.034 μM と強い活性を示す化合物を見出したが、血漿中半減期が短く、このままでは動物を使って薬効を試験できない。生体内物質が非可逆的に攻撃・開環する可能性を低減することが重要である。そこで、スルホニル基を電子求引性が弱いカルバモイル基に変えた。その結果、血漿中半減期が 60 分以上と、生体内における安定性が大幅に向上した。

しかし、IC_{50} が 5.4 μM と活性は大きく低下してしまったので、その回復をめざしてさらに最適化を進めた。具体的には S_2’ site との親和性を指標に、複合体モデルをもとに分子設計し、prime site 芳香環を修飾、変換した化合物をさまざまに合成した。

それらの中から、強い活性と生体内における安定性を併せ持ち、*in vivo* 皮膚炎モデルにおいて、研究初期の化合物に比べ、低い投与量で有効な化合物を見出した。それらについては、合成化学的手法と X 線結晶構造解析を組み合わせる方法で立体化学を決定し、さらに、目的とする立体化学の化合物を、効率よく合成する手法を確立した。

Table 12 本研究のまとめ



化合物	ヒトキマーゼ に対するIC$_{50}$ (μM)	マウス血中半減期 T$_{1/2}$ (min)	有意な抑制を示す 投与量(mg/kg)
2c	0.13	7	50 （腹腔内）
4c	0.034	18	2.0（経口）
13	5.4	>60	10 （経口）
(6*S*1'*R*)-**25d**	0.17	54	0.4（経口）

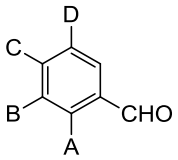
本研究によって、臨床試験を実施した化合物の供給も果たしており、「安全性が高く、経口投与が可能なアトピー性皮膚炎治療薬」の創製に合成化学的見地から貢献した。

実験の部

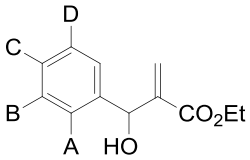
General Methods

^{1}H NMR spectra were recorded on Brucker ARX-400 or Brucker Avance III (400 MHz) spectrometer in the indicated solvent. Chemical shifts and coupling constants in ^{1}H NMR signals were elucidated by the aid of analyzing software, ACD Spectrus Processor (2012 Release). Mass spectra (ESI) were recorded on Agilent G1956A MSD spectrometer system. High resolution mass spectra (FAB) were recorded on JEOL the Mstation JMS-700. Specific rotations were recorded on Jasco DIP-1020. Melting points were recorded on Stanford Research Systems OptiMelt MPA-100. Chemical reagents and solvents were purchased from Sigma-Aldrich Co. LLC., Tokyo Chemical Industry Co., LTD., Wako Pure Chemical Industries LTD., Kanto Chemical Co., Inc. or Nacalai Tesque, and used without purification. Flash column chromatography was performed using Merck Silica Gel 60 (230-400 mesh) or Purif-Pack SI 30 μm supplied by Shoko Scientific. Preparative TLC was performed using Merck PTLC Glass Plates Si 60 20 x 20 cm, 0.5 mm or 1 mm thick.]

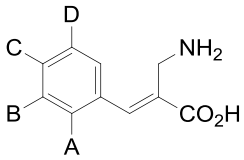
Table 13 Structures of compounds **5a-e**, **6a-e** and **7a-e**



5



6



7

5-7	A	B	C	D
a	H	H	H	H
b	OMe	H	H	Cl
c	Cl	H	H	H
d	H	Cl	H	H
e	H	H	Cl	H

Ethyl 2-(1'-hydroxylbenzyl)propenoate (6a)

To a mixture of **5a** (2.00 g) and ethyl acrylate (3.28 mL) were added 1,4-diazabicyclo[2.2.2]octane (2.11 g), lanthanum trifluoromethanesulfonate (550 mg) and triethanolamine (1.25 mL), and the mixture was stirred at room temperature for 3 days. Water and saturated potassium hydrogen sulfate aqueous solution were added to the reaction mixture, and the resulting mixture was extracted with ethyl acetate. The organic layer was successively washed with water and brine, dried over anhydrous sodium sulfate, then concentrated *in vacuo* to give **6a** (3.64 g, 94.0%). ^{1}H NMR ($CDCl_3$) δ: 7.41-7.31 (4H, m), 7.31-7. 27 (1H, m), 6.35-6.33 (1H, m), 5.83-5.79 (1H, m), 5.60-5.54 (1H, m), 4.18 (2H, q, *J* = 7.1 Hz), 1.24 (3H, t, *J* = 7.1 Hz); MS (ESI): 189 $(M\text{-}OH)^+$. This was employed for the next step without further purification.

Ethyl 2-[[(5'-chloro-2'-methoxy)phenyl]-hydroxymethyl]propenoate (6b)

Ester **6b** was prepared from **5b** in a similar manner to **6a**.^{1}H NMR ($CDCl_3$) δ: 7.37 (1H, d, *J* = 2.6 Hz), 7.22 (1H, dd, *J* = 8.7, 2.7 Hz), 6.80 (1H, d, *J* = 8.8 Hz), 6.31 (1H, dd, *J* = 1.3, 1.3 Hz), 5.86-5.80 (1H, m), 5.68 (1H, dd, *J* = 1.3, 1.3 Hz), 4.22 (2H, q, *J* = 7.1 Hz), 3.81 (3H, s), 1.28 (3H, t, *J* = 7.2 Hz); MS (ESI): 253 $(M\text{-}OH)^+$. This was employed for the next step without further purification.

Ethyl 2-[(2'-cholorophenyl)-hydroxymethyl]propenoate (6c)

^{1}H NMR ($CDCl_3$) δ: 7.56 (1H, d, *J* = 7.8 Hz), 7.36 (1H, d, *J* = 7.8 Hz), 7.31 (1H, dd, *J* = 7.8, 7.8 Hz), 7.25 (1H, dd, *J* = 7.8, 7.8 Hz), 6.34 (1H, s), 5.98 (1H, s), 5.58 (1H, s), 4.23 (2H, q, *J* = 7.2 Hz), 1.28 (3H, t, *J* = 7.2 Hz); MS (ESI): 233 $(M\text{-}OH)^+$. This was employed for the next step without further purification.

Ethyl 2-[(3'-cholorophenyl)-hydroxymethyl]propenoate (6d)

^{1}H NMR ($CDCl_3$) δ: 7.44-7.35 (1H, m), 7.30-7.24 (3H, m), 6.36 (1H, dd, *J* = 1.1, 1.1 Hz), 5.82 (1H, dd, *J* = 1.1, 1.1 Hz), 5.54-5.50 (1H, m), 4.19 (2H, q, *J* = 7.2 Hz), 1.26 (3H, t, *J* = 7.2 Hz); MS (ESI): 233 $(M\text{-}OH)^+$. This was employed for the next step without further purification.

Ethyl 2-[(4'-cholorophenyl)-hydroxymethyl]propenoate (6e)

Ester **4b** was prepared from **3b** in a similar manner to **4a**. ^{1}H NMR ($CDCl_3$) δ: 7.32 (4H, s), 6.34 (1H, dd, *J* = 1.1, 1.1 Hz), 5.80 (1H, dd, *J* = 1.1, 1.1 Hz), 5.54-5.52 (1H, m), 4.18 (2H, q, *J* = 7.2 Hz), 1.26 (3H, t, *J* = 7.2 Hz); MS (ESI): 233 $(M\text{-}OH)^+$. This was employed for the next step without further purification.

(*E*)-2-(Aminomethyl)-3-phenylpropenoic acid (7a)

To a solution of **6a** (3.64 g) in methylene chloride (30 mL) were added triethylamine (2.58 mL) and acetyl chloride (1.25 mL) under ice cooling, and the mixture was stirred at that temperature for 1 hour. Water was added to the reaction mixture, and the resulting mixture was extracted with methylene chloride. The organic layer was successively washed with saturated potassium hydrogen sulfate aqueous solution, saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

The residue (3.77 g) was diluted with dimethyl sulfoxide (25 mL), then sodium azide (1.48 g) was added to the solution, and the mixture was stirred at room temperature for 30 minutes. Water was added to the reaction mixture, and the resulting mixture was extracted with diethyl ether. The organic layer was successively washed with water and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

The residue (3.29 g) was diluted with tetrahydrofuran (40 mL), then triphenylphosphine

(3.73 g) and water (600 μL) were added to the mixture, and the resulting mixture was stirred at room temperature for 14 hours. Next, tetrahydrofuran was distilled off *in vacuo*.

To the resulted aqueous mixture, ethanol (40 mL) and 2.0 M sodium hydroxide aqueous solution (20 mL) were added, and the mixture was stirred at room temperature for 2 hours. Ethanol was distilled off *in vacuo*, and the resulted aqueous layer was washed with ethyl acetate. Then, the resulted aqueous layer was neutralized with 2.0 M hydrochloric acid. The precipitates were collected by filtration, washed with diethyl ether, and dried *in vacuo* to give **7a** (640 mg, 25.4%). ^{1}H NMR (acetic acid-*d*4): δ 8.16 (1H, s), 7.43-7.56 (5H, m), 4.23 (2H, s); MS (ESI): 178 $(M+H)^+$. This was employed for the next step without further purification.

(*E*)-2-(Aminomethyl)-3-(5'-chloro-2'-methoxy)propenoic acid (7b)

Acid **7b** was prepared from **6b** in a similar manner to **7a**. ^{1}H NMR (acetic acid-*d*4) δ: 8.07 (1H, s), 7.41 (1H, dd, *J* = 2.6, 8.8 Hz), 7.34 (1H, d, *J* = 2.6 Hz), 7.03 (1H, d, *J* = 8.8 Hz), 4.07 (2H, s); MS (ESI): 242 $(M+H)^+$. This was employed for the next step without further purification.

(*E*)-2-(Aminomethyl)-3-(2'-cholorophenyl)propenoic acid (7c)

^{1}H NMR (DMSO-*d*6): δ 8.37 (2H, br), 7.53 (1H, s), 7.52-7.47 (1H, m), 7.44-7.28 (3H, m), 3.57 (2H, s); MS (ESI): 212 $(M+H)^+$. This was employed for the next step without further purification.

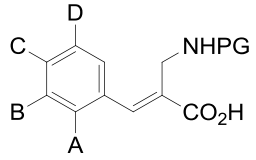
(*E*)-2-(Aminomethyl)-3-(3'-cholorophenyl)propenoic acid (7d)

^{1}H NMR (acetic acid-*d*4): δ 8.06 (1H, s), 7.49-7.42 (3H, m), 7.35-7.40 (1H, m), 4.16 (2H, s); MS (ESI): 212 $(M+H)^+$. This was employed for the next step without further purification.

(*E*)-2-(Aminomethyl)-3-(4'-cholorophenyl)propenoic acid (7e)

^{1}H NMR (acetic acid-*d*4): δ 8.07 (1H, s), 7.51-7.41 (4H, m), 4.17 (2H, s); MS (ESI): 212 $(M+H)^+$. This was employed for the next step without further purification.

Table 14 Structures of compounds **8a-e**



8

8	A	B	C	D	PG
a	H	H	H	H	Boc
b	OMe	H	H	Cl	CF_3CO
c	Cl	H	H	H	CF_3CO
d	H	Cl	H	H	Boc
e	H	H	Cl	H	Boc

(*E*)-2-[[(*tert*-Butoxycarbonyl)amino]methyl]-3-phenylpropenoic acid (8a)

To a suspension of **7a** (450 mg) in tetrahydrofuran (10 mL) were added 2.0 M sodium hydroxide aqueous solution (2.5 mL) and di-*tert*-butyl dicarbonate (610 mg), and the mixture was stirred at room temperature for 1 hour. Next, tetrahydrofuran was distilled off *in vacuo*, and the resulted aqueous mixture was adjusted pH to approximately 4 by adding saturated potassium hydrogen sulfate aqueous solution. The mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 4 / 1), then the precipitates were collected by filtration to give **8a** (700 mg, quaant.). ^{1}H NMR ($CDCl_3$): δ 7.98-7.73 (1H, br), 7.60-7.30 (5H, br), 6.75 (0.5H, br), 5.14 (0.5H, br), 4.25 (2H, d, J = 5.9 Hz), 1.60-1.15 (9H, br); MS: 278 $(M+H)^+$. This was employed for the next

step without further purification.

(*E*)-2-[[(*tert*-Butoxycarbonyl)amino]methyl]-3-(3'-chlorophenyl)propenoic acid (8d)

Acid **8d** was prepared from **7d** in a similar manner to **8a**. ^{1}H NMR ($CDCl_3$) δ: 7.85-7.65 (2H, m), 7.51-7.35 (2H, m), 6.80 (1H, br), 5.10 (1H, br), 4.21 (2H, d, J = 3.5Hz), 1.25 (9H, br); MS: 334 $(M+Na)^+$. This was employed for the next step without further purification.

(*E*)-2-[[(*tert*-Butoxycarbonyl)amino]methyl]-3-(4'-chlorophenyl)propenoic acid (8e)

^{1}H NMR ($CDCl_3$) δ: 7.82-7.70 (1H, m), 7.48-7.22 (3H, m), 6.77 (1H, br), 5.14 (1H, br), 4.21 (2H, d, J = 6.3 Hz), 1.28 (9H, br); MS: 334 $(M+Na)^+$. This was employed for the next step without further purification.

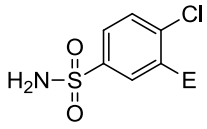
(*E*)-3-[(5"-Chloro-2"-methoxy)phenyl]-2-[(2',2',2'-trifluroacetamino)methyl]propenoic acid (8b)

To a suspension of **7b** (1.00 g) in tetrahydrofuran (10 mL) was added trifluoroacetic anhydride (1.0 mL) under ice cooling, and the mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated *in vacuo*, and ethyl acetate was added to the residue. The resulting mixture was successively washed with saturated potassium hydrogen sulfate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 4 / 1), then the precipitates were collected by filtration to give **8b** (1.20 g, 85.7%). ^{1}H NMR ($CDCl_3$) δ: 8.01 (1H, s), 7.39 (1H, d, J = 2.4 Hz), 7.35 (1H, dd, J = 8.8, 2.4 Hz), 7.01 (1H, br), 6.88 (1H, d, J = 8.8 Hz), 4.33 (2H, d, J = 6.0 Hz), 3.84 (3H, s); MS (ESI): 360 $(M+Na)^+$. This was employed for the next step without further purification.

(*E*)-3-(2''-Chlorophenyl)-2-[(2',2',2'-trifluroacetamino)methyl]propenoic acid (8c)

Acid **8c** was prepared from **7c** in a similar manner to **8b**. ^{1}H NMR (DMSO-*d*6) δ: 12.9 (1H, br), 9.59 (1H, br), 7.79 (1H, s), 7.59-7.53 (1H, m), 7.52-7.48 (1H, m), 7.47-7.38 (2H, m), 4.08 (2H, d, J = 4.7 Hz); MS (ESI): 308 $(M+H)^+$. This was employed for the next step without further purification.

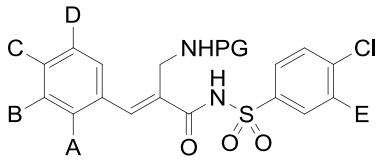
Table 15 Structures of compounds **9a-c**



9

9	E
a	H
b	$NHCOCF_3$
c	CO_2t-Bu

Table 16 Structures of compounds **10a-g**



10

10	A	B	C	D	PG	E
a	H	H	H	H	Boc	H
b	OMe	H	H	Cl	CF_3CO	H
C	OMe	H	H	Cl	CF_3CO	$NHCOCF_3$
d	Cl	H	H	H	CF_3CO	H
e	H	Cl	H	H	Boc	H
f	H	H	Cl	H	Boc	H
g	OMe	H	H	Cl	CF_3CO	CO_2t-Bu

(*E*)-2-[[(*tert*-Butoxycarbonyl)amino]methyl]-*N*-[(4'-chlorophenyl)sulfonyl]-3-phenylpropenamide (10a)

To a solution of **8a** (789 mg) and **9a** (551mg) in methylene chloride (15 mL) were added 4-*N*,*N*-dimethylaminopyridine (350 mg) and 1-[(3'-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (830 mg), and the mixture was stirred at room temperature for 14 hours. Then, ethyl acetate was added to the reaction mixture. The resulting mixture was successively washed with water, saturated potassium hydrogen sulfate aqueous solution, water and brine, dried over anhydrous sodium sulfate, then concentrated *in vacuo* to give **10a** (1.39 g, quantitative). ^{1}H NMR ($CDCl_3$) δ: 12.00-11.12 (1H, br), 8.08 (2H, d, J = 8.5 Hz), 7.88 (1H, s), 7.50 (2H, d, J = 8.5 Hz), 7.44-7.35 (3H, m), 7.21 (2H, d, J = 7.0 Hz), 4.93 (1H, t, J = 6.9 Hz), 4.12 (2H, d, J = 6.9 Hz), 1.51 (9H, s); MS: 395(M-*tert*-Bu)$^+$. This was employed for the next step without further purification.

(*E*)-3-[(5"-Chloro-2"-methoxy)phenyl]-*N*-[(4"'-chlorophenyl)sulfonyl]-2-[(2',2',2'-trifluoroacetamido)methyl]propenamide (10b)

Amide **10b** was prepared from **8b** and **9a** in a similar manner to **10a**. ^{1}H NMR ($CDCl_3$) δ: 9.56-9.29 (1H, br), 8.06 (2H, d, J = 8.5 Hz), 7.54 (1H, s), 7.54 (2H, d, J = 8.5 Hz), 7.35 (1H, dd, J = 8.9, 2.4 Hz), 7.23 (1H, d, J = 2.4 Hz), 7.07-6.97 (1H, m), 6.90 (1H, d, J = 8.9 Hz), 4.21 (2H, d, J = 6.3 Hz), 3.84 (3H, s); MS: 511(M+H)$^+$. This was employed for the next step without further purification.

(*E*)-3-[(5"-Chloro-2"-methoxy)phenyl]-*N*-[[[4"'-chloro-3-(2"",2"",2""-trifluoroacetamido)]phenyl]sulfonyl]-2-[(2',2',2'-trifluoroacetamido)methyl]propenamide (10c)

^{1}H NMR ($CDCl_3$) δ: 8.99 (1H, d, *J* = 2.0 Hz), 8.49 (1H, s), 7.99 (1H, dd, *J* = 8.5, 2.0 Hz), 7.65 (1H, d, *J* = 8.5 Hz), 7.55 (1H, s), 7.33 (1H, dd, *J* = 8.9, 2.4 Hz), 7.24 (1H, d, *J* = 2.4 Hz), 7.10-7.06 (1H, m), 6.87 (1H, d, *J* = 8.9 Hz), 4.22 (2H, d, *J* = 6.1 Hz), 3.89 (3H, s). This was employed for the next step without further purification.

(*E*)-3-(2”-Chlorophenyl)-*N*-[(4”’-chlorophenyl)sulfonyl]-2-[(2’,2’,2’-trifluoroacetamido)methyl]propenamide (10d)

^{1}H NMR ($CDCl_3$) δ: 9.88-9.14 (1H, m), 8.06 (2H, d, *J* = 8.8 Hz), 7.60 (1H, s), 7.54 (2H, d, *J* = 8.8 Hz), 7.37-7.33 (3H, m), 6.93 (1H, t, *J* = 6.1 Hz), 4.21 (2H, d, *J* = 6.1 Hz). This was employed for the next step without further purification.

(*E*)-2-[[(*tert*-Butoxycarbonyl)amino]methyl]-3-(4’-chlorophenyl)-*N*-[(3”-chlorophenyl)sulfonyl] propenamide (10e)

^{1}H NMR ($CDCl_3$) δ: 8.08 (2H, d, *J* = 8.6 Hz), 7.78 (1H, s), 7.50 (2H, d, *J* = 8.6 Hz), 7.39-7.30 (2H, m), 7.19 (1H, s), 7.14-7.05 (1H, m), 4.90 (1H, t, *J* = 6.6 Hz), 4.09 (2H, d, *J* = 6.6 Hz), 1.51 (9H, s). This was employed for the next step without further purification.

(*E*)-2-[[(*tert*-Butoxycarbonyl)amino]methyl]-3-(4’-chlorophenyl)-*N*-[(4”-chlorophenyl)sulfonyl] propenamide (10f)

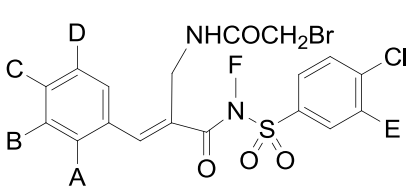
^{1}H NMR ($CDCl_3$) δ: 8.07 (2H, d, *J* = 6.2 Hz), 7.78 (1H, s), 7.50 (2H, d, *J* = 6.2 Hz), 7.38 (2H, d, *J* = 8.1 Hz), 7.16 (2H, d, *J* = 8.1 Hz), 4.91 (1H, t, *J* = 7.1 Hz), 4.10 (2H, d, *J* = 7.1 Hz), 1.50 (9H, s). This was employed for the next step without further purification.

(*E*)-*N*-[[4”’-Chloro-3”’-(*tert*-butoxycarbonyl)phenyl]sulfonyl]-3-[(5”-chloro-2”-methoxy)p

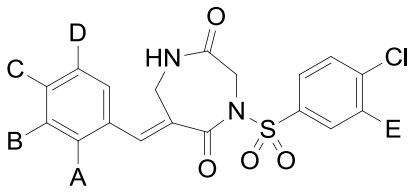
henyl]-2-[(2',2',2'-trifluoroacetamido)methyl]propenamide (10g)

^{1}H NMR ($CDCl_3$) δ: 8.39 (1H, d, *J* = 2.2 Hz), 8.12 (1H, dd, *J* = 8.5, 2.2 Hz), 7.60 (1H, d, *J* = 8.5 Hz), 7.56 (1H, s), 7.34 (1H, dd, *J* = 8.9, 2.4 Hz), 7.22 (1H, d, *J* = 2.4 Hz), 7.15-7.10 (1H, m), 6.88 (1H, d, *J* = 8.9 Hz), 4.20 (2H, d, *J* = 6.1 Hz), 3.82 (3H, s). This was employed for the next step without further purification.

Table 17 Structures of compounds **11a-g** and **12a-g**



11



12

11 or **12**	A	B	C	D	E	F
a	H	H	H	H	H	H
b	OMe	H	H	Cl	H	H
c	OMe	H	H	Cl	NH_2	Na
d	Cl	H	H	H	H	Na
e	H	Cl	H	H	H	H
f	H	H	Cl	H	H	H
g	OMe	H	H	Cl	CO_2t-Bu	H

(*E*)-2-[(2'-Bromoacetamido)methyl]-*N*-[(4"-chlorophenyl)sulfonyl]-3-phenylpropenamide (11a)

1.0 M hydrogen chloride solution in acetic acid (20 mL) was added to **10a** (1.37 g), and the mixture was stirred at room temperature for 90 minutes. Then, the reaction mixture was diluted with diethyl ether, and stirred at 0 °C for 30 minutes. The precipitates were collected by filtration to give colorless solids (880 mg).

To a biphasic mixture of the above-mentioned solid (880 mg) in methylene chloride (12 mL) and water (6.0 mL) were added triethylamine (700 μL) and bromoacthyl bromide (220 μL) at 0 °C, and the mixture was stirred at room temperature for 20 minutes.

Another portion of triethylamine (350 μL) and bromoacetyl bromide (110 μL) was added to the mixture, and the resulting mixture was stirred at room temperature. Then, the reaction mixture was diluted with ethyl acetate, and the mixture was successively washed with saturated sodium hydrogen carbonate aqueous solution, water, saturated potassium hydrogen sulfate aqueous solution, water, and brine. The organic layer was dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was triturated with a mixture of ethyl acetate and hexane, and the insoluble solid was collected by filtration to give **11a** (640 mg, 44.6%). ^{1}H NMR ($CDCl_3$) δ: 7.98-7.93 (2H, m), 7.48-7.43 (3H, m), 7.39-7.32 (5H, m), 7.07 (1H, s), 5.22 (2H, s), 4.30 (2H, s). This was employed for the next step without further purification.

(*E*)-2-[(2'-Bromoacetamido)methyl]-3-(3"-chlorophenyl)-*N*-[(4"'-chlorophenyl)sulfonyl]propenamide (11e)

Amide **11e** was prepared from **10e** in a similar manner to **11a**.^{1}H NMR (DMSO-*d*6) δ: 8.46 (1H, t, *J* = 5.1 Hz), 7.97 (2H, d, *J* = 8.6 Hz), 7.72 (2H, d, *J* = 8.6 Hz), 7.58 (1H, s), 7.52 (1H, s), 7.48-7.44 (3H, m), 3.98 (2H, d, *J* = 5.1 Hz), 3.79-3.73 (2H, m). This was employed for the next step without further purification.

(*E*)-2-[(2'-Bromoacetamido)methyl]-3-(4"-chlorophenyl)-*N*-[(4"'-chlorophenyl)sulfonyl]propenamide (11f)

^{1}H NMR (DMSO-*d*6) δ: 8.46 (1H, t, *J* = 4.6 Hz), 7.97 (2H, d, *J* = 8.6 Hz), 7.72 (2H, d, *J* = 8.6 Hz), 7.54-7.48 (4H, m), 3.99 (2H, d, *J* = 4.6 Hz), 3.76 (2H, s). This was employed for the

next step without further purification.

(*E*)-6-Benzylidene-4-[(4'-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (12a)

To a solution of **11a** (640 mg) in *N*,*N*-dimethylformamide (90 mL) was added 60% dispersion of sodium hydride in mineral oil (58 mg), and the mixture was stirred at room temperature for 20 minutes and at 60 °C for 14 hours. After cooling, acetic acid was added to the reaction mixture, and the resulting mixture was concentrated *in vacuo*. The residue was diluted with ethyl acetate, and the mixture was successively washed with saturated sodium hydrogen carbonate aqueous solution, water, saturated potassium hydrogen sulfate aqueous solution, water and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 5 / 1), then the precipitates were collected by filtration to give **12a** (354 mg, 66.8%). ^{1}H NMR (DMSO-*d*6) δ: 8.07 (1H, t, *J* = 4.1 Hz), 7.96 (2H, d, *J* = 7.9 Hz), 7.74 (2H, d, *J* = 7.9 Hz), 7.54 (1H, s), 7.49-7.38 (5H, m), 4.71 (2H, s), 4.29 (2H, d, *J* = 4.1 Hz). This was employed for the next step without further purification.

(*E*)-6-(3"-Chlorobenzylidene)-4-[(4'-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (12e)

Diazepane **12e** was prepared from **11e** in a similar manner to **12a**. ^{1}H NMR ($CDCl_3$) δ: 8.02 (2H, d, *J* = 8.6 Hz), 7.59 (1H, s), 7.52 (2H, d, *J* = 8.6 Hz), 7.42-7.33 (2H, m), 7.25-7.22 (1H, m), 7.17-7.11 (1H, m), 6.02-5.92 (1H, m), 4.70 (2H, s), 4.31 (1H, d, *J* = 4.6 Hz), 4.32 (1H, d, *J* = 4.6 Hz). This was employed for the next step without further purification.

(*E*)-6-(4"-Chlorobenzylidene)-4-[(4'-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (12f)

^{1}H NMR ($CDCl_3$) δ: 8.02 (2H, d, *J* = 7.5 Hz), 7.60 (1H, s), 7.52 (2H, d, *J* = 7.5 Hz), 7.40

(2H, d, *J* = 7.4 Hz), 7.20 (2H, d, *J* = 7.4 Hz), 5.84-5.93 (1H, m), 4.70 (2H, s), 4.31 (2H, d, *J* = 3.6 Hz). This was employed for the next step without further purification.

(*E*)-2-[(2'-Bromoacetamido)methyl]-3-[(5"-chloro-2"-methoxy)phenyl]-*N*-[(4"'-chlorophenyl)sulfonyl]propenamide (11b)

To a solution of **10b** (1.01 g) in methanol (9.0 mL) was added 2.0 M sodium hydroxide aqueous solution (2.2 mL), and the mixture was stirred at room temperature for 2 hours. Methanol was distilled off *in vacuo*.

The resulted aqueous solution was diluted with methylene chloride (15 mL), then bromoacetyl chloride (180 μL) was added under ice cooling, and the mixture was stirred at that temperature for 20 minutes. Next, methylene chloride was distilled off *in vacuo*, and the resulted aqueous solution was extracted with ethyl acetate. The organic layer was successively washed with saturated sodium hydrogen carbonate aqueous solution, brine, saturated potassium hydrogen sulfate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated i*n vacuo* to give **11b** (930 mg, 87.8%). This was employed for the next step without further purification.

(*E*)-2-[(2'-Bromoacetamido)methyl]-*N*-[[3"'-(*tert*-butoxycarbonyl)-4"'-chlorophenyl]sulfonyl]-3-[(5"-chloro-2"-methoxy)phenyl]propenamide (11g)

Amide **11g** was prepared from **10g** in a similar manner to **11b**. Yield: 96.9%. This was employed for the next step without further purification.

(*E*)-6-[(2"-Chloro-2"-methoxy)benzylidene]-4-[(4'-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (12b)

To a solution of **11b** (915 mg) in *N*,*N*-dimethylformamide (50 mL) was added 60% dispersion of sodium hydride in mineral oil (80 mg), and the mixture was stirred at 60 to 80 °C for 19 hours. After cooling to room temperature, acetic acid (1.0 mL) was added to the reaction mixture, and the mixture was concentrated *in vacuo*. Ethyl acetate was added to the residue, and the solution was successively washed with saturated sodium hydrogen carbonate aqueous solution, water, saturated potassium hydrogen sulfate aqueous solution and brine, dried over anhydrous sodium sulfate, then concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 5 / 1), then the precipitates were collected by filtration to give **12b** (373 mg, 48.0%). ^{1}H NMR (DMSO-*d*6) δ: 8.07-8.02 (1H, m), 7.95 (2H, d, *J* = 8.6 Hz), 7.75 (2H, d, *J* = 8.6 Hz), 7.54 (1H, s), 7.47 (1H, dd, *J* = 8.8, 2.5 Hz), 7.27 (1H, d, *J* = 2.5 Hz), 7.11 (1H, d, *J* = 8.8 Hz), 4.72 (2H, s), 4.18 (2H, d, *J* = 4.2 Hz), 3.80 (3H, s); MS (ESI): 455(M+H)$^+$. This was employed for the next step without further purification.

(*E*)-4-[[[3'-(*tert*-Butoxycalbonyl)-4'-chloro]phenyl]sulfonyl]-6-[(5"-chloro-2"-methoxy)benzylidene]-1,4-diazepan-2,5-dione (12g)

Diazepane **12g** was prepared from **11g** in a similar manner to **12b**. ^{1}H NMR ($CDCl_3$) δ: 8.36 (1H, d, *J* = 2.2 Hz), 8.07 (1H, dd, *J* = 8.5, 2.2 Hz), 7.67 (1H, s), 7.58 (1H, d, *J* = 8.5 Hz), 7.32 (1H, dd, *J* = 8.9, 2.4 Hz), 7.06 (1H, d, *J* = 2.4 Hz), 6.85 (1H, d, *J* = 8.9 Hz), 6.12 (1H, t, *J* = 3.7 Hz), 4.69 (2H, s), 4.20 (2H, d, *J* = 3.7), 3.81 (3H, s); MS (ESI): 499 (M+H)$^+$. This was employed for the next step without further purification.

(*E*)-4-[[(3'-Amino-4'-chloro)phenyl]sulfonyl]-6-[(5"-chloro-2"-methoxy)benzylidene]-1,4-diazepan-2,5-dione (12c)

To a solution of **10c** (2.25 g) in methanol (20 mL) was added 2 M sodium hydroxide aqueous solution (5.8 mL), and the mixture was stirred at room temperature for 23 hours and at 60 °C another 7 hours. Then methanol was distilled off *in vacuo*.

The resulted aqueous solution was diluted with methylene chloride (20 mL), then bromoacetyl chloride (0.35 mL) was added under ice cooling, and the resulting mixture was stirred at that temperature for 20 minutes. Next, methylene chloride was distilled off in vacuo, and the resulted aqueous solution was extracted with ethyl acetate. The organic layer was successively washed with saturated potassium hydrogen sulfate aqueous solution, water, saturated sodium hydrogen carbonate aqueous solution and brine, and dried over anhydrous sodium sulfate. Then, *N*,*N*-dimethylformamide (40 mL) was added to the organic layer, and ethyl acetate was distilled off *in vacuo* to give a solution of **11c** as a sodium salt in *N*,*N*-dimethylformamide.

The above-mentioned solution was stirred at 60 °C for 60 hours, and concentrated in vacuo. The residue was diluted with ethyl acetate. The mixture was successively washed with saturated potassium hydrogen sulfate aqueous solution and brine. The organic layer was dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 5 / 1), then the precipitates were collected by filtration to give **12c** (1.07 g, 62.9% from **10c**). ^{1}H NMR (DMSO-*d*6) δ: 11.26-11.04 (1H, m), 8.04 (1H, t, *J* = 4.3 Hz), 7.62 (1H, d, *J* = 8.1 Hz), 7.57 (1H, d, *J* = 2.0 Hz), 7.55 (1H, s), 7.47 (1H, dd, *J* = 8.1, 2.5 Hz), 7.32 (1H, dd, *J* = 9.2, 2.0 Hz), 7.28 (1H, d, *J* = 2.5 Hz), 7.11 (1H, d, *J* = 9.2 Hz), 4.68 (2H, s), 4.17 (2H, d, *J* = 4.3 Hz), 3.80 (3H, s); MS (ESI): 471 $(M+H)^+$.This was employed for the next step without further purification.

(*E*)-6-(2''-Chlorobenzylidene)-4-[(4'-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (12d)

Diazepane **12d** was prepared from **10d** in a similar manner to **12c**. ^{1}H NMR ($CDCl_3$) δ: 8.04 (2H, d, *J* = 8.6 Hz), 7.73 (1H, s), 7.53 (2H, d, *J* = 8.6 Hz), 7.46 (1H, dd, *J* = 7.6, 1.0 Hz), 7.38-7.38 (2H, m), 7.15 (1H, dd, *J* = 7.1, 1.0 Hz), 5.91-5.84 (1H, m), 4.71 (2H, s), 4.17 (2H, d, *J* = 4.6 Hz). This was employed for the next step without further purification.

6-Benzyl-4-[(4'-chlorobenzene)sulfonyl]-1,4-diazepan-2,5-dione (4a)

To a solution of **12a** (350 mg) in tetrahydrofuran (20 mL) was added 5% platinum, sulfide, on carbon (35 mg), and the mixture was stirred under hydrogen atmosphere at room temperature for 15 hours. Next, the catalyst was filtered out through Celite while being washed with tetrahydrofuran, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 3 / 2 to 2 / 1), then the product was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 5 / 1), then the precipitates were collected by filtration to give **4a** (255 mg, 73.2%). ^{1}H NMR ($CDCl_3$) δ: 7.96 (2H, d, *J* = 8.6 Hz), 7.51 (2H, d, *J* = 8.6 Hz), 7.32-7.21 (3H, m), 7.11 (2H, d, *J* = 6.8 Hz), 5.87 (1H, br), 4.97 (1H, d, *J* = 17.6 Hz), 4.42 (1H, d, *J* = 17.6 Hz), 3.39-3.12 (4H, m), 2.55 (1H, dd, *J* = 14.4, 8.9 Hz); MS (ESI): 393 $(M+H)^+$; HRMS (FAB): calcd for $C_{18}H_{18}ClN_2O_4S^+$ 393.0670 $(M+H)^+$, found 393.0658.

6-[(5"-Chloro-2"-methoxy)benzyl]-4-[(4'-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (4c)

Diazepane **4c** was prepared from **12b** in a similar manner to **4a**. ^{1}H NMR ($CDCl_3$): δ 7.96 (2H, d, *J* = 8.7 Hz), 7.52 (2H, d, *J* = 8.7 Hz), 7.19 (1H, dd, *J* = 8.8, 2.6 Hz), 7.01 (1H, d, *J* = 2.6 Hz), 6.77 (1H, d, *J* = 8.8 Hz), 5.71 (1H, br), 5.00 (1H, d, *J* = 17.7 Hz), 4.39 (1H, d, *J* = 17.7 Hz), 3.79 (3H, s), 3.50-3.40 (1H, m), 3.26-3.09 (3H, m), 2.52 (1H, dd, *J* = 14.1, 9.1 Hz); MS (ESI):

457 $(M+H)^+$; HRMS (FAB): calcd for $C_{19}H_{19}Cl_2N_2O_5S^+$ 457.0386 $(M+H)^+$, found 457.0381.

4-[[(3'-Amino-4'-chloro)phenyl]sulfonyl]-6-[(5"-chloro-2"-methoxy)benzyl]-1,4-diazepan-2,5-dione hydrochloride (4d)

Diazepane **4d**, which was finally obtained as a hydrochloride salt by treatment with 4.0 M hydrogen chloride solution in 1,4-dioxane, was prepared from **12c** in a similar manner to **4a**. ^{1}H NMR (DMSO-*d*6) δ: 7.85-7.80 (1H, br), 7.44 (1H, d, *J* = 8.3 Hz), 7.40 (1H, d, *J* = 2.3 Hz), 7.28-7.23 (2H, m), 7.00-6.94 (2H, m), 4.86 (1H, d, *J* = 17.5 Hz), 4.47 (1H, d, *J* = 17.5 Hz), 3.76 (3H, s), 3.72-3.65 (1H, m), 3.02-2.98 (2H, m), 2.85 (1H, dd, *J* = 14.2, 4.2 Hz), 2.55-2.45 (1H, m); MS (ESI): 472 $(M+H)^+$; HRMS (FAB): found 490.0623. This suggested the formation of hydrolyzed product of **2i** [calculated HRMS for $C_{19}H_{22}Cl_2N_3O_6S^+$: 490.0601 $(M+H)^+$]. It was considered that hydrolysis proceeded during the preservation.

6-(2"-Chlorobenzyl)-4-[(4'-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (4e)

Diazepane **4e** was prepared from **12d** in a similar manner to **4a**.^{1}H NMR ($CDCl_3$) δ: 7.96 (2H, d, *J* = 8.7 Hz), 7.52 (2H, d, *J* = 8.7 Hz), 7.36 (1H, dd, *J* = 5.8, 2.4 Hz), 7.25-7.16 (3H, m), 5.97 (1H, br), 4.99 (1H, d, *J* = 17.7 Hz), 4.41 (1H, d, *J* = 17.7 Hz), 3.57-3.48 (1H, m), 3.34-3.18 (3H, m), 2.72 (1H, dd, *J* = 14.2, 8.2 Hz); MS (ESI): 427 $(M+H)^+$; HRMS (FAB): calcd for $C_{18}H_{17}Cl_2N_2O_4S^+$ 427.0281 $(M+H)^+$, found 427.0295.

6-(3"-Chlorobenzyl)-4-[(4'-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (4f)

^{1}H NMR ($CDCl_3$) δ: 7.96 (2H, d, *J* = 8.5 Hz), 7.52 (2H, d, *J* = 8.5 Hz), 7.23 (1H, d, *J* = 4.3 Hz), 7.23 (1H, d, *J* = 4.3 Hz), 7.11 (1H, s), 7.01 (1H, dd, *J* = 4.3, 4.3 Hz), 5.67 (1H, br), 5.00 (1H, d, *J* = 17.7 Hz), 4.42 (1H, d, *J* = 17.7 Hz), 3.39-3.25 (2H, m), 3.23-3.14 (2H, m), 2.53 (1H,

dd, J = 14.4, 8.4 Hz); MS (ESI): 427 $(M+H)^+$; HRMS (FAB): calcd for $C_{18}H_{17}Cl_2N_2O_4S^+$ 427.0281 $(M+H)^+$, found 427.0261.

6-(4”-Chlorobenzyl)-4-[(4’-chlorophenyl)sulfonyl]-1,4-diazepan-2,5-dione (4g)

^{1}H NMR ($CDCl_3$) δ: 7.95 (2H, d, J = 8.7 Hz), 7.51 (2H, d, J = 8.7 Hz), 7.27 (2H, d, J = 8.3 Hz), 7.06 (2H, d, J = 8.3 Hz), 5.68 (1H, br), 4.98 (1H, d, J = 17.7 Hz), 4.41 (1H, d, J = 17.7 Hz), 3.36-3.24 (2H, m), 3.22-3.13 (2H, m), 2.54 (1H, dd, J = 14.3, 8.0 Hz); MS (ESI): 427 $(M+H)^+$; HRMS (FAB): calcd for $C_{18}H_{17}Cl_2N_2O_4S^+$ 427.0281 $(M+H)^+$, found 427.0261.

4-[(4’-Chlorophenyl)sulfonyl]-6-(2”-methoxybenzyl)-1,4-diazepan-2,5-dione (4b)

To a solution of **4c** (40.0 mg) in acetic acid (2.0 mL) was added 5% palladium carbon (60.0 mg), and the mixture was stirred under hydrogen atmosphere at room temperature for 22 hours. Next, the catalyst was filtered off through Celite while being washed with ethyl acetate, and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC (developed by diethyl ether) to give **4b** (9.10 mg, 24.6%). ^{1}H NMR ($CDCl_3$) δ: 7.97 (2H, d, J = 8.8 Hz), 7.51 (2H, d, J = 8.8 Hz), 7.22 (1H, dd, J = 7.1, 1.5 Hz), 7.03 (1H, dd, J = 7.1, 1.5 Hz), 6.90-6.84 (2H, m), 5.67 (1H, br), 4.98 (1H, d, J = 17.6 Hz), 4.40 (1H, d, J = 17.6 Hz), 3.81 (3H, s), 3.54-3.42 (1H, m), 3.25-3.08 (3H, m), 2.56 (1H, dd, J = 14.0, 9.1 Hz); MS (ESI): 423 $(M+H)^+$; HRMS (FAB): calcd for $C_{19}H_{20}ClN_2O_5S^+$ 423.0776 $(M+H)^+$, found 423.0761.

6-[(5”-Chloro-2”-methoxy)benzyl]-4-[[(3’-carboxy-4’-chloro)phenyl]sulfonyl]-1,4-diazepan-2,5-dione (4h)

To a solution of a product (590 mg), which was prepared from **12g** in a similar manner to **4a**, in methylene chloride (5.0 mL) was added trifluoroacetic acid (5.0 mL), and the mixture was

stirred at room temperature for 90 minutes. Then, the reaction mixture was concentrated *in vacuo*, the residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate/hexane = 5/1), then the precipitate was collected by filtration to give **4h** (403 mg, 76.0%). ^{1}H NMR (DMSO-*d*6) δ: 13.99 (1H, br), 8.28 (1H, d, *J* = 2.3 Hz), 8.04 (1H, dd, *J* = 8.5, 2.3 Hz), 7.88-7.83 (1H, m), 7.85 (1H, d, *J* = 8.5 Hz), 7.28-7.21 (2H, m), 6.97 (1H, d, *J* = 8.5 Hz), 4.88 (1H, d, *J* = 17.5 Hz), 4.53 (1H, d, *J* = 17.5 Hz), 3.75 (3H, s), 3.72-3.61 (1H, m), 3.03-2.99 (2H, m), 2.84 (1H, dd, *J* = 14.3, 4.8 Hz), 2.57-2.50 (1H, m); MS (ESI): 501 $(M+H)^+$; HRMS (FAB): calcd for $C_{20}H_{18}Cl_2N_2NaO_7S^+$ 523.0104 $(M+Na)^+$, found 523.0094.

(*E*)-2-[(*N*-*tert*-Butoxycarbonyl)aminomethyl]-3-[(5'-chloro-2'-methoxy)phenyl]propenoic acid (14)

Acid **14** was prepared from **7b** in a similar manner to **8a**. ^{1}H NMR ($CDCl_3$) δ: 7.93 (0.5H, br), 7.78 (0.5H, br), 7.42 (0.5H, br), 7.30 (1H, dd, *J* = 8.8, 2.4 Hz), 7.19 (0.5H, br), 6.84 (1H, d, *J*= 8.8 Hz), 6.76 (0.5H, br), 5.12 (0.5H, br), 4.14 (2H, br), 3.84 (3H, s), 1.55-1.15 (9H, m); MS (ESI): 364 $(M+Na)^+$. This was employed for the next step without further purification.

(*E*)-2-[[(*tert*-Butoxycarbonyl)amino]methyl]-3-[(5'-chloro-2'-methoxy)phenyl]-*N*-[[(4"-chlorophenyl)amino]carbonyl]propenamide (16)

To a solution of **15** (1.90 g) in *N*,*N*-dimethylformamide (35 mL) was added 60% dispersion of sodium hydride in mineral oil (460 mg), and the mixture was stirred at room temperature for 1 hour. Next, to the reaction mixture was added a solution, which was prepared by stirring a mixture of 1,1'-carbonyldiimidazole (1.9 g) and **14** (4.00 g) in tetrahydrofuran (35 mL) at room temperature for 30 minutes, under ice cooling, and the resulting mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with ethyl acetate and saturated

potassium hydrogen sulfate aqueous solution, and the layers were separated. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1) to give **16** (2.60 g, 45.1%). ^{1}H NMR ($CDCl_3$) δ: 10.79 (1H, br), 9.46 (1H, br), 7.70 (1H, s), 7.50 (2H, d, *J* = 8.8 Hz), 7.33 (1H, dd, *J* = 8.8, 2.5 Hz), 7.30-7.20 (3H, m), 6.87 (1H, d, *J* = 8.8 Hz), 4.92 (1H, br), 4.15 (2H, d, *J* = 6.4 Hz), 3.84 (3H, s), 1.46 (9H, s). This was employed for the next step without further purification.

(*E*)-2-[(2'-Bromoacetamido)methyl]-3-[(5"-chloro-2"-methoxy)phenyl]-*N*-[[(4"'-chlorophenyl)amino]carbonyl]propenamide (17)

1.0 M hydrogen chloride solution in acetic acid (15 mL) was added to **16** (2.60 g), and the mixture was stirred at room temperature for 1 hour. Acetic acid was distilled off *in vacuo*.

The residue was diluted with methylene chloride (50 mL) and water (10 mL), then bromoacetyl chloride (500 μL) and triethylamine (1.7 mL) were added to the mixture under ice cooling, and the biphasic mixture was stirred at that temperature for 20 minutes. Then, ethyl acetate and water were added to the reaction mixture, and the resulting mixture was separated into two layers. The organic layer was successively washed with saturated sodium hydrogen carbonate aqueous solution, water, saturated potassium hydrogen sulfate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was tritulated with ethyl acetate, and the insoluble solid was collected by filtration to give **17** (2.50 g, 92.3%). ^{1}H NMR (DMSO-*d*6) δ: 10.8 (1H, s), 10.69 (1H, s), 8.55 (1H, br), 7.65-7.55 (3H, m), 7.51 (1H, s), 7.45 (1H, dd, *J* = 9.0, 2.2 Hz), 7.4 (2H, d, *J* = 8.8 Hz), 7.12 (1H, d, *J* = 9.0 Hz), 4.09 (2H, d, *J* = 5.0 Hz), 3.86 (2H, s), 3.82 (3H, s). This was employed for the next step without further purification.

(*E*)-6-[(2”-Chloro-2”-methoxy)benzylidene]-4-[[(4’-chlorophenyl)amino]carbonyl]-1,4-diazepan-2,5-dione (18)

To a solution of **17** (2.08 g) in *N*,*N*-dimethylformamide (150 mL) was added 60% dispersion of sodium hydride in mineral oil (150 mg). The mixture was stirred at room temperature for 30 minutes, then warmed to 60 °C, and stirred for 1 hour. Next, acetic acid (500 μL) was added to the reaction mixture, and the mixture was concentrated *in vacuo*. The residue was diluted with ethyl acetate. The resulted solution was successively washed with saturated sodium hydrogen carbonate aqueous solution, water, saturated potassium hydrogen sulfate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 5 / 1), then the precipitates were collected by filtration to give **18** (1.26 g, 72.0%). ^{1}H NMR ($CDCl_3$) δ: 11.23 (1H, s), 7.52 (2H, d, *J* = 8.8 Hz), 7.48 (1H, s), 7.35 (1H, dd, *J* = 8.8, 2.5 Hz), 7.29 (2H, d, *J* = 8.8 Hz), 7.14 (1H, d, *J* = 2.5 Hz), 6.89 (1H, d, *J* = 8.8 Hz), 5.90-5.84 (1H, m), 4.74 (2H, s), 4.30 (2H, dd, *J* = 3.3, 1.9 Hz), 3.87 (3H, s); MS (ESI): 434 $(M+H)^+$. This was employed for the next step without further purification.

(*E*)-6-[(2”-Chloro-2”-methoxy)benzy]-4-[[(4’-chlorophenyl)amino]carbonyl]-1,4-diazepan-2,5-dione (13)

To a solution of **18** (42.0 mg) in tetrahydrofuran (3.0 mL) was added 5% platinum, sulfided, on carbon (20.0 mg), and the mixture was stirred under hydrogen atmosphere at room temperature for 18 hours. Next, the catalyst was filtered out through Celite while being washed with tetrahydrofuran, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 2 / 3 to 1 / 2), and the product was

dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 5 / 1), then the precipitates were collected by filtration to give **13** (15.7 mg, 37.2%). ^{1}H NMR (DMSO-*d*6) δ: 11.08 (1H, s), 7.75 (1H, d, *J* = 3.5 Hz), 7.57 (2H, d, *J* = 8.8 Hz), 7.39 (2H, d, *J* = 8.8 Hz), 7.36 (1H, d, *J* = 2.5 Hz), 7.27 (1H, dd, *J* = 8.8, 2.5 Hz), 7.01 (1H, d, *J* = 8.8 Hz), 4.78 (1H, d, *J* = 17.3 Hz), 4.63 (1H, d, *J* = 17.3 Hz), 4.00-3.90 (1H, m), 3.80 (3H, s), 3.23 (1H, dd, *J* = 12.9, 12.9 Hz), 3.08-2.99 (2H, m), 2.67 (1H, dd, *J* = 14.4, 9.0 Hz); MS (ESI): 436 $(M+H)^+$; HRMS (FAB): calcd for $C_{20}H_{20}Cl_2N_3O_4^+$ 436.0825 $(M+H)^+$, found 436.0798.

Ethyl 2-[(*E*)-2'-(aminomethyl)-3'-[(5"-chloro-2"-methoxy)phenyl]propenamido]acetate hydrochloride (20)

To a solution of **14** (123 g) in methylene chloride (400 mL) were added glycine ethyl ester hydrochloride (51.0 g), 1-hydroxybenzotriazole (49.0 g), triethylamine (53 mL) and 1-[(3'-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (76.0 g) under ice cooling, and the mixture was stirred at room temperature for 2 hours. Water was added to the reaction mixture, then the precipitates were filtered off while being washed with ethyl acetate, and the filtrate was extracted with ethyl acetate. The organic layer was successively washed with saturated potassium hydrogen sulfate aqueous solution, water, saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 5 / 1), then the precipitates were collected by filtration to give solids (132 g, 85.9%). ^{1}H NMR ($CDCl_3$) δ: 7.64-7.50 (2H, m), 7.27 (1H, dd, *J* = 8.9, 2.1 Hz), 7.16 (1H, d, *J* = 2.1 Hz), 6.84 (1H, d, *J* = 8.9 Hz), 5.04-4.95 (1H, m), 4.23 (2H, q, *J* = 7.3 Hz), 4.11 (2H, d, *J* = 5.6 Hz), 4.09 (2H, d, *J* = 6.7 Hz), 3.82 (3H, s), 1.43 (9H, s), 1.30 (3H, t, *J* = 7.2 Hz).

4.0 M hydrogen chloride solution in ethyl acetate (350 mL) was added to the

above-mentioned product (132 g), and the mixture was stirred at room temperature for 20 minutes. Diethyl ether was added to the reaction mixture, then the precipitates were collected by filtration to give **20** (109 g, 97.1%). ^{1}H NMR (DMSO-*d*6) δ: 9.06 (1H, t, *J* = 5.8 Hz), 8.15-7.76 (2H, m), 7.60-7.58 (1H, m), 7.50-7.46 (2H, m), 7.15 (1H, d, *J* = 9.7 Hz), 4.13 (2H, q, *J* = 7.2 Hz), 3.96 (2H, d, *J* = 5.8 Hz), 3.84 (3H, s), 3.67 (2H, s), 1.22 (3H, t, *J* = 7.2 Hz); MS (ESI): 327 $(M+H)^+$. This was employed for the next step without further purification.

(*E*)-2-[3'-[(5'''-Chloro-2'''-methoxy)phenyl]-2'-[[(2'',4'',5''-trimethoxybenzyl)amino]methyl]propenamido]acetic acid hydrochloride (21)

To a solution of **20** (55.8 g) in tetrahydrofuran (800 mL) were added 2,4,6-trimethoxybenzaldehyde (30.5 g) and triethylamine (21.4 mL), and the mixture was stirred at room temperature for 20 minutes. Next, sodium triacetoxy borohydride (50.0 g) was added to the mixture, and the resulting mixture was stirred at room temperature for 1 hour. Then, water was added to the reaction mixture, and tetrahydrofuran was distilled off *in vacuo*. The resulted aqueous solution was basified with sodium hydroxide aqueous solution to pH 10, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and the solvent was partially distilled off *in vacuo*. 4.0 M hydrogen chloride solution in ethyl acetate was added to the resulted solution, and the mixture was stirred under ice cooling for 30 minutes. The precipitates were collected by filtration to give a product (75.7 g, 90.7%). A similar synthesis was performed again to give the same product (72.8 g, 87.3%). ^{1}H NMR (DMSO-*d*6) δ: 9.12 (1H, br), 8.58 (1H, br), 7.59 (1H, s), 7.48 (1H, d, *J* = 8.9 Hz), 7.32 (1H, s), 7.13 (1H, d, *J* = 8.9 Hz), 6.23 (2H, s), 4.13 (2H, q, *J* = 7.1 Hz), 3.96 (2H, d, *J* = 5.7 Hz), 4.01-3.92 (4H, m), 3.82 (3H, s), 3.79 (3H, s), 3.75 (6H, s), 1.21 (3H, t, *J* = 7.1 Hz); MS (ESI): 507 $(M+H)^+$.

To a solution of the above-mentioned product (148.5 g) in methanol (300 mL) was added 2 M sodium hydroxide aqueous solution (300 mL), and the mixture was stirred at room temperature for 2 hours. The reaction mixture was neutralized with 6.0 M hydrochloric acid (100 mL), and methanol was distilled off *in vacuo*. The seed crystals, which were previously prepared in a similar manner to this way, were added to the resulted solution, and the mixture was stirred under ice cooling for 30 minutes. The precipitates were collected by filtration to give **21** (143.1 g, quantitative). ^{1}H NMR (DMSO-*d*6) δ: 9.15-9.03 (1H, m), 7.57 (1H, s), 7.46 (1H, dd, *J* = 8.9, 2.5 Hz), 7.32 (1H, d, *J* = 2.5 Hz), 7.12 (1H, d, *J* = 8.9 Hz), 6.23 (2H, s), 3.97-3.91 (2H, m), 3.91-3.87 (2H, m), 3.81 (3H, s), 3.79 (3H, s), 3.75 (6H, s), 3.74-3.71 (2H, m); MS (ESI): 479 $(M+H)^+$. This was employed for the next step without further purification.

6-[(5”-Chloro-2”-methoxy)benzyl]-1-(2’,4’,6’-trimethoxybenzyl)-1,4-diazepan-2,5-dione (22)

A solution of **21** (53.0 g) and 1-hydroxybenzotriazole (14.0 g) in *N*,*N*-dimethylformamide (1 L) was dropwisely added to a solution of 1-[(3’-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (24.0 g) and triethylamine (17 mL) in *N*,*N*-dimethylformamide (500 mL) over 2 hours. Then, an insoluble substance was filtered out while being washed ethyl acetate, and the filtrate was concentrated *in vacuo*. Ethyl acetate and 1.0 M hydrochloric acid were added to the residue, and the mixture was stirred for 30 minutes. An insoluble solid was collected by filtration, and washed with water and ethyl acetate. Ethyl acetate and 1.0 M sodium hydroxide aqueous solution were added to the solid, and stirred for 30 minutes. An insoluble solid was collected by filtration, and washed with water and ethyl acetate. A mixture of tetrahydrofuran and methanol (1 / 1) was added to the solid, and the mixture was stirred under heating at reflux for 30 minutes. The mixture was cooled to room temperature, and filtered through Celite

while being washed with mixture of tetrahydrofuran and methanol (1 / 1). The filtrate was concentrated *in vacuo*. The residue was recrystallized from ethyl acetate to give the product (22.1 g, 43.3%). ^{1}H NMR ($CDCl_3$) δ: 7.60 (1H, s), 7.24 (1H, dd, *J* = 8.6, 2.5 Hz), 6.76-6.69 (2H, m), 6.12-6.03 (1H, m), 5.75 (2H, s), 4.52 (2H, s), 4.23 (2H, s), 4.05 (2H, d, *J* = 6.1 Hz), 3.79 (3H, s), 3.76 (3H, s), 3.52 (6H, s).

To a solution of the above-mentioned product (16.3 g) in tetrahydrofuran (600 mL) was added 2% platinum, sulfide, on carbon (7.3 g), and the mixture was stirred under hydrogen atmosphere at room temperature for 60 hours. Next, the catalyst was filtered out through Celite while being washed with tetrahydrofuran, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (chloroform / ethyl acetate / methanol = 7 / 1 / 0.5) to give **22** (13.1 g, 80.0%). ^{1}H NMR ($CDCl_3$) δ: 7.14 (1H, dd, *J* = 8.6, 2.5 Hz), 6.89 (1H, d, *J* = 2.5 Hz), 6.70 (1H, d, *J* = 8.6 Hz), 5.99 (2H, s), 5.98-5.93 (1H, m), 4.81 (1H, d, *J* = 13.7 Hz), 4.26 (1H, d, *J* = 13.7 Hz), 4.22 (1H, dd, *J* = 15.7, 3.5 Hz), 3.84 (3H, s), 3.74-3.67 (1H, m), 3.72 (3H, s) 3.64 (6H, s), 3.38 (1H, dd, *J* = 15.7, 12.0 Hz), 3.12 (1H, dd, *J* = 13.0, 3.3 Hz), 2.95 (1H, dd, *J* = 15.0, 4.8 Hz), 2.40-2.54 (2H, m); MS (ESI): 461 $(M+H)^+$. This was employed for the next step without further purification.

(*S*)-6-[(5”-Chloro-2”-methoxy)benzyl]-1-(2’,4’,6’-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(*S*)-22] and (*R*)-6-[(5”-Chloro-2”-methoxy)benzyl]-1-(2’,4’,6’-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(*R*)-22]

Racemic mixture of **22** was separated by HPLC equipped with chiral stationary phase (Daicel Chemical Industries, CHIRALCEL OD-H; 25 cm x 5 cmϕ) by eluting a mixture of mobile acetonitrile and trifluoroacetic acid (1000 / 1) to give products. The each product was diluted with tetrahydrofuran, and the resulted suspension was stirred at 60 °C until the solution

became clear. Then, the solution was gradually cooled to room temperature, and the precipitates were collected by filtration to give each (*S*)-**22** or (*R*)-**22** including tetrahydrofuran as residual solvent. The ratio of the number of molecules was estimated to be 2 : 3 by ^{1}H NMR analysis. ^{1}H NMR ($CDCl_3$) δ: 7.14 (1H, dd, *J* = 2.4, 8.5 Hz), 6.88 (1H, d, *J* = 2.4 Hz), 6.70 (1H, d, *J* = 8.5 Hz), 5.98 (2H, s), 5.98-5.93 (1H, m), 4.81 (1H, d, *J* = 13.4 Hz), 4.26 (1H, d, *J* = 13.4 Hz), 4.22 (1H, dd, *J* = 15.2, 3.5 Hz), 3.83 (3H, s), 3.77-3.73 (4H, m), 3.72 (3H, s), 3.63 (6H, s), 3.39 (1H, dd, *J* = 15.2, 12.4 Hz), 3.12 (1H, dd, *J* = 3.4, 13.2 Hz), 2.94 (1H, dd, *J* = 4.7, 15.2 Hz), 2.54-2.38 (2H, m); MS (ESI): 461 $(M+H)^+$; $[\alpha]^{20}_D$ +120.0 (c = 0.1, methanol) for (*S*)-**22** and –111.6 for (*R*)-**22**. This was employed for the next step without further purification.

6-[(5'''-Chloro-2'''-methoxy)benzyl]-4-[(4''-nitrophenoxy)carbonyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione (23a)

To a solution of **22** (2.69 g) in tetrahydrofuran (160 mL) was added 1.59 M solution of *n*-butyllithium in hexane (3.7 mL) at –78 °C, and the mixture was stirred at that temperature for 20 minutes. Next, a solution of *p*-nitrophenyl chloroformate (1.3 g) in tetrahydrofuran (10 mL) was added to the mixture at –78 °C, and the resulting mixture was stirred at that temperature for 1 hour. Then, saturated potassium hydrogen sulfate aqueous solution was added to the reaction mixture, and tetrahydrofuran was distilled off *in vacuo*, and the resulted aqueous solution was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give **23a** (4.05 g, quantitative). ^{1}H NMR ($CDCl_3$) δ: 8.27 (2H, d, *J* = 9.2 Hz), 7.36 (2H, d, *J* = 9.2 Hz), 7.17 (1H, dd, *J* = 8.6, 2.5 Hz), 6.89-6.97 (1H, m), 6.73 (1H, d, *J* = 8.6 Hz), 6.05 (2H, s), 4.96 (1H, d, *J* = 17.3 Hz), 4.70 (1H, d, *J* = 13.7 Hz), 4.48 (1H, d, *J* = 13.7 Hz), 4.46 (1H, d, *J* = 17.3 Hz), 3.83 (3H, s), 3.77 (3H, s), 3.69 (6H, s), 3.34-3.51 (2H, m), 3.02-3.25 (2H, m), 2.34-2.53 (1H, m). This was employed for the next step

without further purification.

6-[(5'''-Chloro-2'''-methoxy)benzyl]-4-[(2''-chlorophenoxy)carbonyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione (23b)

Diazepane **23b** was prepared from **22** and 2-chlorophenyl chloroformate in a similar manner to **23a** with purification by silica gel column chromatography (hexane / ethyl acetate / methanol = 1 / 2 / 0 to 3 / 3 / 1). ^{1}H NMR ($CDCl_3$) δ: 7.44-7.40 (1H, m), 7.33-7.12 (4H, m), 6.93 (1H, d, *J* = 2.6 Hz), 6.73 (1H, d, *J* = 8.8 Hz), 6.05 (2H, s), 5.06 (1H, d, *J* = 17.6 Hz), 4.83 (1H, d, *J* = 13.7 Hz), 4.44 (1H, d, *J* = 17.6 Hz), 4.34 (1H, d, *J* = 13.7 Hz), 3.83 (3H, s), 3.77 (3H, s), 3.7 (6H, s), 3.57-3.45 (1H, m), 3.29-3.14 (2H, m), 3.07 (1H, dd, *J* = 14.1, 3.8 Hz), 2.38 (1H, dd, *J* = 14.1, 9.8 Hz); MS (ESI): 617 $(M+H)^+$. This was employed for the next step without further purification.

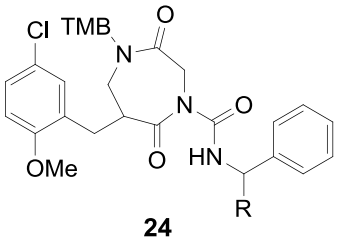
(*R*)-6-[(5'''-Chloro-2'''-methoxy)benzyl]-4-[(2''-chlorophenoxy)carbonyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(*R*)-23b]

Diazepane (*R*)-**23b** was prepared from (*R*)-**22** and 2-chlorophenyl chloroformate in a similar manner to **23a** with purification by silica gel column chromatography (hexane/ethyl acetate = 1 / 2, then ethyl acetate/methanol = 10 / 1), however, 1,2-dimethoxyethane was used as reaction solvent due to low solubility of (*R*)-**22** in tetrahydrofuran. (*R*)-**23b** showed a ^{1}H NMR spectrum identical to **23b**. MS (ESI): 617 $(M+H)^+$; $[\alpha]^{20}_D$ –39.4(c = 0.1, methanol). This was employed for the next step without further purification.

(*S*)-6-[(5'''-Chloro-2'''-methoxy)benzyl]-4-[(2''-chlorophenoxy)carbonyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(*S*)-23b]

Diazepane (*S*)-**23b** was prepared from (*S*)-**22** and 2-chlorophenyl chloroformate in a similar manner to **23a** with purification by silica gel column chromatography (hexane / ethyl acetate = 1 / 2, then ethyl acetate/methanol = 5 / 1). (*S*)-**23b** showed a ^{1}H NMR spectrum identical to **23b**. MS (ESI): 617 $(M+H)^+$; $[\alpha]^{20}_D$ +33.3 (c = 0.1, methanol). This was employed for the next step without further purification.

Table 18 Structures of compounds **24a-c**



24

24	R
a	H
b	Et
c	*n*-Pr

6-[(5”-Chloro-2”-methoxy)benzyl]-4-[(benzylamino)carbonyl]-1-(2’,4’,6’-trimethoxybenzyl)-1,4-diazepan-2,5-dione (24a)

To a solution of **23a** (100 mg) in *N*,*N*-dimethylformamide (5.0 mL) was added benzylamine (17.5 μL) at 0 °C, and the mixture was stirred at 0 °C for 3 hours. Then, water was added to the reaction mixture, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1 to 1 / 99) to give **24a** (60.7 mg, 64.0%). ^{1}H NMR (DMSO-*d*6) δ: 9.37 (1H, t, *J* = 5.6 Hz), 7.36-7.25 (5H, m), 7.18 (1H, dd, *J* = 8.6, 2.5 Hz), 6.92 (1H, d, *J* = 2.5 Hz), 6.75 (1H, d, *J* = 8.6

Hz), 6.06 (2H, s), 5.38 (1H, d, *J* = 17.3 Hz), 4.82 (1H, d, *J* = 13.7 Hz), 4.50 (2H, d, *J* = 5.6 Hz), 4.30 (1H, d, *J* = 13.7 Hz), 4.27 (1H, d, *J* = 17.3 Hz), 3.84 (3H, s), 3.78 (3H, s), 3.66 (6H, s), 3.60-3.47 (1H, m), 3.11-2.84 (3H, m), 2.38 (1H, dd, *J* = 14.0, 8.9 Hz). This was employed for the next step without further purification.

6-[(5'''-Chloro-2'''-methoxy)benzyl]-4-[[(1''-phenylpropyl)amino]carbonyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione (24b)

Diazepane **24b** was prepared in a similar manner to **24a**. ^{1}H NMR (DMSO-*d*6) δ: 9.48(0.5H, d, *J* = 7.6 Hz), 9.46 (0.5H, d, *J* = 7.6 Hz), 7.26 (5H, m), 7.21-7.15 (1H, m), 6.94 (0.5H, d, *J* = 2.5 Hz), 6.92 (0.5H, d, *J* = 2.5 Hz), 6.76 (0.5H, d, *J* = 8.6 Hz), 6.75 (0.5H, d, *J* = 8.6 Hz), 6.08 (1H, s), 5.99 (1H, s), 5.33 (0.5H, d, *J* = 17.8 Hz), 5.32 (0.5H, d, *J* = 17.8 Hz), 4.88-4.75 (2H, m), 4.32 (0.5H, d, *J* = 13.7 Hz), 4.27-4.16 (1.5H, m), 3.84 (1.5H, s), 3.83 (1.5H, s), 3.78 (1.5H, m), 3.77 (1.5H, m), 3.71 (3H, s), 3.52 (3H, s), 3.61-3.42 (1H, m), 3.14-2.92 (3H, m), 2.40 (1H, dd, *J* = 13.7, 9.7 Hz), 1.92-1.75 (2H, m), 0.90 (3H, dd, *J* = 7.4, 7.4 Hz). ^{1}H NMR spectrum of **24b** was assigned as mixture of two diastereomers. When each signal of corresponding proton was separated, the number of proton was assigned to be 0.5. ^{1}H NMR spectra of following diastereomers were assigned in the same manner. This was employed for the next step without further purification.

6-[(5'''-Chloro-2'''-methoxy)benzyl]-4-[[(1''-phenylbutyl)amino]carbonyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione (24c)

^{1}H NMR (DMSO-*d*6) δ: 9.46 (0.5H, d, *J* = 7.6 Hz), 9.44 (0.5H, d, *J* = 7.6 Hz), 7.34-7.23 (5H, m), 7.22-7.16 (1H, m), 6.94 (0.5H, d, *J* = 2.5 Hz), 6.92 (0.5H, d, *J* = 2.5 Hz), 6.76 (0.5H, d, *J* = 8.6 Hz), 6.75 (0.5H, d, *J* = 8.6 Hz), 6.08 (1H, s, *J* = 4.2 Hz), 5.99 (1H, s), 5.32 (1H, d, *J* = 17.8

Hz), 4.94-4.75 (2H, m), 4.33 (0.5H, d, *J* = 13.7 Hz), 4.27-4.17 (1.5H, m), 3.85 (1.5H, s), 3.83 (1.5H, s), 3.78 (1.5H, s), 3.76 (1.5H, s), 3.7 (3H, s), 3.51 (3H, s), 3.54-3.49 (1H, m), 3.15-2.92 (3H, m), 2.40 (1H, dd, *J* = 13.7, 9.2 Hz), 1.78 (2H, m), 1.27 (2H, m), 0.96-0.85 (3H, m). This was employed for the next step without further purification.

6-[(5'-Chloro-2'-methoxy)benzyl]-4-[(benzylamino)carbonyl]-1,4-diazepan-2,5-dione (19a)

A mixture of **24a** (58.8 mg) and 1.0 M hydrogen chloride solution in acetic acid (3.0 mL) was stirred at room temperature for 15 hours. Then, the mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1 to 1 / 99) to give **19a** (37.0 mg, 90.2%). ^{1}H NMR ($CDCl_3$) δ: 9.37(1H, br), 7.39-7.29 (5H, m), 7.21 (1H, dd, *J* = 8.7, 2.6 Hz), 7.11 (1H, d, *J* = 2.6 Hz), 6.80 (1H, d, 8.7 Hz), 5.76 (1H, br), 5.45 (1H, d, *J* = 17.5 Hz), 4.51 (2H, d, *J* = 5.5 Hz), 4.14 (1H, d, *J* = 17.5 Hz), 3.83 (3H, s), 3.75-3.68 (1H, m), 3.36-3.31 (2H, m), 3.16 (1H, dd, *J* = 13.9, 5.4 Hz), 2.58 (1H, dd, *J* = 13.9, 8.2 Hz); MS (ESI): 416 $(M+H)^+$; HRMS (FAB): calcd for $C_{21}H_{23}ClN_3O_4^+$ 416.1372 $(M+H)^+$, found 416.1396.

6-[(5''-Chloro-2''-methoxy)benzyl]-4-[[(1'-phenylpropyl)amino]carbonyl]-1,4-diazepan-2,5-dione (19b)

Diazepane **19b** was prepared from **24b** in a similar manner to **19a**. ^{1}H NMR ($CDCl_3$) δ: 9.49 (1H, d, *J* = 7.7 Hz), 7.39-7.21 (6H, m), 7.13 (1H, d, *J* = 2.5 Hz), 6.81 (0.5H, d, *J* = 8.7 Hz), 6.80 (0.5H, d, *J* = 8.8 Hz), 5.64 (0.5H, br), 5.60 (0.5H, br), 5.40 (0.5H, d, *J* = 17.6Hz), 5.38 (0.5H, d, *J* = 17.6 Hz), 4.85-4.76 (1H, m), 4.12 (0.5H, d, *J* = 17.6 Hz), 4.07 (0.5H, d, *J* = 17.6 Hz), 3.84 (1.5H, s), 3.82 (1.5H, s), 3.72-3.62 (1H, m), 3.35-3.27 (2H, m), 3.19 (1H, dd, *J* = 13.9, 5.2 Hz), 2.65-2.59 (1H, m), 1.95-1.79 (2H, m), 0.95-0.86 (3H, m); MS (ESI): 444 $(M+H)^+$; HRMS

(FAB): calcd for $C_{23}H_{26}ClN_3NaO_4^+$ 466.1504 $(M+Na)^+$, found 466.1515.

6-[(5”-Chloro-2”-methoxy)benzyl]-4-[[(1’-phenylbutyl)amino]carbonyl]-1,4-diazepan-2,5-dione (19c)

^{1}H NMR ($CDCl_3$) δ: 9.48 (1H, d, *J* = 7.5 Hz), 7.39-7.20 (6H, m), 7.13 (1H, d, *J* = 2.6 Hz), 6.81 (0.5H, d, *J* = 8.8 Hz), 6.80 (0.5H, d, *J* = 8.8 Hz), 5.76 (0.5H, br), 5.71 (0.5H, br), 5.39 (0.5H, *J* = d, 17.5 Hz), 5.37 (0.5H, d, *J* = 17.5 Hz), 4.93-4.84 (1H, m), 4.11 (0.5H, d, *J* = 17.5 Hz), 4.06 (0.5H, d, *J* = 17.5 Hz), 3.84 (1.5H, s), 3.82 (1.5H, s), 3.72-3.62 (1H, m), 3.38-3.28 (2H, m), 3.19 (1H, dd, *J* = 14.0, 5.2 Hz), 2.66-2.59 (1H, m), 1.91-1.71 (2H, m), 1.45-1.23 (2H, m), 0.98-0.87 (3H, m); MS (ESI): 458 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{28}ClN_3NaO_4^+$ 480.1661 $(M+Na)^+$, found 480.1653.

***tert*-Butyl 3-[1’-(1”,3”-dioxoisoindolin-2”-yl)propyl]benzoate (28a)**

To a suspension of copper (I) iodide (3.10 g) in diethyl ether (70 mL) was added 0.89 M solution of ethylmagnesium bromide in tetrahydrofuran (3.5 mL) at -23 °C, and the mixture was stirred at that temperature for 20 minutes. Next, a solution of **26a** (3.00 g) in diethylether (10 mL) was added to the mixture, and the resulting mixture was stirred at –23 °C for 30 minutes. Then, saturated ammonium chloride aqueous solution and 28% ammonia aqueous solution were added to the reaction mixture. The resulting mixture was stirred at room temperature for 40 minutes, and extracted with ethyl acetate. The organic layer was successively washed with water and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

To a solution of the residue (3.08 g) in tetrahydrofuran (70 mL) were added phthalimide (3.80 g), triphenylphosphine (6.90 g) and diethyl azodicarboxylate (4.1 mL), and the mixture was stirred at room temperature for 1 hour. Then, the mixture was concentrated *in vacuo*, and

the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 7 / 1 to 6 / 1) to give **28a** (2.61 g, 49.3%). ^{1}H NMR ($CDCl_3$) δ: 8.12 (1H, s), 7.89 (1H, d, *J* = 7.6 Hz), 7.78-7.85 (2H, m), 7.77-7.64 (3H, m), 7.38 (1H, dd, *J* = 7.6, 7.6 Hz), 5.29 (1H, dd, *J* = 9.7, 6.6 Hz), 2.67-2.51 (1H, m), 2.41-2.28 (1H, m), 1.59 (9H, s), 0.98 (3H, dd, *J* = 7.4, 7.4 Hz). This was employed for the next step without further purification.

***tert*-Butyl 4-[1'-(1",3"-dioxoisoindolin-2"-yl)propyl]benzoate (28c)**

Ester **28c** was prepared from **26c** in a similar manner to **28a**. ^{1}H NMR ($CDCl_3$) δ: 7.95 (2H, d, *J* = 7.8 Hz), 7.83-7.80 (2H, m), 7.72-7.68 (2H, m), 7.57 (2H, d, *J* = 7.8 Hz), 5.28 (1H, dd, *J* = 9.7, 6.6 Hz), 2.64-2.49 (1H, m), 2.43-2.27 (1H, m), 1.56 (9H, s), 0.98 (3H, dd, *J* = 7.1, 7.1 Hz). This was employed for the next step without further purification.

***tert*-Butyl 3-(1'-aminopropyl)benzoate hydrochloride (29a)**

To a solution of **28a** (550 mg) in methanol (3.0 mL) was added hydrazine monohydrate (90 mL), and the mixture was stirred at reflux for 1 hour. After cooling to room temperature, precipitates were removed by filtration while being washed with methanol. The filtrate was concentrated *in vacuo*, and the residue was diluted with ethyl acetate. The solution was successively washed with saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The yellow oil (289 mg) was dissolved in ethyl acetate, and 4.0 M hydrogen chloride solution in 1,4-dioxane (320 μL) was added to the mixture. The precipitates were collected by filtration while being washed with ethyl acetate to give **29a** (229 mg, 56.0%). ^{1}H NMR (DMSO-*d*6) δ: 8.05 (1H, s), 8.01 (1H, d, *J* = 7.7 Hz), 7.65 (1H, d, *J* = 7.7 Hz), 7.57 (1H, dd, *J* = 7.7, 7.7 Hz), 4.84 (3H, br), 4.26 (1H, dd, *J* = 9.1, 6.0 Hz), 2.12-1.92 (2H, m), 1.61 (9H, s), 0.90 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 219

$(M\text{-}NH_2)^+$. This was employed for the next step without further purification.

***tert*-Butyl 4-(1'-aminopropyl)benzoate hydrochloride (29c)**

Ester **29c** was prepared from **28c** in a similar manner to **29a**. ^{1}H NMR (DMSO-*d*6) δ: 8.04 (2H, d, *J* = 8.4 Hz), 7.51 (2H, d, *J* = 8.4 Hz), 4.84 (3H, br), 4.25 (1H, dd, *J* = 9.0, 6.0 Hz), 2.10-1.90 (2H, m), 1.60 (9H, s), 0.90 (3H, dd, *J* = 7.4, 7.4 Hz); MS (ESI): 219 $(M\text{-}NH_2)^+$. This was employed for the next step without further purification.

***tert*-Butyl (2-nitro-5-propionyl)benzoate (32b)**

To a solution of **30b** (3.98 g) in dimethoxyethane (80 mL) were added tripotassium phosphate (3.61 g), 2-(di-*tert*-butylphosphino)-2'-methylbiphenyl (480 mg), tris(dibenzylideneacetone)dipalladium(0) (352 mg) and 1-nitropropane (2.75 mL), and the mixrure was stirred at room temperature for a few minutes. Then, the mixture was warmed to 90 °C, and stirred at this temperature for 15 hours. Then, an insoluble substance was removed by filtration through Celite while being washed with ethyl acetate, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 9 / 1 to 7 / 3) to give a product (3.78 g, 78.9%).

To a solution of the product (3.65 g) in a mixture of 1,4-dioxane (150 mL) and water (10 mL) was added potassium *tert*-butoxide (1.45 g), and the resulting mixture was stirred at room temperature for 5 hours. Then, 1.0 M hydrochloric acid was added to the reaction mixture at 0 °C, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 9 / 1 to 8 / 2) to give **32b** (2.10 mg, 63.8%). ^{1}H NMR ($CDCl_3$) δ: 8.28 (1H, d, *J* = 1.8 Hz), 8.13 (1H, dd, *J* = 8.6, 1.8 Hz),

7.86 (1H, d, *J* = 8.6 Hz), 3.04 (2H, q, *J* = 7.1 Hz), 1.56 (9H, s), 1.25 (3H, t, *J* = 7.1 Hz). This was employed for the next step without further purification.

***tert*-Butyl (2-nitro-4-propionyl)benzoate (32d)**

Ester **32d** was prepared from **30d** in a similar manner to **32b**.^{1}H NMR ($CDCl_3$) δ: 8.41 (1H, d, *J* = 1.5 Hz), 8.2 (1H, dd, *J* = 7.9, 1.5 Hz), 7.8 (1H, d, *J* = 7.9 Hz), 3.04 (2H, q, *J* = 7.2 Hz), 1.58 (9H, s), 1.26 (3H, t, *J* = 7.2 Hz). This was employed for the next step without further purification.

***tert*-Butyl [2-amino-5-(1'-aminopropyl)]benzoate dihydrochloride (29b)**

To a solution of **32b** (350 mg) in ethanol (10 mL) were added hydroxylamine hydrochloride (96.0 mg) and sodium acetate (154 mg), and the mixture was stirred at reflux for 20 hour. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*, and the residue was diluted with ethyl acetate. The resulted solution was successively washed with water, saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

To a solution of the residue (125 mg) in ethanol (15 mL) was added 10% palladium on carbon (37 mg), and the mixture was stirred under hydrogen atmosphere at a pressure of 4 atm at room temperature for 6 hours. The catalyst was filtered out through Celite while being washed with ethanol, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and 4.0 M hydrogen chloride solution in ethyl acetate (600 μL) was added to the solution. The precipitate was collected by filtration while being washed with ethyl acetate to give **29b** (121 mg, 30.5%). ^{1}H NMR (DMSO-*d*6) δ: 8.22 (3H, br), 7.67 (1H, d, *J* = 1.2 Hz), 7.30 (1H, dd, *J* = 8.6, 1.2 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 5.50-4.40 (3H, m), 3.95-3.86 (1H, m),

1.94-1.83 (1H, m), 1.79-1.65 (1H, m), 1.51 (9H, s), 0.72 (3H, dd, *J* = 7.4, 7.4 Hz); MS (ESI): 234 $(M\text{-}NH_2)^+$. This was employed for the next step without further purification.

***tert*-Butyl [2-amino-4-(1'-aminopropyl)]benzoate dihydrochloride (29d)**

Ester **29d** was prepared from **32d** in a similar manner to **29b**.^{1}H NMR ($CDCl_3$) δ: 7.44 (1H, d, *J* = 8.3 Hz), 6.58 (1H, d, *J* = 1.6 Hz), 6.54 (1H, dd, *J* = 8.3,1.6 Hz), 5.70-5.60 (3H, m), 3.68 (1H, dd, *J* = 6.7, 6.7 Hz), 1.68-1.59 (2H, m), 1.56 (9H, s), 0.85 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 234 $(M\text{-}NH_2)^+$. This was employed for the next step without further purification.

(6*R,1"*R**)-4-[[[1"-[3"'-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R**,1'*R**)-33a: fast moving isomer] and (6*R**,1"*S**)-4-[[[1"-[3"'-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R**,1'*S**)-33a: slow moving isomer]**

To a solution of **23a** (445 mg) in *N*,*N*-dimethylformamide (5.0 mL) were added *tert*-butyl 3-(1-aminopropyl)benzoate hydrochloride **29a** (200 mg) and triethylamine (105 μL) at 0 °C, and the mixture was stirred at 0 °C for 1 hour. Then, the reaction mixture was diluted with ethyl acetate. The mixture was successively washed with water, saturated potassium hydrogen sulfate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 2) to give the (6*R**,1'*R**)-**33a** (fast moving isomer, 120 mg, 23.4%) and (6*R**,1'*S**)-**33a** (slow moving isomer, 78.0 mg, 15.2%).

(6*R**,1'*R**)-**33a**: ^{1}H NMR ($CDCl_3$) δ: 9.49 (1H, d, *J* = 7.3 Hz), 7.89 (1H, s), 7.85 (1H, d, *J* = 7.6 Hz), 7.43 (1H, d, *J* = 7.6 Hz), 7.35 (1H, dd, *J* = 7.6, 7.6 Hz), 7.16 (1H, dd, *J* = 8.8, 2.6 Hz),

6.89 (1H, d, *J* = 2.6 Hz), 6.73 (1H, d, *J* = 8.8 Hz), 6.06 (2H, s), 5.28 (1H, d, *J* = 17.4 Hz), 4.82 (1H, ddd, *J* = 7.3, 7.3, 7.3 Hz), 4.76 (1H, d, *J* = 13.8 Hz), 4.31 (1H, d, *J* = 13.8 Hz), 4.19 (1H, d, *J* = 17.4 Hz), 3.82 (3H, s), 3.76 (3H, s), 3.69 (6H, s), 3.57-3.43 (1H, m), 3.10 (1H, dd, *J* = 14.0, 4.4 Hz), 3.05-2.96 (2H, m), 2.37 (1H, dd, *J* = 14.0, 9.6 Hz), 1.90-1.78 (2H, m), 1.57 (9H, s), 0.89 (3H, dd, *J* = 7.3, 7.3 Hz). This was employed for the next step without further purification.

(6*R**,1'*S**)-**33a**: ^{1}H NMR ($CDCl_3$) δ: 9.48 (1H, d, *J* = 7.5 Hz), 7.92-7.88 (2H, m), 7.43 (1H, d, *J* = 7.7 Hz), 7.36 (1H, dd, *J* = 7.7, 7.7 Hz), 7.18 (1H, dd, *J* = 8.7, 2.6 Hz), 6.92 (1H, d, *J* = 2.6 Hz), 6.75 (1H, d, *J* = 8.7 Hz), 5.99 (2H, s), 5.29 (1H, d, *J* = 17.4 Hz), 4.85 (1H, ddd, *J* = 7.5, 7.5, 7.5 Hz), 4.76 (1H, d, *J* = 13.8 Hz), 4.257 (1H, d, *J* = 13.8 Hz), 4.252 (1H, d, *J* = 17.4 Hz), 3.81 (3H, s), 3.77 (3H, s), 3.60-3.48 (1H, m), 3.54 (6H, s), 3.10 (1H, dd, *J* = 13.8, 4.8 Hz), 3.00-2.94 (2H, m), 2.40 (1H, dd, *J* = 13.8, 9.2 Hz), 1.94-1.76 (2H, m), 1.60 (9H, s), 0.90 (3H, dd, *J* = 7.5, 7.5 Hz). This was employed for the next step without further purification.

(6*R,1''*R**)-4-[[[1''-[[4'''-Amino-3'''-(*tert*-butoxycarbonyl)]phenyl]propyl]amino]carbonyl]-6-[(5''''-chloro-2''''-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R**,1'*R**)-33b] and (6*R**,1''*S**)-4-[[[1''-[[4'''-Amino-3'''-(*tert*-butoxycarbonyl)]phenyl]propyl]amino]carbonyl]-6-[(5''''-chloro-2''''-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R**,1'*S**)-33b]**

Diazepane (6*R**,1'*R**)-**33b** and (6*R**,1'*S**)-**33b** were prepared from **23a** and **29b** in a similar manner to (6*R**,1'*R**)-**33a** and (6*R**,1'*S**)-**33a**.

It was impossible to separate into (6*R**,1'*R**)-**33b** and (6*R**,1'*S**)-**33b** by silica gel column chromatography. Rf = 0.27 (developed by hexane / ethyl acetate = 1 / 2). The mixture was employed for the next step.

(6*R,1”*R**)-4-[[[1”-[4”’-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5””-chloro-2””-methoxy)benzyl]-1-(2’,4’,6’-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R**,1’*R**)-33c] and (6*R**,1”*S**)-4-[[[1”-[4”’-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5””-chloro-2””-methoxy)benzyl]-1-(2’,4’,6’-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R**,1’*S**)-33c]**

(6*R**,1’*R**)-**33c**: ^{1}H NMR (DMSO-*d*6) δ: 9.52 (1H, d, *J* = 7.6 Hz), 7.95 (2H, d, *J* = 8.6 Hz), 7.33 (2H, d, *J* = 8.6 Hz), 7.19 (1H, dd, *J* = 8.6, 2.5 Hz), 6.93 (1H, d, *J* = 2.5 Hz), 6.75 (1H, d, *J* = 8.6 Hz), 6.08 (2H, s), 5.31 (1H, d, *J* = 17.8 Hz), 4.84 (1H, ddd, *J* = 7.6, 7.6, 7.6 Hz), 4.78 (1H, d, *J* = 13.7 Hz), 4.34 (1H, d, *J* = 13.7 Hz), 4.20 (1H, d, *J* = 17.8 Hz), 3.85 (3H, s), 3.77 (3H, s), 3.71 (6H, s), 3.50-3.49 (1H, m), 3.11 (1H, dd, *J* = 13.7, 4.6 Hz), 3.07-2.98 (2H, m), 2.41 (1H, dd, *J* = 13.7, 9.2 Hz), 1.90-1.77 (2H, m), 1.58 (9H, s), 0.90 (3H, dd, *J* = 7.4, 7.4 Hz). This was employed for the next step without further purification.

(6*R**,1’*S**)-**33c**: ^{1}H NMR (DMSO-*d*6) δ: 9.51 (1H, d, *J* = 7.6 Hz), 7.96 (2H, d, *J* = 7.7 Hz), 7.31 (2H, d, *J* = 7.7 Hz), 7.19 (1H, dd, *J* = 8.6, 2.5 Hz), 6.94 (1H, d, *J* = 2.5 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 6.01 (2H, s), 5.29 (1H, d, *J* = 17.3 Hz), 4.86 (1H, ddd, *J* = 7.6, 7.6, 7.6 Hz), 4.81 (1H, d, *J* = 13.7 Hz), 4.25 (1H, d, *J* = 17.3 Hz), 4.22 (1H, d, *J* = 13.7 Hz), 3.83 (3H, s), 3.78 (3H, s), 3.54 (6H, s), 3.59-3.50 (1H, m), 3.11 (1H, dd, *J* = 14.0, 4.8 Hz), 3.03-2.92 (2H, m), 2.42 (1H, dd, *J* = 14.0 Hz, 8.9 Hz), 1.90-1.75 (2H, m), 1.60 (9H, s), 0.90 (2H, dd, *J* = 7.4, 7.4 Hz). This was employed for the next step without further purification.

(6*R,1”*R**)-4-[[[1”-[[3”’-Amino-4”’-(*tert*-butoxycarbonyl)]phenyl]propyl]amino]carbonyl]-6-[(5””-chloro-2””-methoxy)benzyl]-1-(2’,4’,6’-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R**,1’*R**)-33d] and (6*R**,1”*S**)-4-[[[1”-[[3”’-Amino-4”’-(*tert*-**

butoxycarbonyl)]phenyl]propyl]amino]carbonyl]-6-[(5””-chloro-2””-methoxy)benzyl]-1-(2’,4’,6’-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R,1’*S**)-33d]**

(6*R**,1’*R**)-**33d**: Rf = 0.42 (developed by hexane / ethyl acetate = 1 / 2). This was employed for the next step without further purification.

(6*R**,1’*S**)-**33d**: Rf = 0.29 (hexane / ethyl acetate = 1 / 2). This was employed for the next step without further purification.

(6*R,1’*R**)-4-[[[1’-(3”-Carboxylphenyl)propyl]amino]carbonyl]-6-[(5””-chloro-2””-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R**,1’*R**)-25a]**

1.0 M hydrogen chloride solution in acetic acid (1.5 mL) was added to (6*R**,1”*R**)-**33a** (115 mg), and the mixture was stirred at room temperature for 24 hours. Then, the mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane / ethyl acetate / methanol = 3 / 3 / 1) to give (6*R**,1’*R**)-**25a** (19.0 mg, 25.5%). ^{1}H NMR (DMSO-*d*6) δ: 13.01(1H, br), 9.48 (1H, d, *J* = 7.3 Hz), 7.86 (1H, s), 7.82 (1H, d, *J* = 7.7 Hz), 7.67 (1H, d, *J* = 3.5 Hz), 7.55 (1H, d, *J* = 7.7 Hz), 7.46 (1H, dd, *J* = 7.7, 7.7 Hz), 7.33 (1H, d, *J* = 2.6 Hz), 7.27 (1H, dd, *J* = 8.8, 2.6 Hz), 7.00 (1H, d, *J* = 8.8 Hz), 4.79-4.69 (2H, m), 4.49 (1H, d, *J* = 17.2 Hz), 3.91-3.81 (1H, m), 3.79 (3H, s), 3.16 (1H, dd, *J* = 12.6, 12.6 Hz), 3.05-2.96 (2H, m), 2.67 (1H, dd, *J* = 14.3, 9.3 Hz), 1.89-1.74 (2H, m), 0.84 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 488 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{26}ClN_3NaO_6^+$ 510.1402 $(M+Na)^+$, found 510.1388.

(6*R,1’*R**)-4-[[[1’-[(4”-Amino-3”-carboxyl)phenyl]propyl]amino]carbonyl]-6-[(5””-chloro-2””-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R**,1’*R**)-25b]**

Diazepane (6*R**,1’*R**)-**25b** was prepared from (6*R**,1”*R**)-**33b** in a similar manner to

(6*R**,1'*R**)-**25a**.^{1}H NMR (DMSO-*d*6) δ: 9.33 (1H, d, *J* = 7.7 Hz), 7.68 (1H, d, *J* = 3.2 Hz), 7.60 (1H, d, *J* = 2.4 Hz), 7.33 (1H, d, *J* = 2.8 Hz), 7.27 (1H, dd, *J* = 8.9, 2.8 Hz), 7.17 (1H, dd, *J* = 8.5, 2.4 Hz), 7.00 (1H, d, *J* = 8.5 Hz), 6.71 (1H, d, *J* = 8.9 Hz), 4.76 (1H, d, *J* = 17.0 Hz), 4.54-4.45 (2H, m), 3.90-3.80 (1H, m), 3.78 (3H, s), 3.14 (1H, dd, *J* = 12.7, 12.7 Hz), 3.06-2.89 (2H, m), 2.65 (1H, dd, *J* = 14.2, 9.3 Hz), 1.82-1.64 (2H, m), 0.80 (3H, dd, *J* = 7.3, 7.3 Hz); HRMS (FAB): calcd for $C_{24}H_{27}ClN_4NaO_6^+$ 525.1511 $(M+Na)^+$, found 525.1482.

(6*R,1'*R**)-4-[[[1'-(4"-Carboxylphenyl)propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R**,1'*R**)-25c]**

^{1}H NMR (DMSO-*d*6) δ: 13.05-12.95 (1H, br), 9.36 (1H, d, *J* = 7.3 Hz), 7.70-7.63 (2H, m), 7.31 (1H, d, *J* = 2.7 Hz), 7.25 (1H, dd, *J* = 8.7, 2.7 Hz), 7.11-7.02 (2H, m), 6.99 (1H, d, *J* = 8.7 Hz), 6.48-6.42 (1H, m), 4.86 (1H, ddd, *J* = 7.3, 7.3, 7.3 Hz), 4.76 (1H, d, *J* = 17.4 Hz), 4.51 (1H, d, *J* = 17.4 Hz), 3.90-3.79 (1H, m), 3.78 (3H, s) 3.18-3.07 (1H, m), 3.04-2.90 (2H, m), 2.63-2.55 (1H, m), 1.90-1.78 (2H, m), 0.85 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 488 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{26}ClN_3NaO_6^+$ 510.1402 $(M+Na)^+$, found 510.1403.

(6*R,1'*R**)-4-[[[1'-[(3"-Amino-4"-carboxyl)phenyl]propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R**,1'*R**)-25d]**

^{1}H NMR (DMSO-*d*6) δ: 9.39 (1H, d, *J* = 7.6 Hz), 7.65 (1H, d, *J* = 3.7 Hz), 7.61 (1H, d, *J* = 8.3 Hz), 7.30 (1H, d, *J* = 2.6 Hz), 7.24 (1H, dd, *J* = 8.7, 2.6 Hz), 6.97 (1H, d, *J* = 8.7 Hz), 6.60 (1H, s), 6.40 (1H, d, *J* = 8.3 Hz), 5.72 (2H, s), 4.73 (1H, d, *J* = 17.1 Hz), 4.54-4.45 (2H, m), 3.87-3.80 (1H, m), 3.76 (3H, s), 3.12 (1H, dd, *J* = 12.6, 12.6 Hz), 3.00-2.91 (2H, m), 2.67-2.60 (1H, m), 1.77-1.49 (2H, m), 0.80 (3H, dd, 7.2, 7.2 Hz); MS (ESI): 503 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{27}ClN_4NaO_6^+$ 525.1511 $(M+Na)^+$, found 525.1503.

(6*R,1'*S**)-4-[[[1'-(3"-Carboxylphenyl)propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R**,1'*S**)-25a]**

^{1}H NMR (DMSO-*d*6) δ: 12.92 (1H, br), 9.45 (1H, d, *J* = 7.4 Hz), 7.86 (1H, s), 7.83 (1H, d, *J* = 7.6 Hz), 7.64 (1H, d, *J* = 3.2 Hz), 7.56 (1H, d, *J* = 7.6 Hz), 7.47 (1H, dd, *J* = 7.6, 7.6 Hz), 7.33 (1H, d, *J* = 2.4 Hz), 7.27 (1H, dd, *J* = 8.9, 2.4 Hz), 7.01 (1H, d, *J* = 8.9 Hz), 4.80-4.72 (2H, m), 4.52 (1H, d, *J* = 17.2 Hz), 3.92-3.82 (1H, m), 3.79 (3H, s), 3.12 (1H, dd, J = 12.7, 12.7 Hz), 3.03-2.94 (2H, m), 2.65 (1H, dd, *J* = 14.2, 9.0 Hz), 1.87-1.71 (2H, m), 0.84 (3H, dd, *J* = 7.2, 7.2 Hz); MS (ESI): 488 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{26}ClN_3NaO_6^+$ 510.1402 $(M+Na)^+$, found 510.1422.

(6*R,1'*S**)-4-[[[1'-[(4"-Amino-3"-carboxyl)phenyl]propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R**,1'*S**)-25b]**

^{1}H NMR (DMSO-*d*6) δ: 9.31 (1H, d, *J* = 7.7 Hz), 7.65 (1H, d, *J* = 3.7 Hz), 7.59 (1H, d, *J* = 2.0 Hz), 7.33 (1H, d, *J* = 2.6 Hz), 7.27 (1H, dd, *J* = 8.7, 2.6 Hz), 7.18 (1H, dd, *J* = 8.5, 2.0 Hz), 7.00 (1H, d, *J* = 8.5 Hz), 6.72 (1H, d, *J* = 8.7 Hz), 4.77 (1H, d, *J* = 17.5 Hz), 4.55-4.48 (2H, m), 3.91-3.81 (1H, m), 3.79 (3H, s), 3.10 (1H, dd, *J* = 12.7, 12.7 Hz), 3.02-2.92 (2H, m), 2.63 (1H, dd, *J* = 14.6, 8.9 Hz), 1.82-1.64 (2H, m), 0.79 (3H, dd, *J* = 7.3, 7.3 Hz); HRMS (FAB): calcd for $C_{24}H_{27}ClN_4NaO_6^+$ 525.1511 $(M+Na)^+$, found 525.1541

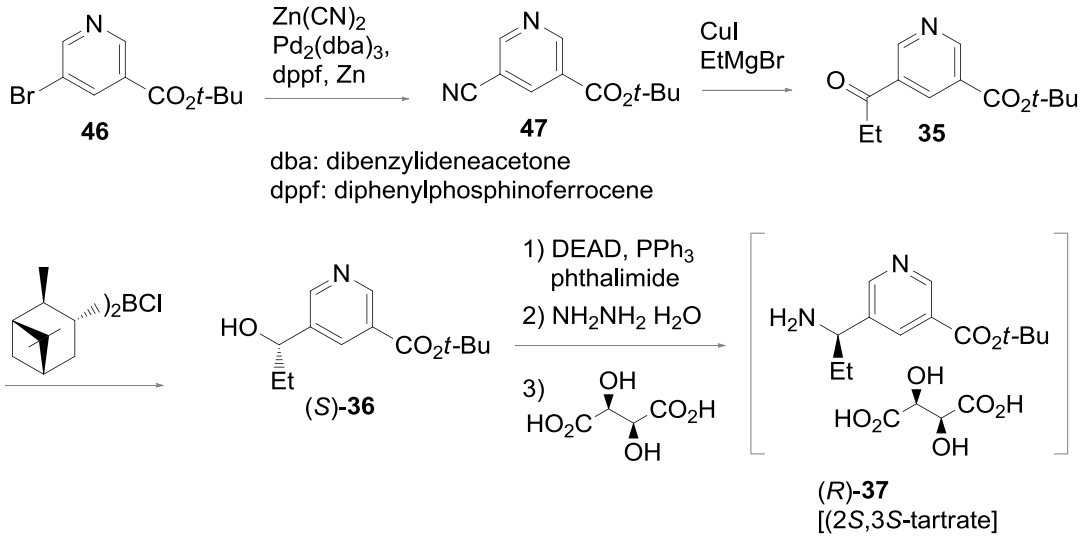
(6*R,1'*S**)-4-[[[1'-(4"-Carboxylphenyl)propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R**,1'*S**)-25c]**

^{1}H NMR (DMSO-*d*6) δ: 12.71 (1H, br), 9.43 (1H, d, *J* = 7.5 Hz), 7.88 (2H, d, *J* = 8.2 Hz), 7.63 (1H, d, *J* = 3.6 Hz), 7.4 (2H, d, *J* = 8.2 Hz), 7.31 (1H, d, *J* = 2.6 Hz), 7.24 (1H, dd, *J* = 8.9,

2.6 Hz), 6.98 (1H, d, *J* = 8.9 Hz), 4.79-4.69 (2H, m), 4.50 (1H, d, *J* = 17.1 Hz), 3.90-3.80 (1H, m), 3.76 (3H, s), 3.11 (1H, dd, *J* = 13.0, 13.0 Hz), 3.00-2.91 (2H, m), 2.63 (1H, dd, *J* = 14.4, 9.0 Hz), 1.84-1.69 (2H, m), 0.81 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 488 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{26}ClN_3NaO_6^+$ 510.1402 $(M+Na)^+$, found 510.1420.

(6*R,1'*S**)-4-[[[1'-[(3"-Amino-4"-carboxyl)phenyl]propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R**,1'*S**)-25d]**

^{1}H NMR (DMSO-*d*6) δ: 9.37 (1H, d, *J* = 7.7 Hz), 7.63 (1H, br), 7.62 (1H, d, *J* = 7.6 Hz), 7.30 (1H, d, *J* = 2.6 Hz), 7.24 (1H, dd, *J* = 8.7, 2.6 Hz), 6.98 (1H, d, *J* = 8.7 Hz), 6.57 (1H, s), 6.39 (1H, d, *J* = 7.6 Hz), 5.72 (2H, s), 4.76 (1H, d, *J* = 17.0 Hz), 4.55-4.46 (2H, m), 3.90-3.81 (1H, m), 3.76 (3H, s), 3.10 (1H, dd, *J* = 12.7, 12.7 Hz), 3.00-2.91 (2H, m), 2.65-2.55 (1H, m), 1.74-1.65 (2H, m), 0.79 (3H, dd, *J* = 7.2, 7.2 Hz); MS (ESI): 503 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{27}ClN_4NaO_6^+$ 525.1511 $(M+Na)^+$, found 525.1541.



Scheme 11 Synthesis of (*R*)-**37**

***tert*-Butyl 5-cyanopyridine-3-carboxylate (47)**

To a solution of **46** (500 mg) in dimethylacetamide (2.5 mL) were added zinc cyanide (136 mg), tris(dibenzylideneacetone)dipalladium(0) (35 mg), 1,1'-bis(diphenylphosphino)ferrocene (43 mg) and zinc powder (15 mg), and the mixture was stirred at 120 °C for 3.5 hours. Then, an insoluble substance was removed by filtration while being washed with ethyl acetate, and the filtrate was diluted with ethyl acetate. The solution was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane /ethyl acetate = 3 / 1) to give **47** (295 mg, 74.5%). ^{1}H NMR (CDCl3) δ: 9.33 (1H, d, *J* = 1.8 Hz), 9.00 (1H, d, *J* = 2.2 Hz), 8.49 (1H, dd, *J* = 2.2, 1.8 Hz), 1.62 (9H, s); MS (ESI): 149 (M-*tert*-Bu)$^+$. This was employed for the next step without further purification.

***tert*-Butyl 5-propionylpyridine-3-carboxylate (35)**

To a suspension of cupper iodide (1.00 g) in tetrahydrofuran (18 mL) was added 0.86 M solution of ethylmagnesium bromide in tetrahydrofuran (12.3 mL) at –20 °C, and the resulting mixture was stirred under ice cooling for 30 minutes. Then, the mixture was cooled to –20 °C, and a solution of **47** (900 mg) in tetrahydrofuran (9.0 mL) was added to the mixture. After stirring at –20 °C for 30 minutes, saturated ammonium hydrogen chloride aqueous solution was added to the reaction mixture, and the resulting mixture was stirred at room temperature for 1 hour. Then, the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 3 / 1) to give **35** (550 mg, 53.0%). ^{1}H NMR (CDCl3) δ: 9.30 (1H, d, *J* = 1.8 Hz), 9.28 (1H, d, *J* = 2.2 Hz), 8.72 (1H, dd, *J* = 2.2, 1.8 Hz), 3.05 (2H, q, *J* = 7.3 Hz), 1.63 (9H, s), 1.27 (3H, t, *J* = 7.3 Hz); MS (ESI): 180

(M-*tert*-Bu)$^+$. This was employed for the next step without further purification.

***tert*-Butyl (*S*)-5-(1'-hydroxypropyl)pyridine-3-carboxylate [(*S*)-36]**

To a solution of **35** (630 mg) in tetrahydrofuran (3.2 ml) was dropwisely added 60% solution of (–)-B-chlorodiisopinocampheylborane in hexane (3.2 mL) at –20 °C, and the mixture was stirred at –20 °C for 18 hours. Then, ether and water were added to the reaction mixture, and the layers were separated. The organic layer was extracted with water and 1.0 M hydrochloric acid. Then, the combined aqueous layer was neutralized by sodium hydrogen carbonate, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give (*S*)-**36** (440 mg, 69.3 %, 90%ee). The enantiomeric excess was determined by HPLC on chiral stationary phase (analytical column: CHIRALCEL AD-H of Daicel Chemical Industries, mobile phase: hexane / 2-propanol = 9 / 1, flow rate: 1.0 mL / min, minor peak: t_R = 6.8 min, major peak: t_R = 9.0 min, peak area ratio: major peak / minor peak = 95.0 / 5.0). MS (ESI): 182 (M-*tert*-Bu)$^+$. This was employed for the next step without further purification.

***tert*-Butyl (*R*)-5-(1'-aminopropyl)pyridine-3-carboxylate D-tartrate [(*R*)-37]**

To a solution of (*S*)-**36** (490 mg) in tetrahydrofuran (10 mL) were added triphenylphosphine (1.08 g), diethyl azodicarboxylate (650 μL) and phtalimide (460 mg) under cooling at 0 °C, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated *in vacuo*, and 1.0 M sodium hydroxide aqueous solution and ethyl acetate were added to the residue, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography

(chloroform / ethyl acetate = 6 / 1) to give a product (640 mg, 84.6%).

To a solution of the product (640 mg) in acetonitrile (3.2 mL) was added hydrazine monohydrate (420 μL) at 60 °C, then the mixture was stirred at that temperature for 4 hours. Then, water was added to the mixture, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

The residue was diluted with ethyl acetate (3.7 mL), and 1.0 M solution of D-tartaric acid in ethanol (1.56 mL) was added to the solution. Then, the precipitate was collected by filtration while being washed with ethanol to give (*R*)-**37** (330 mg, 50.9%, 98.0% ee). The enantiomeric excess was determined by HPLC on chiral stationary phase (analytical column: CHIRALCEL AD-H of Daicel Chemical Industries; mobile phase: hexane / 2-propanol / diethylamine = 95 / 5 / 0.05, flow rate: 1.0 mL / min, major peak: t_R = 16.1 min, minor peak: t_R = 18.6 min, peak area ratio: major peak / minor peak = 99.0 / 1.0). ^{1}H NMR (DMSO-*d*6) δ: 8.99 (1H, d, *J* = 2.0 Hz), 8.81 (1H, d, *J* = 2.0 Hz), 8.33 (1H, dd, *J* = 2.0, 2.0 Hz), 7.75-6.95 (3H, br), 4.18 (1H, dd, *J* = 6.4, 6.4 Hz), 3.86 (2H, s), 1.95-1.71 (2H, m), 1.57 (9H, s), 0.78 (3H, dd, *J* = 7.4, 7.4 Hz); MS (ESI): 237 $(M+H)^+$; $[\alpha]^{20}_D$ −14.5 (c = 0.1, methanol); melting point: 147-149 °C. This was employed for the next step without further purification.

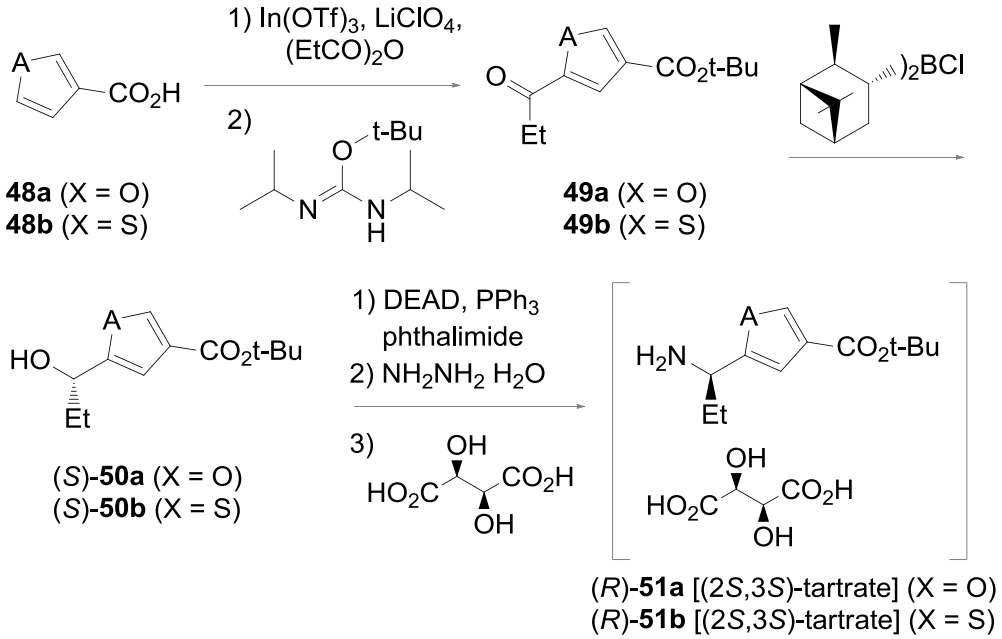
tert-Butyl (*S*)-5-(1'-hydroxypropyl)furan-2-carboxylic acid [(*S*)-39]

(1*S*,2*R*)-2-(dibutylamino)-1-phenylpropan-1-ol (81.0 mg) was diluted with a mixture of hexane (5.0 mL) and toluene (5.0 mL). Aldehyde **38** (1.00 g) was added to the solution and the resulting mixture was stirred at room temperature for 30 minutes. Then, 1.0 M solution of diethylzinc in hexane (11.3 mL) was added to the mixture under ice cooling, and the resulting mixture was stirred at that temperature for 16 hours. Then, saturated ammonium chloride

aqueous solution was added to the reaction mixture, the resulting mixture was stirred at room temperature for 20 minutes, and 1.0 M hydrochloric acid was added. The resulting mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give (*S*)-**39** (1.53 g, quantitative, 87.0% ee). The enantiomeric excess was determined by HPLC on chiral stationery phase (analytical column: CHIRALPAK AD-H of Daicel Chemical Industries, mobile phase: hexane / 2-propanol = 98 / 2, flow rate: 1.0 mL / min, minor peak: t_R = 18.8 min, major peak: t_R = 19.9 min, peak area ration: major peak / minor peak = 93.5 / 6.5). ^{1}H NMR ($CDCl_3$) δ: 6.98 (1H, d, *J* = 3.4 Hz), 6.30 (1H, d, *J* = 3.4 Hz), 4.65 (1H, br), 1.99-1.80 (2H, m), 1.55 (9H, s), 0.96 (3H, dd, *J* = 7.4, 7.4 Hz). This was employed for the next step without further purification.

***tert*-Butyl (*R*)-5-(1'-aminopropyl)furan-2-carboxylate D-tartrate [(*R*)-40]**

Ester (*R*)-**40** (97% ee) was prepared from (*S*)-**39** in a similar manner to (*R*)-**37**. The enantiomeric excess was determined by HPLC on chiral stationary phase in the same analytical condition to that in (*S*)-**39** (minor peak: t_R = 11.5 min, major peak: t_R = 12.8 min, peak area ratio: major peak / minor peak = 98.5 / 1.5). ^{1}H NMR (DMSO-*d*6) δ: 8.25-7.30 (3H, br), 7.16 (1H, d, *J* = 3.5 Hz), 6.59 (1H, d, *J* = 3.5 Hz), 4.15 (1H, dd, *J* = 6.8, 6.8 Hz), 3.95 (2H, s), 1.89-1.70 (2H, m), 1.51 (9H, s), 0.85 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 226 $(M+H)^+$; $[\alpha]^{20}_D$ –14.1 (c = 0.1, methanol); melting point: 142-144 °C. This was employed for the next step without further purification.



Scheme 12 Syntheses of (*R*)-**51a** and (*R*)-**51b**

***tert*-Butyl 5-propionylfuran-3-carboxylate (49a)**

To a solution of **48a** (1.12 g) in nitromethane (10 mL) were added indium (III) trifluoromethylsulfonate[49] (56 mg), lithium perchlorate (1.06 g) and propionic anhydride (1.28 mL), and the mixture was stirred at 50 °C for 3 hours. Then, water was added to the reaction mixture, and the layers were separated, and the aqueous layer was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

To a solution of the residue (1.10 g) in methylene chloride (22 mL) was added *N*,*N*'-diisopropyl-O-*tert*-butylisourea (8.0 mL), and the mixture was stirred at reflux for 3 hours. Precipitates were filtered out while being washed with diethyl ether, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 5 / 1). Then, hexane was added to the product, and the precipitates were collected by filtration while being washed with diethyl ether to give **49a** (0.71 g, 31.7%). ^{1}H

NMR ($CDCl_3$) δ: 8.02 (1H, s), 7.39 (1H, s), 2.85 (2H, q, *J* = 7.3 Hz), 1.56 (9H, s), 1.21 (3H, t, *J* = 7.3 Hz); MS (ESI): 225 $(M+H)^+$. This was employed for the next step without further purification.

***tert*-Butyl 5-propionylthiophene-3-carboxylate (49b)**

Ester **49b** was prepared from **48b** in a similar manner to **49a**. ^1H NMR ($CDCl_3$) δ: 8.23 (1H, s), 8.01 (1H, s), 2.95 (2H, q, *J* = 7.3 Hz), 1.57 (9H, s), 1.24 (3H, t, *J* = 7.3 Hz); MS (ESI): 241 $(M+H)^+$. This was employed for the next step without further purification.

***tert*-Butyl (*S*)-5-(1'-hydroxypropyl)furan-3-carboxylate [(*S*)-50a]**

Ester (*S*)-**50a** (91.0% ee) was prepared from **49a** in a similar manner to (*S*)-**36**. The enantiomeric excess was determined by HPLC on chiral stationary phase in the same analytical condition to that in (*S*)-**39** (major peak: t_R = 25.9 min, minor peak: t_R = 28.0 min, peak area ratio: major peak / minor peak = 95.5 / 4.5). ^1H NMR ($CDCl_3$) δ: 7.86 (1H, s), 6.53 (1H, s), 4.63-4.55 (1H, m), 4.11-4.02 (1H, m), 1.75-1.71 (1H, m), 1.71-1.67 (1H, m), 1.54 (9H, s), 1.00-0.93 (3H, m). This was employed for the next step without further purification.

***tert*-Butyl (*S*)-5-(1'-hydroxypropyl)thiophene-3-carboxylate [(*S*)-50b]**

Ester (*S*)-**50b** (77.0% ee) was prepared from **49b** in a similar manner to (*S*)-**36**. The enantiomeric excess was determined by HPLC on chiral stationary phase in the same analytical condition to that in (*S*)-**39** (minor peak: t_R = 10.6 min, major peak: t_R = 11.4 min, peak area ratio: major peak / minor peak = 88.5 / 11.5). ^1H NMR ($CDCl_3$) δ: 7.86 (1H, d, *J* = 0.8 Hz), 6.53 (1H, d, *J* = 0.8 Hz), 4.59 (1H, ddd, *J* = 6.3, 6.3, 6.3 Hz), 4.10-4.03 (1H, m), 1.96-1.78 (2H, m), 1.56 (9H, s), 0.96 (1H, dd, *J* = 7.3, 7.3 Hz). This was employed for the next step without further

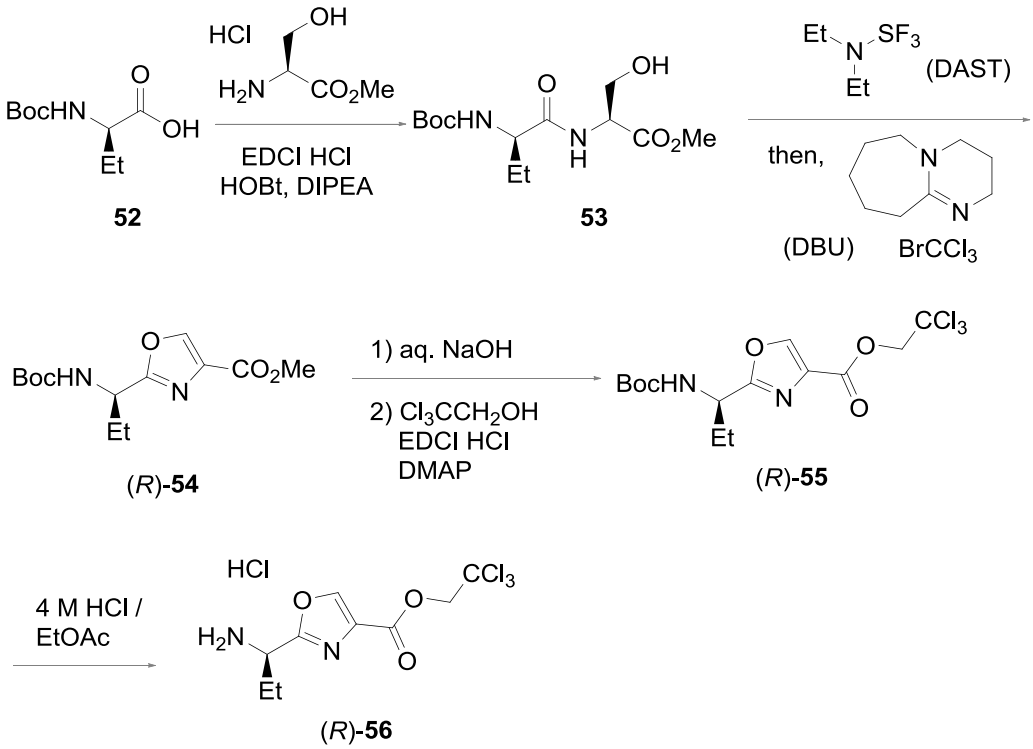
purification.

***tert*-Butyl (*R*)-5-(1'-aminopropyl)furan-3-carboxylate D-tartrate [(*R*)-51a]**

Ester (*R*)-**51a** was prepared from (*S*)-**50a** in a similar manner to (*R*)-**37**. ^{1}H NMR (DMSO-*d*6) δ: 8.28 (1H, s), 6.65 (1H, s), 4.12 (1H, dd, *J* = 6.3, 6.3 Hz), 3.91 (2H, s), 1.89-1.72 (2H, m), 1.50 (9H, s), 0.82 (3H, dd, *J* = 7.4, 7.4 Hz); MS (ESI): 209 $(M\text{-}NH_2)^+$. This was employed for the next step without further purification.

***tert*-Butyl (*R*)-5-(1'-aminopropyl)thiophene-3-carboxylate D-tartrate [(*R*)-51b]**

Ester (*R*)-**51b** (93.0% ee) was prepared from (*S*)-**50b** in a similar manner to (*R*)-**37**. The enantiomeric excess was determined by HPLC on chiral stationary phase in the same analytical condition to that in (*S*)-**39** (major peak: t_R = 13.7 min, minor peak: t_R = 14.6 min, peak area ratio: major peak / minor peak = 96.5 / 3.5). ^{1}H NMR (DMSO-*d*6) δ: 8.17 (1H, s), 7.41 (1H, s), 4.35 (1H, dd, *J* = 7.8, 7.8 Hz), 3.93 (2H, s), 1.92-1.70 (2H, m), 1.51 (9H, s), 0.84 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 225 $(M+H)^+$. This was employed for the next step without further purification.



Scheme 13 Synthesis of (*R*)-**56**

Methyl (3*S*,2'*R*)-2-[2'-[(*tert*-butoxycarbonyl)amino]butanamido]-3-hydroxypropanoate (53)

To a solution of **52** (5.00 g) in methylene chloride (100 mL) were added L-serine methyl ester hydrochloride (5.7 g), 1-hydroxybenzotriazole (5.0 g), *N*,*N*-diisopropylethylamine (6.4 mL) and 1-[3'-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (7.1 g), and the mixture was stirred at room temperature for 3 hours. The reaction mixture was diluted with ethyl acetate. Then, the mixture was successively washed with water, saturated potassium hydrogen sulfate aqueous solution, saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. Then, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 4 / 6 to 3 / 7) to give **53** (5.27 g, 70.4%). ^{1}H NMR ($CDCl_3$) δ: 7.00 (1H, d, J = 7.3 Hz), 5.01 (1H, d, J = 6.5 Hz),

4.71-4.58 (1H, m), 4.08-3.98 (2H, m), 3.99-3.90 (1H, m), 3.79 (3H, s), 2.86-2.77 (1H, m), 1.97-1.83 (1H, m), 1.75-1.63 (1H, m), 1.45 (9H, s), 0.99 (3H, dd, J = 7.5, 7.5 Hz); MS (ESI): 327 $(M+Na)^+$. This was employed for the next step without further purification.

Methyl (*R*)-2-[1'-[(*tert*-butoxycarbonyl)amino]propyl]oxazole-4-carboxylate [(*R*)-54]

To a solution of **53** (2.46 g) in methylene chloride (50 mL) cooled at –20 °C was dropwisely added *N*,*N*- diethylaminosulfur trifluoride[50] (1.17 mL), and the mixture was stirred at that temperature for 1 hour. Then, bromotrichloromethane (2.8 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (4.2 mL) were added to the mixture. After stirring at 0 °C for 6 hours, saturated sodium hydrogen carbonate aqueous solution was added to the mixture, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. Then, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 8 / 2 to 7 / 3) to give (*R*)-**54** (1.00 g, 43.5%). ^{1}H NMR ($CDCl_3$) δ: 8.18 (1H, s), 5.28-5.15 (1H, m), 4.97-4.79 (1H, m), 3.91 (3H, s), 2.04-1.76 (2H, m), 1.45 (9H, s), 0.93 (3H, dd, J = 7.5, 7.5 Hz). This was employed for the next step without further purification.

2,2,2-Trichloroethyl (*R*)-2-[1'-[(*tert*-butoxycarbonyl)amino]propyl]oxazole-4-carboxylate [(*R*)-55]

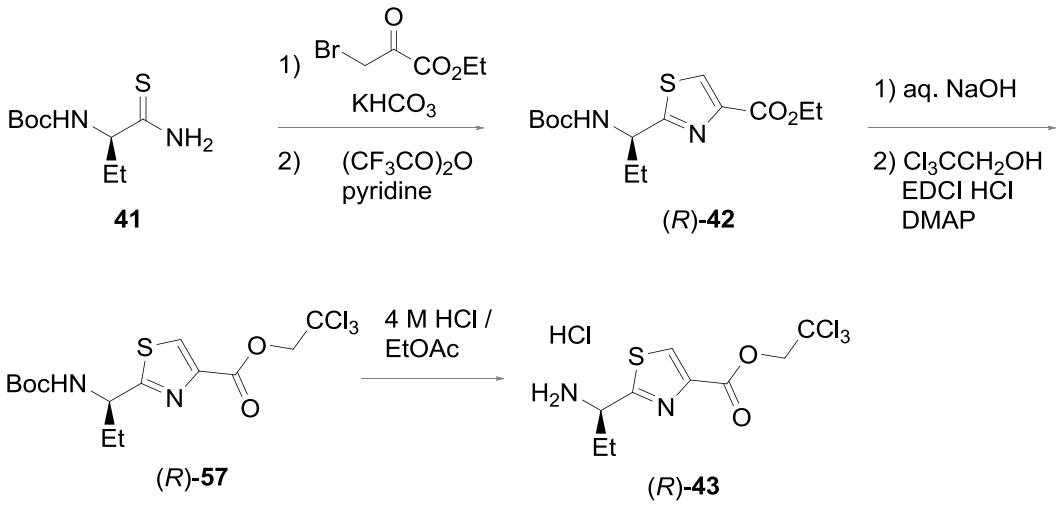
To a solution of (*R*)-**54** (670 mg) in methanol (10 mL) was added 4.0 M sodium hydroxide aqueous solution (1.18 mL), and the mixture was stirred at room temperature for 3 hours. Methanol was distilled off *in vacuo*, and the residue was diluted with water, and the resulted solution was washed with ethyl acetate. The aqueous layer was acidified with 6.0 M hydrochloric acid to pH 4, and the resulting mixture was extracted with ethyl acetate. The

organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

To a solution of the residue (520 mg) in methylene chloride (10 mL) were added 1-[(3'-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (440 mg), 4-dimethylaminopyridine (23 mg) and 2,2,2-trichloroethanol (280 μL), and the mixture was stirred at room temperature for 2.5 hours. The reaction mixture was concentrated *in vacuo*. The residue was diluted with ethyl acetate. Then, the solution was successively washed with water, saturated potassium hydrogen sulfate aqueous solution, saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. Then, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 6 / 1 to 3 / 1) to (*R*)-**55** (0.46 g, 48.6%). ^{1}H NMR ($CDCl_3$) δ: 8.28 (1H, s), 5.26-5.16 (1H, m), 5.03-4.86(3H, m), 2.04-1.92 (1H, m), 1.01-1.80 (1H, m), 1.43 (9H, s), 0.95 (6H, dd, *J* = 7.5, 7.5 Hz); MS (ESI): 424 $(M+Na)^+$. This was employed for the next step without further purification.

2,2,2-Trichloroethyl (*R*)-2-(1'-Aminopropyl)oxazole-4-carboxylate hydrochloride [(*R*)-56]

4.0 M Hydrogen chloride solution in ethyl acetate (5.0 mL) was added to (*R*)-**55** (750 mg), and the mixture stirred at room temperature for 2 hours. Then, the reaction mixture was concentrated *in vacuo* to give (*R*)-**56** (510 mg, 80.8%). ^{1}H NMR ($CDCl_3$) δ: 9.50 - 9.37 (2H, m), 8.39 (1H, s), 4.97-4.89 (2H, m), 4.84-4.75 (1H, m), 2.37-2.28 (2H, m), 1.11 (3H, dd, *J* = 7.5, 7.5 Hz); MS (ESI): 301 $(M+H)^+$. This was employed for the next step without further purification.



Scheme 14 Synthesis of (*R*)-**43**

Ethyl (*R*)-2-[1'-[(*tert*-butoxycarbonyl)amino]propyl]thiazole-4-carboxylate [(*R*)-42]

To a solution of **41** (2.82 g) in dimethoxyethane (60 mL) cooled at 0 °C were added potassium hydrogen carbonate (10.3 g) and ethyl bromopyruvate (4.9 mL), and the mixture was stirred at that temperature for 30 minutes. Then, the mixture was warmed to room temperature. After stirring at room temperature for 18 hours, the reaction mixture was concentrated *in vacuo*. The residue was diluted with chloroform, and the solution was successively washed with water and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

To a solution of the residue in dimethoxyethane (60 mL) cooled at 0 °C were added trifluoroacetic anhydride (3.6 mL) and pyridine (4.7 mL), and the mixture was stirred at that temperature for 1 hour. Then, the reaction mixture was concentrated *in vacuo*, and the residue was diluted with ethyl acetate. Then, the solution was successively washed with saturated potassium hydrogen sulfate aqueous solution, saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, then concentrated *in vacuo*. Then, the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 9 / 1 to 17 / 3) to give (*R*)-**42** (4.25 g, quantitative). ^{1}H NMR ($CDCl_3$) δ: 8.08 (1H, s), 5.16-5.16 (1H, m),

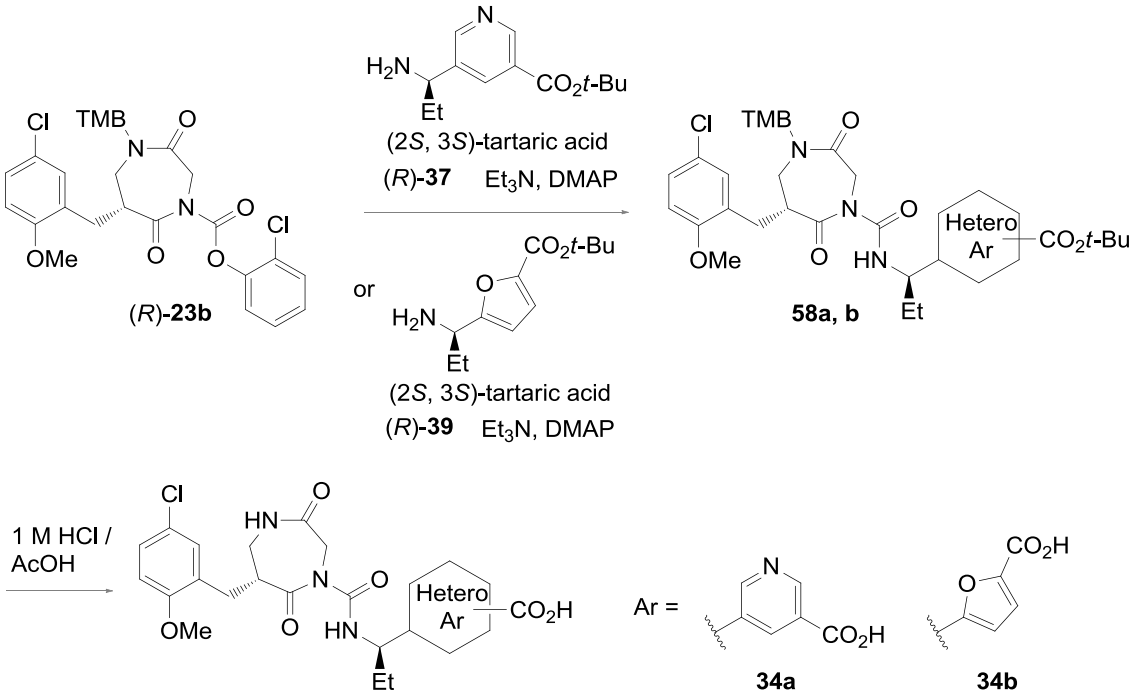
5.00-4.88 (1H, m), 4.42 (2H, q, J = 7.3 Hz), 2.21-2.08 (1H, m), 1.93-1.79 (1H, m), 1.45 (9H, s), 1.40 (3H, t, J = 7.3 Hz), 0.98 (3H, dd, J = 7.3, 7.3 Hz); MS (ESI): 337 $(M+Na)^+$. This was employed for the next step without further purification.

2,2,2-Trichloroethyl (*R*)-2-[1'-[(*tert*-butoxycarbonyl)amino]propyl]thiazole-4-carboxylate [(*R*)-57]

Ester (*R*)-**57** was prepared from (*R*)-**42** in a similar manner to (*R*)-**55**. ^{1}H NMR (DMSO-*d*6) δ: 8.23 (1H, s), 5.29-5.14 (1H, m), 5.02-4.91 (1H, m), 4.19-4.09 (2H, m), 2.25-2.08 (1H, m), 1.95-1.79 (1H, m), 1.45 (9H, s), 1.00 (3H, dd, J = 7.3, 7.3 Hz); MS (ESI): 439 $(M+Na)^+$. This was employed for the next step without further purification

2,2,2-Trichloroethyl (*R*)-2-(1'-Aminopropyl)thiazole-4-carboxylate hydrochloride [(*R*)-43]

Ester (*R*)-**43** was prepared from (*R*)-**57** in a similar manner to (*R*)-**56**. ^{1}H NMR ($CDCl_3$) δ: 9.43-9.30 (3H, m), 8.37 (1H, s), 5.00-4.93 (3H, m), 2.42-2.27 (2H, m), 1.10 (3H, dd, J = 7.3, 7.3 Hz); MS (ESI): 316 $(M+H)^+$; $[\alpha]^{20}_D$ +4.7 (c = 0.1, methanol); melting point: 166-168 °C. This was employed for the next step without further purification.



Scheme 15 Syntheses of **34a** and **34b**

(6*R*,1"*R*)-4-[[[1"-[5""-(*tert*-Butoxycarbonyl)pyridine-3""-yl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione (58a)

To a solution of (*R*)-**23b** (500 mg) in *N*,*N*-dimethylformamide (2.5 mL) were added (*R*)-**37** (313 mg), triethylamine (113 μL) and 4-dimethylaminopyridine (99.0 mg) under ice cooling, and the mixture was stirred at that temperature for 20 hours. Then, water and ethyl acetate were added to the reaction mixture, and the layers were separated. Then, the organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1 to 1 / 2) to give **58a** (518 mg, 88.2%). ^{1}H NMR ($CDCl_3$) δ: 9.57 (1H, d, *J* = 7.2 Hz), 9.04 (1H, d, *J* = 2.0 Hz), 8.69 (1H, d, *J* = 2.0 Hz), 8.14 (1H, dd, *J* = 2.0, 2.0 Hz), 7.18 (1H, dd, *J* = 8.9, 2.8 Hz), 6.90 (1H, d, *J* = 2.8 Hz), 6.75 (1H, d, *J* = 8.9 Hz), 6.08 (2H, s), 5.25 (1H, d, *J* = 17.0 Hz), 4.86

(1H, ddd, *J* = 7.2, 7.2, 7.2 Hz), 4.75 (1H, d, *J* = 13.8 Hz), 4.35 (1H, d, *J* = 13.8 Hz), 4.22 (1H, d, *J* = 17.0 Hz), 3.84 (3H, s,), 3.78 (3H, s), 3.71 (6H, s), 3.58-3.49 (1H, m), 3.13 (1H, dd, *J* = 13.8, 4.5 Hz), 3.06-2.99 (2H, m), 2.39 (1H, dd, *J* = 13.8, 9.5 Hz), 1.93-1.81 (2H, m), 1.61 (9H, s), 0.95 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 725 $(M+H)^+$. This was employed for the next step without further purification.

(6*R*,1"*R*)-4-[[[1"-[5""-(*tert*-Butoxycarbonyl)furan-2""-yl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione (58b)

Diazepane **58b** was prepared from (*R*)-**23b** and (*R*)-**40** in a similar manner to **58a**. tR in HPLC = 5.4 min (analytical column: YMC-Pack Pro C18; mobile phase: gradient solvent system of acetonitrile / water / trifluoroacetic acid = 5 / 95 / 0.1 (solvent A)- acetonitrile / trifluoroacetic acid = 100 / 0.09 (solvent B); gradient condition: solvent A / solvent B = 100 / 0 to 10 / 90 (0-3.5 min), 10 / 90 to 2 / 98 (3.5-4.0 min), 2/98 (4.0-4.9 min), 2 / 98 to 100 / 0 (4.9-6.3 min); flow rate: 1.0 mL / min); MS (ESI): 715 $(M+H)^+$. This was employed for the next step without further purification.

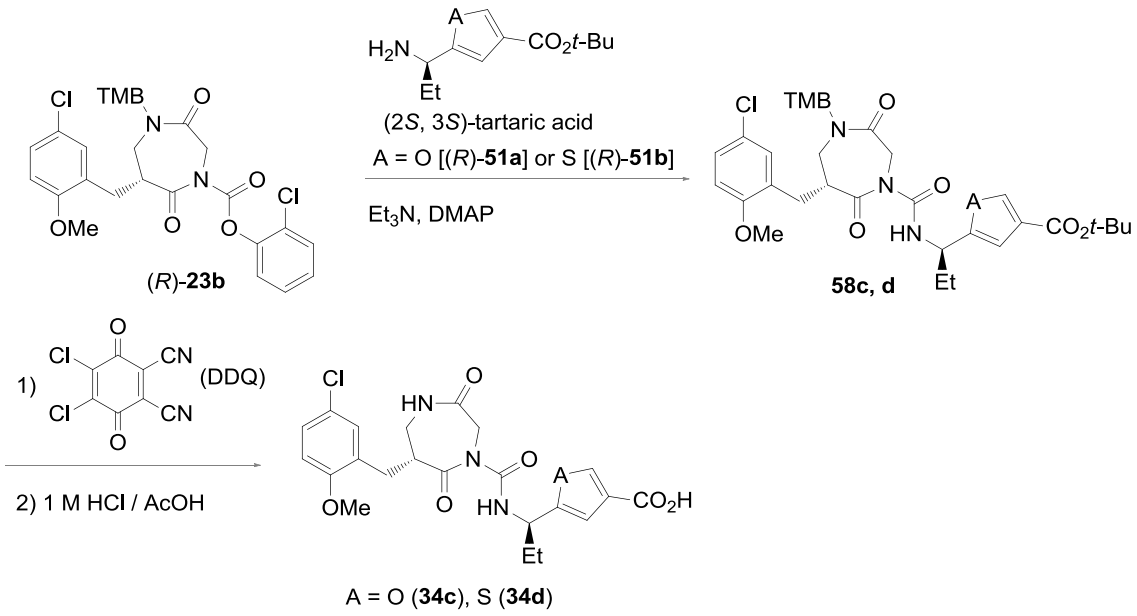
(6*R*,1'*R*)-4-[[[1'-(5"-Carboxylpyridine-3"-yl)propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1,4-diazepan-2,5-dione (34a)

1.0 M hydrogen chloride solution in acetic acid (5.0 mL) was added to **58a** (516 mg), and the resulting mixture was stirred at room temperature for 14 hours. Then, the mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (ethyl acetate / acetic acid = 20 / 1, then ethyl acetate / acetic acid / methanol = 20 / 1 / 2) to give **34a** (118 mg, 33.9%). ^{1}H NMR (DMSO-*d*6) δ: 9.48 (1H, d, *J* = 7.1 Hz), 8.93 (1H, d, *J* = 1.8 Hz), 8.73-8.69 (1H, m), 8.19-8.15 (1H, m), 7.67 (1H, d, *J* = 3.8 Hz), 7.34 (1H, d, *J* = 2.7 Hz), 7.27

(1H, dd, *J* = 8.7, 2.7 Hz), 7.01 (1H, d, *J* = 8.7 Hz), 4.79 (1H, ddd, *J* = 7.1, 7.1, 7.1 Hz), 4.69 (1H, d, *J* = 17.1 Hz), 4.49 (1H, d, *J* = 17.1 Hz), 3.92-3.80 (1H, m), 3.79 (3H, s), 3.17 (1H, dd, *J* = 12.9, 12.9 Hz), 3.07-2.97 (2H, m), 2.78-2.65 (1H, m), 1.95-1.78 (2H, m), 0.87 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 489 $(M+H)^+$; HRMS (FAB): calcd for $C_{23}H_{25}ClN_4NaO_6^+$ 511.1355 $(M+Na)^+$, found 511.134; $[\alpha]^{20}_D$ –32.6 (c = 0.1, methanol).

(6*R*,1'*R*)-4-[[[1'-(5"-Carboxylfuran-2"-yl)propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione (34b)

Diazepane **34b** was prepared from **58b** in a similar manner to **34a**. ^{1}H NMR (DMSO-*d*6) δ: 9.35 (1H, d, *J* = 7.1 Hz), 7.69-7.60 (1H, m), 7.30 (1H, s), 7.24 (1H, d, *J* = 8.7 Hz), 7.11 (1H, d, *J* = 3.4 Hz), 6.97 (1H, d, *J* = 8.7 Hz), 6.46 (1H, d, *J* = 3.4 Hz), 4.85 (1H, ddd, *J* = 7.1, 7.1, 7.1 Hz), 4.74 (1H, d, *J* = 17.3 Hz), 4.50 (1H, d, *J* = 17.3 Hz), 3.89-3.78 (1H, m), 3.76 (3H, s), 3.17-2.88 (3H, m), 2.65-2.57 (1H, m), 1.89-1.77 (2H, m), 0.84 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 478 $(M+H)^+$; HRMS (FAB): calcd for $C_{22}H_{24}ClN_3NaO_7^+$ 500.1195 $(M+Na)^+$, found 500.1194; $[\alpha]^{20}_D$ –47.4 (c = 0.1, methanol).



Scheme 16 Syntheses of **34c** and **34d**

(6*R*,1"*R*)-4-[[[1"-[4"'-(*tert*-Butoxycarbonyl)furan-2"'-yl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione (58c)

Diazepane **58c** was prepared from (*R*)-**23b** and (*R*)-**51a** in a similar manner to **58a**.^{1}H NMR ($CDCl_3$) δ: 9.36 (1H, d, *J* = 7.3 Hz), 7.83 (1H, d, *J* = 0.8 Hz), 7.18 (1H, dd, *J* = 8.5, 2.4 Hz), 6.89 (1H, d, J = 2.4 Hz), 6.75 (1H, d, *J* = 8.5 Hz), 6.49 (1H, d, *J* = 0.8 Hz), 6.07 (2H, s), 5.33 (1H, d, *J* = 17.5 Hz), 4.94 (1H, ddd, *J* = 7.3, 7.3, 7.3 Hz), 4.78 (1H, d, *J* = 13.8 Hz), 4.32 (1H, d, *J* = 13.8 Hz), 4.24 (1H, d, *J* = 17.5 Hz), 3.83 (3H, s), 3.77 (3H, s), 3.70 (6H, s), 3.58-3.47 (1H, m), 3.10 (1H, dd, *J* = 13.8, 4.5 Hz), 3.05-2.99 (2H, m), 2.37 (1H, dd, *J* = 13.8, 9.5 Hz), 1.96-1.78 (2H, m), 1.53 (9H, s), 0.91 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 715 $(M+H)^+$. This was employed for the next step without further purification.

(6*R*,1"*R*)-4-[[[1"-[4"'-(*tert*-Butoxycarbonyl)thiophene-2"'-yl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione

(58d)

^{1}H NMR ($CDCl_3$) δ: 9.41 (1H, d, *J* = 8.1 Hz), 7.87 (1H, d, *J* = 1.2 Hz), 7.31 (1H, d, *J* = 1.2 Hz), 7.18 (1H, dd, *J* = 8.5, 2.8 Hz), 6.89 (1H, d, *J* = 2.8 Hz), 6.75 (1H, d, *J* = 8.5 Hz), 6.07 (2H, s), 5.32 (1H, d, *J* = 17.0 Hz), 5.13-5.03 (1H, m), 4.77 (1H, d, *J* = 13.8 Hz), 4.33 (1H, d, *J* = 13.8 Hz), 4.24 (1H, d, *J* = 17.0 Hz), 3.84 (3H, s), 3.77 (3H, s), 3.70 (6H, s), 3.59-3.47 (1H, m), 3.10 (1H, dd, *J* = 14.2, 4.5 Hz), 3.05-2.98 (2H, m), 2.37 (1H, dd, *J* = 14.2, 9.5 Hz), 1.97-1.87 (2H, m), 1.55 (9H, s), 0.96 (3H, dd, *J* = 7.5, 7.5 Hz); MS (ESI): 730 $(M+H)^+$. This was employed for the next step without further purification.

(6*R*,1'*R*)-4-[[[1'-(4"-Carboxylfuran-2"-yl)propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione (34c)

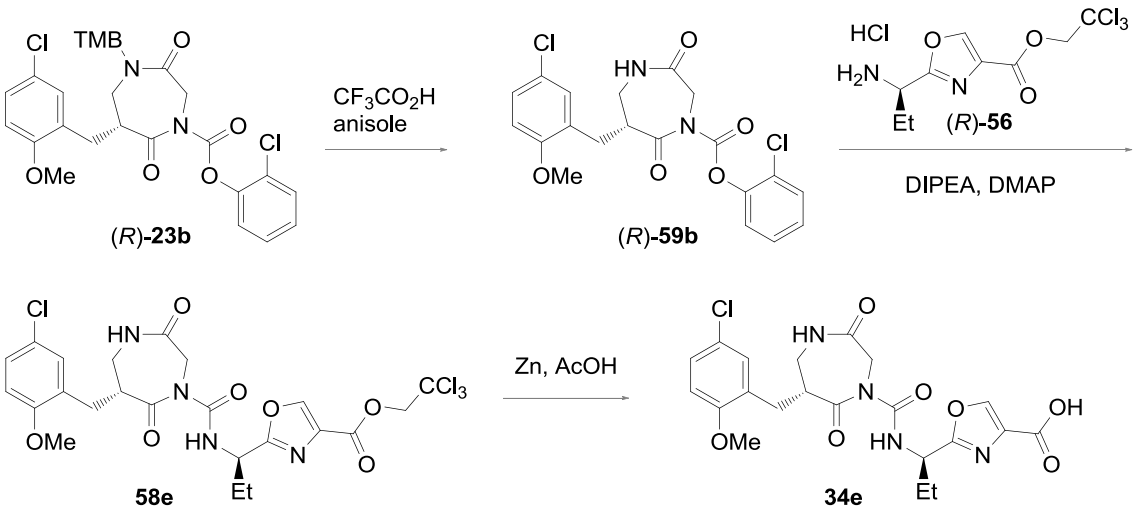
To a biphasic mixture of **58c** (176 mg) in methylene chloride (3.5 mL) and water (0.4 mL) were added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (109 mg), and the resulting mixture was stirred at room temperature for 24 hours. Then, water and chloroform were added to the reaction mixture, and the layers were separated. Then, the organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 2) to give a product (113 mg, 84.9%). ^{1}H NMR ($CDCl_3$) δ: 9.38 (1H, d, *J* = 7.3 Hz), 7.84 (1H, s), 7.21 (1H, dd, *J* = 8.9, 2.4 Hz), 7.10 (1H, d, *J* = 2.4 Hz), 6.80 (1H, d, *J* = 8.9 Hz), 6.50 (1H, s), 5.84-5.76 (1H, m), 5.39 (1H, d, *J* = 17.5 Hz), 4.93 (1H, ddd, *J* = 7.3, 7.3, 7.3 Hz), 4.12 (1H, d, *J* = 17.5 Hz), 3.83 (3H, s), 3.74-3.64 (1H, m), 3.36-3.25 (2H, m), 3.19 (1H, dd, *J* = 14.2, 4.9 Hz), 2.58 (1H, dd, *J* = 14.2, 8.7 Hz), 1.96-1.86 (2H, s), 1.53 (9H, s), 0.94 (3H, dd, *J* = 7.5, 7.5 Hz). This was employed for the next step without further purification.

1.0 M hydrogen chloride solution in acetic acid (1.1 mL) was added to the

above-mentioned product (106 mg), the mixture was stirred at room temperature for 4 hours. Then, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 1 / 1), then the precipitate was collected by filtration while being washed with diethyl ether to give **34c** (78.0 mg, 82.1%). ^{1}H NMR (DMSO-*d*6) δ: 12.71-12.59 (1H, br), 9.36 (1H, d, *J* = 7.1 Hz), 8.20 (1H, s), 7.69 (1H, d, *J* = 3.9 Hz), 7.33 (1H, d, *J* = 2.6 Hz), 7.27 (1H, dd, *J* = 8.8, 2.6 Hz), 7.01 (1H, d, *J* = 8.8 Hz), 6.54 (1H, s), 4.85 (1H, ddd, *J* = 7.1, 7.1, 7.1 Hz), 4.78 (1H, d, *J* = 17.1 Hz), 4.52 (1H, d, *J* = 17.1 Hz), 3.91-3.81 (1H, m), 3.79 (3H, s) 3.15 (1H, dd, *J* = 12.5, 12.5 Hz), 3.07-2.92 (2H, m), 2.68-2.57 (1H, m), 1.89-1.75 (2H, m), 0.86 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 478 $(M+H)^+$; HRMS (FAB): calcd for $C_{22}H_{24}ClN_3NaO_7^+$ 500.1195 $(M+Na)^+$, found 500.1190; $[\alpha]^{20}_D$ –92.3 (c = 0.1, methanol).

(6*R*,1'*R*)-4-[[[(*R*)-1'-(4"-Carboxylthiophene-2"-yl)propyl]amino]carbonyl]-(*R*)-6-[(5""-chloro-2""-methoxy)benzyl]-1,4-diazepan-2,5-dione (34d)

Diazepane **34d** was prepared from **58d** in a similar manner to **34c**. ^{1}H NMR (DMSO-*d*6) δ: 12.80-12.65 (1H, br), 9.42 (1H, d, *J* = 7.1 Hz), 8.09 (1H, s), 7.69 (1H, d, *J* = 3.5 Hz), 7.33 (1H, d, *J* = 2.6 Hz), 7.30-7.24 (2H, m), 7.01 (1H, d, *J* = 8.8 Hz), 4.97 (1H, ddd, *J* = 7.1, 7.1, 7.1 Hz), 4.77 (1H, d, *J* = 17.1 Hz), 4.53 (1H, d, *J* = 17.1 Hz), 3.93-3.84 (1H, m), 3.79 (3H, s) 3.15 (1H, dd, *J* = 12.5, 12.5 Hz), 3.07-2.94 (2H, m), 2.68-2.58 (1H, m), 1.94-1.85 (2H, m), 0.89 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 494 $(M+H)^+$; HRMS (FAB): calcd for $C_{22}H_{24}ClN_3NaO_6S^+$ 516.0967 $(M+Na)^+$, found 516.0092; $[\alpha]^{20}_D$ –74.3 (c = 0.1, methanol).



Scheme 17 Synthesis of **34e**

(*R*)-6-[(5”-Chloro-2”-methoxy)benzyl]-4-[(2’-chlorophenoxy)carbonyl]-1,4-diazepan-2,5-dione [(*R*)-59b]

Anisole (2.3 mL) and trifluoroacetic acid (10 mL) were added to (*R*)-**23b** (1.29 g), and the mixture was stirred at room temperature for 2 hours. Then, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1 to 1 / 99). The solid was tritulated with diethyl ether, and collected by filtration while being washed with diethyl ether to give (*R*)-**59b** (595 mg, 65.1%). ^{1}H NMR ($CDCl_3$) δ: 7.91 (1H, d, *J* = 4.5 Hz), 7.63-7.60 (1H, m), 7.45-7.41 (2H, m), 7.39-7.32 (2H, m), 7.28 (1H, dd, *J* = 2.6, 8.9 Hz), 7.03 (1H, d, *J* = 8.9 Hz), 4.83 (1H, d, *J* = 17.5 Hz), 4.50 (1H, d, *J* = 17.5 Hz), 3.89-3.83 (1H, m), 3.81 (3H, s), 3.30-3.22 (1H, m), 3.13-2.96 (2H, m), 2.63 (1H, dd, *J* = 8.9, 14.2 Hz); MS (ESI): 438 $(M+H)^+$. This was employed for the next step without further purification.

(6*R*,1’*R*)-6-[(5””-Chloro-2””-methoxy)benzyl]-4-[[[1’-[4”-[(2”’,2”’,2”’-trichloroethoxy)calbonyl]oxazole-2”-yl]propyl]amino]carbonyl]-1,4-diazepan-2,5-dione

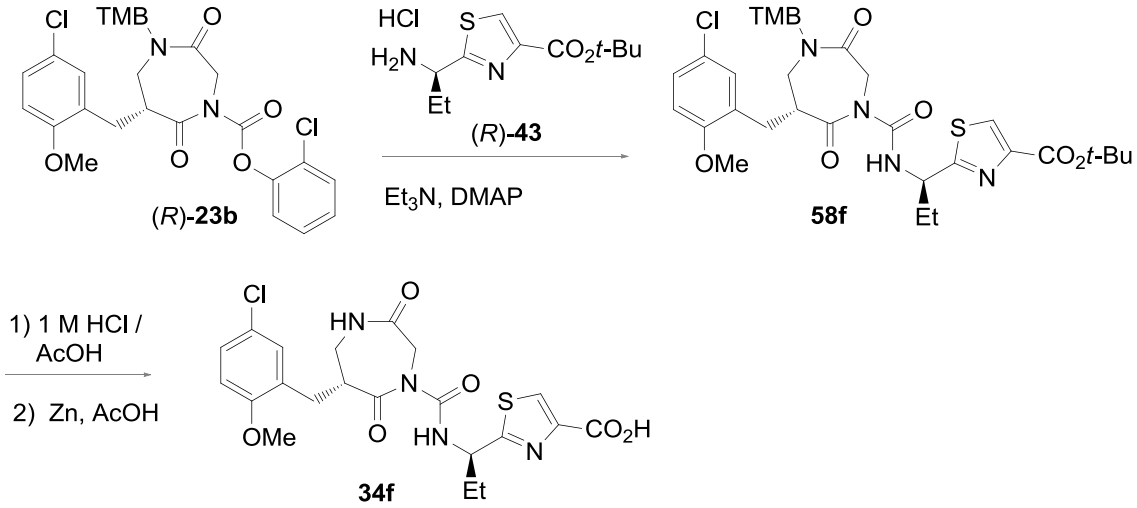
(58e)

To a solution of (*R*)-**59b** (189 mg) in *N*,*N*-dimethylformamide (430 μL), were added (*R*)-**56** (109 mg) and 4-dimethylaminopyridine (53 mg) under ice cooling, and the mixture was stirred at that temperature for 15 hours. Then, water was added to the reaction mixture, and the resulting mixture was extracted with ethyl acetate. Then, the organic layer was successively washed with saturated potassium hydrogen sulfate aqueous solution, saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by preparative TLC (developed by chloroform / ethyl acetate / methanol = 10 / 10 / 1) to give **58e** (97.0 mg, 36.8%). tR in HPLC = 4.1 min (analytical column: YMC-Pack Pro C18; mobile phase: gradient solvent system of acetonitrile / water / trifluoroacetic acid = 5 / 95 / 0.1 (solvent A)- acetonitrile / trifluoroacetic acid = 100 / 0.09 (solvent B); gradient condition: solvent A / solvent B = 100 / 0 to 10 / 90 (0-3.5 min), 10 / 90 to 2 / 98 (3.5-4.0 min), 2 / 98 (4.0-4.9 min); flow rate: 1.0 mL/min). MS (ESI): 611 $(M+H)^+$; $[\alpha]^{20}_D$ –63.8 (c = 0.1, methanol). This was employed for the next step without further purification.

(6*R*,1'*R*)-4-[[[1'-(4"-Carboxyloxazole-2"-yl)propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione (34e)

To a solution of **58e** (97.0 mg) in acetic acid (2.0 mL) was added zinc (200 mg), and the mixture was stirred at room temperature for 3 hours. Then, insoluble materials removed by filtration while being washed with ethyl acetate, and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC (developed by chloroform / ethyl acetate / methanol / acetic acid = 5 / 5 / 1 / 0.1) to give **34e** (7.0 mg, 9.2%). ^{1}H NMR ($CDCl_3$) δ: 9.53 (1 H, d, *J* = 6.9 Hz), 7.69 (1H, s), 7.34 (1H, s), 7.27 (1H, d, *J* = 8.5 Hz), 7.01 (1H, d, *J* = 8.5 Hz), 4.99-4.92 (1H, m), 4.76 (1H, d, *J* = 17.0 Hz), 4.56 (1H, d, *J* = 17.0 Hz), 3.92-3.84 (1H, m), 3.79

(3H, s), 3.50-3.36 (1H, m), 3.25-3.10 (1H, m), 3.04-2.92 (2H, m), 2.69-2.60 (1H, m), 1.99-1.80 (2H, m), 0.90-0.81 (3H, m); MS (ESI): 479 $(M+H)^+$; HRMS (FAB): calcd for $C_{21}H_{23}ClN_4NaO_7^+$ 501.1147 $(M+Na)^+$, found 501.1133; $[\alpha]^{20}_D$ –52.8 (c = 0.1, methanol)..



Scheme 18 Synthesis of **34f**

(6*R*,1"*R*)-6-[(5""""-Chloro-2""""-methoxy)benzyl]-4-[[[1"-[4"'-[(2"",2"",2""-trichloroethoxy)calbonyl]thiazole-2-yl]propyl]amino]carbonyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione (58f)

Diazepane **59f** was prepared from (*R*)-**23b** and (*R*)-**43** in a similar manner to **58a**. ^{1}H NMR ($CDCl_3$) δ: 9.68 (1H, d, *J* = 7.3 Hz), 8.23 (1H, s), 7.19 (1H, dd, *J* = 8.9 Hz, 2.8 Hz), 6.92 (1H, d, *J* = 2.8 Hz), 6.76 (1H, d, *J* = 8.9 Hz), 6.07 (2H, s), 5.28 (1H, d, *J* = 17.5 Hz), 5.27-5.19 (1H, m), 5.01-4.95 (2H, m), 4.78 (1H, d, *J* = 13.8 Hz), 4.34 (1H, d, *J* = 13.8 Hz), 4.29 (1H, d, *J* = 17.5 Hz), 3.84 (3H, s), 3.78 (3H, s), 3.71 (6H, s), 3.61-3.51 (1H, m), 3.12 (1H, dd, *J* = 13.6, 4.7 Hz), 3.07-3.02 (2H, m), 2.42 (1H, dd, *J* = 13.6, 9.1 Hz), 2.28-2.16 (2H, m), 1.01 (3H, dd, *J* = 7.5, 7.5 Hz); MS (ESI): 807 $(M+H)^+$. This was employed for the next step without further purification.

(6*R*,1'*R*)-4-[[[1'-(4"-Carboxylthiazole-2"-yl)propyl]amino]carbonyl]6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione (34f)

1.0 M hydrogen chloride solution in acetic acid (2.0 mL) and anisole (89 μL) were added to **58f** (46.0 mg), and the resulting mixture was stirred at room temperature for 40 hours. Then, the mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (chloroform / ethyl acetate / methanol = 15 / 15 / 1) to give a product. ^{1}H NMR ($CDCl_3$) δ: 9.70 (1H, d, *J* = 7.3 Hz), 8.23 (1H, s), 7.22 (1H, dd, *J* = 8.9, 2.4 Hz), 7.13 (1H, d, *J* = 2.4 Hz), 6.81 (1H, d, *J* = 8.9 Hz), 5.72-5.69 (1H, m), 5.35 (1H, d, *J* = 17.5 Hz), 5.23 (1H, ddd, *J* = 7.3, 7.3, 7.3 Hz), 4.99 (2H, s), 4.15 (1H, d, *J* = 17.5 Hz), 3.84 (3H, s), 3.76-3.67 (1H, m), 3.38-3.31 (2H, m), 3.21 (1H, dd, *J* = 13.8, 5.7 Hz), 2.63 (1H, dd, *J* = 13.8, 8.5 Hz), 2.28-2.16 (2H, m), 1.04 (3H, dd, *J* = 7.3, 7.3 Hz). This was employed for the next step without further purification.

To a solution of the above-mentioned product (42.0 mg) in acetic acid (800 μL), was added zinc (80 mg), and the mixture was stirred at room temperature for 25 hours. Then, insoluble materials were removed by filtration while being washed with ethyl acetate, and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC (developed by chloroform / ethyl acetate / methanol = 10 / 10 / 1) to give **34f** (14.0 mg, 49.6%). ^{1}H NMR ($CDCl_3$) δ: 9.59 (1H, d, *J* = 6.9 Hz), 7.71 (1H, s), 7.40-7.31 (1H, m), 7.28 (1H, dd, *J* = 2.4, 8.5 Hz), 7.01 (1H, d, *J* = 8.5 Hz), 5.19-5.04 (1H, m), 4.80-4.67 (1H, m), 4.67-4.50 (1H, m), 3.94-3.84 (1H, m), 3.80 (3H, s), 3.48-3.37 (1H, m), 3.23-3.15 (1H, m), 3.03-2.95 (2H, m), 2.74-2.62 (1H, m), 2.08-1.91 (2H, m), 0.94-0.89 (3H, m); MS (ESI): 495 $(M+H)^+$; HRMS (FAB): calcd for $C_{21}H_{23}ClN_4NaO_6S^+$ 517.0919 $(M+Na)^+$, found 517.0928; $[\alpha]^{20}_D$ –45.8 (c = 0.1, methanol),

(*R*)-2-[1'-[6"-[(5"'-Chloro-2"'-methoxy)benzyl]-3',7'-dioxo-1',4'-diazepan]carboxamido]butanoic acid (45)

To a solution of **23b** (3.00 g) in *N*,*N*-dimethylformamide (75 mL) were added *tert*-butyl (*R*)-2-aminobutanoate (700 mg) and triethylamine (450 μL) under ice cooling, and the mixture was stirred at that temperature for 3 hours. Then, water was added to the reaction mixture, and the resulting mixture was extracted with a mixture of hexane and ethyl acetate (1 / 1). Then, the organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1 to 1 / 99) to give a product (1.20 g, 38.1%). ^{1}H NMR ($CDCl_3$) δ: 9.50 (0.5H, d, *J* = 7.1 Hz), 9.47 (0.5H, d, *J* = 7.1 Hz), 7.19-7.13 (1H, m, M18), 6.91 (0.5H, d, *J* = 2.5 Hz), 6.88 (0.5H, d, *J* = 2.5 Hz), 6.74 (0.5H, d, *J* = 8.6 Hz), 6.73 (0.5H, d, *J* = 8.6 Hz), 5.35 (0.5H, d, *J* = 17.3 Hz), 5.30 (0.5H, d, *J* = 17.3 Hz), 4.77 (1H, dd, *J* = 13.7, 7.6 Hz), 4.44-4.35 (1H, m), 4.35-4.25 (1H, m), 3.69 (1.5H, s), 3.68 (1.5H, s), 3.58-3.45 (1H, m), 3.18-3.08 (1H, m, M09), 3.05-2.94 (2H, m, M08), 2.44-2.32 (1H, m, M07), 1.94-1.82 (1H, m, M06), 1.82-1.70 (1H, m), 1.51-1.41 (9H, m), 0.92 (3H, dd, *J* = 7.4, 7.4 Hz). This was employed for the next step without further purification.

To a solution of the above-mentioned product (1.20 g) in methylene chloride (15 mL) cooled at 0 °C was added trifluoroacetic acid (15 mL), and the mixture was stirred at room temperature for 7.5 hours. Then, the reaction mixture was concentrated *in vacuo*, and the residue was purified with Florisil (chloroform / methanol = 5 / 1) to give a reddish brown product. The product was diluted with ethyl acetate. Then, the solution was washed with water, and extracted with saturated sodium hydrogen carbonate aqueous solution. The sodium hydrogen carbonate aqueous layer was acidified with hydrochloric acid to pH 4, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give **45** (638 mg, 58.0%). ^{1}H

NMR ($CDCl_3$) δ: 13.15-12.59 (1H, m), 9.40 (0.5H, d, *J* = 7.1 Hz), 9.36 (0.5H, d, *J* = 7.1 Hz), 7.70-7.62 (1H, m), 7.32-7.29 (1H, m), 7.24 (1H, dd, *J* = 8.9, 2.8 Hz), 6.98 (1H, d, *J* = 8.9 Hz), 4.78 (0.5H, d, *J* = 17.3 Hz), 4.77 (0.5H, d, *J* = 17.3 Hz), 4.51 (1H, d, *J* = 17.3 Hz), 4.27-4.16 (1H, m), 3.91-3.81 (1H, m), 3.77 (3H, s), 3.16-3.05 (1H, m), 3.05-2.90 (2H, m), 2.68-2.57 (1H, m), 1.85-1.63 (2H, m), 0.87-0.78 (3H, m). ^{1}H NMR spectrum of **45** was assigned as mixture of two diastereomers. If each signal of correspond protons was separated, the number of proton was assigned as 0.5. NMR spectra of following diastereomers were assigned in the same manner. MS (ESI): 412 $(M+H)^+$. This was employed for the next step without further purification.

(2*R*,6"*R*)-2-[1'-[6"-[(5""-Chloro-2""-methoxy)benzyl]-3',7'-dioxo-1',4'-diazepan]carboxamido]butanoic acid [(*R*)-45]

Acid (*R*)-**45** was prepared from (*R*)-**23b** in a similar manner to **45**. ^{1}H NMR (DMSO-*d*6) δ: 13.13-12.81 (1H, m), 9.40 (1H, d, *J* = 7.3 Hz), 7.75-7.69 (1H, m), 7.35 (1H, d, *J* = 2.4 Hz), 7.28 (1H, dd, *J* = 8.9, 2.4 Hz), 7.01 (1H, d, *J* = 8.9 Hz), 4.81 (1H, d, *J* = 17.5 Hz), 4.55 (1H, d, *J* = 17.5 Hz), 4.28-4.21 (1H, m), 3.95-3.84 (1H, m), 3.80 (3H, s), 3.14 (1H, dd, *J* = 12.8, 12.8 Hz), 3.06-2.93 (2H, m), 2.69-2.59 (1H, m), 1.87-1.68 (2H, m), 0.87 (1H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 412 $(M+H)^+$. This was employed for the next step without further purification.

(1"*R*)-4-[[[[1"-[(3""-Carboxylphenyl)amino]-1"-oxo]2'-butyl]amino]carbonyl]-6-[(5""-Chloro-2""-methoxy)benzyl]-1,4-diazepan-2,5-dione (44a)

To a solution of **45** (50.2 mg) in methylene chloride (3.0 mL) were added *tret*-butyl 2-aminobenzoate (47.1 mg), triethylamine (250 μL) and 25% solution of *n*-propyl phosphoric acid anhydride in ethyl acetate (370 μL), and the mixture was stirred at room temperature for 7 hours. The reaction mixture was concentrated *in vacuo*, and ethyl acetate was added to the

residue. The resulted solution was successively washed with water, saturated potassium hydrogen sulfate aqueous solution, brine, saturated sodium hydrogen carbonate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The solid was tritulated with a mixture of diethyl ether and hexane (1 / 1), and collected by filtration while being washed with the solution.

1 M hydrogen chloride solution in acetic acid (3.0 mL) was added to the above-mentioned solid, and the mixture was stirred at room temperature for 3 hours. The reaction mixture was concentrated *in vacuo*, and ethyl acetate was added to the residue. An insoluble substance was filtered out while being washed with ethyl acetate, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 1 / 1), then the precipitates were collected by filtration while being washed with diethyl ether to give **44a** (52.3 mg, 80.8%). ^{1}H NMR (DMSO-*d*6) δ: 13.10-12.47 (1H, br), 10.35 (0.5H, s), 10.33 (0.5H, s), 9.48 (0.5H, d, *J* = 7.2 Hz), 9.43 (0.5H, d, *J* = 7.3 Hz), 8.22-8.20 (1H, m), 7.78 (1H, d, *J* = 7.9 Hz), 7.65 (1H, s), 7.61 (1H, d, *J* = 7.9 Hz), 7.41 (1H, dd, *J* = 7.9, 7.9 Hz), 7.31 (1H, s), 7.24 (0.5H, d, 8.8Hz), 7.23 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 4.78 (0.5H, d, *J* = 17.2 Hz), 4.77 (0.5H, d, *J* = 17.2 Hz), 4.51 (1H, d, *J* = 17.2 Hz), 4.49-4.39 (1H, m), 3.90-3.81 (1H, m), 3.77 (3H, s), 3.18-3.07 (1H, m), 3.05-2.91 (2H, m), 2.69-2.60 (1H, m), 1.89-1.67 (2H, m), 0.92-0.80 (3H, m); MS (ESI): 531 $(M+H)^+$; HRMS (FAB): calcd for $C_{25}H_{27}ClN_4NaO_7^+$ 553.1460 $(M+Na)^+$, found 553.1480.

(1”*R*)-4-[[[[1”-[(4”’-Carboxylphenyl)amino]-1”-oxo]2’-butyl]amino]carbonyl]-6-[(5”’-chloro-2””-methoxy)benzyl]-1,4-diazepan-2,5-dione (44b)

Diazepane **44b** was prepared in a similar manner to **44a**. ^{1}H NMR (DMSO-*d*6) δ: 12.88-12.35 (1H, br), 10.47 (0.5H, s), 10.45 (0.5H, s), 9.47 (0.5H, d, *J* = 7.2 Hz), 9.43 (0.5H, d,

J = 7.3 Hz), 7.87 (2H, d, *J* = 8.6 Hz), 7.68 (2H, d, *J* = 8.6 Hz), 7.70-7.64 (1H, m), 7.31 (1H, s), 7.248 (0.5H, d, *J* = 8.8 Hz), 7.242 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 4.77 (1H, d, *J* = 17.1 Hz), 4.51 (1H, d, *J* = 17.1 Hz), 4.48-4.40 (1H, m), 3.92-3.81 (1H, m), 3.77 (3H, s), 3.18-3.08 (1H, m), 3.05-2.92 (2H, m), 2.65-2.59 (1H, m), 1.85-1.65 (2H, m), 0.90-0.85 (3H, m); MS (ESI): 531 $(M+H)^+$; HRMS (FAB): calcd for $C_{25}H_{27}ClN_4NaO_7^+$ 553.1460 $(M+Na)^+$, found 553.1442.

(1”*R*)-4-[[[[1”-[(3”’-Aminophenyl)amino]-1”-oxo]2’-butyl]amino]carbonyl]-6-[(5””-chloro-2””-methoxy)benzyl]-1,4-diazepan-2,5-dione (44c)

^{1}H NMR (DMSO-*d*6) δ: 9.83 (0.5H, s), 9.81 (0.5H, s), 9.45 (0.5H, d, *J* = 7.4 Hz), 9.41 (0.5H, d, *J* = 7.4 Hz), 7.65 (1H, d, *J* = 2.9 Hz), 7.316 (0.5H, s), 7.311 (0.5H, s), 7.24 (0.5H, d, *J* = 8.8 Hz), 7.23 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 6.91-6.87 (2H, m), 6.64 (0.5H, d, *J* = 7.8 Hz), 6.23 (0.5H, d, *J* = 7.8 Hz), 5.02 (2H, s), 4.78 (0.5H, d, *J* = 17.1 Hz), 4.77 (0.5H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz), 4.45-4.37 (1H, m), 3.92-3.81 (1H, m), 3.77 (3H, s), 3.19-3.08 (1H, m), 3.07-2.91 (2H, m), 2.68-2.60 (1H, m), 1.82-1.65 (2H, m), 0.89-0.82 (3H, m); MS (ESI): 502 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{28}ClN_5NaO_5^+$ 524.1671 $(M+Na)^+$, found 524.1632.

(1”*R*)-4-[[[[1”-[(4”’-Aminophenyl)amino]-1”-oxo]2’-butyl]amino]carbonyl]-6-[(5””-chloro-2””-methoxy)benzyl]-1,4-diazepan-2,5-dione (44d)

^{1}H NMR (DMSO-*d*6) δ: 9.72 (0.5H, s), 9.70 (0.5H, s), 9.43 (0.5H, d, *J* = 7.4 Hz), 9.39 (0.5H, d, *J* = 7.5 Hz), 7.65 (1H, d, *J* = 3.2 Hz), 7.31 (0.5H, s), 7.30 (0.5H, s), 7.24 (0.5H, d, *J* = 8.9 Hz), 7.23 (0.5H, d, *J* = 8.9 Hz), 7.17 (2H, d, *J* = 8.6 Hz), 6.98 (1H, d, *J* = 8.9 Hz), 6.48 (1H, d, *J* = 8.6 Hz), 6.46 (1H, d, *J* = 8.6 Hz), 4.847 (1H, s), 4.842 (1H, s), 4.79 (0.5H, d, *J* = 17.1 Hz),

4.77 (0.5H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz), 4.39-4.32 (1H, m), 3.91-3.81 (1H, m), 3.77 (3H, s), 3.18-3.07 (1H, m), 3.03-2.92 (2H, m), 2.68-2.60 (1H, m), 1.80-1.62 (2H, m), 0.89-0.81 (3H, m); MS (ESI): 502 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{28}ClN_5NaO_5^+$ 524.1671 $(M+Na)^+$, found 524.1688.

(1”*R*)-4-[[[[1”-[[(4”’-Amino-3”’-carboxyl)phenyl]amino]-1”-oxo]2’-butyl]amino]carbonyl]-6-[(5””-Chloro-2””-methoxy)benzyl]-1,4-diazepan-2,5-dione (44e)

^{1}H NMR (DMSO-*d*6) δ: 9.92 (0.5H, s), 9.89 (0.5H, s), 9.45 (0.5H, d, *J* = 7.4 Hz), 9.40 (0.5H, d, *J* = 7.4 Hz), 7.95-7.92 (1H, m), 7.66 (0.5H, s), 7.61 (0.5H, s), 7.427 (0.5H, d, *J* = 8.7 Hz), 7.421 (0.5H, d, *J* = 8.7 Hz), 7.31 (1H, s), 7.24 (0.5H, d, *J* = 8.8 Hz), 7.23 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 6.71 (1H, d, *J* = 8.7 Hz), 4.78 (0.5H, d, *J* = 17.1 Hz), 4.77 (0.5H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz), 4.39-4.32 (1H, m), 3.91-3.79 (1H, m), 3.77 (3H, s), 3.17-3.07 (1H, m), 3.03-2.92 (2H, m), 2.68-2.60 (1H, m), 1.83-1.65 (2H, m), 0.89-0.81 (3H, m); MS (ESI): 546 $(M+H)^+$; HRMS (FAB): calcd for $C_{25}H_{28}ClN_5NaO_7^+$ 568.1569 $(M+Na)^+$, found 568.1561.

(1”*R*)-4-[[[[1”-[[(5”’-Amino-3”’-carboxyl)phenyl]amino]-1”-oxo]2’-butyl]amino]carbonyl]-6-[(5””-Chloro-2””-methoxy)benzyl]-1,4-diazepan-2,5-dione (44f)

^{1}H NMR (DMSO-*d*6) δ: 12.73-11.90 (1H, br), 10.10 (0.5H, s), 10.00 (0.5H, s), 9.46 (0.5H, d, *J* = 7.3 Hz), 9.42 (0.5H, d, *J* = 7.4 Hz), 7.65 (1H, d, *J* = 3.2 Hz), 7.32-7.22 (3H, m), 7.10 (0.5H, s), 7.09 (0.5H, s), 6.99 (0.5H, s), 6.97 (0.5H, s), 6.87 (0.5H, s), 6.86 (0.5H, s), 5.32 (2H, br), 4.78 (1H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz), 4.42-4.38 (1H, m), 3.90-3.82 (1H, m), 3.77 (3H, s), 3.17-3.07 (1H, m), 3.04-2.93 (2H, m), 2.67-2.62 (1H, m), 1.85-1.60 (2H, m), 0.88-0.81 (3H, m); MS (ESI): 546 $(M+H)^+$; HRMS (FAB): calcd for $C_{25}H_{28}ClN_5NaO_7^+$

568.1569 $(M+Na)^+$, found 568.1544.

(1''*R*)-4-[[[[1''-[(5'''-Carboxyl-1'''*H*-pyrrol-3'''-yl)amino]-1''-oxo]2'-butyl]amino]carbonyl]-6-[(5''''-chloro-2''''-methoxy)benzyl]-1,4-diazepan-2,5-dione (44g)

^{1}H NMR (DMSO-*d*6) δ: 12.30-12.10 (1H, br), 11.46 (1H, s), 10.10 (0.5H, s), 10.07 (0.5H, s), 9.44 (0.5H, d, *J* = 7.4 Hz), 9.39 (0.5H, d, *J* = 7.4 Hz), 7.659 (0.5H, s), 7.651 (0.5H, s), 7.315 (0.5H, s), 7.310 (0.5H, s), 7.24 (0.5H, d, *J* = 8.9 Hz), 7.23 (0.5H, d, *J* = 8.9 Hz), 7.15 (1H, s), 6.98 (1H, d, *J* = 8.9 Hz), 6.64-6.58 (1H, m), 4.78 (0.5H, d, *J* = 17.1 Hz), 4.76 (0.5H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz) 4.37-4.29 (1H, m), 3.91-3.80 (1H, m), 3.77 (3H, s), 3.12 (0.5H, dd, *J* = 13.2, 13.2 Hz), 3.11 (0.5H, dd, *J* = 12.8, 12.8 Hz), 3.03-2.92 (2H, m), 2.66-2.51 (1H, m), 1.80-1.62 (2H, m), 0.86-0.80 (3H, m); MS (ESI): 520 $(M+H)^+$; HRMS (FAB): calcd for $C_{23}H_{26}ClN_5NaO_7^+$ 542.1413 $(M+Na)^+$, found 542.1384.

(1''*R*)-6-[(5''''-Chloro-2''''-methoxy)benzyl]-4-[[[[1''-[(2'''*H*-tetrazol-5'''-yl)amino]-1''-oxo]2'-butyl]amino]carbonyl]--1,4-diazepan-2,5-dione (44h)

To a solution of **45** (52.1 mg) in methylene chloride (3.0 mL) were added 5-amino-1*H*-tetrazole monohydrate (130 mg), triethylamine (1.3 mL) and 25% solution of *n*-propyl phosphoric acid anhydride in acetate (1.9 mL), and the mixture was stirred at room temperature for 7 days. The reaction mixture was concentrated *in vacuo*, and the residue was diluted with ethyl acetate. The solution was washed with water, and extracted with saturated sodium hydrogen carbonate aqueous solution. Then, the aqueous solution was acidified with 6.0 M hydrochloric acid to pH 4, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and hexane was added to

the solution (ethyl acetate / hexane = 4 / 1), then the precipitate was collected by filtration while being washed with diethyl ether to give **44h** (11.7 mg, 19.3%). ^{1}H NMR (DMSO-*d*6) δ: 15.90 (1H, br), 12.10 (1H, br), 9.52-9.47 (1H, m), 7.70-7.64 (1H, m), 7.31 (1H, s), 7.249 (0.5H, d, *J* = 8.8 Hz), 7.243 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 4.75 (1H, d, *J* = 17.2 Hz), 4.56-4.46 (1H, m), 4.52 (1H, d, *J* = 17.2 Hz), 3.94-3.84 (1H, m), 3.77 (3H, s), 3.18-2.90 (3H, m), 2.68-2.58 (1H, m), 1.89-1.63 (2H, m), 0.90-0.75 (3H, m); MS (ESI): 479 $(M+H)^+$; HRMS (FAB): calcd for $C_{19}H_{23}ClN_8NaO_5^+$ 501.1372 $(M+Na)^+$, found 501.1397.

(6R,1”*R*)-6-[(5””-Chloro-2””-methoxy)benzyl]-4-[[[[1”-[(2”’*H*-tetrazol-5”’-yl)amino]-1”-oxo]2’-butyl]amino]carbonyl]--1,4-diazepan-2,5-dione [(6*R*,1’*R*)-44h]

Diazepane (6*R*,1’*R*)-**44h** was prepared from (*R*)-**45** in a similar manner to **44h**. ^{1}H NMR ($CDCl_3$) δ: 9.46 (1H, d, *J* = 7.7 Hz), 7.74-7.68 (1H, m), 7.34 (1H, s), 7.27 (1H, d, *J* = 8.5 Hz), 7.01 (1H, d, *J* = 8.5 Hz), 4.80 (1H, d, *J* = 17.5 Hz), 4.57-4.45 (2H, m), 3.93-3.85 (1H, m), 3.80 (3H, s), 3.20-3.09 (1H, m, *J* = 14.2 Hz), 3.06-2.91 (2H, m), 2.69-2.60 (1H, m), 1.87-1.66 (2H, m, *J* = 7.3 Hz), 0.89 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 479 $(M+H)^+$; HRMS (FAB): calcd for $C_{19}H_{23}ClN_8NaO_5^+$ 501.1372 $(M+Na)^+$, found 501.1379; $[\alpha]^{20}_D$ –45.0 (c = 0.1, MeOH).

(*S*)-*tert*-Butyl 3-(1’-hydroxypropyl)benzoate [(*S*)-27a]

To a solution of **26a** (3.00 g) in methylene chloride (30 mL) were added (*S*)-(+)-mandelic acid (1.10 g), 1.01 M solution of diethylzinc in hexane (44 mL) and titanium tetraisopropoxide (6.0 mL) at 0 °C, and the mixture was stirred at room temperature for 16 hours. Then, 1.0 M hydrochloric acid was slowly added to the reaction mixture at 0 °C, and the precipitate was removed by filtration. The filtrate was extracted with ethyl acetate. The organic layer was successively washed with saturated sodium hydrogen carbonate aqueous solution, water,

saturated potassium hydrogen sulfate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give (*S*)-**27a** (3.20 g, 93.0%, 70.0% ee). The enantiomeric excess was determined by HPLC on chiral stationary phase (analytical column: CHIRALCEL AD-H of Daicel Chemical Industries; mobile phase: hexane / ethanol = 8/2, flow rate: 1.0 mL / min, minor peak: t_R = 6.4 min, major peak: t_R = 7.0 min, peak area ratio: major peak / minor peak = 85.0 / 15.0). ^{1}H NMR ($CDCl_3$) δ: 8.08 (1H, s), 8.03 (1H, d, *J* = 7.7 Hz), 7.62 (1H, d, *J* = 7.7 Hz), 7.47 (1H, dd, *J* = 7.7, 7.7 Hz), 4.73-4.69 (1H, m), 1.90-1.75 (2H, m), 1.29-1.27 (9H, m), 0.94 (3H, dd, *J* = 7.5, 7.5 Hz); $[\alpha]^{20}_D$ –30.7 (c = 0.1, methanol). This was employed for the next step without further purification.

(*R*)-*tert*-Butyl 3-(1'-aminopropyl)benzoate L-tartrate [(*R*)-29a]

To a solution of (*S*)-**27a** (3.20 g) in tetrahydrofuran (80 mL) were added phthalimide (3.30 g), triphenylphosphine (5.80 g) and diethyl azodicarboxylate (3.5 mL) at 0 °C, and the mixture was stirred at room temperature for 2 hours. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane / ethyl acetate = 5 / 1) to give a product (3.20 g, 64.6%). ^{1}H NMR ($CDCl_3$) δ: 8.11 (1H, s), 7.89 (1H, d, *J* = 7.5 Hz), 7.77-7.85 (2H, m), 7.76-7.62 (3H, m), 7.38 (1H, dd, *J* = 7.5, 7.5 Hz), 5.30 (1H, dd, *J* = 9.7, 6.6 Hz), 2.66-2.50 (1H, m), 2.42-2.28 (1H, m), 1.59 (9H, s), 0.98 (3H, dd, *J* = 7.4, 7.4 Hz). This was employed for the next step without further purification.

To a solution of the above-mentioned product (3.20 g) in methanol (25 mL) was added hydrazine monohydrate (1.6 mL), and the mixture was stirred under heating at reflux for 2 hours. The precipitate was filtered out while being washed with methanol, and the filtrate was concentrated *in vacuo*. The residue was diluted with ethyl acetate, and the solution was successively washed with water and brine. The organic layer was dried over anhydrous sodium

sulfate, and concentrated *in vacuo*.

The residue (2.00 g) was diluted with methanol (10 mL), and L-tartaric acid (1.30 g) was added to the solution, and the mixture was concentrated *in vacuo*. The residue was recrystallized from ethyl acetate / ethanol (2 / 1) to give (*R*)-**29a** (1.20 g, 35.5%, 98.0% ee).

The enantiomeric excess was determined by HPLC on chiral stationary phase in the same analytical condition to that in (*R*)-**38** (major peak: t_R = 7.3 min, minor peak: t_R = 8.4 min, peak area ratio: major peak / minor peak = 99.0 / 1.0). ^{1}H NMR (DMSO-*d*6) δ: 8.05 (1H, s), 8.01 (1H, d, *J* = 7.7 Hz), 7.65 (1H, d, *J* = 7.7 Hz), 7.57 (1H, dd, *J* = 7.7, 7.7 Hz), 4.38 (2H, s), 4.26 (1H, dd, *J* = 9.1, 6.0 Hz), 2.12-1.92 (2H, m), 1.61 (9H, s), 0.90 (3H, dd, *J* = 7.3, 7.3 Hz); MS (ESI): 219 $(M\text{-}NH_2)^+$; $[\alpha]^{20}_D$ +5.4 (c = 0.1, methanol); melting point: 146-147 °C. This was employed for the next step without further purification.

(*S*)-*tert*-Butyl 3-(1'-aminopropyl)benzoate D-tartrate [(*S*)-29a]

To a solution of **26a** (2.90 g) in methylene chloride (30 mL) were added (*R*)-(+)-phenyllactic acid[51] (1.00 g), titanium tetraisopropoxide (5.8 mL) and 1.01 M solution of diethylzinc in hexane (42 mL) at 0 °C, and the mixture was stirred at room temperature for 16 hours. 1.0 M hydrochloric acid aqueous solution was added to the reaction mixture at 0 °C, and the precipitate was filtered out while being washed with methylene chloride. The filtrate was extracted with ethyl acetate. The organic layer was successively washed with saturated sodium hydrogen carbonate aqueous solution, water, saturated potassium hydrogen sulfate aqueous solution and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

Then, (*S*)-**29** (520 mg, 9.6%, 98.0% ee) was prepared from the above-mentioned product (3.10 g) in a similar manner to (*R*)-**29**. The enantiomeric excess was determined by HPLC on chiral stationary phase in the same analytical condition to that in (*R*)-**38** (minor peak: t_R = 7.4

min, major peak: t_R = 8.4 min, peak area ratio: major peak / minor peak = 99.0 / 1.0). (*S*)-**26** showed a ^{1}H NMR spectrum identical to (*R*)-**29**. MS (ESI): 219 $(M\text{-}NH_2)^+$. This was employed for the next step without further purification.

(6*R*,1"*R*)-4-[[[1"-[3""-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R*,1'*R*)-33a]

(6*S*,1"*R*)-4-[[[1"-[3""-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*S*,1'*R*)-33a]

To a solution of **23a** (1.30 g) in *N*,*N*-dimethyl formamide (13 mL) were added (*R*)-**29a** (820 mg, 98.0% ee) and triethylamine (0.30 mL) under ice cooling, and the resulting mixture was stirred under ice-cooling for 1 hour. Then, the reaction mixture was diluted with ethyl acetate, and the mixture was successively washed with saturated potassium hydrogen sulfate aqueous solution, water and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1 to 2 / 3) to give (6*R*,1'*R*)-**33a** (300 mg, 20.0%) and (6*S*,1'*R*)-**33a** (400 mg, 26.7%). ^{1}H NMR spectra of (6*R*,1'*R*)-**33a** and (6*S*,1'*R*)-**33a** were identical with those of (6*R**,1'*R**)-**33a** and (6*R**,1'*S**)-**33a**, respectively. These were employed for the next step without further purification.

(6*S*,1"*S*)-4-[[[1"-[3""-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*S*,1'*S*)-33a]

(6*R*,1"*S*)-4-[[[1"-[3""-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R*,1'*S*)-33a]

Diazepanes (6*S*,1'*S*)-**33a** and (6*R*,1'*S*)-**33a** were prepared from **23a** and (*S*)-**29a** (98% ee) in a similar manner to (6*R*,1'*R*)-**33a** and (6*S*,1'*R*)-**33a**. ^{1}H NMR spectra of (6*S*,1'*S*)-**33a** and (6*R*,1'*S*)-**33a** were identical with those of (6*R**,1'*R**)-**33a** and (6*R**,1'*S**)-**33a**, respectively. These were employed for the next step without further purification.

(6*R*,1'*R*)-4-[[[1'-(3"-Carboxylphenyl)propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R*,1'*R*)-25a]

A mixture of (6*R*,1'*R*)-**33a** (415 mg) and 1.0 M hydrogen chloride solution in acetic acid (4.0 mL) was stirred at room temperature for 24 hours. Then, the reaction mixture was concentrated *in vacuo*. The residue was purified by Florisil (tetrahydrofuran) and silica gel column chromatography (chloroform / ethyl acetate / methanol / acetic acid = 8 / 8 / 1/ 0.08) to give (6*R*,1'*R*)-**25a** (131 mg, 46.9%). Diazepane (6*R*,1'*R*)-**25a** showed a ^{1}H NMR spectrum identical to (6*R**,1'*R**)-**25a**. MS (ESI): 488 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{26}ClN_3NaO_6^+$ 510.1402 $(M+Na)^+$, found 510.1393; $[\alpha]^{20}_D$ –60.7 (c = 0.1, methanol).

(6*S*,1'*S*)-4-[[[1'-(3"-Carboxylphenyl)propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*S*,1'*S*)-25a]

Diazepane (6*S*,1'*S*)-**25a** was prepared in a similar manner to (6*R*,1'*R*)-**25a**, and showed a ^{1}H NMR spectrum identical to (6*R**,1'*R**)-**25a**. MS (ESI): 488 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{26}ClN_3NaO_6^+$ 510.1402 $(M+Na)^+$, found 510.1421; $[\alpha]^{20}_D$ +70.5 (c = 0.1, methanol),

(6*S*,1'*R*)-4-[[[1'-(3"-Carboxylphenyl)propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*S*,1'*R*)-25a]

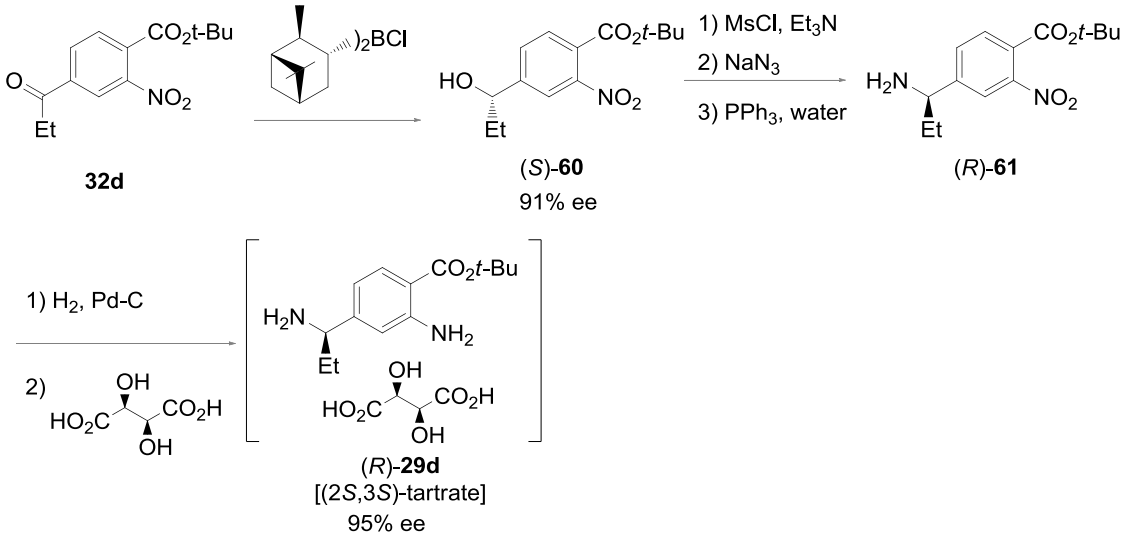
Diazepane (6*S*,1'*R*)-**25a** showed a ^{1}H NMR spectrum identical to (6*R**,1'*S**)-**25a**. MS (ESI): 488 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{26}ClN_3NaO_6^+$ 510.1402 $(M+Na)^+$, found 510.1408; $[\alpha]^{20}_D$ +40.6 (c = 0.1, methanol).

(6*R*,1'*S*)-4-[[[1'-(3"-Carboxylphenyl)propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R*,1'*S*)-25a]

Diazepane (6*R*,1'*S*)-**25a** showed a ^{1}H NMR spectrum identical to (6*R**,1'*S**)-**25a**. MS (ESI): 488 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{26}ClN_3NaO_6^+$ 510.1402 $(M+Na)^+$, found 510.1426; $[\alpha]^{20}_D$ –40.2 (c = 0.1, methanol).

(6*R*,1"*R*)-4-[[[1"-[3"'-(*tert*-Butoxycarbonyl)phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R*,1'*R*)-33a]

To a solution of (*R*)-**23b** (90.0 mg) in *N*,*N*-dimethylformamide (300 μL) was added 4-dimethylaminopyridine (18.0 mg) at 0 °C, and the mixture was stirred at 0 °C for 30 minutes. Then, (*R*)-**29a** (59.0 mg) and triethylamine (50 μL) were added to the mixture, and the resulting mixture was stirred at 0 °C for 8 hours. Then, the reaction mixture was diluted with ethyl acetate. The resulting mixture was successively washed with saturated potassium hydrogen sulfate aqueous solution, water and brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1 to 1 / 2) to give (6*R*,1'*R*)-**33a**. A ^{1}H NMR spectrum of (6*R*,1'*R*)-**33a** was identical with that of (6*R**,1'*R**)-**33a**.



Scheme 19 Synthesis of (*R*)-**29d**

***tert*-Butyl (*S*)-4-(1'-hydroxypropyl)-2-nitrobenzoate [(*S*)-60]**

Ester (*S*)-**60** (91.0% ee) was prepared from **32d** in a similar manner to (*S*)-**36**. The enantiomeric excess was determined by HPLC on chiral stationary phase in the same analytical condition to that in (*S*)-**36** (major peak: t_R = 7.6 min, minor peak: t_R = 8.5 min, peak area ratio: major peak / minor peak = 95.5 / 4.5). ^{1}H NMR ($CDCl_3$) δ: 7.81 (1H, s), 7.71 (1H, d, *J* = 7.9 Hz), 7.6 (1H, d, *J* = 7.9 Hz), 4.79-4.70 (1H, m), 1.85-1.75 (2H, m), 1.56 (9H, s), 1.00-0.90 (3H, m); $[\alpha]^{20}_D$ –13.8 (c = 0.1, methanol). This was employed for the next step without further purification.

***tert*-Butyl (1'*R*)-4-(1'-aminopropyl)-2-nitrobenzoate hydrochloride [(*R*)-61]**

To a solution of (*S*)-**60** (109 g) in tetrahydrofuran (436 mL) were added triethylamine (108 mL) and methanesulfonyl chloride (36 mL) under ice cooling, and the mixture was stirred for 15 minutes. Then, water was added to the reaction mixture, and the resulting mixture was

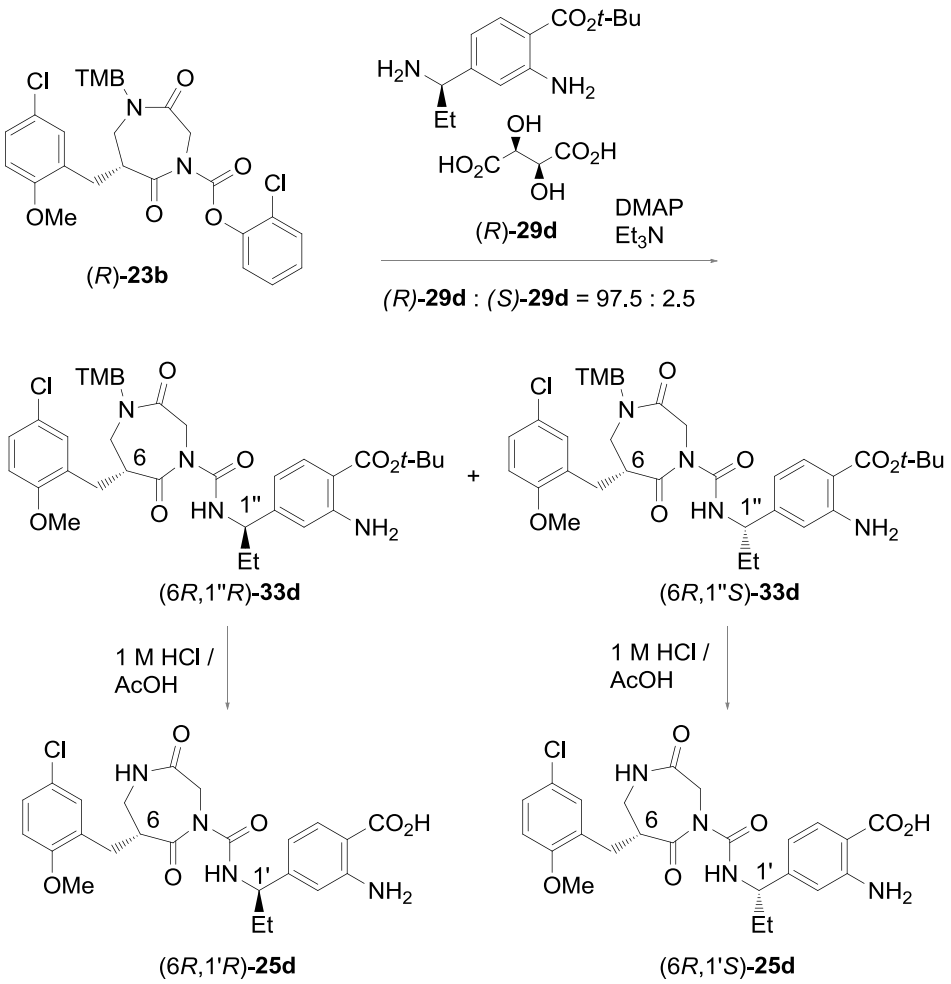
extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*.

To a solution of the residue (137 g) in *N*,*N*-dimethylformamide (685 mL) was added sodium azide (16.3 g) under ice cooling, and the mixture was stirred at room temperature for 1 hour. Then, water was added to the reaction mixture, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated i*n vacuo*.

To a solution of the residue (139 g) in tetrahydrofuran (1.4 L) were added water (70 mL) and triphenylphosphine (119 g) under ice cooling, and the mixture was stirred at 50 °C for 20 hours. Then, the reaction mixture was concentrated *in vacuo*, and toluene and 0.5 M hydrochloric acid were added to the residue, and the layers were separated. Hexane was added to the organic layer, and the resulted organic solution was extracted with 0.5 M hydrochloric acid. The aqueous layer was basified with sodium hydroxide aqueous solution to pH 10, and the resulted aqueous solution was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was diluted with ethyl acetate, and 4.0 M hydrogen chloride solution in ethyl acetate (110 mL) was added to the solution, and the precipitate was collected by filtration while being washed with ethyl acetate. The solid was recrystallized from *N*,*N*-dimethylformamide / ethyl acetate (1 / 10) to give (*R*)-**61** (45.8 g, 42.2%). ^{1}H NMR (DMSO-*d*6) δ: 8.66 (3H, br), 8.22 (1H, s), 7.96-7.89 (2H, m), 4.38 (1H, dd, *J* = 8.9, 5.8 Hz), 2.04-1.93 (1H, m), 1.92-1.80 (1H, m), 1.50 (9H, s), 0.77 (3H, dd, *J* = 7.4, 7.4 Hz); MS (ESI): 281 $(M+H)^+$. This was employed for the next step without further purification.

tert-Butyl (1'_R_)-2-amino-4-(1'-aminopropyl)benzoate D-tartrate [(_R_)-29d]

To a solution of (*R*)-**61** (4.66 g) in ethanol (150 mL) was added 10% palladium on carbon (1.0 g), and the mixture was stirred under hydrogen atmosphere at room temperature for 8 hours. The catalyst was filtered out through Celite while being washed with ethyl acetate, and the filtrate was concentrated *in vacuo*. Then, 1.0 M solution of D-tartaric acid in ethanol (16.6 mL) was added to the residue, and the suspension was stirred at reflux until dissolving. Next, ethyl acetate was added to the clear solution, the resulting mixture was cooled to room temperature, and the crystals were collected by filtration while being washed with ethanol to give (*R*)-**29d** (5.42 g, 75.7%, 95.0% ee). The enantiomeric excess was determined by HPLC on chiral stationary phase (analytical column: CHIRALCEL AD-H of Daicel Chemical Industries, mobile phase: hexane / ethanol / diethylamine = 95 / 5 / 0.05, flow: 1.0 mL/min, minor peak: t_R = 19.4 min, major peak: t_R = 21.0 min, peak area ratio: major peak / minor peak = 97.5 / 2.5). ^{1}H NMR ($CDCl_3$) δ: 7.67 (1H, d, *J* = 8.2 Hz), 6.73 (1H, d, *J* = 1.4 Hz), 6.67 (2H, s), 6.58 (1H, dd, *J* = 8.2, 1.4 Hz), 3.96-3.89 (1H, m), 3.85 (2H, s), 1.92-1.64 (2H, m), 1.52 (9H, s), 0.76 (3H, dd, *J* = 7.4, 7.4 Hz); MS (ESI): 234 $(M\text{-}NH_2)^+$. This was employed for the next step without further purification.



Scheme 20 Syntheses of (6*R*,1'*R*)-**25d** and (6*R*,1'*S*)-**25d**

(6*R*,1"*R*)-4-[[[1"-[[3"'-Amino-4"'-(*tert*-butoxycarbonyl)]phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R*,1'*R*)-33d]

(6*R*,1"*S*)-4-[[[1"-[[3"'-Amino-4"'-(*tert*-butoxycarbonyl)]phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*R*,1'*S*)-33d]

To a solution of (*R*)-**23b** (564 mg) in *N*,*N*-dimethylformamide (50 mL) were added (*R*)-**29d** (366 mg, 95.0% ee), 4-dimethylaminopyridine (111 mg) and triethylamine (254 mL), and the

mixture was stirred at room temperature for 3 days. Then, saturated potassium hydrogen sulfate aqueous solution and water were added to the reaction mixture, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane / ethyl acetate = 1 / 1 to 1 / 99) to give (6*R*,1'*R*)-**33d** (485 mg, 71.3%) and (6*R*,1''*S*)-**33d** (13.0 mg, 1.9%). Diazepane (6*R*,1'*R*)-**33d** and (6*R*,1'*S*)-**33d** showed ^{1}H NMR spectra identical to (6*R**,1'*R**)-**33d** and (6*R**,1'*S**)-**33d**, respectively. These were employed for the next step without further purification.

(6*S*,1"*S*)-4-[[[1"-[[3"'-Amino-4"'-(*tert*-butoxycarbonyl)]phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*S*,1'*S*)-33d]

(6*S*,1"*R*)-4-[[[1"-[[3"'-Amino-4"'-(*tert*-butoxycarbonyl)]phenyl]propyl]amino]carbonyl]-6-[(5""-chloro-2""-methoxy)benzyl]-1-(2',4',6'-trimethoxybenzyl)-1,4-diazepan-2,5-dione [(6*S*,1'*R*)-33d]

Diazepanes (6*S*,1'*S*)-**33d** and (6*S*,1'*R*)-**33d** were prepared from (*S*)-**23b** and (*R*)-**29d** (95.0% ee) in a similar manner to (6*R*,1'*R*)-**33d** and (6*R*,1'*S*)-**33d**. ^{1}H NMR spectra of (6*S*,1'*S*)-**33d** and (6*S*,1'*R*)-**33d** were identical with those of (6*R**,1'*R**)-**33d** and (6*R**,1'*S**)-**33d**, respectively. These were employed for the next step without further purification.

(6*R*,1'*R*)-4-[[[1'-[(3"-Amino-4"-carboxyl)phenyl]propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R*,1'*R*)-25d]

1.0 M hydrogen chloride solution in acetic acid (10 mL) was added to (6*R*,1'*R*)-**33d** (484 mg), and the mixture was stirred at room temperature for 14 hours. Then, the reaction mixture

was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (chloroform / ethyl acetate / methanol / acetic acid = 7.5 / 7.5 / 1 / 0.1). The product was dissolved in ethyl acetate, and hexane was added to the solution (ethyl acetate / hexane = 1 / 1), then the precipitate was collected by filtration while being washed with diethyl ether to give (6*R*,1'*R*)-**25d** (166 mg, 50.4%). Diazepane (6*R*,1'*R*)-**25d** showed a ^{1}H NMR spectrum identical to (6*R**,1'*R**)-**25d**. MS (ESI): 503 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{27}ClN_4NaO_6^+$ 525.1511 $(M+Na)^+$, found 525.1485; $[\alpha]^{20}_D$ –16.0 (c = 0.1, methanol).

(6*R*,1'*S*)-4-[[[1'-[(3"-Amino-4"-carboxyl)phenyl]propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*S*,1'*S*)-25d]

Diazepane (6*S*,1'*S*)-**25d** was prepared in a similar manner to (6*R*,1'*R*)-**25d**, and showed a ^{1}H NMR spectrum identical to (6*R**,1'*R**)-**25d**. MS (ESI): 503 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{27}ClN_4NaO_6^+$ 525.1511 $(M+Na)^+$, found 525.1530; $[\alpha]^{20}_D$ +25.1 (c = 0.1, methanol).

(6*S*,1'*R*)-4-[[[1'-[(3"-Amino-4"-carboxyl)phenyl]propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*S*,1'*R*)-25d]

Diazepane (6*S*,1'*R*)-**25d** showed a ^{1}H NMR spectum identical to (6*R**,1'*S**)-**25d**. MS (ESI): 503 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{27}ClN_4NaO_6^+$ 525.1511 $(M+Na)^+$, found 525.1520; $[\alpha]^{20}_D$ +49.1 (c = 0.1, methanol)

(6*R*,1'*S*)-4-[[[1'-[(3"-Amino-4"-carboxyl)phenyl]propyl]amino]carbonyl]-6-[(5"'-chloro-2"'-methoxy)benzyl]-1,4-diazepan-2,5-dione [(6*R*,1'*S*)-25d]

Diazepane (6*R*,1'*S*)-**25d** showed a ^{1}H NMR spectrum identical to (6*R**,1'*S**)-**25d**. MS (ESI): 503 $(M+H)^+$; HRMS (FAB): calcd for $C_{24}H_{27}ClN_4NaO_6^+$ 525.1511 $(M+Na)^+$, found

525.1489; $[\alpha]^{20}_{D}$ –42.3 (c = 0.1, methanol).

Preparation of single crystal of (*R*)-29a

Ester (*R*)-**29a** (10 mg) was dissolved in a mixture of methanol (0.5 mL) and water (0.2 mL), and the solution was allowed to stand at room temperature for 16 hours. The precipitates were collected by filtration to give the crystal of (*R*)-**29a**.

Preparation of single crystal of (6*R,1'*S**)-33a**

Diazepane (6*R**,1'*S**)-**33a** (8 mg) was dissolved in a mixture of 2-propanol (0.6 mL), methanol (0.6 mL) and water (0.12 mL), and the solution was allowed to stand at room temperature for 24 hours. The precipitates were collected by filtration to give the crystal of (6*R**,1'*S**)-**33a**.

引用文献

1. Schwartz, B. L.; Austen, F. K. *J. Invest. Dermatol.* **1980**, *74*, 349.
2. Hoit, D. B.; Shao, Y.; Kinoshita, A.; Gabel, M.; Husain, A.; Walsh, A. R. *J. Clin. Invest.* **1995**, *95*, 1519.
3. Tomimori, Y.; Muto, T.; Fukami, H.; Saito, K.; Horikawa, C.; Turuoka, N.; Yamashiro, K.; Saito, M.; Sugiura, N.; Sumida, M.; Kakutani, S.; Fukuda, Y. *Biochem. Biophys. Res. Commn.* **2002**, *290*, 1478.
4. Lindstedt, A. K.; Wang, Y.; Shiota, N.; Saarinen, J.; Hyytiainen, M.; Kokkoken, O. J.; Keski-Oja, J.; Kovanen, T. P. *FASEB J.* **2001**, *15*, 1377.
5. Irani, A. A.; Bradford, T. R.; Kepley, C. L.; Schechter, N. M.; Schwartz, L. B. *J. Histochem. Cytochem.* **1989**, *37*, 1509.
6. Gilfillan, M. A.; Tkaczyk, C. *Nat. Rev. Immunol.* **2006**, *6*, 218.
7. Tomimori, Y.; Tsuruoka, N.; Fukami, H.; Saito, K.; Horikawa, C.; Saito, M.; Muto, T.; Sugiura, N.; Yamashiro, K.; Sumida, M.; Kakutani, A.; Fukuda, Y. *Biochemical Pharmacology* **2002**, *64*, 1187.
8. Mao, Q. X.; Shirakawa, T.; Yoshikawa, T.; Yoshikawa, K.; Kawai, M.; Sasaki, S.; Enomoto, T.; Hashimoto, T.; Furuyama, J.; Hopkin, M. J.; Morimoto, K. *Lancet* **1996**, *348*, 581.
9. Tanaka, K.; Sugiura, H.; Uehara, M.; Sato, H.; Hashimoto-Tamaoki, T.; Furuyama, J. *Clin. Exp. Allergy* **1999**, *29*, 800.
10. Weidinger, S.; Rummler, L.; Klopp, N.; Wagenpfeil, S.; Baurecht, J. H.; Fischer, G.; Holle, R.; Gauger, A.; Schafer, T.; Jakob, T.; Ollert, M.; Behrendt, H.; Wichmann, E. H.; Ring, J.; Illig, T. *Allergy* **2005**, *60*, 1256.
11. Badertscher, K.; Bronnimann, M.; Karlen, S.; Braathen, L. R.; Yawalkar, N. *Arch. Dermatol. Res.* **2005**, *296*, 503.
12. 高井真司 日本薬理学雑誌 **2003**, *122*, 111.
13. Caughey, G. H.; Zerweck, E. H.; Vanderslice, P. *J. Biol. Chem.* **1991**, *266*, 12956.
14. Pereira, P. J.; Wang, Z. M.; Rubin, H.; Huber, R.; Bode, W.; Schechter, N. M.; Strobl, S. *J. Mol. Biol.* **1999**, *286*, 163.
15. Perola, E.; Herman, L.; Weiss, J. *J. Chem. Inf. Model.* **2012**, *52*, 1027.
16. Turk, B. *Nat. Rev. Drug Discov.* **2006**, *5*, 785.
17. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.
18. Kato, Y.; Kido, H.; Fukusen, N.; Katsunuma, N. *J Biochem.* **1988**, *103*, 820.
19. Bastos, M.; Maeji, J. N.; Abeles, H. R. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 6738.
20. Eda, M.; Ashimori, A.; Akahoshi, F.; Yoshimura, T.; Inoue, Y.; Fukaya, C.; Nakajima, M.; Fukuyama, H.; Imada, T.; Nakamura, N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 913.

21. Doggrell, S. A. *Expert Opin. Ther. Pat.* **2008**, *18*, 485.
22. Green Cross Corp. (Akahoshi, F.; Ashimori, A.; Yoshimura, T.; Eda, M.; Sakashita, H.; Nakajima, M.; Imada, T.) *WO-09818794*, **1998**.
23. Teijin Ltd. (Tuchiya, N.; Matsumoto, Y.; Saito, H.; Mizuno, H.) *WO-200153291*, **2001**.
24. Mitsubishi-Tokyo Pharmaceuticals Inc. (Aoyama, Y.; Seki, M.: Matsuda, H.; Usui, Y.; Abe, Y.; Shimada, M.; Yamamoto, M.) *WO-200123349*, **2001**.
25. Toa Eiyo Ltd. (Satoh, S.; Tatsui, A.; Hasegawa, T.; Yamada, H.; Kazayama, S.; Morita, T.; Masaki, H.; Takahashi, A.) *WO-200222595*, **2002**.
26. Niwata, S.; Fukami, H.; Sumida, M.; Ito, A.; Kakutani, S.; Saitoh, M.; Suzuki, K.; Imoto, M.; Shibata, T.; Imajo, S.; Kiso, Y.; Tanaka, T.; Nakazato, H.; Ihihara, T.; Takai, S.; Yamamoto, D.; Shiota, N.; Miyazaki, M.; Okunishi, H.; Kinishita, A.; Urata, H.; Arakawa, K. *J. Med. Chem.* **1997**, *40*, 2156.
27. Fukami, H.; Imajo, S.; Ito, A.; Kakutani, S.; Shibata, H,; Sumida, M.; Tanaka, T.; Niwata, S.; Saitoh, M.; Kiso, Y.; Miyazaki, M.; Okunishi, H.; Urata, H.; Arakawa, K. *Drug Design and Discovery* **2000**, *17*, 69.
28. Tomimori, Y.; Muto, T.; Fukami, H.; Saito, K.; Horikawa, C.; Turuoka, N.; Saito, M.; Sugiura, N.; Yamashiro, K.; Sumida, M.; Kakutani, S.; Fukuda, Y. *Lab. Invest.* **2002**, *82*, 789.
29. Watanabe, N.; Tomimori, Y.; Saito, K.; Miura, K.; Wada, A.; Tsudzuki, M.; Fukuda, Y. *Int. Arch. Allergy Immunol.* **2002**, *128*, 229.
30. ハプテン反復塗布マウスにおける効果（未発表）　アスビオファーマ（株）免疫・炎症疾患フィールド
31. マウス静脈内投与後の血漿中半減期（未発表）　アスビオファーマ（株）創薬技術ファンクション
32. Tanaka, T.; Muto, T.; Maruoka, H.; Imajo, S.; Fukami, H.; Tomimori, Y.; Fukuda, Y.; Nakatsuka, T. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 3431.
33. Ogata, A.; Fujieda, Y.; Terakawa, M.; Muto, T.; Tanaka, T.; Maruoka, H.; Nagahira, K.; Fukuda, Y.; Tomimori, Y.; Watanabe, N. *Int. Immunopharmacol.* **2011**, *11*, 1628.
34. Dahl, G.; Akerud, T. *Drug Discov. Today* **2013**, *18*, 697.
35. Maruoka, H.; Muto, T.; Tanaka, T.; Imajo, S.; Tomimori, Y.; Fukuda, Y.; Nakatsuka, T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3435.
36. Tanaka, T.; Sugawara, H.; Maruoka, H.; Imajo, S.; Muto, T. *Bioorg. Med. Chem.* **2013**, *21,* 4233.
37. Brown, H. C.; Chandrasekharan, J.; Ramachandran, P. V. *J. Am. Chem. Soc.* **1988**, *110*, 1539.
38. Soai, K.; Yokoyama, S.; Hayasaka, T. *J. Org. Chem.* **1991**, *56*, 4264.

39. Bauer, T.; Tarasiuk, J. *Tetrahedron Lett.* **2002**, *43*, 687.
40. Greco, M. N.; Hawkins, M. J.; Powell, E. T.; Almond, J., H. R.; de Garavilla, L.; Hall, J.; Minor, L. K.; Wang, Y.; Corcoran, T. W.; Cera, E. D.; Cantwell, A. M.; Savvides, S. N.; Damiano, B. P.; Maryanoff, B. E. *J. Med. Chem.* **2007**, *50*, 1727.
41. Arooj, M.; Kim, S.; Sakkiah, S.; Cao, G. P.; Lee, Y.; Lee, K. W. *PLOS ONE* **2013**, *8*, e62740.
42. Matsumoto, Y.; Kakuda, S.; Koizumi, M.; Mizuno, T.; Muroga, Y.; Kawamura, T.; Takimoto-Kaminura, M. *J. Synchrotron Rad.* **2013**, *20*, 914.
43. Lo, H. Y.; Nemoto, P. A.; Kim, J. M.; Hao, M.; Qian, K. C.; Farrow, N. A.; Albaugh, D. R.; Fowler, D. M.; Schneiderman, R. D.; August, E. M.; Martin, L.; Hill-Drzewi, M.; Pullen, S. S.; Takahashi, H.; Lombaert, S. D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4533.
44. 上村みどり *CICSJ Bulletin* **2013**, *31*, 104.
45. Ingallinella, P.; Fattori, D.; Altamura, S.; SteinKuhler, C.; Koch, U.; Cicero, D.; Bazzo, R.; Cortese, R.; Bianchi, E.; Pessi, A. *Biochemistry* **2002**, *41*, 5483.
46. Maibaum, J.; Stutz, S.; Goschke, R.; Rigollier, P.; Yamaguchi, Y.; Cumin, F.; Rahuel, J.; Baum, H.; Cohen, N.; Schnell, C. R.; Fuhrer, W.; Gruetter, M. G.; Schilling, W.; Wood, J. M. *J. Med. Chem.* **2007**, *50*, 4832.
47. Sellner, H.; Cottens, S.; Cumin, F.; Ehrhardt, C.; Kosaka, T.; Lorthiois, E.; Ostermann, N.; Webb, R. L.; Rigel, D. F.; Wagner, T.; Maibaum, J. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1787.
48. Terakawa, M.; Fujieda, Y.; Tomimori, Y.; Muto, T.; Tanaka, T.; Maruoka, H.; Nagahira, K.; Ogata, A.; Nakatsuka, T.; Fukuda, Y. *Eur. J. Pharmacol.* **2008**, *601*, 186.
49. Chapman, C. J.; Frost, C. G.; Hartley, J. P.; Whittle, A. J. *Tetrahedron Lett.* **2001**, *42*, 773.
50. Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165.
51. Bauer, T.; Gajewiak, J. *Tetrahedron Lett.* **2005**, *16*, 851.

謝辞

本論文の執筆をご指導頂いた慶應義塾大学薬学部 有機化学講座 須貝 威教授、貴重なご助言を賜った医薬品化学講座 増野匡彦教授、生命機能物理学講座 大澤匡範教授に厚く御御礼申上げます。

本研究を推進するにあたり、終始温かいご指導を賜りました武藤 毅博士、中塚 隆博士、福田好晃博士、深見治一博士に心より深く感謝いたします。

本研究の成果は、共同研究者であった丸岡 博博士、X線結晶構造解析を行っていただいた菅原 肇博士、小山嘉晃氏、複合体モデルを作成していただいた今城精一博士、酵素阻害活性を測定していただいた冨森嘉晃博士ら多くの方々のご協力の賜物であり、ここに心より深く感謝いたします。

本研究に際して、御協力、御助言いただきました第一三共株式会社創薬化学研究所玉木和彦博士、アスビオファーマ株式会社の諸兄に心より深く感謝いたします。

本学位論文執筆のきっかけを与えて下さった慶應義塾大学理工学部応用化学科 中田雅也教授に心より御礼申上げます。

最後に、健康に気遣い、笑顔で激励を続けてくれた家族に心より深く感謝いたします。