学位論文

ペプチド性天然物ベラクトシン A をプロトタイプとした
高活性プロテアソーム阻害剤の創製研究

北海道大学大学院生命科学院

生命科学専攻生命医薬科学コース
創薬有機化学研究室

川村　周平

謝辞

本研究を行うに際し、終始御懇篤なる御指導と御鞭撻を賜りました北海道大学大学院薬学研究科　周東智　教授に心より感謝致します。

本研究を行うにあたり、有益なる御助言、御討論をして頂きました北海道大学大学院薬学研究科　有澤　光弘　准教授に深く感謝致します

本論文の審査をして頂き、有益なる御助言を賜りました北海道大学大学院薬学研究科　松田　彰　教授、市川　聡　准教授、阿部　洋　准教授に深く感謝致します。

薬理活性評価をして頂きました静岡県立大学大学院薬学研究科　浅井　章良　教授、海野　雄加　特任助教に深く感謝致します。

ドッキングシミュレーション及び分子モデリングに関し、有益な御助言を賜りました産業技術総合研究所広川　貴次　博士、亀田　倫史　博士に深く感謝致します。

プロテアソームの X 線結晶構造解析をして頂きましたミュンヘン工科大学 Michael Groll 博士、Anja List 博士に深く感謝致します。

単結晶 X 線結晶構造解析をして頂きましたリガク X 線研究所　山野　昭人　博士に深く感謝致します。

薬理活性評価をして頂きました愛知学院大学大学院薬学研究科　故佐々木　琢磨　教授、田中　基裕　准教授に深く感謝致します。

各種機器分析を行って頂きました北海道大学機器分析センターの皆様に深く感謝致します。

日々活発な御討論をして頂きました北海道大学大学院薬学研究院創薬有機化学研究室に御関係する皆様に深く感謝致します。

共に励まし合い、心の支えとなって下さった北海道大学薬学部同期生を始めとする友人たちに深く感謝致します。

最後に著者の学生生活を経済的、精神的に支えて下さった家族ならびに親戚の皆様に心より感謝致します。

2014 年　3 月　　川村　周平

略語表

本論文中、以下の略語を使用した。

Ac	acetyl
Ala	alanine
AMC	aminomethylcoumarin
ATP	adenosine 5'-triphosphate
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bu	butyl
Bz	benzoyl
calcd	calculated
CBS	Corey-Bakshi-Shibata
Cbz	benzyloxycarbonyl
ChT-L	chymotrypsin-like
C-L	caspase-like
conc.	concentrated
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIBAL	diisobutylalminium hydride
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	*N*, *N*-dimethylformamide
DMP	Dess-Martin periodinane
DPPA	diphenylphosphoryl azide
DMSO	dimethyl sulfoxide
dr	diastereomeric ratio
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	enantiomeric excess
EI	electron impact ionization
eq., equiv.	equivalent
Et	ethyl
FAB	fast atom bombardment
FDA	food and drug administration
Fmoc	9-fluorenylmethoxycarbonyl
Gly	glycine
HBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
LDA	lithium diisopropylamide
Leu	leucine
LiHMDS	lithium hexamethyldisilazide
LMP7	large multifunctional peptidase 7
LRMS	low-resolution mass spectrometry
m-CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
MHC	major histocompatibility complex
mp	melting point
MS	mass spectrometry
MTPA	α-methoxy-α-(trifluoromethyl)phenylacetic acid
n-	normal
NaHMDS	sodium hexamethyldisilazide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
ox.	oxidation
PGME	phenylglycine methyl ester
Ph	phenyl
Phe	phenylalanine
Piv	pivaloyl
ppm	parts per million
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
Rf	retardation factor
rt.	room temperature
t-	tertialy-
sat.	saturated
sec-	secondary-
Su	succinimide
Suc	succinyl
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TEAA	triethylammonium acetate
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
Thr	threonine

T-L	trypsin-like
TMS	trimethylsilyl
Trp	tryptophan
quant.	quantitative yield

目次

序論

癌は先進諸国における主要な死因の一つであり、より効果的な治療薬の開発を目指して日夜研究が行われている。筆者は、近年新たな抗がん薬の標的分子として注目されているプロテアソームに対して阻害作用を有する天然物ベラクトシン A をプロトタイプとした新規プロテアソーム阻害剤の開発及び合理的創薬方法論の開発研究を行ったので、以下にその研究成果を述べる。

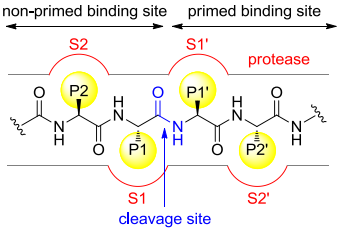
1. プロテアソームの構造と機能

細胞内タンパク質は、主としてユビキチン-プロテアソーム系[1] (Figure 0-1a) またはオートファジー[2]によって分解される。オートファジーがオートファゴソーム形成によるバルク分解系[2]であるのに対して、ユビキチンプロテアソーム系はポリユビキチン化されたタンパク質を選択的に分解する。[1]ユビキチン-プロテアソーム系はタンパク質の代謝回転を制御することで、シグナル伝達[3]、免疫応答、小胞体ストレス応答[4]、細胞周期の進行[5]等の広範な細胞機能に関わっている。

ユビキチン-プロテアソーム系においてタンパク質分解を担うのは 26S プロテアソームであり、これは 20S プロテアソームと 19S 調節サブユニットにより構成される巨大なタンパク質複合体である (Figure 0-1b)。[6]19S 調節サブユニットは、ポリユビキチン鎖の認識、ATP 依存的な基質タンパク質の解きほぐし（アンフォールディング）及び 20S プロテアソーム内腔へ送り込み（トランスロケーション）、基質タンパク質からのポリユビキチン鎖の切り離し等の役割を担っている。[6]20S プロテアソームはそれぞれ 7 個のサブユニットからなるα-リングとβ-リングにより構築される円筒状の複合体であり、β-リングを構築するサブユニットのうちβ1、β2 及びβ5 の 3 つのサブユニットがプロテアーゼ活性を有している (Figure 0-1b)。[6]これら 3 つのサブユニットは、主として S1 ポケット*の構造の違いによって異なる基質特異性を有しており、β1 はカスパーゼ様活性（酸性アミノ酸残基の C 末端側で切断, C-L 活性）、β2 はトリプシン様活性（塩基性アミノ酸残基の C 末端側で切断, T-L 活性）、β5 はキモトリプシン様活性（疎水性アミノ酸残基の C 末端側で切断, ChT-L 活性）をそれぞれ示すことが分かっている。[6]基質タンパク質は 20S プロテアソーム内腔を通過する際に、これらのサブユニットによって分解を受け、短いペプチド鎖となる。[7]この短鎖ペプチドは細胞内ペプチダーゼにより更なる分解を受ける他、MHC class I 分子により提示される抗原ペプチドとしても重要である。[8]

---

* 一般にプロテアーゼの基質結合ポケット及び基質ペプチドの各アミノ酸残基は、基質ペプチドの切断部位を境に下図に示すように表される。



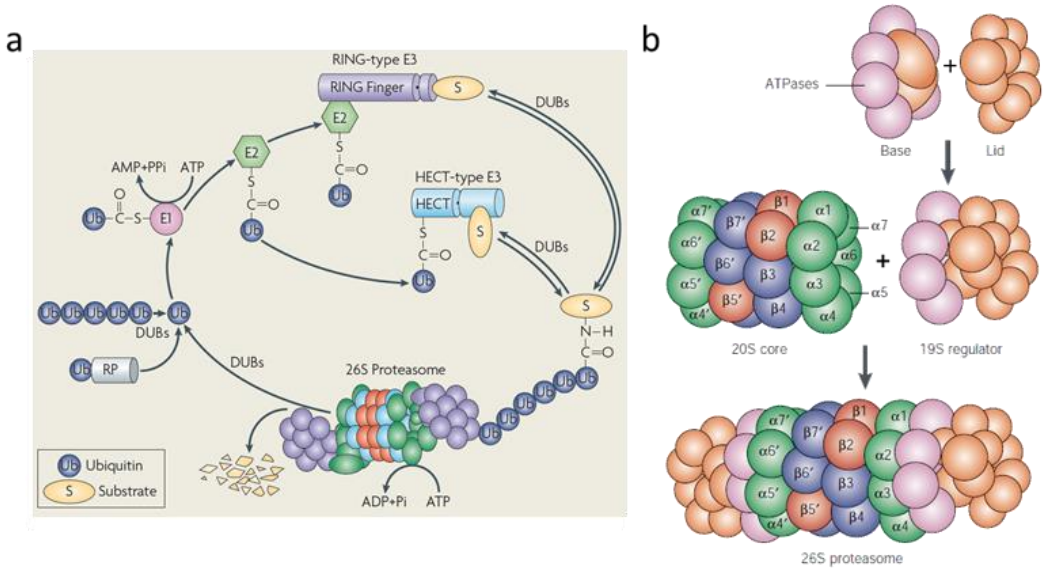


Figure 0-1. (a) ユビキチン-プロテアソーム系[6] (b) 20S プロテアソームと調節サブユニット[9]

2. 組織特異的プロテアソーム

脊椎動物では、β1, β2, β5 の 3 種の触媒サブユニットからなる構成型プロテアソームに加えて、免疫プロテアソーム[10]及び胸腺プロテアソーム[11]と呼ばれる 2 種の組織特異的プロテアソームが存在することが報告されている。これら組織特異的プロテアソームでは、触媒サブユニットの構成が異なっており、免疫プロテアソームはβ1i, β2i, β5i、[10, 12]胸腺プロテアソームはβ1i, β2i, β5t[11]の 3 種の触媒サブユニットによって構成される。免疫プロテアソームはリンパ球や単球をはじめとする造血細胞において構成的に発現している他、INF-γによって誘導される。[10, 13]免疫プロテアソームでは、構成型プロテアソームに比べて C-L 活性が弱く、T-L 及び ChT-L 活性が強くなっており、効率的に MHC-class I 分子により提示される抗原ペプチドを切り出す*[14]ことが分かっており、免疫応答において重要な役割を果たす。[4a, 15]一方、胸腺プロテアソームは胸腺皮質上皮細胞に特異的に発現しており、構成型プロテアソームに比べて ChT-L 活性が弱く[11]ユニークな抗原ペプチドの切り出しによって T 細胞の正の選択において重要な役割を果たしていると考えられている。†[11, 14, 16]

3. プロテアソーム阻害剤とその生理活性

プロテアソームは、I-κB,[17] p53,[18] p27[19]等の細胞周期の進行やアポトーシスに関わるタンパク質の分解に関与していることや、変性タンパク質の分解による小胞体ストレスの軽減[4b]等の役割を担っていることから、抗がん薬の新規ターゲットとして注目されることとなった。プロテアソーム阻害剤ボルテゾミブが、それまで有効な治療法のなかった再発又は難治性の多発性骨髄腫を対象疾患として有効性が示され、2003 年に FDA により認可された (Figure 0-2)。[20]しかしながら、抗がん薬において常に重大な問題となる耐性や、末梢神経障害をはじめとする重篤な副作用の発現についても数多く報告されており、[21]ボルテゾミブに代わる新たなプロテアソーム阻害剤の開発を目指して盛んに研究が行われている。2012 年には、カルフィルゾミブが多発性骨髄腫を対象疾患として認可され、[22]2013 年現在、サリノスポラミド A (マリゾミブ, NPI-0052),[23]

* MHC class I 分子は C 末端部に疎水性または塩基性のアミノ酸残基を有するペプチドと親和性が高い。細胞質にはアミノペプチダーゼが存在するが、カルボキシペプチダーゼは存在しないため、C 末端のアミノ酸残基はプロテアソームによる切断によって規定される。

† 胸腺において T 細胞は、胸腺上皮細胞による自己 MHC・ペプチド複合体を認識する TCR 発現 T 細胞の選択（正の選択）と、主としてマクロファージや樹状細胞による自己 MHC 分子に結合した自己抗原を認識する T 細胞の排除（負の選択）を経て成熟する。正の選択を担う胸腺上皮細胞がユニークな抗原ペプチドを提示することは正の選択と負の選択の区別に重要であると考えられている。

CEP-18770,[24] MLN-9708,[25] ONX-0912[26]の 4 種のプロテアソーム阻害剤について多発性骨髄腫を対象疾患として臨床試験が行われている (Figure 0-2)。[27]

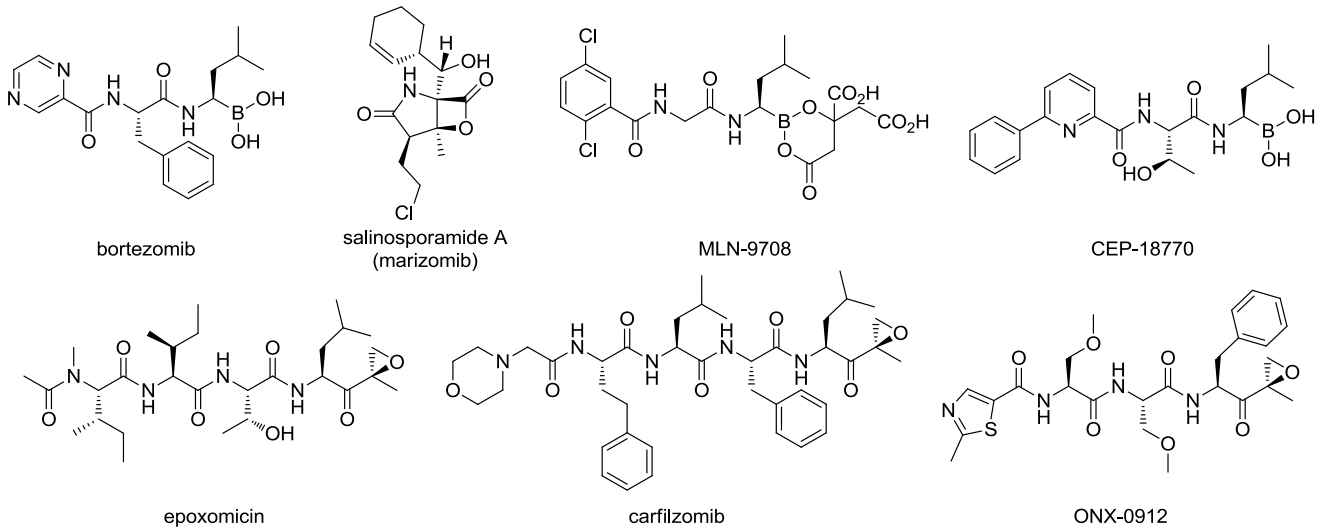


Figure 0-2. 代表的なプロテアソーム阻害剤

先述したように、プロテアソームは C-L (β1), T-L (β2), ChT-L (β5) の 3 種のプロテアーゼ活性を有している。基質タンパク質により異なるが、一般に ChT-L 活性がタンパク質分解に最も重要であり、[28]上述のプロテアソーム阻害剤は全て ChT-L 活性を強力に阻害する。ChT-L 活性 (β5 及びβ5i サブユニット) の選択的阻害により細胞増殖抑制作用が見られるが、[29]加えて C-L 及び T-L 活性を阻害することでその作用が増強されることが報告されている。[30]

構成型プロテアソームあるいは免疫プロテアソームに選択的なプロテアソーム阻害剤もこれまでにいくつか報告されている (Figure 0-3)。[4a, 29, 31]特に免疫プロテアソーム選択的阻害剤 PR-957 はβ5i サブユニットを選択的に阻害することで細胞増殖抑制作用を示すことなくサイトカインの産生を抑制し、優れた免疫抑制作用を示すことが報告されており、現在前臨床試験が行われている。[4a]

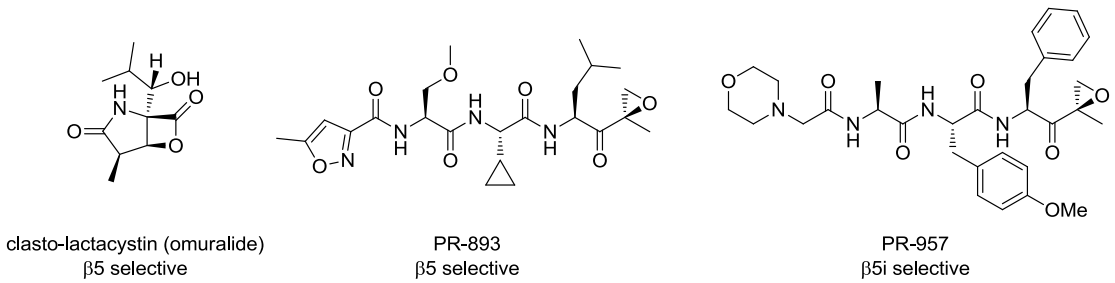


Figure 0-3. 構成型あるいは免疫プロテアソーム選択的阻害剤

4. ベラクトシン誘導体

ベラクトシン A は 2000 年に浅井らにより報告された放線菌由来のペプチド性天然物であり、プロテアソーム阻害作用（ChT-L 活性選択的）に基づくがん細胞増殖抑制作用を有している (Figure 0-4)。[32]ベラクトシン誘導体はその立体的に歪んだβ-ラクトン部がプロテアソーム活性残基である N 末端 Thr 残基のヒドロキシ基と反応してエステル結合を形成することで、プロテアソームに対する共有結合性阻害剤として働くことが、

ホモベラクトシン C 誘導体とプロテアソームの共結晶の X 線結晶構造解析により知られている (Figure 0-5)。[33]更に、X 線結晶構造解析により、ベラクトシン誘導体は既知のプロテアソーム阻害剤とは異なる結合部位を有することが分かった。[33a]すなわち、他のプロテアソーム阻害剤がいずれも基質 P 領域結合部 (non-primed substrate binding site) に結合するのに対して、ベラクトシン誘導体は主として基質 P'領域結合部 (primed substrate binding site) に結合する (Figure 0-5)。[33-34]従って、ベラクトシン誘導体をリード化合物とすることで、基質 P'領域結合部を対象とする構造活性相関研究が可能となると共に、他のプロテアソームとは結合部位の異なる阻害剤の開発が可能となるため、新規プロテアソーム阻害剤開発におけるリード化合物として魅力的である。

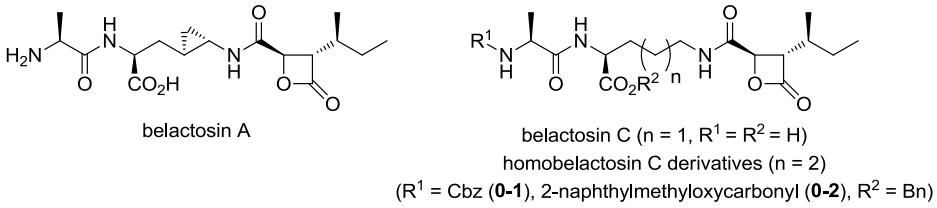


Figure 0-4. ベラクトシン誘導体

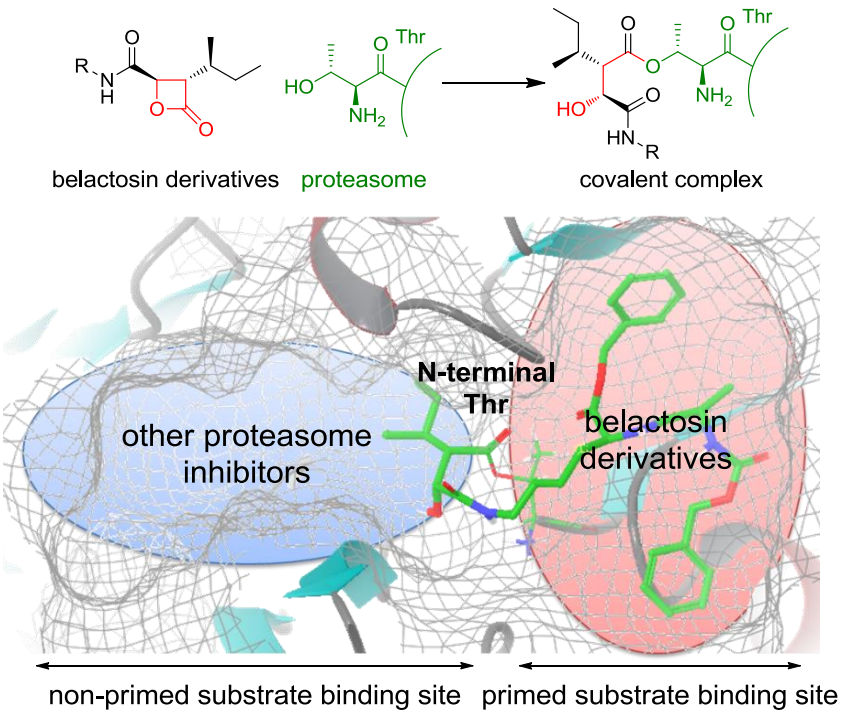


Figure 0-5. ホモベラクトシン C 誘導体 **0-1** とプロテアソームの共結晶の X 線結晶構造解析[33a]

先述の通り、ベラクトシン A はペプチド性の天然物であり、L-アラニン、2-アミノシクロプロピルアラニン及びβ-ラクトン部から成る。当研究室の吉田は、ベラクトシン A のプロテアソーム阻害活性がシクロプロパン構造により規定されるアラニン部とβ-ラクトン部の三次元的空間配置に依存するものと考え、2-アミノシクロプロピルアラニン部の立体配置に着目した三次元的構造活性相関研究を展開し (Figure 0-6)、非天然型のシスシクロプロパン骨格を有する *cis*/L-*anti* 異性体*__0-7__ がトランスシクロプロパン骨格を有するベラクトシ

* シスシクロプロパン骨格を有し、カルボキシ基部が L-アミノ酸の立体配置でシクロプロパン環に対する相対立体配置がアンチであることを示す。他の異性体についても同様に表記した。

ン A に比べて 2 倍程度強いプロテアソーム阻害活性を示すことを見出した。*[35]

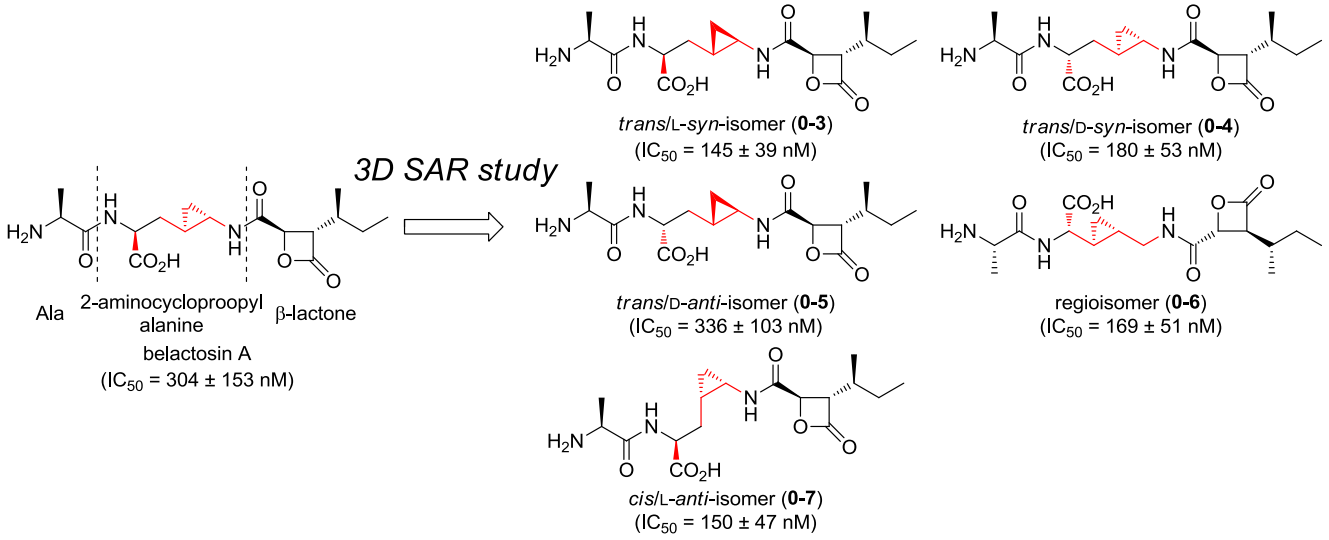


Figure 0-6. ベラクトシン A の三次元的構造活性相関研究

更に、これらの化合物の合成中間体である N 末端部に Cbz 基、カルボキシ基部にビニル基を有する化合物 **0-8 – 0-10** はより強力なプロテアソーム阻害活性を示し、N 末端部及びカルボキシ基部の疎水性置換基がプロテアソームとの相互作用に重要であることが示唆された (Figure 0-7)。[36]

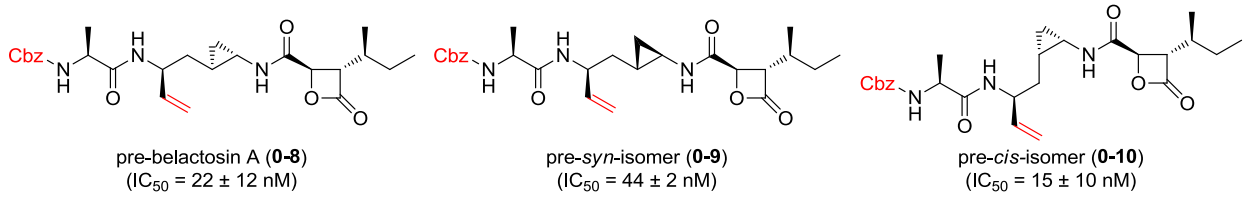


Figure 0-7. ベラクトシン A 及びその立体異性体の合成中間体

5. 本研究の概要

以上の知見を基に、筆者はベラクトシン誘導体をリード化合物とした構造活性相関研究により、プロテアソーム基質 P'領域結合部位における構造活性相関を明らかにすると共に、強力なプロテアソーム阻害活性及び細胞増殖抑制作用を示す誘導体を見出すことを目指して研究を展開した。

第一章では、pre-*cis* 異性体†**0-10** をリード化合物とした系統的構造活性相関研究について述べる。これまでに知られていなかったベラクトシン誘導体の免疫プロテアソームに対する阻害活性や、プロテアソーム阻害活性の可逆性、化学的及び生物学的安定性等についても検討し、ベラクトシン誘導体による細胞増殖抑制作用について考察する。第二章では、新たな創薬方法論として共有結合性化合物であるベラクトシン誘導体を用いて遷移状態近傍における結合様式解析法について述べる。共有結合性化合物の標的タンパク質との結合における非共有結合性相互作用の寄与について考察すると共に、ドッキングを応用した計算化学的手法と、配座制御による有機化学的手法の両面から遷移状態結合配座について議論する。第三章では、解析したベラ

* 本論文において、特に断りのない限りプロテアソーム阻害活性は ChT-L 活性に対する阻害活性を指す。
† *cis* 異性体の合成中間体であることを示す。他の異性体の合成中間体についても同様に表記した。

クトシン誘導体の活性配座に基づいた理論的“scaffold hopping”*による非ペプチド性阻害剤の創製について述べる。創薬研究における“scaffold hopping”の有用性についてリガンド効率の観点から考察する。第四章では、ベラクトシン誘導体の物理学的及び生物学的安定性の改善を目指した研究について述べる。更に、化合物の構造及び配座と安定性の関係について議論する。第五章及び第六章では、それぞれ新たに開発した非ペプチド性阻害剤とボロン酸型及びエポキシケトン型プロテアソーム阻害剤とのハイブリッド化合物の合成について述べると共に、結合部位の違いを活かした理論的なハイブリッド化合物の創製とその生物活性についてまとめる。

* ある活性を有する化合物の構造を基に、その活性を保持して分子骨格の大きく異なる化合物を見出すこと。詳細については第三章を参照。

第一章　ベラクトシン A シス型異性体の構造活性相関研究[37]

第一節　ベラクトシン A シス型異性体の誘導体の設計

序論で述べたように、当研究室の吉田は *cis*/L-*anti* 異性体 **0-7** に比べてその合成中間体である N 末端部に Cbz 基、カルボキシ基部にビニル基を有する pre-*cis* 異性体 **0-10** がより強力なプロテアソーム阻害活性を有することを見出した。[36]そこで、筆者は N 末端部及びカルボキシ基部の二つの置換基がプロテアソーム阻害活性に重要であると考え、これら二つの部位における構造活性相関を系統的に検討することとした (Figure 1-1)。

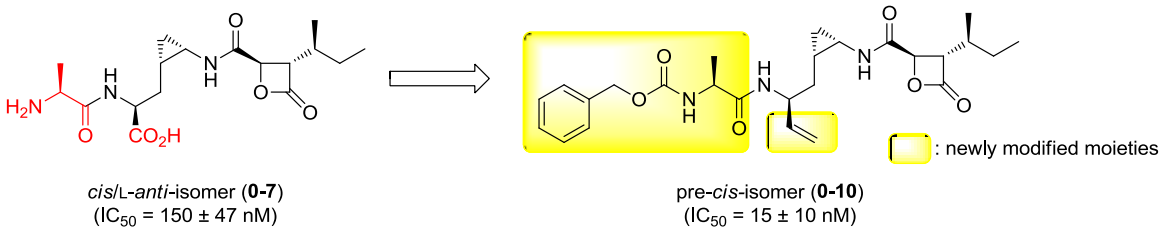
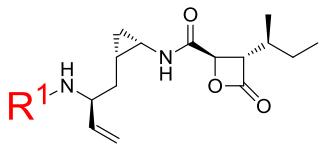


Figure 1-1. 構造活性相関研究のリード化合物と修飾部位

1. N 末端部置換誘導体の設計

はじめに、カルボキシ基部をビニル基に固定し、N 末端部置換基の構造活性相関について調べた。系統的構造活性相関研究により基質 P'領域結合部における構造活性相関を明らかにすべく、アラニル基の代わりに、直接アシル基を導入した化合物 **1-1 – 1-4**、他の N-Cbz-アミノアシル基で置き換えた化合物 **1-5 – 1-9**、アラニンアミノ基上の置換基を検討した化合物 **1-10 – 1-14** の 3 種類の化合物群を設計した (Figure 1-2)。



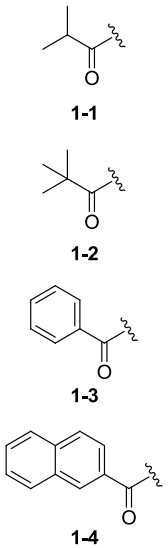
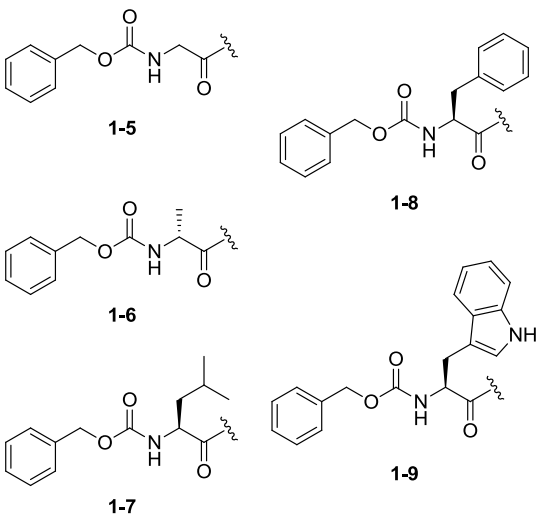
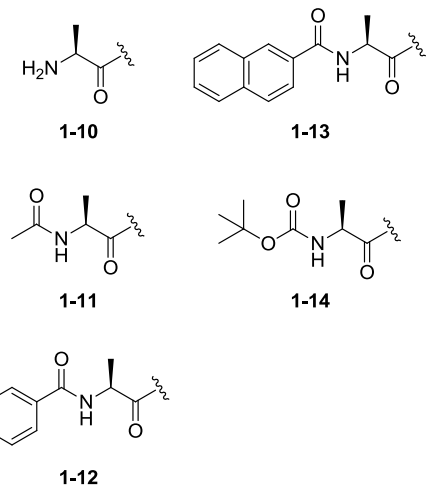
acyl groups	N-Cbz-amino acyl groups	N-substituted alanyl groups
		

Figure 1-2. 設計した N 末端部置換誘導体

2. カルボキシ基部置換誘導体の設計

次いで、N 末端部置換基を Cbz-Ala 基に固定し、カルボキシ基部に種々の疎水性置換基を有する化合物 **1-15 – 1-23** を設計・合成することで、カルボキシ基部の構造活性相関を検証することとした (Figure 1-3)。

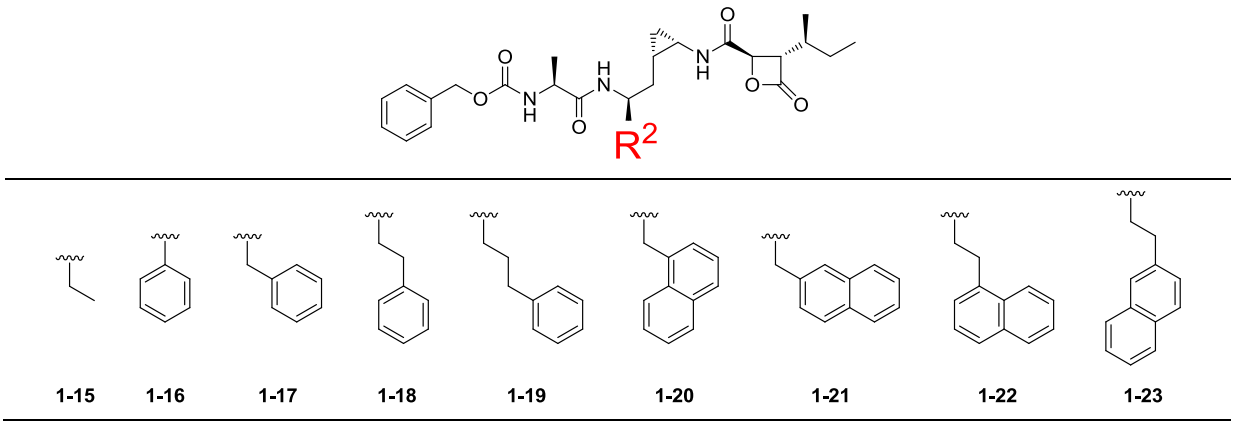


Figure 1-3. 設計したカルボキシ基部置換誘導体

3. 二置換誘導体の設計

最後に、N 末端部及びカルボキシ基部置換誘導体により得られた知見を基に、化合物 **1-24 – 1-25** を設計・合成し、カルボキシ基部置換基をフェネチル基に固定して N 末端部置換基を再検討することにした (Figure 1-4)。

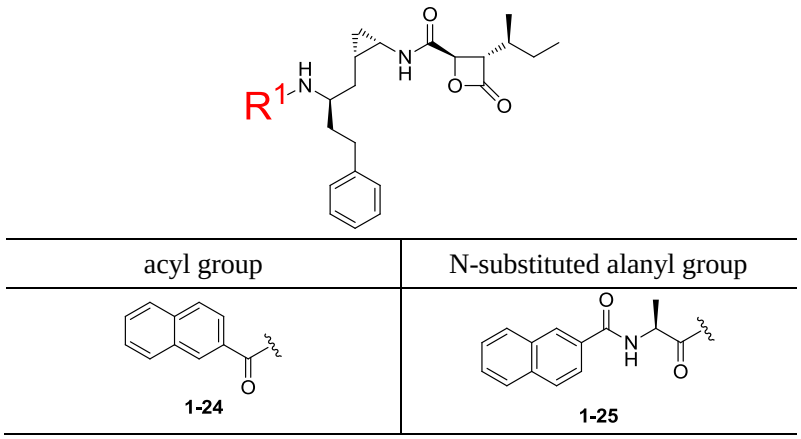


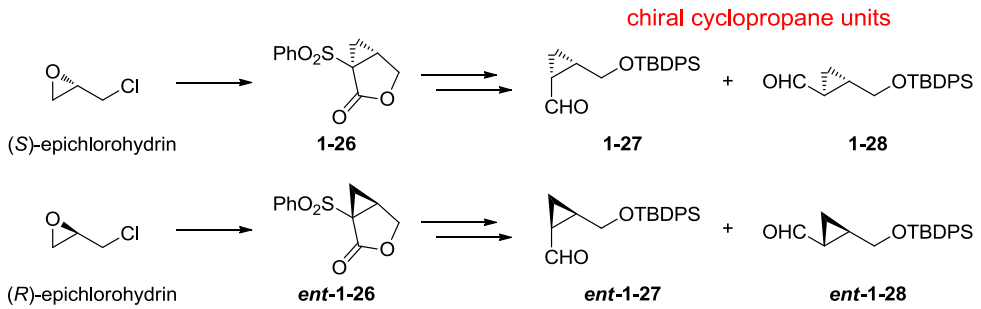
Figure 1-4. 設計した二置換誘導体

第二節　ベラクトシン A シス型異性体の誘導体の合成

1. ベラクトシン誘導体の合成計画

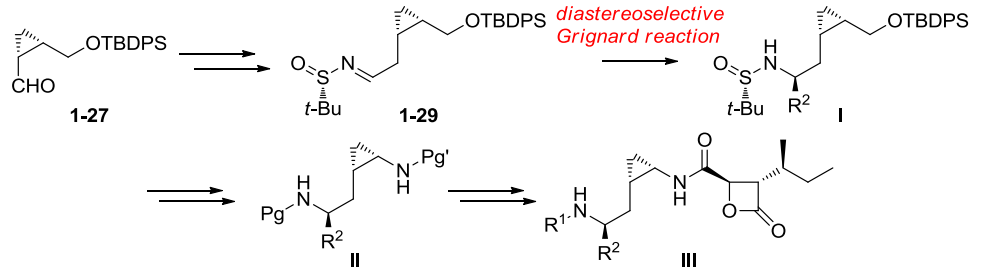
当研究室では、三次元的構造活性相関研究を指向して化合物 **1-27** とそのトランス異性体 **1-28**、及びそれぞれのエナンチオマーである ***ent*-1-27**, ***ent*-1-28** から成る光学活性シクロプロパンユニットを設計し、その系統的合成法を開発した (Scheme 1-1)。[38]これらの光学活性シクロプロパンユニットは一連の立体化学的多様性を有するシクロプロパン化合物の合成に極めて有効であり、[38-39]先に述べたベラクトシン A の立体及び位置異性体もこれらのユニットから合成された。[35-36]

Scheme 1-1. 当研究室で開発した光学活性シクロプロパンユニットとその系統的合成法[38]



本研究において、全ての標的化合物はシスシクロプロパンユニット **1-27** から合成可能である。Scheme 1-2 に示すように、当研究室の吉田の方法[35]に従ってシクロプロパンユニット **1-27** を(*S*)-*t*-ブタンスルフィニルイミン **1-29** へと変換した後、ジアステレオ選択的 Grignard 反応[40]によりカルボキシ基部置換基 (R^2) を導入した中間体 **I** を合成する。この中間体 **I** から Curtius 転位を経て中間体 **II** を得た後、N 末端部置換基 (R^1) 及びβ-ラクトンユニット[41]を縮合して、N 末端部及びカルボキシ基部に望みの置換基を有する標的化合物 **III** を合成することを計画した。

Scheme 1-2. 標的化合物の合成計画



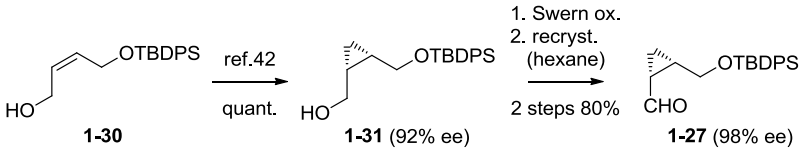
2. N 末端部置換誘導体の合成

多数の誘導体を合成するため、大量のシスシクロプロパンユニット **1-27** を高い光学純度で合成する必要があった。*そこで、Charette らにより報告されたアリルアルコール **1-30** のエナンチオ選択的 Simmons-Smith 反応を利用することを考えた。[42]この反応は大スケールでの合成に適しており、シクロプロピルメチルアルコ

* 上述の光学活性シクロプロパンユニットの合成法は高い光学純度 (> 98% ee) で 4 種のユニットを系統的に合成でき、各種立体異性体を合成して三次元的構造活性相関研究を展開する際には非常に有用であるが、トランス体の生成が優先するためシス体の大量合成には不向きであった。

ール **1-31** を定量的に 92% ee のエナンチオマー過剰率で与え、一度に 30 g 以上合成することが可能であった。更に、Swern 酸化により **1-31** のヒドロキシメチル基を酸化してシスシクロプロパンユニット **1-27** とした後、再結晶することで、98% ee までエナンチオマー過剰率を高めることが可能であることが分かり、光学活性シスシクロプロパンユニットの大量合成法を確立することが出来た。

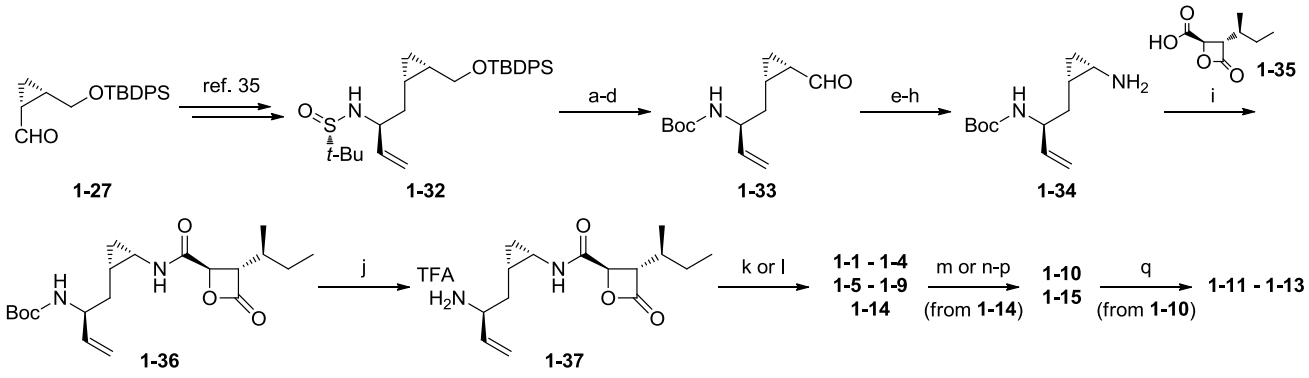
Scheme 1-3. 光学活性シクロプロパンユニット **1-27** の合成



1-30 **1-31** (92% ee) **1-27** (98% ee)

合成したシスシクロプロパンユニット **1-27** から Scheme 1-4 に示すように N 末端部置換誘導体 **1-1** – **1-14** を合成した。当研究室の吉田の方法[35]に従ってシスシクロプロパンユニット **1-27** から合成した化合物 **1-32** の *t*-ブタンスルフィニル基を酸加水分解により除去した後、生じたアミノ基を Boc 基で保護し、次いで TBDPS 基を TBAF を用いて除去した後、生じたアルコールの Swern 酸化によりアルデヒド **1-33** を得た。更に Pinnick 酸化によりカルボン酸へと酸化した後、DPPA を用いて酸アジドへと変換し、Curtius 転位により対応するイソシアネートとした後、塩基性条件下で加水分解してアミン **1-34** を得た。これに対して Armstrong らの方法に従って合成したβ-ラクトンユニット **1-35**[41]を縮合して化合物 **1-36** を得た。**1-36** の Boc 基を除去してアミン **1-37** とした後、アミノ基をそれぞれ対応する酸塩化物によりアシル化して標的化合物 **1-1** – **1-4** を、また混合酸無水物法により種々のアミノ酸を縮合して標的化合物 **1-5** – **1-9** 及び **1-14** をそれぞれ合成した。**1-14** の Boc 基を除去して標的化合物 **1-10** を合成し、更にそのアミノ基をそれぞれ対応する酸塩化物によりアシル化して標的化合物 **1-11** – **1-13** を合成した。

Scheme 1-4. N 末端部置換誘導体 **1-1** – **1-14** 及びカルボキシ基部置換誘導体 **1-15** の合成 [a]



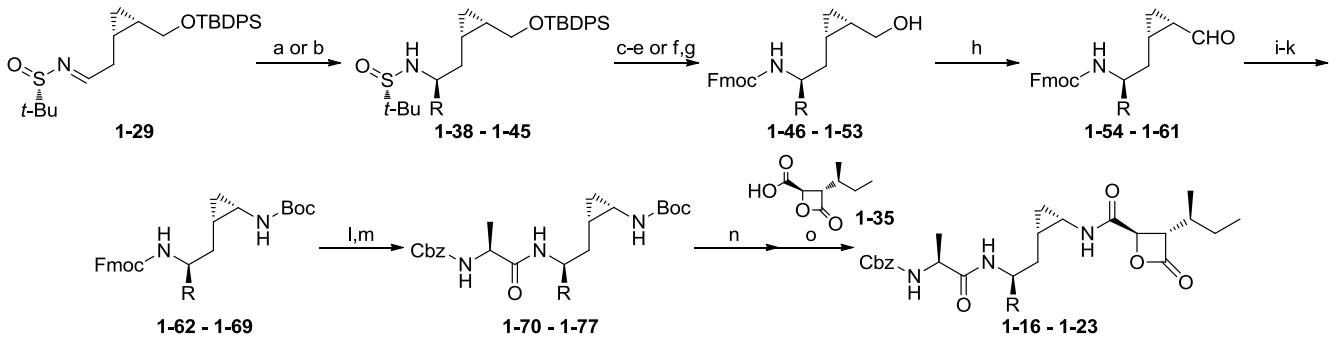
[a]Reagents and conditions: (a) HCl, THF/H_2O, 0 °C; (b) Boc_2O, Et_3N, THF; (c) TBAF, THF; (d) Swern ox., 66% (4 steps); (e) Pinnick ox.; (f) DPPA, Et_3N, CH_2Cl_2, 0 °C to rt; (g) toluene, reflux; (h) KOH, THF/H_2O; (i) **1-35**, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, 48% (5 steps); (j) TFA, CH_2Cl_2, 0 °C to rt; (k) RCl, Et_3N, CH_2Cl_2, 0 °C, yields (2 steps) were as follows respectively, **1-1** (R = isopropanoyl) quant., **1-2** (R = Piv) 81%, **1-3** (R = Bz) 78%, **1-4** (R = 2-naphthoyl) 92%; (l) R-OH, PivCl, Et_3N, CH_2Cl_2, 0 °C, yields (2 steps) were as follows respectively, **1-5** (R = Cbz-Gly) 96%, **1-6** (R = Cbz-D-Ala) quant., **1-7** (R = Cbz-Leu) 64%, **1-8** (R = Cbz-Phe) 66%, **1-9** (R = Cbz-Trp) 65%, **1-14** (R = Boc-Ala) quant.; (m) TFA, CH_2Cl_2, 0 °C, quant. (**1-10**); (n) Pd/C, H_2, THF; (o) TFA, CH_2Cl_2, 0 °C; (p) CbzCl, Et_3N, CH_2Cl_2, 0 °C, 66% (3 steps, **1-15**); (q) RCl, Et_3N, CH_2Cl_2, 0 °C, yields were as follows respectively, **1-11** (R = Ac) 59%, **1-12** (R

= Bz) quant., **1-13** (R = 2-naphthoyl) 95%.

3. カルボキシ基部置換誘導体の合成

カルボキシ基部置換誘導体 **1-16** – **1-23** は、当研究室の吉田による pre-*cis* 異性体 **0-10** の合成法[35-36]に従い、それぞれ対応する Grignard 試薬を用いてカルボキシ基部置換基を導入して合成した (Scheme 1-5)。*カルボキシ基部にエチル基を有する標的化合物 **1-15** は **1-14** のビニル基を還元した後、Boc 基を除去して生じたアミノ基を Cbz 化して合成した (Scheme 1-4)。

Scheme 1-5. カルボキシ基部置換誘導体 **1-16** – **1-23** の合成 [a]



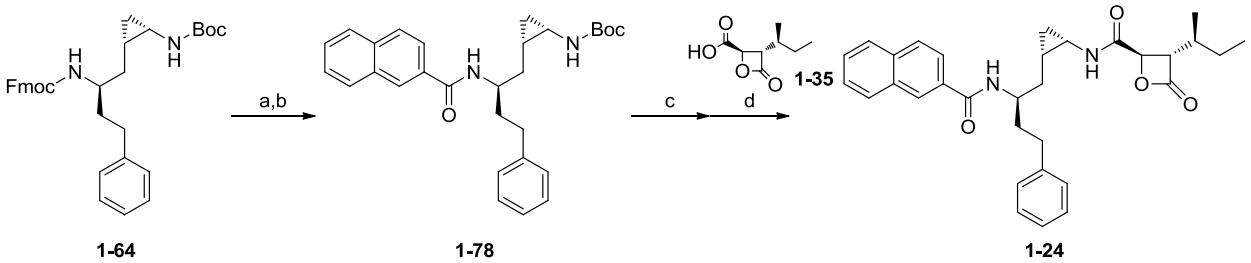
[a]Reagents and conditions: (a) RMgX, toluene, reflux; (b) RMgX, CH_2Cl_2; (c) HCl, THF/H_2O, 0 °C; (d) FmocOSu, Na_2CO_3, THF/H_2O; (e) HCl, AcOEt/MeOH, yields (4 steps) were as follows, respectively, **1-46** (R = Ph) 60%, **1-47** (R = Bn) 56%, **1-48** (R = phenethyl) 35%, **1-49** (R = phenylpropyl) 26%; (f) HCl, AcOEt/MeOH; (g) FmocOSu, Na_2CO_3, THF/H_2O, yields (3 steps) were as follows respectively, **1-52** (R = 1-naphthylethyl) 36%, **1-53** (R = 2-naphthylethyl) 48%; (h) DMP, CH_2Cl_2, yields (4 steps) were as follows respectively, **1-58** (R = 1-naphthylmethyl) 20%, **1-59** (R = 2-naphthylmethyl) 20%; (i) Pinnick ox.; (j) DPPA, Et_3N, CH_2Cl_2, 0 °C to rt; (k) *t*-BuOH, reflux, yields (4 steps for **1-62** – **1-65**, **1-68**, **1-69** and 3 steps for **1-66**, **1-67**) were as follows respectively, **1-62** (R = Ph) 70%, **1-63** (R = Bn) 74%, **1-64** (R = phenethyl) 70%, **1-65** (R = phenylpropyl) 62%, **1-66** (R = 1-naphthylmethyl) 64%, **1-67** (R = 2-naphthylmethyl) 64%, **1-68** (R = 1-naphthylethyl) 59%, **1-69** (R = 2-naphthylethyl) 52%; (l) K_2CO_3, MeOH; (m) Cbz-Ala-OH, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, yields (2 steps) were as follows respectively, **1-70** (R = Ph) 74%, **1-71** (R = Bn) 70%, **1-72** (R = phenethyl) 84%, **1-73** (R = phenylpropyl) 88%, **1-74** (R = 1-naphthylmethyl) 97%, **1-75** (R = 2-naphthylmethyl) 89%, **1-76** (R = 1-naphthylethyl) 94%, **1-77** (R = 2-naphthylethyl) 98%; (n) TFA, CH_2Cl_2; (o) **1-35**, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, yields (2 steps) were as follows respectively, **1-16** (R = Ph) 100%, **1-17** (R = Bn) 100%, **1-18** (R = phenethyl) 86%, **1-19** (R = phenylpropyl) 87%, **1-20** (R = 1-naphthylmethyl) 74%, **1-21** (R = 2-naphthylmethyl) 82%, **1-22** (R = 1-naphthylethyl) 76%, **1-23** (R = 2-naphthylethyl) 83%.

4. 二置換誘導体の合成

N 末端部に 2-ナフトイル基を有する標的化合物 **1-24** は、化合物 **1-64** から Scheme 1-6 に示すように合成した。

* Grignard 反応により導入したカルボキシ基部置換基の絶対立体配置は改良 Mosher 法[43]により決定した（実験の部参照）。

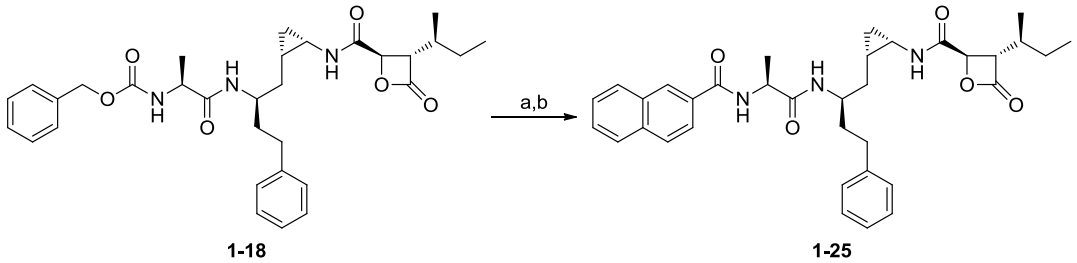
Scheme 1-6. 標的化合物 **1-24** の合成 [a]



[a]Reagents and conditions: (a) K_2CO_3, MeOH; (b) 2-naphthoyl chloride, Et_3N, CH_2Cl_2, 0 °C; (c) TFA, CH_2Cl_2; (d) **1-35**, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, 48% (4 steps).

N 末端部に 2-ナフトイルアラニル基を有する標的化合物 **1-25** は、化合物 **1-18** から Scheme 1-7 に示すように合成した。

Scheme 1-7. 標的化合物 **1-25** の合成 [a]



[a]Reagents and conditions: (a) Pd/C, H_2, TFA/THF, 0 °C; (b) 2-naphthoyl chloride, Et_3N, CH_2Cl_2 0 °C, 76% (2 steps).

第三節　ベラクトシン A シス型異性体の誘導体の生物活性

1. N 末端部置換誘導体のプロテアソーム阻害活性

合成した標的化合物のヒト 20S プロテアソーム ChT-L 活性に対する阻害活性を蛍光基質 Suc-LLVY-AMC を用いて測定した。N 末端部置換誘導体 **1-1** – **1-14** のプロテアソーム阻害活性を Table 1-1 に示す。*これらの N 末端部置換誘導体の IC_{50} は 25 – 300 nM にわたり、N 末端部置換基がプロテアソーム阻害活性に大きく影響していることが分かる。

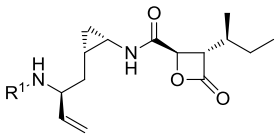
N 末端部にアシル基を導入した化合物 **1-1** – **1-4** のうち、立体的に小さなアシル基を有する **1-1** – **1-3** のプロテアソーム阻害活性は弱かったが (IC_{50} = 130 – 230 nM)、2-ナフトイル基を有する化合物 **1-4** はリード化合物である **0-10** と同等のプロテアソーム阻害活性 (IC_{50} = 60 nM) を示した。

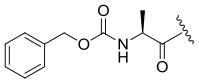
リード化合物 **0-10** のアラニン部を他のアミノ酸残基で置き換えた、化合物 **1-5** – **1-9** は、リード化合物と同等のプロテアソーム阻害活性を示し (IC_{50} = 28 – 57 nM)、アミノ酸側鎖構造はプロテアソーム阻害活性にあまり重要でないことが示唆された。

リード化合物 **0-10** のアラニン部アミノ基上の置換基を変換した化合物 **1-10** – **1-14** のうち、嵩高い疎水性置換基を有する化合物 **1-12** – **1-14** は強いプロテアソーム阻害活性 (IC_{50} = 25 – 79 nM) を示したが、置換基を持たない化合物 **1-10** (IC_{50} = 300 nM) 及び小さなアセチル基を有する化合物 **1-11** (IC_{50} = 230 nM) では、大きなプロテアソーム阻害活性の低下がみられた。

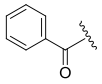
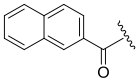
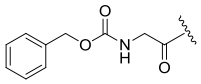
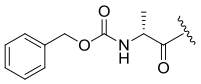
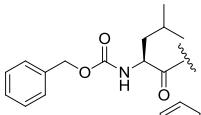
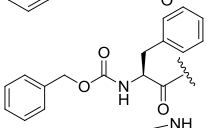
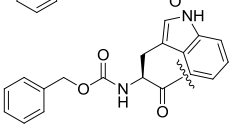
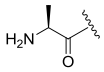
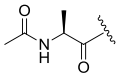
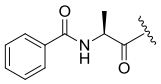
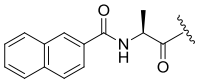
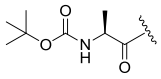
これらの N 末端部置換誘導体による系統的構造活性相関研究により、ベラクトシン誘導体のアラニン構造自体はプロテアソームとの相互作用においてあまり重要ではないが (**0-10** vs. **1-4** – **1-9**)、アミノ基上の疎水性置換基をプロテアソームの疎水性結合ポケットへと配置するためのリンカー部として機能していることが示唆された (**1-2** vs. **1-14**)。アラニン構造を持たない N 末端部に 2-ナフトイル基を有する化合物 **1-4** が強いプロテアソーム阻害活性を示したのは、2-ナフトイル基が大きいためプロテアソーム疎水性ポケットに達することが可能であるためと考えられる。また、化合物 **1-14** に示されるように、疎水性置換基としては、芳香族のみならず脂肪族置換基も効果的に相互作用することが分かった。これら一連の N 末端部置換誘導体の中では、N-(2-ナフトイル)アラニル基を有する化合物 **1-13** が最も強力なプロテアソーム阻害活性を示した (IC_{50} = 25 nM)。

Table 1-1. N 末端部置換誘導体 **1-1** – **1-14** のプロテアソーム阻害活性



compound number	R^1	ChT-L activity, IC_{50} [nM][a]
0-10		49 ± 9.5
1-1		230 ± 40
1-2		210 ± 25

* アッセイ系が異なるため、化合物 **0-10** とベラクトシン A の IC_{50} 値が序論で示した値よりも高くなっている。

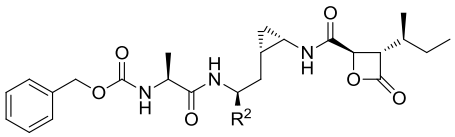
1-3		130 ± 13
1-4		60 ± 12
1-5		41 ± 11
1-6		57 ± 12
1-7		47 ± 9.2
1-8		30 ± 3.1
1-9		28 ± 2.6
1-10		300 ± 70
1-11		230 ± 35
1-12		79 ± 13
1-13		25 ± 1.7
1-14		77 ± 12
belactosin A		1440
bortezomib		4.5

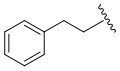
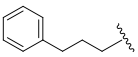
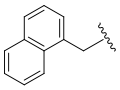
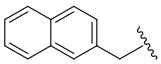
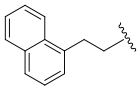
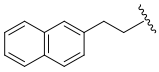
[a]Based on three experiments

2. カルボキシ基部置換誘導体のプロテアソーム阻害活性

カルボキシ基部置換誘導体 **1-15** – **1-23** のプロテアソーム阻害活性を Table 1-2 に示す。これらのカルボキシ基部置換誘導体の IC_{50} 値は 5.7 – 200 nM にわたり、カルボキシ基部置換基がプロテアソーム阻害活性に大きく影響することが分かる。リード化合物 **0-10** のビニル基をエチル基で置き換えた化合物 **1-15** は **0-10** と同等のプロテアソーム阻害活性 (IC_{50} = 38 nM) を示し、これら不飽和度の異なる二つの置換基が生物学的に等価であることが分かった。カルボキシ基部にフェニル基を有する化合物 **1-16** (IC_{50} = 90 nM) ではプロテアソーム阻害活性の低下がみられたが、フェニル基との間にアルキルリンカーを導入した化合物 **1-17** – **1-19** ではプロテアソーム阻害活性は大きく向上し、中でもフェネチル基を有する化合物 **1-18** は IC_{50} 値が 5.7 nM と、臨床で用いられているプロテアソーム阻害剤ボルテゾミブ (IC_{50} = 4.5 nM) と同等の強力なプロテアソーム阻害活性を示した。また、ナフタレン環を有する化合物 **1-20** – **1-23** では、対応するアルキルリンカー長の等しいベンゼン環を有する化合物に比べてプロテアソーム阻害活性が低下しており (**1-17** vs. **1-20** – **1-21**, **1-18** vs. **1-22** – **1-23**)、ナフタレン環よりもベンゼン環の方が効果的な相互作用が可能であることが分かった。

Table 1-2. カルボキシ基部置換誘導体 **1-15** – **1-23** のプロテアソーム阻害活性



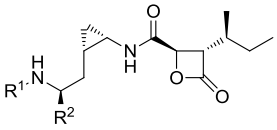
compound number	R^2	ChT-L activity, IC_{50} [nM][a]
0-10		49 ± 9.5
1-15		38 ± 11
1-16		90 ± 20
1-17		14 ± 1.2
1-18		5.7 ± 1.2
1-19		35 ± 9.1
1-20		130 ± 17
1-21		200 ± 44
1-22		63 ± 10
1-23		29 ± 7.8
belactosin A		1440
bortezomib		4.5

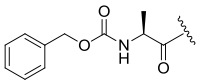
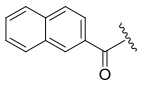
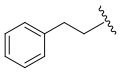
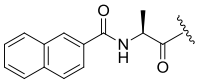
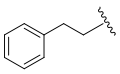
[a]Based on three experiments.

3. 二置換誘導体のプロテアソーム阻害活性

上述の構造活性相関研究から、カルボキシ基部にフェネチル基を有し、N 末端部に 2-ナフトイル基を有する化合物 **1-24** 及び N-(2-ナフトイル)アラニル基を有する化合物 **1-25** を設計・合成し、そのプロテアソーム阻害活性を評価した (Table 3)。いずれも対応するカルボキシ基部にビニル基を有する化合物に比べて強いプロテアソーム阻害活性を示した (**1-4** vs. **1-24**, **1-13** vs. **1-25**)。

Table 1-3. 二置換誘導体 **1-24** 及び **1-25** のプロテアソーム阻害活性



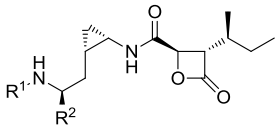
compound number	R^1	R^2	ChT-L activity, IC_{50} [nM][a]
0-10			49 ± 9.5
1-24			33 ± 8.4
1-25			10 ± 1.0
belactosin A			1440
Bortezomib			4.5

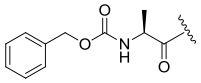
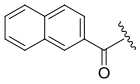
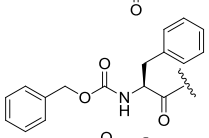
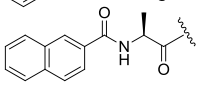
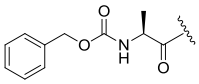
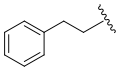
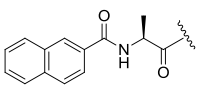
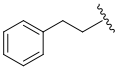
[a]Based on three experiments

4. ベラクトシン誘導体の C-L 活性, T-L 活性及び他のプロテアーゼに対する阻害活性

合成した標的化合物のうち、化合物 **1-4**, **1-8**, **1-13**, **1-18**, **1-25** についてヒト 20S プロテアソーム C-L 活性及び T-L 活性に対する阻害活性を、それぞれ蛍光基質 Ac-*n*LP*n*LD-AMC*及び Ac-RLR-AMC を用いて評価した。Table 1-4 に示すように、ベラクトシン誘導体は ChT-L 活性選択的な阻害剤であり、600 nM 及び 1300 nM 以上の高濃度においてのみ C-L 及び T-L 活性を阻害することが分かった。

Table 1-4. ChT-L, C-L 及び T-L 活性に対する阻害活性と細胞増殖抑制作用



compound number	R^1	R^2	IC_{50} [nM][a]			cell growth IC_{50} [μM]
			ChT-L activity	C-L activity	T-L activity	HCT116
0-10			49 ± 9.5	1600	> 3000	0.91
1-4			60 ± 1.2	670	1900	11.2
1-8			30 ± 3.1	1780	2190	4.16
1-13			25 ± 1.7	690	1320	2.61
1-18			5.7 ± 1.2	2420	1920	1.82
1-25			10 ± 1.0	> 3000	> 3000	0.79
bortezomib			4.5	130	1880	0.01

[a]Based on three experiments

* *n*L はノルロイシンを表す。

ボロン酸型のプロテアソーム阻害剤であるボルテゾミブは、多くのセリンプロテアーゼに対して阻害活性を示すことが報告されており、これが主要な用量制限因子である末梢神経障害を引き起こす原因となっていると考えられている。[44]そこで、最も強力なプロテアソーム阻害活性を示した化合物 **1-18** について他のプロテアーゼに対する阻害活性を調べた。Figure 1-5 に示すように、化合物 **1-18** はシステインプロテアーゼであるカスパーゼ 3、カテプシン B 及びセリンプロテアーゼであるトリプシンに対して全く阻害活性を示さず、プロテアソームに対して高度に選択的な阻害剤であることが分かった。

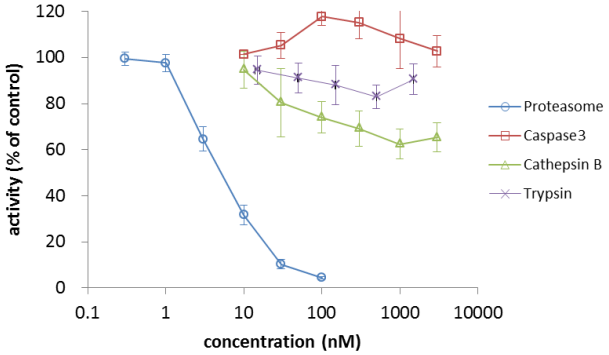


Figure 1-5. 化合物 **1-18** のカスパーゼ 3、カテプシン B 及びトリプシンに対する阻害活性

5. ベラクトシン誘導体の免疫プロテアソーム阻害活性

近年、免疫プロテアソーム阻害活性の細胞増殖抑制作用や免疫抑制作用における重要性が報告されており、[4a, 29]プロテアソーム阻害剤の免疫プロテアソーム阻害活性が注目されている。しかしながら、ベラクトシン誘導体の免疫プロテアソーム阻害活性についてはこれまでに報告がなかった。そこで、ベラクトシン A と最も強力なプロテアソーム阻害活性を示した化合物 **1-18** についてβ5i サブユニットに対する阻害活性を Kuhn らにより報告された active-site ELISA 法[45]により評価した。コントロールとしては、それぞれ非選択的、β5 選択的、β5i 選択的なプロテアソーム阻害剤であるボルテゾミブ、ラクタシスチン、PR-957 を用いた。[31a, 31e]Figure 1-6 に示すように、ベラクトシン A 及び化合物 **1-18** はβ5 及びβ5i サブユニットをほぼ等しく阻害しており、ボルテゾミブと同様に非選択的阻害剤であることが分かった。これは、基質 P'領域結合部に結合する化合物の免疫プロテアソーム阻害活性の初めての報告である。

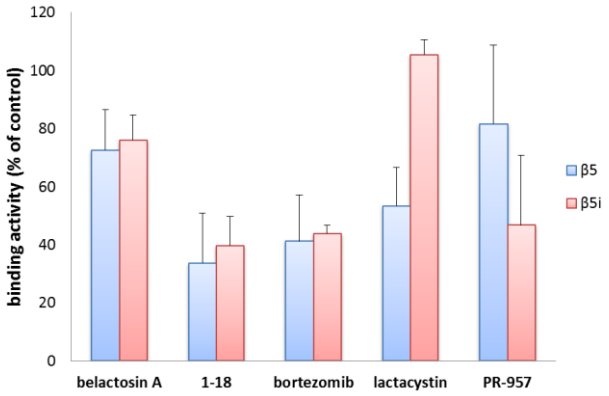


Figure 1-6. ベラクトシン A 及び化合物 **1-18** の免疫プロテアソーム阻害活性

6. ベラクトシン誘導体の細胞増殖抑制作用

合成した標的化合物のうち、化合物 **1-4**, **1-8**, **1-13**, **1-18**, **1-25** について HCT116 細胞に対する細胞増殖抑制作用を調べた。Table 1-4 に示すように、これらの化合物は IC_{50} 値 0.79 – 11.2 μM で、細胞増殖抑制作用を示した。更に、これらの化合物で処理した HCT116 細胞の細胞周期を解析したところ、ボルテゾミブと同様に細胞周期の進行を G2/M 期で停止させることが分かった (Figure 1-7)。*

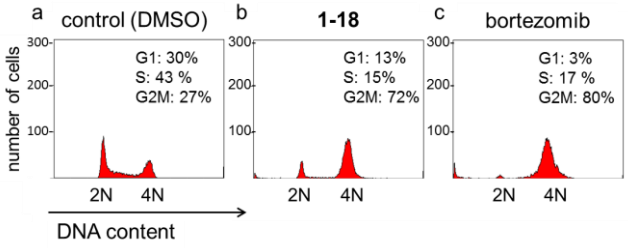


Figure 1-7. 化合物 **1-18** で処理した HCT116 細胞の細胞周期解析

また、これらの化合物の細胞系でのプロテアソーム阻害活性をウエスタンブロット法により解析した。がん抑制遺伝子産物である p53 はプロテアソームにより分解されるタンパク質の一つである。[18, 46]Figure 1-8 に示すように、一連のベラクトシン誘導体で処理した HCT116 細胞では p53 の蓄積が見られた。また、これらの細胞ではアポトーシスのマーカーである poly(ADP-ribose) polymerase (PARP) の切断による cleaved PARP の蓄積がみられ、アポトーシス経路が活性化されていることが分かった。

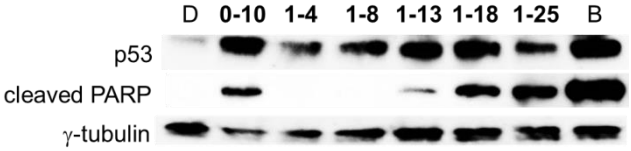


Figure 1-8. ベラクトシン誘導体で処理した HCT116 細胞中の p53 及び cleaved PARP の検出 (D, DMSO; B, bortezomib)

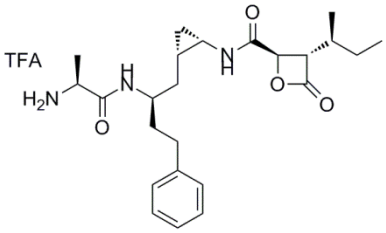
以上のことから、ベラクトシン誘導体は細胞系でプロテアソームを阻害し、G2/M 期で細胞周期の進行を停止させると共にアポトーシスを引き起こすことで細胞増殖抑制作用を示していることが明らかになった。

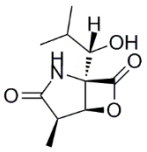
7. ベラクトシン誘導体の化学的及び生物学的安定性

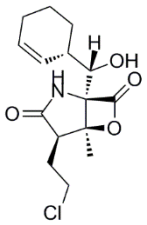
一連のベラクトシン誘導体は水溶性が低く、水溶液中での安定性評価が困難であったため、化合物 **1-18** の Cbz 基を除去した化合物 **1-79**†について、0.1 M triethylammonium acetate (TEAA) 緩衝液 (pH 7.4) 及びヒト AB 型血清中での 37 °C における半減期を、HPLC で残存率の経時変化を追跡して測定した。Table 1-5 に示すように、化合物 **1-79** は pH 7.4 の TEAA 緩衝液中で半減期が 10 時間であり、他のβ-ラクトン系のプロテアソーム阻害剤であるオオムラライドやサリノスポラミド A に比べて化学的にかなり安定であることが分かった。[47]しかしながら、ヒト AB 型血清中での半減期は 2.3 分と極めて短く、生理的条件下では極めて不安定であることが分かった。

* 他の化合物についても同様の結果が得られた（実験の部参照）。
† 合成については実験の部を参照

Table 1-5. ベラクトシン誘導体 **1-79** の 0.1 M TEAA 緩衝液 (pH 7.4) 及びヒト AB 型血清中での半減期







	1-79	omuralide	salinosporamide A
$t_{1/2}$ in pH 7.4 buffer at 37 °C	10 h	13 min[a]	56 min[a]
$t_{1/2}$ in human AB serum at 37 °C	2.3 min		

[a]From ref. 47

8. ベラクトシン誘導体のプロテアソーム阻害作用の可逆性

ベラクトシン誘導体はプロテアソーム活性残基である N 末端 Thr 残基のヒドロキシ基をアシル化することでプロテアソームに対する共有結合性阻害剤として働くが、もし生じたエステル結合が加水分解されるのであれば、その阻害作用は可逆的であると考えられる (Figure 1-9)。これまで、ベラクトシン誘導体のプロテアソーム阻害作用が可逆的であるか不可逆的であるかについては知られていなかった。

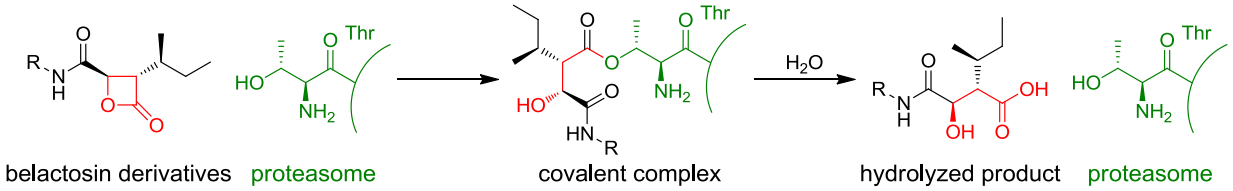


Figure 1-9. ベラクトシン誘導体のプロテアソーム阻害様式とプロテアソームの再賦活化

そこで、ベラクトシン誘導体のプロテアソーム阻害活性の可逆性を調べるため、プロテアソームを化合物 **1-18** とプレインキュベーションした後、半透膜を用いて化合物を除去し、除去操作前後でのプロテアソーム活性を評価した。コントロールとして、可逆的プロテアソーム阻害剤であるボルテゾミブ及び不可逆的プロテアソーム阻害剤であるカルフィルゾミブについても同様に実験を行った。[25a, 31e, 47]Figure 1-10 に示すように、化合物 **1-18** で処理した場合、除去操作後にプロテアソームの再賦活化がみられ、プロテアソームとの間に形成したエステル結合がゆっくりと加水分解されていることが示唆された。以上の実験により、ベラクトシン誘導体は、ボルテゾミブほど解離速度が速くはないものの、可逆的なプロテアソーム阻害剤であることが分かった。

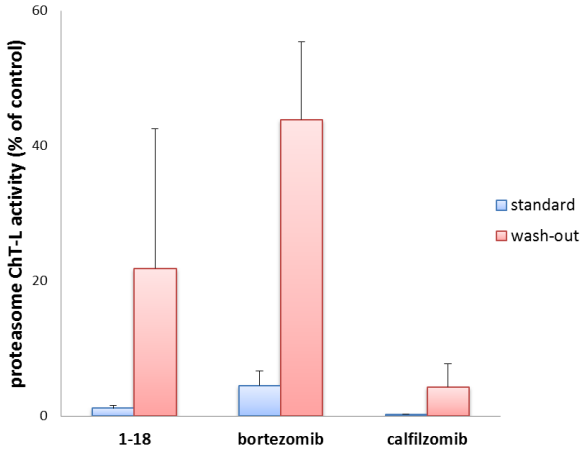


Figure 1-10. プロテアソーム阻害作用の可逆性試験

9. ベラクトシン誘導体の細胞増殖抑制作用についての考察

上述のように、一連のベラクトシン誘導体は、プロテアソーム阻害活性に基づき細胞増殖抑制作用を示す。しかしながら、ボルテゾミブがプロテアソーム阻害活性 (IC_{50} = 4.5 nM) とほぼ同等の細胞増殖抑制作用 (IC_{50} = 10 nM) を示すのに対して、ベラクトシン誘導体の細胞増殖抑制作用 (IC_{50} = 790 – 11200 nM) はプロテアソーム阻害活性 (IC_{50} = 5.7 – 60 nM) に比べて著しく低い (Table 1-4)。先の実験結果から、これは、ベラクトシン誘導体が生理学的条件下で極めて不安定であり、且つベラクトシン誘導体のプロテアソーム阻害活性が可逆的であるために、細胞系でプロテアソームを持続的に阻害することができないためと考えられる。*

10. ベラクトシン誘導体とプロテアソームの共結晶の X 線結晶構造解析

最も高いプロテアソーム阻害活性を示した化合物 **1-18** とプロテアソームの共結晶について X 線結晶構造解析を行い、共有結合状態における結合様式を検証した。Figure 1-11 に示すように、化合物 **1-18** はこれまでに報告されているホモベラクトシン C 誘導体と同様に、[33]プロテアソーム基質 P'領域結合部に結合していることが分かった。また、β-ラクトン部は、プロテアソームとの共有結合形成により開環しており、歪みを解消するように配座が変化していることが分かる。これは、シクロプロパン構造を有するベラクトシン誘導体の X 線結晶構造解析の初めての例である。

* ベラクトシン誘導体よりも不安定であったサリノスポラミド A はプロテアソーム阻害作用が不可逆的であるためにプロテアソーム阻害活性 (IC_{50} = 0.7 nM) とほぼ同等の強力な細胞増殖抑制作用 (IC_{50} = 9.5 nM) を示すことが報告されている。[48]

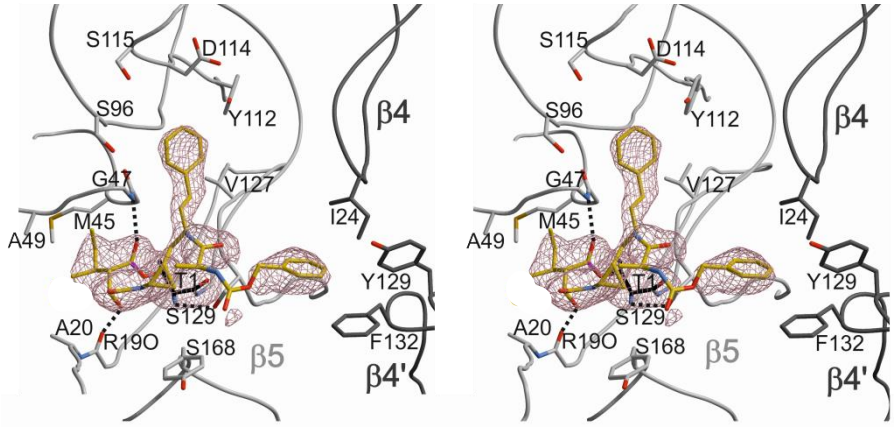


Figure 1-11. 化合物 **1-18** のβ5 サブユニットへの結合様式 (PDB code 4J70)

以上、筆者はベラクトシン A シス型異性体の系統的構造活性相関研究により、基質 P'領域結合部における構造活性相関を明らかにすると共に、ボルテゾミブと同等の強力なプロテアソーム阻害活性を有する誘導体 **1-18** を見出した。更に、ベラクトシン誘導体は共有結合性阻害剤でありながら、そのプロテアソーム阻害作用は可逆的であることを明らかにし、ベラクトシン誘導体の細胞増殖抑制作用がプロテアソーム阻害活性に比べて低い理由について考察した。

第二章　ベラクトシン A シス型異性体の誘導体の活性配座解析[49]

第一節　共有結合性化合物の活性配座解析

1. 共有結合性化合物の非共有結合状態

共有結合性化合物は、標的タンパク質と安定な共有結合を形成することで、強力かつ持続的な作用を示すことから、多くの共有結合性化合物が臨床で用いられている。[50]共有結合性阻害剤とその標的タンパク質の結合過程の概略を Figure 2-1 に示す。共有結合性阻害剤が標的タンパク質と共有結合を形成するためには、はじめに、標的タンパク質に非共有結合性相互作用によって認識され、非共有結合性複合体 (P･I) を形成する必要がある。次いで、共有結合性阻害剤の反応点と標的タンパク質の反応点が近接した高エネルギーな遷移状態 (P‥I) を経由して共有結合性複合体 (P-I) の形成に至る。[50a]

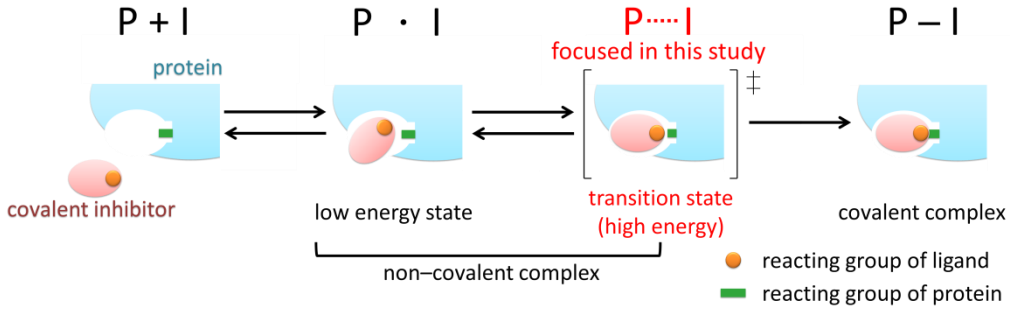


Figure 2-1. 共有結合性化合物の標的タンパク質との結合過程

共有結合性阻害剤が標的タンパク質と安定な共有結合を形成する場合、*非共有結合性相互作用は遷移状態を含めた非共有結合性複合体を安定化することで阻害活性に寄与していると考えられる。†この場合、共有結合性阻害剤の活性配座は非共有結合状態における結合配座であり、構造情報に基づいた理論的な構造活性相関研究を展開するためには、非共有結合性複合体の構造を解析する必要がある。安定な非共有結合性複合体 (P･I) を形成する化合物の場合には、非共有結合性化合物の場合と同様にドッキングシミュレーションや X 線結晶構造解析による解析が可能である。一方、非共有結合性複合体 (P･I) が不安定であり、その阻害活性が共有結合形成能に高度に依存している化合物‡では、活性配座が高エネルギーで不安定な遷移状態 (P‥I) 近傍における結合配座となるため、従来のドッキングシミュレーションや X 線結晶構造解析による解析は困難である。従って、共有結合性化合物の遷移状態近傍における結合様式を解析する手法の開発は、より合理的な共有結合性阻害剤の開発を可能にし、創薬化学の発展に寄与するものと考えられる。

2. ベラクトシン A シス型異性体の誘導体の活性配座

第一章において述べたように、筆者はベラクトシン A シス型異性体の系統的構造活性相関研究により、強力なプロテアソーム阻害活性を有する誘導体 **1-18** を見出し、その共有結合状態におけるプロテアソーム結合

---

* 標的タンパク質と反応してヘミアセタールを形成する共有結合性阻害剤のように、共有結合形成が可逆的な場合には、共有結合状態においても非共有結合性相互作用は重要な役割を果たすと考えられる。
† 本論文においては簡単のため、遷移状態も非共有結合状態に含める。
‡ 共有結合性化合物が標的タンパク質と不可逆な共有結合を形成する場合、その阻害活性は非共有結合性複合体の安定性と共有結合形成能（共有結合形成の活性化エネルギーの大きさ）によって決まる。従って、共有結合性化合物と標的タンパク質の非共有結合性複合体が阻害活性を示すのに十分な安定性を有していない場合、阻害活性は共有結合形成能に大きく依存することとなる。

配座を X 線結晶構造解析により明らかにした。しかしながら、結晶中における熱運動の大きさの指標となる *B*-factor[51]が共有結合状態の **1-18** 構造部において高く、とりわけ構造活性相関研究よりプロテアソーム阻害活性に重要であることが明らかな N 末端部及びカルボキシ基部を含む部分では 80 Å^2 以上と極めて高かった (Figure 2-2)。従って、共有結合状態においてはこれら二つの疎水性置換基を含む領域は、プロテアソーム阻害活性に極めて重要であるにも関わらず自由度が高く、プロテアソームと効果的に相互作用していないことが示唆された。

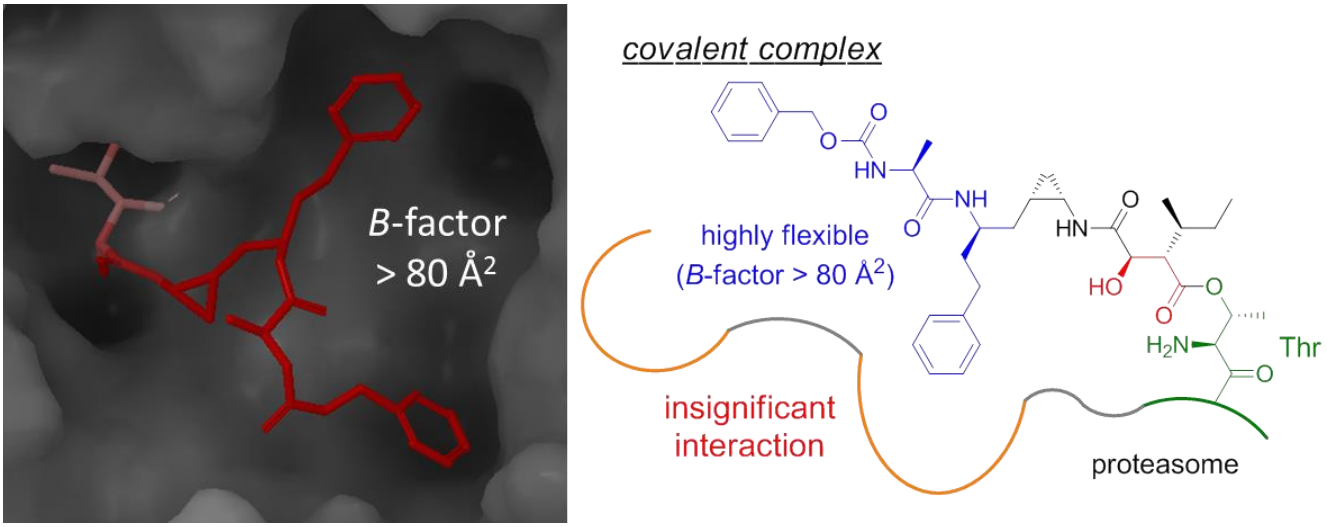


Figure 2-2. 化合物 **1-18** とプロテアソームの共結晶の X 線結晶構造（結晶構造中の **1-18** は *B*-factor の値に対応して色付けられており、値が高いほど赤く、低いほど青くなる）

ベラクトシン誘導体がプロテアソームと共有結合を形成する際には、歪んだβ-ラクトン環の開環を伴うため、歪みの解消を駆動力として、β-ラクトン部を中心に大きな構造変化が起こると推定される。従って、N 末端部及びカルボキシ基部置換基を含む領域は非共有結合状態を安定化することで、プロテアソーム阻害活性に寄与しているが、一度共有結合が形成されると、β-ラクトン部の開環により引き起こされた構造変化によってプロテアソームとの相互作用が維持できなくなっているものと理解できる。

ベラクトシン誘導体のプロテアソーム阻害活性における非共有結合性複合体 (P･I) の寄与を調べるため、化合物 **1-18** のβ-ラクトン部をβ-ラクタムで置き換えた化合物 **2-1** を設計・合成し、そのプロテアソーム阻害活性を評価した (Figure 2-3)。[*]化合物 **2-1** と化合物 **1-18** の高い構造類似性のため、これらの化合物とプロテアソームとの非共有結合性複合体 (P･I) の安定性は同程度と考えられるが、[†]β-ラクタムはβ-ラクトンに比べて十分に化学的に安定であるため、化合物 **2-1** の共有結合形成能は化合物 **1-18** と比べて著しく低い。Figure 2-3 に示すように、化合物 **1-18** が強力なプロテアソーム阻害活性 (IC_{50} = 5.7 nM) を示すのに対し、そのβ-ラクタム共役体である化合物 **2-1** は全くプロテアソーム阻害活性を示さなかった (IC_{50} > 10000 nM)。このことから、ベラクトシン誘導体とプロテアソームの非共有結合性複合体 (P･I) は、プロテアソーム阻害活性を示すほどには安定ではなく、ベラクトシン誘導体のプロテアソーム阻害活性はその共有結合形成能に完全に依存することが示唆された。

[*] 合成については実験の部を参照。
[†] 水素結合アクセプターから水素結合ドナーへの変換は大きな構造変化とみなし得るが、プロテアソームがプロテアーゼであることを考えると、ラクタムとしたことにより大きく不利な相互作用が生じるとは考えにくい。

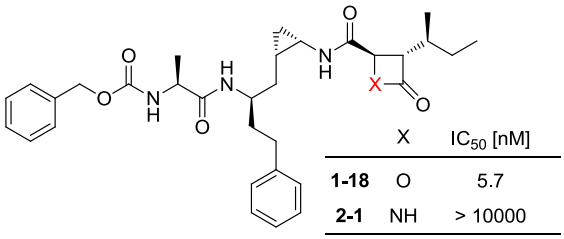


Figure 2-3. 化合物 **1-18** のβ-ラクタム類縁体 **2-1** の構造とプロテアソーム阻害活性

上述の結果より、Figure 2-4 に示すように、ベラクトシン誘導体の N 末端部及びカルボキシ基部の二つの疎水性置換基は、プロテアソームとの共有結合形成の遷移状態を安定化し、共有結合の形成を促進することでプロテアソーム阻害活性に寄与しているものと考えられる。従って、ベラクトシン誘導体の活性配座は、共有結合形成の遷移状態近傍における結合配座であると考えられる。

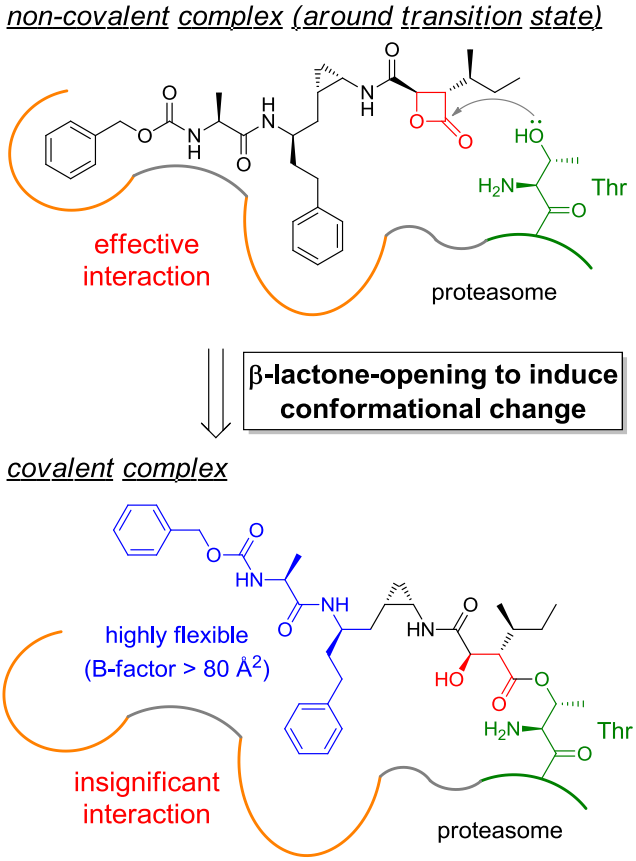


Figure 2-4. 化合物 **1-18** のプロテアソームとの共有結合形成に伴う仮想の結合状態の変化

以上の考察より、筆者はベラクトシン誘導体のプロテアソームとの共有結合形成の遷移状態近傍における結合配座を明らかにすることを計画した。活性配座探索にしばしば用いられる手法である、配座制限誘導体の合成による有機化学的アプローチと、[52]遷移状態近傍における結合配座解析のための、新たなドッキングコンセプトに基づく計算化学的アプローチによって、実験化学及び理論化学の両面からベラクトシン誘導体の活性配座を検証することとした。

第二節　活性配座解析を指向した配座制限誘導体の設計と配座解析

1. シクロプロパン歪みを利用した配座制限誘導体の設計

シクロプロパンのそれぞれの炭素原子はエクリプス配座に固定されている。従って、シス配置にある置換基間には 1,3-アリル歪み様の大きな立体反発があり、これをシクロプロパン歪みと呼ぶ。[39c, 53]従って、Figure 2-5 に示すように、シクロプロパン上の置換基の配座はシクロプロパン歪みが最小となるように制御される。

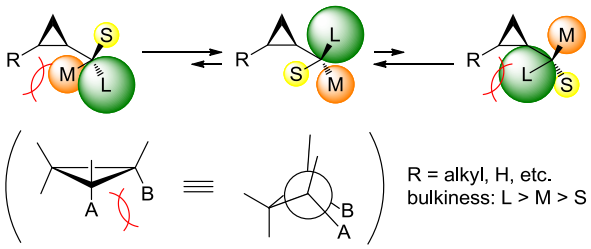


Figure 2-5. シクロプロパン歪み

Figure 2-6a に示すように、化合物 **1-18** においては、シクロプロパン環の炭素原子 (C1) と隣接位炭素原子 (C1') 間の結合の回転は、シクロプロパン歪みの大きな配座 C を避けるように制限され、プロテアソーム阻害活性に重要な N 末端部及びカルボキシ基部を含む領域 X が、シクロプロパン環と反対側を向く配座 A (*anti*) と、同じ側を向く配座 B (*syn*) が安定配座であると考えられる。これら二つの想定される安定配座間では、活性に必須のβ-ラクトン部と領域 X の三次元的空間配置が大きく異なるため、いずれか一方が活性配座であると予想される。

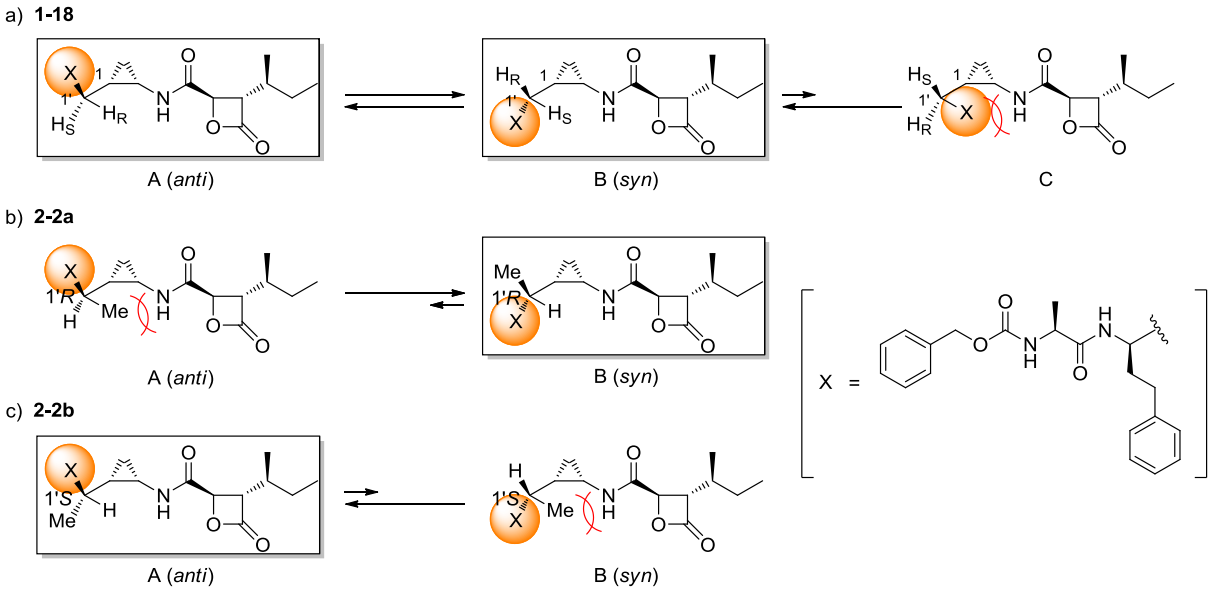


Figure 2-6. 化合物 **1-18** 及びその配座制限誘導体 **2-2a** 及び **2-2b** の推定安定配座

それに対して、Figure 2-6b 及び Figure 2-6c に示すように、隣接位炭素原子 (C1') 上の *pro-R* の水素原子をメチル基で置換した化合物 **2-2a** では配座 A (*anti*) が、*pro-S* の水素原子をメチル基で置換した化合物 **2-2b** では配座 B (*syn*) が、それぞれ導入したメチル基に起因する大きなシクロプロパン歪みによって不安定となる。

このため、化合物 **2-2a** 及び **2-2b** の安定配座はそれぞれ配座 B (*syn*) 及び配座 A (*anti*) となる。活性配座に制限された誘導体が活性を保持する一方で、活性配座とは異なる配座に制限された誘導体では大きく活性が低下すると考えられる。従って、これらの配座制限誘導体を合成し、そのプロテアソーム阻害活性を評価することで、活性配座における領域 X とシクロプロパン環の位置関係が *syn* または *anti* のいずれであるか明らかにすることが可能であると考えられる。

Figure 2-7 に示すように、一連のベラクトシン A シス型異性体の活性配座に関する知見を得るため、化合物 **1-18**, **1-24** 及び **1-25** の配座制限誘導体 **2-2**a – **2-4a** 及び **2-2b** – **2-4b** を設計した。

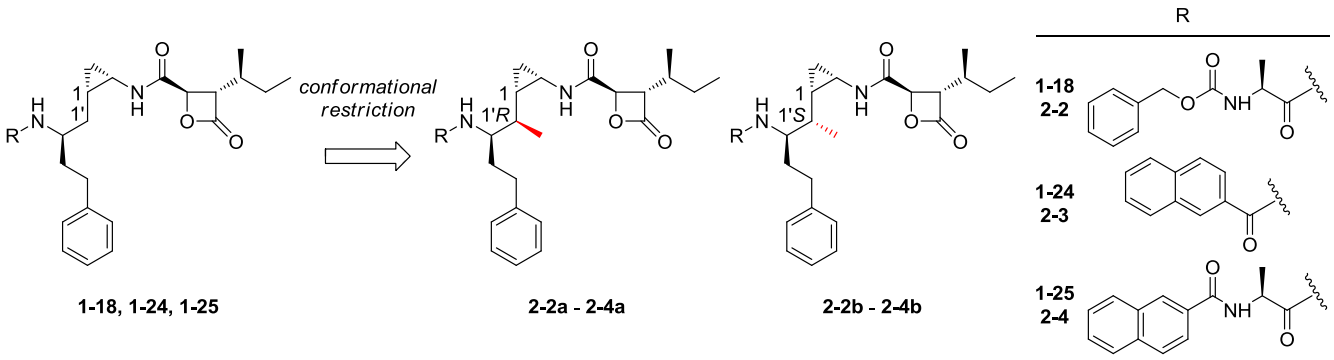


Figure 2-7. 設計したシクロプロパン歪みを利用した配座制限誘導体

2. 分子力学計算による配座制限誘導体の配座解析

MacroModel 9.9 (Schrödinger, LLC) を用いた分子力学計算によって、ベラクトシン A シス型異性体の誘導体 **1-18** とその配座制限誘導体 **2-2a** 及び **2-2b** の立体配座を解析した (Figure 2-8)。化合物 **1-18** については推定通り Figure 2-8a に示す *anti* 及び *syn* 配座の二つの安定配座が得られ、*anti* 配座が *syn* 配座に比べて僅かに安定であることが分かった (ΔE = 1.2 kcal/mol)。また、先に述べたように、これら二つの配座異性体間では、プロテアソーム阻害活性に必須なβ-ラクトン部 (緑色) と N 末端部及びカルボキシ基部を含む領域 X (橙色) の三次元的空間配置が大きく異なることが見て取れる。また、配座制限誘導体 **2-2a** 及び **2-2b** についても Figure 2-8b に示すように、それぞれ推定通り *syn* 及び *anti* 配座が最安定配座として得られ、*anti* 及び *syn* 配座異性体とのエネルギー差がそれぞれ 2.4 kca/mol 及び 7.1 kcal/mol であることが分かった。[*]

以上、分子力学計算による一連の誘導体の配座解析結果は、シクロプロパン歪みを利用した配座制限誘導体の設計を支持するものであり、これらの配座制限誘導体を合成することとした。

[*] 化合物 **1-24**, **1-25** とその配座制限誘導体 **2-3a** – **2-4a** 及び **2-3b** – **2-4b** についても同様の計算結果が得られた（実験の部参照）。

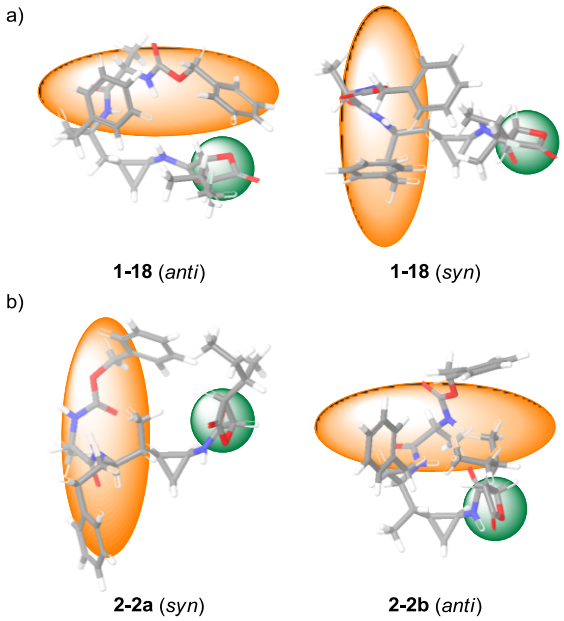


Figure 2-8. 分子力学計算により得られた化合物 **1-18** とその配座制限誘導体 **2-2a** 及び **2-2b** の安定配座

3. 配座制限誘導体の NOE 実験及び X 線結晶構造解析による配座解析

合成した配座制限誘導体 **2-2a** 及び **2-2b**（配座制限誘導体の合成については本章第五節参照）とこれらの親化合物である **1-18** について重クロロホルム中における最安定配座を NOE 実験により調べた (Figure 2-9)。**2-2b** については、H_d の照射で H_a において NOE が観測されたことから、*syn* 配座をとっていることが示唆された。一方で **2-2c** については、H_b 及び H_c の照射で H_a において NOE が観測されたことから *anti* 配座をとっていることが示唆された。更に、親化合物である **1-18** については H_a の照射で H_b 及び H_c において NOE が観測されたことから、*anti* 配座をとっていることが示唆された。これらの NOE 実験による配座解析結果は分子力学計算により予想された最安定配座を支持する。

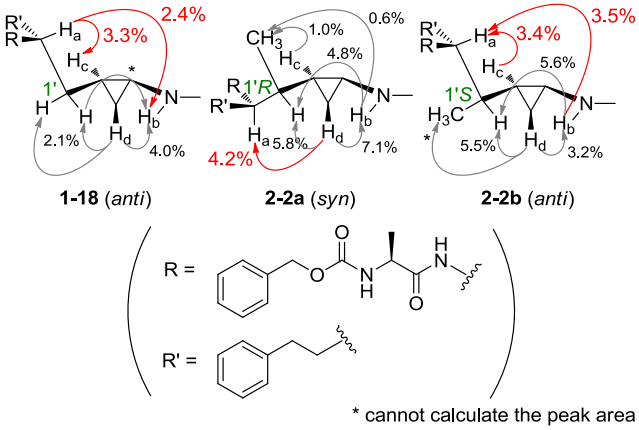


Figure 2-9. NOE 測定による配座制限誘導体 **2-a** 及び **2-b** とその親化合物 **1-18** の配座解析

更に、配座制限誘導体 **2-2b** については単結晶が得られ、X 線結晶構造解析により結晶中 *anti* 配座をとっていることが分かった (Figure 2-10)。

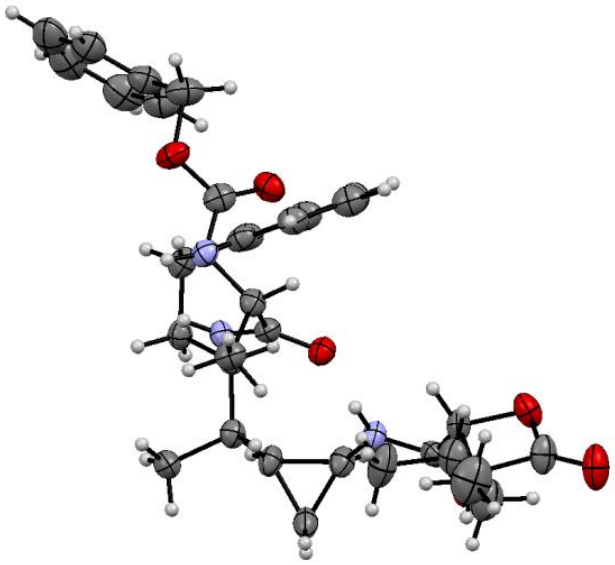


Figure 2-10. 配座制限誘導体 **2-2b** の X 線結晶構造解析

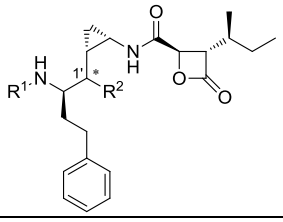
以上、これらの NOE 測定及び X 線結晶構造解析による配座解析により、設計・合成した一連の配座制限誘導体が実際に分子力学計算により予想された最安定配座を有することが明かとなり、期待通りシクロプロパン歪みを利用した配座制御法が機能していることが確かめられた。従って、一連の配座制限誘導体のプロテアソーム阻害活性を評価することで、ベラクトシン A シス型異性体の誘導体の活性配座に関する知見が得られるものと期待される。

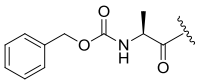
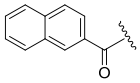
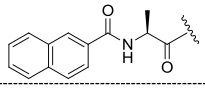
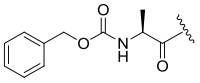
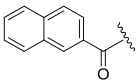
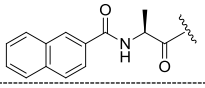
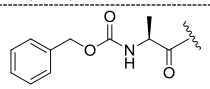
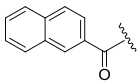
第三節　配座制限誘導体の生物活性

1. 配座制限誘導体のプロテアソーム阻害活性及び細胞増殖抑制作用

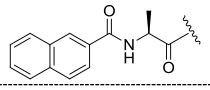
合成した配座制限誘導体 **2-2a** – **2-4a** 及び **2-2b** – **2-4b** のヒト 20S プロテアソーム ChT-L 活性に対する阻害活性を蛍光基質 Suc-LLVY-AMC を用いて測定した。Table 2-1 に示すように、これらの配座制限誘導体のプロテアソーム阻害活性は、それぞれ対応するシクロプロパン隣接位炭素原子 (C1') 上にメチル基を持たない親化合物 **1-18**, **1-24** 及び **1-25** のプロテアソーム阻害活性よりも低かった。しかしながら、C1'-立体異性体間でプロテアソーム阻害活性を比較すると、全ての異性体間で 1'*R*-異性体が対応する 1'*S*-異性体よりも有意に高いプロテアソーム阻害活性を有することが分かった。従って、ベラクトシン A シス型異性体の N 末端部及びカルボキシ基部を含む領域は、活性配座において 1'*R*-異性体同様、*syn* 配座であると考えられる。[*]

Table 2-1. 配座制限誘導体 **2-2a** – **2-4a** 及び **2-2b** – **2-4b** のプロテアソーム阻害活性及び細胞増殖抑制作用



compound number	R^1	R^2	conformation	proteasome IC_{50} (nM)[a]	cell growth IC_{50} (μM)[a]		
				ChT-L activity	HS-Sultan	KB	HCT116
1-18		H	*syn/anti*	5.7 ± 1.2	0.87	2.6	1.8
1-24				33 ± 8.4			
1-25				10 ± 1.0			
2-2a		*R*-CH_3	*syn*	47 ± 2.9	0.31	1.7	0.63
2-3a				140 ± 36			
2-4a				42 ± 5.8			
2-2b		*S*-CH_3	*anti*	280 ± 85	1.8	> 10	> 10
2-3b				750 ± 100			

[*] 1'*R*-異性体のプロテアソーム阻害活性がそれぞれ対応する親化合物のプロテアソーム阻害活性と比べて低かった理由としては二通り考えられる。一つは、導入した C1'-メチル基がプロテアソームと相互作用する際に立体障害となっている可能性である。もう一つは、活性配座が同じ *syn* 配座でも 1'*R*-異性体の最安定配座に一致していない可能性である。配座制限誘導体ではメチル基を導入することで配座を制御しているため、最安定配座と異なる場合のエネルギー損失が大きい。

2-4b		630 ± 47
belactosin A		1440

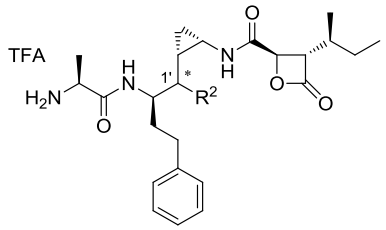
[a]Based on three experiments.

また、合成した配座制限誘導体のうち、**2-2a** 及び **2-2b** について HS-Sultan, KB 及び HCT116 細胞に対する細胞増殖抑制作用を測定した。Table 2-1 に示すように、*anti* 配座に制御した配座制限誘導体 **2-2b** の細胞増殖抑制作用は、親化合物である **1-18** に比べて低かった。これは親化合物に比べて著しく低いプロテアソーム阻害活性 (IC_{50} = 280 nM) により説明される。しかしながら、*syn* 配座に制御した配座制限誘導体 **2-2a** は、そのプロテアソーム阻害活性 (IC_{50} = 47 nM) が親化合物 **1-18** (IC_{50} = 5.7 nM) より有意に低いにも関わらず、全ての細胞株に対して **1-18** と同等あるいはより強力な細胞増殖抑制作用を示した。

2. 配座制限誘導体の化学的及び生物学的安定性

一連の配座制限誘導体は水溶性が低く、水溶液中での安定性評価が困難であったため、それぞれ化合物 **2-2a** 及び **2-2b** の Cbz 基を除去した水溶性誘導体 **2-5a** 及び **2-5b** について、0.1 M triethylammonium acetate (TEAA) 緩衝液 (pH 7.4) 及びヒト AB 型血清中での 37 °C における半減期を、HPLC で残存率の経時変化を追跡して測定した (Table 2-2)。[*]興味深いことに、**2-5a** 及び **2-5b** は化合物 **1-79** に比べて化学的、生物学的に安定であり、C1'-メチル基の導入による配座制御によって安定性が改善された可能性が示唆された。配座制限誘導体 **2-2a** が親化合物 **1-18** よりもプロテアソーム阻害活性が低いにも関わらず、より強力な細胞増殖抑制作用を示したのは細胞系での安定性の違いによるのかもしれない。

Table 2-2. 配座制限誘導体 **2-5a** 及び **2-5b** の 0.1 M TEAA 緩衝液 (pH 7.4) 及びヒト AB 型血清中での半減期



compound number	R^2	conformation	$t_{1/2}$ at 37 °C	
			0.1 M TEAA buffer (h)	human AB serum (min)
1-79	H	*syn*/*anti*	10	2.3
2-5a	*R*-CH_3	*syn*	14	4.2
2-5b	*S*-CH_3	*anti*	21	4.7

[*] 化合物 **2-5a** 及び **2-5b** の合成については実験の部を参照。

第四節　遷移状態近傍における結合様式のドッキングによる解析

1. 遷移状態近傍における結合様式解析のためのドッキングコンセプト

上述のように、ベラクトシン誘導体はプロテアソームとの共有結合形成に伴うβ-ラクトン環の開環により、歪みの解消を駆動力としてβ-ラクトン部を中心に大きな配座変化が起こるため、X 線結晶構造解析により解析した共有結合状態における結合様式から遷移状態近傍における結合様式を推測するのは困難である。そこで、Figure 2-11 に概略を示した新たなドッキングコンセプトに基づく計算化学的手法によって遷移状態近傍における結合様式を解析することを計画した。

共有結合性阻害剤と標的タンパク質の共有結合形成の遷移状態近傍において、共有結合性阻害剤及び標的タンパク質の反応点の原子間距離はファンデルワールス半径の和よりも小さい。通常のドッキングプログラムは安定な非共有結合性複合体 (P･I) を予測するものであるため、このような原子間距離が極めて近接した部分を含む不安定な遷移状態近傍における複合体 (P‥I) を予測することはできない。従って、共有結合性阻害剤を単純にアポタンパク質 (Figure 2-11B) に対してドッキングすると、安定な非共有結合性複合体 (P･I) (Figure 2-11C) が計算結果として得られ、遷移状態における結合様式を解析することは出来ない。そこで、筆者は共有結合性阻害剤の遷移状態近傍における結合様式解析法として、Figure 2-11 中に緑色の矢印で示したドッキング法を考案した。まず、アポタンパク質 (Figure 2-11B) の構造のうち、共有結合性阻害剤と反応する活性基を除去し、ドッキングにおいて共有結合性阻害剤の活性基が大きな立体反発を伴うことなく占めることのできる空間をつくる (Figure 2-11D)。次いで、X 線結晶構造解析により明らかにした共有結合性複合体 (P-I) (Figure 2-11A) における結合様式から、遷移状態近傍において共有結合性阻害剤の活性基が占める空間及びその配向を想定する (Figure 2-11E)。これを基に、タンパク質の活性基の除去によってつくった空間を適切な三次元的空間配置で共有結合性阻害剤の活性基が占めるように条件付けしてドッキングすることで、遷移状態近傍における複合体 (P‥I) (Figure 2-11F) の構造を解析することが出来ると考えられる。

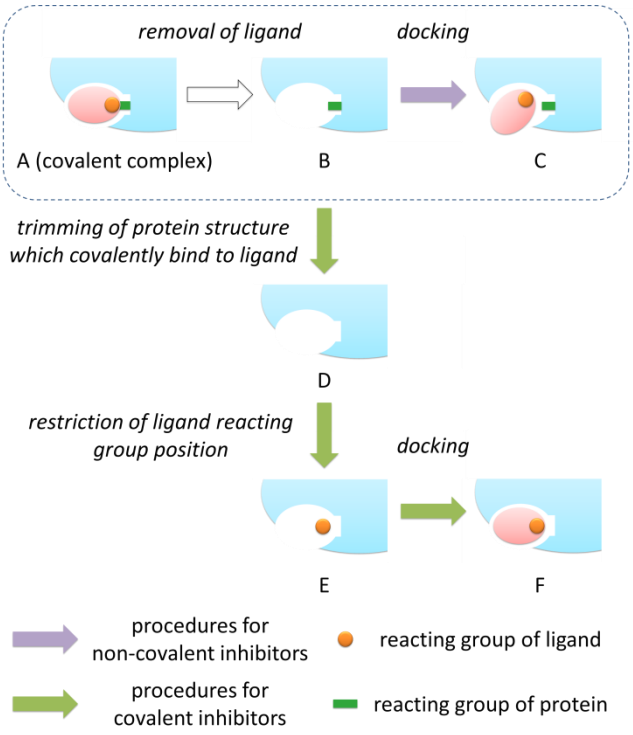


Figure 2-11. 共有結合の遷移状態近傍における非共有結合状態解析のためのドッキングコンセプト

2. プロテアソームモデルの構築

上述のコンセプトに基づきベラクトシン誘導体の遷移状態近傍における結合状態を解析するため、ドッキングに必要なプロテアソームモデルを構築した。このドッキング法においては、共有結合性複合体の構造から、遷移状態近傍における共有結合性阻害剤の活性基の大まかな位置及び配向を合理的に予測する必要がある。しかしながら、ベラクトシン誘導体の活性基であるβ-ラクトンは、共有結合形成に伴いその配座が大きく変化するため (Figure 2-12a)、ベラクトシン誘導体とプロテアソームの共有結合性複合体の構造からその遷移状態近傍における位置及び配向を推測することは困難であった (Figure 2-12a)。

サリノスポラミド A は、ベラクトシン誘導体と同じく活性基としてβ–ラクトンを有する共有結合性のプロテアソーム阻害剤である。[23]このため、プロテアソームとの共有結合形成の遷移状態近傍におけるβ-ラクトン環の位置及び配向はベラクトシン誘導体とサリノスポラミド A で類似しているものと考えられる。サリノスポラミド A において活性基であるβ-ラクトン環はγ-ラクタム環と縮環構造をとっており、プロテアソームとの共有結合形成に伴うβ-ラクトン環の開環による配座変化はγ-ラクタム環によって制限される (Figure 2-12b)。[54]しかしながら、β-ラクトン環の開環により生じたヒドロキシ基が即座にγ-ラクタム環の置換基上のクロロ基を置換してエーテル環を形成するため、β-ラクトン環の開環直後の共有結合状態の構造は解析されていない (Figure 2-12b)。[54]そこで、フルオロサリノスポラミド A とプロテアソームの共結晶[55]に着目した。フルオロサリノスポラミド A はγ-ラクタム環の置換基上にクロロ基の代わりにフルオロ基を持つため、その低い脱離能のためにエーテル環の形成が遅く、β-ラクトン環の開環直後の共有結合状態 (Figure 2-12c*) の構造が解析されていた。[55]この共有結合性複合体におけるフルオロサリノスポラミド A 及びプロテアソームの構造は十分に遷移状態近傍におけるこれらの構造を反映するものであると考えられる。

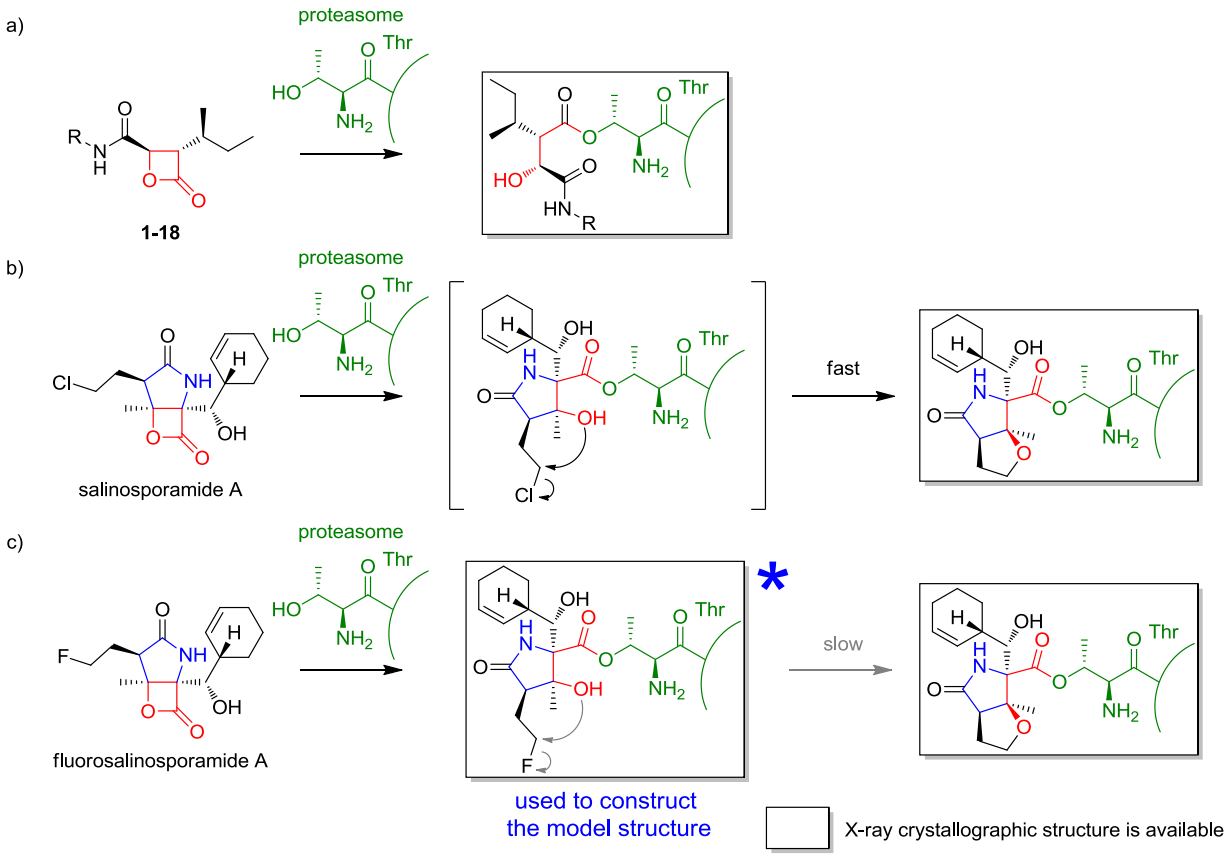


Figure 2-12. ベラクトシン誘導体 **1-18**、サリノスポラミド A 及びフルオロサリノスポラミド A のプロテアソームとの結合に伴う構造変化

そこで、Figure 2-13 に示すように、この共結晶の構造 (Figure 2-13a) から遷移状態近傍におけるβ-ラクトンの三次元的空間配置を推測するとともに、ドッキングに必要なプロテアソームモデルを構築することとした。まず、フルオロサリノスポラミド A とプロテアソームの間に形成されたエステル結合を切断した。続いて、プロテアソーム活性残基である Thr の側鎖を除去し、ドッキングにおいてβ-ラクトンが立体反発を受けることなく Thr 残基に近接した状態をとることが出来るようにした (Figure 2-13b)。次に、フルオロサリノスポラミド A のヒドロキシ基の酸素原子とカルボニル基の炭素原子を結び、分子力学計算を行うことでβ-ラクトン環を構築した (Figure 2-13c)。最後にβ-ラクトン環以外のフルオロサリノスポラミド A の構造を除去してドッキングに必要なプロテアソームモデルを得た (Figure 2-13d)。このモデル中のβ-ラクトンの空間配置を遷移状態近傍の複合体における仮想のβ-ラクトンの空間配置とし、これを中心にβ-ラクトン部の位置及び配向を制限した状態でドッキングを行うことで、ベラクトシン誘導体の遷移状態近傍における結合様式を解析することとした。

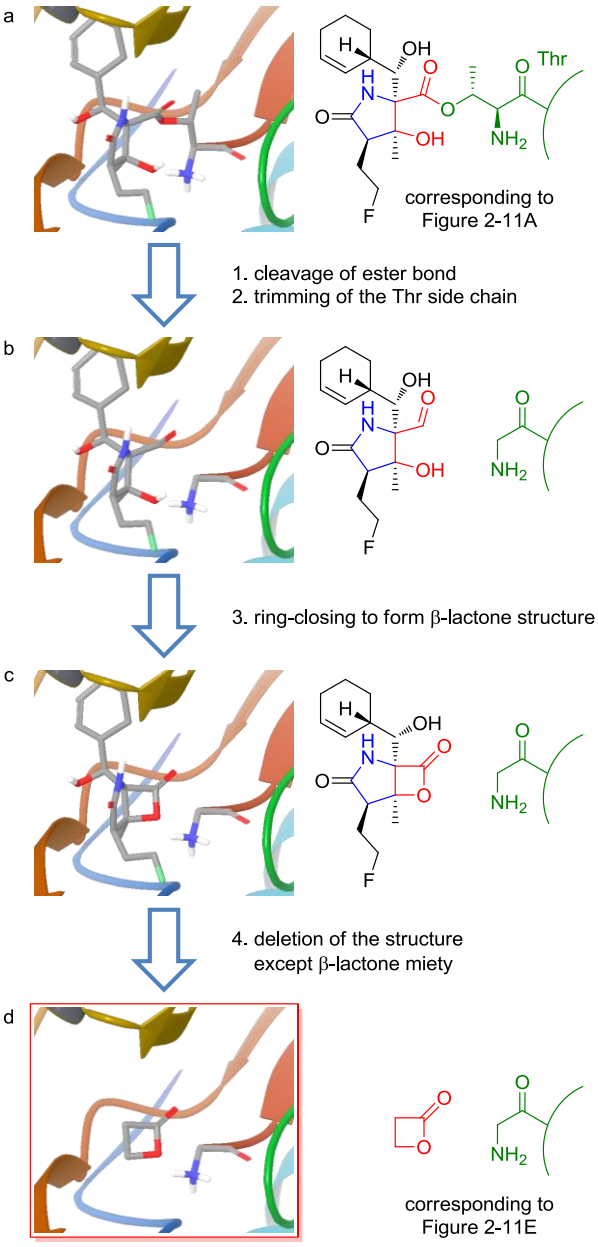


Figure 2-13. ドッキングのためのプロテアソームモデルの構築

3. サリノスポラミドA誘導体のドッキング

ドッキングコンセプト及び構築したプロテアソームモデルを評価するため、Glide 5.7 (Schrödinger LLC) により Figure 2-14 に示した文献既知のサリノスポラミドA誘導体[56]をドッキングし、Prime 3.0 (Schrödinger LLC) を用いて MM-GBSA 法[57]により結合エネルギー (Prime MM-GBSA ΔG bind) を計算して、実験値である pIC_{50} との相関を検証した。*

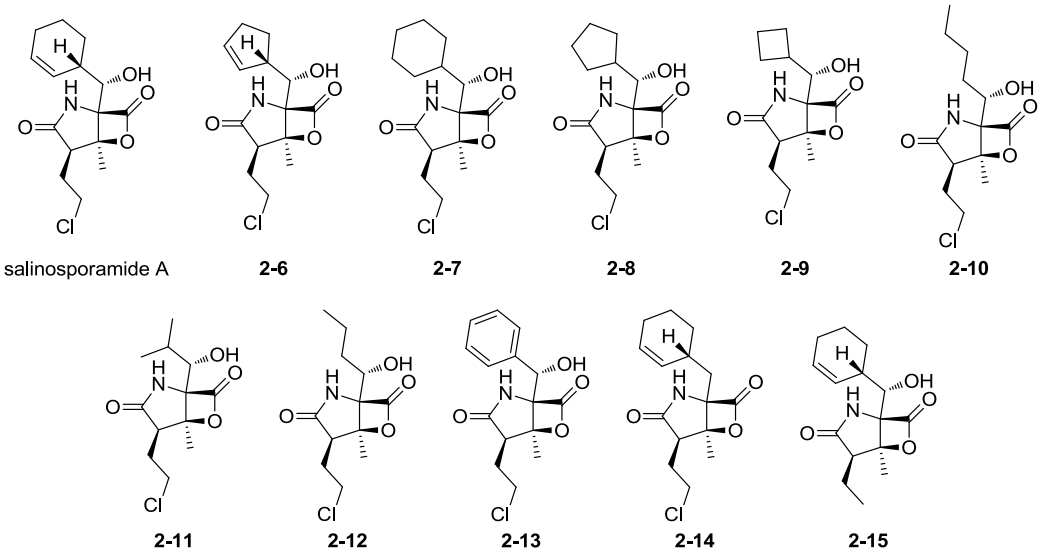


Figure 2-14. ドッキングに用いたサリノスポラミドA誘導体の構造

Figure 2-15a に示すように、計算により求めた結合エネルギー (Prime MM-GBSA ΔG bind) は pIC_{50} と良好な相関を示した (r = 0.82)。また、これらのサリノスポラミド誘導体はそれぞれ異なる疎水性置換基を有するため、clogP と pIC_{50} の相関も調べた。しかしながら、Figure 2-15b に示すようにこれらの間に全く相関は見られず (r = 0.096)、計算により求めた結合エネルギーが単に化合物の疎水性の大小を反映しているのではなく、遷移状態近傍におけるプロテアソームとの特異的な疎水性相互作用の大小を反映していることが分かる。†これは、計算により求めた共有結合性化合物の遷移状態近傍における非共有結合性相互作用に基づく結合エネルギーと実験的に求めた阻害活性の良好な相関を示した初めての例である。

以上、考案したドッキングコンセプトに基づき構築したプロテアソームモデルを用いて計算したサリノスポラミドA誘導体の遷移状態近傍における非共有結合性相互作用エネルギーとプロテアソーム阻害活性が良い相関を示したことから、遷移状態近傍における複合体を解析するドッキングコンセプト及び構築したプロテアソームモデルは妥当なものであると考えられる。

* これらのサリノスポラミド誘導体は分子量が小さく配座の自由度が小さいため高い精度でドッキング及び結合エネルギーの計算を行えるものと考えられる。加えて、酵母 20S プロテアソームに対する阻害活性が同一条件で測定されており、高活性な誘導体から低活性な誘導体まで揃っていることから (IC_{50} = 1.9 – 1290 nM) ドッキング法の評価に適している。

† 各々のサリノスポラミドA誘導体の IC_{50}, pIC_{50}, Prime MM-GBSA ΔG bind 及び clogP 値については実験の部を参照。

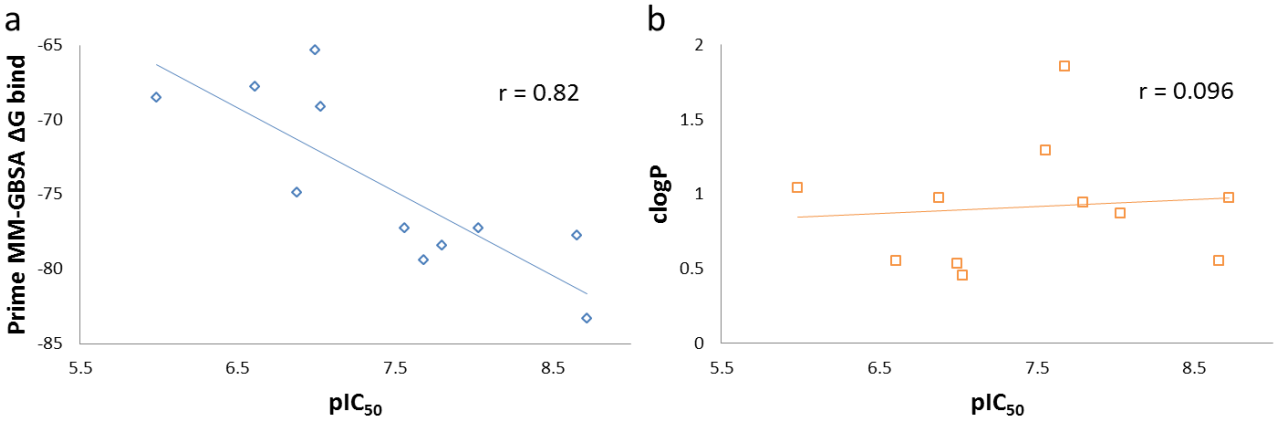


Figure 2-15. pIC_{50} と結合エネルギー (Prime MM-GBSA ΔG bind) (a) 及び clogP (b) との相関

4. ベラクトシン A シス型異性体の誘導体の遷移状態近傍における結合様式

構築したプロテアソームモデルを用いてベラクトシン A シス型異性体の誘導体 **1-18** とその配座制限誘導体 **2-2a** 及び **2-2b** について Glide 5.7 (Schrödinger LLC) を用いてドッキングを行い、これらの誘導体の遷移状態近傍における結合様式を解析した。ドッキングにより予測された化合物 **1-18** の遷移状態近傍における結合様式を Figure 2-16a に示す。この結合配座において、プロテアソーム阻害活性に重要な N 末端部及びカルボキシ基部置換基を含む領域 X は *syn* 配座であるが (Figure 2-16a)、これは一連の配座制限誘導体を用いた実験化学的手法により得られた活性配座に関する知見と一致し、計算化学的手法により解析した遷移状態近傍における結合配座の妥当性を支持する。想定通り、ドッキングにより解析した **1-18** の遷移状態近傍における結合様式は、X 線結晶構造解析による共有結合状態の結合様式とは異なり、とりわけ N 末端部及びカルボキシ基部置換基を含む領域 X において大きな差異がみられる (Figure 2-16a)。また、N 末端部及びカルボキシ基部置換基の二つのベンゼン環は共有結合状態に比べてプロテアソーム表面の結合部位とより近接した状態にあり、遷移状態近傍においてプロテアソームとより効果的に相互作用していることが伺える。以上の解析結果は、Figure 2-4 に示したベラクトシン誘導体の共有結合形成に伴う結合様式の変化に関する仮説を支持するものである。

ドッキングにより予測した *syn* 配座に制御した配座制限誘導体 **2-2a** の遷移状態近傍における結合様式を Figure 2-16b に示すが、**1-18** と同様の結合様式であることが分かる (Figure 2-16d)。一方で、*anti* 配座に制御した配座制限誘導体 **2-2b** のドッキングでは、Figure 2-16c に示すような結合配座が得られ、**1-18** と同様の結合様式は得られなかった。これは配座制限誘導体 **2-2a** (IC_{50} = 47 nM) が親化合物のプロテアソーム阻害活性 (IC_{50} = 5.7 nM) をある程度保持したのに対し、配座制限誘導体 **2-2b** のプロテアソーム阻害活性は著しく低かった (IC_{50} = 280 nM) ことに合致する。このことからも、ドッキングにより解析した遷移状態近傍における結合様式が妥当なものであることが示唆される。

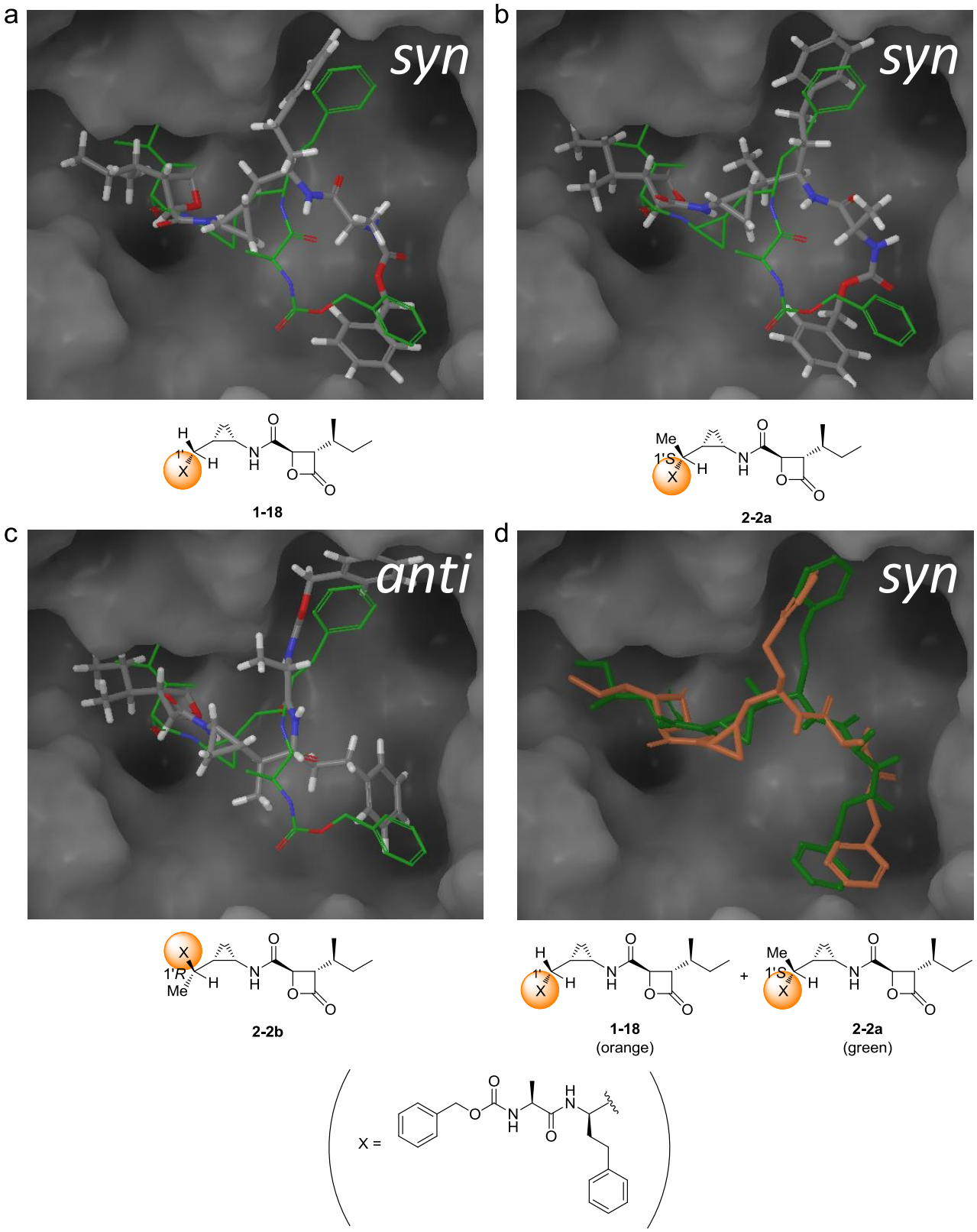


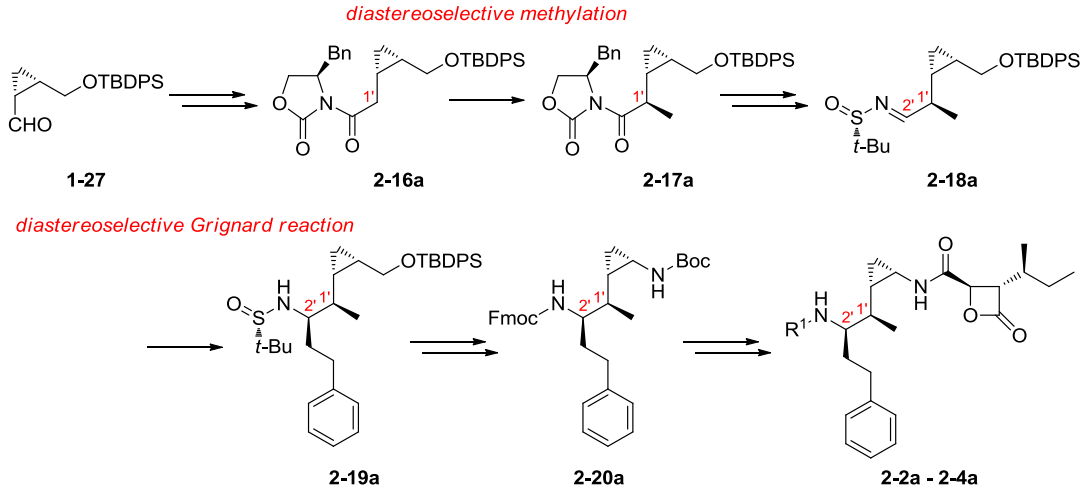
Figure 2-16. (a-c) ドッキングにより解析したベラクトシン A シス型異性体の誘導体 **1-18** (a, gray tube) とその配座制限誘導体 **2-2a** (b, gray tube) 及び **2-2b** (c, gray tube) の遷移状態近傍における結合様式と X 線結晶構造解析により明らかにした化合物 **1-18** の共有結合状態における結合様式 (green wire) (d) 化合物 **1-18** と配座制限誘導体 **2-2a** のドッキング結果の重ね合わせ (**1-18**, orange tube; **2-2a**, green tube)

第五節　配座制限誘導体の合成

1. 配座制限誘導体の合成計画

配座制限誘導体 **2-2a** – **2-4a** の合成計画を Scheme 2-1 に示す。この合成においては C1'及び C2'炭素原子における不斉構築が鍵となる。C2'炭素原子の不斉は、第一章で述べた親化合物 **1-18**, **1-24** 及び **1-25** の合成と同様に、光学活性 *t*-ブタンスルフィニルイミン **2-18a** に対するジアステレオ選択的 Grignard 反応[40]により構築する。基質である光学活性 *t*-ブタンスルフィニルイミン **2-18a** は配座制御型シクロプロパンユニット **2-17a** から合成する。配座制御型シクロプロパンユニット **2-17a** の合成、即ち 1'-位の不斉は、光学活性オキサゾリジノンを不斉補助基とした[58]化合物 **2-16a** のジアステレオ選択的メチル化反応により構築し、基質である **2-16a** は光学活性シスシクロプロパンユニット **1-27** から合成する。配座制限誘導体 **2-2b** – **2-4b** についても同様に合成することとした。

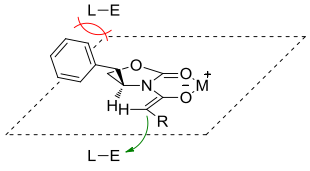
Scheme 2-1. 配座制限誘導体 **2-2a** – **2-4a** の合成計画



2. 配座制限誘導体の合成

光学活性シスシクロプロパンユニット **1-27** を出発物質として、一連の配座制限誘導体 **2-2a** – **2-4a** の合成における鍵中間体 **2-20a** を Scheme 2-2 に示すように合成した。シクロプロパンユニット **1-27** を基質として、$MeOCH_2PPh_3Cl$ を用いた Wittig 反応と続くエノールエーテルの酸加水分解により対応するアルデヒドを得た。このアルデヒドを Pinnick 酸化により酸化してカルボン酸 **2-21** を得た。混合酸無水物法によりカルボン酸 **2-21** を(4*R*)-4-ベンジル-2-オキサゾリジノン[58]と縮合してジアステレオ選択的メチル化反応の基質 **2-16a** を合成した。これを THF 中-78 °C において NaHMDS 及び CH_3I で処理して配座制御型シクロプロパンユニット **2-17a** を得た (dr 97:3)。[*,†]**2-17a** を DIBAL で処理して不斉補助基であるオキサゾリジノン部を還元的に除去してア

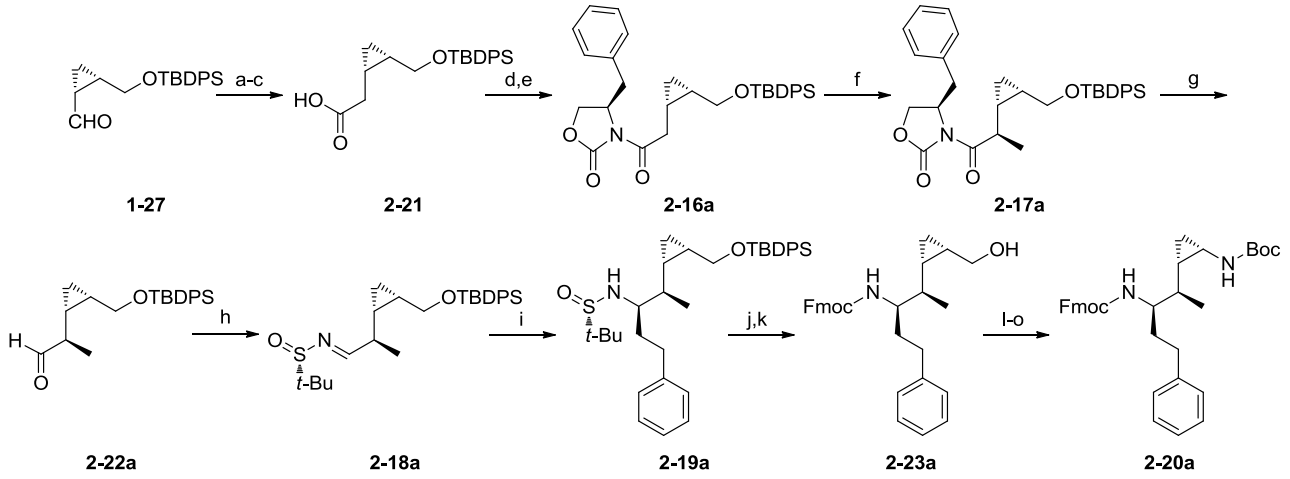
[*] オキサゾリジノンを不斉補助基として用いることで、金属カチオンとの錯体形成によって配座が固定されたエノラートを生じる。このエノラートの二つの反応面のうち片方はオキサゾリジノン上の置換基によって立体的に遮蔽されるため、他方の面からの反応が優先的に進行し、対応する立体配置を有する生成物が選択的に得られる（下図）。[58]



（次ページに続く）

ルデヒド **2-22a** とし、更に(*S*)-*t*-BuSONH$_2$[40]と脱水縮合してジアステレオ選択的 Grignard 反応[40]の基質である(*S*)-*t*-ブタンスルフィニルイミン **2-18a** を得た。**2-18a** をジクロロメタン中室温において PhCH$_2$CH$_2$MgCl で処理し、アルキル化生成物 **2-19a** を得た。‡尚、このジアステレオ選択的 Grignard 反応において粗生成物の ^{1}H-NMR スペクトル上ジアステレオマーの生成は見られなかった。*t*-ブタンスルフィニル基及び TBDPS 基を酸性条件下除去した後、生じたアミノ基を Fmoc 基で保護して化合物 **2-23a** を得た。**2-23a** のヒドロキシメチル基を Dess-Martin 酸化及び Pinnick 酸化により段階的にカルボキシ基へと酸化した後、DPPA で処理して酸アジドへと変換し、更に *t*-ブタノール中加熱環流して Curtius 転位と続くイソシアネートの加溶媒分解により鍵中間体 **2-20a** を得た。

Scheme 2-2. 配座制御型シクロプロパンユニット **2-17a** と続く鍵中間体 **2-20a** の合成 [a]



[a]Reagents and conditions: (a) MeOCH$_2$PPh$_3$Cl, NaHMDS, THF, 0 °C; (b) HCl, THF/H$_2$O; (c) Pinnick ox., 90% (3 steps from **1-27**); (d) PivCl, Et$_3$N, CH$_2$Cl$_2$, 0 °C; (e) (4*R*)-4-benzyl-2-oxazolidinone, *n*-BuLi, THF, -78 °C, 83% (2 steps from **2-21**); (f) CH$_3$I, NaHMDS, THF, -78 °C, 83% (dr 97:3); (g) DIBAL, THF, -78 °C, 69%; (h) (*S*)-*t*-BuSONH$_2$, CuSO$_4$, CH$_2$Cl$_2$, 96%; (i) PhCH$_2$CH$_2$MgCl, CH$_2$Cl$_2$; (j) HCl, AcOEt/MeOH; (k) FmocOSu, Na$_2$CO$_3$, THF/H$_2$O, 66% (3 steps from **2-18a**); (l) Dess-Martin ox.; (m) Pinnick ox.; (n) DPPA, Et$_3$N, CH$_2$Cl$_2$, 0 °C to rt; (o) *t*-BuOH, reflux, 72% (4 steps from **2-23a**).

鍵中間体 **2-20a** から配座制限誘導体 **2-2a** – **2-4a** を Scheme 2-3 に示すように合成した。化合物 **2-20a** の Fmoc 基をメタノール中 K$_2$CO$_3$ で処理することで除去した後、生じたアミノ基に対して Cbz-Ala-OH を混合酸無水物法により縮合して化合物 **2-24a** を得た。**2-24a** の Boc 基を酸性条件下にて除去した後、生じたアミノ基に対してβ-ラクトンユニット **1-35**[41]を混合酸無水物法により縮合して配座制限誘導体 **2-2a** を得た。**2-2a** の Cbz 基を水素化分解により除去し、生じたアミノ基を 2-ナフトイルクロリドを用いてアシル化して配座制限誘導体 **2-4a** を得た。配座制限誘導体 **2-3b** は鍵中間体 **2-20a** から **2-2a** と同様に合成した。

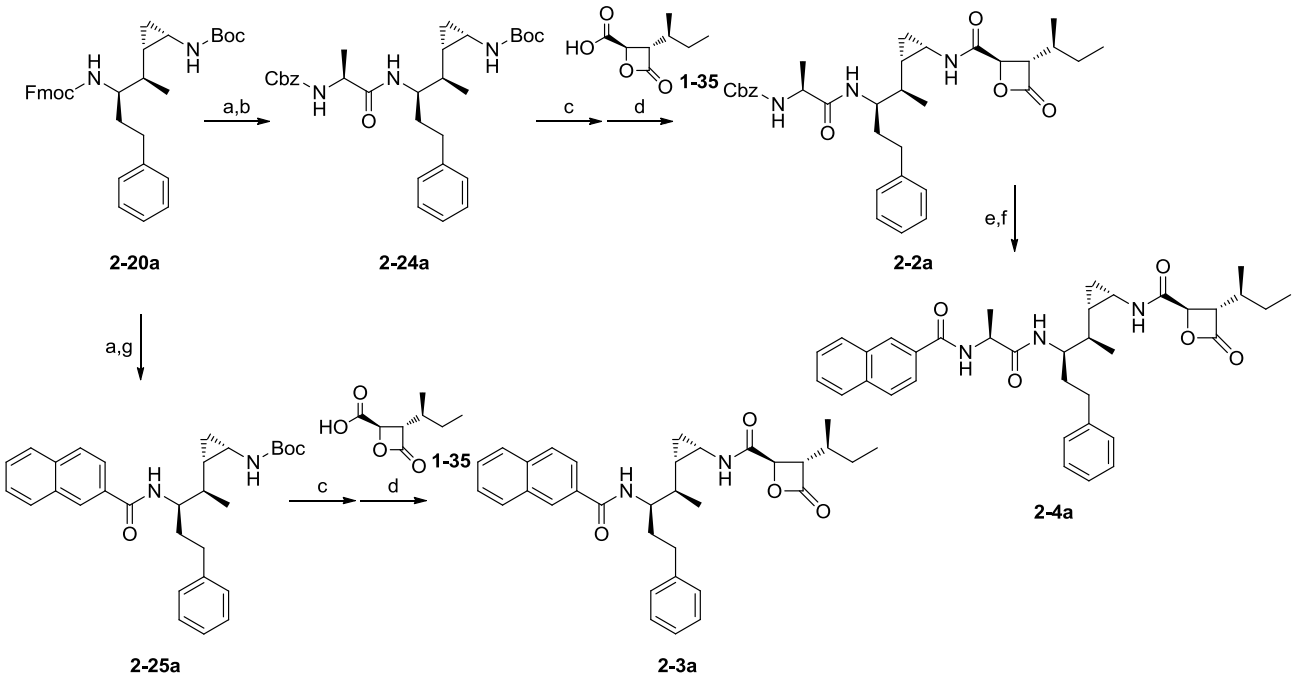
（前ページから続く）

* ここで生じたジアステレオマーに由来する副生成物は化合物 **2-23a** の段階において容易に除去できた。

† 導入したメチル基の絶対立体配置は PGME 法[59]により決定した（実験の部参照）。

‡ 導入したフェネチル基の絶対立体配置は改良 Mosher 法[43]により決定した（実験の部参照）。

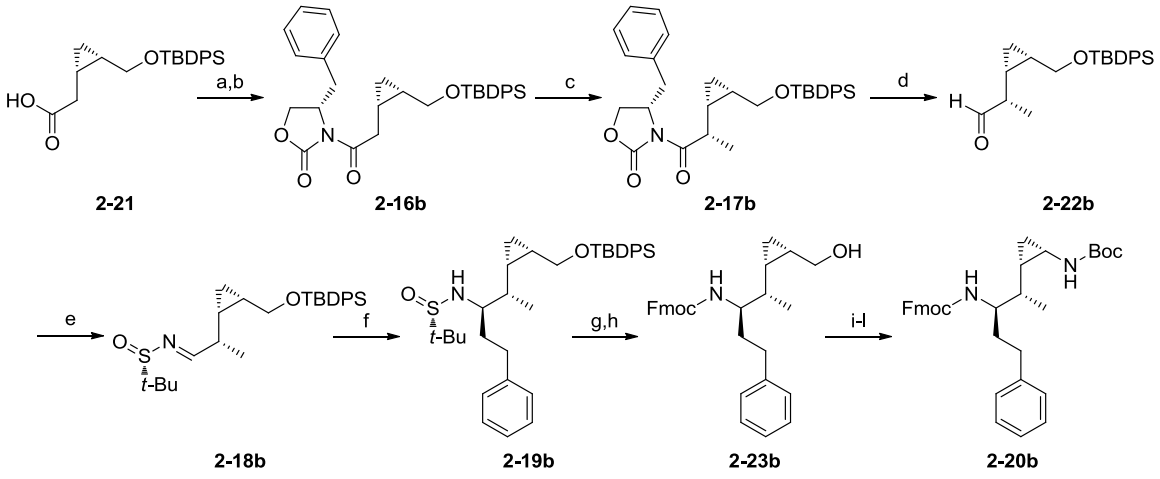
Scheme 2-3. 配座制限誘導体 **2-2a** – **2-4a** の合成 [a]



[a]Reagents and conditions: (a) K_2CO_3, MeOH; (b) Cbz-Ala-OPiv, Et_3N, CH_2Cl_2, 0 °C to rt, 100% (2 steps from **2-20a**); (c) TFA/CH_2Cl_2; (d) PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, (**2-2a**, 2 steps 82% from **2-24a**; **2-3a**, 2steps 91% from **2-25a**); (e) Pd/C, H_2, TFA/THF, 0 °C; (f) 2-naphthoyl chloride, Et_3N, CH_2Cl_2, 0 °C, 70% (2 steps from **2-2a**); (g) 2-naphthoyl chloride, Et_3N, CH_2Cl_2, 0 °C, 100% (2 steps from **2-20a**)

配座制限誘導体 **2-2b** – **2-4b** の合成における鍵中間体 **2-20b** は、カルボン酸 **2-21** から **2-20a** と同様に合成した (Scheme 2-4)。

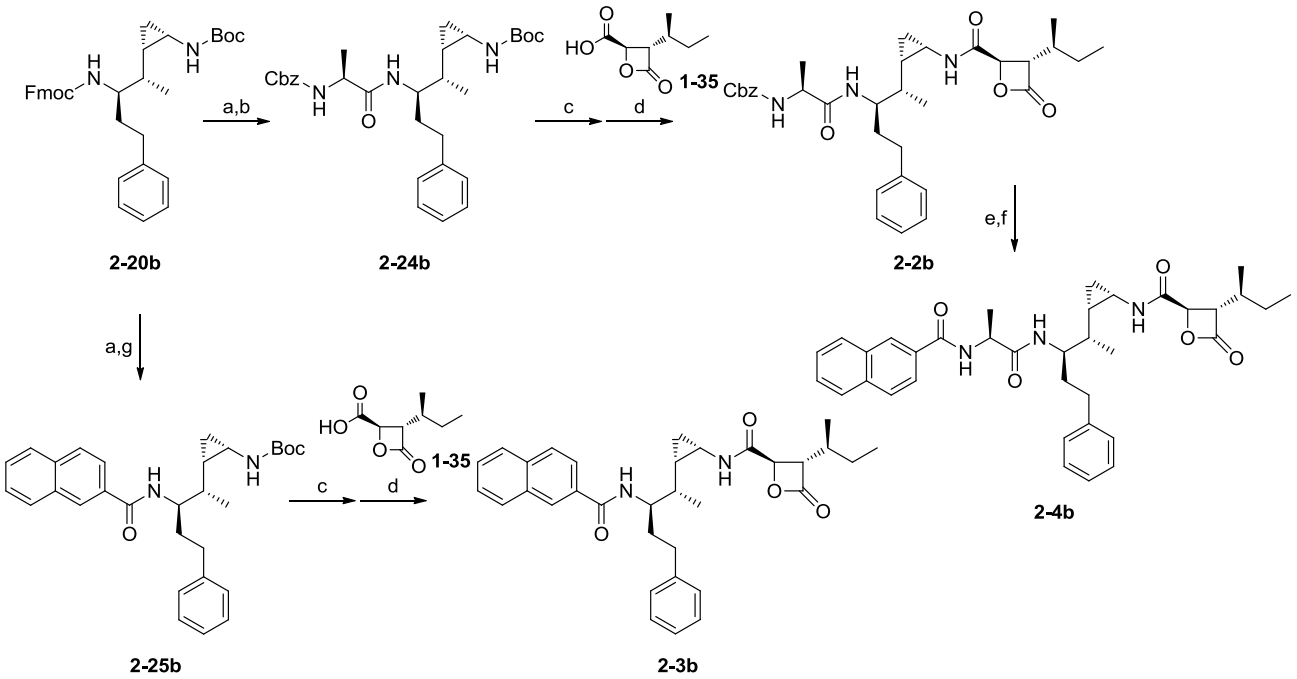
Scheme 2-4. 配座制御型シクロプロパンユニット **2-17b** と続く鍵中間体 **2-20b** の合成 [a]



[a]Reagents and conditions: (a) PivCl, Et_3N, CH_2Cl_2, 0 °C; (b) (4*S*)-4-benzyl-2-oxazolidinone, *n*-BuLi, THF, -78 °C, 89% (2 steps from **2-21**); (c) CH_3I, NaHMDS, THF, -78 °C, 79% (dr 99:1); (d) DIBAL, THF, -78 °C, 56%; (e) (*S*)-*t*-$BuSONH_2$, $CuSO_4$, CH_2Cl_2, 89%; (f) $PhCH_2CH_2MgCl$, CH_2Cl_2; (g) HCl, AcOEt/MeOH; (h) FmocOSu, Na_2CO_3, THF/H_2O, 65% (3 steps from **2-18b**); (i) Dess-Martin ox.; (j) Pinnick ox.; (k) DPPA, Et_3N, CH_2Cl_2, 0 °C to rt; (l) *t*-BuOH, reflux, 62% (4 steps from **2-23b**).

配座制限誘導体 **2-2b** – **2-4b** は鍵中間体 **2-20b** から **2-2a** – **2-4a** と同様に合成した (Scheme 2-5)。

Scheme 2-5. 配座制限誘導体 **2-2b** – **2-4b** の合成 [a]



[a]Reagents and conditions: (a) K_2CO_3, MeOH; (b) Cbz-Ala-OPiv, Et_3N, CH_2Cl_2, 0 °C to rt, 99% (2 steps from **2-20b**); (c) TFA/CH_2Cl_2; (d) PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, (**2-2b**, 2 steps 94% from **2-24b**; **2-3b**, 2 steps 94% from **2-25b**); (e) Pd/C, H_2, TFA/THF, 0 °C; (f) 2-naphthoyl chloride, Et_3N, CH_2Cl_2, 0 °C, 56% (2 steps from **2-2b**); (g) 2-naphthoyl chloride, Et_3N, CH_2Cl_2, 0 °C, 96% (2 steps from **2-20b**)

以上に述べた配座制限誘導体の合成において、2'-位の不斉を構築するジアステレオ選択的 Grignard 反応の選択性が 1'-位の立体配置によって大きく影響されることが分かった。即ち、1'*R* 配置の基質 **2-18a** に対する Grignard 反応が単一ジアステレオマーを与えたのに対して、1'*R* 配置の基質 **2-18b** に対する Grignard 反応はジアステレオマー比 5:1 で異性体混合物を与えた。[*]この C1'-異性体間での立体選択性の違いは Figure 2-17 に示す反応機構によって説明できると考えられる。*t*-ブタンスルフィニルイミンに対する Grignard 反応はスルフィニル基の酸素原子が Mg^{2+}イオンに配位し六員環状の遷移状態を経由して進行すると考えられており、嵩高い *t*-ブチル基がエクアトリアル位を占める六員環遷移状態を経由して生成する立体異性体が優先して得られる (Ellman モデル)。[40]基質が 1'*S*-異性体 **2-18b** である場合、Figure 2-17 に示すように優先する六員環遷移状態において C1'-メチル基が Grignard 試薬の接近する面を向いていることから、C1'-メチル基の立体効果によって **2-18b** に対する Grignard 反応の立体選択性が低下していると考えられる。

[*] Grignard 反応の立体選択性は粗生成物の ^{1}H-NMR スペクトルより算出した。

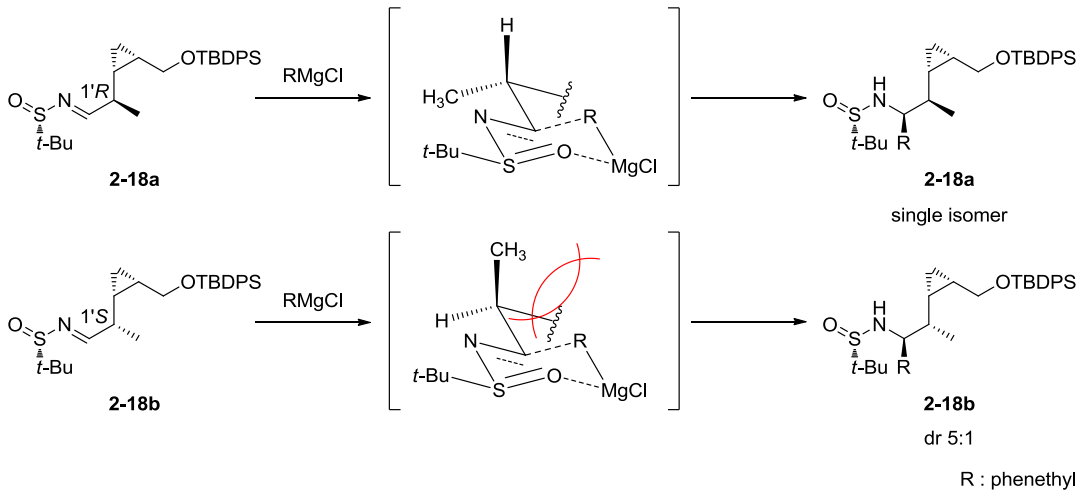


Figure 2-17. ジアステレオ選択的 Grignard 反応の立体選択性発現機構

以上、筆者はベラクトシン A シス型異性体の誘導体の遷移状態近傍における結合様式を、シクロプロパン歪みを利用した配座制限誘導体の合成による有機化学的アプローチと、新たなドッキングコンセプトに基づく計算化学的アプローチを組み合わせることで解析した。更に、一連のサリノスポラミド A 誘導体を用いて、ドッキングにより得られた遷移状態近傍における複合体の結合エネルギーと実際の阻害活性が良好な相関を示すことを明らかにした。これは、考案したドッキング法の有用性を示すのみならず、共有結合性阻害剤開発における遷移状態結合様式の解析の重要性を示すものである。

第三章　“Scaffold Hopping”による非ペプチド性プロテアソーム阻害剤の開発[60]

第一節　“Scaffold Hopping”

創薬研究においては、リード化合物の標的分子に対する生物学的作用のみならず、代謝安定性、膜透過性、水溶性、毒性プロファイルや合成容易性といった他の多くの性質を最適化しなければならない。[61]リード化合物に対する置換基の導入あるいは修飾は比較的容易に検討可能であり、これらの諸性質の最適化に一般的に用いられる方法である。しかしながら、とりわけリード化合物の骨格構造が改善すべき性質の原因となっている場合には置換基の変換のみでは問題の解決は難しい場合も多く、飛躍的な構造変換、即ち“scaffold hopping”が必要となる。[62]例えば、これまでに多くの優れた生理活性を有する天然物や生理活性ペプチドが発見されているが、その複雑な骨格構造やペプチド骨格に由来する低い膜透過性及び代謝安定性のために、医薬としての利用はしばしば制限される。[63]従って、これらの天然物や生理活性ペプチドをリード化合物としてドラッグライクな化合物を効率的に創製するためには、理論的な“scaffold hopping”のための方法論の開発が必須となる。

“Scaffold hopping”という言葉は比較的新しく、1999 年に Schneider らによって、“*identification of isofunctional molecular structures with significantly different molecular backbones*”として定義された。**[64]**2012 年、Sun らは構造変化の大きさに基づき“scaffold hopping”を、1° hop: heterocycle replacement, 2° hop: ring opening or closure, 3° hop: peptidomimetics 及び 4° hop: topology-based hopping の四種類に分類している (Figure 3-1)。**[62e]**一般に、構造変化の大きな高次の“scaffold hopping”になるほど分子特性は大きく変化するが、成功率はこれに反比例する。従って、ファーマコフォアモデルに基づき完全に分子骨格を変換する“topology-based hopping”は、創薬研究において大きな可能性を秘めているものの、その成功例は極めて少ない。**[62e]**

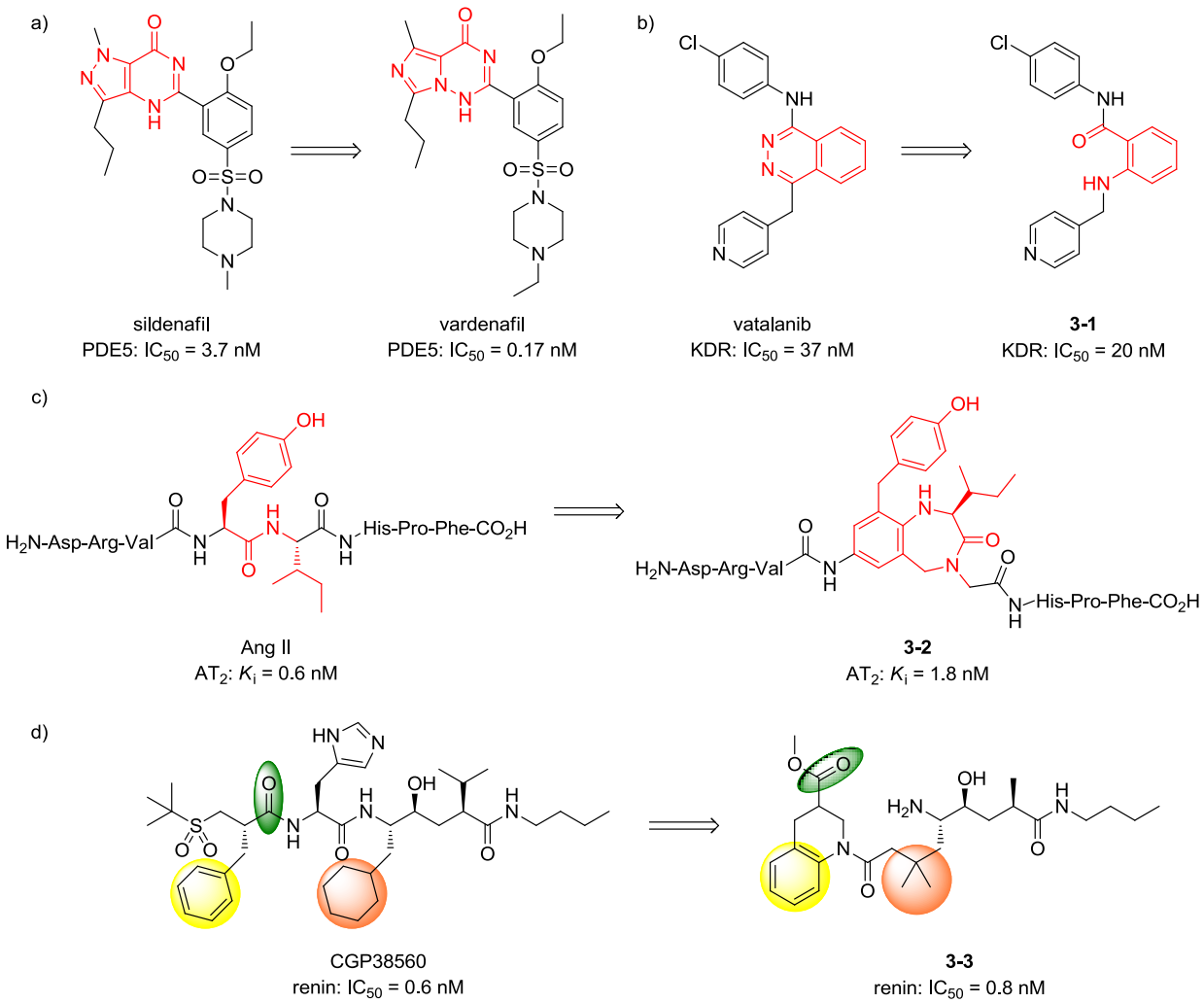


Figure 3-1. Sun らの分類[62e]による四種類の“scaffold hopping”の例 (a) 1° hop: heterocycle replacement[65] (b) 2° hop: ring opening or closure[66] (c) 3° hop: peptidomimetics[67] (d) 4° hop: topology-based hopping[68]

第二節　“Topology-Based Scaffold Hopping”による非ペプチド性化合物の設計

1. ベラクトシン誘導体の骨格構造

序論及び第一章で述べたように、一連の構造活性相関研究により、ペプチド性天然物ベラクトシン A をリード化合物として高いプロテアソーム阻害活性を有するベラクトシン誘導体 **1-18** を見出すことに成功した (Figure 3-2)。しかしながら、**1-18** はその多数の不斉点からなるペプチド骨格のために合成が煩雑である（全 26 工程）という問題があった。また、ペプチド骨格は一般に膜透過性や代謝安定性を低下させる。[63b]そこで、**1-18** の多数の不斉点からなるペプチド骨格を“scaffold hopping”によりアキラルな非ペプチド骨格へと変換し、よりドラッグライクなプロテアソーム阻害剤を創出することを計画した。

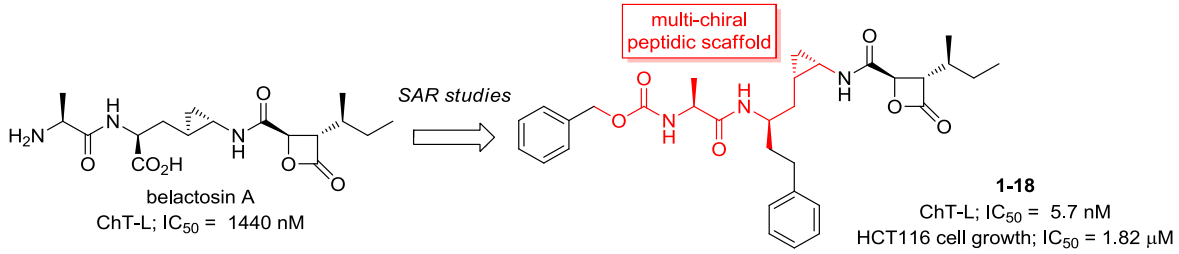


Figure 3-2. ペプチド性天然物ベラクトシン A から見出した高活性なプロテアソーム阻害剤 **1-18**

2. ファーマコフォアモデルの構築と化合物の設計

第二章で述べたように、筆者は化合物 **1-18** の遷移状態近傍における結合状態を解析した (Figure 3-3b, green tube)。更に、ホモベラクトシン C 誘導体 **0-1** 及び **0-2** のプロテアソームとの共結晶の構造が Meijere らによって報告されている (Figure 3-3b: **0-1**, yellow wire; **0-2**, orange wire)。[33]興味深いことに、これらのいずれの複合体においても Figure 3-3a 中赤で示したペプチド骨格はプロテアソームと水素結合を形成しておらず、ペプチド骨格が非ペプチド骨格で置き換えることが可能であると考えられる (Figure 3-3b)。また、これらの複合体において、ペプチド骨格は N 末端部の疎水性芳香環をプロテアソーム結合部位に配置するために屈曲しており、短縮・簡略化が可能であることが示唆された (Figure 3-3b)。

これらの構造情報と第一章で述べた構造活性相関研究から、共有結合形成に必須のβ-ラクトン部とカルボキシ基部及び N 末端部の二つの疎水性芳香環のみがプロテアソーム阻害活性に重要であると考え、Figure 3-3c に示したファーマコフォアモデルを構築した。このファーマコフォアモデルを基にして“topology-based scaffold hopping”により非ペプチド性化合物 **3-4** – **3-11** を設計した (Figure 3-3a)。[*]二つのベンゼン環を対称に配置することでアキラルな骨格構造とした。設計した化合物の Figure 3-3b に示したベラクトシン誘導体 **1-18** 及びホモベラクトシン C 誘導体 **0-1**, **0-2**[33]の結合配座に対するフレキシブルアライメント (MOE 2012.10)[†] の結果を Figure 3-3d に示す。全ての化合物が、プロテアソームとの間に立体反発を生じることなくファーマコフォアモデルを満たすことが可能であることが分かり、強いプロテアソーム阻害活性を有することが期待される。

[*] 合成容易性、配座柔軟性及び水溶性を考慮して骨格部分に窒素原子又は酸素原子を導入することとし、アミン型化合物 **3-4** – **3-7** 及びエーテル型化合物 **3-8** – **3-11** を設計した。

[†] 鋳型構造に対して化合物の配座を変化させながら重なりが大きくなるように重ね合わせる。ここでは、ベラクトシン誘導体 **1-18** 及びホモベラクトシン C 誘導体 **0-1**, **0-2** の結合配座を鋳型構造とし、これに設計した一連の化合物を重ね合わせた。

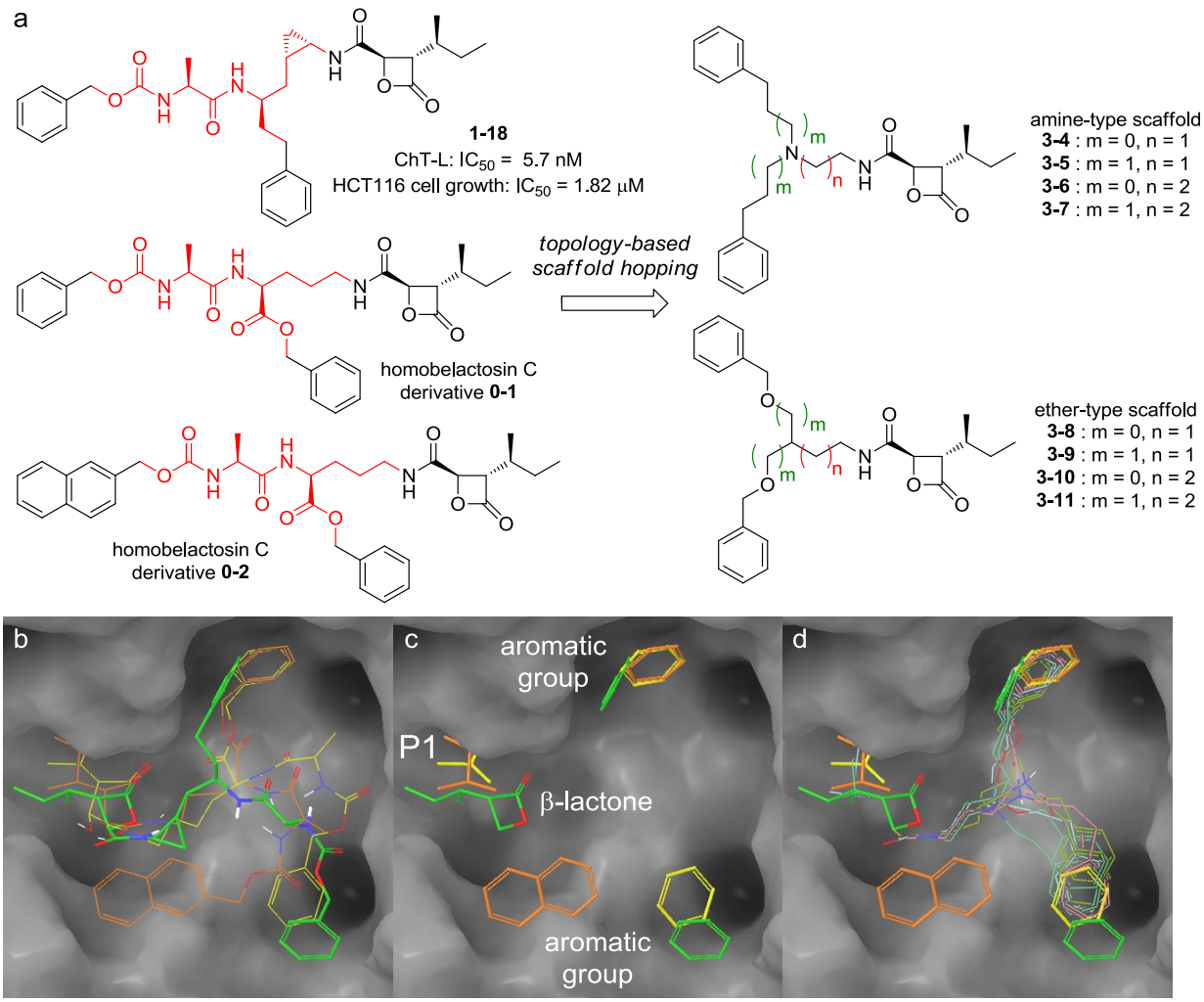


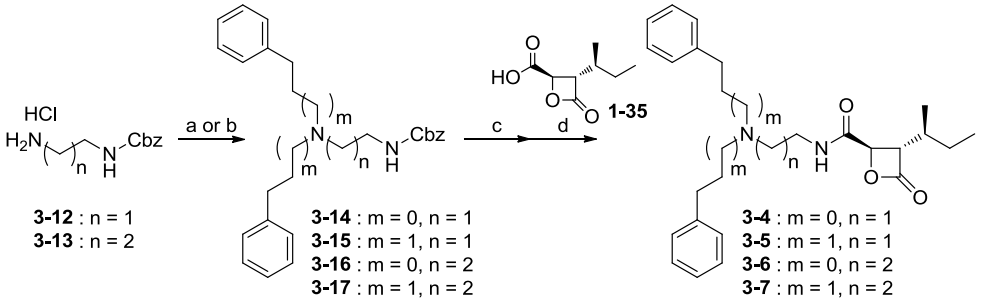
Figure 3-3. “Topology-based scaffold hopping”による非ペプチド性化合物の設計 (a) ベラクトシン誘導体 **1-18** 及びホモベラクトシンC誘導体 **0-1**, **0-2**[33]の構造と、新たに設計した非ペプチド性化合物 **3-4** – **3-11** の構造 (b) ホモベラクトシンC誘導体 **0-1** 及び **0-2** の共有結合様式 (**0-1**, yellow wire; **0-2**, orange wire)[33] と、解析した **1-18** の遷移状態近傍における結合様式 (green tube) (c) 構築したファーマコフォアモデル (d) 設計した非ペプチド性化合物の Figure 3-3b に示した **1-18**, **0-1** 及び **0-2** の結合配座に対するフレキシブルアライメント (**3-4** and **3-8**, aquamarine; **3-5** and **3-9**, turquoise; **3-6** and **3-10**, yellow green; **3-7** and **3-11**, pink)

第三節　非ペプチド性化合物の合成

1. アミン型化合物の合成

アミン型化合物 **3-4** – **3-7** の合成を Scheme 3-1 に示す。アミン **3-12** をメタノール中 $PhCH_2CHO$ 及び $NaBH_3CN$ で処理することで還元的アルキル化反応成績体 **3-14** を得た。**3-14** の Cbz 基を水素化分解により除去した後、生じたアミノ基に対して混合酸無水物法によりβ-ラクトンユニット **1-35**[41]を縮合して標的化合物 **3-4** を得た。他の標的化合物についても同様に合成した。

Scheme 3-1. アミン型化合物 3-4 – 3-7 の合成 [a]

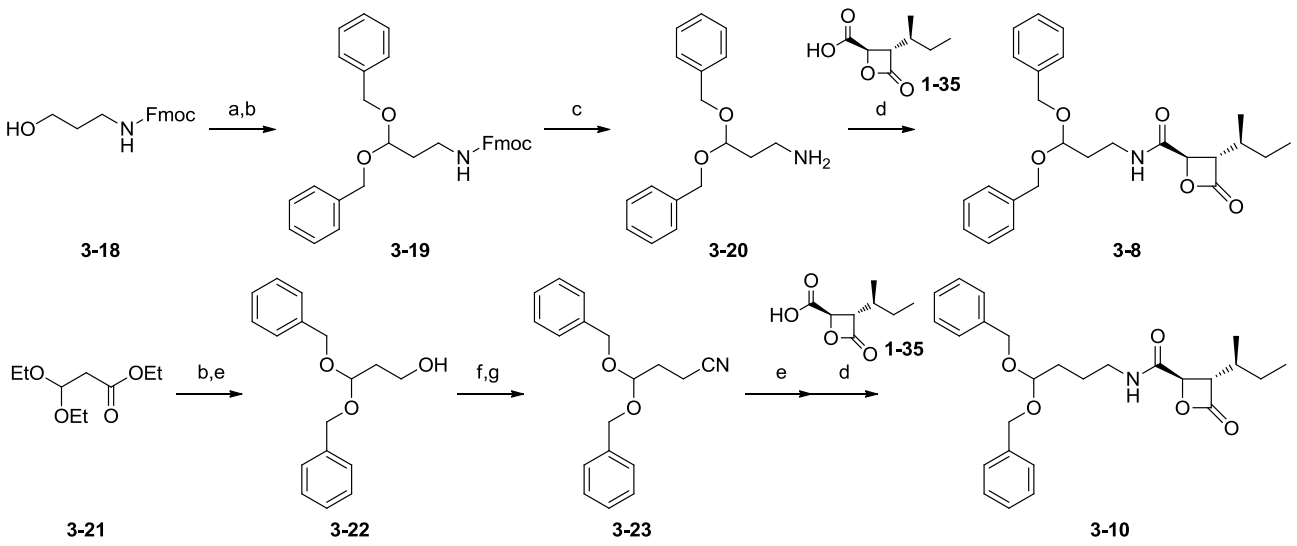


[a]Reagents and conditions: (a) phenylacetaldehyde, $NaBH_3CN$, MeOH, 96% for **3-14**, 86% for **3-16**; (b) benzylacetaldehyde, $NaBH_3CN$, MeOH, quant. for **3-15**, 90% for **3-17**; (c) H_2, $Pd(OH)_2$/C, MeOH; (d) **1-35**, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, yields were as follows (2 steps), 90% for **3-4**, quant. for **3-5**, 83% for **3-6**, 67% for **3-7**.

2. エーテル型化合物の合成

エーテル型化合物 **3-8** 及び **3-10** の合成を Scheme 3-2 に示す。Dess-Martin 酸化によりアルコール **3-18** をアルデヒドへと酸化した後、ベンジルアルコールと脱水縮合してアセタール **3-19** を得た。**3-19** の Fmoc 基をメタノール中 K_2CO_3 で処理して除去した後、生じたアミノ基に対して混合酸無水物法によりβ-ラクトンユニット **1-35**[41]を縮合して標的化合物 **3-8** を得た。エチルアセタール **3-21** をアセタール交換によりベンジルアセタールとした後、エチルエステル部を $LiAlH_4$ で還元してアルコール **3-22** を得た。**3-22** のヒドロキシ基を MsCl によりメシル化した後、DMSO 中 NaCN で処理してニトリル **3-23** を得た。**3-23** のシアノ基を $LiAlH_4$ で還元し、生じたアミノ基に対して混合酸無水物法によりβ-ラクトンユニット **1-35**[41]を縮合して標的化合物 **3-10** を得た。

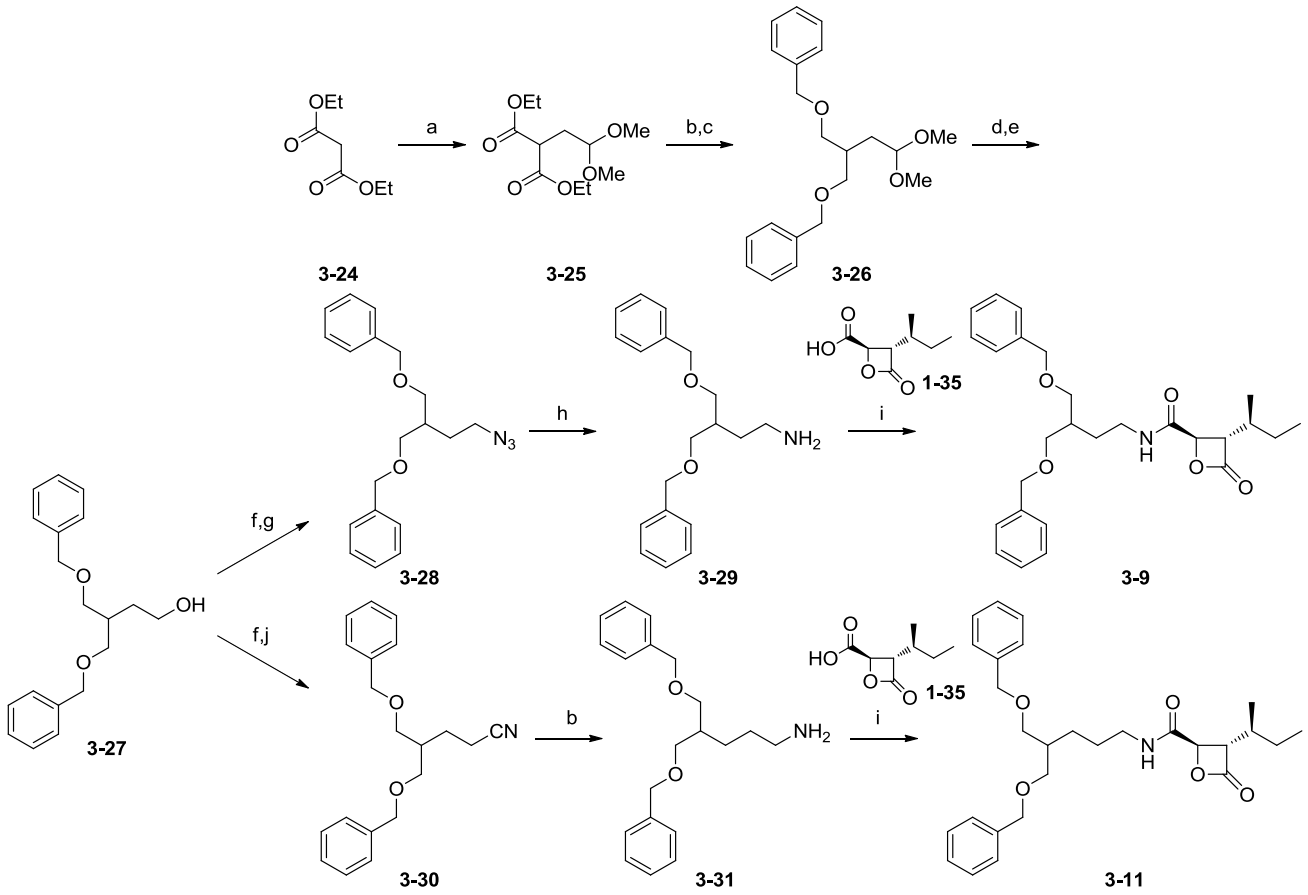
Scheme 3-2. エーテル型化合物 **3-8** 及び **3-10** の合成 [a]



[a]Reagents and conditions: (a) Dess-Martin periodinane, CH_2Cl_2; (b) BnOH, *p*-TsOH, benzene, reflux, 2 steps 57% for **3-19**; (c) K_2CO_3, MeOH, 97%; (d) **1-35**, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, 95% for **3-8**, 2 steps quant. for **3-10**; (e) $LiAlH_4$, Et_2O, 0 °C to rt, 2 steps 60% for **3-22**; (f) MsCl, Et_3N, CH_2Cl_2, 0 °C; (g) NaCN, DMSO, 60 °C, 2 steps 92%.

エーテル型化合物 **3-9** 及び **3-11** の合成を Scheme 3-3 に示す。マロン酸ジエチル **3-24** をエタノール中 NaOEt 及び $BrCH_2CH(OMe)_2$ で処理してアルキル化し、化合物 **3-25** を得た。**3-25** のエチルエステルを $LiAlH_4$ で還元して対応するジオールとし、これを DMF 中 BnBr 及び NaH で処理してベンジルエーテル **3-26** を得た。**3-26** のジメチルアセタールを加水分解して対応するアルデヒドとした後、これをメタノール中 $NaBH_4$ で処理してアルコール **3-27** を得た。**3-27** のヒドロキシ基を MsCl によりメシル化した後、これを NaN_3 あるいは NaCN で処理することでそれぞれアジド **3-28** 及びニトリル **3-30** を得た。**3-28** のアジド基を Staudinger 反応により還元してアミン **3-29** とし、このアミノ基に対して混合酸無水物法によりβ-ラクトンユニット **1-35**[41]を縮合して標的化合物 **3-9** を得た。また、ニトリル **3-30** のシアノ基を $LiAlH_4$ で還元してアミン **3-31** とし、このアミノ基に対して混合酸無水物法によりβ-ラクトンユニット **1-35**[41]を縮合して標的化合物 **3-11** を得た。

Scheme 3-3. エーテル型化合物 **3-9** 及び **3-11** の合成 [a]



[a]Reagents and conditions: (a) bromoacetaldehyde dimethyl acetal, NaOEt, EtOH, rt to reflux, 59% (b) $LiAlH_4$, Et_2O, 0 °C to rt; (c) NaH, BnBr, DMF, 0 °C, to rt, 2 steps 64%; (d) AcOH, H_2O; (e) $NaBH_4$, MeOH, 0 °C to rt, 2 steps 97%; (f) MsCl, Et_3N, CH_2Cl_2, 0 °C; (g) NaN_3, DMF, 70 °C, 2 steps 91%; (h) PPh_3, H_2O/THF, rt to reflux, quant.; (i) **1-35**, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, quant. for **3-9**, 2 steps quant. for **3-11**; (j) NaCN, DMSO, 60 °C, 2 steps 98%.

以上に示したように、設計した非ペプチド性化合物 **3-4** – **3-11** は、その骨格に不斉点を持たないためいずれも短工程で容易に合成可能であった。

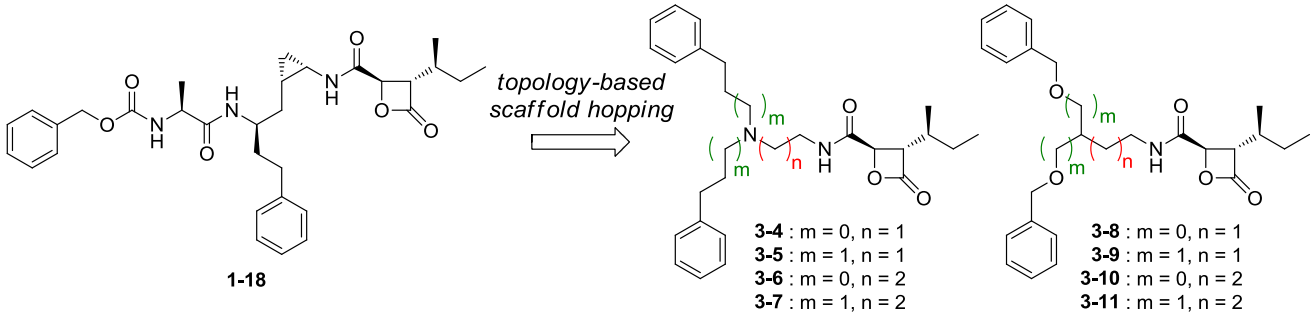
第四節　非ペプチド性化合物の生物活性

1. 非ペプチド性化合物のプロテアソーム阻害活性及び細胞増殖抑制作用

合成した非ペプチド性化合物 **3-4** – **3-11** のヒト 20S プロテアソーム ChT-L 活性に対する阻害活性を蛍光基質 Suc-LLVY-AMC を用いて測定した (Table 3-1)。アミン型化合物 **3-4** – **3-7** は、一連の構造活性相関研究のリード化合物であるペプチド性天然物ベラクトシン A (IC_{50} = 1440 nM) よりも強力なプロテアソーム阻害活性を有することが分かった (IC_{50} = 175 – 393 nM)。更に、エーテル型化合物 **3-8** – **3-11** は、親化合物 **1-18** の多数の不斉点からなるペプチド骨格に比べて高度に簡略化されたアキラルな非ペプチド骨格を有するにも関わらず、強力なプロテアソーム阻害活性を示した (IC_{50} = 26 – 56 nM)。これらの非ペプチド性化合物では、骨格のアルキル鎖長はプロテアソーム阻害活性にあまり大きな影響を与えなかった。これはいずれのアルキル鎖長を有する化合物もファーマコフォアを満たすことを示しており、Figure 2-d に示した計算結果と一致する。

更に、これらの非ペプチド性プロテアソーム阻害剤の HCT116 細胞に対する細胞増殖抑制作用を評価した。Table 3-1 に示すように、**3-5** と **3-9** 以外の全ての化合物が 10 μM 以下の濃度で細胞増殖抑制作用を示し、中でもエーテル型化合物 **3-10** は親化合物である **1-18** とほぼ同等の細胞増殖抑制作用を示した。

Table 3-1. 非ペプチド性化合物 **3-4** – **3-11** のプロテアソーム阻害活性及び細胞増殖抑制作用とその諸性質



compound number	ChT-L activity			molecular properties compared with **1-18**			HCT116 cell growth
	IC_{50} [nM][a]	BEI[b]	SEI[c]	molecular weight	tPSA[d]	total synthetic steps	IC_{50} [μM][a]
3-4	175 ± 32	37	26	- 141	- 64	- 16	8.90
3-5	393 ± 24	33	25	- 113	- 64	- 16	> 10
3-6	215 ± 40	35	26	- 127	- 64	- 16	2.20
3-7	352 ± 25	32	25	- 99	- 64	- 16	3.80
3-8	28 ± 2.2	41	23	- 138	- 49	- 15	4.10
3-9	29 ± 13	38	23	- 110	- 49	- 10	> 10
3-10	26 ± 6.1	40	24	- 124	- 49	- 13	1.80
3-11	56 ± 16	36	22	- 96	- 49	- 10	4.20
1-18	5.7 ± 1.2	34	15	-	-	-	1.82

[a]Based on three experiments. [b]pIC_{50} per molecular weight (kDa). [c]pIC_{50} per polar surface area (PSA) normalized to 100 $Å^2$. [d]Calculated by ChemBioDraw Ultra 12.0.

2. 結合効率指数 (BEI) 及び表面結合効率指数 (SEI) の変化

“Scaffold hopping”による分子特性の変化を検証するため、一連の化合物の結合効率指数 (BEI) 及び表面積

結合効率指数 (SEI) を計算した (Table 3-1)。BEI 及び SEI は、それぞれ分子量 (kDa) 及び極性表面積 (polar surface area, PSA) 100 Å^2 当たりの pIC$_{50}$ 値を示す。[69]分子量及び極性表面積は、化合物の膜透過性やバイオアベイラビリティとの相関がよく知られている物理化学的性質であり、[70]標的に対する活性値とこれらの物理化学的性質のバランスを示す BEI 及び SEI は効果的なドラッグライクネスの指標である。[69]Abad-Zapatcro らは、ドラッグライクな開発候補化合物を見出すためには、リード化合物の最適化において BEI 及び SEI を同時に改善していくことが重要であると報告している。[69]

“Topology-based scaffold hopping”により新たに見出した非ペプチド性プロテアソーム阻害剤 **3-4** – **3-11** と、第一章で述べた親化合物 **1-18** と共通のペプチド骨格を有する一連のベラクトシン誘導体の BEI – SEI プロット図を Figure 3-4 に示す。*同一のペプチド骨格を有する一連のベラクトシン誘導体を比較すると、BEI は大きく変化しているものの (BEI = 25 – 43)、SEI はほとんど変化していないことが分かる (SEI = 13 – 16)。これは、置換基の変換はプロテアソーム阻害活性に大きく影響するものの、骨格構造に由来する分子特性に対して与える影響は小さいことを示す。一方で、“scaffold hopping”により新たに見出した非ペプチド性化合物 **3-4** – **3-11** は、ペプチド骨格を持たないために極性表面積が小さく、親化合物 **1-18** と比べて顕著に高い SEI を示した。更に、構造が簡略化されたこれらの化合物の分子量は親化合物 **1-18** と比べて小さいために、**3-5** 及び **3-7** 以外の全ての化合物が **1-18** よりも高い BEI を示した。これらの優れた BEI 及び SEI は、新たに見出した非ペプチド性プロテアソーム阻害剤が親化合物 **1-18** よりも、優れたリード化合物であることを示すものである。

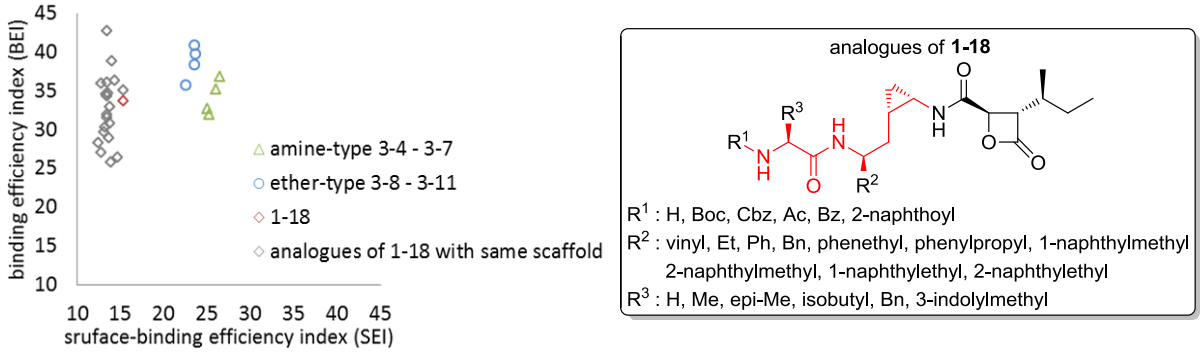


Figure 3-4. ベラクトシン誘導体と非ペプチド性化合物の BEI 及び SEI

以上、筆者はベラクトシン A シス型異性体の誘導体 **1-18** を親化合物とした“topology-based scaffold hopping”により、**1-18** の多数の不斉点からなるペプチド骨格をアキラルな非ペプチド骨格へと変換することに成功した。新たに見出した一連の非ペプチド性プロテアソーム阻害剤は、合成が容易である上に親化合物 **1-18** よりも優れた BEI 及び SEI を示すことから、更なる構造最適化研究におけるより有用なリード化合物であると考えられる。本研究は“topology-based scaffold hopping”が所望の生物活性を損なうことなく分子特性を変換するのに極めて効果的であり、天然物やペプチドをリード化合物とした創薬研究において非常に有用であることを示すものである。

また、一連の構造活性相関研究によって、低活性なペプチド性天然物ベラクトシン A (IC$_{50}$ = 1440 nM) から大きな構造簡略化を伴って極めて高活性な非ペプチド性プロテアソーム阻害剤 **3-10** (IC$_{50}$ = 26 nM) を見出したことは特筆すべき成果である。

* 各々のベラクトシン誘導体の BEI 及び SEI については実験の部を参照。

第四章　ベラクトシン A シス型異性体の安定化誘導体の開発[71]

第一節　安定化を指向した誘導体の設計

化合物の化学的及び生物学的安定性はその化学構造により規定されるため、構造変換によって調節することが可能である。[72]創薬研究においては、一般に不安定な化合物は生体内で迅速に分解を受けて望みの生理活性を発現することが出来ないうえ、非標的タンパク質と共有結合を形成して毒性の発現に繋がる場合もあることから、適切な安定性を有する化合物を開発する必要がある。[73]化合物の化学的及び生物学的安定性は、不安定な部位の立体的及び電子的性質を変化させて安定化することで改善することができる。更に、化合物が酵素によって分解を受ける場合には、酵素に対する親和性を低下させることで安定性を改善することができるため、化合物の分子サイズ、疎水性、電子的性質や配座などの分子特性の変換が有効である。

第一章で述べたように、ベラクトシン A シス型異性体の誘導体 **1-18** の細胞増殖抑制作用 (IC_{50} = 1.8 μM) はそのプロテアソーム阻害活性 (IC_{50} = 5.7 nM) に比べて著しく低かったが、その原因はベラクトシン誘導体が生理学的条件下極めて不安定である (Table 1-5) ためであると考えられる。そこで、細胞増殖抑制作用の改善を目指し、安定なベラクトシン誘導体を開発することを計画した。

1. 立体効果を利用したβ-ラクトン部の安定化

ベラクトシン誘導体の分解は、主に不安定なβ-ラクトン部の加水分解によるものと考えられる。そこで、立体効果を利用してβ-ラクトン部の加水分解に対する反応性を低下させることで、より安定な誘導体を創出することを計画した。一般に、カルボニル基の求核剤との反応性は、カルボニル基周辺を立体的に遮蔽することで低下するため、[74]Figure 4-1a に示すように、β-ラクトンカルボニルα-位に種々の置換基を有する一連の誘導体 **4-1** – **4-3** を設計した。α-炭素上の置換基の立体的嵩高さは Figure 4-1a に示すように **4-1** < **1-18** < **4-2** < **4-3** であると考えられ、この順にβ-ラクトン部の安定性が増すと期待される。*

* ベラクトシン誘導体はそのβ-ラクトン部がプロテアソームの活性残基である Thr 側鎖のヒドロキシ基と反応して共有結合を形成することでプロテアソーム阻害活性を示すため、β-ラクトン部の反応性の低下はプロテアソーム阻害活性の低下に繋がると考えられる。しかしながら、これらの誘導体において、新たにβ-ラクトンカルボニルα-位に導入したメチル基は、Thr のヒドロキシ基が接近する面とは反対側の置換基であるため、プロテアソーム阻害活性をある程度保持して安定化が実現することを期待した。

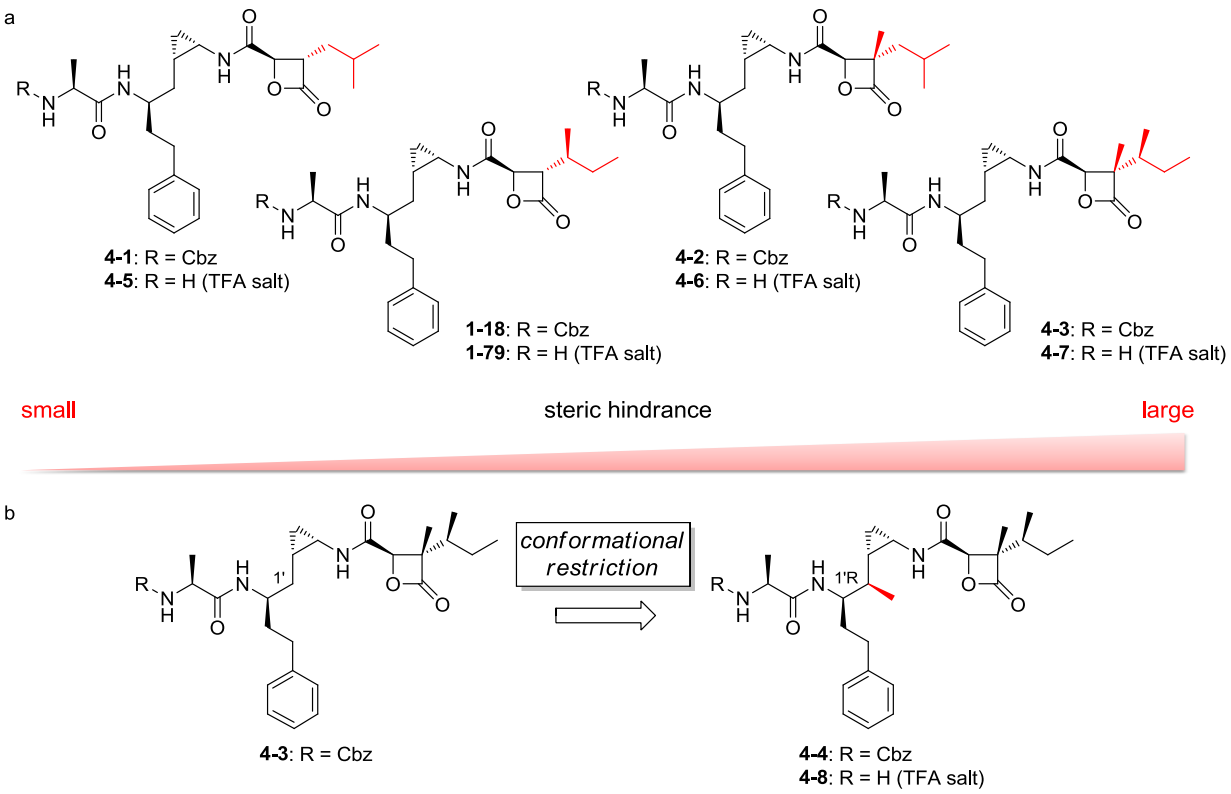


Figure 4-1. 設計した誘導体の構造 (a) β-ラクトン部の立体効果による安定化 (b) 配座制御による安定化

2. 配座制御による安定化

第一章で述べたように、ベラクトシン誘導体 **1-79** は pH 7.4 TEAA 緩衝液中での半減期は 10 時間とそれほど不安定ではなかったのに対し、ヒト AB 型血清中での半減期が 2.3 分と生理学的条件下極めて不安定であった。このことから、ベラクトシン誘導体は生理学的条件下酵素による分解を受けると考えられる。そこで、配座制御による分解酵素への親和性低下を期待して、第二章で述べた C1'上へのメチル基の導入によるシクロプロパン歪みを利用した配座制御により、化合物 **4-3** を親化合物として活性配座である *syn* 配座に制御した配座制限誘導体 **4-4** を設計した (Figure 4-1b)。このシクロプロパン歪みを利用した配座制御は、メチル基の導入という小さな構造変化によって効果的に実現するため、配座と安定性の関係を検証するのに適している。

3. 安定性評価のための水溶性誘導体

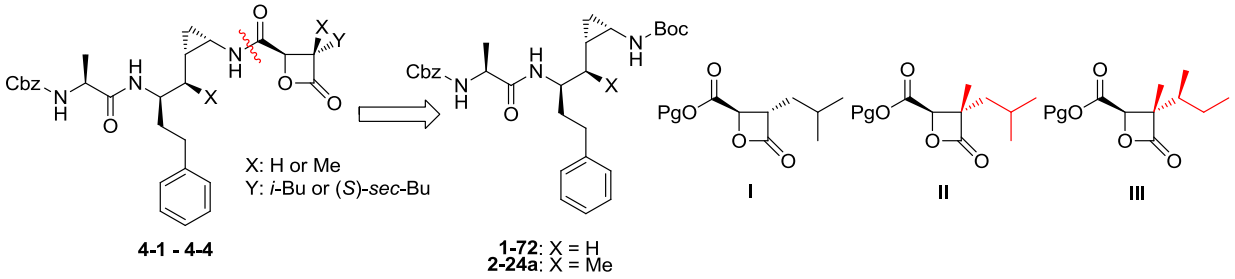
一連の設計した誘導体は水溶性が低いと考えられる。そこで、設計した誘導体の化学的及び生物学的安定性を評価して化学構造と安定性の関係を検証するために、各々の誘導体について N 末端部の Cbz 基を持たない水溶性の高い誘導体 **4-5** – **4-8** を合成することにした (Figure 4-1)。

第二節　設計した誘導体の合成

1. 設計した誘導体の合成計画

標的化合物 **4-1** – **4-4** は、**1-72** または **2-24a** に対してそれぞれ対応するβ-ラクトンユニットを縮合して合成できると考えられる。**1-72** 及び **2-24a** の合成についてはそれぞれ第一章及び第二章で述べた (Scheme 1-5 及び Scheme 2-3)。β-ラクトンユニット **I** – **III** の合成においては、とりわけ **II** 及び **III** のβ-ラクトンカルボニルα-位の第四級不斉炭素の構築が鍵となる。

Scheme 4-1. 設計した誘導体 **4-1** – **4-4** の合成計画



2. β-ラクトンユニットの合成

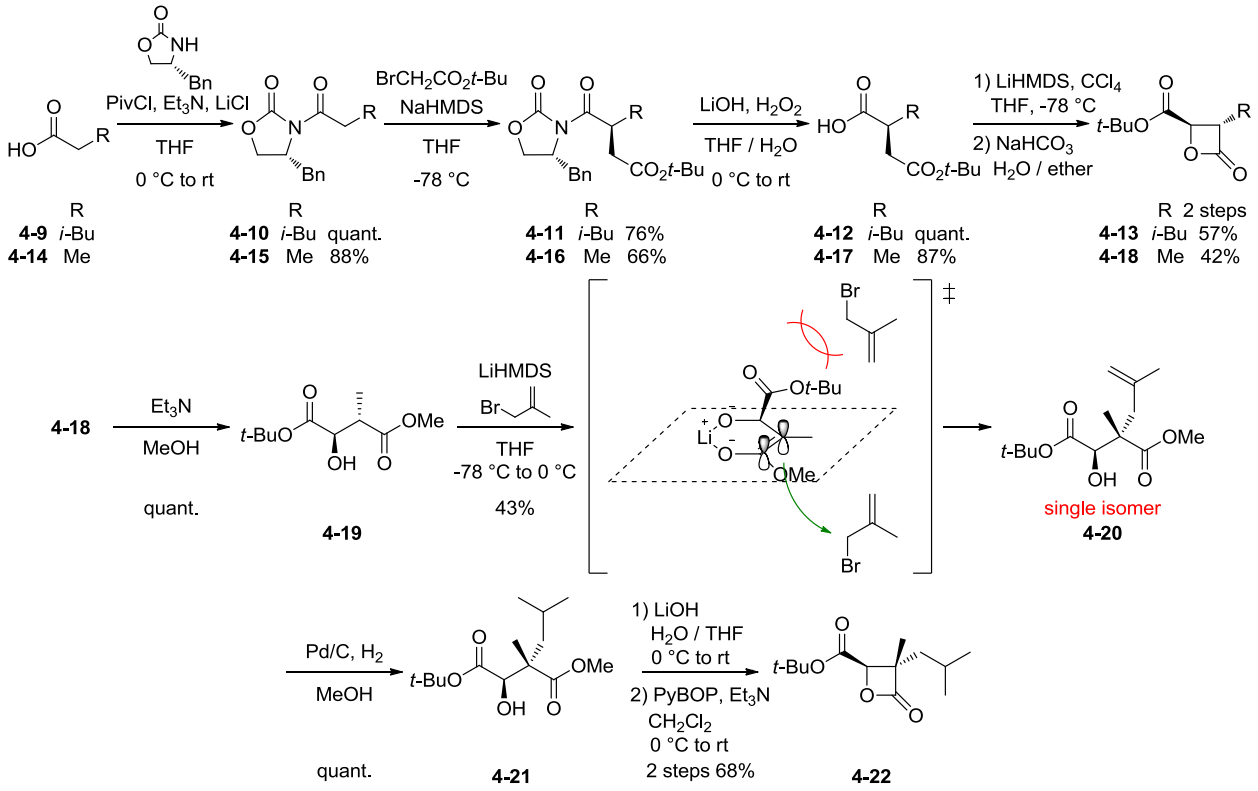
β-ラクトンユニット **I** 及び **II** の合成を Scheme 4-2 に示す。β-ラクトンユニット **I** は Armstrong らの報告したβ-ラクトンユニット **1-35** の合成法[41]と同様の方法により合成した。即ち、4-メチルペンタン酸 **4-9** と(4*R*)-4-ベンジル-2-オキサゾリジノンを、LiCl を添加剤として用いた混合酸無水物法[75]により縮合して化合物 **4-10** を得た。**4-10** を THF 中-78 °C において NaHMDS 及び $BrCH_2CO_2t$-Bu で処理することで、カルボニルα-位に対してジアステレオ選択的にアルキル基を導入して化合物 **4-11** を得た。*[58, 76] **4-11** のオキサゾリジノン部を LiOH 及び H_2O_2 を用いた加水分解[77]により除去してカルボン酸 **4-12** を得た。**4-12** を THF 中-78 °C において LiHMDS 及び CCl_4 で処理することで、*t*-ブチルエステルのカルボニルα-位に対してジアステレオ選択的にクロロ基を導入し、†[78]続く塩基性二相系条件下での閉環反応によりβ-ラクトンユニット **4-13** を得た (ユニット **I**, Pg = *t*-Bu)。‡

β-ラクトンユニット **II** はプロピオン酸 **4-14** を出発物質として **4-13** と同様に合成したβ-ラクトン **4-18** から合成した。**4-18** をメタノリシスにより開環して化合物 **4-19** を得た。**4-19** を THF 中-78 °C において LiHMDS 及び 3-ブロモ-2-メチルプロパンで処理後 0 °C に昇温することで、メチルエステルα-位をジアステレオ選択的にアルキル化し、第四級不斉炭素を有する化合物 **4-20** を単一のジアステレオマーとして得た。§この反応はジアニオンが Li にキレートした六員環状の遷移状態を経由して進行すると考えられ、Scheme 4-2 に示すように嵩高い *t*-ブチルエステルの立体効果に基づき高いジアステレオ選択性が発現すると考えられる。[79] **4-20** の二重結合を接触水素化により還元して化合物 **4-21** を得た。**4-21** のメチルエステルを選択的に加水分解した後、ジクロロメタン中 PyBOP[80]で処理して閉環反応を行いβ-ラクトンユニット **4-22** を得た (ユニット II, Pg = *t*-Bu)。尚、β-ラクトンユニット **4-22** の相対立体配置は、**4-22** の NOE 実験により決定した (Figure 4-2a)。**

---

* 本反応において、ジアステレオマーの生成は粗生成物の ^{1}H-NMR スペクトル上見られなかった。
† この反応は、ジアニオンが Li にキレートした七員環状の遷移状態を経由して進行すると考えられている。[76]
‡ 本反応において、ジアステレオマーの生成は粗生成物の ^{1}H-NMR スペクトル上見られなかった。
§ 本反応においては、**4-20** のヒドロキシ基が TMS 化された生成物が 50%以上の収率で得られた。
** 絶対立体配置は既知化合物 **4-17** の比旋光度を測定して決定した。

Scheme 4-2. β-ラクトンユニット **I** 及び **II** の合成



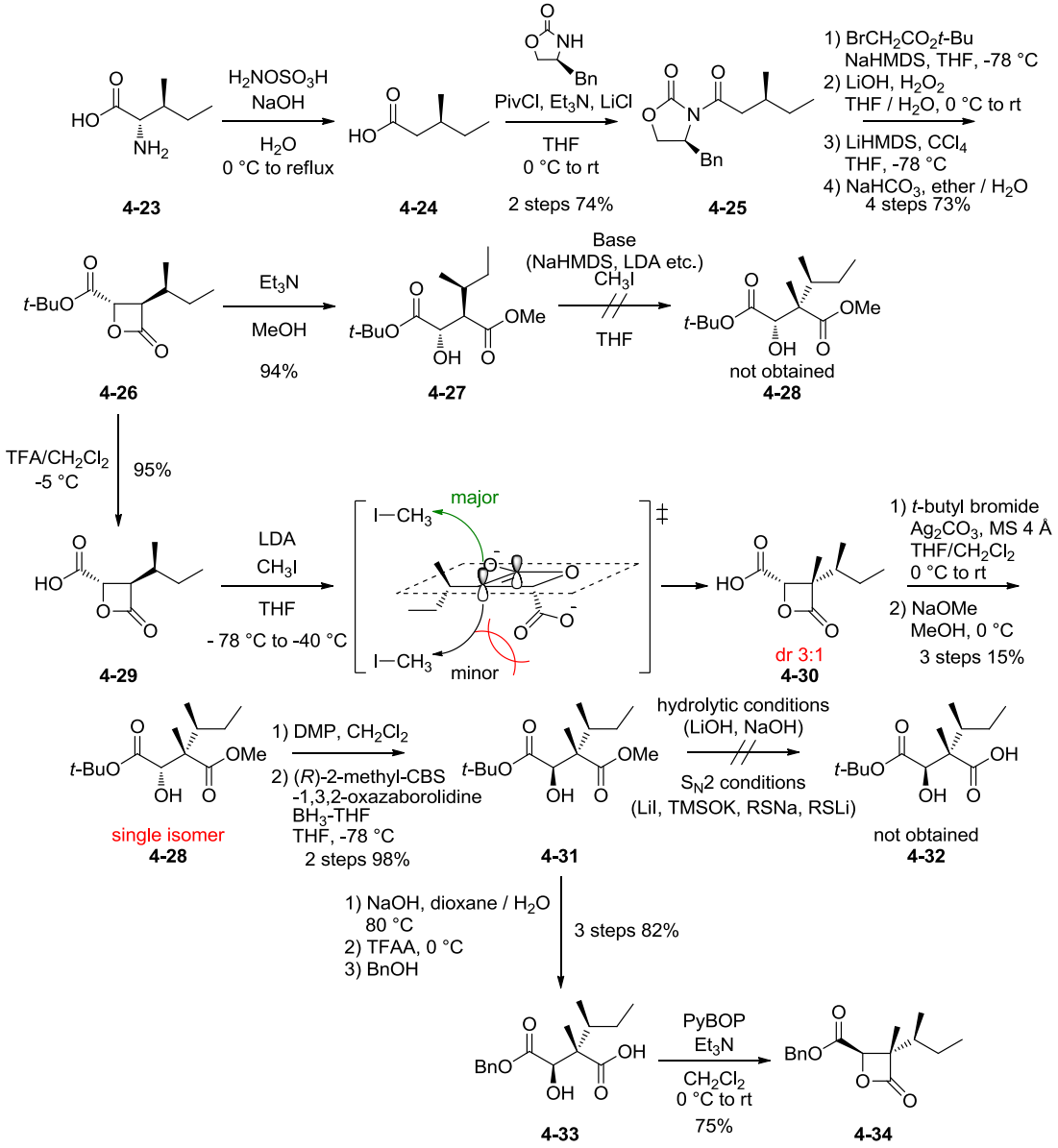
β-ラクトンユニット **III** の合成を Scheme 4-3 に示す。L-イソロイシン **4-23** の脱アミノ化[81]によりカルボン酸 **4-24** を得た。このカルボン酸 **4-24** を出発物質として **4-19** の合成と同様にアルコール **4-27** を合成した。このアルコール **4-27** を基質として、上述の **4-19** のジアステレオ選択的アルキル化反応と同様のメチル化反応を種々検討したが、反応は全く進行せず **4-28** は得られなかった。この原因は、嵩高い(*S*)-*sec*-ブチル基の立体効果のために、**4-27** から生じるジアニオンの反応性が低いためと考えられる。そこで、β-ラクトン **4-29** を基質としてジアステレオ選択的メチル化反応を検討することとした。[82]β-ラクトン **4-29** は、β-ラクトン **4-26** の *t*-ブチル基を酸性条件下除去して合成した。**4-29** を THF 中-78 °C において LDA 及び MeI で処理した後-40 °C に昇温することで、収率は低いもののジアステレオ選択的にメチル化が進行し、第四級不斉炭素を有する化合物 **4-30** が得られた (dr 3:1)。この反応においては、Scheme 4-3 に示すようにβ-ラクトン環上のカルボキシ基の立体効果に基づいて立体選択性が規定されているものと考えられる。**4-30** のカルボキシ基を *t*-ブチル基で再び保護した後、メタノリシスによりβ-ラクトン環を開環して化合物 **4-28** を単一ジアステレオマーとして得た。**4-28** の第二級アルコール部を Dess-Martin 酸化により酸化した後、生じたカルボニル基を(*R*)-2-メチル-CBS-1,3,2-オキサゾリジノン[83]を用いた CBS 還元によりジアステレオ選択的に還元することで、**4-28** の立体異性体 **4-31** を単一ジアステレオマーとして得た。*続いて、**4-31** のメチルエステルの選択的な脱保護によりモノカルボン酸 **4-32** を得るべく種々条件を検討したが、加水分解及び S_N2 条件いずれにおいても *t*-ブチルエステルの脱保護が優先して進行し、**4-32** は全く得られなかった。そこで、**4-32** のメチルエステル及び *t*-ブチルエステルを加水分解してジカルボン酸とした後、無水トリフルオロ酢酸 (TFAA) で処理することで環状酸無水物とし、更にベンジルアルコールで加溶媒分解することでモノカルボン酸 **4-33** を得た。†[84]最後に、

* CBS 還元のジアステレオ選択性は粗生成物の ^{1}H-NMR スペクトルより 99:1 であり、生じたジアステレオマーはカラムクロマトグラフィーによる精製で除いた。

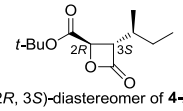
† この環状酸無水物の加溶媒分解は位置特異的に進行し、位置異性体の生成は粗生成物の ^{1}H-NMR スペクトル上見られなかった。

4-33 をジクロロメタン中 PyBOP[80]で処理して閉環反応を行いβ-ラクトンユニット **4-34** を得た (ユニット **III**, Pg = Bn)。尚、β-ラクトンユニット **4-34** の相対立体配置は **4-34** の NOE 実験により決定した (Figure 4-2b)。*

Scheme 4-3. β-ラクトンユニット **III** の合成



* 絶対立体配置は、化合物 **4-26** の比旋光度及び ^{1}H-NMR スペクトルが Armstrong らにより合成されたβ-ラクトンユニット **1-35** の合成中間体である (2*R*, 3*S*)-ジアステレオマー[41]のものと異なっており、かつβ-ラクトン環上のプロトン間の *J* 値がほぼ一致し (**4-26**, *J* = 4.5 Hz; diastereomer, *J* = 4.3 Hz[41])、トランス配置のβ-ラクトンであると考えられることから決定した。



(2*R*, 3*S*)-diastereomer of **4-26**
$J_{2H,3H}$ = 4.3 Hz

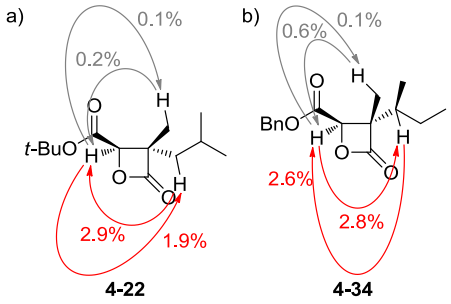
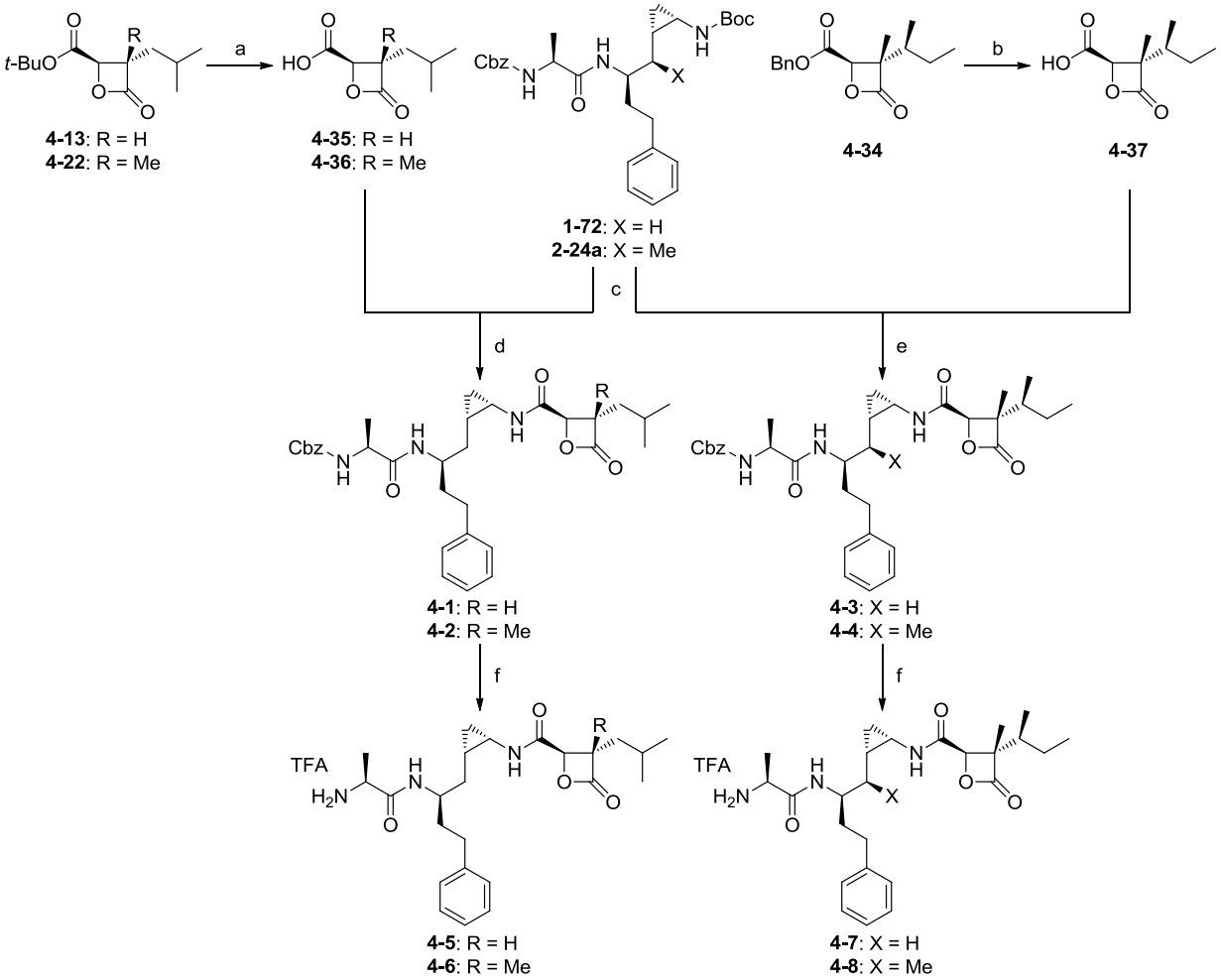


Figure 4-2. β-ラクトンユニット **4-22** 及び **4-34** の NOE 実験

3. 設計した誘導体の合成

標的化合物 **4-1** – **4-8** の合成を Scheme 4-4 に示す。β-ラクトンユニット **4-13**, **4-22** 及び **4-34** の保護基を除去してそれぞれ対応するカルボン酸とした後、**1-72** あるいは **2-24a** の Boc 基を除去して得たアミンと縮合することで、標的化合物 **4-1** – **4-4** を合成した。また **4-1** – **4-4** の Cbz 基を水素化分解により除去して、標的化合物 **4-5** – **4-8** を合成した。

Scheme 4-4. 設計した誘導体 **4-1** – **4-8** の合成 [a]



[a]Reagents and conditions: (a) TFA/CH_2Cl_2, -5 °C; (b) Pd/C, H_2, THF, quant.; (c) TFA/CH_2Cl_2; (d) PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, 62% (**4-1**, 2 steps from **1-72**), 100% (**4-2**, 2 steps from **1-72**); (e) EDC·HCl, HOAt, Et_3N, CH_2Cl_2, 0 °C, quant. (**4-3**, 2 steps from **1-72**), 91% (**4-4**, 2 steps from **2-24a**); (f) Pd/C, H_2, TFA/THF, 0 °C, quant. (**4-5** – **4-8**)

第三節　合成した誘導体の安定性及び生物活性

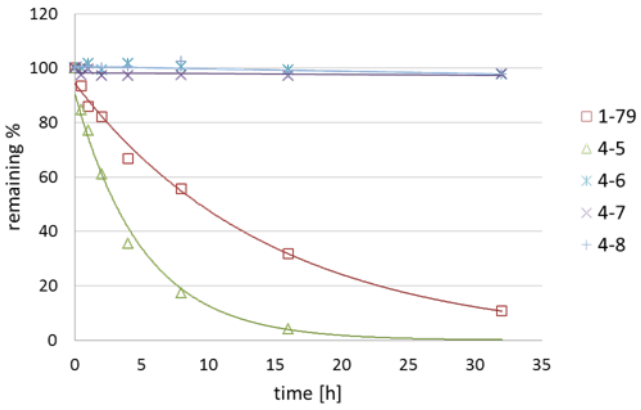
1. 合成した誘導体の化学的及び生物学的安定性

合成した誘導体 **4-5** – **4-8** の 37 °C における 0.1 M TEAA 緩衝液 (pH 7.4) 中及びヒト AB 型血清中における残存率の経時変化を HPLC で測定し、各化合物のそれぞれの条件下での半減期を求めた (Figure 4-3)。0.1 M TEAA 緩衝液中での化合物の安定性は **4-5** < **1-79** < **4-6** – **4-8** の順となり、設計時の想定通り Figure 4-1 に示したβ-ラクトンカルボニルα-位の置換基の立体的嵩高さの順と一致していることが分かった。**4-6** – **4-8** のβ-ラクトンカルボニルα-位に第四級炭素を有する誘導体は、本条件下では非常に安定であり全く分解されなかった。

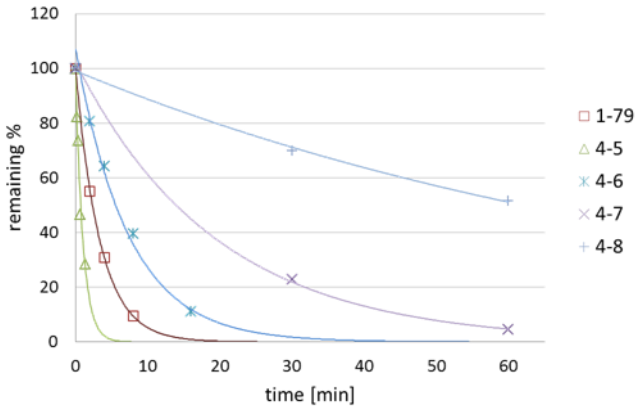
ヒト AB 型血清中での安定性も **4-5** < **1-79** < **4-6** < **4-7** < **4-8** の順となり、Figure 4-1 に示したβ-ラクトンカルボニルα-位の置換基の立体的嵩高さの順と一致した。しかしながら、これらの化合物のヒト AB 型血清中における半減期は 0.1 M TEAA 緩衝液中での半減期に比べて極めて短く、ヒト AB 型血清中では酵素によって分解されていることが示唆された。また、**4-7** の配座制限誘導体である **4-8** は、**4-5** ($t_{1/2}$ = 14 min) に比べて非常に安定であった ($t_{1/2}$ = 63 min)。この安定性の差異は、設計時に期待した通り配座制御によって分解酵素に対する親和性が低下したことによるものと考えられ、配座柔軟性と生物学的安定性の関係を示す例として興味深い。

以上の水溶性誘導体 **4-5** – **4-8** を用いた安定性試験の結果から、リード化合物である **1-18** に比べて新たに設計・合成した誘導体 **4-4** は化学的及び生物学的に安定であると考えられる。

a



b



c

compound number	$t_{1/2}$ at 37 °C	
	0.1 M TEAA buffer (pH 7.4) [h]	human AB serum [min]
1-79	10	2.3
4-5	3.5	0.73
4-6	ND	5.0
4-7	ND	14
4-8	ND	63

Figure 4-3. 標的化合物 **1-79** 及び **4-5** – **4-8** の化学的及び生物学的安定性 (a) HPLC で測定した **1-79** 及び **4-5** – **4-8** の 0.1 M TEAA 緩衝液 (pH 7.4) 中での残存率の経時変化 (b) HPLC で測定した **1-79** 及び **4-5** – **4-8** のヒト AB 型血清中での残存率の経時変化 (c) それぞれの条件における **1-79** 及び **4-5** – **4-8** の半減期

2. 合成した誘導体のプロテアソーム阻害活性及び細胞増殖抑制作用

合成した誘導体 **4-1** – **4-4** のヒト 20S プロテアソーム ChT-L 活性に対する阻害活性を蛍光基質 Suc-LLVY-AMC を用いて測定した。Table 4-1 に示すように、β-ラクトンカルボニルα-位に第四級炭素原子を有する誘導体 **4-2** – **4-4** のプロテアソーム阻害活性 (何れも IC_{50} = 1.3 μM) は、リード化合物である **1-18** のプロテアソーム阻害活性 (IC_{50} = 5.7 nM) と比べて極めて低かった。第二章で述べたように、ベラクトシン誘導体のプロテアソーム阻害活性はその共有結合形成能に高度に依存しているため、安定なβ-ラクトンを有するこれらの誘導体ではプロテアソーム阻害活性が大きく低下したと考えられる。また、β-ラクトンカルボニルα-位に導入したメチル基の立体効果がプロテアソームとの相互作用において不利に働いている可能性も考えられる。**4-1** のプロテアソーム阻害活性 (IC_{50} = 72 nM) が **1-18** に比べて低いのは、イソブチル基 (**4-1**) よりも (*S*)-*sec*-ブチル基 (**1-18**) の方がプロテアソーム S1 ポケットと効果的に相互作用するためと考えられる。

また、合成した誘導体 **4-1** – **4-4** の HCT116 細胞に対する細胞増殖抑制作用を評価した。Table 4-1 に示すように、**4-3** 以外の誘導体は **1-18** (IC_{50} = 1.8 μM) と同等の細胞増殖抑制作用を示した (IC_{50} = 3.8 – 5.2 μM)。プロテアソーム阻害活性が著しく低下した **4-2** 及び **4-4** が **1-18** と同等の細胞増殖抑制作用を示したのは、**4-2** 及び **4-4** が **1-18** に比べて細胞評価系で安定であるためと考えることができる。

Table 4-1. 合成した誘導体 **4-1** – **4-4** のプロテアソーム阻害活性及び細胞増殖抑制作用

compound number	IC_{50} [μM][a]	
	ChT-L activity	cell growth (HCT116)
1-18	0.0057	1.8
4-1	0.072	5.2
4-2	1.3	3.8
4-3	1.3	> 10
4-4	1.3	4.0

[a]Based on three experiments

以上、筆者はβ-ラクトンカルボニル炭素の立体的遮蔽及びシクロプロパン歪みを利用した配座制御によって、ベラクトシン A シス型異性体の誘導体 **1-18** の安定化誘導体 **4-4** を見出すことに成功した。しかしながら、**4-4** のプロテアソーム阻害活性 (IC_{50} = 1.3 μM) はリード化合物である **1-18** (IC_{50} = 5.7 nM) に比べて著しく低く、プロテアソーム阻害活性を保持してベラクトシン誘導体を安定化することは困難であることが示唆された。

第五章　ペプチドボロン酸とのハイブリッド化合物の創製

第一節　ハイブリッド化合物の設計

第一章で述べたように、ベラクトシン A シス型異性体の誘導体 **1-18** の細胞増殖抑制作用 (IC_{50} = 1.8 μM) はそのプロテアソーム阻害活性 (IC_{50} = 5.7 nM) に比べて著しく低かった。ベラクトシン誘導体の活性基であるβ-ラクトンは生理学的条件下極めて不安定であり容易に加水分解を受けるため、ベラクトシン誘導体は細胞系で安定に存在できず、その細胞増殖抑制作用が低いものと考えられる。第四章では、β-ラクトンのカルボニル基を立体的に遮蔽することで、β-ラクトンの安定性を高めた安定化誘導体 **4-4** を見出すことに成功したが、プロテアソーム阻害活性もβ-ラクトンの反応性の低下に伴って低下してしまい、強い細胞増殖抑制作用を示す誘導体を見出すことは出来なかった。以上のことから、強い細胞増殖抑制作用を示す誘導体を見出すためには、活性基であるβ-ラクトンを生理学的条件下で安定な別の活性基に変換する必要があると考えた。

1. ペプチドボロン酸型プロテアソーム阻害剤

ペプチドボロン酸は代表的なプロテアソーム阻害剤のクラスであり、臨床で用いられているボルテゾミブ[20]や現在臨床試験が行われている CEP-18770[24]及び MLN-9708[25]はいずれもこのクラスのプロテアソーム阻害剤である。 (Figure 5-1)。ペプチドボロン酸型プロテアソーム阻害剤は、その活性基であるボロン酸がプロテアソーム活性残基である Thr 残基のヒドロキシ基とボロン酸エステルを形成することでプロテアソームに対する共有結合性阻害剤として働く。[34b, 85]ボロン酸は生理学的条件下で安定であり、多くの高い細胞増殖抑制作用を有するペプチドボロン酸型プロテアソーム阻害剤が知られている。[24-25, 86]そこで、ベラクトシン誘導体の活性基をβ-ラクトンからボロン酸へと変換することを計画した。

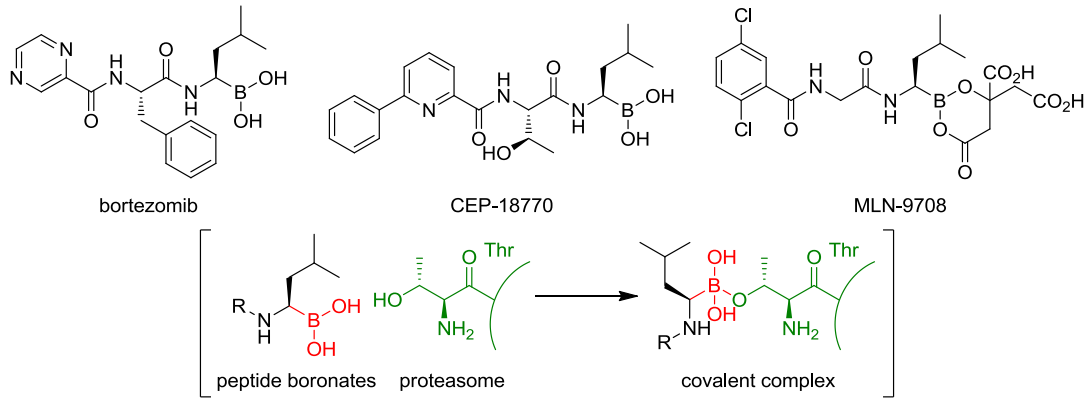


Figure 5-1. 代表的なペプチドボロン酸型プロテアソーム阻害剤とそのプロテアソーム阻害様式

2. ハイブリッド化合物の設計

ペプチドボロン酸型プロテアソーム阻害剤であるボルテゾミブ及びベラクトシン A シス型異性体の誘導体 **1-18** とプロテアソームの共結晶の X 線結晶構造[37, 85]を Figure 5-3 に重ねて示す。序論で述べたように、ベラクトシン誘導体は他のプロテアソーム阻害剤とは異なる結合部位を有しており、ボルテゾミブが P 領域結合部 (non-primed binding site) に結合するのに対して、ベラクトシン誘導体 **1-18** はβ-ラクトン部以外は P'領域結合部 (primed binding site) に結合していることが分かる。更に、ボルテゾミブの P2 側鎖はプロテアソーム結合状態において、ベラクトシン誘導体の結合部位である P'領域結合部の方向を向いていることが分かる。

従って、ペプチドボロン酸の P2 側鎖に対してベラクトシン誘導体の構造を連結することで、P'領域結合部と P 領域結合部の両方と強力に相互作用するベラクトシン誘導体とペプチドボロン酸のハイブリッド化合物の創製が可能であると考えた。

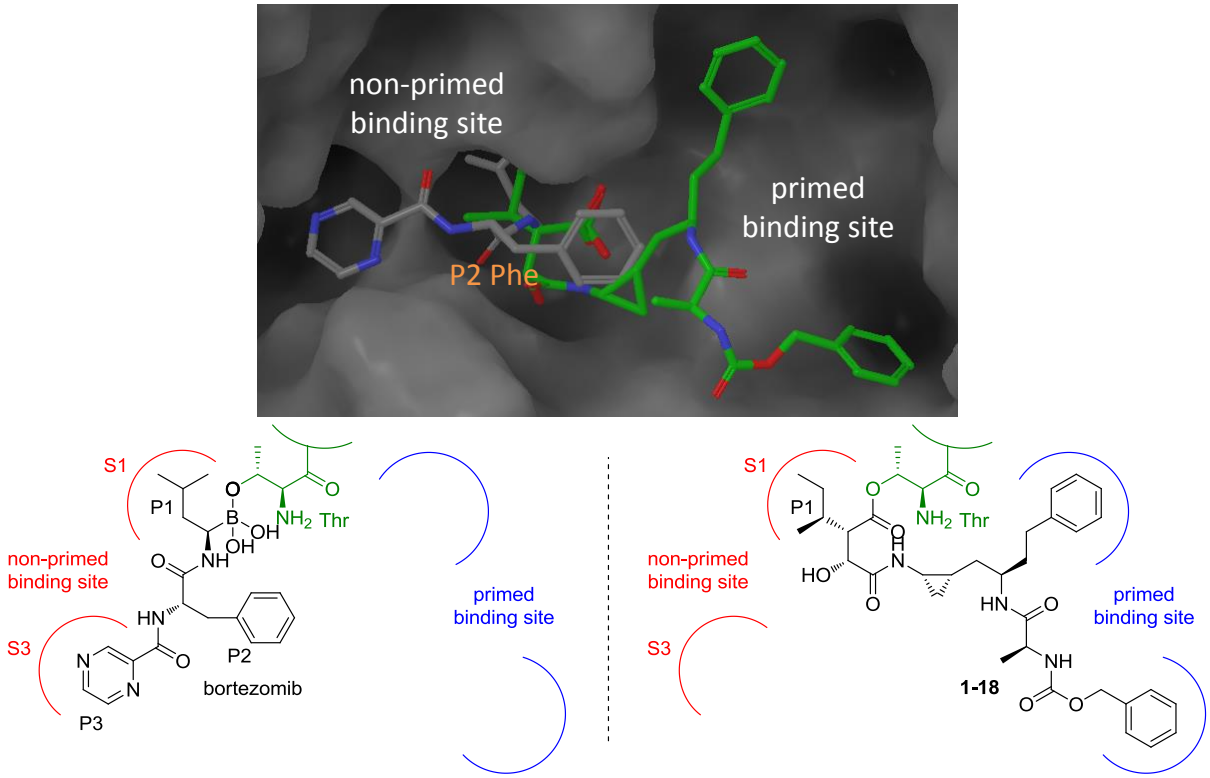


Figure 5-2. ボルテゾミブ (gray tube) 又はベラクトシン誘導体 **1-18** (green tube) とプロテアソームとの共結晶の構造の重ね合わせ

ベラクトシン A シス型異性体の誘導体 **1-18** はペプチド骨格を有している。このため、同様にペプチド骨格を有するペプチドボロン酸とのハイブリッド化合物は多数のペプチド結合を持つこととなり、膜透過性や代謝安定性上の問題が懸念される。[63b]そこで、第三章で述べた **1-18** を親化合物とした"topology-based scaffold hopping"により見出した、非ペプチド性プロテアソーム阻害剤 **3-9** の構造を基にしてハイブリッド化合物を設計した。Figure 5-3 に示すように、**3-9** 由来の非ペプチド構造 (n = 0 – 2) をペプチドボロン酸と P2 側鎖を介してアミド結合で繋ぐことで、P2 残基として Asp (m = 1) を有する化合物 **5-1a** – **5-3a** と Glu (m = 2) を有する化合物 **5-1b** – **5-3b** を設計した。*

* ペプチドボロン酸のピナンジオールエステルはプロドラッグとして働き、対応するペプチドボロン酸と同等のプロテアソーム阻害活性を示すことが知られている。[85d, 87]

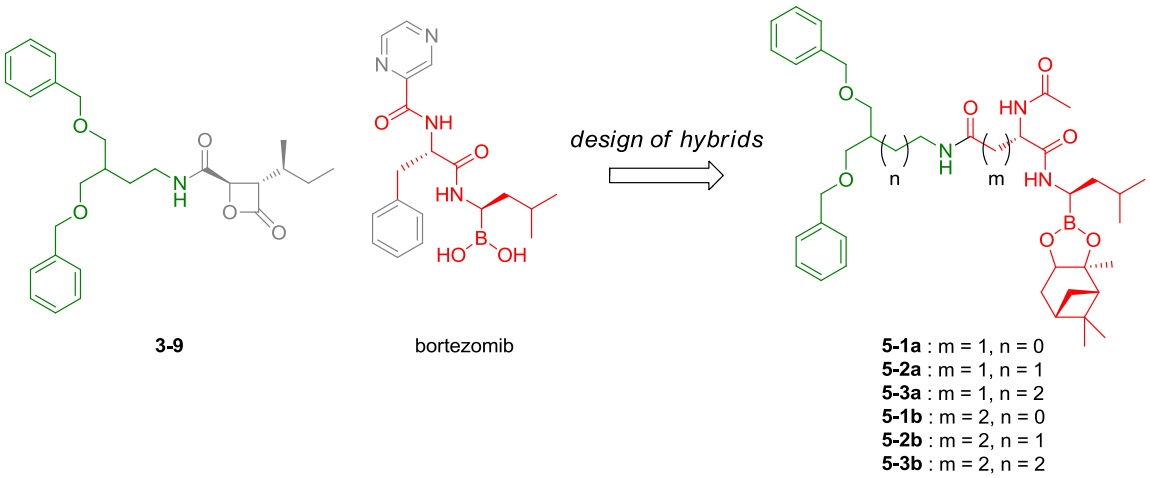


Figure 5-3. 設計したハイブリッド化合物 **5-1a** – **5-3a** 及び **5-1b** – **5-3b**

分子設計の妥当性を計算化学的に検証するため、設計したハイブリッド化合物 **5-1a** – **5-3a** 及び **5-1b** – **5-3b** を、第二章で述べた共有結合性化合物のドッキング法に基づいてプロテアソームとドッキングした。*化合物 **5-2a** のドッキング結果を Figure 5-4 に示す。†予測された結合様式において、**5-2a** の非ペプチド構造部及びペプチドボロン酸部はそれぞれ P'領域結合部及び P 領域結合部と期待した通りに相互作用しており、ハイブリッド化合物の分子設計が妥当なものであることが示唆された。

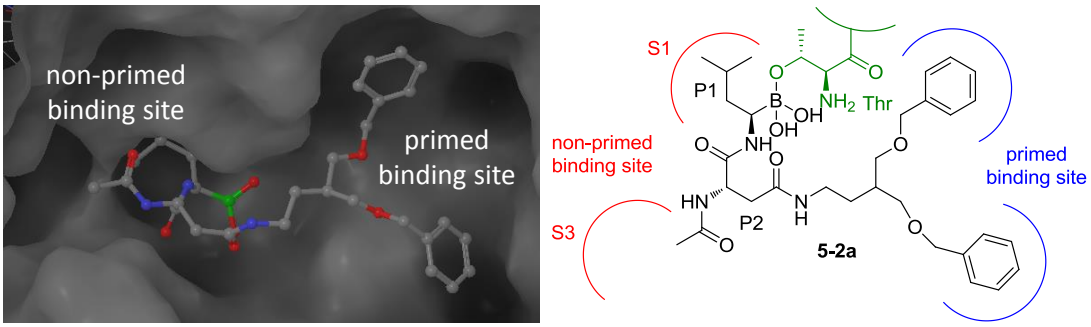


Figure 5-4. ドッキングにより予測された **5-2a** のプロテアソーム結合様式

3. P3 部置換誘導体の設計

上述のハイブリッド化合物 **5-1a** – **5-3a** 及び **5-1b** – **5-3b** のうち、後述するように最もプロテアソーム阻害活性及び細胞増殖抑制作用の高かった **5-2a** をリード化合物として P3 部の構造活性相関を検証した。‡Figure 5-1 に示した既知のペプチドボロン酸型プロテアソーム阻害剤の構造を参考にして Figure 5-5 に示した **5-4a** – **5-7a** を設計した。

---

* ボルテゾミブとプロテアソームの共結晶の X 線結晶構造を用いて、第二章で述べたドッキング法に基づいてプロテアソームモデルを構築してドッキングを行った。詳細については実験の部を参照。

† 他の化合物のドッキング結果については実験の部を参照。

‡ **5-2a** においてはアセチル部が P3 部に相当する。

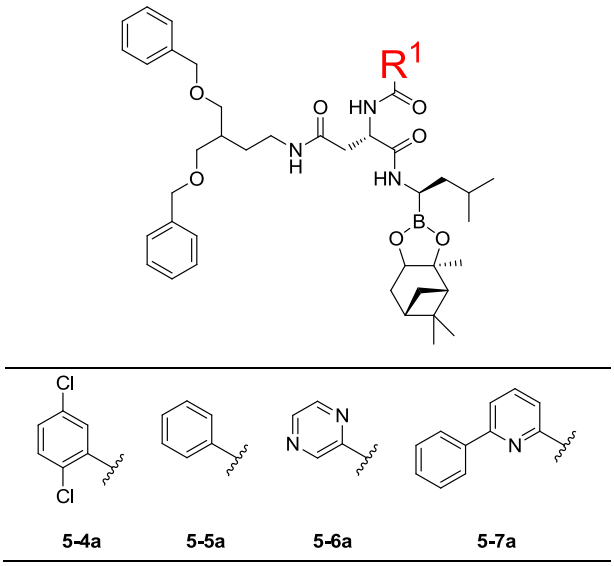


Figure 5-5. 設計した P3 部置換誘導体 **5-4a** – **5-7a**

4. 非ペプチド部置換誘導体の設計

一連のハイブリッド化合物における非ペプチド部の生物活性に対する寄与を検証するため、**5-2a** をリード化合物として非ペプチド部置換誘導体 **5-8a** 及び **5-9a** を設計した。

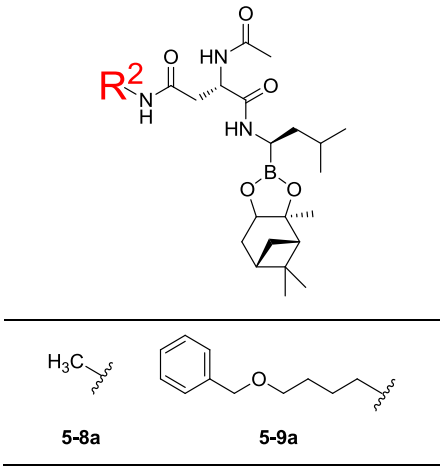


Figure 5-6. 設計した非ペプチド部置換誘導体 **5-8a** – **5-9a**

第二節　ハイブリッド化合物の合成

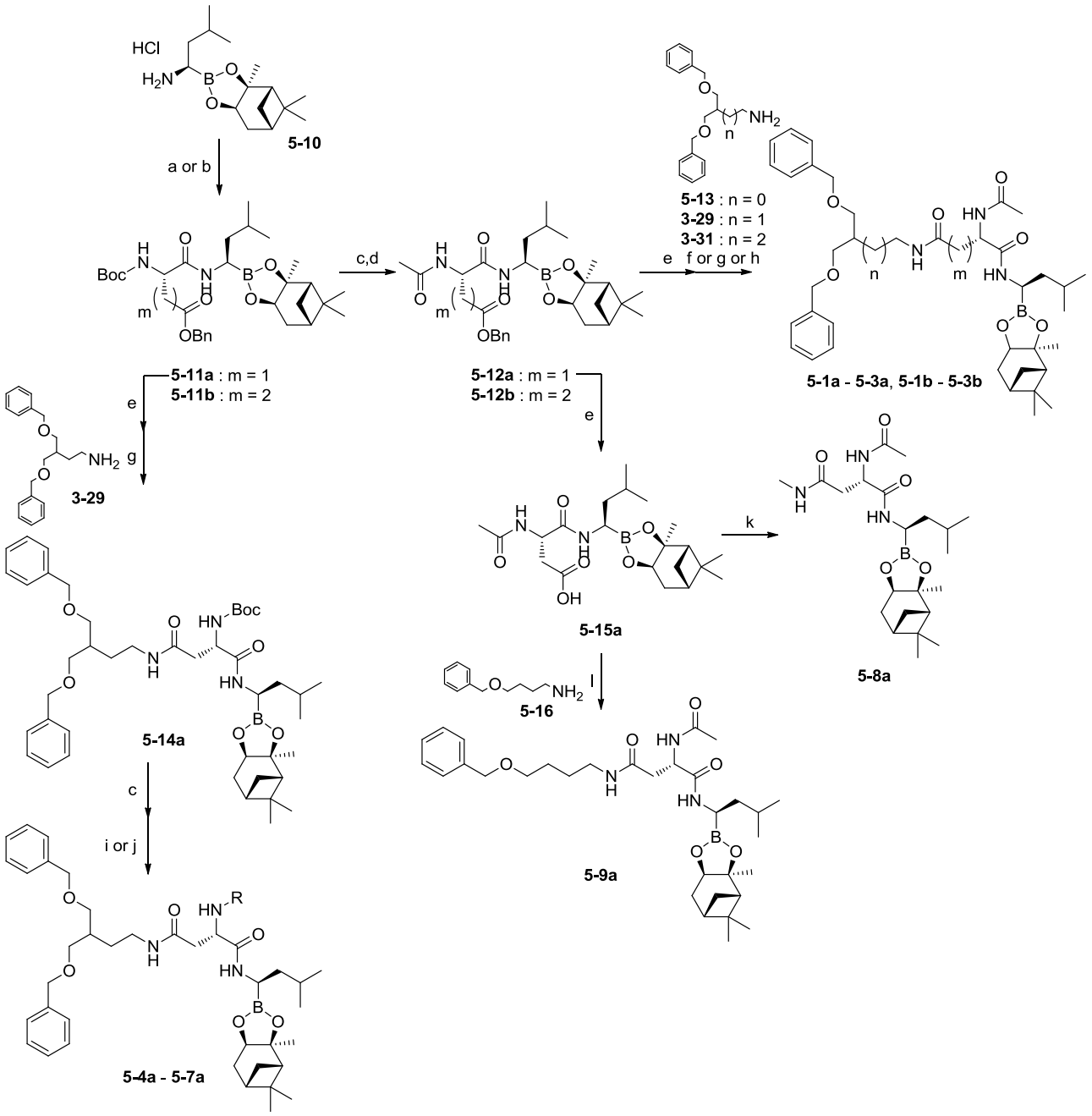
設計したハイブリッド化合物 **5-1a** – **5-9a** 及び **5-1b** – **5-3b** の合成を Scheme 5-1 に示す。ロイシンボロン酸のピナンジオールエステル **5-10**[*][88]を Boc-Asp(OBn)-OH 又は Boc-Glu(OBn)-OH と混合酸無水物法により縮合してそれぞれ **5-11a** 及び **5-11b** を得た。**5-11a** 及び **5-11b** の Boc 基をジクロロメタン中 TFA で処理して除去し、生じたアミノ基を無水酢酸によりアセチル化してそれぞれ **5-12a** 及び **5-12b** を得た。**5-12a** 及び **5-12b** のベンジル基を水素化分解により除去した後、生じたカルボキシ基に対してアミン **5-13**,[†] **3-29** 又は **3-31** を縮合してそれぞれ標的化合物 **5-1a** – **5-3a** 及び **5-1b** – **5-3b** を得た。

化合物 **5-11a** のベンジル基を水素化分解により除去した後、生じたカルボキシ基に対してアミン **3-29** を縮合して **5-14a** を得た。**5-14a** の Boc 基をジクロロメタン中 TFA で処理して除去した後、生じたアミノ基に対してそれぞれ対応するカルボン酸もしくは酸塩化物を縮合することで標的化合物 **5-4a** – **5-7a** を得た。また、化合物 **5-12a** のベンジル基を水素化分解により除去した後、生じたカルボキシ基に対してメチルアミンもしくはアミン **5-16**[‡]を縮合することでそれぞれ標的化合物 **5-8a** 及び **5-9a** を得た。

また、これらのペプチドボロン酸のピナンジオールエステルは、順相シリカゲルカラムクロマトグラフィーによる精製条件下不安定であり部分的に加水分解されるため、全ての標的化合物は逆相カラムクロマトグラフィーにより精製した。

[*] ピナンジオールエステル **5-10** の合成については実験の部を参照。
[†] アミン **5-13** の合成については実験の部を参照。
[‡] アミン **5-16** の合成については実験の部を参照。

Scheme 5-1. ハイブリッド化合物 **5-1a** – **5-9a** 及び **5-1b** – **5-3b** の合成 [a]



[a]Reagents and conditions: (a) Boc-Asp(OBn)-OH, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt; (b) Boc-Glu(OBn)-OH, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt; (c) TFA, CH_2Cl_2, 0 °C to rt; (d) Ac_2O, Et_3N, CH_2Cl_2, 0 °C, 68% for **5-12a** (3 steps from **5-10**), 84% for **5-12b** (3 steps from **5-10**); (e) Pd/C, H_2, THF; (f) **5-13**, EDC, HOAt, DIEA, CH_2Cl_2, -18 °C to rt, 68% for **5-1a** (2 steps from **5-12a**), 78% for **5-1b** (2 steps from **5-12b**); (g) **3-29**, EDC, HOAt, DIEA, CH_2Cl_2, -18 °C to rt, 77% for **5-2a** (2 steps from **5-12a**), 84% for **5-2b** (2 steps from **5-12b**); (h) **3-31**, EDC, HOAt, DIEA, CH_2Cl_2, -18 °C to rt, 71% for **5-3a** (2 steps from **5-12a**), 75% for **5-3b** (2 steps from **5-12b**); (i) R-Cl, Et_3N, CH_2Cl_2, 0 °C, 49% for **5-4a** (R = 2,5-dichlorobenzoyl, 5 steps from **5-10**), 53% for **5-5a** (R = benzoyl, 5 steps from **5-10**); (j) R-OH, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, 47% for **5-6a** (R = pyrazine-2-carbonyl, 5 steps from **5-10**), 54% for **5-7a** (R = 6-phenylpyridine-2-carbonyl, 5 steps from **5-10**); (k) CH_3NH_2･HCl, EDC, HOAt, DIEA, CH_2Cl_2, 0 °C to rt, 2 steps 18% from **5-12a**; (l) 4-(benzyloxy)butan-1-amine･HCl, EDC, HOAt, DIEA, CH_2Cl_2, -18 °C to rt, 2 steps 76% from **5-12a**.

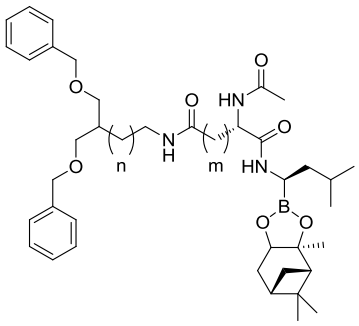
第三節　ハイブリッド化合物の生物活性

1. ハイブリッド化合物のプロテアソーム阻害活性及び細胞増殖抑制作用

合成した標的化合物のヒト 20S プロテアソーム ChT-L 活性, C-L 活性及び T-L 活性に対する阻害作用をそれぞれ蛍光基質 Suc-LLVY-AMC, Ac-*n*LP*n*LD-AMC 及び Ac-RLR-AMC を用いて測定した。ハイブリッド化合物 **5-1a** – **5-3a** 及び **5-1b** – **5-3b** のプロテアソーム阻害活性を Table 5-1 に示す。これら全てのハイブリッド化合物はプロテアソーム ChT-L 活性を強力に阻害し、その IC_{50} 値は 2.7 – 5.7 nM とボルテゾミブ (IC_{50} = 7.0 nM) と同等またはそれ以下であった。P2 残基として Asp (m = 1) を有する化合物 **5-1a** – **5-3a** についてはボルテゾミブ (C-L activity, IC_{50} = 120 nM; T-L activity, IC_{50} = 2000 nM) と同様のサブユニット選択性を示し、C-L 活性及び T-L 活性に対する IC_{50} 値はそれぞれ 120 – 150 nM 及び 1100 – 2700 nM であった。一方、P2 残基として Glu (m = 2) を有する化合物 **5-1b** – **5-3b** については、T-L 活性に対する IC_{50} 値は 2400 – 3000 nM と **5-1a** – **5-3a** と同等であったが、C-L 活性に対する IC_{50} 値は 46 – 76 nM と **5-1a** – **5-3a** に比べて低かった。このことから、C-L 活性を担うβ-1 サブユニットの結合部位においては P2 残基として Asp よりも Glu の方が好ましいことが示唆された。また、これらの化合物においては、非ペプチド部のアルキル鎖長 (n = 0 – 2) はプロテアソーム阻害活性に対してあまり影響しなかった。非ペプチド部の骨格構造が柔軟であるため、どのアルキル鎖長においても二つのベンゼン環をプロテアソーム結合ポケットに配置することが出来ると考えられる。

また、合成した標的化合物の HCT116 細胞に対する細胞増殖抑制作用を評価した。ハイブリッド化合物 **5-1a** – **5-3a** 及び **5-1b** – **5-3b** の HCT116 細胞に対する細胞増殖抑制作用を Table 5-1 に示す。期待した通り、全てのハイブリッド化合物は強力な細胞増殖抑制作用を示した。興味深いことに、P2 残基として Asp を有する化合物 **5-1a** – **5-3a** (IC_{50} = 32 – 45 nM) は Glu を有する **5-1b** – **5-3b** (IC_{50} = 64 – 110 nM) よりも強力な細胞増殖を示すことが分かった。両者のプロテアソーム阻害活性に大きな違いは見られないため、膜透過性や代謝安定性が異なる可能性が考えられる。

Table 5-1. ハイブリッド化合物 **5-1a** – **5-3a** 及び **5-1b** – **5-3b** のプロテアソーム阻害活性及び細胞増殖抑制作用



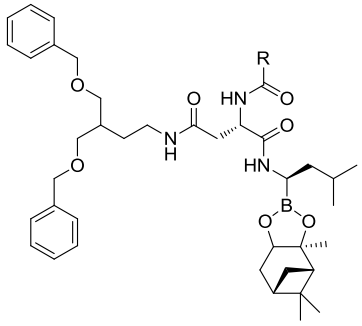
compound number	m	n	IC_{50} [nM][a]			
			ChT-L activity	C-L activity	T-L activity	HCT116 cell growth
5-1a		0	3.9 ± 2.2	140 ± 11	1100 ± 130	45.4
5-2a	1 (Asp)	1	2.7 ± 1.0	150 ± 24	2700 ± 330	31.8
5-3a		2	4.1 ± 1.1	120 ± 23	2700 ± 720	43.0
5-1b		0	5.1 ± 1.8	75 ± 8.7	2400 ± 880	63.6
5-2b	2 (Glu)	1	5.7 ± 3.6	76 ± 6.0	2500 ± 1700	110.3
5-3b		2	4.1 ± 2.8	46 ± 3.9	3000 ± 610	67.2
bortezomib			7.0 ± 0.5	120 ± 10	2000 ± 790	5.0

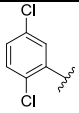
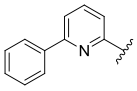
[a]Based on three experiments

2. P3 部置換誘導体のプロテアソーム阻害活性及び細胞増殖抑制作用

最も強いプロテアソーム阻害活性及び細胞増殖抑制作用を示したハイブリッド化合物 **5-2a** をリード化合物として、P3 部の構造活性相関を調べた。P3 部置換誘導体 **5-4a** – **5-7a** のプロテアソーム阻害活性及び細胞増殖抑制作用を Table 5-2 に示す。P3 置換基の変換によってプロテアソーム阻害活性は向上しなかったが、P3 部に 2,5-ジクロロフェニル基を有する化合物 **5-4a** 及びビアリール (3-フェニル-ピリジン-2-イル) 基を有する化合物 **5-7a** は ChT-L 活性に対して高い選択性を示した。特に、化合物 **5-7a** は ChT-L 活性を強力に阻害するものの (IC_{50} = 7.5 nM) C-L 活性に対する阻害活性は非常に低かった (IC_{50} = 1000 nM)。同じ P3 置換基を有するペプチドボロン酸型プロテアソーム阻害剤 CEP18770 は C-L 活性を強力に阻害するため (IC_{50} = 70 nM)、[24]一連のハイブリッド化合物においては、非ペプチド部のプロテアソームとの相互作用によって P3 置換基のプロテアソームによる認識が変化している可能性が示唆された。

Table 5-2. P3 部置換誘導体 **5-4a** – **5-7a** のプロテアソーム阻害活性及び細胞増殖抑制作用



compound number	R	IC_{50} [nM][a]			
		ChT-L activity	C-L activity	T-L activity	HCT116 cell growth
5-4a		5.9 ± 0.5	250 ± 140	> 10000	140
5-5a		7.8 ± 2.6	130 ± 6.2	2700 ± 380	60.0
5-6a		7.1 ± 4.2	210 ± 73	3300 ± 600	45.0
5-7a		7.5 ± 0.9	1000 ± 890	> 10000	260
5-2a		2.7 ± 1.0	150 ± 24	2700 ± 330	31.8
bortezomib		7.0 ± 0.5	120 ± 10	2000 ± 790	5.0

[a]Based on three experiments

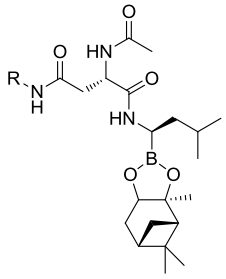
3. 非ペプチド部置換誘導体のプロテアソーム阻害活性及び細胞増殖抑制作用

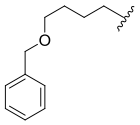
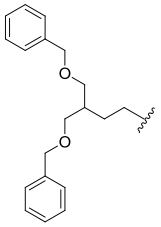
ハイブリッド化合物 **5-2a** をリード化合物として、非ペプチド部の構造活性相関を検証した。非ペプチド部

置換誘導体 **5-8a** 及び **5-9a** のプロテアソーム阻害活性及び細胞増殖抑制作用を Table 5-3 に示す。非ペプチド部を持たない **5-8a** と比較して、ベンゼン環が一つの非ペプチド部を有する **5-9a** 及びベンゼン環が二つの非ペプチド部を有する **5-2a** はそれぞれ 10 倍及び 20 倍強力に ChT-L 活性を阻害した。このことから、非ペプチド部は期待した通りプロテアソームと相互作用して阻害活性に寄与していることが分かる。

非ペプチド部を持たない化合物 **5-8a** はほとんど細胞増殖抑制作用を示さなかった (IC_{50} = 2500 nM)。これは **5-8a** のプロテアソーム阻害活性が低いため、あるいは極性が高いために膜透過性が問題となっていると解釈できる。また、ベンゼン環が一つの非ペプチド部を有する **5-9a** は、ベンゼン環が二つの非ペプチド部を有する **5-2a** と同等のプロテアソーム阻害活性を示すにも関わらず、その細胞増殖抑制作用は IC_{50} 値が 130 nM と **5-2a** に比べて低かった (IC_{50} = 32 nM)。この差異は膜透過性や代謝安定性、あるいは後述するプロテアソーム阻害作用の可逆性の違いによるものと考えられる。

Table 5-3. 非ペプチド部置換誘導体 **5-8a** 及び **5-9a** のプロテアソーム阻害活性及び細胞増殖抑制作用



compound number	R	IC_{50} [nM][a]			
		ChT-L activity	C-L activity	T-L activity	HCT116 cell growth
5-8a		54 ± 3.8	500 ± 64	> 10000	2500
5-9a		4.8 ± 1.0	170 ± 114	2170 ± 150	130
5-2a		2.7 ± 1.0	150 ± 24	2700 ± 330	31.8

[a]Based on three experiments

4. ハイブリッド化合物の免疫プロテアソーム阻害活性

上述の構造活性相関研究により見出した強力なプロテアソーム阻害活性及び細胞増殖抑制作用を有するハイブリッド化合物 **5-2a** 及び **5-9a** について、β5i サブユニットに対する阻害活性を Kuhn らにより報告された active-site ELISA 法[45]により測定した。コントロールとしては、それぞれ非選択的、β5 選択的、β5i 選択的なプロテアソーム阻害剤であるボルテゾミブ、ラクタシスチン、PR-957 を用いた。[31a, 31e]Figure 5-7 に示すように、ハイブリッド化合物である **5-2a** 及び **5-9a** はペプチドボロン酸型プロテアソーム阻害剤であるボルテゾミブに比べてβ5i 選択的性が高いことが分かった。この選択性の違いは、非ペプチド部のプロテアソームとの相互作用に基づくものであると考えられる。

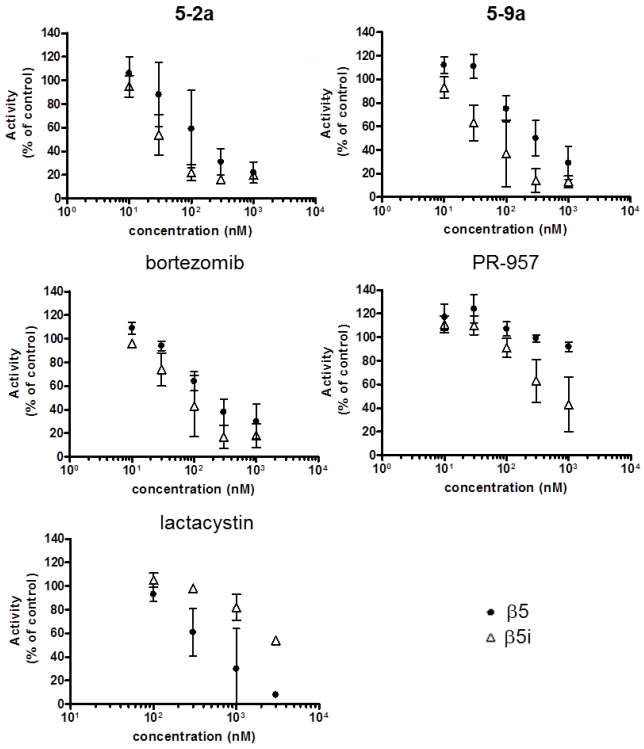


Figure 5-7. ハイブリッド化合物 **5-2a** 及び **5-9a** の免疫プロテアソーム阻害活性

5. ハイブリッド化合物による細胞増殖抑制作用の発現機構

ハイブリッド化合物 **5-2a** 又は **5-9a** で処理した HCT116 細胞の細胞周期を解析した。Figure 5-8 に示すように、これらのハイブリッド化合物はボルテゾミブと同様に細胞周期の進行を G2/M 期で停止させることが分かった (Figure 5-8)。

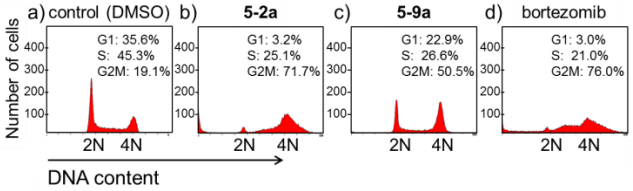


Figure 5-8. ハイブリッド化合物 **5-2a** 又は **5-9a** で処理した HCT116 細胞の細胞周期解析

また、ハイブリッド化合物 **5-2a** 又は **5-9a** で処理した HCT116 細胞中の p53 及び cleaved PARP をウエスタンブロット法により検出した。Figure 5-9 に示すように、これらの化合物で処理した細胞ではプロテアソーム基質である p53 の蓄積がみられた。更に、これらの細胞では poly(ADP-ribose) polymerase (PARP) の切断により生じるアポトーシスマーカーである cleaved PARP の蓄積がみられ、アポトーシス経路が活性化されていることが分かる。

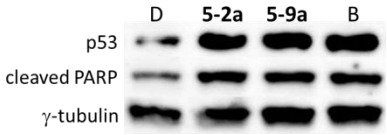


Figure 5-9. ハイブリッド化合物 **5-2a** 又は **5-9a** で処理した HCT116 細胞中の p53 及び cleaved PARP の検出 (D, DMSO; B, bortezomib)

以上のことから、これらのハイブリッド化合物はボルテゾミブと同様に、細胞系でプロテアソームを阻害し、G2/M 期で細胞周期の進行を停止させると共にアポトーシスを引き起こすことで細胞増殖抑制作用を示していると考えられる。

6. ハイブリッド化合物の他のプロテアーゼに対する阻害活性

一般に、ボロン酸を活性基として有するプロテアソーム阻害剤ではセリンプロテアーゼに対する阻害作用が問題となる。[34b, 44]ボルテゾミブによる化学療法において主要な用量制限因子となる末梢神経障害もセリンプロテアーゼに対する阻害作用に起因するものと考えられている。[44]そこで、ハイブリッド化合物 **5-2a** 及び **5-9a** のセリンプロテアーゼ（カテプシン A 及びカテプシン G）及びシステインプロテアーゼ（カテプシン B）に対する阻害作用を評価した。Table 5-4 に示すように、ボルテゾミブは Arastu-Kapur らにより報告[44]された通りセリンプロテアーゼであるカテプシン A 及びカテプシン G に対して阻害活性を示し、カルフィルゾミブはシステインプロテアーゼであるカテプシン B に対して阻害活性を示した。一方で、ハイブリッド化合物 **5-2a** 及び **5-9a** はいずれのプロテアーゼに対しても全く阻害活性を示さなかった。これらのハイブリッド化合物は P2 側鎖に大きな非ペプチド構造を有するために高いプロテアソーム選択性を示すと考えられる。従って、これらのハイブリッド化合物をリード化合物とすることで、ボルテゾミブと比べて副作用の少ないプロテアソーム阻害剤を開発できると考えられる。

Table 5-4. ハイブリッド化合物 **5-2a** 及び **5-9a** のカテプシン A、B 及び G に対する阻害活性

compound number	IC_{50} [μM][a]		
	cathepsin A	cathepsin B	cathepsin G
5-2a	> 30	> 30	> 30
5-9a	> 30	> 30	> 30
bortezomib	9.2	> 30	3.1
carfilzomib	> 30	11	> 30

[a]Based on three experiments

7. ハイブリッド化合物のプロテアソーム阻害作用の可逆性

先に述べたように、ボロン酸を活性基として有するプロテアソーム阻害剤は、プロテアソーム活性残基である N 末端 Thr 残基のヒドロキシ基とボロン酸エステルを形成することで共有結合性阻害剤として働く。しかしながら、このボロン酸とヒドロキシ基の間に生じる共有結合は配位結合であり可逆的である。ペプチドボロン酸型プロテアソーム阻害剤のうち、MLN9708 とボルテゾミブについてプロテアソームβ5 サブユニットからの解離半減期がそれぞれ 18 分及び 110 分であると報告されている[25a]ことから、ペプチドボロン酸は可逆的なプロテアソーム阻害剤であることが分かる。またこれら二つのペプチドボロン酸の解離半減期が大きく異なることから、ボロン酸を活性基として有するプロテアソーム阻害剤の解離半減期は、活性基以外の構造により大きく影響を受けると考えられる。

ハイブリッド化合物の非ペプチド部の解離半減期に対する影響を検証するため、プロテアソームをハイブリッド化合物 **5-2a** 又は **5-9a** とプレインキュベーションした後、半透膜を用いて化合物を除去し、その前後でのプロテアソーム ChT-L 活性を評価した。コントロールとして、可逆的プロテアソーム阻害剤であるボルテゾミブ及び不可逆的プロテアソーム阻害剤であるカルフィルゾミブについても同様に実験を行った。[25a, 31e, 47]Figure 5-10 に示すように、ハイブリッド化合物 **5-9a** はボルテゾミブと同様にプロテアソームを可逆的に阻

害したが、ハイブリッド化合物 **5-2a** はエポキシケトンを活性基として有するカルフィルゾミブと同様に不可逆的にプロテアソームを阻害することが分かった。この二つの化合物間の差異は非ペプチド部のプロテアソームとの相互作用の違いに起因し、より多くの疎水性相互作用部位を有する **5-2a** ではプロテアソーム阻害作用が不可逆的になったと考えられる。“Drug-target residence time”[*]は *in vivo* での有効性や標的選択性を大きく左右する因子であるため、[89]不可逆的な阻害作用を示す **5-2a** は *in vivo* で持続的に薬効を発現し且つ低毒性なプロテアソーム阻害剤開発のためのリード化合物として魅力的であると考えられる。

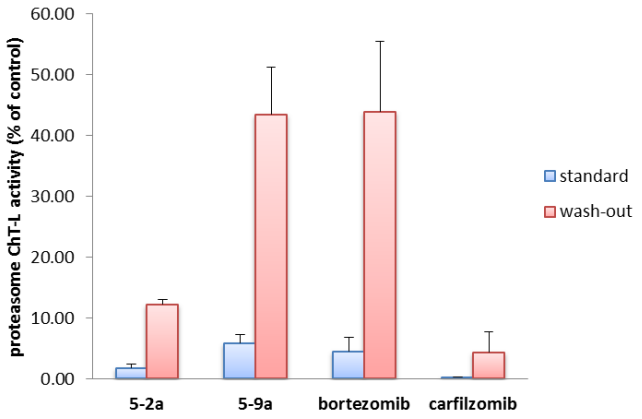


Figure 5-10. ハイブリッド化合物 **5-2a** 及び **5-9a** のプロテアソーム阻害作用の可逆性試験

以上、筆者は第三章で述べた非ペプチド性プロテアソーム阻害剤とペプチドボロン酸のハイブリッド化合物を結合部位の違いに基づく合理的な分子設計により創出した。一連のハイブリッド化合物は強力なプロテアソーム阻害活性及び細胞増殖抑制作用を有する。また、これらのハイブリッド化合物はボルテゾミブと同じくボロン酸を活性基として有するにも関わらず、セリンプロテアーゼを含む他のプロテアーゼに対して阻害活性を示さず高いプロテアソーム選択性を有しており、更にそのプロテアソーム阻害作用は不可逆的であることが分かった。従って、一連のハイブリッド化合物は従来のペプチドボロン酸とは性質の異なる新たなケモタイプのプロテアソーム阻害剤であり、その高いプロテアソーム選択性及びプロテアソーム阻害作用の不可逆性から、ボルテゾミブにおいて問題となるオフターゲット毒性を示さない新たな抗がん薬の開発におけるリード化合物として魅力的である。

[*] “Drug-target residence time”とは、リガンドが標的分子と結合した後解離するまでの時間のことである。

第六章　ペプチドエポキシケトンとのハイブリッド化合物の創製

第一節　ハイブリッド化合物の設計

1. ペプチドエポキシケトン型プロテアソーム阻害剤

ペプチドエポキシケトンは代表的なプロテアソーム阻害剤のクラスであり、[31a, 34b, 90]臨床で用いられているカルフィルゾミブ[22]や現在臨床試験が行われている ONX-0912、[26]更に免疫プロテアソーム選択的阻害剤である PR-957[4a]はいずれもこのクラスのプロテアソーム阻害剤である。ペプチドエポキシケトン型プロテアソーム阻害剤は、その活性基であるエポキシケトンがプロテアソーム活性残基である Thr 残基のヒドロキシ基とヘミアセタールを形成した後、アミノ基と反応してモルフォリン環を形成することでプロテアソームに対する共有結合性阻害剤として働く。[34b, 91]エポキシケトンとプロテアソーム Thr 残基の反応により形成されるモルフォリン環は安定であるため、ペプチドエポキシケトン型プロテアソーム阻害剤はプロテアソームを不可逆的に阻害する。[31e]

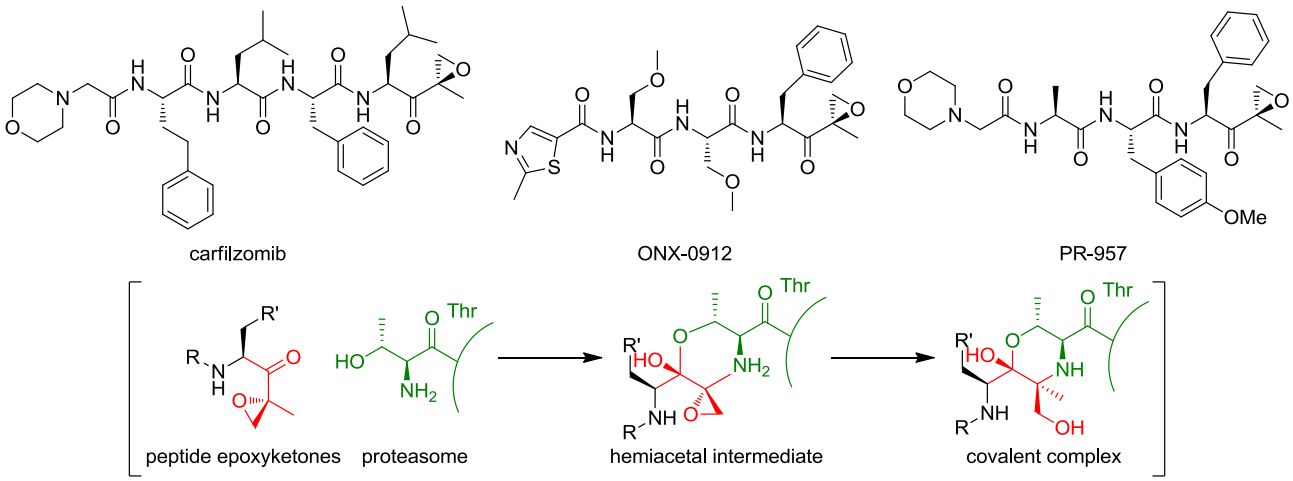


Figure 6-1. 代表的なペプチドエポキシケトン型プロテアソーム阻害剤とそのプロテアソーム阻害様式

2. ハイブリッド化合物の設計

ペプチドエポキシケトン型プロテアソーム阻害剤であるエポキソマイシン及びベラクトシン A シス型異性体の誘導体 **1-18** とプロテアソームの共結晶の X 線結晶構造[37, 91]を Figure 6-2 に重ねて示す。序論で述べたように、ベラクトシン誘導体は他のプロテアソーム阻害剤とは異なる結合部位を有しており、エポキソマイシンが P 領域結合部に結合するのに対して、ベラクトシン誘導体 **1-18** はβ-ラクトン部位以外は P’領域結合部に結合していることが分かる。また、エポキソマイシンの P2 側鎖はプロテアソーム結合状態において、ベラクトシン誘導体の結合部位である P’領域結合部の方向を向いていることが分かる。従って、ペプチドエポキシケトンの P2 側鎖に対してベラクトシン誘導体の構造を連結することで、P’領域結合部と P 領域結合部の両方と強力に相互作用するベラクトシン誘導体とペプチドエポキシケトンのハイブリッド化合物の創製が可能であると考えた。

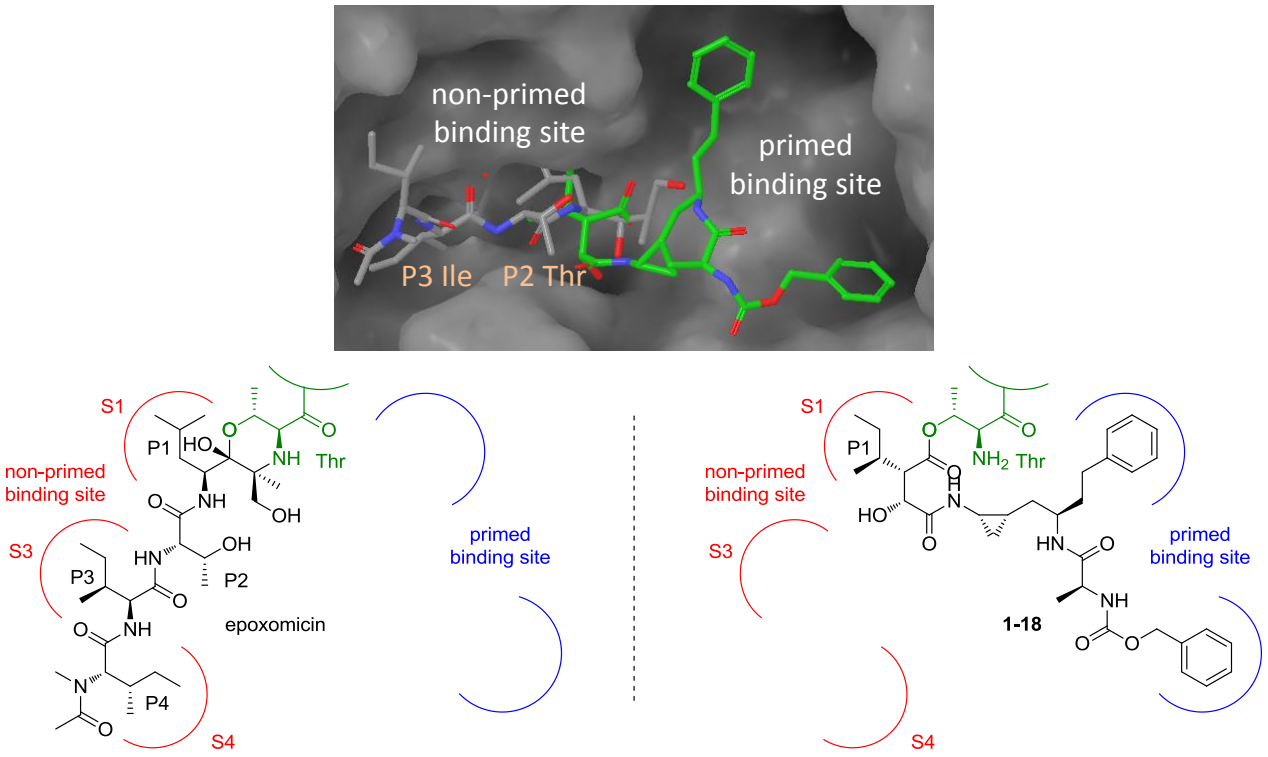


Figure 6-2. エポキソマイシン (gray tube) 又はベラクトシン誘導体 **1-18** (green tube) とプロテアソームとの共結晶の構造の重ね合わせ

ベラクトシン A シス型異性体の誘導体 **1-18** はペプチド骨格を有している。このため、同様にペプチド骨格を有するペプチドエポキシケトンとのハイブリッド化合物は多数のペプチド結合を持つこととなり、膜透過性や代謝安定性上の問題が懸念される。[63b]そこで、第三章で述べた **1-18** を親化合物とした“topology-based scaffold hopping”により見出した、非ペプチド性プロテアソーム阻害剤 **3-9** の構造を基にしてハイブリッド化合物を設計することとした。これまでに知られている全てのエポキシケトン型プロテアソーム阻害剤は S3 ポケットと相互作用する P3 側鎖を有しているが、[92]ハイブリッド化合物では非ペプチド部と P’領域結合部との相互作用によって P3 側鎖と S3 ポケットの相互作用の欠損を補うことが出来ると考え、Figure 6-3 に示した P3 以降のペプチド部を持たないハイブリッド化合物 **6-1** 及び **6-2** を設計した。

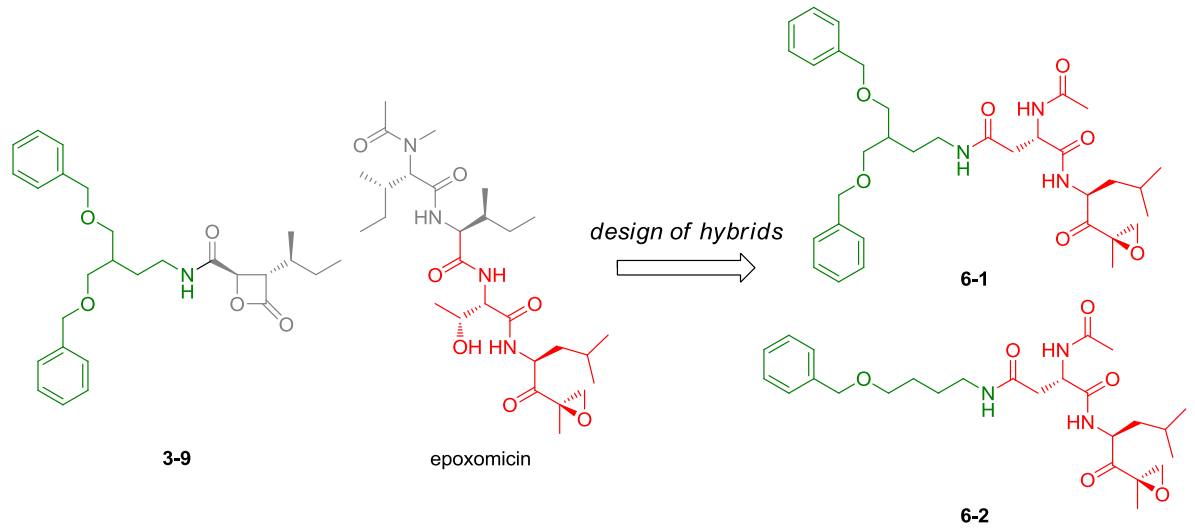


Figure 6-3. 設計したハイブリッド化合物 **6-1** 及び **6-2**

分子設計の妥当性を検証するため、設計したハイブリッド化合物 **6-1** 及び **6-2** の結合様式をコンピュータシミュレーションにより解析した (Figure 6-4)。*予測された結合様式において、**6-1** 及び **6-2** の非ペプチド部及びペプチドエポキシケトン部はそれぞれ P'領域結合部及び P 領域結合部と期待した通りに相互作用しており、ハイブリッド化合物の分子設計が妥当なものであることが示唆された。

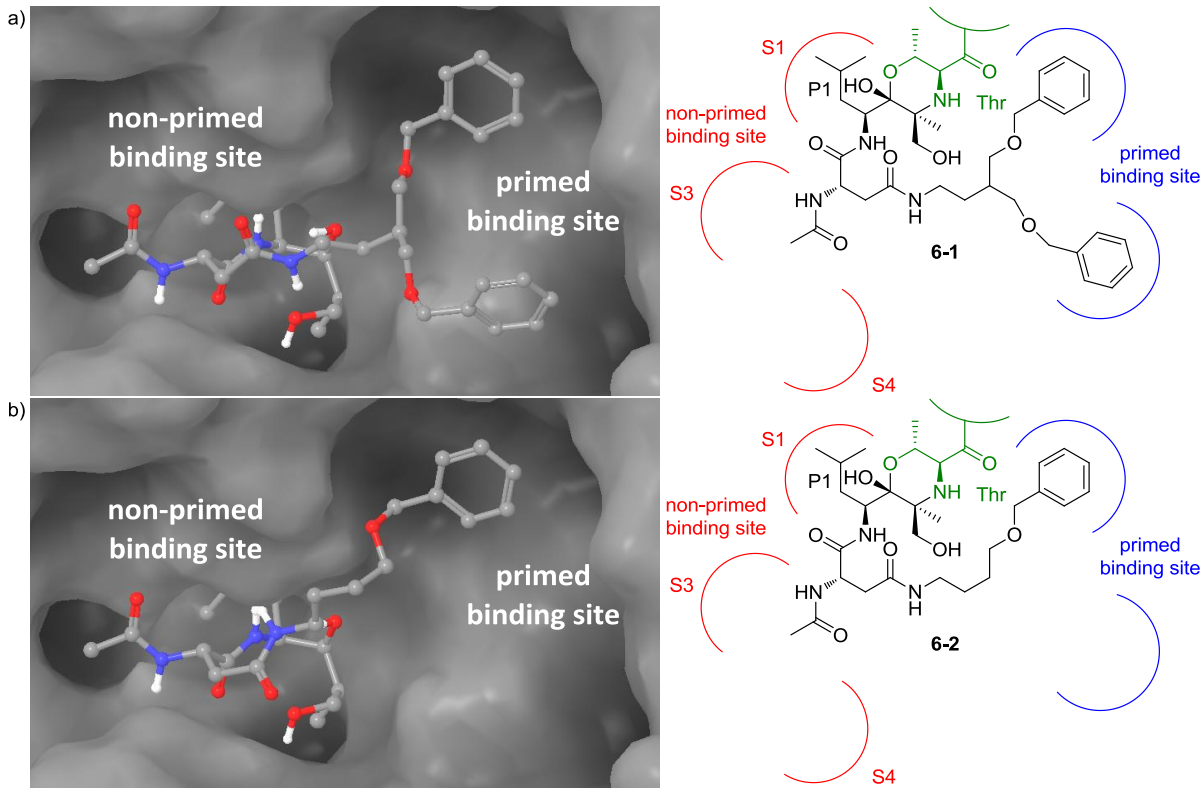


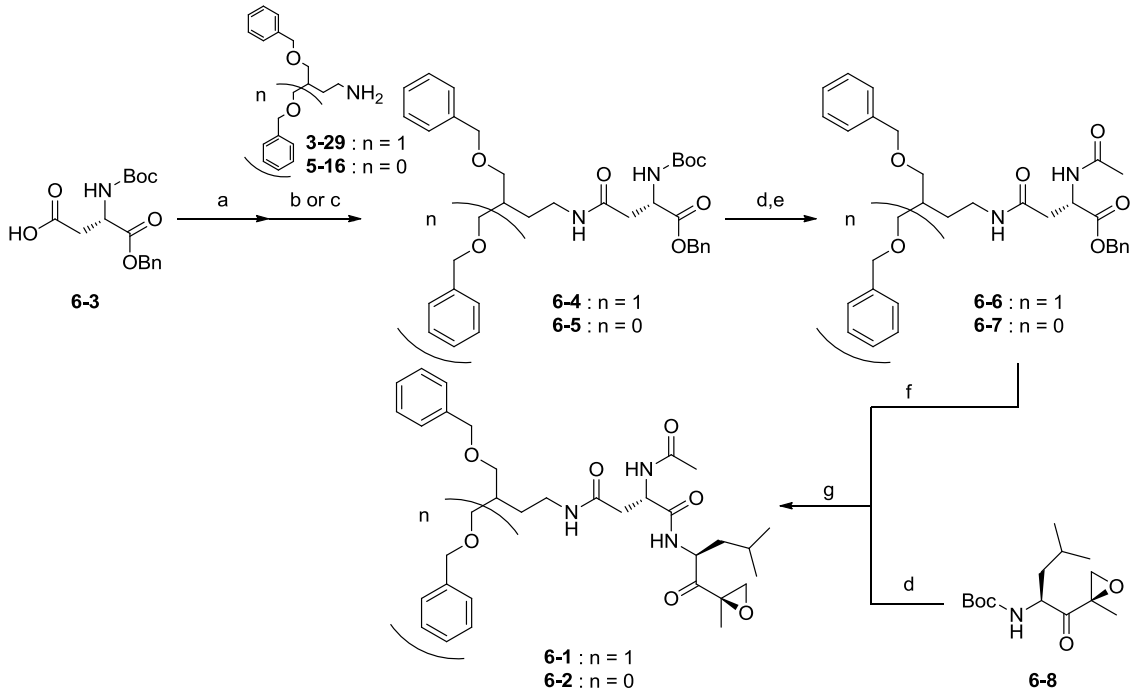
Figure 6-4. コンピュータシミュレーションにより予測されたハイブリッド化合物 **6-1** 及び **6-2** のプロテアソーム結合様式

* 計算方法については実験の部を参照。

第二節　ハイブリッド化合物の合成

設計したハイブリッド化合物 **6-1** 及び **6-2** の合成を Scheme 6-1 に示す。Boc-Asp-OBn **6-3** のカルボキシ基に対してアミン **3-29** 又は **5-16** を混合酸無水物法により縮合してそれぞれ化合物 **6-4** 及び **6-5** を得た。化合物 **6-4** 及び **6-5** の Boc 基をジクロロメタン中 TFA で処理して除去した後、生じたアミノ基を無水酢酸を用いてアセチル化してそれぞれ化合物 **6-6** 及び **6-7** を得た。化合物 **6-6** 及び **6-7** のベンジル基を 1,4-シクロヘキサジエンを用いた水素転移反応により除去した後、生じたカルボキシ基に対して文献既知のエポキシケトンユニット **6-8**[93]の Boc 基を除去して得たアミンを縮合してそれぞれ標的化合物 **6-1** 及び **6-2** を得た。

Scheme 6-1. ハイブリッド化合物 **6-1** 及び **6-2** の合成 [a]



[a]Reagents and conditions: (a) PivCl, Et_3N, CH_2Cl_2, 0 °C to rt; (b) **3-29**, Et_3N, CH_2Cl_2, 0 °C to rt; (c) **5-16**, Et_3N, CH_2Cl_2, 0 °C to rt; (d) TFA, CH_2Cl_2; (e) Ac_2O, Et_3N, CH_2Cl_2, 0 °C to rt; (f) 1,4-cyclohexadiene, Pd/C, EtOH; (g) HBTU, HOBt, DIEA, THF, - 5 °C, 60% for **6-1** (6 steps), 60% for **6-2** (6 steps).

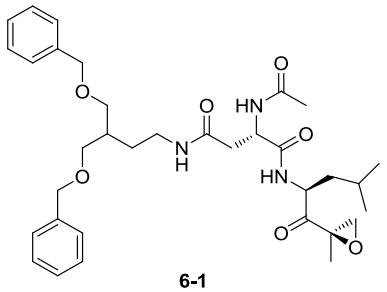
第三節　ハイブリッド化合物の生物活性

合成したハイブリッド化合物 **6-1** 及び **6-2** のヒト 20S プロテアソーム ChT-L 活性に対する阻害作用を蛍光基質 Suc-LLVY-AMC を用いて測定した (Table 6-1)。ハイブリッド化合物 **6-1** 及び **6-2** のプロテアソーム ChT-L 活性に対する IC_{50} 値はそれぞれ 2.3 μM 及び 5.7 μM であり、設計時の期待通り P3 部以降のペプチド部を持たないにも関わらずプロテアソーム阻害活性を示した。

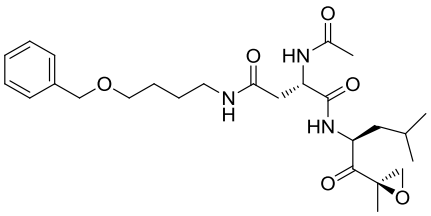
また、ハイブリッド化合物 **6-1** 及び **6-2** の HCT116 細胞に対する細胞増殖抑制作用を評価した (Table 6-1)。ハイブリッド化合物 **6-1** 及び **6-2** の HCT116 細胞に対する IC_{50} 値はそれぞれ 1.9 μM 及び 2.9 μM であり、プロテアソーム阻害作用と同等の細胞増殖抑制作用を示した。

従って、これらのハイブリッド化合物は活性基としてエポキシケトンを有し、P'領域結合部及び P 領域結合部の両方に結合部位を有する新たなクラスのプロテアソーム阻害剤の開発におけるリード化合物として有用である。

Table 6-1. ハイブリッド化合物 **6-1** 及び **6-2** のプロテアソーム阻害活性及び細胞増殖抑制作用



6-1



6-2

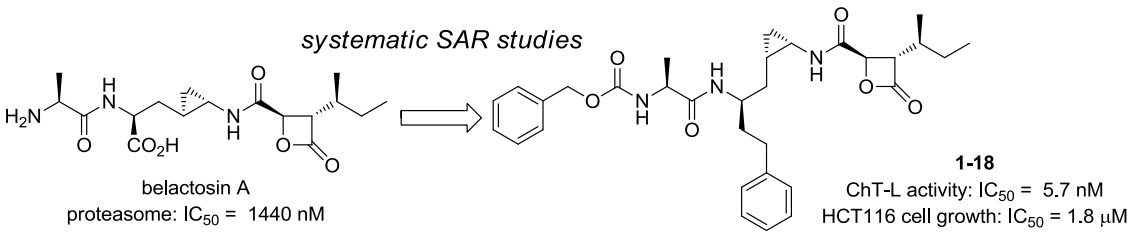
compound number	IC_{50} [μM][a]	
	ChT-L activity	HCT116 cell growth
6-1	2.3 ± 0.1	1.9
6-2	5.7 ± 0.4	2.9

[a]Based on three experiments

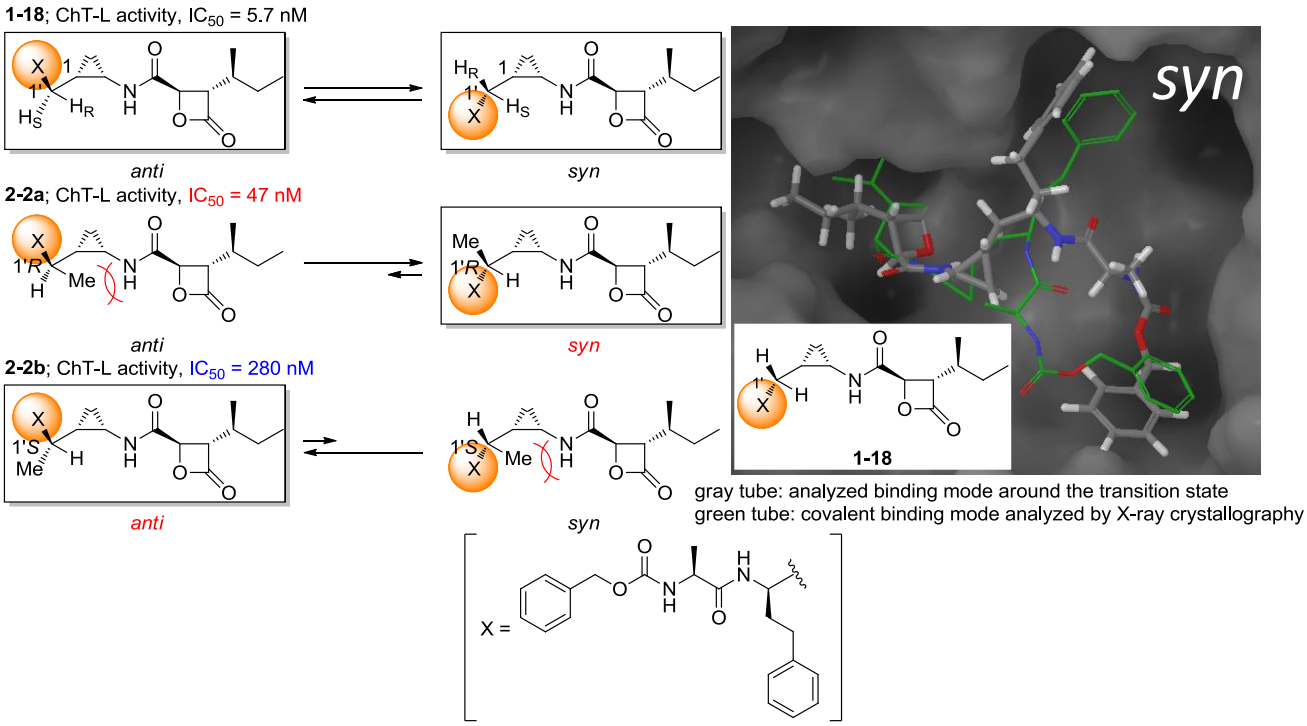
以上、筆者は第三章で述べた非ペプチド性プロテアソーム阻害剤とペプチドエポキシケトンのハイブリッド化合物を結合部位の違いに基づく合理的な分子設計により創出した。これらのハイブリッド化合物は従来のペプチドエポキシケトン型プロテアソーム阻害剤とは構造的に異なり、プロテアソームの P'領域結合部及び P 領域結合部の両方と相互作用する新たなケモタイプのプロテアソーム阻害剤であると考えられる。

結語

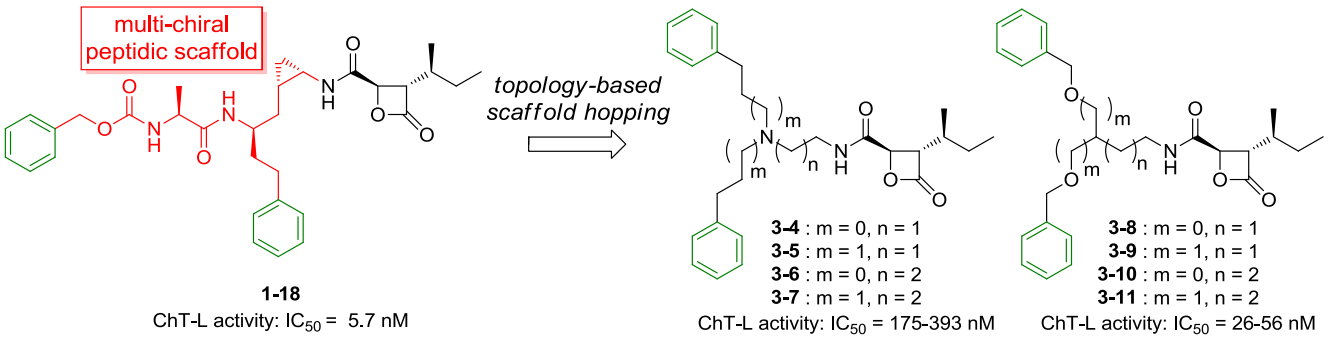
1. ベラクトシン A シス型異性体の系統的構造活性相関研究により、プロテアソームの基質 P'領域結合部における構造活性相関を明らかにすると共に、ボルテゾミブと同等の強力なプロテアソーム阻害活性を有する誘導体 **1-18** を見出した。



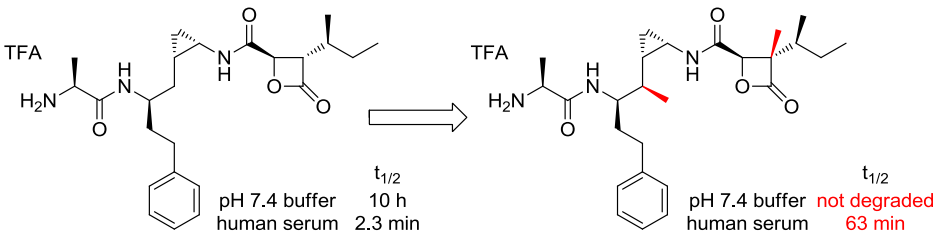
2. ベラクトシン A シス型異性体の誘導体の遷移状態近傍における結合様式を、シクロプロパン歪みを利用した配座制限誘導体の合成による有機化学的アプローチと、新たなドッキングコンセプトに基づく計算化学的アプローチを組み合わせて解析した。



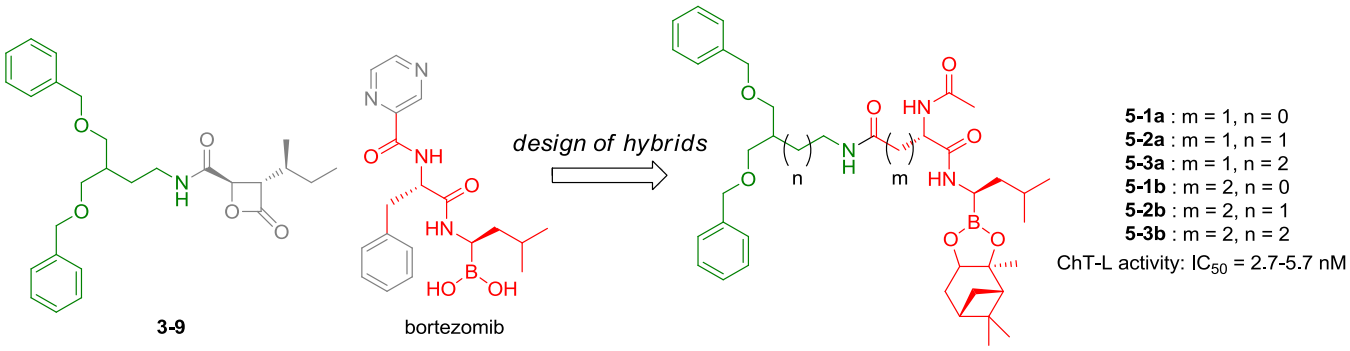
3. ベラクトシン A シス型異性体の誘導体 **1-18** を親化合物とした"topology-based scaffold hopping"により、その多数の不斉点からなるペプチド骨格をアキラルな非ペプチド骨格へと変換した非ペプチド性プロテアソーム阻害剤を開発した。



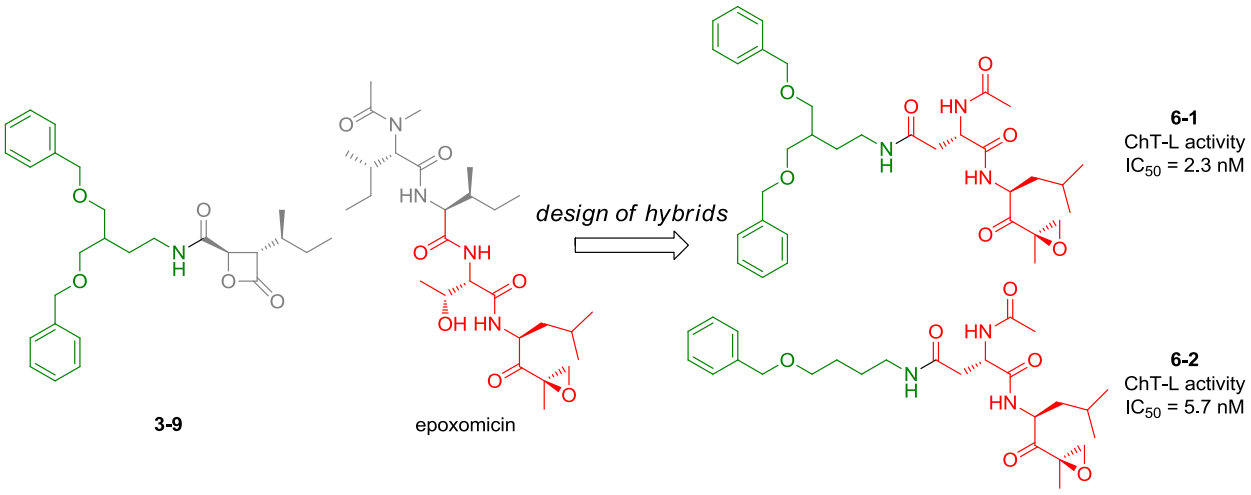
4. ベラクトシン A シス型異性体の誘導体 **1-18** をリード化合物として、β-ラクトンカルボニル炭素の立体的遮蔽及びシクロプロパン歪みを利用した配座制御によって安定化誘導体 **4-4** を見出した。



5. ベラクトシン誘導体 **1-18** を親化合物として開発した非ペプチド性プロテアソーム阻害剤とペプチドボロン酸型プロテアソーム阻害剤のハイブリッドに基づき、強力なプロテアソーム阻害活性及び細胞増殖抑制作用を有する阻害剤を、両者の結合部位の違いに基づく合理的な分子設計により創出した。



6. ベラクトシン誘導体 **1-18** を親化合物として開発した非ペプチド性プロテアソーム阻害剤とペプチドエポキシケトン型プロテアソーム阻害剤のハイブリッド化合物を、両者の結合部位の違いに基づく合理的な分子設計により創出した。


design of hybrids
3-9
epoxomicin
6-1
ChT-L activity
IC50 = 2.3 nM
6-2
ChT-L activity
IC50 = 5.7 nM

実験の部

共通事項

測定機器

NMR スペクトルは JEOL JNM-AL-400、JEOL JMM-ECX-400P、JEOL JMM-ECA-500 を用いて測定した。^{1}H-NMR スペクトルの化学シフトは、測定溶媒として $CDCl_3$ を用いた場合にはテトラメチルシランを内部標準としたときのδ値を ppm で表示した。ただし、測定溶媒として CD_3OD を用いた場合には $CD_2\underline{H}$-OD (3.31 ppm)、DMSO-d_6 を用いた場合には $CD_2\underline{H}$-S (O)-CD_3 (2.50 ppm) を標準とした。^{13}C-NMR スペクトルの化学シフトは、測定溶媒として $CDCl_3$ を用いた場合には $CDCl_3$ (77.0 ppm)、CD_3OD を用いた場合には CD_3OD (49.0 ppm) を標準とした。シグナルの多重度は、s: singlet、d: doublet、t: triplet、q: quartet、sept: septet、m: multiplet、br: broad の略号を用いて示した。スピン結合定数は *J* 値 (Hz) で表示した。また、シグナルの帰属は ^{1}H-^{1}H-COSY 及び ^{1}H-^{13}C-COSY スペクトルに基づいて行った。

質量分析は JEOL JMS-700TZ、JMS-HX110 及び JEOL FABmate を用いて行った。

比旋光度は JASCO P-1030 を用いて測定した。溶媒には市販の吸光分析用の溶媒を用いた。

高速液体クロマトグラフィー (HPLC) には以下のシステムを用いた。JASCO 880-PU (pump), JASCO 875-UV (detector), JASCO 802-SC (system controller), JASCO 807-IT (recorder)

実験材料

反応溶媒として使用したジクロロメタンは五酸化二リンより蒸留したものを用いた。エーテルは金属ナトリウムにより蒸留したものを用いた。THF は市販の無水のものをそのまま用いた。その他の試薬及び溶媒については特に記載のない限り、市販の一級または特級のものをそのまま用いた。アルゴンガス、水素ガスは市販のものをそのまま用いた。Pd/C は 10% Pd/ C 粉末（含水品）PE-TYPE (N.E. Chemcat Co.) を使用した。TLC は Merck silica gel $60F_{254}$ を使用した。順相シリカゲルクロマトグラフィーには Silica Gel 60 (Kanto Chemical Co.) または Silica Gel 60N (Kanto Chemical Co.) を使用した。NH シリカゲルカラムクロマトグラフィーには Chromatorex (Fuji Silysia Chemical Ltd.) を用いた。セライトろ過には Celite 545 (Kanto Chemical Co.) を使用した。

Synthetic procedures for 1-1 – 1-14

(1*R*,2*S*)-2-(*t*-Butyldiphenylsilyloxymethyl)cyclopropanecarbaldehyde **1-27**

A solution of oxalyl chloride (11.0 ml, 131 mmol, 1.5 equiv) in DCM (170 ml) was cooled to -78 °C. A solution of DMSO (18.6 ml, 262 mmol, 3.0 equiv) in DCM (140 ml) was added *via cannula* over 1 h, followed by a solution of cyclopropane alcohol **1-31** (29.7 g, 87.2 mmol) in DCM (170 ml) over 2 h. After 1 h, triethylamine (72.9 ml, 523 mmol, 6.0 equiv) was added. After 30 min, the reaction mixture was quenched with sat. NH_4Cl, warmed to rt and diluted with water. The layers were separated and the aqueous layer was extracted with DCM, the combined organic layers were washed with 1 M HCl, sat. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure to give the crude product as a pale yellow solid. Recrystallization from *n*-hexane furnished the title compound **1-27** (23.6 g, 69.8 mmol, 80%, 98% ee) as a colorless needle. Aldehyde **1-27** was converted to cyclopropane alcohol **1-31** ($NaBH_4$/ MeOH) and the ee value was determined by HPLC analysis (Chiralcel OD, *n*-hexane/ *i*-PrOH 97:3, 0.5 ml/ min, -18 °C, 264 nm, retention times: 12.7 min (minor), 14.6 min (major)). $[\alpha]^{22}_D$ -9.48 (*c* 1.18, $CHCl_3$) [lit. (for *ent*-**1-27**) $[\alpha]^{23}_D$ +5.8 (*c* 1.10, $CHCl_3$)][38]; mp 78 °C (lit. (for *ent*-**1-27**) 81-82 °C)[38]; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.36 (d, *J* = 5.7 Hz, 1H, aldehyde H), 7.69-7.60 (m, 4H, aromatic), 7.48-7.35 (m, 6H, aromatic), 4.00 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2O), 3.66 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 2.00-1.91 (m, 1H, cyclopropyl CH), 1.82-1.72 (m, 1H, cyclopropyl CH), 1.32-1.24 (m, 1H, cyclopropyl CH_2), 1.24-1.16 (m, 1H, cyclopropyl CH_2), 1.03 (s, 9H, CCH_3); LRMS (ESI) *m/z* 361.16 [$(M+Na)^+$]. ^{1}H-NMR, $[\alpha]^{22}_D$ data and melting point are in agreement with those reported (for *ent*-**1-27**) by Kazuta.[38]

Aldehyde **1-33**

To a solution of sulfinamide **1-32** (1.38 g, 2.86 mmol) in THF (30 ml) was added 12 M HCl (2.9 ml) at 0 °C. After 20 min at 0 °C, the reaction mixture was neutralized with sat. $NaHCO_3$, diluted with water and concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with AcOEt, the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the crude product as a pale yellow oil.

To a solution of the oil in THF (30 ml) was added Boc_2O (2.0 ml, 8.57 mmol, 3.0 equiv) and triethylamine (1.4 ml, 9.71 mmol, 3.4 equiv). After 27 h at rt, the solvent was removed under reduced pressure and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 21:1) to yield the *N*-Boc product as a pale yellow oil.

To a solution of the oil in THF (23 ml) was added TBAF (9.13 ml, 9.13 mmol, 4.0 equiv). After 7 h, the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 2:1) to yield the corresponding alcohol as a white solid.

A solution of oxalyl chloride (484 μl, 5.72 mmol, 2.0 equiv) in DCM (10 ml) was cooled to -78 °C. A solution of DMSO (813 μl, 11.4 mmol, 4.0 equiv) in DCM (4.0 ml) was added *via cannula*. After 1 h, a solution of the aforementioned alcohol in DCM (6.0 ml) was added *via cannula*. After 2 h , Triethylamine (3.19 ml, 22.9 mmol, 8.0 equiv) was added. After 40 min, the reaction mixture was warmed to rt and stirred for 5 h. The reaction mixture was quenched with sat. NH_4Cl and diluted with AcOEt. The layers were separated and the organic layer was washed with 1

M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 8:1) to yield aldehyde **1-33** (452 mg, 1.89 mmol, 4 steps 66%) as a colorless oil. $[\alpha]^{21}_D$ 39.00 (*c* 1.60, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.55 (d, *J* = 4.6 Hz, 1H, aldehyde H), 5.86-5.71 (m, 1H, vinyl CH), 5.18 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 5.12 (d, *J* = 10.3 Hz, 1H, vinyl CH_2), 4.60 (br, 1H, NH), 4.22 (br, 1H, CHN), 2.03-1.95 (m, 1H, cyclopropyl CH), 1.90-1.79 (m, 1H, C$\underline{H}_2$CHN), 1.74-1.65 (m, 1H, C$\underline{H}_2$CHN), 1.60-1.51 (m, 1H, cyclopropyl CH), 1.45 (s, 9H, CCH_3), 1.28-1.21 (m, 1H, cyclopropyl CH_2), 1.21-1.14 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 201.3, 155.3, 138.2, 114.9, 79.4, 52.8, 32.8, 28.3, 27.0, 21.6, 14.3; LRMS (ESI) *m/z* 262.14 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{13}H_{21}NO_3Na$: 262.1419 $[(M+Na)^+]$, found: 262.1417.

Key intermediate **1-36**

To a solution of aldehyde **1-33** (449 mg, 1.88 mmol) in 80% aqueous *t*-BuOH (20 ml) was added NaH_2PO_4-$2H_2O$ (439 mg, 2.81 mmol, 1.5 equiv), 2-methyl-2-butene (1.59 ml, 15.0 mmol, 8.0 equiv) and $NaClO_2$ (678 mg, 7.50 mmol, 4.0 equiv). After 2 h at rt, the reaction mixture was diluted with AcOEt, washed with 1 M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the crude product as a pale yellow viscous oil.

To a solution of the viscous oil in DCM (10 ml) was added triethylamine (524 μl, 3.76 mmol, 2.0 equiv) and DPPA (810 μl, 3.76 mmol, 2.0 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was warmed to rt and stirred for 18 h. Then the mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the crude product as a pale yellow oil.

A solution of the oil in toluene (200 ml) was refluxed for 13 h and the solvent was removed under reduced pressure. To the residue in THF (10 ml) was added 4 M KOH (30 ml). After 1 h at rt, the reaction mixture was concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with DCM, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by NH-silica gel column chromatography (*n*-hexane/ AcOEt 5:1-3:1-1:1-0:1) to yield amine **1-34** as a white solid.

To a solution of β-lactone unit **1-35** (874 mg, 5.08 mmol, 2.7 equiv) in DCM (20 ml) was added triethylamine (707 μl, 5.08 mmol, 2.7 equiv) and PivCl (626 μl, 5.08 mmol, 2.7 equiv) at 0 °C. After 30 min, a solution of amine **1-34** in DCM (30 ml) was added and the resulting mixture was warmed to rt. After 1 h at rt, the reaction mixture was diluted with AcOEt, washed with 1M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:1) to yield key intermediate **1-36** (343 mg, 0.902 mmol, 5 steps 48%) as a colorless oil. $[\alpha]^{21}_D$ 9.64 (*c* 0.34, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.00 (br, 1H, amide NH), 5.79 (ddd, *J* = 17.2, 10.3, 6.3 Hz, 1H, vinyl CH), 5.27 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 5.19 (d, *J* = 10.3 Hz, 1H, vinyl CH_2), 4.72 (br, 1H, carbamate NH), 4.65 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.12-4.04 (m, 1H, CHN), 3.81 (br, 1H, OCOCH), 2.91-2.82 (m, 1H, cyclopropyl CH), 2.02-1.92 (m, 1H, C$\underline{H}$$CH_3$), 1.74-1.61 (m, 2H, C$\underline{H}_2$$CH_3$ and C$\underline{H}_2$CHN), 1.50-1.40 (m, 10H, C$\underline{H}_2$CHN and CC$\underline{H}_3$), 1.38-1.27 (m, 1H, C$\underline{H}_2$$CH_3$), 1.12-0.98 (m, 5H, cyclopropyl CH, cyclopropyl CH_2 (1H) and CHC$\underline{H}_3$), 0.94 (dd, *J* = 7.4, 6.9 Hz, 3H, CH_2C$\underline{H}_3$), 0.34-0.25 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.8, 169.4, 155.5, 137.4, 116.2, 79.7, 70.7, 62.4, 53.3, 33.7, 33.6, 28.4, 28.3, 26.6, 26.6, 16.3, 13.7, 12.3, 11.1; LRMS (ESI) *m/z* 403.22 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{20}H_{32}N_2O_5Na$: 403.2203 $[(M+Na)^+]$, found: 403.2208.

General procedure for the preparation of the target compounds **1-1** – **1-4**

To a solution of key intermediate **1-36** (1.0 equiv) in DCM (0.1 M) was added an equivalent amount of TFA at 0 °C. After 15 min at 0 °C, the reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was concentrated *in vacuo* to give the crude product as a pale yellow oil.

To a solution of the oil in DCM (0.1 M) was added triethylamine (2.2 equiv) and acyl chloride (1.2 equiv) at 0 °C and stirred at 0 °C until the starting material disappeared on TLC. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/AcOEt 3:2) to yield the corresponding target compound.

Target compound **1-1**

Target compound **1-1** (27.6 mg, 0.0788 mmol, 2 steps quant.) was obtained as a colorless viscous oil. $[\alpha]^{22}_{D}$ 16.89 (*c* 0.96, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.46 (br, 1H, NHCO), 6.29 (d, *J* = 6.9 Hz, 1H, isopropanoyl NH), 5.81 (ddd, *J* = 17.2, 9.2, 5.7 Hz, 1H, vinyl CH), 5.22 (dd, *J* = 17.2, 1.1 Hz, 1H, vinyl CH_2), 5.16 (dd, *J* = 9.2, 1.1 Hz, 1H, vinyl CH_2), 4.77 (d, *J* = 4.6 Hz, 1H, NHCOC<u>H</u>), 4.41-4.31 (m, 1H, NCH), 3.69 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOC<u>H</u>), 2.90-2.81 (m, 1H, cyclopropyl CH), 2.45 (sept, *J* = 6.9 Hz, 1H, isopropyl CH), 2.05-1.94 (m, 1H, C<u>H</u>CH_3), 1.91-1.83 (m, 1H, NCHC<u>H</u>$_2$), 1.68-1.57 (m, 1H, C<u>H</u>$_2CH_3$), 1.38-1.21 (m, 2H, C<u>H</u>$_2CH_3$ and NCHC<u>H</u>$_2$), 1.17 (d, *J* = 6.9 Hz, 6H, isopropyl CH_3), 1.12-1.04 (m, 4H, cyclopropyl CH_2 (1H) and CHC<u>H</u>$_3$), 1.04-0.95 (m, 1H, cyclopropyl CH), 0.92 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H</u>$_3$), 0.31-0.22 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.1, 170.3, 169.4, 137.4, 115.8, 70.4, 62.5, 51.8, 35.4, 33.4, 32.4, 26.7, 26.6, 19.6, 19.6, 16.2, 14.0, 12.1, 11.0; LRMS (EI) *m/z* 350 (M^+); HRMS (EI) calcd for $C_{19}H_{30}N_2O_4$: 350.2206 (M^+), found: 350.2205.

Target compound **1-2**

Target compound **1-2** (17.3 mg, 0.0475 mmol, 2 steps 81%) was obtained as a white solid. $[\alpha]^{22}_{D}$ 9.01 (*c* 0.82, $CHCl_3$); mp 103-104 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.60 (br, 1H, NHCO), 6.16 (d, *J* = 6.9 Hz, 1H, PivN<u>H</u>), 5.83 (ddd, *J* = 17.2, 10.3, 5.7 Hz, 1H, vinyl CH), 5.24 (dd, *J* = 17.2, 1.1 Hz, 1H, vinyl CH_2), 5.20 (dd, *J* = 10.3, 1.1 Hz, 1H, vinyl CH_2), 4.79 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.37-4.28 (m, 1H, PivNHC<u>H</u>), 3.75 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.88-2.82 (m, 1H, cyclopropyl CH), 2.04-1.94 (m, 1H, C<u>H</u>CH_3), 1.87-1.79 (m, 1H, NCHC<u>H</u>$_2$), 1.67-1.56 (m, 1H, C<u>H</u>$_2CH_3$), 1.38-1.27 (m, 2H, NCHC<u>H</u>$_2$ and C<u>H</u>$_2CH_3$), 1.22 (s, 9H, CCH_3), 1.11-1.03 (m, 4H, cyclopropyl CH_2 and CHC<u>H</u>$_3$), 1.03-0.95 (m, 1H, cyclopropyl CH), 0.92 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H</u>$_3$), 0.29-0.21 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 178.5, 170.2, 169.6, 137.2, 116.3, 70.4, 62.3, 52.2, 38.6, 33.4, 32.7, 27.5, 26.7, 26.6, 16.2, 13.6, 12.6, 11.0; LRMS (EI) *m/z* 364 (M^+); HRMS (EI) calcd for $C_{20}H_{32}N_2O_4$: 364.2362 (M^+), found: 364.2361.

Target compound **1-3**

Target compound **1-3** (15.5 mg, 0.0403 mmol, 2 steps 78%) was obtained as a colorless viscous oil. $[\alpha]^{22}_{D}$ 21.86 (*c* 1.01, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.94-7.89 (m, 2H, aromatic), 7.54-7.48 (m, 1H, aromatic), 7.47-7.42 (m, 2H, aromatic), 7.24 (br, 1H, NCOCH), 7.08 (d, *J* = 6.9 Hz, 1H, BzN<u>H</u>), 5.90 (ddd, *J* = 17.2, 10.3, 5.7 Hz, 1H, vinyl CH), 5.30 (dd, *J* = 17.2, 1.1 Hz, 1H, vinyl CH_2), 5.20 (dd, *J* = 10.3, 1.1 Hz, 1H, vinyl CH_2), 4.77 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.70-4.61 (m, 1H, BzNHC<u>H</u>), 3.71 (dd, *J* = 7.4, 4,6 Hz, 1H, OCOCH), 2.88-2.81 (m, 1H, cyclopropyl CH), 2.04-1.90 (m, 2H, C<u>H</u>CH_3 and NCHC<u>H</u>$_2$), 1.70-1.58 (m, 1H, C<u>H</u>$_2CH_3$), 1.47-1.37 (m, 1H, NCHC<u>H</u>$_2$), 1.37-1.26 (m, 1H,

$C\underline{H}_2CH_3$), 1.13-1.00 (m, 5H, cyclopropyl CH, cyclopropyl CH_2 (1H), $CHC\underline{H}_3$), 0.91 (dd, *J* = 7.4, 7.4 Hz, 3H, $CH_2C\underline{H}_3$), 0.35-0.26 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.3, 169.4, 167.2, 137.5, 134.0, 131.6, 128.5, 127.2, 115.9, 70.5, 62.7, 52.4, 33.5, 32.2, 26.8, 26.6, 16.2, 14.1, 12.0, 11.0; LRMS (EI) *m/z* 384 (M^+); HRMS (EI) calcd for $C_{22}H_{28}N_2O_4$: 384.2049 (M^+), found: 384.2056.

Target compound **1-4**

Target compound **1-4** (30.5 mg, 0.0702 mmol, 2 steps 92%) was obtained as a colorless viscous oil. $[\alpha]^{21}_D$ 42.42 (*c* 0.89, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.47 (br, 1H, aromatic), 8.02-7.83 (m, 4H, aromatic), 7.61-7.50 (m, 2H, aromatic), 7.34 (br, 2H, NH), 5.95 (ddd, *J* = 17.2, 10.4, 5.9 Hz, 1H, vinyl CH), 5.34 (dd, *J* = 17.2, 1.4 Hz, 1H, vinyl CH_2), 5.23 (dd, *J* = 10.4, 1.4 Hz, 1H, vinyl CH_2), 4.81 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.76-4.66 (m, 1H, CONHC$\underline{H}$), 3.74 (dd, *J* = 7.7, 4.5 Hz, 1H, OCOC$\underline{H}$), 2.90-2.82 (m, 1H, cyclopropyl CH), 2.08-1.93 (m, 2H, C$\underline{H}CH_3$ and NCHC$\underline{H}_2$), 1.72-1.59 (m, 1H, $C\underline{H}_2CH_3$), 1.50-1.39 (m, 1H, NCHC$\underline{H}_2$), 1.39-1.24 (m, 1H, $C\underline{H}_2CH_3$), 1.15-1.03 (m, 5H, cyclopropyl $C\underline{H}_2$ (1H), cyclopropyl C$\underline{H}$ and $CHC\underline{H}_3$), 0.91 (dd, *J* = 7.7, 7.2 Hz, 3H, $CH_2C\underline{H}_3$), 0.37-0.28 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.3, 169.4, 167.3, 137.5, 134.8, 132.6, 131.2, 129.0, 128.3, 127.8, 127.7, 127.6, 126.6, 123.7, 115.8, 70.5, 62.7, 52.5, 33.5, 32.1, 26.8, 26.7, 16.3, 14.2, 12.0, 11.0; LRMS (EI) *m/z* 434 (M^+); HRMS (EI) calcd for $C_{26}H_{30}N_2O_4$: 434.2206 (M^+), found: 434.2212.

General procedure for the preparation of the target compounds **1-6** – **1-9** and **1-14**

To a solution of the *N*-protected amino acid (3.0 equiv) in DCM (0.1 M) was added triethylamine (3.0 equiv) and PivCl (3.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding acid anhydride.

To a solution of key intermediate **1-36** (1.0 equiv) in DCM (0.1 M) was added an equivalent amount of TFA at 0 °C. After 15 min at 0 °C, the reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was concentrated *in vacuo* to give the crude product as a pale yellow oil.

To a solution of the oil in DCM (0.1 M) was added triethylamine (2.0 equiv) and a solution of the aforementioned acid anhydride (3.0 equiv) in DCM (0.1 M) at 0 °C. The reaction mixture was warmed to rt and stirred until the starting material disappeared on TLC. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography to yield the corresponding target compound.

Target compound **1-6**

The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:1) to yield target compound **1-6** (28.5 mg, 0.0587 mmol, 2 steps quant.) as a colorless viscous oil. $[\alpha]^{19}_D$ 14.69 (*c* 0.99, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.44-7.28 (m, 5H, aromatic), 7.08 (br, 2H, amide NH), 5.76 (ddd, *J* = 17.2, 10.3, 5.7 Hz, 1H, vinyl CH), 5.63 (d, *J* = 6.9 Hz, 1H, carbamate NH), 5.23-5.02 (m, 4H, vinyl CH_2 and benzyl CH_2), 4.66 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.44-4.25 (m, 2H, NCH and D-Ala α-H), 3.64 (br, 1H, OCOCH), 2.78 (br, 1H, cyclopropyl CH), 2.02-1.92 (m, 1H, C$\underline{H}CH_3$), 1.89-1.77 (m, 1H, NCHC$\underline{H}_2$), 1.69-1.56 (m, 1H, $C\underline{H}_2CH_3$), 1.42 (d, *J* = 6.9 Hz, 3H, D-Ala CH_3), 1.36-1.17 (m, 2H, $C\underline{H}_2CH_3$ and NCHC$\underline{H}_2$), 1.11-0.99 (m, 4H, cyclopropyl CH_2 (1H) and $CHC\underline{H}_3$), 0.99-0.83 (m, 4H, cyclopropyl CH and $CH_2C\underline{H}_3$), 0.26 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.4, 170.4, 169.4, 155.9, 137.2, 136.3,

128.5, 128.1, 128.0, 115.5, 70.6, 66.8, 62.9, 51.8, 50.5, 33.6, 31.8, 26.8, 26.6, 18.9, 16.3, 14.2, 11.5, 11.0; LRMS (ESI) *m/z* 508.24 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{26}H_{35}N_3O_6Na$: 508.2418 [$(M+Na)^+$], found: 508.2414.

Target compound **1-5**

The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:1) to yield target compound **1-5** (19.4 mg, 0.0411 mmol, 2 steps 96%) as a colorless viscous oil. $[\alpha]^{18}_D$ 5.92 (*c* 0.52, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.39-7.27 (m, 5H, aromatic), 6.91 (br, 2H, amide NH), 5.75 (ddd, *J* = 17.2, 10.3, 5.7 Hz, 1H, vinyl CH), 5.63 (br, 1H, carbamate NH), 5.22-5.03 (m, 4H, vinyl CH_2 and benzyl CH_2), 4.62 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.48-4.37 (m, 1H, NCH), 4.00-3.82 (m, 2H, Gly α-H), 7.21 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.80 (br, 1H, cyclopropyl CH), 2.01-1.90 (m, 1H, C<u>H</u>CH_3), 1.87-1.74 (m, 1H, NCHC<u>H</u>$_2$), 1.66-1.54 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.37-1.16 (m, 2H, C<u>H</u>$_2$$CH_3$ and NCHC<u>H</u>$_2$), 1.06-0.79 (m, 8H, cyclopropyl CH_2 (1H), cyclopropyl CH, CHC<u>H</u>$_3$ and CH_2C<u>H</u>$_3$), 0.31-0.17 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.3, 169.3, 168.9, 156.5, 137.3, 136.3, 128.5, 128.1, 128.0, 115.5, 70.6, 67.0, 62.9, 51.7, 44.4, 33.6, 31.9, 26.8, 26.6, 16.3, 14.3, 11.3, 11.0; LRMS (ESI) *m/z* 494.22 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{25}H_{33}N_3O_6Na$: 494.2262 [$(M+Na)^+$], found: 494.2260.

Target compound **1-7**

The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 2:1) to yield target compound **1-7** (19.7 mg, 0.0373 mmol, 2 steps 64%) as a colorless viscous oil. $[\alpha]^{21}_D$ -2.18 (*c* 0.62, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.27 (m, 5H, aromatic), 6.99 (br, 2H, amide NH), 5.78 (ddd, *J* = 17.2, 10.3, 5.2 Hz, 1H, vinyl CH), 5.52 (d, *J* = 8.6 Hz, 1H, carbamate NH), 5.16 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 5.12 (d, *J* = 10.3 Hz, 1H, vinyl CH_2), 5.09 (s, 2H, benzyl CH_2), 4.65 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.48-4.36 (m, 1H, NCH), 4.36-4.19 (m, 1H, Leu α-H), 3.63 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.81 (br, 1H, cyclopropyl CH), 2.02-1.91 (m, 1H, C<u>H</u>CH_3), 1.91-1.78 (m, 1H, NCHC<u>H</u>$_2$), 1.78-1.49 (m, 4H, Leu CH, C<u>H</u>$_2$$CH_3$ (1H) and Leu CH_2), 1.32-1.25 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.20-1.10 (m, 1H, NCHC<u>H</u>$_2$), 1.1-0.99 (m, 4H, cyclopropyl CH and CHC<u>H</u>$_3$), 0.99-0.73 (m, 10H, cyclopropyl CH_2, Leu CH_3 and CH_2C<u>H</u>$_3$), 0.30-0.17 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.4, 170.4, 169.4, 156.2, 137.4, 136.4, 128.5, 128.4, 128.1, 127.9, 115.2, 70.7, 66.8, 62.9, 53.5, 51.5, 42.0, 33.7, 31.8, 26.9, 26.7, 24.7, 22.9, 22.0, 16.3, 14.4, 11,3, 11.0; LRMS (EI) *m/z* 527 (M^+); HRMS (EI) calcd for $C_{29}H_{41}N_3O_6$: 527.2995 (M^+), found: 527.3000.

Target compound **1-8**

The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:2) to yield target compound **1-8** (22.3 mg, 0.0397 mmol, 2 steps 66%) as a colorless viscous oil. $[\alpha]^{21}_D$ 13.14 (*c* 1.04, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40-7.16 (m, 10H, aromatic), 7.02 (br, 1H, amide NH), 6.47 (d, *J* = 6.9 Hz, 1H, amide NH), 5.66 (d, *J* = 8.0 Hz, 1H, carbamate NH), 5.61 (ddd, *J* = 17.2, 10.3, 5.7 Hz, 1H, vinyl CH), 5.07 (s, 2H, benzyl CH_2), 5.03 (d, *J* = 10.3 Hz, 1H, vinyl CH_2), 4.95 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 4.64 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.54-4.43 (m, 1H, Phe α-H), 4.43-4.31 (m, 1H, NCH), 3.64 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 3.15-3.00 (m, 2H, Phe CH_2), 2.74 (br, 1H, cyclopropyl CH), 2.01-1.91 (m, 1H, C<u>H</u>CH_3), 1.81-1.72 (m, 1H, NCHC<u>H</u>$_2$), 1.67-1.56 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.35-1.23 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.21-1.10 (m, 1H, NCHC<u>H</u>$_2$), 1.09-0.95 (m, 4H, cyclopropyl CH_2 and $CHCH_3$), 0.94-0.81 (m, 4H, cyclopropyl CH and CH_2C<u>H</u>$_3$), 0.26-0.14 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.7, 170.3, 169.5, 155.9, 136.9, 136.6, 136.4, 129.4, 128.6, 128.4, 128.0, 127.9, 126.9, 115.6, 70.8, 66.8, 62.9, 56.4, 51.6, 39.0, 33.6, 32.0, 26.7, 26.6, 16.3, 14.2, 11.5, 11.0; LRMS (EI) *m/z* 561 (M^+); HRMS (EI) calcd for $C_{32}H_{39}N_3O_6$: 561.2839

(M^+), found: 561.2843.

Target compound **1-9**

The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:1) to yield target compound **1-9** (17.7 mg, 0.0295 mmol, 2 steps 65%) as a colorless viscous oil. $[\alpha]^{22}_D$ 14.22 (*c* 0.82, $CHCl_3$); Spectroscopic data for major rotamer (rotamer ratio 10:3): ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.18 (br, 1H, indole NH), 7.68 (d, *J* = 7.4 Hz, 1H, aromatic), 7.23-7.43 (m, 6H, aromatic), 7.19 (dd, *J* = 7.4, 7.4 Hz, 1H, aromatic), 7.05-7.16 (m, 1H, aromatic), 7.02 (d, *J* = 2.3 Hz, 1H, aromatic), 6.98 (br, 1H, amide NH), 6.21 (d, *J* = 5.7 Hz, 1H, amide NH), 5.72 (d, *J* = 8.0 Hz, 1H, carbamate NH), 5.53 (ddd, *J* = 17.2, 10.3, 5.7 Hz, 1H, vinyl CH), 5.10 (s, 2H, benzyl CH_2), 4.99 (d, *J* = 10.3 Hz, 1H, vinyl CH), 4.91 (d, *J* = 17.2 Hz, 1H, vinyl CH), 4.63 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.61-4.52 (m, 1H, Trp α-H), 4.34-4.24 (m, 1H, NCH), 3.66 (dd, *J* = 6.9, 4.6 Hz, 1H, OCOCH), 3.38 (dd, *J* = 14.3, 5.2 Hz, 1H, Trp CH_2), 3.16 (dd, *J* = 14.3, 6.9 Hz, 1H, Trp CH_2), 2.62 (br, 1H, cyclopropyl CH), 2.01-1.89 (m, 1H, C<u>H</u>CH_3), 1.70-1.48 (m, 2H, NCHC<u>H</u>$_2$ and C<u>H</u>$_2$$CH_3$), 1.39-1.23 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.23-1.09 (m, 1H, NCHC<u>H</u>$_2$), 1.02 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 0.96-0.76 (m, 4H, cyclopropyl CH_2 and CH_2C<u>H</u>$_3$), 0.76-0.60 (m, 1H, cyclopropyl CH), 0.06 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.1, 170.1, 169.6, 156.0, 136.7, 136.4, 136.2, 128.5, 128.5, 128.1, 128.0, 127.4, 123.2, 122.3, 120.0, 119.0, 115.7, 111.1, 110.6, 70.8, 66.8, 62.8, 55.5, 51.7, 33.6, 32.1, 28.7, 26.6, 26.4, 16.3, 13.7, 11.7, 11.0; Spectroscopic data for minor rotamer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.18 (br, 1H, indole NH), 8.05 (br, 1H, amide NH), 7.71 (br, 1H, aromatic), 7.38-7.27 (m, 6H, aromatic), 7.19 (dd, *J* = 7.4, 7.4 Hz, 1H, aromatic), 7.16-7.05 (m, 1H, aromatic), 7.02 (d, *J* = 2.3 Hz, 1H, aromatic), 6.72 (br, 1H, amide NH), 5.83 (d, *J* = 7.4 Hz, 1H, carbamate NH), 5.57 (br, 1H, vinyl CH), 5.08 (s, 2H, benzyl CH_2), 4.99 (d, *J* = 10.3 Hz, 1H, vinyl CH_2), 4.95 (br, 1H, NCOCH), 4.91 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 4.69 (br, 1H, Trp α-H), 4.39 (br, 1H, NCH), 3.66 (dd, *J* = 6.9, 4.6 Hz, 1H, OCOCH), 3.43 (br, 1H, Trp CH_2), 3.19 (br, 1H, Trp CH_2), 2.29 (br, 1H, cyclopropyl CH), 2.01-1.89 (m, 1H, C<u>H</u>CH_3), 1.70-1.48 (m, 2H, NCHC<u>H</u>$_2$ and C<u>H</u>$_2$$CH_3$), 1.34-1.24 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.22-1.12 (m, 1H, NCHC<u>H</u>$_2$), 1.02 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 0.89 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H</u>$_3$), 0.75-0.65 (m, 1H, cyclopropyl CH_2), 0.41 (br, 1H, cyclopropyl CH), 0.08 (br, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 601.34 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{34}H_{41}N_4O_6$: 601.3026 [$(M+H)^+$], found: 601.3015.

Target compound **1-14**

The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:2) to yield target compound **1-14** (285 mg, 0.627 mmol, 2 steps quant.) as a colorless viscous oil. $[\alpha]^{22}_D$ -2.69 (*c* 1.17, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.17 (br, 1H, amid NH), 6.95 (d, *J* = 5.4 Hz, 1H, amide NH), 5.81 (ddd, *J* = 17.2, 10.4, 5.4 Hz, 1H, vinyl CH), 5.28 (d, *J* = 6.3 Hz, 1H, carbamate NH), 5.20 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 5.14 (d, *J* = 10.4 Hz, 1H, vinyl CH_2), 4.69 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.47-4.35 (m, 1H, NCH), 4.25 (br, 1H, Ala α-H), 3.68 (dd, *J* = 7.7, 4.5 Hz, 1H, OCOCH), 2.90-2.76 (m, 1H, cyclopropyl CH), 2.07-1.92 (m, 1H, C<u>H</u>CH_3), 1.89-1.77 (m, 1H, NCHC<u>H</u>$_2$), 1.72-1.56 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.51-1.17 (m, 14H, CCH_3, Ala CH_3, C<u>H</u>$_2$$CH_3$ (1H) and NCHC<u>H</u>$_2$ (1H)), 1.14-0.84 (m, 8H, cyclopropyl CH_2 (1H), cyclopropyl CH, CHC<u>H</u>$_3$ and CH_2C<u>H</u>$_3$), 0.36-0.22 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.7, 170.2, 169.4, 155.4, 137.3, 115.4, 79.8, 70.6, 62.7, 51.6, 50.0, 33.6, 32.2, 28.3, 26.8, 26.6, 18.8, 16.3, 14.1, 11.6, 11.0; LRMS (ESI) *m/z* 452.29 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{23}H_{38}N_3O_6$: 452.2761 [$(M+H)^+$], found: 452.2779.

Target compound **1-10**

To a solution of compound **1-14** (24.5 mg, 0.0543 mmol) in DCM (270 µl) was added TFA (270 µl) at 0 °C. The reaction mixture was concentrated *in vacuo* to give target compound **1-10** (25.3 mg, 0.0544 mmol, quant.) as a colorless viscous oil. $[\alpha]^{22}_{D}$ -5.03 (*c* 1.27, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.29 (br, 1H, amide NH), 8.25 (br, 3H, Ala NH_3), 7.43 (br, 1H, amide NH), 5.75 (ddd, *J* = 17.2, 10.3, 5.2 Hz, 1H, vinyl CH), 5.12 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 5.08 (d, *J* = 10.3 Hz, 1H, vinyl CH_2), 4.71 (d, *J* = 4.0 Hz, 1H, NCOCH), 4.39 (br, 1H, NCH), 4.22 (br, 1H, Ala α-H), 3.59 (dd, *J* = 7.4, 4.0 Hz, 1H, OCOCH), 2.81 (br, 1H, cyclopropyl CH), 2.04-1.91 (m, 1H, C<u>H</u>CH_3), 1.78-1.41 (m, 5H, NCHC<u>H</u>$_2$ (1H), C<u>H</u>$_2CH_3$ (1H) and Ala CH_3), 1.41-1.20 (m, 2H, NCHC<u>H</u>$_2$ and C<u>H</u>$_2CH_3$), 1.11-0.81 (m, 8H, cyclopropyl CH, cyclopropyl CH_2 (1H), CHC<u>H</u>$_3$ and CH_2C<u>H</u>$_3$), 0.34 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.1, 169.9, 137.0, 115.1, 70.6, 62.9, 52.3, 49.6, 33.5, 31.7, 27.3, 26.6, 17.3, 16.1, 14.7, 10.9, 10.5; LRMS (ESI) *m/z* 352.23 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{18}H_{30}N_3O_4$: 352.2236 [$(M+H)^+$], found: 352.2224.

General procedure for the preparation of the target compounds **1-11** – **1-13**

To a solution of target compound **1-10** (1.0 equiv) in DCM (0.1 M) was added triethylamine (2.2 equiv) and acyl chloride (1.2 equiv) at 0 °C and stirred at the same temperature until the starting material disappeared on TLC. The reaction mixture was diluted with AcOEt, washed with 1N HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography to yield the corresponding target compound.

Target compound **1-11**

The crude product was purified by flash column chromatography ($CHCl_3$/ MeOH 15:1) to yield target compound **1-11** (9.2 mg, 0.0234 mmol, 59%) as a colorless viscous oil. $[\alpha]^{22}_{D}$ -8.17 (*c* 0.71, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.29 (br, 1H, amide NH), 7.12 (d, *J* = 7.2 Hz, 1H, amide NH), 6.68 (d, *J* = 7.7 Hz, 1H, Ala NH), 5.82 (ddd, *J* = 17.2, 10.4, 5.9 Hz, 1H, vinyl CH), 5.23 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 5.17 (d, *J* = 10.4 Hz, 1H, vinyl CH_2), 4.66 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.63-4.52 (m, 1H, Ala α-H), 4.40-4.29 (m, 1H, NCH), 3.72 (dd, *J* = 7.7, 4.5 Hz, 1H, OCOCH), 2.93-2.82 (m, 1H, cyclopropyl CH), 2.07-1.87 (m, 5H, acetyl CH_3, C<u>H</u>CH_3 and NCHC<u>H</u>$_2$), 1.72-1.61 (m, 1H, C<u>H</u>$_2CH_3$), 1.46-1.27 (m, 4H, Ala CH_3 and C<u>H</u>$_2CH_3$), 1.27-1.16 (m, 1H, NCHC<u>H</u>$_2$), 1.13-1.02 (m, 4H, CHC<u>H</u>$_3$ and cyclopropyl CH_2), 1.02-0.89 (m, 4H, cyclopropyl CH and CH_2C<u>H</u>$_3$), 0.35-0.21 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.4, 170.3, 170.1, 170.0, 136.9, 115.9, 71.3, 62.9, 52.2, 48.8, 33.7, 32.4, 26.8, 26.7, 23.1, 19.2, 16.3, 14.2, 11.7, 11.0; LRMS (EI) *m/z* 393 (M^+); HRMS (EI) calcd for $C_{20}H_{31}N_3O_5$: 393.2264 (M^+), found: 393.2268.

Target compound **1-12**

The crude product was purified by flash column chromatography (*n*-hexane/AcOEt 1:2) to yield target compound **1-12** (18.4 mg, 0.0404 mmol, quant.) as a colorless viscous oil. $[\alpha]^{22}_{D}$ 7.69 (*c* 0.65, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.86 (d, *J* = 7.4 Hz, 2H, aromatic), 7.50 (t, *J* = 7.4 Hz, 1H, aromatic), 7.43 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.37 (d, *J* = 7.4 Hz, 1H, amide NH), 7.27 (d, *J* = 6.9 Hz, 1H, Ala NH), 7.18 (br, 1H, amide NH), 5.83 (ddd, *J* = 17.2, 10.3, 5.7 Hz, 1H, vinyl CH), 5.25 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 5.17 (d, *J* = 10.3 Hz, 1H, vinyl CH_2), 4.91-4.80 (m, 1H, Ala α-H), 4.64 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.46-4.35 (m, 1H, NCH), 3.70 (dd, *J* = 8.0, 4.6 Hz, 1H,

OCOCH), 2.91-2.78 (m, 1H, cyclopropyl CH), 2.02-1.93 (m, 1H, C<u>H</u>CH_3), 1.93-1.83 (m, 1H, NCHC<u>H_2</u>), 1.69-1.58 (m, 1H, C<u>H_2</u>CH_3), 1.52 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.38-1.17 (m, 2H, NCHC<u>H_2</u> and C<u>H_2</u>CH_3), 1.11-0.82 (m, 8H, cyclopropyl CH_2 (1H), cyclopropyl CH, CHC<u>H_3</u> and CH_2C<u>H_3</u>), 0.32-0.20 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.3, 170.3, 169.8, 166.9, 137.1, 133.8, 131.6, 128.5, 127.2, 115.7, 71.0, 62.9, 52.2, 49.2, 33.7, 32.2, 26.9, 26.6, 19.2, 16.3, 14.3, 11.5, 11.0; LRMS (EI) *m/z* 455 (M^+); HRMS (EI) calcd for $C_{25}H_{33}N_3O_5$: 455.2420 (M^+), found: 455.2424.

Target compound **1-13**

The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:2) to yield target compound **1-13** (19.2 mg, 0.0380 mmol, 95%) as a colorless viscous oil. $[\alpha]^{20}_D$ 29.36 (*c* 0.65, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.42 (s, 1H, aromatic), 7.98-7.83 (m, 4H, aromatic), 7.62-7.48 (m, 3H, aromatic and Ala NH), 7.44 (d, *J* = 7.4 Hz, 1H, amide NH), 7.21 (br, 1H, amide NH), 5.85 (ddd, *J* = 17.2, 10.3, 5.7 Hz, 1H, vinyl CH), 5.28 (d, *J* = 17.2 Hz, 1H, vinyl CH_2), 5.19 (d, *J* = 5.2 Hz, 1H, vinyl CH_2), 5.00-4.90 (m, 1H, Ala α-H), 4.63 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.48-4.37 (m, 1H, NCH), 3.71 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.91-2.79 (m, 1H, cyclopropyl CH), 2.00-1.86 (m, 2H, C<u>H</u>CH_3 and NCHC<u>H_2</u>), 1.66-1.52 (m, 4H, C<u>H_2</u>CH_3 (1H) and Ala CH_3), 1.35-1.18 (m, 2H, NCHC<u>H_2</u> and C<u>H_2</u>CH_3), 1.08-0.94 (m, 5H, cyclopropyl CH_2 (1H), cyclopropyl CH and CHC<u>H_3</u>), 0.89 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H_3</u>), 0.31-0.20 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.4, 170.3, 169.8, 166.9, 137.1, 134.8, 132.6, 131.0, 129.0, 128.3, 127.8, 127.7, 126.7, 123.8, 115.8, 71.1, 62.9, 52.2, 49.3, 33.6, 32.2, 26.9, 26.6, 19.3, 16.3, 14.3, 11.5, 11.0; LRMS (EI) *m/z* 505 (M^+); HRMS (EI) calcd for $C_{29}H_{35}N_3O_5$: 505.2577 (M^+), found: 505.2579.

Synthetic procedures for 1-15 – 1-23

Target compound **1-15**

To a solution of compound **1-14** (41.7 mg, 0.0923 mmol) in THF (1.0 ml) was added Pd/C (42 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 90 min. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the crude product as a colorless oil.

A solution of the oil in DCM (500 µl) was cooled to 0 °C. TFA (500 µl) was added and the resulting mixture was stirred at 0 °C for 90 min. The reaction mixture was concentrated *in vacuo* to give the crude product as a pale yellow oil.

To a solution of the oil in DCM (1.0 ml) was added triethylamine (40 µl, 0.277 mmol, 3.0 equiv) and CbzCl (40 µl, 0.277 mmol, 3.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:1) to yield target compound **1-15** (29.6 mg, 0.0607 mmol, 3 steps 66%) as a white solid. $[\alpha]^{19}_D$ 8.00 (*c* 0.50, $CHCl_3$); mp 119-120 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40-7.27 (m, 5H, aromatic), 7.17 (br, 1H, amide NH), 6.68 (d, *J* = 6.9 Hz, 1H, amide NH), 5.68 (d, *J* = 6.9 Hz, 1H, Ala NH), 5.10 (s, 2H, Cbz CH_2), 4.67 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.38-4.20 (m, 1H, Ala α-H), 3.86-3.74 (m, 1H, NCH), 3.65 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.78 (br, 1H, cyclopropyl CH), 2.04-1.92 (m, 1H, C<u>H</u>CH_3), 1.80-1.68 (m, 1H, NCHC<u>H_2</u>), 1.68-1.56 (m, 2H, NCHC<u>H_2</u>CH_3 and C<u>H_2</u>CH_3), 1.45-1.21 (m, 5H, Ala CH_3, NCHC<u>H_2</u>CH_3 and C<u>H_2</u>CH_3), 1.20-1.09 (m, 1H, NCHC<u>H_2</u>), 1.09-0.99 (m, 4H, CHC<u>H_3</u> and cyclopropyl CH_2), 0.99-0.71 (m, 7H, CH_2C<u>H_3</u>,

NCHCH$_2$C<u>H$_3$</u> and cyclopropyl CH), 0.27-0.15 (m, 1H, cyclopropyl CH$_2$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.7, 170.3, 169.4, 155.9, 136.4, 128.4, 128.1, 127.9, 70.4, 66.7, 62.8, 51.2, 50.6, 33.6, 31.9, 27.5, 27.0, 26.6, 19.1, 16.3, 14.6, 11.6, 11.0, 10.1; LRMS (ESI) *m/z* 510.26 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{26}H_{37}N_3O_6Na$: 510.2575 [(M+Na)$^+$], found: 510.2570.

<u>Alcohol **1-46**</u>

To a refluxing solution of imine **1-29** (700 mg, 1.54 mmol) in toluene (190 ml) was added PhMgCl (0.922 ml, 1.84 mmol, 1.2 equiv, 2.0 M in THF) and the resulting mixture was refluxed for 10 min. The reaction mixture was cooled to 0 °C, quenched with sat. NH_4Cl and diluted with AcOEt. The layers were separated and the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:1) to yield sulfinamide **1-38** (dr 7:1) as a yellow oil.

To a solution of sulfinamide **1-38** in THF (15 ml) was added 12 M HCl (1.5 ml) at 0 °C. After 10 min at 0 °C, the reaction mixture was neutralized with sat. $NaHCO_3$, diluted with water and concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with AcOEt, the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the crude product as a yellow oil.

To a solution of the oil in 50% aqueous THF (13 ml) was added Na_2CO_3 (139 mg, 1.31 mmol, 1.0 equiv) and FmocOSu (882 mg, 2.61 mmol, 2.0 equiv) and stirred at rt for 36 h. The reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude prodcut was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield the *N*-Fmoc product as a pale yellow solid.

To a solution of the solid in MeOH (10 ml) was added 4M HCl/ AcOEt (1.0 ml) and stirred at rt for 2 h. The reaction mixture was cooled to 0 °C and neutralized with sat. $NaHCO_3$, diluted with water and concentrated *in vacuo* to remove MeOH. The residual aqueous solution was extracted with AcOEt, the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:1) to yield alcohol **1-46** (382 mg, 0.924 mmol, 4 steps 60%, single isomer) as a white solid. $[\alpha]^{23}_D$ -21.52 (*c* 1.22, $CHCl_3$); mp 90-92 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.73 (d, *J* = 7.4 Hz, 2H, aromatic), 7.57 (br, 2H, aromatic), 7.43-7.17 (m, 9H, aromatic), 6.20 (br, 1H, carbamate NH), 4.73 (br, 1H, NCH), 4.46 (dd, *J* = 9.7, 6.3 Hz, 1H, Fmoc CH$_2$), 4.37 (dd, *J* = 9.7, 6.3 Hz, 1H, Fmoc CH$_2$), 4.18 (br, 1H, Fmoc CH), 4.06-3.91 (m, 1H, C<u>H$_2$</u>OH), 3.36-3.21 (m, 1H, C<u>H$_2$</u>OH), 1.99 (br, 2H, NCHC<u>H$_2$</u> and OH), 1.69-1.56 (m, 1H, NCHC<u>H$_2$</u>), 1.20-1.09 (m, 1H, cyclopropyl CH), 0.85-0.73 (m, 1H, cyclopropyl CH), 0.70-0.62 (m, 1H, cyclopropyl CH$_2$), -0.05--0.15 (m, 1H, cyclopropyl CH$_2$); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 156.3, 144.2, 144.2, 141.5, 141.4, 128.6, 127.6, 127.3, 127.1, 127.0, 126.3, 125.1, 125.1, 119.9, 66.4, 62.6, 56.6, 47.6, 35.4, 18.0, 13.6, 8.1; LRMS (FAB) *m/z* 414.2 [(M+H)$^+$]; HRMS (FAB) calcd for $C_{27}H_{28}NO_3$: 414.2069 [(M+H)$^+$], found: 414.2063.

<u>Alcohol **1-47**</u>

Alcohol **1-47** (368 mg, 0.860 mmol, 4 steps 56%, a white solid, single isomer) was prepared from imine **1-29** (700 mg, 1.54 mmol) as described for alcohol **1-46** using BnMgBr (1.5 equiv, 1.0 M in THF) instead of PhMgCl. $[\alpha]^{24}_D$ -4.60 (*c* 0.35, $CHCl_3$); mp 109-111 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.74 (d, *J* = 7.4 Hz, 2H, aromatic), 7.56 (dd, *J* = 6.9, 6.9 Hz, 2H, aromatic), 7.37 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.32-7.05 (m, 7H, aromatic), 5.59 (br, 1H, carbamate NH), 4.41 (br, 2H, Fmoc CH$_2$), 4.18 (dd, *J* = 6.9 Hz, 6.3 Hz, 1H, Fmoc CH), 3.88 (br, 2H, NCH and

C$\underline{H_2}$OH), 3.27-3.14 (m, 1H, C$\underline{H_2}$OH), 2.81 (br, 2H, benzyl CH_2), 1.74 (br, 1H, OH), 1.67 (br, 1H, NCHC$\underline{H_2}$), 1.36-1.23 (m, 1H, NCHC$\underline{H_2}$), 1.15-1.04 (m, 1H, cyclopropyl CH), 0.78 (br, 1H, cyclopropyl CH), 0.71-0.61 (m, 1H, cyclopropyl CH_2), -0.10--0.19 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 156.4, 144.3, 144.2, 141.5, 141.4, 138.3, 129.4, 128.4, 127.6, 127.0, 127.0, 126.4, 125.1, 119.9, 66.2, 62.6, 53.4, 47.6, 41.6, 32.4, 18.0, 13.4, 8.2; LRMS (FAB) *m/z* 428.3 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{28}H_{30}NO_3$: 428.2226 [$(M+H)^+$], found: 428.2241.

Alcohol **1-48**

Alcohol **1-48** (238 mg, 0.539 mmol, 4 steps 35%, a white solid, single isomer) was prepared from imine **1-29** (700 mg, 1.54 mmol) as described for alcohol **1-46** using phenethylmagnesium chloride (2.0 equiv, 1.0 M in THF) instead of PhMgCl. $[\alpha]^{25}_D$ -8.01 (*c* 1.27, $CHCl_3$); mp 162-163 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.75 (d, *J* = 7.4 Hz, 2H, aromatic), 7.64-7.55 (m, 2H, aromatic), 7.37 (dd, *J* = 8.0, 6.9 Hz, 2H, aromatic), 7.33-7.20 (m, 4H, aromatic), 7.20-7.10 (m, 3H, aromatic), 5.34 (br, 1H, carbamate NH), 4.57-4.40 (m, 2H, Fmoc CH_2), 4.20 (dd, *J* = 6.3, 6.3 Hz, 1H, Fmoc CH), 3.86 (br, 1H, C$\underline{H_2}$OH), 3.73 (br, 1H, NCH), 3.33-3.20 (m, 1H, C$\underline{H_2}$OH), 2.65 (br, 2H, benzyl CH_2), 1.86 (br, 1H, BnCH_2), 1.81 (br, 1H, OH), 1.67 (br, 2H, NCHC$\underline{H_2}$ and BnCH_2), 1.41-1.31 (m, 1H, NCHCH_2), 1.13-0.99 (m, 1H, cyclopropyl CH), 0.76 (br, 1H, cyclopropyl CH), 0.70-0.62 (m, 1H, cyclopropyl CH_2), -0.05--0.18 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 156.6, 144.3, 144.2, 142.0, 141.5, 141.5, 128.4, 128.4, 127.7, 127.6, 127.1, 127.0, 125.9, 125.0, 119.9, 66.1, 62.6, 52.3, 47.7, 37.3, 33.7, 32.4, 18.0, 13.2, 8.3; LRMS (FAB) *m/z* 442.29 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{29}H_{32}NO_3$: 442.2382 [$(M+H)^+$], found: 442.2366.

Alcohol **1-49**

Alcohol **1-49** (201 mg, 0.456 mmol, 4 steps 26%, a white amorphous solid, single isomer) was prepared from imine **1-29** (800 mg, 1.76 mmol) as described for alcohol **1-46** using 3-phenylpropylmagnesium bromide (3.0 equiv, 0.3 M in THF, freshly prepared from 3-phenylpropyl bromide) instead of PhMgCl. $[\alpha]^{24}_D$ -5.27 (*c* 0.42, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.74 (d, *J* = 7.4 Hz, 2H, aromatic), 7.59 (d, *J* = 7.4 Hz, 2H, aromatic), 7.37 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.31-7.18 (m, 4H, aromatic), 7.18-7.08 (m, 3H, aromatic), 5.23 (br, 1H, carbamate NH), 4.56-4.35 (m, 2H, Fmoc CH_2), 4.19 (dd, *J* = 6.3, 6.3 Hz, 1H, Fmoc CH), 3.85 (br, 1H, C$\underline{H_2}$OH), 3.69 (br, 1H, NCH), 3.31-3.18 (m, 1H, C$\underline{H_2}$OH), 2.59 (br, 2H, benzyl CH_2), 1.86 (br, 1H, OH), 1.66 (br, 2H, BnCH_2), 1.58 (br, 2H, NCHC$\underline{H_2}$ and BnCH_2C$\underline{H_2}$), 1.40 (br, 1H, BnCH_2C$\underline{H_2}$), 1.35-1.24 (m, 1H, NCHC$\underline{H_2}$), 1.11-1.00 (m, 1H, cyclopropyl CH), 0.74 (br, 1H, cyclopropyl CH), 0.69-0.60 (m, 1H, cyclopropyl CH_2), -0.10--0.18 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 156.6, 144.3, 144.2, 142.2, 141.5, 141.4, 128.5, 128.3, 127.6, 127.6, 127.1, 127.0, 125.8, 125.0, 119.9, 66.1, 62.6, 52.2, 47.7, 35.7, 35.0, 33.8, 27.6, 18.0, 13.2, 8.3; LRMS (FAB) *m/z* 456 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{30}H_{34}NO_3$: 456.2539 [$(M+H)^+$], found: 456.2523.

Alcohol **1-52**

To a solution of imine **1-29** (600 mg, 1.32 mmol) in DCM (130 ml) was added 2-(1-naphthyl)ethylmagnesium bromide (6.59 ml, 6.59 mmol, 5.0 equiv, 1.0 M in THF, freshly prepared from 2-(1-naphthyl)ethyl bromide). After 20 min at rt, the reaction mixture was poured into ice-cold sat. NH_4Cl with stirring. The resulting mixture was concentrated *in vacuo* to remove DCM and the residual aqueous solution was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 5:1-2:1) to yield sulfinamide **1-44** (dr 3:1)

as a colorless viscous oil.

To a solution of sulfinamide **1-44** in MeOH (15 ml) was added 4 M HCl/ AcOEt (1.5 ml) and the resulting mixture was stirred at rt for 2 h. The reaction mixture was concentrated *in vacuo* to give the corresponding amino alcohol as a yellow oil.

To a solution of the oil in 50% aqueous THF (15 ml) was added $NaCO_3$ (140 mg, 1.32 mmol, 1.0 equiv) and FmocOSu (534 mg, 1.58 mmol, 1.2 equiv) at rt. After 23 h at rt, the reaction mixture was concentrated *in vacuo* to remove THF and the residual aqueous solution was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was recrystallized from AcOEt/ *n*-hexane to yield alcohol **1-52** (234 mg, 0.475 mmol, 3 steps 36%, single isomer) as a colorless needle. $[\alpha]^{20}_D$ -10.25 (*c* 0.33, $CHCl_3$); mp 113-115 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.97 (d, *J* = 7.4 Hz, 1H, aromatic), 7.81 (d, *J* = 7.4 Hz, 1H, aromatic), 7.78-7.53 (m, 5H, aromatic), 7.53-7.20 (m, 8H, aromatic), 5.46 (br, 1H, carbamate NH), 4.61-4.41 (m, 2H, Fmoc CH_2), 4.20 (br, 1H, Fmoc CH), 3.85 (br, 2H, NCH and C$\underline{H_2}$OH), 3.25 (br, 1H, C$\underline{H_2}$OH), 3.13 (br, 1H, NpC$\underline{H_2}$), 3.06 (br, 1H, NpC$\underline{H_2}$), 1.99 (br, 1H, NpCH$_2$C$\underline{H_2}$), 1.88 (br, 1H, OH), 1.78 (br, 1H, NpCH$_2$C$\underline{H_2}$), 1.66 (br, 1H, NCHC$\underline{H_2}$), 1.44-1.32 (m, 1H, NCHCH$_2$),1.12-1.00 (m, 1H, cyclopropyl CH), 0.76 (br, 1H, cyclopropyl CH), 0.70-0.59 (m, 1H, cyclopropyl CH_2), -0.06--0.17 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 156.7, 144.2, 144.1, 141.5, 141.4, 138.1, 134.0, 131.9, 128.8, 127.6, 127.6, 127.1, 127.0, 126.7, 125.8, 125.8, 125.6, 125.4, 125.0, 123.6, 119.9, 66.1, 62.5, 52.7, 47.7, 36.8, 33.7, 29.5, 17.9, 13.2, 8.3; LRMS (ESI) *m/z* 514.23 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{33}H_{33}NO_3Na$: 514.2353 [(M+Na)$^+$], found: 514.2346.

Alcohol **1-53**

To a solution of imine **1-29** (600 mg, 1.32 mmol) in DCM (150 ml) was added 2-(2-naphthyl)ethylmagnesium bromide (19.8 ml, 3.95 mmol, 3.0 equiv, 0.2 M in THF, freshly prepared from 2-(2-naphthyl)ethyl bromide). After 1 h at rt, the reaction mixture was poured into ice-cold sat NH_4Cl with stirring. The resulting mixture was concentrated *in vacuo* to remove DCM and the residual aqueous solution was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 10:1-3:1) to yield sulfinamide **1-45** (dr 6:1) as a colorless oil.

To a solution of sulfinamide **1-45** in MeOH (15 ml) was added 4 M HCl/ AcOEt (1.5 ml). After 2 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amino alcohol as a yellow oil.

To a solution of the oil in 50% aqueous THF (15 ml) was added Na_2CO_3 (140 mg, 1.32 mmol, 1.0 equiv) and FmocOSu (533 mg, 1.58 mmol, 1.2 equiv). After 19 h at rt, the reaction mixture was concentrated *in vacuo* to remove THF and the residual aqueous solution was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:1) to yield alcohol **1-53** (311 mg, 0.634 mmol, 3 steps 48%, single isomer) as a white solid. $[\alpha]^{20}_D$ -11.51 (*c* 0.44, $CHCl_3$); mp 140-141 °C; ^{1}H-NMR (400 MHz, $CDCl_3$, 328 K) δ 7.83-7.65 (m, 5H, aromatic), 7.65-7.49 (m, 3H, aromatic), 7.45-7.18 (m, 7H, aromatic), 5.46 (br, 1H, carbamate NH), 4.58-4.36 (m, 2H, Fmoc CH_2), 4.16 (dd, *J* = 6.3, 6.3 Hz, 1H, Fmoc CH), 3.94-3.67 (m, 2H, C$\underline{H_2}$OH and NCH), 3.32-3.17 (m, 1H, C$\underline{H_2}$OH), 2.78 (br, 2H, NpCH$_2$), 1.94 (br, 2H, NpCH$_2$C$\underline{H_2}$ and OH), 1.76 (br, 1H, NpCH$_2$C$\underline{H_2}$), 1.64 (br, 1H, NCHC$\underline{H_2}$), 1.44-1.30 (m, 1H, NCHC$\underline{H_2}$), 1.12-0.97 (m, 1H, cyclopropyl CH), 0.75 (br, 1H, cyclopropyl CH), 0.69-0.57 (m, 1H, cyclopropyl CH_2), -0.06--0.21 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ

156.6, 144.2, 144.1, 141.5, 141.4, 139.4, 133.7, 132.1, 127.9, 127.6, 127.6, 127.4, 127.2, 127.0, 127.0, 126.3, 125.9, 125.1, 125.0, 119.9, 66.0, 62.5, 52.3, 47.6, 37.2, 33.6, 32.5, 17.9, 13.2, 8.3; LRMS (ESI) *m/z* 514.23 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{33}NO_3Na$: 514.2353 [$(M+Na)^+$], found: 514.2346.

Aldehyde **1-58**

To a solution of imine **1-29** (500 mg, 1.10 mmol) in DCM (150 ml) was added 1-naphthylmethylmagnesium chloride (19.8 ml, 3.95 mmol, 3.0 equiv, 0.2 M in diethyl ether, freshly prepared from 1-naphthylmethyl chloride). After 20 min at rt, the reaction mixture was poured into ice-cold sat. NH_4Cl with stirring. The resulting mixture was concentrated *in vacuo* and the residual aqueous solution was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 10:1-5:1-2:1) to yield sulfinamide **1-42** (dr 2:1) as a white amorphous solid.

To a solution of sulfinamide **1-42** in MeOH (15 ml) was added 4 M HCl/ AcOEt (1.5 ml). After 2 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amino alcohol as a yellow oil.

To a solution of the oil in 50% aqueous THF (15 ml) was added Na_2CO_3 (117 mg, 1.10 mmol, 1.0 equiv) and FmocOSu (445 mg, 1.32 mmol, 1.2 equiv). After 16 h at rt, the reaction mixture was concentrated *in vacuo* to remove THF and the residual aqueous solution was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:1) to yield alcohol **1-50** as a white amorphous solid.

To a solution of alcohol **1-50** in DCM (5.0 ml) was added DMP (788 mg, 1.65 mmol, 1.5 equiv). After 14 h at rt, the reaction was quenched with a solution of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (1:3), extracted with $CHCl_3$, the organic layer was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 5:1) to yield aldehyde **1-58** (105 mg, 0.220 mmol, 4 steps 20%, single isomer) as a white amorphous solid. $[\alpha]^{21}_D$ 10.37 (*c* 0.41, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 9.45 (br, 1H, aldehyde H), 8.04 (d, *J* = 8.0 Hz, 1H, aromatic), 7.88-7.01 (m, 14H, aromatic), 5.26 (br, 1H, carbamate NH), 5.12 (br, 1H, NCH), 4.46 (br, 1H, Fmoc CH_2), 4.40 (br, 1H, Fmoc CH_2), 4.13 (dd, *J* = 6.3, 5.7 Hz, 1H, Fmoc CH), 2.68 (br, 2H, $NpCH_2$), 2.03 (br, 2H, $NCHC\underline{H_2}$), 1.85 (br, 1H, cyclopropyl CH), 1.34 (br, 1H, cyclopropyl CH), 1.03 (br, 2H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 200.9, 155.8, 144.0, 143.9, 141.4, 141.3, 136.5, 132.9, 132.8, 128.4, 127.6, 127.0, 126.2, 125.6, 124.8, 124.5, 122.9, 119.9, 66.5, 52.3, 47.4, 34.1, 27.1, 21.7, 14.1, 13.9; LRMS (ESI) *m/z* 498.20 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{29}NO_3Na$: 498.2040 [$(M+Na)^+$], found: 498.2045.

Aldehyde **1-59**

To a solution of imine **1-29** (600 mg, 1.32 mmol) in DCM (130 ml) was added 2-naphthylmethylmagnesium chloride (19.8 ml, 4.00 mmol, 3.0 equiv, 0.2 M in diethyl ether, freshly prepared from 2-naphthylmethyl chloride). After 10 min at rt, the reaction mixture was poured into ice-cold sat. NH_4Cl and the resulting mixture was concentrated *in vacuo* to remove DCM. The residual aqueous solution was extracted with AcOEt and the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield sulfinamide **1-43** (dr 4:3) as a yellow oil.

To a solution of sulfinamide **1-43** in MeOH (15 ml) was added 4 M HCl/ AcOEt (1.5 ml). After 3 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amino alcohol as a yellow oil.

To a solution of the oil in 50% aqueous THF (15 ml) was added Na_2CO_3 (140 mg, 1.32 mmol, 1.0 equiv) and FmocOSu (534 mg, 1.58 mmol, 1.2 equiv). After 18 h at rt, the reaction mixture was concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with AcOEt, the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:1) to yield alcohol **1-51** as a colorless viscous oil.

To a solution of alcohol **1-51** in DCM (10 ml) was added DMP (942 mg, 1.98 mmol, 1.5 equiv). After 14 h at rt, the reaction was quenched with a solution of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (1:3), extracted with $CHCl_3$, the organic layer was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 7:1) to yield aldehyde **1-59** (126 mg, 0.264 mmol, 4 steps 20%, single isomer) as a white amorphous solid. $[\alpha]^{21}_D$ -9.62 (*c* 0.45, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, 328 K) δ 9.52 (br, 1H, aldehyde H), 8.13-7.14 (m, 15H, aromatic), 5.59 (br, 1H, carbamate NH), 5.43 (br, 1H, NCH), 4.66-4.27 (m, 2H, Fmoc CH_2), 4.14 (dd, *J* = 6.3, 6.3 Hz, 1H, Fmoc CH), 2.59 (br, 2H, NpCH_2), 2.37 (br, 2H, NCHC$\underline{H_2}$), 1.85 (br, 1H, cyclopropyl CH), 1.37 (br, 1H, cyclopropyl CH), 0.98 (br, 1H, cyclopropyl CH_2), 0.94 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 201.2, 156.1, 144.0, 143.9, 141.4, 141.4, 134.0, 133.7, 133.5, 131.0, 129.7, 129.5, 128.2, 127.6, 127.0, 127.0, 126.3, 124.9, 124.7, 123.6, 119.9, 66.6, 52.2, 47.4, 33.2, 27.3, 22.5, 21.2, 14.0; LRMS (ESI) *m/z* 498.20 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{29}NO_3Na$: 498.2040 [$(M+Na)^+$], found: 498.2046.

Diprotected amine **1-62**

To a solution of alcohol **1-46** (314 mg, 0.760 mmol) in DCM (10 ml) was added DMP (645 mg, 1.52 mmol, 2.0 equiv) and stirred at rt for 2 h. The reaction was quenched with a solution of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (1:3), extracted with $CHCl_3$, the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding aldehyde as a white solid.

To a suspension of the aldehyde in 80% aqueous *t*-BuOH (10 ml) was added THF (8.0 ml), NaH_2PO_4-$2H_2O$ (178 mg, 1.14 mmol, 1.5 equiv), 2-methyl-2-butene (644 μl, 6.08 mmol, 8.0 equiv) and $NaClO_2$ (275 mg, 3.04 mmol, 4.0 equiv). After 5 h at rt, the reaction mixture was diluted with AcOEt, washed with 1M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a yellow oil.

To a solution of the carboxylic acid in DCM (10 ml) was added triethylamine (414 μl, 3.04 mmol, 4.0 equiv) and DPPA (491 μl, 2.28 mmol, 3.0 equiv) at 0 °C. After 20 min at 0 °C, the reaction mixture was warmed to rt and stirred for 15 h. The reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 5:1) to yield the corresponding acyl azide as a white solid.

A solution of the acyl azide in *t*-BuOH (10 ml) was refluxed for 17 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 4:1) to yield diprotected amine **1-62** (265 mg, 0.532 mmol, 4 steps 70%) as a white solid. $[\alpha]^{24}_D$ -38.03 (*c* 0.96, $CHCl_3$); mp 154-155 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.82-7.56 (m, 4H, aromatic), 7.39-7.17 (m, 9H, aromatic), 6.82 (br, 1H, carbamate NH), 4.76 (br, 2H, carbamate NH and NCH), 4.35 (br, 2H, Fmoc CH_2), 4.19 (br, 1H, Fmoc CH), 2.66 (br, 1H, cyclopropyl CH), 1.85 (br, 1H, NCHC$\underline{H_2}$), 1.68 (br, 1H, NCHC$\underline{H_2}$), 1.50 (s, 9H, CCH_3), 0.88 (br, 2H, cyclopropyl CH and cyclopropyl CH_2), 0.05 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 157.3, 156.3, 144.2, 143.7, 141.3, 128.5, 127.5, 127.1, 127.0, 126.1, 125.3, 119.8, 79.9, 66.6, 55.8, 47.5, 34.0, 28.4, 27.8, 15.4, 11.3; LRMS

(FAB) *m/z* 499.3 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{31}H_{35}N_2O_4$: 499.2597 [$(M+H)^+$], found: 499.2589.

Diprotected amine **1-63**

Diprotected amine **1-63** (186 mg, 0.363 mmol, 4 steps 74%, a white solid) was prepared from alcohol **1-47** (210 mg, 0.491 mmol) as described for diprotected amine **1-62**. $[\alpha]^{25}_D$ -34.19 (*c* 1.39, $CHCl_3$); mp 105-106 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.74 (d, *J* = 8.0 Hz, 2H, aromatic), 7.63 (br, 2H, aromatic), 7.37 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.31-7.21 (m, 4H, aromatic), 7.21-7.08 (m, 3H, aromatic), 5.95 (br, 1H, carbamate NH), 4.69 (br, 1H, carbamate NH), 4.42 (br, 1H, Fmoc CH_2), 4.34 (br, 1H, Fmoc CH_2), 4.21 (dd, *J* = 7.4, 6.9 Hz, 1H, Fmoc CH), 3.95 (br, 1H, NCH), 2.87 (br, 1H, benzyl CH_2), 2.75 (br, 1H, benzyl CH_2), 2.61 (br, 1H, cyclopropyl CH), 1.48-1.32 (m, 11H, NCHC$\underline{H}_2$ and CCH_3), 0.94-0.74 (m, 2H, cyclopropyl CH and cyclopropyl CH_2), 0.10-0.01 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 157.1, 156.4, 144.3, 144.3, 141.4, 141.4, 138.2, 129.5, 128.3, 127.6, 127.0, 126.3, 125.3, 119.9, 79.7, 66.4, 52.8, 47.6, 41.9, 31.0, 28.4, 27.7, 15.2, 11.5; LRMS (FAB) *m/z* 513.3 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{32}H_{37}N_2O_4$: 513.2753 [$(M+H)^+$], found: 513.2747.

Diprotected amine **1-64**

Diprotected amine **1-64** (162 mg, 0.308 mmol, 4 steps 70%, a white solid) was prepared from alcohol **1-48** (194 mg, 0.439 mmol) as described for diprotected amine **1-62**. $[\alpha]^{24}_D$ -30.76 (*c* 1.50, $CHCl_3$); mp 116-118 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.74 (d, *J* = 7.4 Hz, 2H, aromatic), 7.65 (br, 2H, aromatic), 7.36 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.32-7.20 (m, 4H, aromatic), 7.20-7.08 (m, 3H, aromatic), 5.73 (br, 1H, carbamate NH), 4.73 (br, 1H, carbamate NH), 4.42 (br, 2H, Fmoc CH_2), 4.22 (dd, *J* = 6.9, 6.9 Hz, 1H, Fmoc CH), 3.78 (br, 1H, NCH), 2.63 (br, 2H, benzyl CH_2), 2.61 (br, 1H, cyclopropyl CH), 1.83 (br, 1H, $BnCH_2$), 1.75 (br, 1H, $BnCH_2$), 1.63-1.38 (m, 11H, NCHC$\underline{H}_2$ and CCH_3), 0.87 (br, 1H, cyclopropyl CH_2), 0.82 (br, 1H, cyclopropyl CH), 0.08 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 157.1, 156.6, 144.2, 142.0, 141.4, 128.4, 128.3, 127.6, 127.0, 125.8, 125.2, 119.9, 79.7, 66.3, 51.4, 47.6, 37.6, 32.2, 32.0, 28.4, 27.5, 14.9, 11.7; LRMS (FAB) *m/z* 527.3 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{33}H_{39}N_2O_4$: 527.2910 [$(M+H)^+$], found: 527.2913.

Diprotected amine **1-65**

Diprotected amine **1-65** (125 mg, 0.231 mmol, 4 steps 62%, a white solid) was prepared from alcohol **1-49** (169 mg, 0.372 mmol) as described for diprotected amine **1-62**. $[\alpha]^{25}_D$ -24.14 (*c* 1.48, $CHCl_3$); mp 98-99 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.74 (d, *J* = 7.4 Hz, 2H, aromatic), 7.64 (d, *J* = 6.3 Hz, 2H, aromatic), 7.36 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.31-7.21 (m, 4H, aromatic), 7.18-7.10 (m, 3H, aromatic), 5.62 (br, 1H, carbamate NH), 4.72 (br, 1H, carbamate NH), 4.41 (br, 2H, Fmoc CH_2), 4.22 (dd, *J* = 6.9, 6.9 Hz, 1H, Fmoc CH), 3.75 (br, 1H, NCH), 2.60 (br, 3H, cyclopropyl CH and benzyl CH_2), 1.66 (br, 2H, $BnCH_2$), 1.61-1.36 (m, 13H, $BnCH_2C\underline{H}_2$, NCHC$\underline{H}_2$ and CCH_3), 0.87 (br, 1H, cyclopropyl CH_2), 0.80 (br, 1H, cyclopropyl CH), 0.07 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 157.1, 156.5, 144.3, 142.3, 141.5, 128.4, 128.3, 127.6, 127.0, 125.8, 125.3, 119.9, 79.7, 66.3, 51.4, 47.6, 35.8, 35.4, 32.1, 28.4, 27.5, 14.9, 11.7; LRMS (FAB) *m/z* 541 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{34}H_{41}N_2O_4$: 541.3066 [$(M+H)^+$], found: 541.3088.

Diprotected amine **1-66**

To a suspension of aldehyde **1-58** (102 mg, 0.214 mmol) in 80% aqueous *t*-BuOH (2.0 ml) was added THF (1.6 ml),

NaH_2PO_4-$2H_2O$ (50.1 mg, 0.321 mmol, 1.5 equiv), 2-methyl-2-butene (182 µl, 1.71 mmol, 8.0 equiv) and $NaClO_2$ (77.5 mg, 0.856 mmol, 4.0 equiv). After 2 h at rt, the reaction mixture was diluted with AcOEt, washed with 1 M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a white solid.

To a solution of the solid in DCM (5.0 ml) was added triethylamine (60 µl, 0.428 mmol, 2.0 equiv) and DPPA (138 µl, 0.642 mmol, 3.0 equiv) at 0 °C. After 20 min at 0 °C, the reaction mixture was warmed to rt and stirred for 5 h. The reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 5:1) to yield the correspoinding acyl azide as a white solid.

A solution of the solid in *t*-BuOH (10 ml) was heated under reflux for 20 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (DCM/ MeOH 99:1-9:1) to yield diprotected amine **1-66** (77.1 mg, 0.137 mmol, 3 steps 64%) as a white solid. $[\alpha]^{20}_D$ -9.75 (*c* 0.38, $CHCl_3$); mp 173-174 °C; ^{1}H-NMR (400 MHz, $CDCl_3$, 328 K) δ 8.05 (d, *J* = 8.2 Hz, 1H, aromatic), 7.86-7.08 (m, 15H, aromatic and carbamate NH), 5.32 (br, 1H, NCH), 4.76 (br, 1H, carbamate NH), 4.33 (br, 2H, Fmoc CH_2), 4.19 (br, 1H, Fmoc CH), 2.75 (br, 2H, NpCH_2), 2.70 (br, 1H, cyclopropyl CH), 1.87 (br, 1H, NCHC$\underline{H}_2$), 1.67 (br, 1H, NCHC$\underline{H}_2$), 1.54 (s, 9H, CCH_3), 1.02-0.79 (br, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.12-0.00 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 157.4, 156.4, 144.2, 141.3, 138.5, 133.0, 132.8, 130.1, 128.4, 127.5, 126.9, 126.7, 126.0, 125.3, 125.2, 124.3, 123.3, 119.8, 66.6, 52.7, 47.5, 33.0, 28.4, 28.0, 15.6, 13.7, 11.2; LRMS (ESI) *m/z* 585.27 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{36}H_{38}N_2O_4Na$: 585.2724 [$(M+Na)^+$], found: 585.2717.

Diprotected amine **1-67**

Diprotected amine **1-67** (83.1 mg, 0.169 mmol, 3 steps 64%, a colorless viscous oil) was prepared from aldehyde **1-59** (126 mg, 0.264 mmol) as described for diprotected amine **1-66**. $[\alpha]^{20}_D$ 1.08 (*c* 0.29, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, 328 K) δ 8.20 (d, *J* = 7.7 Hz, 1H, aromatic), 7.87-7.09 (m, 14H, aromatic), 6.78 (br, 1H, carbamate NH), 5.62 (br, 1H, NCH), 5.11 (br, 1H, carbamate NH), 4.33 (br, 2H, Fmoc CH_2), 4.16 (br, 1H, Fmoc CH), 2.73 (br, 1H, cyclopropyl CH), 2.64 (br, 2H, NpCH_2), 2.17 (br, 2H, NCHCH_2), 1.54 (s, 9H, CCH_3), 0.92 (br, 1H, cyclopropyl CH), 0.86 (br, 1H, cyclopropyl CH_2), 0.02 (br, 1H, cyclopropyl CH); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 157.6, 156.6, 144.2, 144.2, 141.3, 134.7, 133.8, 133.6, 131.1, 129.8, 129.3, 127.8, 127.5, 126.9, 126.9, 125.8, 125.3, 125.3, 124.4, 124.0, 119.8, 66.7, 52.8, 47.5, 32.1, 28.5, 28.3, 21.3, 16.2, 11.3; LRMS (ESI) *m/z* 585.27 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{36}H_{38}N_2O_4Na$: 585.2724 [$(M+Na)^+$], found: 585.2720.

Diprotected amine **1-68**

To a solution of alcohol **1-52** (232 mg, 0.471 mmol) in DCM (5.0 ml) was added DMP (300 mg, 0.707 mmol, 1.5 equiv). After 2 h at rt, the reaction was quenched with a solution of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (1:3), extracted with $CHCl_3$, the organic layer was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding aldehyde as a white solid.

To a suspension of the solid in 80% aqueous *t*-BuOH (5.0 ml) was added THF (4.0 ml), NaH_2PO_4-$2H_2O$ (110 mg, 0.707 mmol, 1.5 equiv), 2-methyl-2-butene (400 µl, 3.77 mmol, 8.0 equiv) and $NaClO_2$ (171 mg, 1.89 mmol, 4.0 equiv). After 2 h at rt, the reaction mixture was diluted with AcOEt, washed with 1 M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a white solid.

To a solution of the solid in DCM (5.0 ml) was added triethylamine (131 µl, 0.943 mmol, 2.0 equiv) and DPPA (305 µl, 1.41 mmol, 3.0 equiv) at 0 °C. After 20 min at 0 °C, the reaction mixture was warmed to rt and stirred for 4 h. The reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt/ DCM 5:1:0-0:0:1) to yield the corresponding acyl azide as a white solid.

A solution of the solid in *t*-BuOH (10 ml) was heated under reflux for 20 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (DCM/ MeOH 99:1) to yield diprotected amine **1-68** (160 mg, 0.278 mmol, 4 steps 59%) as a white solid. $[\alpha]^{20}_D$ -10.35 (*c* 0.34, $CHCl_3$); mp 133-134 °C; ^{1}H-NMR (400 MHz, $CDCl_3$, 328 K) δ 7.97 (d, *J* = 6.3 Hz, 1H, aromatic), 7.88-7.54 (m, 6H, aromatic), 7.54-7.16 (m, 8H, aromatic), 5.88 (br, 1H, carbamate NH), 4.73 (br, 1H, carbamate NH), 4.45 (br, 2H, Fmoc CH_2), 4.24 (br, 1H, Fmoc CH), 3.90 (br, 1H, NCH), 3.10 (br, 2H, NpCH_2), 2.61 (br, 1H, cyclopropyl CH), 1.96 (br, 1H, Np$CH_2C\underline{H}_2$), 1.83 (br, 1H, Np$CH_2C\underline{H}_2$), 1.55 (br, 2H, NCHC$\underline{H}_2$), 1.46 (s, 9H, CCH_3), 0.86 (br, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.08 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 157.1, 156.7, 144.2, 141.4, 138.1, 134.0, 131.8, 128.8, 127.6, 127.0, 126.6, 125.9, 125.8, 125.6, 125.4, 125.2, 123.6, 119.9, 79.7, 66.4, 51.8, 47.6, 37.2, 32.0, 29.4, 28.4, 27.6, 14.9, 11.6; LRMS (ESI) *m/z* 599.29 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{37}H_{40}N_2O_4Na$: 599.2880 [$(M+Na)^+$], found: 599.2874.

Diprotected amine **1-69**

Diprotected amine **1-69** (188 mg, 0.325 mmol, 4 steps 52%, a white solid) was prepared from alcohol **1-53** (307 mg, 0.625 mmol) as described for diprotected amine **1-68**. $[\alpha]^{21}_D$ -21.38 (*c* 0.37, $CHCl_3$); mp 172-173 °C; ^{1}H-NMR (400 MHz, $CDCl_3$, 328 K) δ 7.82-7.18 (m, 15H, aromatic), 5.80 (br, 1H, carbamate NH), 4.73 (br, 1H, carbamate NH), 4.42 (br, 2H, Fmoc CH_2), 4.21 (dd, *J* = 6.8, 6.8 Hz, 1H, Fmoc CH), 3.83 (br, 1H, NCH), 2.80 (br, 2H, NpCH_2), 2.60 (br, 1H, cyclopropyl CH), 1.91 (br, 1H, Np$CH_2C\underline{H}_2$), 1.83 (br, 1H, Np$CH_2C\underline{H}_2$), 1.68-1.34 (m, 11H, NCHC$\underline{H}_2$ and CCH_3), 0.86 (br, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.08 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 157.1, 156.6, 144.2, 141.4, 139.5, 133.8, 132.1, 127.9, 127.6, 127.6, 127.4, 127.2, 127.0, 126.3, 125.9, 125.2, 125.1, 119.9, 79.7, 66.3, 51.5, 47.6, 37.5, 32.4, 32.0, 28.4, 27.5, 14.9, 11.7; LRMS (ESI) *m/z* 599.29 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{37}H_{40}N_2O_4Na$: 599.2880 [$(M+Na)^+$], found: 599.2873.

Carbamate **1-70**

To a solution of Cbz-Ala (194 mg, 0.867 mmol, 3.0 equiv) in DCM (10 ml) was added triethylamine (121 µl, 0.867 mmol, 3.0 equiv) and PivCl (107 µl, 0.867 mmol, 3.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding acid anhydride as a colorless oil.

To a solution of diprotected amine **1-62** (144 mg, 0.289 mmol, 1.0 equiv) in MeOH (5 ml) was added K_2CO_3 (39.9 mg, 0.289 mmol, 1.0 equiv). After 23 h at rt, the reaction mixture was concentrated *in vacuo* and the crude product was purified by NH-silica gel column chromatography (*n*-hexane/ AcOEt 1:1) to yield the corresponding amine as a white solid.

To a solution of the amine in DCM (2.5 ml) was added triethylamine (121 µl, 0.716 mmol, 3.0 equiv) and a solution of the aforementioned acid anhydride in DCM (1.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 17 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine,

dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:2) to yield carbamate **1-70** (103 mg, 0.214 mmol, 2 steps 74%) as a white solid. $[\alpha]^{23}_D$ -59.69 (*c* 0.42, $CHCl_3$); mp 132 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 8.17 (br, 1H, amide NH), 7.38-7.15 (m, 10H, aromatic), 5.73 (br, 1H, Ala NH), 5.10 (s, 2H, Cbz CH_2), 5.05-4.97 (m, 1H, NCH), 4.73 (br, 1H, carbamate NH), 4.47 (br, 1H, Ala α-H), 2.64 (br, 1H, cyclopropyl CH), 2.06-1.94 (m, 1H, NCHC$\underline{H}_2$), 1.58-1.37 (m, 13H, NCHC$\underline{H}_2$ (1H), Ala CH_3 and CCH_3), 0.95-0.78 (m, 2H, cyclopropyl CH and cyclopropyl CH_2), 0.06-0.00 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 172.5, 157.6, 155.8, 143.5, 136.8, 128.5, 128.4, 128.0, 128.0, 127.0, 125.9, 80.5, 66.7, 53.7, 50.7, 33.1, 28.4, 28.1, 19.6, 16.0, 10.7; LRMS (FAB) *m/z* 482.3 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{27}H_{36}N_3O_5$: 482.2655 [$(M+H)^+$], found: 482.2644.

Carbamate **1-71**

Carbamate **1-71** (97.9 mg, 0.198 mmol, 2 steps 70%, a white solid) was prepared from diprotected amine **1-63** (145 mg, 0.282 mmol) as described for carbamate **1-70**. $[\alpha]^{22}_D$ -38.69 (*c* 0.43, $CHCl_3$); mp 123-124 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.41-7.11 (m, 11H, aromatic and amide NH), 5.74 (br, 1H, Ala NH), 5.16-5.05 (m, 2H, Cbz CH_2), 4.66 (br, 1H, carbamate NH), 4.37 (br, 1H, Ala α-H), 4.27-4.16 (m, 1H, NCH), 2.85 (dd, *J* = 13.7, 5.2 Hz, 1H, benzyl CH_2), 2.68 (dd, *J* = 13.7, 7.4 Hz, 1H, benzyl CH_2), 2.54 (br, 1H, cyclopropyl CH), 1.76-1.63 (m, 1H, NCHC$\underline{H}_2$), 1.48 (s, 3H, CCH_3), 1.44-1.30 (m, 9H, CCH_3 (6H) and Ala CH_3), 1.20 (br, 1H, NCHC$\underline{H}_2$), 0.88-0.78 (m, 1H, cyclopropyl CH_2), 0.63 (br, 1H, cyclopropyl CH), 0.04--0.06 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 172.7, 157.4, 155.7, 138.1, 136.8, 129.6, 128.4, 128.3, 128.1, 128.0, 126.3, 80.3, 66.7, 50.8, 50.8, 41.9, 29.5, 28.3, 28.0, 19.7, 15.7, 10.7; LRMS (FAB) *m/z* 496 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{28}H_{38}N_3O_5$: 496.2811 [$(M+H)^+$], found: 496.2808.

Carbamate **1-72**

Carbamate **1-72** (99.9 mg, 0.196 mmol, 2 steps 84%, a white solid) was prepared from diprotected amine **1-64** (123 mg, 0.233 mmol) as described for carbamate **1-70**. $[\alpha]^{23}_D$ -26.27 (*c* 0.47, $CHCl_3$); mp 102-103 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.38-7.11 (m, 11H, aromatic and amide NH), 5.76 (br, 1H, Ala NH), 5.10 (s, 2H, Cbz CH_2), 4.71 (br, 1H, carbamate NH), 4.44-4.33 (m, 1H, Ala α-H), 4.15-4.04 (m, 1H, NCH), 2.66-2.52 (m, 3H, cyclopropyl CH and benzyl CH_2), 1.85-1.62 (m, 3H, NCHC$\underline{H}_2$ (1H) and BnCH_2), 1.49 (s, 3H, CCH_3), 1.47-1.35 (m, 9H, CCH_3 (6H) and Ala CH_3), 1.35-1.19 (m, 1H, NCHC$\underline{H}_2$), 0.92-0.81 (m, 1H, cyclopropyl CH_2), 0.79-0.68 (m, 1H, cyclopropyl CH), 0.06--0.02 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 172.8, 157.4, 155.8, 142.1, 136.8, 128.4, 128.4, 128.4, 128.1, 128.0, 125.8, 80.3, 66.7, 51.0, 49.1, 38.0, 32.1, 30.5, 28.4, 28.0, 19.7, 15.5, 10.7; LRMS (FAB) *m/z* 510 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{29}H_{40}N_3O_5$: 510.2968 [$(M+H)^+$], found: 510.2979.

Carbamate **1-73**

Carbamate **1-73** (86.9 mg, 0.166 mmol, 2 steps 88%, a white solid) was prepared from diprotected amine **1-73** (102 mg, 0.189 mmol) as described for carbamate **1-74**. $[\alpha]^{23}_D$ -26.27 (*c* 0.47, $CHCl_3$); mp 106-107 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.36-7.19 (m, 8H, aromatic and amide NH), 7.17-7.10 (m, 3H, aromatic), 5.76 (br, 1H, carbamate NH), 5.09 (s, 2H, Cbz CH_2), 4.69 (br, 1H, carbamate NH), 4.42-4.31 (m, 1H, Ala α-H), 4.11-4.00 (m, 1H, NCH), 2.66-2.50 (m, 3H, benzyl CH_2 and cyclopropyl CH), 1.79-1.65 (m, 1H, NCHC$\underline{H}_2$), 1.65-1.56 (m, 2H, BnCH_2), 1.56-1.46 (m, 1H, BnCH_2CH_2), 1.46-1.31 (m, 13H, CCH_3, Ala CH_3 and Bn$CH_2C\underline{H}_2$ (1H)), 1.21 (br, 1H, NCHC$\underline{H}_2$),

0.93-0.79 (m, 1H, cyclopropyl CH_2), 0.76-0.65 (m, 1H, cyclopropyl CH), 0.05--0.05 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 172.7, 157.4, 155.8, 142.3, 136.8, 128.4, 128.3, 128.2, 128.0, 128.0, 125.7, 80.3, 66.7, 51.0, 49.0, 35.8, 36.0, 30.4, 28.3, 28.0, 27.3, 19.7, 15.4, 10.8; LRMS (FAB) *m/z* 524 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{30}H_{42}N_3O_5$: 524.3124 [$(M+H)^+$], found: 524.3121.

Carbamate **1-74**

To a solution of Cbz-Ala (91.7 mg, 0.411 mmol, 3.0 equiv) in DCM (30 ml) was added triethylamine (57.2 μl, 0.411 mmol, 3.0 equiv) and PivCl (50.6 μl, 0.411 mmol, 3.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding acid anhydride as a colorless oil.

To a suspension of diprotected amine **1-66** (77.0 mg, 0.137 mmol, 1.0 equiv) in MeOH (10 ml) was added THF (6.0 ml) and K_2CO_3 (22.7 mg, 0.164 mmol, 1.2 equiv). After 17 h at rt, the reaction mixture was concentrated *in vacuo*. The crude product was purified by NH-silica gel column chromatography (*n*-hexane/ AcOEt 4:1-0:1) to yield the corresponding amine as a white solid.

To a solution of the solid in DCM (1.0 ml) was added triethylamine (57.2 μl, 0.411 mmol, 3.0 equiv) and a solution of the aforementioned acid anhydride in DCM (500 μl) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 16 h. The reaction mixture was diluted with AcOEt, washed with 1M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 2:1) to yield carbamate **1-74** (218 mg, 0.399 mmol, 2 steps 97%) as a colorless viscous oil. $[\alpha]^{22}_D$ -51.68 (*c* 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 8.51 (br, 1H, amide NH), 8.05 (d, *J* = 8.6 Hz, 1H, aromatic), 7.73 (d, *J* = 8.6 Hz, 1H, aromatic), 7.59 (d, *J* = 8.6 Hz, 1H, aromatic), 7.52-7.44 (m, 1H, aromatic), 7.44-7.37 (m, 1H, aromatic), 7.37-7.18 (m, 7H, aromatic), 5.72 (br, 1H, Ala NH), 5.55-5.46 (m, 1H, NCH), 5.09 (s, 2H, Cbz CH_2), 4.78 (br, 1H, carbamate NH), 4.49 (br, 1H, Ala α-H), 2.76 (br, 2H, $NpCH_2$), 2.67 (br, 1H, cyclopropyl CH), 2.02-1.90 (m, 1H, NCHC$\underline{H}_2$), 1.52 (br, 10H, NCHC$\underline{H}_2$ (1H) and CCH_3), 1.41 (d, *J* = 7.4 Hz, 3H, Ala CH_3), 0.90 (br, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.02 (br, 1H, cyclopropyl CH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.4, 157.7, 155.6, 138.2, 136.6, 132.8, 132.5, 130.0, 128.4, 128.2, 128.0, 127.9, 126.5, 125.9, 125.1, 124.3, 123.0, 80.6, 66.5, 50.8, 50.2, 31.6, 28.3, 28.1, 19.9, 16.1, 13.7, 10.4; LRMS (ESI) *m/z* 568.28 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{39}N_3O_5Na$: 568.2782 [$(M+Na)^+$], found: 568.2778.

Carbamate **1-75**

Carbamate **1-75** (64.2 mg, 0.118 mmol, 2 steps 89%, a colorless viscous oil) was prepared from diprotected amine **1-67** (74.4 mg, 0.132 mmol) as described for carbamate **1-74**. $[\alpha]^{22}_D$ -37.61 (*c* 1.02, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 8.26 (br, 1H, amide NH), 8.11 (br, 1H, aromatic), 7.73 (d, *J* = 9.2 Hz, 1H, aromatic), 7.61 (d, *J* = 8.0 Hz, 1H, aromatic), 7.43-7.17 (m, 9H, aromatic), 5.77 (br, 1H, NCH), 5.71 (br, 1H, Ala NH), 5.05 (s, 2H, Cbz CH_2), 5.01 (br, 1H, carbamate NH), 4.47 (br, 1H, Ala α-H), 2.72 (br, 1H, cyclopropyl CH), 2.64 (br, 2H, $NpCH_2$), 2.18-2.00 (m, 2H, NCHC$\underline{H}_2$), 1.55 (s, 9H, CCH_3), 1.32 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 0.91 (br, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.03 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 172.6, 158.0, 155.7, 136.8, 134.5, 133.7, 133.5, 131.1, 129.9, 129.2, 128.5, 128.4, 127.9, 127.7, 125.6, 124.3, 123.8, 80.6, 66.6, 51.5, 50.5, 30.6, 28.8, 28.6, 21.2, 19.5, 16.9, 10.4; LRMS (ESI) *m/z* 568.28 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{39}N_3O_5Na$: 568.2782 [$(M+Na)^+$], found: 568.2776.

Carbamate **1-76**

Carbamate **1-76** (75.3 mg, 0.135 mmol, 2 steps 94%, a white solid) was prepared from diprotected amine **1-68** (82.6 mg, 0.143 mmol) as described for carbamate **1-74**. $[\alpha]^{22}_{D}$ -30.66 (*c* 1.34, $CHCl_3$); mp 162-163 °C; ^{1}H-NMR (400 MHz, $CDCl_3$, 328 K) δ 7.96 (d, *J* = 8.2 Hz, 1H, aromatic), 7.86-7.76 (m, 1H, aromatic), 7.67 (d, *J* = 8.2 Hz, 1H, aromatic), 7.57-7.18 (m, 9H, aromatic), 5.83 (br, 1H, Ala NH), 5.11 (s, 2H, Cbz CH_2), 4.75 (br, 1H, carbamate NH), 4.52-4.37 (m, 1H, Ala α-H), 4.29-4.15 (m, 1H, NCH), 3.17-2.95 (m, 2H, $NpCH_2$), 2.58 (br, 1H, cyclopropyl CH), 1.99-1.86 (m, 1H, $NpCH_2C\underline{H}_2$), 1.86-1.70 (m, 2H, $NpCH_2C\underline{H}_2$ and $NCHC\underline{H}_2$), 1.49 (d, *J* = 7.2 Hz, 3H, Ala CH_3), 1.45-1.18 (m, 10H, $NCHC\underline{H}_2$ (1H) and CCH_3), 0.94-0.82 (m, 1H, cyclopropyl CH_2), 0.82-0.69 (m, 1H, cyclopropyl CH), 0.10-0.00 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 173.0, 157.5, 155.8, 138.2, 136.7, 134.0, 131.9, 128.8, 128.4, 128.0, 128.0, 126.6, 125.9, 125.8, 125.6, 125.4, 123.7, 80.3, 66.7, 51.1, 49.4, 37.5, 30.5, 29.3, 28.3, 28.0, 19.7, 15.5, 10.7; LRMS (ESI) *m/z* 582.29 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{33}H_{41}N_3O_5Na$: 582.2938 $[(M+Na)^+]$, found: 582.2934.

Carbamate **1-77**

Carbamate **1-77** (194 mg, 0.347 mmol, 2 steps 98%, a white solid) was prepared from diprotected amine **1-69** (204 mg, 0.354 mmol) as described for carbamate **1-74**. $[\alpha]^{22}_{D}$ -25.90 (*c* 0.43, $CHCl_3$); mp 133-135 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.80-7.70 (m, 3H, aromatic), 7.58 (br, 1H, aromatic), 7.46-7.21 (m, 9H, amide NH and aromatic), 5.83 (br, 1H, Ala NH), 5.10 (s, 2H, Cbz CH_2), 4.81 (br, 1H, carbamate NH), 4.41 (br, 1H, Ala α-H), 4.14 (br, 1H, NCH), 2.85-2.71 (m, 2H, $NpCH_2$), 2.57 (br, 1H, cyclopropyl CH), 1.94-1.82 (m, 1H, $NpCH_2C\underline{H}_2$), 1.82-1.70 (m, 2H, $NpCH_2C\underline{H}_2$ and $NCHC\underline{H}_2$), 1.45 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.41 (s, 9H, CCH_3), 1.36-1.22 (m, 1H, $NCHC\underline{H}_2$), 0.93-0.80 (m, 1H, cyclopropyl CH_2), 0.74 (br, 1H, cyclopropyl CH), 0.08--0.01 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 172.9, 157.4, 155.8, 139.5, 136.7, 133.7, 132.1, 128.4, 128.0, 127.9, 127.9, 127.6, 127.4, 127.2, 126.3, 125.8, 125.1, 80.2, 66.7, 51.0, 49.1, 37.8, 32.2, 30.4, 28.3, 27.9, 19.6, 15.4, 10.7; LRMS (ESI) *m/z* 582.29 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{33}H_{41}N_3O_5Na$: 582.2938 $[(M+Na)^+]$, found: 582.2935.

Target compound **1-16**

To a solution of carbamate **1-70** (92.9 mg, 0.193 mmol, 1.0 equiv) in DCM (3.0 ml) was added TFA (3.0 ml). After 10 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a brown oil.

To a solution of β-lactone unit **1-35** (66.5 mg, 0.386 mmol, 2.0 equiv) in DCM (4.0 ml) was added triethylamine (54 µl, 0.386 mmol, 2.0 equiv) and PivCl (48 µl, 0.386 mmol, 2.0 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (5.0 ml) was added triethylamine (81 µl, 0.579 mmol, 3.0 equiv) and a solution of the acid anhydride in DCM (4.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 20 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 5:4) to yield target compound **1-16** (103 mg, 0.193 mmol, 2 steps 100%) as a white solid. $[\alpha]^{19}_{D}$ -23.70 (*c* 1.44, $CHCl_3$); mp 87-89 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.2 Hz, 1H, amide NH), 7.42-7.17 (m, 10H, aromatic), 7.14 (br, 1H, amide NH), 5.73 (d, *J* = 7.7 Hz, 1H, Ala NH), 5.07 (s, 2H, Cbz CH_2), 4.93-4.82 (m, 1H, NCH), 4.67 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.45-4.33 (m, 1H, Ala α-H), 3.61 (dd, *J* = 7.7, 4.5

Hz, 1H, OCOCH), 2.80 (br, 1H, cyclopropyl CH), 2.12-1.89 (m, 2H, NCHC$\underline{H}_2$ and C$\underline{H}$CH$_3$), 1.71-1.57 (m, 1H, C$\underline{H}_2$CH$_3$), 1.47-1.21 (m, 5H, Ala CH$_3$, NCHC$\underline{H}_2$ and C$\underline{H}_2$CH$_3$), 1.05 (d, *J* = 6.8 Hz, 3H, CHC$\underline{H}_3$), 1.01-0.80 (m, 5H, cyclopropyl CH$_2$ (1H), cyclopropyl CH and CH$_2$C$\underline{H}_3$), 0.30-0.15 (m, 1H, cyclopropyl CH$_2$); ^{13}C-NMR (100 MHz, CDCl$_3$) δ 172.3, 170.4, 169.2, 155.8, 141.8, 136.4, 128.7, 128.4, 128.0, 127.8, 127.5, 126.1, 70.8, 66.6, 62.8, 53.9, 50.3, 33.9, 33.6, 27.1, 26.5, 19.0, 16.3, 14.8, 11.0, 10.9; LRMS (ESI) *m/z* 558.26 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{30}H_{37}N_3O_6Na$: 558.2575 [(M+Na)$^+$], found: 558.2568.

Target compound **1-17**

Target compound **1-17** (97.1 mg, 0.177 mmol, 2 steps 100%, a white solid) was prepared from carbamate **1-71** (87.5 mg, 0.177 mmol) as described for target compound **1-16**. $[\alpha]^{19}_D$ -39.33 (*c* 1.05, CHCl$_3$); mp 79-82 °C; ^{1}H-NMR (400 MHz, CDCl$_3$) δ 7.42-7.02 (m, 10 H, aromatic), 6.99 (br, 1H, amide NH), 6.91 (d, *J* = 7.2 Hz, 1H, amide NH), 5.77 (d, *J* = 7.2 Hz, 1H, Ala α-H), 5.19-4.99 (m, 2H, Cbz CH$_2$), 4.57 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.39-4.20 (m, 1H, Ala α-H), 4.20-4.07 (m, 1H, NCH), 3.22 (dd, *J* = 7.2, 4.5 Hz, 1H, OCOCH), 3.01-2.82 (m, 1H, benzyl CH$_2$), 2.82-2.60 (m, 2H, benzyl CH$_2$ and cyclopropyl CH), 1.98-1.81 (m, 1H, C$\underline{H}$CH$_3$), 1.81-1.65 (m, 1H, NCHC$\underline{H}_2$), 1.65-1.51 (m, 1H, C$\underline{H}_2$CH$_3$), 1.40-1.19 (m, 4H, C$\underline{H}_2$CH$_3$ and Ala CH$_3$), 1.19-1.06 (m, 1H, NCHC$\underline{H}_2$), 1.06-0.82 (m, 8H, CHC$\underline{H}_3$, CH$_2$C$\underline{H}_3$, cyclopropyl CH$_2$ (1H) and cyclopropyl CH), 0.29-0.13 (m, 1H, cyclopropyl CH$_2$); ^{13}C-NMR (100 MHz, CDCl$_3$) δ 172.6, 170.3, 169.1, 155.7, 137.3, 136.4, 129.5, 128.4, 128.3, 128.0, 127.9, 126.5, 70.4, 66.6, 62.6, 50.6, 50.4, 40.2, 33.6, 30.8, 27.2, 26.6, 19.3, 15.9, 14.9, 11.2, 11.0; LRMS (ESI) *m/z* 572.27 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{31}H_{39}N_3O_6Na$: 572.2731 [(M+Na)$^+$], found: 572.2728.

Target compound **1-18**

Target compound **1-18** (86.5 mg, 0.154 mmol, 2 steps 86%, a white solid) was prepared from carbamate **1-72** (90.9 mg, 0.178 mmol) as described for target compound **1-16**. $[\alpha]^{19}_D$ 3.56 (*c* 1.54, CHCl$_3$); mp 129-131 °C; ^{1}H-NMR (500 MHz, CDCl$_3$) δ 7.39-7.21 (m, 8H, amide NH and aromatic), 7.21-7.06 (m, 3H, aromatic), 6.87 (d, *J* = 7.4 Hz, 1H, amide NH), 5.75 (d, *J* = 7.4 Hz, 1H, Ala NH), 5.09 (s, 2H, Cbz CH$_2$), 4.66 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.36-4.22 (m, 1H, Ala α-H), 3.98-3.86 (m, 1H, NCH), 3.64 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.77 (br, 1H, cyclopropyl CH), 2.62 (br, 2H, benzyl CH$_2$), 2.02-1.82 (m, 2H, C$\underline{H}$CH$_3$ and BnCH$_2$), 1.79-1.65 (m, 2H, BnCH$_2$ and NCHC$\underline{H}_2$), 1.67-1.53 (m, 1H, C$\underline{H}_2$CH$_3$), 1.40 (d, *J* = 6.9 Hz, 3H, Ala CH$_3$), 1.34-1.23 (m, 1H, C$\underline{H}_2$CH$_3$), 1.23-1.10 (m, 1H, NCHC$\underline{H}_2$), 1.08-0.96 (m, 4H, cyclopropyl C$\underline{H}_2$ and CHC$\underline{H}_3$), 0.96-0.81 (m, 4H, cyclopropyl CH and CH$_2$C$\underline{H}_3$), 0.27-0.16 (m, 1H, cyclopropyl CH$_2$); ^{13}C-NMR (125 MHz, CDCl$_3$) δ 172.7, 170.3, 169.5, 155.9, 141.3, 136.3, 128.4, 128.4, 128.2, 128.0, 127.8, 126.0, 70.7, 66.7, 62.7, 50.6, 49.7, 36.7, 33.5, 32.5, 32.3, 26.9, 26.6, 19.1, 16.2, 14.4, 11.6, 11.0; LRMS (ESI) *m/z* 586.29 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{32}H_{41}N_3O_6Na$: 586.2888 [(M+Na)$^+$], found: 586.2882.

Target compound **1-19**

Target compound **1-19** (80.5 mg, 0.139 mmol, 2 steps 87%, a white solid) was prepared from carbamate **1-73** (84.0 mg, 0.160 mmol) as described for target compound **1-16**. $[\alpha]^{19}_D$ 3.38 (*c* 0.99, CHCl$_3$); mp 120-121 °C; ^{1}H-NMR (500 MHz, CDCl$_3$) δ 7.41-7.06 (m, 11H, amide NH and aromatic), 6.78 (d, *J* = 7.4 Hz, 1H, amide NH), 5.75 (d, *J* = 6.9 Hz, 1H, Ala NH), 5.08 (s, 2H, Cbz CH$_2$), 4.64 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.36-4.22 (m, 1H, Ala α-H), 3.87 (br, 1H, NCH), 3.60 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.76 (br, 1H, cyclopropyl CH), 2.60 (br, 2H, benzyl CH$_2$), 1.97-1.86 (m, 1H, C$\underline{H}$CH$_3$), 1.73-1.49 (m, 5H, NCHC$\underline{H}_2$ (1H), BnC$\underline{H}_2$, BnCH$_2$C$\underline{H}_2$ (1H) and C$\underline{H}_2$CH$_3$ (1H)), 1.46-1.31 (m, 4H,

$BnCH_2C\underline{H}_2$ (1H) and Ala CH_3), 1.30-1.19 (m, 1H, $C\underline{H}_2CH_3$), 1.19-1.07 (m, 1H, $NCHC\underline{H}_2$), 1.13-0.94 (m, 4H, cyclopropyl CH_2 (1H) and $CHC\underline{H}_3$), 0.94-0.80 (m, 4H, $CH_2C\underline{H}_3$ and cyclopropyl CH), 0.26-0.13 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.7, 170.3, 169.5, 155.8, 141.8, 136.3, 128.4, 128.3, 128.3, 128.0, 127.8, 125.8, 70.7, 66.7, 62.7, 50.5, 49.6, 35.5, 34.2, 33.5, 32.5, 27.5, 26.9, 26.6, 19.2, 16.2, 14.5, 11.6, 11.0; LRMS (ESI) *m/z* 600.30 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{43}N_3O_6Na$: 600.3044 [$(M+Na)^+$], found: 600.3041.

Target compound **1-20**

To a solution of carbamate **1-74** (19.8 mg, 0.0363 mmol, 1.0 equiv) in DCM (400 µl) was added TFA (400 µl) at rt. After 2 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow viscous oil.

To a solution of β-lactone unit **1-35** (12.5 mg, 0.0726 mmol, 2.0 equiv) in DCM (1.0 ml) was added triethylamine (10 µl, 0.0726 mmol, 2.0 equiv) and PivCl (8.9 µl, 0.0726 mmol, 2.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (15 µl, 0.109 mmol, 3.0 equiv) and a solution of the acid anhydride in DCM (1.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 17 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 2:1) to yield target compound **1-20** (16.0 mg, 0.0267 mmol, 2 steps 74%) as a white amorphous solid. $[\alpha]^{19}_D$ -8.26 (*c* 0.48, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.2 Hz, 1H, aromatic), 7.86 (br, 1H, amide NH), 7.80 (d, *J* = 7.7 Hz, 1H, aromatic), 7.65 (d, *J* = 8.6 Hz, 1H, aromatic), 7.58-7.41 (m, 2H, aromatic), 7.41-7.17 (m, 7H, aromatic), 6.93 (br, 1H, amide NH), 5.60 (d, *J* = 6.8 Hz, 1H, Ala NH), 5.52-5.38 (m, 1H, NCH), 5.09 (s, 2H, benzyl CH_2), 4.72 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.49-4.34 (m, 1H, Ala α-H), 3.69 (dd, *J* = 7.7, 4.5 Hz, 1H, OCOCH), 2.85 (br, 1H, cyclopropyl CH), 2.75 (br, 2H, $NpCH_2$), 2.21-1.95 (m, 2H, $NCHC\underline{H}_2$ and $C\underline{H}CH_3$), 1.80-1.64 (m, 1H, $C\underline{H}_2CH_3$), 1.48-1.26 (m, 5H, $NCHC\underline{H}_2$ (1H), $NpCH_2$ and Ala CH_3), 1.14 (d, *J* = 6.3 Hz, 3H, $CHC\underline{H}_3$), 1.06-0.88 (m, 5H, cyclopropyl CH_2 (1H), cyclopropyl CH and $CH_2C\underline{H}_3$), 0.31-0.15 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.1, 170.6, 169.0, 155.8, 136.5, 136.5, 132.8, 132.6, 131.1, 128.4, 128.3, 128.0, 127.9, 126.9, 126.3, 125.6, 124.4, 122.4, 70.9, 66.7, 63.1, 50.9, 50.3, 33.9, 32.8, 27.4, 26.7, 19.1, 16.4, 15.2, 14.0, 11.0, 10.9; LRMS (ESI) *m/z* 622.29 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{35}H_{41}N_3O_6Na$: 622.2888 [$(M+Na)^+$], found: 522.2878.

Target compound **1-21**

Target compound **1-21** (17.1 mg, 0.0285 mmol, 2 steps 82%, a colorless viscous oil) was prepared from carbamate **1-75** (18.9 mg, 0.0346 mmol) as described for target compound **1-20**. $[\alpha]^{19}_D$ -30.58 (*c* 0.50, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.03 (br, 2H, aromatic), 7.86 (d, *J* = 7.7 Hz, 1H, aromatic), 7.72 (d, *J* = 8.6 Hz, 1H, aromatic), 7.56-7.17 (m, 10H, aromatic (8H) and amide NH (2H)), 5.82-5.50 (m, 2H, Ala NH and NCH), 5.18-4.98 (m, 2H, benzyl CH_2), 4.77 (d, *J* = 4.5 Hz, 1H, NCH), 4.27 (br, 1H, Ala α-H), 3.86 (dd, *J* = 7.7, 4.5 Hz, 1H, OCOCH), 2.93 (br, 1H, cyclopropyl CH), 2.76-2.52 (m, 3H, $NCHC\underline{H}_2$ (1H) and $NpCH_2$), 2.10-1.94 (m, 1H, $C\underline{H}CH_3$), 1.86-1.63 (m, 2H, $NCHC\underline{H}_2$ and $C\underline{H}_2CH_3$), 1.46-1.26 (m, 4H, $C\underline{H}_2CH_3$ (1H) and Ala CH_3), 1.10 (d, *J* = 6.8 Hz, 3H, $CHCH_3$), 1.03-0.91 (m, 4H, cyclopropyl CH_2 (1H) and $CH_2C\underline{H}_3$), 0.91-0.76 (m, 1H, cyclopropyl CH), 0.25-0.14 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.5, 170.5, 170.0, 156.0, 136.4, 129.6, 128.6, 128.4, 128.1, 127.8, 126.4, 124.9, 123.6, 71.2, 66.8, 62.5, 51.5, 50.4, 33.6, 29.7, 27.3, 26.7, 21.4, 18.5, 16.3, 15.2, 12.5, 11.1; LRMS (ESI) *m/z* 622.29

[(M+Na)$^+$]; HRMS (ESI) calcd for $C_{35}H_{41}N_3O_6Na$: 622.2888 [(M+Na)$^+$], found: 622.2874.

Target compound **1-22**

Target compound **1-22** (22.3 mg, 0.0363 mmol, 2 steps 76%, a colorless viscous oil) was prepared from carbamate **1-76** (26.6 mg, 0.0475 mmol) as described for target compound **1-20**. $[\alpha]^{19}_D$ 0.66 (*c* 0.81, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.2 Hz, 1H, aromatic), 7.85 (d, *J* = 7.7 Hz, 1H, aromatic), 7.71 (d, *J* = 8.2 Hz, 1H, aromatic), 7.57-7.43 (m, 2H, aromatic), 7.43-7.19 (m, 7H, aromatic), 7.06 (br, 1H, amide NH), 6.92 (d, *J* = 8.2 Hz, 1H, amide NH), 5.69 (d, *J* = 6.8 Hz, 1H, Ala NH), 5.10 (s, 2H, benzyl CH_2), 4.65 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.41-4.26 (m, 1H, Ala α-H), 4.16-4.00 (m, 1H, NCH), 3.60 (dd, *J* = 7.2, 4.5 Hz, 1H, OCOCH), 3.20-2.95 (m, 2H, $NpCH_2$), 2.77 (br, 1H, cyclopropyl CH), 2.10-1.86 (m, 2H, $NpCH_2C\underline{H}_2$ and $C\underline{H}CH_3$), 1.86-1.72 (m, 2H, $NpCH_2C\underline{H}_2$ and $NCHC\underline{H}_2$), 1.65-1.50 (m, 1H, $C\underline{H}_2CH_3$), 1.44 (d, *J* = 7.2 Hz, 3H, Ala CH_3), 1.36-1.10 (m, 2H, $NCHC\underline{H}_2$ and $C\underline{H}_2CH_3$), 1.10-0.75 (m, 8H, $CHC\underline{H}_3$, $CH_2C\underline{H}_3$, cyclopropyl CH_2 (1H) and cyclopropyl CH), 0.30-0.15 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.9, 170.3, 169.3, 155.9, 137.5, 136.3, 133.8, 131.5, 128.9, 128.4, 128.1, 127.9, 126.8, 126.0, 125.9, 125.6, 125.5, 123.4, 70.7, 66.8, 62.9, 50.7, 50.0, 36.4, 33.6, 32.4, 29.6, 27.0, 26.6, 19.1, 16.2, 14.6, 11.4, 11.0; LRMS (ESI) *m/z* 636.30 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{36}H_{43}N_3O_6Na$: 636.3044 [(M+Na)$^+$], found: 636.3036.

Target compound **1-77**

Target compound **1-77** (55.3 mg, 0.0901 mmol, 2 steps 83%, a colorless viscous oil) was prepared from carbamate **1-77** (60.8 mg, 0.109 mmol) as described for target compound **1-20**. $[\alpha]^{19}_D$ 4.87 (*c* 1.68, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.83-7.71 (m, 3H, aromatic), 7.58 (br, 1H, aromatic), 7.49-7.37 (m, 2H, aromatic), 7.37-7.21 (m, 6H, aromatic), 7.18 (br, 1H, amide NH), 6.91 (d, *J* = 7.4 Hz, 1H, amide NH), 5.74 (d, *J* = 6.9 Hz, 1H, Ala NH), 5.09 (s, 2H, benzyl CH_2), 4.65 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.39-4.24 (m, 1H, Ala α-H), 3.98 (br, 1H, NCH), 3.63 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.78 (br, 3H, cyclopropyl CH and $NpCH_2$), 2.02-1.89 (m, 2H, $NpCH_2C\underline{H}_2$ and $C\underline{H}CH_3$), 1.83-1.70 (m, 2H, $NpCH_2C\underline{H}_2$ and $NCHC\underline{H}_2$), 1.65-1.52 (m, 1H, $C\underline{H}_2CH_3$), 1.40 (d, *J* = 7.4 Hz, 3H, Ala CH_3), 1.35-1.12 (m, 2H, $NCHC\underline{H}_2$ and $C\underline{H}_2CH_3$), 1.08-0.78 (m, 8H, $CHC\underline{H}_3$, $CH_2C\underline{H}_3$, cyclopropyl CH_2 (1H) and cyclopropyl CH), 0.28-0.16 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 170.3, 169.4, 155.9, 138.8, 136.3, 133.5, 132.0, 128.4, 128.0, 127.8, 127.6, 127.3, 127.0, 126.3, 126.0, 125.2, 70.7, 66.7, 62.8, 50.6, 49.8, 36.8, 33.5, 32.5, 26.9, 26.6, 19.1, 16.2, 14.5, 11.5, 11.0; LRMS (ESI) *m/z* 636.31 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{36}H_{43}N_3O_6Na$: 636.3044 [(M+Na)$^+$], found: 636.3031.

Synthetic procedures for 1-24 and 1-25

Target compound **1-24**

To a solution of diprotected amine **1-64** (23.3 mg, 0.0442 mmol) in MeOH (1.0 ml) was added K_2CO_3 (12.2 mg, 0.0885 mmol) and stirred at rt for 23 h. The reaction mxiture was concentrated *in vacuo* and the crude product was purified by NH-silica gel column chromatography (*n*-hexane/ AcOEt/ MeOH 1:1:0-0:1:0-0:1:1) to yield the corresponding amine as a colorless viscous oil.

To a solution of the oil in DCM (2.0 ml) was added triethylamine (11 μl, 0.0779 mmol) and 2-Naphthoyl chloride (14.8 mg, 0.0779 mmol) at 0 °C. After 3 h, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was

purified by silica gel column chromatography (*n*-hexane/ AcOEt = 4:1) to yield carbamate **1-78** as a white solid.

To a solution of carbamate **1-78** in DCM (1.0 ml) was added TFA (0.5 ml). After 2 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow oil.

To a solution of β-lactone unit **1-35** (12.2 mg, 0.0710 mmol) in DCM (1.0 ml) was added triethylamine (9.9 µl, 0.0710 mmol) and PivCl (8.7 µl, 0.0710 mmol) at 0 °C. After 1 h at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (22 µl, 0.156 mmol) and a solution of the acid anhydride in DCM at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred at rt for 11 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by HPLC (Mightysil Si 60 250-20 (5 µm), *n*-hexane/ AcOEt 58:42) to yield target compound **1-24** (10.8 mg, 0.0211 mmol, 4 steps 48%) as a colorless viscous oil. $[\alpha]^{19}_D$ 26.89 (*c* 0.23, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H, aromatic), 7.95-7.83 (m, 3H, aromatic), 7.83-7.77 (m, 1H, aromatic), 7.61-7.51 (m, 2H, aromatic), 7.49 (br, 1H, amide NH), 7.35-7.17 (m, 5H, aromatic), 6.90 (d, *J* = 8.0 Hz, 1H, amide NH), 4.82 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.30-4.19 (m, 1H, NCH), 3.72 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.90-2.74 (m, 3H, benzyl CH_2 and cyclopropyl CH), 2.17-2.06 (m, 1H, $BnCH_2$), 2.06-1.84 (m, 3H, $BnC\underline{H}_2$, $C\underline{H}CH_3$ and $NCHC\underline{H}_2$), 1.71-1.56 (m, 1H, $C\underline{H}_2CH_3$), 1.48-1.37 (m, 1H, $NCHC\underline{H}_2$), 1.37-1.25 (m, 1H, $C\underline{H}_2CH_3$), 1.15-1.02 (m, 5H, $CHC\underline{H}_3$, cyclopropyl CH_2 (1H) and cyclopropyl CH), 0.89 (dd, *J* = 7.4, 7.4 Hz, 3H, $CH_2C\underline{H}_3$), 0.32-0.23 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.3, 169.4, 167.5, 141.5, 137.8, 132.6, 131.1, 129.0, 128.7, 128.3, 128.3, 127.7, 127.6, 127.6, 126.7, 126.2, 123.6, 70.5, 62.6, 50.9, 36.3, 33.5, 33.1, 32.7, 26.8, 26.7, 16.2, 14.3, 12.4, 11.0; LRMS (ESI) *m/z* 535.26 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{36}N_2O_4Na$: 535.2567 [$(M+Na)^+$], found: 535.2567.

Target compound **1-25**

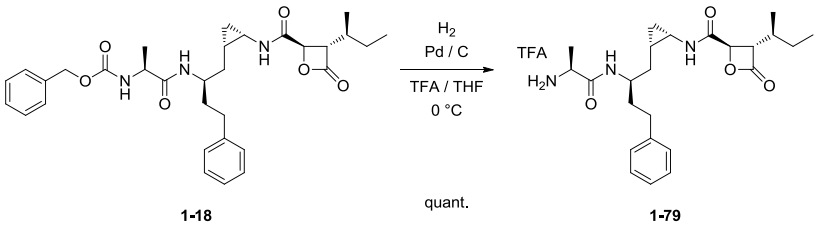
To a solution of compound **1-18** (25.6 mg, 0.0454 mmol) in THF (1.0 ml) was added TFA (1.0 ml) and Pd/C (30 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 70 min. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding amine as a colorless oil.

To a solution of the oil in DCM (1.0 ml) was added triethylamine (19 µl, 0.136 mmol) and 2-Naphthoyl chloride (26.0 mg, 0.136 mmol) at 0 °C. After 1 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 5:4) to yield target compound **1-25** (20.2 mg, 0.0346 mmol, 2 steps 76%) as a colorless viscous oil. $[\alpha]^{19}_D$ 43.28 (*c* 0.17, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H, aromatic), 7.95-7.81 (m, 4H, aromatic), 7.60-7.48 (m, 3H, aromatic and amide NH), 7.39 (d, *J* = 2.9 Hz, 1H, amide NH), 7.31 (d, *J* = 8.0 Hz, 1H, amide NH), 7.29-7.22 (m, 2H, aromatic), 7.22-7.12 (m, 3H, aromatic), 4.96-4.85 (m, 1H, Ala α-H), 4.61 (d, *J* = 4.6 Hz, 1H, NCOCH), 3.92 (br, 1H, NCH), 3.70 (dd, *J* = 8.0, 4.6 Hz, 1H, OCOCH), 2.85-2.62 (m, 3H, benzyl CH_2 and cyclopropyl CH), 2.02-1.87 (m, 2H, $BnCH_2$ and $C\underline{H}CH_3$), 1.85-1.73 (m, 2H, $BnCH_2$ and $NCHC\underline{H}_2$), 1.65-1.53 (m, 4H, $C\underline{H}_2CH_3$ and Ala CH_3), 1.34-1.18 (m, 2H, $C\underline{H}_2CH_3$ and $NCHC\underline{H}_2$), 1.06-0.81 (m, 8H, $CHC\underline{H}_3$, $CH_2C\underline{H}_3$, cyclopropyl CH_2 (1H) and cyclopropyl CH), 0.24-0.16 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 170.3, 170.0, 167.1, 141.3, 134.8, 132.6, 131.0, 129.0, 128.5, 128.3, 128.2, 127.8, 127.7, 126.7, 126.1, 123.8, 71.1, 62.9, 50.2, 49.4, 36.5, 33.6, 33.1, 32.6, 26.9, 26.6, 19.4, 16.2, 14.4, 11.9, 11.0; LRMS (ESI) *m/z*

606.30 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{35}H_{41}N_3O_5Na$: 606.2938 [(M+Na)$^+$], found: 606.2937.

Synthetic procedure for 1-79

Scheme S1-1. Synthesis of **1-79**



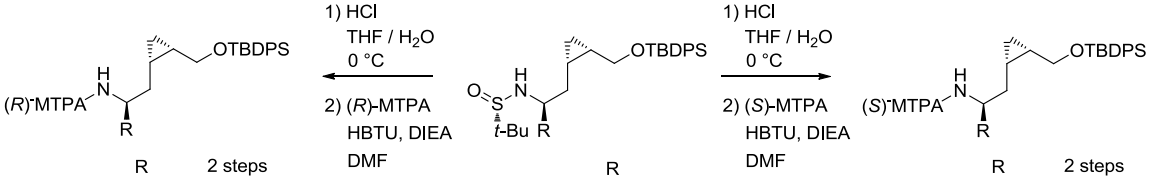
Compound **1-79**

To a solution of **1-18** (17.0 mg, 0.0301 mmol) in DCM (300 μl) was added TFA (300 μl) and Pd/C (20 mg) at 0 °C. The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred at 0 °C under an atmosphere of hydrogen for 2 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to yield compound **1-79** (16.0 mg, 0.0304 mmol, quant.) as a colorless viscous oil. $[\alpha]^{21}_D$ -25.14 (*c* 0.57, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.56 (d, *J* = 8.6 Hz, 1H), 8.53 (br, 3H), 8.32 (d, *J* = 3.4 Hz, 1H), 7.25-7.13 (m, 4H), 7.13-7.04 (m, 1H), 4.78 (d, *J* = 4.6 Hz, 1H), 4.28-4.17 (m, 1H), 4.09-3.98 (m, 1H), 3.67 (dd, *J* = 8.0, 4.6 Hz, 1H), 2.82-2.73 (m, 1H), 2.72-2.62 (m, 1H), 2.58-2.46 (m, 1H), 1.98-1.87 (m, 1H), 1.85-1.69 (m, 2H), 1.69-1.58 (m, 1H), 1.56-1.45 (m, 1H), 1.55 (d, *J* = 6.9 Hz, 3H), 1.44-1.34 (m, 1H), 1.34-1.21 (m, 1H), 1.20-1.09 (m, 1H), 1.02 (d, *J* = 6.9 Hz, 3H), 0.91 (dd, *J* = 7.4, 7.4 Hz, 3H), 0.89-0.79 (m, 1H), 0.42-0.33 (m, 1H); ^{13}C-NMR (125 MHz, THF-d8) δ 172.3, 170.7, 170.0, 143.4, 129.4, 129.1, 126.6, 71.4, 63.8, 50.5, 38.6, 34.8, 33.7, 28.5, 27.7, 18.3, 16.7, 16.0, 11.7, 11.1; LRMS (ESI) *m/z* 430.27 [(M+H)$^+$]; HRMS (ESI) calcd for $C_{24}H_{36}N_3O_4$: 430.2700 [(M+H)$^+$], found: 430.2703; Anal. calcd for $C_{26}H_{35}F_3N_3O_5 \cdot 1.0TFA \cdot 1.5H_2O$: C, 50.37; H, 5.89; N, 6.29. Found: C, 50.59; H, 6.08; N, 6.34.

Determination of the absolute configuration of stereocenters constructed by Grignard reactions

Absolute configuration of the stereocenters constructed by diastereoselective Grignard reactions were determined by modified Mosher's method.[43]

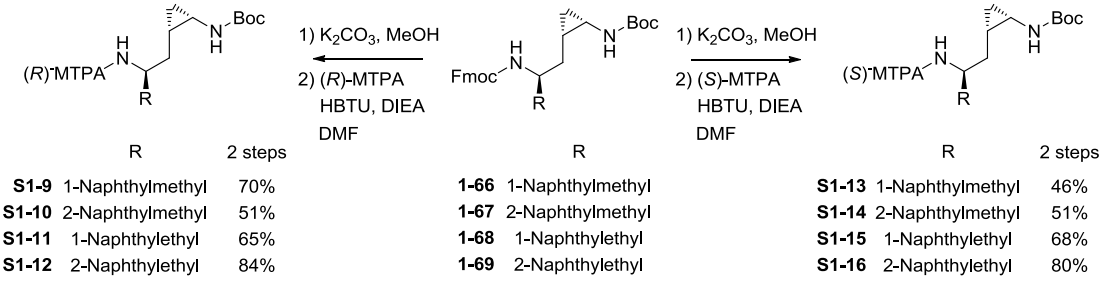
Scheme S1-2. Synthesis of Mosher amide **S1-1** – **S1-16**



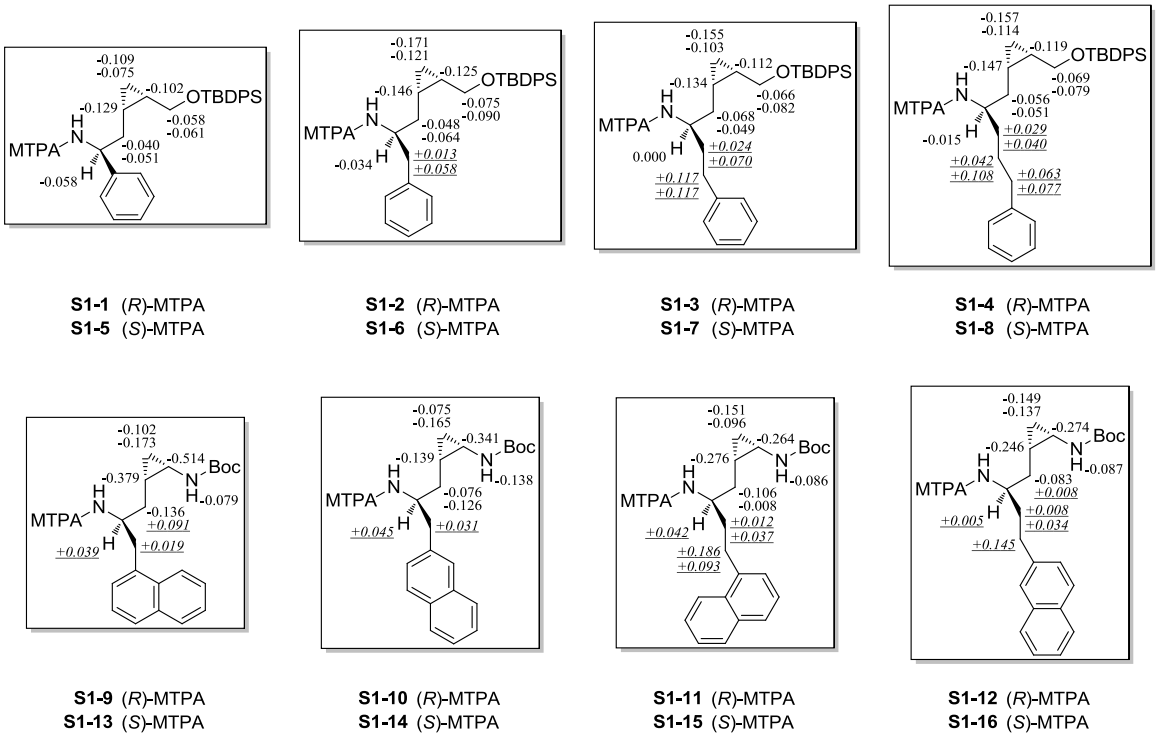
	R	2 steps
S1-1	Ph	80%
S1-2	Bn	73%
S1-3	Phenethyl	72%
S1-4	Phenylpropyl	34%

	R
1-38	Ph
1-39	Bn
1-40	Phenethyl
1-41	Phenylpropyl

	R	2 steps
S1-5	Ph	75%
S1-6	Bn	79%
S1-7	Phenethyl	71%
S1-8	Phenylpropyl	37%



	R	2 steps		R		R	2 steps
S1-9	1-Naphthylmethyl	70%	**1-66**	1-Naphthylmethyl	**S1-13**	1-Naphthylmethyl	46%
S1-10	2-Naphthylmethyl	51%	**1-67**	2-Naphthylmethyl	**S1-14**	2-Naphthylmethyl	51%
S1-11	1-Naphthylethyl	65%	**1-68**	1-Naphthylethyl	**S1-15**	1-Naphthylethyl	68%
S1-12	2-Naphthylethyl	84%	**1-69**	2-Naphthylethyl	**S1-16**	2-Naphthylethyl	80%



S1-1 (*R*)-MTPA **S1-5** (*S*)-MTPA | **S1-2** (*R*)-MTPA **S1-6** (*S*)-MTPA | **S1-3** (*R*)-MTPA **S1-7** (*S*)-MTPA | **S1-4** (*R*)-MTPA **S1-8** (*S*)-MTPA

S1-9 (*R*)-MTPA **S1-13** (*S*)-MTPA | **S1-10** (*R*)-MTPA **S1-14** (*S*)-MTPA | **S1-11** (*R*)-MTPA **S1-15** (*S*)-MTPA | **S1-12** (*R*)-MTPA **S1-16** (*S*)-MTPA

Figure S1-1. Determination of the absolute configuration by the modified Mosher's method ($\Delta\delta = \delta_S - \delta_R$ (ppm; $CDCl_3$))

Mosher amide **S1-1**

To a solution of sulfinamide **1-38** (34.0 mg, 0.0637 mmol) in THF (1.0 ml) was added 12 M HCl (0.10 ml) at 0 °C. After 30 min at 0 °C, the reaction mixture was neutralized with sat. $NaHCO_3$, diluted with water and concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding amine as a colorless oil.

To a solution of HBTU (65.3 mg, 0.172 mmol, 2.7 equiv) in DMF (2.0 ml) was added (*R*)-MTPA (44.7 mg, 0.191 mmol, 3.0 equiv) and DIEA (26.8 µl, 0.191 mmol, 3.0 equiv). After 5 min at rt, a solution of the aforementioned amine in DMF (1.0 ml) was added and the resulting mixture was stirred for 10 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 10:1) to yield Mosher amide **S1-1** (32.9 mg, 0.0509 mmol, 2 steps 80%) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.711-7.634 (7.673) (m, 4H, aromatic), 7.445-7.209 (7.327) (m, 14H, aromatic), 7.209-7.137 (7.173) (m, 2H, aromatic), 7.102 (d, *J* = 8.0 Hz, 1H, amide NH), 5.159-5.073 (5.117) (m, 1H, NCH), 3.828 (dd, *J* = 10.9, 5.2 Hz, 1H, CH_2O), 3.497 (dd, *J* = 10.9, 8.6 Hz, 1H, CH_2O), 3.422 (s, 3H, OCH_3), 2.191-2.075 (2.131) (m, 1H, $NCHCH_2$), 1.600-1.480 (1.538) (m, 1H, $NCHCH_2$),

1.166-1.066 (1.114) (m, 1H, cyclopropyl CH), 1.044 (s, 9H, CCH_3), 0.823-0.710 (0.770) (m, 1H, cyclopropyl CH), 0.648-0.558 (0.605) (m, 1H, cyclopropyl CH_2), -0.063--0.150 (-0.107) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 668.28 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{38}H_{42}NO_3F_3SiNa$: 668.2778 [$(M+Na)^+$], found: 668.2779.

Mosher amide **S1-2**

Mosher amide **S1-2** (29.6 mg, 0.0449 mmol, 2 steps 73%, a colorless oil) was prepared from sulfinamide **1-39** (33.6 mg, 0.0615 mmol) as described for Mosher amide **S1-1**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.709-7.643 (7.676) (m, 4H, aromatic), 7.456-7.148 (7.302) (m, 14H, aromatic), 7.139-7.069 (7.104) (m, 2H, aromatic), 6.755 (d, *J* = 8.6 Hz, 1H, amide NH), 4.437-4.324 (4.378) (m, 1H, NCH), 3.814 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2O), 3.480 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 3.237 (s, 3H, OCH_3), 2.908 (dd, *J* = 13.7, 5.7 Hz, 1H, benzyl CH_2), 2.739 (dd, *J* = 13.7, 8.0 Hz, 1H, benzyl CH_2), 1.904-1.819 (1.860) (m, 1H, $NCHCH_2$), 1.330-1.233 (1.279) (m, 1H, $NCHCH_2$), 1.194-1.103 (1.148) (m, 1H, cyclopropyl CH), 1.043 (s, 9H, CCH_3), 0.946-0.845 (0.892) (m, 1H, cyclopropyl CH), 0.743-0.659 (0.699) (m, 1H, cyclopropyl CH_2), -0.013--0.070 (-0.040) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 682.29 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{39}H_{44}NO_3F_3SiNa$: 682.2935 [$(M+Na)^+$], found: 682.2937.

Mosher amide **S1-3**

Mosher amide **S1-3** (40.0 mg, 0.0594 mmol, 2 steps 72%, a colorless oil) was prepared from sulfinamide **1-40** (46.3 mg, 0.0824 mmol) as described for Mosher amide **S1-1**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.747-7.635 (7.691) (m, 4H, aromatic), 7.603-7.502 (7.553) (m, 2H, aromatic), 7.444-7.322 (7.383) (m, 9H, aromatic), 7.255 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.215-7.125 (7.170) (m, 1H, aromatic), 7.087 (d, *J* = 7.4 Hz, 2H, aromatic), 6.690 (d, *J* = 9.2 Hz, 1H, amide NH), 4.181-4.065 (4.126) (m, 1H, NCH), 3.831 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2O), 3.495 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 3.390 (s, 3H, OCH_3), 2.524 (dd, *J* = 8.0, 8.0 Hz, 2H, benzyl CH_2), 1.940-1.814 (1.871) (m, 2H, $NCHC\underline{H}_2$ and $BnCH_2$), 1.796-1.678 (1.733) (m, 1H, $BnCH_2$), 1.290-1.184 (1.231) (m, 1H, $NCHC\underline{H}_2$), 1.184-1.090 (1.138) (m, 1H, cyclopropyl CH), 1.052 (s, 9H, CCH_3), 0.902-0.791 (0.843) (m, 1H, cyclopropyl CH), 0.739-0.633 (0.687) (m, 1H, cyclopropyl CH_2), -0.012--0.081 (-0.044) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 696.31 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{40}H_{46}NO_3F_3SiNa$: 696.3091 [$(M+Na)^+$], found: 696.3092.

Mosher amide **S1-4**

Mosher amide **S1-4** (11.2 mg, 0.0163 mmol, 2 steps 34%, a colorless oil) was prepared from sulfinamide **1-41** (27.6 mg, 0.0479 mmol) as described for Mosher amide **S1-1**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.701-7.625 (7.663) (m, 4H, aromatic), 7.518 (d, *J* = 7.4 Hz, 2H, aromatic), 7.461-7.306 (7.384) (m, 9H, aromatic), 7.295-7.217 (7.256) (m, 2H, aromatic), 7.217-7.117 (7.167) (m, 1H, aromatic), 7.074 (d, *J* = 6.9 Hz, 2H, aromatic), 6.583 (d, *J* = 9.2 Hz, 1H, amide NH), 4.170-4.051 (4.103) (m, 1H, NCH), 3.809 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2O), 3.484 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 3.380 (s, 3H, OCH_3), 2.666-2.469 (2.602 and 2.517) (m, 2H, benzyl CH_2), 1.853-1.741 (1.796) (m, 1H, $NCHC\underline{H}_2$), 1.709-1.476 (1.595, 1.572 and 1.521) (m, 3H, $BnCH_2C\underline{H}_2$ (1H) and $BnCH_2$), 1.476-1.364 (1.429) (m, 1H, $BnCH_2C\underline{H}_2$), 1.209-1.088 (1.162 and 1.129) (m, 2H, $NCHC\underline{H}_2$ and cyclopropyl CH), 1.042 (s, 9H, CCH_3), 0.867-0.746 (0.815) (m, 1H, cyclopropyl CH), 0.724-0.616 (0.668) (m, 1H, cyclopropyl CH_2), -0.024--0.124 (-0.071) (m, 1H, cyclopropyl CH_2); LRMS (FAB) *m/z* 710.4 [$(M+Na)^+$]; HRMS (FAB) calcd for $C_{41}H_{48}NO_3F_3SiNa$: 710.3253 [$(M+Na)^+$], found: 710.3272.

Mosher amide **S1-5**

To a solution of sulfinamide **1-38** (27.3 mg, 0.0512 mmol) in THF (1.0 ml) was added 12 M HCl (0.10 ml) at 0 °C. After 30 min at 0 °C, the reaction mixture was neutralized with sat. $NaHCO_3$, diluted with water, concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding amine as a colorless oil.

To a solution of HBTU (52.4 mg, 0.138 mmol, 2.7 equiv) in DMF (2.0 ml) was added (*S*)-MTPA (36.0 mg, 0.154 mmol, 3.0 equiv) and DIEA (21.6 μl, 0.154 mmol, 3.0 equiv). After 5 min at rt, a solution of the aforementioned amine in DMF (1.0 ml) was added and the resulting mixture was stirred for 11 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 10:1) to yield Mosher amide **S1-5** (24.8 mg, 0.0384 mmol, 2 steps 75%) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.654 (d, *J* = 7.4 Hz, 4H, aromatic), 7.588-7.519 (7.549) (m, 2H, aromatic), 7.439-7.289 (7.364) (m, 11H, aromatic), 7.289-7.224 (7.257) (m, 3H, aromatic), 7.044 (d, *J* = 8.0 Hz, 1H, amide NH), 5.185-5.090 (5.136) (m, 1H, NCH), 3.770 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2O), 3.436 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 3.378 (s, 3H, OCH_3), 2.140-2.042 (2.091) (m, 1H, NCHC$\underline{H}_2$), 1.535-1.443 (1.487) (m, 1H, NCHC$\underline{H}_2$), 1.105-0.948 (1.039 and 1.012) (m, 10H, CCH_3 and cyclopropyl CH), 0.714-0.577 (0.641) (m, 1H, cyclopropyl CH), 0.577-0.481 (0.530) (m, 1H, cyclopropyl CH_2), -0.167--0.266 (-0.216) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 668.28 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{38}H_{42}NO_3F_3SiNa$: 668.2778 [$(M+Na)^+$], found: 668.2782.

Mosher amide **S1-6**

Mosher amide **S1-6** (41.1 mg, 0.0623 mmol, 2 steps 79%, a colorless oil) was prepared from sulfinamdie **1-39** (43.2 mg, 0.0789 mmol) as described for Mosher amide **S1-5**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.652 (dd, *J* = 6.3, 6.3 Hz, 4H, aromatic), 7.484 (d, *J* = 6.9 Hz, 2H, aromatic), 7.454-7.149 (7.302) (m, 14H, aromatic), 6.545 (d, *J* = 8.6 Hz, 1H, amide NH), 4.399-4.297 (4.344) (m, 1H, NCH), 3.739 (dd, *J* = 11.5, 6.3 Hz, 1H, CH_2O), 3.390 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 3.183 (s, 3H, OCH_3), 2.921 (dd, *J* = 13.7, 6.3 Hz, 1H, benzyl CH_2), 2.797 (dd, *J* = 13.7, 7.4 Hz, 1H, benzyl CH_2), 1.866-1.760 (1.812) (m, 1H, NCHC$\underline{H}_2$), 1.285-1.170 (1.215) (m, 1H, NCHC$\underline{H}_2$), 1.110-0.978 (1.035 and 1.023) (m, 10H, CCH_3 and cyclopropyl CH), 0.797-0.696 (0.746) (m, 1H, cyclopropyl CH), 0.617-0.541 (0.578) (m, 1H, cyclopropyl CH_2), -0.174--0.252 (-0.211) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 682.30 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{39}H_{44}NO_3F_3SiNa$: 682.2935 [$(M+Na)^+$], found: 682.2936.

Mosher amide **S1-7**

Mosher amide **S1-7** (28.6 mg, 0.0424 mmol, 2 steps 71%, a colorless oil) was prepared from sulfinamide **1-40** (33.6 mg, 0.0598 mmol) as described for Mosher amide **S1-5**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.700-7.638 (7.669) (m, 4H, aromatic), 7.522 (d, *J* = 8.0 Hz, 2H, aromatic), 7.450-7.306 (7.378) (m, 9H, aromatic), 7.306-7.215 (7.261) (m, 2H, aromatic), 7.215-7.137 (7.176) (m, 3H, aromatic), 6.686 (d, *J* = 9.2 Hz, 1H, amide NH), 4.175-4.063 (4.126) (m, 1H, NCH), 3.765 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2O), 3.465-3.363 (3.413) (m, 4H, CH_2O (1H) and OCH_3), 2.714-2.562 (2.641) (m, 2H, benzyl CH_2), 1.959-1.709 (1.895 and 1.803) (m, 3H, NCHC$\underline{H}_2$ (1H) and $BnCH_2$), 1.235-1.135 (1.182) (m, 1H, NCHC$\underline{H}_2$), 1.091-0.984 (1.046 and 1.026) (m, 10H, CCH_3 and cyclopropyl CH), 0.765-0.653 (0.709) (m, 1H, cyclopropyl CH), 0.628-0.543 (0.584) (m, 1H, cyclopropyl CH_2), -0.166--0.241 (-0.199) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 696.31 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{40}H_{46}NO_3F_3SiNa$: 696.3091 [$(M+Na)^+$], found: 696.3094.

Mosher amide **S1-8**

Mosher amide **S1-8** (18.3 mg, 0.0266 mmol, 2 steps 37%, a colorless oil) was prepared from sulfinamide **1-41** (41.4 mg, 0.0719 mmol) as described for Mosher amide **S1-5**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.697-7.616 (7.657) (m, 4H, aromatic), 7.507 (d, *J* = 8.0 Hz, 2H, aromatic), 7.464-7.303 (7.384) (m, 10H, aromatic), 7.303-7.216 (7.260) (m, 1H, aromatic), 7.216-7.119 (7.168) (m, 3H, aromatic), 6.572 (d, *J* = 9.2 Hz, 1H, amide NH), 4.144-4.031 (4.088) (m, 1H, NCH), 3.740 (dd, *J* = 10.9, 5.7 Hz, 1H, CH_2O), 3.456-3.337 (3.405 and 3.378) (m, 4H, CH_2O (1H) and OCH_3), 2.718-2.550 (2.665 and 2.594) (m, 2H, benzyl CH_2), 1.793-1.700 (1.740) (m, 1H, NCHC$\underline{H}_2$), 1.700-1.536 (1.637, 1.629 and 1.601) (m, 3H, BnC$\underline{H}_2$ and $BnCH_2C\underline{H}_2$ (1H)), 1.536-1.394 (1.469) (m, 1H, $BnCH_2C\underline{H}_2$), 1.159-1.069 (1.111) (m, 1H, NCHC$\underline{H}_2$), 1.093-0.951 (1.035 and 1.010) (m, 10H, CCH_3 and cyclopropyl CH), 0.724-0.608 (0.668) (m, 1H, cyclopropyl CH), 0.594-0.507 (0.554) (m, 1H, cyclopropyl CH_2), -0.202--0.257 (-0.228) (m, 1H, cyclopropyl CH_2); LRMS (FAB) *m/z* 710.4 [$(M+Na)^+$]; HRMS (FAB) calcd for $C_{41}H_{48}NO_3F_3SiNa$: 710.3253 [$(M+Na)^+$], found: 710.3247.

Mosher amide **S1-9**

To a solution of diprotected amine **1-66** (8.67 mg, 0.0154 mmol) in MeOH (1.0 ml) was added K_2CO_3 (2.55 mg, 0.0185 mmol, 1.2 equiv). After 22 h at rt, the reaction mixture was concentrated *in vacuo* and the crude product was purified by NH-silica gel column chromatography (*n*-hexane/ AcOEt 1:1-0:1) to yield the corresponding amine as a white amorphous solid.

To a solution of (*R*)-MTPA (10.8 mg, 0.0462 mmol, 3.0 equiv) in DMF (1.0 ml) was added HBTU (15.8 mg, 0.0416 mmol, 2.7 equiv) and DIEA (6.49 μl, 0.0462 mmol, 3.0 equiv). After 5 min at rt, a solution of the aforementioned amine in DMF (1.0 ml) was added and the resulting mixture was stirred for 20 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 5:1) to yield Mosher amide **S1-9** (6.0 mg, 0.0108 mmol, 2 steps 70%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.779 (d, *J* = 5.4 Hz, 1H, amide NH), 8.072 (d, *J* = 8.6 Hz, 1H, aromatic), 7.761 (d, *J* = 7.7 Hz, 1H, aromatic), 7.578-7.196 (7.387) (m, 9H, aromatic), 6.973 (d, *J* = 8.2 Hz, 1H, aromatic), 5.633-5.538 (5.585) (m, 1H, NCH), 4.737 (br, 1H, carbamate NH), 3.616 (s, 3H, OCH_3), 2.767 (br, 2H, $NpCH_2$), 2.644 (br, 1H, cyclopropyl CH), 1.903-1.831 (1.877) (m, 1H, NCHC$\underline{H}_2$), 1.650 (br, 1H, NCHC$\underline{H}_2$), 1.357 (s, 9H, CCH_3), 1.024-0.896 (0.959 and 0.943) (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.102 (br, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 579.24 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{31}H_{35}N_2O_4F_3Na$: 579.2441 [$(M+Na)^+$], found: 579.2435.

Mosher amide **S1-10**

Mosher amide **S1-10** (3.80 mg, 0.00683 mmol, 2 steps 51%, a yellow oil) was prepared from diprotected amine **1-67** (7.54 mg, 0.0134 mmol) as described for Mosher amide **S1-9**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.351 (br, 1H, amide NH), 7.940-6.970 (7.455) (m, 12H aromatic), 5.709 (br, 1H, NCH), 5.313 (br, 1H, carbamate NH), 3.492 (br, 3H, OCH_3), 2.670 (br, 3H, $NpCH_2$ and cyclopropyl CH), 2.287 (br, 1H, NCHC$\underline{H}_2$), 2.175 (br, 1H, NCHC$\underline{H}_2$), 1.441 (s, 9H, CCH_3), 1.009-0.750 (0.921 and 0.844) (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.117 (br, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 579.24 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{31}H_{35}N_2O_4F_3Na$: 579.2441 [$(M+Na)^+$], found: 579.2437.

Mosher amide **S1-11**

Mosher amide **S1-11** (4.70 mg, 0.00824 mmol, 2 steps 65%, a colorless viscous oil) was prepared from diprotected amine **1-68** (7.31 mg, 0.0127 mmol) as described for Mosher amide **S1-9**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.875-7.804 (7.840) (m, 2H, aromatic), 7.702 (d, *J* = 8.2 Hz, 1H, aromatic), 7.648-7.576 (7.621) (m, 2H, aromatic), 7.486-7.431 (7.459) (m, 2H, aromatic, amide NH), 7.401-7.333 (7.367) (m, 5H, aromatic), 7.282-7.203 (7.243) (m, 1H, aromatic), 4.795 (br, 1H, carbamate NH), 4.322-4.189 (4.261) (m, 1H, NCH), 3.473 (s, 3H, OCH_3), 3.120-2.882 (3.001) (m, 2H, $NpCH_2C\underline{H}_2$), 2.644-2.546 (2.602) (m, 1H, cyclopropyl CH), 2.010 (br, 1H, $NpCH_2C\underline{H}_2$), 1.927-1.786 (1.856) (m, 1H, $NpCH_2C\underline{H}_2$), 1.680 (br, 1H, $NCHC\underline{H}_2$), 1.582 (br, 1H, $NCHC\underline{H}_2$), 1.395 (s, 9H, CCH_3), 1.035-0.789 (0.940 and 0.900) (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.244-0.156 (0.199) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 593.26 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{37}N_2O_4F_3Na$: 593.2598 [$(M+Na)^+$], found: 593.2593.

Mosher amide **S1-12**

Mosher amide **S1-12** (7.60 mg, 0.0133 mmol, 2 steps 84%, a colorless viscous oil) was prepared from diprotected amine **1-69** (9.13 mg, 0.0158 mmol) as described for Mosher amide **S1-9**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.829-7.717 (7.773) (m, 3H, aromatic), 7.641-7.552 (7.597) (m, 2H, aromatic), 7.523 (br, 1H, aromatic), 7.480-7.343 (7.412) (m, 6H, aromatic and amide NH), 7.260-7.210 (7.238) (m, 1H, aromatic), 4.809 (br, 1H, carbamate NH), 4.237-4.106 (4.173) (m, 1H, NCH), 3.426 (s, 3H, OCH_3), 2.764-2.639 (2.693) (m, 2H, $NpCH_2$), 2.639-2.542 (2.597) (m, 1H, cyclopropyl CH), 2.037-1.784 (1.962 and 1.869) (m, 2H, $NpCH_2C\underline{H}_2$), 1.679 (br, 1H, $NCHC\underline{H}_2$), 1.588 (br, 1H, $NCHC\underline{H}_2$), 1.396 (s, 9H, CCH_3), 1.006-0.787 (0.939 and 0.901) (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.229-0.125 (0.185) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 593.26 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{37}N_2O_4F_3Na$: 593.2598 [$(M+Na)^+$], found: 593.2591.

Mosher amide **S1-13**

To a solution of diprotected amine **1-66** (7.69 mg, 0.0137 mmol) in MeOH (1.0 ml) was added K_2CO_3 (2.27 mg, 0.0164 mmol, 1.2 equiv). After 22 h at rt, the reaction mixture was concentrated *in vacuo* and the crude product was purified by NH-silica gel column chromatography (*n*-hexane/ AcOEt 1:1-0:1) to yield the corresponding amine as a white amorphous solid.

To a solution of (*S*)-MTPA (9.62 mg, 0.0411 mmol, 3.0 equiv) in DMF (1.0 ml) was added HBTU (14.0 mg, 0.0370 mmol, 2.7 equiv) and DIEA (5.8 μl, 0.0411 mmol, 3.0 equiv). After 5 min at rt, a solution of the aforementioned amine in DMF (1.0 ml) was added and the resulting mixture was stirred for 21 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 5:1) to yield Mosher amide **S1-13** (3.50 mg, 0.00629 mmol, 2 steps 46%) as a white amorphous solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.569 (br, 1H, amide NH), 8.084 (d, *J* = 8.2 Hz, 1H, aromatic), 7.777 (d, *J* = 8.2 Hz, 1H, aromatic), 7.708-7.320 (7.514) (m, 10H, aromatic), 5.680-5.548 (5.624) (m, 1H, NCH), 4.658 (br, 1H, carbamate NH), 3.500 (s, 3H, OCH_3), 2.786 (br, 2H, $NpCH_2$), 2.130 (br, 1H, cyclopropyl CH), 1.741 (br, 2H, $NCHC\underline{H}_2$), 1.488 (s, 9H, CCH_3), 0.811-0.719 (0.770) (m, 1H, cyclopropyl CH_2), 0.580 (br, 1H, cyclopropyl CH), 0.000 (br, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 579.24 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{31}H_{35}N_2O_4F_3Na$: 579.2441 [$(M+Na)^+$], found: 579.2436.

Mosher amide **S1-14**

Mosher amide **S1-14** (3.70 mg, 0.00665 mmol 2 steps 51%, a colorless viscous oil) was prepared from diprotected amine **1-67** (7.34 mg, 0.0130 mmol) as described for Mosher amide **S1-13**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.169 (br, 1H, amide NH), 7.966 (br, 1H, aromatic), 7.864-7.784 (7.819) (m, 1H, aromatic), 7.720-7.226 (7.473) (m, 10H, aromatic), 5.754 (br, 1H, NCH), 5.175 (br, 1H, carbamate NH), 3.306 (br, 3H, OCH_3), 2.701 (br, 2H, $NpCH_2$), 2.329 (br, 1H, cyclopropyl CH), 2.211 (br, 1H, $NCHC\underline{H}_2$), 2.049 (br, 1H, $NCHC\underline{H}_2$), 1.472 (s, 9H, CCH_3), 0.782 (br, 1H, cyclopropyl CH), 0.679 (br, 1H, cyclopropyl CH_2), 0.042 (br, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 579.24 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{31}H_{35}N_2O_4F_3Na$: 579.2441 [$(M+Na)^+$], found: 579.2436.

Mosher amide **S1-15**

Mosher amdie **S1-15** (5.30 mg, 0.00929 mmol, 2 steps 68%, a colorless viscous oil) was prepared from diprotected amine **1-68** (7.88 mg, 0.0137 mmol) as described for Mosher amide **S1-13**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.989 (d, J = 8.2 Hz, 1H, aromatic), 7.887-7.824 (7.853) (m, 1H, aromatic), 7.714 (d, J = 8.6 Hz, 1H, aromatic), 7.612 (br, 2H, aromatic), 7.552-7.300 (7.441) (m, 8H, aromatic and amide NH), 4.709 (br, 1H, carbamate NH), 4.326-4.178 (4.258) (m, 1H, NCH), 3.513 (s, 3H, OCH_3), 3.257-3.033 (3.187 and 3.094) (m, 2H, $NpCH_2$), 2.338 (br, 1H, cyclopropyl CH), 2.022 (br, 1H, $NpCH_2C\underline{H}_2$), 1.958-1.820 (1.893) (m, 1H, $NpCH_2C\underline{H}_2$), 1.574 (br, 2H, $NCHCH_2$), 1.427 (s, 9H, CCH_3), 0.854-0.754 (0.804) (m, 1H, cyclopropyl CH_2), 0.721-0.601 (0.664) (m, 1H, cyclopropyl CH), 0.093-0.009 (0.048) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 593.26 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{37}N_2O_4F_3Na$: 593.2598 [$(M+Na)^+$], found: 593.2593.

Mosher amide **S1-16**

Mosher amide **S1-16** (6.50 mg, 0.0114 mmol, 2 steps 80%, a colorless viscous oil) was prepared from diprotected amine **1-69** (8.22 mg, 0.0143 mmol) as described for Mosher amide **S1-16**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.839-7.735 (7.783) (m, 3H, aromatic), 7.649-7.553 (7.601) (m, 3H, aromatic), 7.484-7.288 (7.386) (m, 7H, aromatic and amide NH), 4.722 (br, 1H, carbamate NH), 4.244-4.095 (4.178) (m, 1H, NCH), 3.482 (s, 3H, OCH_3), 2.898-2.776 (2.838) (m, 2H, $NpCH_2$), 2.323 (br, 1H, cyclopropyl CH), 2.063-1.816 (1.970 and 1.903) (m, 2H, $NpCH_2C\underline{H}_2$), 1.596 (br, 2H, $NCHCH_2$), 1.439 (s, 9H, CCH_3), 0.848-0.736 (0.802) (m, 1H, cyclopropyl CH_2), 0.719-0.577 (0.655) (m, 1H, cyclopropyl CH), 0.106--0.046 (0.036) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 593.26 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{37}N_2O_4F_3Na$: 593.2598 [$(M+Na)^+$], found: 593.2594.

Proteasomes assay

To assess the effect of belactosin derivatives on proteasome, human 20S proteasome (Boston Biochem) were incubated with several inhibitors at 25 °C for 60 min followed by the assay for proteolysis activity with the fluorogenic substrates Suc-LLVY-amc (chymotrypsin-like), Ac-RLR-amc (trypsin-like), and Ac-nLPnLD-amc (caspase-like) as described by Kisselev et al.[94]

Proteasome active site enzyme-linked immunosorbent assay

To determine the proteasome active site selectivity of belactosin derivatives, the active-site ELISA analysis were performed as previously described by Muchamuel et al.[4a] Briefly, 20 nM 20S proteasome or 20 nM 20S

immunoproteasome (Boston Biochem) was preincubated for 5 min in the absence or presence of belactosin derivatives. They were incubated for 1 h with 22.5 nM biotin-(CH2)4-Leu-Leu-Leu-epoxyketone. The samples were denatured by addition of SDS (0.9% final) and heating at 95^{o}C for 5 min. The denatured samples were transferred to a 96-well filter plate (Multiscreen DV; Millipore, Billerica, MA), mixed with streptavidin-sepharose beads (2.5 µl packed beads/well), and incubated for 1 h at 25 °C. The beads were washed 5 times with 150 µl/well of wash buffer (PBS, 1% bovine serum albumin, 0.1% Tween-20) by vacuum filtration. The beads were incubated overnight at 4 °C with the following antibodies diluted into washed buffer: β5 and LMP7 diluted 1:300. The beads were washed 5 times with 150 µl/well of wash buffer and incubated with HRP-conjugated secondary antibody (goat anti-rabbit for β5, goat anti-mouse for LMP7) diluted 1:5000 in wash buffer and incubated 2 h at room temperature. The beads were washed 5 times with 200 µl of wash buffer and developed for chemiluminescence signal using the ECL Plus Western Blotting Detection System (GE Healthcare Life Sciences) following the manufacturer's instructions. For proteasome inhibitor studies, active site probe binding values were represented as the percent of binding relative to DMSO treated sample.

Specificity assay

Inhibitors were incubates with caspase3, cathepsin B, or trypsin in the presence of appropriate fluorogenic peptide substrate at 37 °C for 60 min under the conditions described previously.[32b]

Cell proliferation assay

Cell counting kit-8 (Dojindo Molecular Technologies, Inc.) was used to determine the effects of test compounds on cell proliferation according to the manufacturer's protocol. HCT-116 cells were seeded at 10000 cells/well in a 96-well microtiter plate and treated with belactosin derivatives for 72 h.

Cell cycle analysis

To determine the effect of belactosin derivatives on cell cycle distribution, HCT116 cells (5* 10^5 cells/10-cm dish) were treated with test compounds for 24 h. Thecells were harvested, stained with propidium iodide. DNA content was analyzed by the flowcytometry.

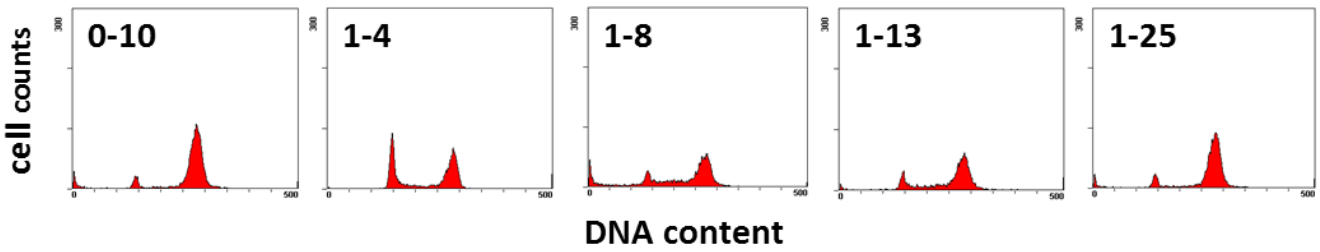


Figure S1-2. Cell cycle distribution analysis of HCT116 cells treated with **0-10**, **1-4**, **1-8**, **1-13** and **1-25**

Immunoblot analysis

The cells treated with belactosin derivativeswere washed twice with phosphate-buffered saline (PBS) and resuspended in 100 μl of RIPA buffer containing protein inhibitor cocktail (Roche Diagnostics, Berkeley, CA). The cell lysates were prepared and subjected to immunoblot analysis using anti-cPARP antibody or anti-p53 antibody.

Stability Testing of 1-79

0.1 M TEAA buffer

Solutions of **1-79** in DMSO (50 μl, 7.5 mM) were diluted with 0.1 M TEAA buffer (950 μl, pH 7.4) and the resulting mixtures were incubated at 37 °C. The time courses were analyzed by RP-HPLC (Mightysil RP-18 GP 250-4.6 (5 μm), MeOH/ H_2O/ TFA 60:40:0.1, 0.8 ml/ min, rt, 210 nm, 15.1 min).

Human AB serum

Solution of **1-79** in DMSO (50 μl, 23 mM) was diluted with human AB serum (950 μl, Sigma-Aldrich) and the resulting mixtures were incubated at 37 °C. At various time points from 0 to 60 min, the reaction mixtures (100 μl) were sampled, which were immediately quenched with CH_3CN (300 μl). The resulting mixtures were centrifuged (10,000 rpm) for 2 min at 4 °C and the supernatants were analyzed by RP-HPLC (Mightysil RP-18 GP 250-4.6 (5 μm), MeOH/ H_2O/ TFA 60:40:0.1, 0.8 ml/ min, rt, 210 nm, 15.1 min).

Reversibility assay

For each reversibility assay, 15 nM 20S proteasome was pre-incubated with each inhibitor at 25 °C for 12 h. 37.5 μM Suc-LLVY-AMC was then added as substrate to start 20S proteasome "standard assay" reaction. After incubation at 25 °C for 1 h, the fluorescence [ex: 390 nm, em: 450 nm] of each reaction was measured by VersaMax Pro microplate reader (Molecular Devices). For each "wash-out assay" reaction, to remove inhibitors unbound to 20S proteasome before beginning the reaction with the substrate, each pre-incubated reaction mixture was loaded onto Microcon® Centrifugal Filter (100 kDa molecular-weight cut off) and washed five times with the assay buffer by centrifuging at 11,000 xg for 5 min. [48]

X-ray crystallographic analysis of proteasome in complex with 1-18

Crystals of the 20S proteasome from *Saccharomyces cerevisiae* were grown in hanging drops at 24 °C .[95] The protein concentration used for crystallization was 40 mg/ml in 10 mM Tris·HCl (pH 7.5) and 1 mM EDTA. Drops were performed by mixing 3 μl of protein and 2 μl of reservoir solution [30 mM MgOAc, 100 mM Mes (pH 7.2), 10% of 2-methyl-2,4-pentanediol (MPD)]. Crystals appeared after two days with a final size of approximately 50×200×500 μm^3 and were incubated at least for 24 h with compound **1-18** (50 mM in DMSO). Subsequently, crystals were soaked in a cryoprotecting buffer (30% MPD_20 mM MgOAc_100 mM Mes, pH 6.9) and cooled in a stream of liquid nitrogen gas at 100 K (Oxford Cryosystems, Oxford, U.K.). Data to 2.8 Å were collected by using synchrotron radiation with λ = 1.0 Å at the X06SA-beamline in SLS/Villingen/Switzerland (Table S1). The X-ray intensities and data reduction

were evaluated by using the XDS program package[96] resulting space group $P2_1$ with cell dimensions of a = 137 Å, b = 302 Å, c = 146 Å, and β = 114°. The anisotropy of diffraction was corrected by an overall anisotropic temperature factor, comparing observed and calculated structure amplitudes by using the program CNS.[97] Electron density was improved by averaging and back transforming the reflections 10 times over the two fold non-crystallographic symmetry axis using the program package MAIN.[98] Conventional crystallographic rigid body, positional and temperature factor refinements were carried out with CNS using coordinates of the yeast 20S proteasome structure as a starting model.[99] For model building the program MAIN was used. The structure was refined to a crystallographic R-factor of 17.2% (free R-factor 22.4%) with rms deviations from target values of 0.013 Å for bonds and 1.66° for angles (Table S1).

Table S1-1. Data collection and refinement statistics

	proteasome:**1-18**
Crystal parameters	
Space group	$P2_1$
Cell constants	a=137.1 Å, b= 301.7 Å, c=146.2 Å, β = 113.7°
Molecules per AU[a]	1
Data collection	
Beamline	SLS, X06DA
Wavelength (Å)	1.0
Resolution range (Å)[b]	40-2.8 (2.9-2.8)
No. observations	875510
No. unique reflections[c]	262724
Completeness (%)[b]	98.6 (92.0)
R_{merge} (%)[b, d]	11.2 (47.4)
I/σ (I)[b]	10.0 (2.6)
Refinement (REFMAC5)	
Resolution range (Å)	15-2.8
No. of reflections	249586
No. atoms	
Protein	49548
Inhibitor	82
Water	1339
R_{work}/R_{free} (%)[e]	0.172 /0.224
R.m.s.deviations[f]	
bond lengths (Å) (°)	0.013/1.657

Average B-factor (Å^2)	51.4
Ramachandran Plot (%)[g]	96.5/3.2/0.3
PDB accession code	4J70

[a] Asymmetric unit

[b] The values in parentheses of resolution range, completeness, R_{merge} and I/σ (I) correspond to the last resolution shell

[c] Friedel pairs were treated as different reflections

[d] $R_{merge}(I) = \Sigma_{hkl}\Sigma_j |[I(hkl)_j - I(hkl)]| / [\Sigma_{hkl} I_{hkl}$, where $I(hkl)_j$ is the measurement of the intensity of reflection hkl and <I(hkl)> is the average intensity

[e] $R = \Sigma_{hkl} | |F_{obs}| - |F_{calc}| | / \Sigma_{hkl} |F_{obs}|$, where R_{free} is calculated without a sigma cut off for a randomly chosen 5% of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections

[f] Deviations from ideal bond lengths/angles

[g] Number of residues in favoured region / allowed region / outlier region

Combustion analysis data for the target compounds.

Table S1-2. Table listing combustion analysis data for the target compounds

1-1	Anal. calcd for $C_{19}H_{30}N_2O_4 \cdot 0.5H_2O$: C, 63.49; H, 8.69; N, 7.79.	Found: C, 63.23; H, 8.44; N, 7.56.
1-2	Anal. calcd for $C_{20}H_{32}N_2O_4$: C, 65.91; H, 8.85; N, 7.69.	Found: C, 66.05; H, 8.98; N, 7.42.
1-3	Anal. calcd for $C_{22}H_{28}N_2O_4$: C, 68.73; H, 7.34; N, 7.29.	Found: C, 68.64; H, 7.47; N, 7.09.
1-4	Anal. calcd for $C_{26}H_{30}N_2O_4 \cdot 1.2H_2O$: C, 68.46; H, 7.16; N, 6.14.	Found: C, 68.53; H, 6.81; N, 5.93.
1-5	Anal. calcd for $C_{25}H_{33}N_3O_6 \cdot 0.1H_2O$: C, 63.44; H, 7.07; N, 8.88.	Found: C, 63.69; H, 7.11; N, 8.48.
1-6	Anal. calcd for $C_{26}H_{35}N_3O_6 \cdot 0.35H_2O$: C, 63.49; H, 7.32: N, 8.54.	Found: C, 63.77; H, 7.25; N, 8.20.
1-7	Anal. calcd for $C_{29}H_{41}N_3O_6 \cdot 0.5H_2O$: C, 64.90; H, 7.89; N, 7.83.	Found: C, 65.28; H, 7.95; N, 7.41.
1-8	Anal. calcd for $C_{32}H_{39}N_3O_6$: C, 68.43; H, 7.00; N, 7.48.	Found: C, 68.16; H, 6.94; N, 7.25.
1-9	Anal. calcd for $C_{34}H_{40}N_4O_6 \cdot 0.515H_2O$: C, 66.95; H, 6.78; N, 9.18.	Found: C, 67.35; H, 6.49; N, 8.78.
1-10	Anal. calcd for $C_{20}H_{30}F_3N_3O_6 \cdot 0.5H_2O$: C, 50.63; H, 6.59; N, 8.86.	Found: C, 50.55; H, 6.52; N, 8.78.
1-11	Anal. calcd for $C_{20}H_{31}N_3O_5 \cdot 0.65H_2O$: C, 59.29; H, 8.03; N, 10.37.	Found: C, 59.49; H, 7.87; N, 10.08.
1-12	Anal. calcd for $C_{25}H_{33}N_3O_5$: C, 65.91; H, 7.30; N, 9.22.	Found: C, 65.63; H, 7.39; N, 8.86.
1-13	Anal. calcd for $C_{29}H_{35}N_3O_5 \cdot 0.4H_2O$: C, 67.92; H, 7.04; N, 8.19.	Found: C, 68.30; H, 7.05; N, 7.78.
1-14	Anal. calcd for $C_{23}H_{37}N_3O_6 \cdot 0.5H_2O$: C, 59.98; H, 8.32; N, 9.12.	Found: C, 60.27; H, 8.28; N, 9.09.
1-15	Anal. calcd for $C_{26}H_{37}N_3O_6 \cdot 0.3H_2O$: C, 63.34; H, 7.69; N, 8.52.	Found: C, 63.28; H, 7.67; N, 8.25.
1-16	Anal. calcd for $C_{30}H_{37}N_3O_6 \cdot 0.3H_2O$: C, 66.60; H, 7.00; N, 7.77.	Found: C, 66.41; H, 6.99; N, 7.65.
1-17	Anal. calcd for $C_{31}H_{39}N_3O_6 \cdot 2.7H_2O$: C, 62.23; H, 7.48; N, 7.02.	Found: C, 62.04; H, 7.15; N, 6.86.
1-18	Anal. calcd for $C_{32}H_{41}N_3O_6 \cdot 0.3H_2O$: C, 67.54; H, 7.37; N, 7.38.	Found: C, 67.47; H, 7.34; N, 7.27.
1-19	Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.3H_2O$: C, 67.97; H, 7.54; N, 7.21.	Found: C, 67.95; H, 7.53; N, 7.14.
1-20	Anal. calcd for $C_{35}H_{41}N_3O_6 \cdot 1.0H_2O$: C, 68.05; H, 7.02; N, 6.80.	Found: C, 68.42; H, 7.01; N, 6.45.
1-21	Anal. calcd for $C_{35}H_{41}N_3O_6 \cdot 2.5H_2O$: C, 65.20; H, 7.19; N, 6.52.	Found: C, 65.06; H, 6.80; N, 6.15.
1-22	Anal. calcd for $C_{36}H_{43}N_3O_6 \cdot 0.72H_2O$: C, 68.99; H, 7.15; N, 6.70.	Found: C, 69.28; H, 7.14; N, 6.40.

1-23	Anal. calcd for $C_{36}H_{43}N_3O_6 \cdot 0.8H_2O$: C, 68.83; H, 7.16; N, 6.69.	Found: C, 68.67; H, 7.03; N, 6.43.
1-24	Anal. calcd for $C_{32}H_{36}N_2O_4 \cdot 0.6H_2O$: C, 73.43; H, 7.16; N, 5.35.	Found: C, 73.83; H, 7.43; N, 4.99.
1-25	Anal. calcd for $C_{35}H_{41}N_3O_5 \cdot 0.4H_2O$: C, 71.14; H, 7.13; N, 7.11.	Found: C, 71.27; H, 7.24; N, 6.76.

Synthetic procedures for 2-2a – 2-4a

Carboxylic acid **2-21**

To a solution of $MeOCH_2PPh_3Cl$ (23.0 g, 67.0 mmol, 3.5 equiv) in THF (200 ml) was added NaHMDS (30 ml, 57.0 mmol, 3.0 equiv, 1.9 M in THF). After 30 min at 0 °C, a solution of aldehyde **1-27** (6.43 g, 19.0 mmol) in THF (50 ml) was added *via cannula*. After 20 min at 0 °C, the reaction was quenched with sat. NH_4Cl, diluted with water and concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with AcOEt, the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 21:1) to yield the corresponding enol ehter as a colorless oil.

To a solution of the oil in THF (430 ml) was added 12 M HCl (40 ml) at 0 °C. After 1 h at 0 °C, the reaction mixture was neutralized with sat. $NaHCO_3$, diluted with water and concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield the corresponding aldehyde as a colorless oil.

To a solution of the oil in 80% aqueous *t*-BuOH (180 ml) was added NaH_2PO_4-$2H_2O$ (3.98 g, 25.5 mmol, 1.5 equiv), 2-methyl-2-butene (14.4 ml, 136 mmol, 8.0 equiv) and $NaClO_2$ (6.15 g, 68.0 mmol, 4.0 equiv). After 40 min at rt, the reaction mixture was diluted with DCM and washed with 1 M HCl. The water layer was extracted with DCM and the combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 19:1-0:1) to yield carboxylic acid **2-21** (6.30 g, 17.1 mmol, 3 steps 90%) as a pale yellow oil. $[\alpha]^{23}_D$ 2.27 (*c* 0.62, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.9 (br, 1H, COOH), 7.74-7.63 (m, 4H, aromatic), 7.48-7.35 (m, 6H, aromatic), 3.97 (dd, *J* = 11.5, 4.6 Hz, 1H, CH_2O), 3.41 (dd, *J* = 11.5, 9.2 Hz, 1H, CH_2O), 2.51 (dd, *J* = 16.0, 5.2 Hz, 1H, $COCH_2$), 2.41 (dd, *J* = 16.0, 8.0 Hz, 1H, $COCH_2$), 1.25 (br, 2H, cyclopropyl CH and cyclopropyl CH), 1.05 (s, 9H, CCH_3), 0.80-0.72 (m, 1H, cyclopropyl CH_2), 0.10-0.02 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 176.4, 135.6, 135.5, 133.1, 129.8, 127.8, 127.8, 64.4, 34.1, 26.7, 19.1, 17.3, 11.8, 8.7; LRMS (ESI) *m/z* 391.16 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{22}H_{28}O_3SiNa$: 391.1700 [$(M+Na)^+$], found: 391.1698.

Imide **2-16a**

To a solution of carboxylic acid **2-21** (3.24 g, 8.78 mmol) in DCM (85 ml) was added triethylamine (1.34 ml, 9.66 mmol, 1.1 equiv) and PivCl (1.19 ml, 9.66 mmol, 1.1 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was diluted with DCM, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding acid anhydride as a pale yellow oil.

To a solution of (4*R*)-4-benzyl-2-oxazolidinone (4.82 g, 27.2 mmol, 3.2 equiv) in THF (85 ml) was added *n*-BuLi (15.3 ml, 25.5 mmol, 3.0 equiv, 1.67 M in *n*-Hexane) at -78 °C. After 1 h at -78 °C, a solution of the aforementioned acid anhydride in THF (20 ml) was added *via cannula*. After 1 h at -78 °C, the reaction was quenched with AcOH (1.7 ml, 30 mmol, 3.4 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 6:1) to yield imide **2-16a** (3.86 g, 7.31

mmol, 2 steps 83%) as a colorless viscous oil. $[\alpha]^{23}_D$ -26.13 (*c* 0.75, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.74-7.65 (m, 4H, aromatic), 7.45-7.35 (m, 6H, aromatic), 7.35-7.29 (m, 2H, aromatic), 7.29-7.23 (m, 1H, aromatic), 7.23-7.16 (m, 2H, aromatic), 4.72-4.61 (m, 1H, NCH), 4.25-4.09 (m, 2H, NCHC$\underline{H}_2$), 3.84 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2O), 3.60 (dd, *J* = 11.5, 8.0 Hz, 1H, CH_2O), 3.29 (dd, *J* = 13.7, 2.9 Hz, 1H, benzyl CH_2), 3.25 (dd, *J* = 17.8, 6.3 Hz, 1H, $COCH_2$), 2.88 (dd, *J* = 17.8, 8.0 Hz, 1H, $COCH_2$), 2.66 (dd, *J* = 13.7, 10.3 Hz, 1H, benzyl CH_2), 1.40-1.30 (m, 1H, cyclopropyl CH), 1.30-1.20 (m, 1H, cyclopropyl CH), 1.05 (s, 9H, CCH_3), 0.82-0.72 (m, 1H, cyclopropyl CH_2), 0.19-0.10 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.1, 153.4, 135.6, 135.3, 133.9, 133.8, 129.5, 129.4, 128.9, 127.6, 127.6, 127.3, 66.2, 64.0, 55.2, 37.9, 35.1, 27.0, 26.8, 19.2, 17.5, 10.9, 8.7; LRMS (ESI) *m/z* 550.24 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{37}NO_4SiNa$: 550.2384 [$(M+Na)^+$], found: 550.2379.

Imide **2-17a**

To a solution of imide **2-16a** (3.86 g, 7.31 mmol) in THF (80 ml) was added NaHMDS (7.70 ml, 14.6 mmol, 2.0 equiv, 1.9 M in THF) at -78 °C. After 1 h at -78 °C, CH_3I (4.55 ml, 73.1 mmol, 10 equiv) was added and the resulting mixture was stirred at -78 °C for 24 h. The reaction was quenched with AcOH (1.3 ml, 21.9 mmol, 3.0 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 7:1) to yield imide **2-17a** (3.27 g, 6.04 mmol, 83%, dr 97:3) as a colorless viscous oil. $[\alpha]^{24}_D$ -47.68 (*c* 0.92, $CHCl_3$); Spectroscopic data for major rotamer (rotamer ratio 4:1): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.77-7.62 (m, 4H, aromatic), 7.52-7.18 (m, 11H, aromatic), 4.71 (ddd, *J* = 10.0, 6.8, 3.2 Hz, 1H, NCH), 4.25-4.13 (m, 2H, $NCHCH_2$), 3.94-3.83 (m, 1H, CH_2O), 3.60-3.46 (m, 2H, CH_2O and C$\underline{H}CH_3$), 3.30 (dd, *J* = 13.6, 3.2 Hz, 1H, benzyl CH_2), 2.77 (dd, *J* = 13.6, 10.0 Hz, 1H, benzyl CH_2), 1.51 (d, *J* = 6.8 Hz, 3H, CHC$\underline{H}_3$), 1.31-1.18 (m, 2H, cyclopropyl CH and cyclopropyl CH), 1.06 (s, 9H, CCH_3), 0.66-0.55 (m, 1H, cyclopropyl CH_2), 0.05--0.04 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.5, 153.1, 135.7, 135.6, 135.6, 135.5, 129.5, 129.4, 128.9, 127.7, 127.6, 127.6, 66.0, 64.1, 55.4, 38.0, 37.1, 26.9, 20.4, 19.2, 18.4, 18.2, 7.6; Spectroscopic data for minor rotamer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.77-7.62 (m, 4H, aromatic), 7.47-7.18 (m, 9H, aromatic), 7.09-7.03 (m, 2H, aromatic), 4.57-4.47 (m, 1H, NCH), 4.11-4.03 (m, 1H, NCHC$\underline{H}_2$), 4.01-3.94 (m, 1H, NCHC$\underline{H}_2$), 3.75 (dd, *J* = 11.3, 7.2 Hz, 1H, CH_2O), 3.64 (dd, *J* = 11.3, 7.2 Hz, 1H, CH_2O), 3.46-3.35 (m, 1H, C$\underline{H}CH_3$), 3.16 (dd, *J* = 13.1, 3.2 Hz, 1H, benzyl CH_2), 2.10 (dd, *J* = 13.1, 10.9 Hz, 1H, benzyl CH_2), 1.40-1.31 (m, 1H, cyclopropyl CH), 1.30 (d, *J* = 6.8 Hz, 3H, $CHCH_3$), 1.29-1.18 (m, 1H, cyclopropyl CH), 1.04 (s, 9H, CCH_3), 0.86-0.76 (m, 1H, cyclopropyl CH_2), 0.15-0.07 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (500 MHz, $CDCl_3$) δ 177.3, 152.9, 135.8, 135.6, 134.3, 134.2, 134.0, 129.2, 128.8, 127.3, 127.3, 127.1, 66.0, 64.5, 55.5, 53.3, 38.1, 37.9, 27.1, 19.3, 18.9, 18.9, 8.8; LRMS (FAB) *m/z* 542.3 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{33}H_{40}NO_4Si$: 542.2727 [$(M+H)^+$], found: 542.2739.

Aldehyde **2-22a**

To a solution of imide **2-17a** (3.27 g, 6.04 mmol, dr 97:3) in DCM (120 ml) was added DIBAL (12.0 ml, 12.1 mmol, 2.0 equiv, 1.0 M in toluene) at -78 °C. After 20 min at -78 °C, the reaction was quenched with MeOH (4.0 ml) and sat. Rochelle's salt (80 ml) was added. The resulting mixture was warmed to rt and vigorously stirred for 1 h. The mixture was diluted with DCM, washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 19:1) to yield the title compound **2-22a** (1.54 g, 4.20 mmol, 69%, dr 97:3) as a colorless oil. $[\alpha]^{24}_D$ -76.03 (*c* 1.17, $CHCl_3$); ^{1}H-NMR (500

MHz, $CDCl_3$) δ 9.27 (d, *J* = 1.7 Hz, 1H, aldehyde H), 7.73-7.64 (m, 4H, aromatic), 7.46-7.35 (m, 6H, aromatic), 3.88 (dd, *J* = 11.5, 5.2 Hz, 1H, CH_2O), 3.49 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 1.95-1.86 (m, 1H, C<u>H</u>CH_3), 1.29 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.28-1.19 (m, 1H, cyclopropyl CH), 1.06 (s, 9H, CCH_3), 0.87-0.72 (m, 2H, cyclopropyl CH and cyclopropyl CH_2), 0.22-0.14 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 204.7, 135.6, 135.5, 133.6, 133.6, 129.7, 127.7, 127.6, 63.5, 46.2, 26.8, 19.2, 17.3, 16.7, 14.2, 7.5; LRMS (ESI) *m/z* 389.1 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{23}H_{30}O_2SiNa$: 389.1913 [$(M+Na)^+$], found: 389.1910.

Imine **2-18a**

To a solution of aldehyde **2-22a** (1.46 g, 3.99 mmol, dr 97:3) in DCM (40 ml) was added (*S*)-*t*-Bu$SONH_2$ (967 mg, 7.98 mmol, 2.0 equiv) and $CuSO_4$ (6.37 g, 39.9 mmol, 10 equiv). After 22 h at rt, the reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield imine **2-18a** (1.80 g, 3.83 mmol, 96%, dr 97:3) as a colorless viscous oil. $[\alpha]^{24}_D$ 21.03 (*c* 0.67, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.05 (d, *J* = 5.2 Hz, 1H, NCH), 7.74-7.64 (m, 4H, aromatic), 7.47-7.36 (m, 6H, aromatic), 3.88 (dd, *J* = 11.5, 5.2 Hz, 1H, CH_2O), 3.55 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 2.25-2.14 (m, 1H, C<u>H</u>CH_3), 1.38 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.30-1.16 (m, 10H, cyclopropyl CH and $SCCH_3$), 1.06 (s, 9H, $SiCCH_3$), 0.96-0.84 (m, 1H, cyclopropyl CH), 0.71-0.61 (m, 1H, cyclopropyl CH_2), 0.10-0.02 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 135.6, 135.5, 133.7, 133.7, 129.6, 127.6, 127.6, 63.6, 56.4, 40.2, 26.8, 22.3, 20.2, 19.2, 18.0, 17.4, 7.9; LRMS (ESI) *m/z* 470.3 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{27}H_{40}NO_2SSi$: 470.2549 [$(M+H)^+$], found: 470.2560.

Alcohol **2-23a**

To a solution of imine **2-18a** (1.80 g, 3.83 mmol, dr 97:3) in DCM (400 ml) was added phenethylmagnesium chloride (7.66 ml, 7.66 mmol, 2.0 equiv, 1.0 M in THF). After 40 min at rt, the reaction mixture was cooled to 0 °C and poured into ice-cold sat. NH_4Cl with stirring. The resulting mixture was diluted with water and concentrated *in vacuo* to remove DCM. The residual aqueous solution was extracted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 5:1-4:1) to yield the corresponding sulfinamide as a colorless viscous oil.

To a solution of the oil in MeOH (30 ml) was added 4 M HCl/ AcOEt (3.0 ml). After 5 h at rt, the reaction mixture was concentrated *in vacuo* to give the corrresponding amino alcohol as a yellow oil.

To a solution of the oil in 50% aqueous THF (30 ml) was added FmocOSu (1.35 g, 4.00 mmol, 1.2 equiv) and Na_2CO_3 (353 mg, 3.33 mmol, 1.0 equiv). After 12 h at rt, the reaction mixture was concentrated *in vacuo* to remove THF. The resulting aqueous solution was extracted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 4:1) to yiled alcohol **2-23a** (1.15 g, 2.53 mmol, 3 steps 66%, single isomer) as a white solid. $[\alpha]^{25}_D$ -7.54 (*c* 0.61, $CHCl_3$); mp 74-75 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.74 (d, *J* = 8.0 Hz, 2H, aromatic), 7.64-7.51 (m, 2H, aromatic), 7.42-7.33 (m, 2H, aromatic), 7.33-7.20 (m, 4H, aromatic), 7.20-7.05 (m, 3H, aromatic), 4.55 (br, 1H, carbamate NH), 4.55-4.42 (m, 2H, Fmoc CH_2), 4.26-4.15 (m, 1H, Fmoc CH), 3.75 (br, 1H, NCH), 3.62 (br, 1H, C<u>H</u>$_2$OH), 3.54 (br, 1H, C<u>H</u>$_2$OH), 2.62 (br, 2H, benzyl CH_2), 1.86 (br, 1H, $BnCH_2$), 1.65 (br, 1H, $BnCH_2$), 1.20 (br, 2H, C<u>H</u>CH_3 and OH), 1.08 (br, 1H, cyclopropyl CH), 0.96 (br, 3H, CH_3), 0.72 (br, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.04 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 156.3, 144.1, 141.9, 141.5,

128.4, 128.4, 127.7, 127.7, 127.0, 127.0, 125.9, 124.9, 120.0, 66.2, 62.8, 56.0, 47.7, 37.6, 34.7, 32.9, 20.4, 17.6, 16.1, 9.4; LRMS (ESI) *m/z* 478.23 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{30}H_{33}NO_3Na$: 478.2353 [$(M+Na)^+$], found: 478.2346.

Diprotected amine **2-20a**

To a solution of alcohol **2-23a** (1.14 g, 2.51 mmol) in DCM (50 ml) was added DMP (1.60 g, 3.76 mmol, 1.5 equiv). After 30 min at rt, the reaction was quenched with a solution of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (1:3), extracted with $CHCl_3$, the organic layer was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding aldehyde as a white amorphous solid.

To a solution of the foam-like solid in THF (20 ml) was added 67% aqueous *t*-BuOH (30 ml), NaH_2PO_4-$2H_2O$ (587 mg, 3.76 mmol, 1.5 equiv), 2-methyl-2-butene (2.13 ml, 20.1 mmol, 8.0 equiv) and $NaClO_2$ (908 mg, 10.0 mmol, 4.0 equiv). After 14 h at rt, the reaction mixture was diluted with DCM, washed with 1 M HCl, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a yellow oil.

To a solution of the oil in DCM (50 ml) was added triethylamine (1.40 ml, 10.0 mmol, 4.0 equiv) and DPPA (1.62 ml, 2.07 g, 7.53 mmol, 3.0 equiv) at 0 °C. After 20 min at 0 °C, the reaction mixture was warmed to rt and stirred for 17 h. The reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 5:1) to yield the corresponding acyl azide as a yellow oil.

A solution of the oil in *t*-BuOH (30 ml) was heated under reflux for 29 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 4:1) to yield diprotected amine **2-20a** (973 mg, 1.80 mmol, 4 steps 72%) as a white amorphous solid. $[\alpha]^{25}_D$ -35.98 (*c* 0.65, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.74 (d, *J* = 8.0 Hz, 2H, aromatic), 7.58 (d, *J* = 7.4 Hz, 2H, aromatic), 7.42-7.33 (m, 2H, aromatic), 7.33-7.20 (m, 4H, aromatic), 7.20-7.06 (m, 3H, aromatic), 4.64-4.43 (m, 3H, Fmoc CH_2 and carbamate NH), 4.38 (br, 1H, carbamate NH), 4.26-4.15 (m, 1H, Fmoc CH), 3.75 (br, 1H, NCH), 2.67 (br, 1H, cyclopropyl CH), 2.61 (br, 2H, benzyl CH_2), 1.82 (br, 1H, $BnCH_2$), 1.65 (br, 1H, $BnCH_2$), 1.45 (s, 9H, CCH_3), 1.18 (br, 1H, C<u>H</u>CH_3), 0.94 (br, 3H, CH_3), 0.88 (br, 1H, cyclopropyl CH_2), 0.65-0.54 (m, 1H, cyclopropyl CH), 0.12 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 156.7, 156.2, 144.1, 144.1, 141.8, 141.5, 141.5, 128.4, 128.3, 127.7, 127.7, 127.1, 127.0, 125.9, 124.9, 124.9, 120.0, 119.9, 79.5, 66.0, 55.7, 47.7, 36.8, 34.8, 32.9, 28.4, 27.2, 21.2, 15.2, 11.5, 6.5; LRMS (ESI) *m/z* 563.29 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{34}H_{40}N_2O_4Na$: 563.2880 [$(M+Na)^+$], found: 563.2872.

Carbamate **2-24a**

To a solution of Cbz-Ala (79.7 mg, 0.357 mmol, 3.0 equiv) in DCM (3.5 ml) was added triethylamine (50 µl, 0.357 mmol, 3.0 equiv) and PivCl (44 µl, 0.357 mmol, 3.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding acid anhydride as a colorless oil.

To a solution of diprotected amine **2-20a** (64.3 mg, 0.119 mmol, 1.0 equiv) in MeOH (1.5 ml) was added K_2CO_3 (32.9 mg, 0.238 mmol, 2.0 equiv). After 14 h at rt, the reaction mixture was concentrated *in vacuo* and the crude product was purified by NH-silica gel column chromatography (*n*-hexane/ AcOEt/ MeOH 3:1:0-0:1:1) to yield the corresponding amine as a pale yellow oil.

To a solution of the oil in DCM (1.0 ml) was added triethylamine (50 µl, 0.357 mmol, 3.0 equiv) and a solution of

the aforementioned acid anhydride in DCM at 0 °C. The reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:2) to yield carbamate **2-24a** (62.3 mg, 0.119 mmol, 2 steps 100%) as a colorless viscous oil. $[\alpha]^{24}_D$ -46.74 (*c* 0.57, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.42-7.22 (m, 7H, aromatic), 7.22-7.06 (m, 3H, aromatic), 7.75 (d, *J* = 8.0 Hz, 1H, amide NH), 5.57 (d, *J* = 5.7 Hz, 1H, carbamate NH), 5.09 (s, 2H, Cbz CH_2), 4.66 (br, 1H, carbamate NH), 4.27-4.15 (m, 1H, Ala α-H), 4.09-4.00 (m, 1H, NCH), 2.72-2.52 (m, 3H, cyclopropyl CH and benzyl CH_2), 1.92-1.81 (m, 1H, $BnCH_2$), 1.74-1.61 (m, 1H, $BnCH_2$), 1.43 (s, 9H, CCH_3), 1.39 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.31-1.18 (m, 1H, C<u>H</u>CH_3), 0.96 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 0.91-0.79 (m, 1H, cyclopropyl CH_2), 0.65-0.52 (m, 1H, cyclopropyl CH), 0.28 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.9, 156.7, 156.1, 141.7, 136.0, 128.5, 128.4, 128.3, 128.2, 128.2, 128.0, 125.9, 79.4, 66.9, 53.6, 50.8, 36.4, 34.1, 32.9, 28.3, 26.8, 20.7, 18.3, 15.5, 11.1; LRMS (ESI) 546.29 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{30}H_{41}N_3O_5Na$: 546.2938 [$(M+Na)^+$], found: 546.2933.

Target compound **2-2a**

To a solution of carbamate **2-24a** (63.5 mg, 0.121 mmol) in DCM (600 μl) was added TFA (600 μl). After 1 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow oil.

To a solution of carboxylic acid **1-35** (41.7 mg, 0.242 mmol, 2.0 equiv) in DCM (2.4 ml) was added triethylamine (34 μl, 0.242 mmol, 2.0 equiv) and PivCl (30 μl, 0.242 mmol, 2.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (51 μl, 0.364 mmol, 3.0 equiv) and a solution of the acid anhydride in DCM at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 19 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 3:2) to yield target compound **2-2a** (57.8 mg, 0.100 mmol, 2 steps 82%) as a white solid. $[\alpha]^{20}_D$ -56.04 (*c* 0.61, $CHCl_3$); mp 63-65 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.39-7.23 (m, 7H, aromatic), 7.23-7.10 (m, 3H, aromatic), 6.35 (d, *J* = 3.4 Hz, 1H, amide NH), 6.12 (d, *J* = 8.6 Hz, 1H, amide NH), 5.39 (d, *J* = 6.9 Hz, 1H, carbamate NH), 5.11 (s, 2H, Cbz CH_2), 4.60 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.24-4.13 (m, 1H, Ala α-H), 4.13-4.01 (m, 1H, NCH), 3.52 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.95-2.84 (m, 1H, cyclopropyl CH), 2.61 (br, 2H, benzyl CH_2), 2.02-1.92 (m, 1H, C<u>H</u>CH_3), 1.90-1.78 (m, 1H, $BnCH_2$), 1.74-1.57 (m, 2H, $BnCH_2$ and C<u>H$_2$</u>CH_3), 1.39 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.37-1.26 (m, 1H, C<u>H$_2$</u>CH_3), 1.11 (br, 1H, C<u>H</u>CH_3), 1.06 (d, *J* = 6.9 Hz, 3H, CHC<u>H$_3$</u>), 1.03-0.96 (m, 1H, cyclopropyl CH_2), 0.93 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>), 0.92 (d, *J* = 6.9 Hz, 3H, CHC<u>H$_3$</u>), 0.80-0.70 (m, 1H, cyclopropyl CH), 0.52-0.39 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.9, 169.5, 169.1, 156.1, 141.5, 136.0, 128.5, 128.4, 128.3, 128.2, 128.1, 126.0, 70.5, 67.1, 62.9, 53.3, 50.8, 36.9, 34.3, 33.6, 32.9, 26.6, 26.3, 20.9, 18.2, 16.2, 15.4, 11.2, 11.0; LRMS (ESI) *m/z* 600.30 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{43}N_3O_6Na$: 600.3044 [$(M+Na)^+$], found: 600.3042.

Target compound **2-4a**

A solution of target compound **2-2a** (36.2 mg, 0.0627 mmol) in THF (1.0 ml) was cooled to 0 °C. TFA (1.0 ml) and Pd/C (35 mg) was added and the flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 1 h. The reaction mixture was filtered through a Celite pad and the filtrate

was concentrated *in vacuo* to give the corresponding amine as a colorless oil.

To a solution of the oil in DCM (2.0 ml) was added triethylamine (29 µl, 0.209 mmol, 3.0 equiv) and 2-naphthoyl chloride (16.0 mg, 0.0837 mmol, 1.2 equiv) at 0 °C. After 1 h at 0 °C, the reaction mxiture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 5:4) to yield target compound **2-4a** (29.1 mg, 0.0487 mmol, 2 steps 70%) as a white solid. $[\alpha]^{20}_D$ -33.69 (*c* 0.28, $CHCl_3$); mp 79-81 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H, aromatic), 7.92-7.80 (m, 4H, aromatic), 7.62-7.48 (m, 2H, aromatic), 7.31-7.15 (m, 6H, aromatic and amide NH), 6.88 (d, *J* = 9.7 Hz, 1H, amide NH), 6.38 (d, *J* = 4.6 Hz, 1H, amide NH), 4.93-4.82 (m, 1H, Ala α-H), 4.52 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.17-4.05 (m, 1H, NCH), 3.48 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.88-2.78 (m, 1H, cyclopropyl CH), 2.78-2.68 (m, 1H, benzyl CH_2), 2.68-2.58 (m, 1H, benzyl CH_2), 1.97-1.85 (m, 2H, C<u>H</u>CH_3 and BnCH_2), 1.85-1.73 (m, 1H, BnCH_2), 1.65-1.52 (m, 4H, Ala CH_3 and C<u>H$_2$</u>CH_3), 1.34-1.19 (m, 1H, CH_2CH_3), 1.19-1.09 (m, 1H, C<u>H</u>CH_3), 1.05-0.95 (m, 1H, cyclopropyl CH_2), 1.00 (d, *J* = 6.3 Hz, 3H, CHC<u>H</u>$_3$), 0.93 (d, *J* = 6.9 Hz, 3H, $CHCH_3$), 0.88 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2CH_3), 0.83-0.72 (m, 1H, cyclopropyl CH), 0.53-0.45 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.1, 169.5, 169.2, 167.5, 141.5, 134.9, 132.5, 130.7, 129.0, 128.6, 128.4, 128.3, 127.9, 127.7, 127.7, 126.9, 126.0, 123.4, 70.5, 62.8, 53.5, 49.7, 37.0, 34.2, 33.6, 33.0, 26.6, 26.3, 20.9, 18.7, 16.2, 15.6, 11.3, 11.0; LRMS (ESI) *m/z* 620.31 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{36}H_{43}N_3O_5Na$: 620.3095 [$(M+Na)^+$], found: 620.3097.

<u>Carbamate **2-25a**</u>

To a solution of diprotected amine **2-20a** (67.2 mg, 0.124 mmol) in MeOH (3.0 ml) was added K_2CO_3 (34.3 mg, 0.248 mmol). After 14 h at rt, the reaction mixture was concentrated *in vacuo* and the crude product was purified by NH-silica gel column chromatography (*n*-hexane/ AcOEt/ MeOH 3:1:0-0:1:1) to yield the corresponding amine as a pale yellow oil.

To a solution of the oil in DCM (3.0 ml) was added triethylamine (17 µl, 0.149 mmol, 1.2 equiv) and 2-Naphthoyl chloride (28.4 mg, 0.149 mmol, 1.2 equiv) at 0 °C. After 2 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 4:1) to yield carbamate **2-25a** (58.7 mg, 0.124 mmol, 2 steps 100%) as a white solid. $[\alpha]^{23}_D$ -40.46 (*c* 1.03, $CHCl_3$); mp 90-92 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H, aromatic), 7.95-7.82 (m, 3H, aromatic), 7.82-7.75 (m, 1H, aromatic), 7.61-7.48 (m, 2H, aromatic), 7.32-7.22 (m, 2H, aromatic), 7.22-7.12 (m, 3H, aromatic), 6.23 (d, *J* = 9.2 Hz, 1H, amide NH), 4.60 (d, *J* = 2.3 Hz, 1H, carbamate NH), 4.48-4.36 (m, 1H, NCH), 2.83-2.63 (m, 3H, benzyl CH_2 and cyclopropyl CH), 2.10-1.97 (m, 1H, BnCH_2), 1.93-1.80 (m, 1H, BnCH_2), 1.54-1.35 (m, 1H, C<u>H</u>CH_3), 1.44 (s, 9H, CCH_3), 1.11 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.06-0.96 (m, 1H, cyclopropyl CH_2), 0.83-0.71 (m, 1H, cyclopropyl CH), 0.31 (br, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.1, 156.8, 141.8, 134.6, 132.6, 132.0, 128.8, 128.5, 128.4, 128.3, 127.7, 127.6, 127.1, 126.7, 125.9, 123.4, 79.5, 54.1, 36.9, 34.2, 33.1, 28.3, 26.8, 20.9, 15.7, 11.6; LRMS (ESI) *m/z* 495.26 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{30}H_{36}N_2O_3Na$: 495.2618 [$(M+Na)^+$], found: 495.2611.

<u>Target compound **2-3a**</u>

To a solution of carbamate **2-25a** (57.1 mg, 0.121 mmol) in DCM (600 µl) was added TFA (600 µl). After 1 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow viscous oil.

To a solution of carboxylic acid **1-35** (41.7 mg, 0.242 mmol, 2.0 equiv) in DCM (2.4 ml) was added triethylamine (34 µl, 0.242 mmol, 2.0 equiv) and PivCl (30 µl, 0.242 mmol, 2.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (50 µl, 0.362 mmol, 3.0 equiv) and a solution of the acid anhydride in DCM at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 19 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 2:1) to yield target compound **2-3a** (58.0 mg, 0.110 mmol, 2 steps 91%) as a white solid. $[\alpha]^{19}_D$ -47.12 (*c* 1.58, $CHCl_3$); mp 78-80 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H, aromatic), 7.95-7.82 (m, 3H, aromatic), 7.78 (dd, *J* = 8.6, 1.7 Hz, 1H, aromatic), 7.61-7.51 (m, 2H, aromatic), 7.31-7.22 (m, 2H, aromatic), 7.22-7.12 (m, 3H, aromatic), 6.39 (d, *J* = 4.0 Hz, 1H, amide NH), 6.17 (d, *J* = 9.7 Hz, 1H, amide NH), 4.61 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.49-4.40 (m, 1H, NCH), 3.54 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 3.00-2.93 (m, 1H, cyclopropyl CH), 2.80-2.70 (m, 2H, benzyl CH_2), 2.08-1.91 (m, 2H, $BnCH_2$ and C<u>H</u>CH_3), 1.91-1.80 (m, 1H, $BnCH_2$), 1.69-1.58 (m, 1H, C<u>H</u>$_2CH_3$), 1.37-1.22 (m, 2H, C<u>H</u>$_2CH_3$ and C<u>H</u>CH_3), 1.22-1.11 (m, 1H, cyclopropyl CH_2), 1.07 (d, *J* = 6.3 Hz, 3H, CHC<u>H</u>$_3$), 1.05 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.00-0.86 (m, 4H, CH_2C<u>H</u>$_3$ and cyclopropyl CH), 0.54-0.46 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.5, 169.1, 167.2, 141.6, 134.7, 132.6, 131.8, 128.8, 128.5, 128.5, 128.3, 127.7, 127.6, 127.1, 126.8, 126.0, 123.4, 70.6, 62.9, 53.8, 37.5, 34.6, 33.6, 33.0, 26.6, 26.4, 21.2, 16.2, 15.6, 11.7, 11.0; LRMS (ESI) *m/z* 549.27 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{38}N_2O_4Na$: 549.2724 [$(M+Na)^+$], found: 549.2719.

Synthetic procedures for 2-2b – 2-4b

<u>Imide **2-16b**</u>

Imide **2-16b** (7.83 mmol, 4.13 g, 2 steps 89%, a colorless viscous oil) was prepared from carboxylic acid **2-21** (3.24 g, 8.78 mmol) as described for imide **2-16a**. $[\alpha]^{23}_D$ 24.77 (*c* 1.05, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.73-7.63 (m, 4H, aromatic), 7.45-7.30 (m, 7H, aromatic), 7.30-7.24 (m, 2H, aromatic), 7.24-7.17 (m, 2H, aromatic), 4.64-4.55 (m, 1H, NCH), 4.11 (dd, *J* = 9.2, 2.9 Hz, 1H, NCHC<u>H</u>$_2$), 4.04 (dd, *J* = 9.2, 8.0 Hz, 1H, NCHC<u>H</u>$_2$), 3.90 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2O), 3.51 (dd, *J* = 11.5, 9.2 Hz, 1H, CH_2O), 3.31 (dd, *J* = 13.7, 3.4 Hz, 1H, benzyl CH_2), 3.15 (dd, *J* = 17.8, 10.3 Hz, 1H, $COCH_2$), 3.00 (dd, *J* = 17.8, 6.3 Hz, 1H, $COCH_2$), 2.76 (dd, *J* = 13.7, 9.7 Hz, 1H, benzyl CH_2), 1.42-1.33 (m, 1H, cyclopropyl CH), 1.33-1.21 (m, 1H, cyclopropyl CH), 1.04 (s, 9H, CCH_3), 0.81-0.73 (m, 1H, cyclopropyl CH_2), 0.18-0.12 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.9, 153.5, 135.5, 135.5, 135.4, 133.8, 133.8, 133.8, 129.6, 129.5, 129.4, 128.9, 127.6, 127.3, 66.1, 64.1, 55.2, 37.8, 34.8, 27.0, 26.8, 19.2, 17.3, 10.8, 8.5; LRMS (ESI) *m/z* 550.24 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{37}NO_4SiNa$: 550.2384 [$(M+Na)^+$], found: 550.2383.

<u>Imide **2-17b**</u>

Imide **2-17b** (3.34 g, 6.17 mmol, 79%, dr 99:1, a white solid) was prepared from imide **2-16b** (4.13 g, 7.83 mmol) as described for imide **2-17a**. $[\alpha]^{23}_D$ 27.12 (*c* 1.52, $CHCl_3$); mp 91-92 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.71-7.63 (m, 2H, aromatic), 7.63-7.55 (m, 2H, aromatic), 7.45-7.23 (m, 9H, aromatic), 7.20-7.13 (m, 2H, aromatic), 4.50-4.39 (m, 1H, NCH), 4.02-3.95 (m, 1H, CH_2O), 3.92 (dd, *J* = 9.1, 2.3 Hz, 1H, NCHC<u>H</u>$_2$), 3.59 (dd, *J* = 9.1, 8.2 Hz, 1H, $NCHCH_2$), 3.48-3.34 (m, 2H, COCH and CH_2O), 3.20 (dd, *J* = 13.1, 2.7 Hz, 1H, benzyl CH_2), 2.69 (dd, *J* = 13.1, 9.5 Hz, 1H,

benzyl CH_2), 1.51-1.41 (m, 1H, cyclopropyl CH), 1.38 (d, *J* = 7.2 Hz, 3H, CHC<u>H</u>$_3$), 1.32-1.20 (m, 1H, cyclopropyl CH), 1.01 (s, 9H, CCH_3), 0.78-0.68 (m, 1H, cyclopropyl CH_2), 0.05--0.04 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 176.7, 153.1, 135.4, 135.4, 135.3, 134.2, 133.8, 129.6, 129.5, 129.4, 128.8, 127.7, 127.6, 127.2, 65.7, 64.8, 54.9, 37.7, 37.4, 26.9, 19.2, 19.0, 18.6, 18.5, 7.6; LRMS (ESI) *m/z* 564.25 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{39}NO_4SiNa$: 564.2541 [$(M+Na)^+$], found: 564.2541.

Aldehyde **2-22b**

Aldehyde **2-22b** (1.28 g, 3.48 mmol, 56%, dr 99:1, a colorless oil) was prepared from imide **2-17b** (3.34 g, 6.17 mmol, dr 99:1) as described for aldehyde **2-22a**. $[\alpha]^{24}_D$ 80.17 (*c* 0.85, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.97 (d, *J* = 1.1 Hz, 1H, aldehyde H), 7.72-7.62 (m, 4H, aromatic), 7.46-7.34 (m, 6H, aromatic), 3.92 (dd, *J* = 11.5, 5.2 Hz, 1H, CH_2O), 3.51 (dd, *J* = 11.5, 9.2 Hz, 1H, CH_2O), 2.04-1.94 (m, 1H, C<u>H</u>CH_3), 1.38-1.26 (m, 1H, cyclopropyl CH), 1.15 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.04 (s, 9H, CCH_3), 0.90-0.81 (m, 1H, cyclopropyl CH), 0.79-0.72 (m, 1H, cyclopropyl CH_2), 0.08-0.02 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 205.1, 135.6, 135.5, 133.6, 133.6, 129.6, 127.7, 127.6, 64.1, 45.7, 26.8, 19.1, 18.5, 17.5, 14.1, 7.7; LRMS (ESI) *m/z* 389.19 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{23}H_{30}O_2SiNa$: 389.1907 [$(M+Na)^+$], found: 389.1907.

Imine **2-18b**

Imine **2-18b** (1.40 g, 2.98 mmol, 89%, dr 99:1, a colorless viscous oil) was prepared from aldehyde **2-22b** (1.23 g, 3.36 mmol, dr 99:1) as described for imine **2-18a**. $[\alpha]^{23}_D$ 221.92 (*c* 0.75, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 4.5 Hz, 1H, NCH), 7.75-7.61 (m, 4H, aromatic), 7.46-7.32 (m, 6H, aromatic), 3.76 (dd, *J* = 11.3, 6.8 Hz, 1H, CH_2O), 3.67 (dd, *J* = 11.3, 6.3 Hz, 1H, CH_2O), 2.29-2.15 (m, 1H, C<u>H</u>CH_3), 1.30-1.10 (m, 13H, cyclopropyl CH, $SCCH_3$ and CHC<u>H</u>$_3$), 1.04 (s, 9H, CCH_3), 0.95-0.83 (m, 1H, cyclopropyl CH), 0.79-0.70 (m, 1H, cyclopropyl CH_2), 0.17-0.09 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.7, 135.6, 135.6, 133.8, 133.7, 129.5, 129.5, 127.6, 63.6, 56.4, 39.7, 26.8, 22.3, 20.2, 19.1, 18.5, 17.8, 7.9; LRMS (ESI) *m/z* 470.3 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{27}H_{40}NO_2SSi$: 470.2549 [$(M+H)^+$], found: 470.2542.

Alcohol **2-23b**

Alcohol **2-23b** (876 mg, 1.92 mmol, 3 steps 65%, a white amorphous solid, single isomer) was prepared from imine **2-18b** (1.23 g, 3.36 mmol, dr 99:1) as described for alcohol **2-23a**. $[\alpha]^{25}_D$ -12.72 (*c* 0.86, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.75 (d, *J* = 7.4 Hz, 2H, aromatic), 7.65-7.56 (m, 2H, aromatic), 7.38 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.34-7.20 (m, 4H, aromatic), 7.20-7.10 (m, 3H, aromatic), 5.65 (d, *J* = 8.6 Hz, 1H, carbamate NH), 4.57 (dd, *J* = 10.9, 6.3 Hz, 1H, Fmoc CH_2), 4.52-4.43 (m, 1H, Fmoc CH_2), 8.40 (dd, *J* = 12.0, 6.3 Hz, 1H, Fmoc CH), 3.99-3.86 (m, 1H, C<u>H</u>$_2$OH), 3.63 (br, 1H, NCH), 3.16 (br, 1H, C<u>H</u>$_2$OH), 2.73-2.52 (m, 2H, benzyl CH_2), 2.37 (br, 1H, OH), 1.93 (br, 1H, $BnCH_2$), 1.58-1.42 (m, 1H, $BnCH_2$), 1.81 (br, 1H, C<u>H</u>CH_3), 1.09-0.98 (m, 1H, cyclopropyl CH), 0.94 (d, *J* = 6.9 Hz, 3H, $CHCH_3$), 0.70-0.52 (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), -0.13--0.26 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 156.9, 144.3, 144.2, 142.2, 141.6, 141.4, 128.4, 127.7, 127.6, 127.1, 127.0, 125.8, 125.0, 119.9, 119.9, 65.9, 62.8, 56.6, 47.7, 36.6, 33.5, 32.6, 21.0, 19.1, 16.5, 6.9; LRMS (ESI) *m/z* 478.23 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{30}H_{33}NO_3Na$: 478.2353 [$(M+Na)^+$], found: 478.2346.

Diprotected amine **2-20b**

Diprotected amine **2-20b** (626 mg, 1.16 mmol, 4 steps 62%, a white amorphous solid) was prepared from alcohol **2-23b** (865 mg, 1.88 mmol) as described for diprotected amine **2-20a**. $[\alpha]^{25}_{D}$ -9.44 (*c* 1.16, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.81-7.66 (m, 4H, aromatic), 7.36 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.32-7.20 (m, 4H, aromatic), 7.20-7.09 (m, 3H, aromatic), 6.56 (br, 1H, carbamate NH), 5.09 (br, 1H, carbamate NH), 4.42 (br, 2H, Fmoc CH_2), 4.30-4.18 (m, 1H, Fmoc CH), 3.66-3.53 (m, 1H, NCH), 2.69 (br, 1H, cyclopropyl CH), 2.63 (br, 2H, benzyl CH_2), 1.95 (br, 1H, $BnCH_2$), 1.64-1.37 (m, 10H, $BnCH_2$ and CCH_3), 1.26 (br, 1H, C<u>H</u>CH_3), 1.00 (d, *J* = 6.3 Hz, 3H, CHC<u>H</u>$_3$), 0.92-0.81 (m, 1H, cyclopropyl CH_2), 0.75 (br, 1H, cyclopropyl CH), 0.13-0.03 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$, 328 K) δ 157.4, 157.1, 144.4, 144.3, 142.3, 141.5, 128.4, 127.6, 127.0, 127.0, 125.8, 125.4, 119.8, 79.8, 66.4, 56.3, 47.7, 34.9, 32.4, 28.7, 28.4, 22.3, 17.7, 10.4, 6.5; LRMS (ESI) *m/z* 563.29 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{34}H_{40}N_2O_4Na$: 563.2880 [$(M+Na)^+$], found: 563.2874.

Carbamate **2-24b**

Carbamate **2-24b** (69.2 mg, 0.132 mmol, 2 steps 99%, a colorless viscous oil) was prepared from diprotected amine **2-20b** (72.1 mg, 0.133 mmol) as described for carbamate **2-24a**. $[\alpha]^{24}_{D}$ -33.08 (*c* 0.62, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, 328 K) δ 7.91 (br, 1H, amide NH), 7.39-7.18 (m, 7H, aromatic), 7.18-7.05 (m, 3H, aromatic), 5.91 (br, 1H, carbamate NH), 5.10 (s, 2H, Cbz CH_2), 4.93 (br, 1H, carbamate NH), 4.54-4.39 (m, 1H, Ala α-H), 3.91-3.78 (m, 1H, NCH), 2.67 (br, 1H, cyclopropyl CH), 2.67-2.47 (m, 2H, benzyl CH_2), 2.05-1.92 (m, 1H, $BnCH_2$), 1.58-1.45 (m, 1H, $BnCH_2$), 1.48 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.40 (s, 9H, CCH_3), 1.25-1.13 (m, 1H, C<u>H</u>CH_3), 1.03 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 0.87-0.77 (m, 1H, cyclopropyl CH_2), 0.77-0.63 (m, 1H, cyclopropyl CH), 0.07-0.00 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$, 328 K) δ 172.9, 157.7, 155.7, 142.2, 136.8, 128.4, 128.3, 128.3, 128.0, 127.9, 125.7, 80.4, 66.6, 54.2, 50.9, 36.0, 33.5, 31.9, 29.1, 28.3, 23.1, 19.8, 18.9, 9.4; LRMS (ESI) *m/z* 546.29 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{30}H_{41}N_3O_5Na$: 546.2938 [$(M+Na)^+$], found: 546.2927.

Target compound **2-2b**

Target compound **2-2b** (71.6 mg, 0.124 mmol, 2 steps 94%, a white solid) was prepared from carbamate **2-24b** (69.2 mg, 0.132 mmol) as described for target compound **2-2a**. $[\alpha]^{20}_{D}$ 0.38 (*c* 0.90, $CHCl_3$); mp 58-60 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.45 (br, 1H, amide NH), 7.37-7.23 (m, 7H, aromatic), 7.23-7.10 (m, 3H, aromatic), 7.06 (d, *J* = 7.4 Hz, 1H, amide NH), 5.67 (d, *J* = 6.9 Hz, 1H, carbamate NH), 5.10 (s, 2H, Cbz CH_2), 4.69 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.37-4.26 (m, 1H, Ala α-H), 3.86-3.74 (m, 1H, NCH), 3.67 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 2.89 (br, 1H, cyclopropyl CH), 2.72-2.48 (m, 2H, benzyl CH_2), 2.02-1.88 (m, 2H, $BnCH_2$ and C<u>H</u>CH_3), 1.64-1.46 (m, 2H, C<u>H$_2$</u>CH_3 and $BnCH_2$), 1.41 (d, *J* = 7.4 Hz, 3H, Ala CH_3), 1.32-1.19 (m, 1H, C<u>H$_2$</u>CH_3), 1.13 (br, 1H, C<u>H</u>CH_3), 1.06-0.92 (m, 7H, CHC<u>H</u>$_3$, CHC<u>H</u>$_3$ and cyclopropyl CH_2), 0.92-0.78 (m, 4H, CH_2C<u>H</u>$_3$ and cyclopropyl CH), 0.21-0.12 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 170.4, 169.6, 155.9, 141.4, 136.3, 128.5, 128.5, 128.2, 128.1, 127.9, 126.1, 70.8, 66.8, 62.6, 54.0, 50.5, 35.8, 33.5, 32.8, 27.9, 26.6, 21.3, 19.0, 16.2, 15.8, 11.3, 11.0; LRMS (ESI) *m/z* 600.30 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{43}N_3O_6Na$: 600.3044 [$(M+Na)^+$], found: 600.3041.

Target compound **2-4b**

Target compound **2-4b** (23.7 mg, 0.0397 mmol, 2 steps 56%, a colorless viscous oil) was prepared from target compound **2-2b** (33.2 mg, 0.0575 mmol) as described for target compound **2-4a**. $[\alpha]^{20}_{D}$ 31.98 (*c* 0.45, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.46 (s, 1H, aromatic), 7.94 (dd, *J* = 8.6, 1.7 Hz, 1H, aromatic), 7.90-7.76 (m, 5H,

aromatic, amide NH and amide NH), 7.64 (d, *J* = 7.4 Hz, 1H, Ala NH), 7.60-7.47 (m, 2H, aromatic), 7.24-7.12 (m, 5H, aromatic), 5.08-4.97 (m, 1H, Ala α-H), 4.67 (d, *J* = 4.6 Hz, 1H, NCOCH), 3.93-3.82 (m, 1H, NCH), 3.77 (dd, *J* = 8.0, 4.6 Hz, 1H, OCOCH), 3.00-2.90 (m, 1H, cyclopropyl CH), 2.85-2.73 (m, 1H, benzyl CH_2), 2.71-2.59 (m, 1H, benzyl CH_2), 2.00-1.88 (m, 2H, $BnCH_2$ and $C\underline{H}CH_3$), 1.82-1.68 (m, 1H, $BnCH_2$), 1.68-1.55 (m, 1H, $C\underline{H}_2CH_3$), 1.61 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.33-1.22 (m, 2H, $C\underline{H}_2CH_3$ and $C\underline{H}CH_3$), 1.08-0.96 (m, 1H, cyclopropyl CH_2), 1.00 (d, *J* = 6.3 Hz, 3H, $CHCH_3$), 0.95 (d, *J* = 6.9 Hz, 3H, $CHCH_3$), 0.92-0.84 (m, 1H, cyclopropyl CH), 0.87 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2CH_3), 0.20-0.12 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.0, 170.4, 170.4, 167.2, 141.5, 134.8, 132.6, 131.0, 129.0, 128.5, 128.3, 128.3, 128.0, 127.7, 127.7, 126.6, 126.1, 123.8, 71.5, 62.7, 54.2, 49.4, 36.5, 33.6, 33.2, 31.4, 27.7, 26.6, 20.9, 20.0, 16.3, 14.8, 11.9, 10.9; LRMS (ESI) *m/z* 620.31 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{36}H_{43}N_3O_5Na$: 620.3095 [$(M+Na)^+$], found: 620.3094.

Carbamate **2-25b**

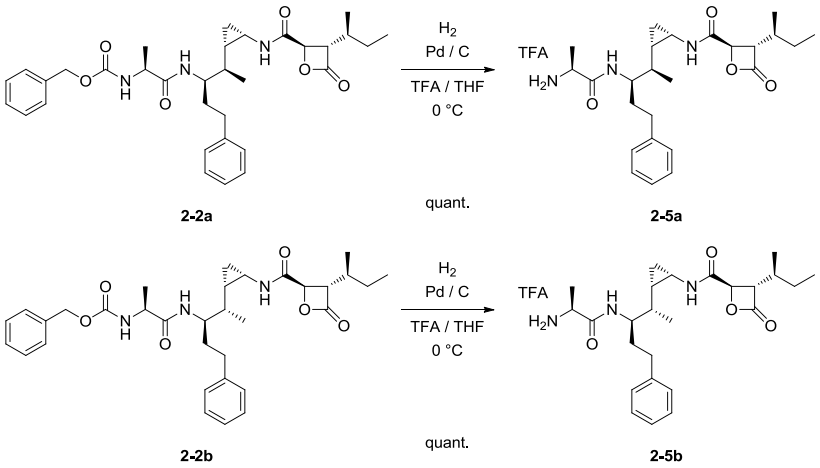
Carbamate **2-25b** (60.6 mg, 0.128 mmol, 2 steps 96%, a white amorphous solid) was prepared from diprotected amine **2-20b** (72.2 mg, 0.134 mmol) as described for carbamate **2-25a**. $[\alpha]^{24}_D$ 17.05 (*c* 0.60, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.67 (d, *J* = 8.6 Hz, 1H, amide NH), 8.62 (s, 1H, aromatic), 8.14 (d, *J* = 8.0 Hz, 1H, aromatic), 7.96-7.83 (m, 3H, aromatic), 7.58-7.48 (m, 2H, aromatic), 7.30-7.7.21 (m, 2H, aromatic), 7.21-7.11 (m, 3H, aromatic), 5.10 (s, 1H, carbamate NH), 4.30-4.19 (m, 1H, NCH), 2.80-2.63 (m, 2H, benzyl CH_2), 2.68-2.58 (m, 1H, cyclopropyl CH), 2.17-2.07 (m, 1H, $BnCH_2$), 1.80-1.68 (m, 1H, $BnCH_2$), 1.52 (s, 9H, CCH_3), 1.44-1.35 (m, 1H, $C\underline{H}CH_3$), 1.12 (d, *J* = 6.9 Hz, 3H, $CHC\underline{H}_3$), 0.94-0.78 (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.16-0.07 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.6, 157.6, 142.5, 134.7, 132.7, 132.0, 129.1, 128.3, 128.0, 127.8, 127.6, 127.2, 126.2, 125.7, 124.7, 80.1, 55.1, 36.0, 33.4, 32.3, 28.6, 28.4, 22.8, 19.0, 10.0; LRMS (ESI) *m/z* 495.26 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{30}H_{36}N_2O_3Na$: 495.2618 [$(M+Na)^+$], found: 495.2609.

Target compound **2-3b**

Target compound **2-3b** (63.4 mg, 0.120 mmol, 2 steps 94%, a colorless viscous oil) was prepared from carbamate **2-25b** (60.6 mg, 0.128 mmol) as described for target compound **2-3a**. $[\alpha]^{19}_D$ 16.53 (*c* 1.56, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.26 (s, 1H, aromatic), 8.10 (br, 1H, amide NH), 7.94-7.81 (m, 4H, aromatic), 7.62-7.50 (m, 2H, aromatic), 7.31-7.24 (m, 2H, aromatic), 7.24-7.13 (m, 4H, aromatic and amide NH), 4.93 (d, *J* = 4.6 Hz, 1H, NCOCH), 4.18-4.08 (m, 1H, NCH), 3.77 (dd, *J* = 4.6 Hz, 2.9 Hz, 1H, OCOCH), 2.93-2.84 (m, 1H, cyclopropyl CH), 2.84-2.75 (m, 2H, benzyl CH_2), 2.13-2.03 (m, 1H, $BnCH_2$), 2.03-1.93 (m, 1H, $C\underline{H}CH_3$), 1.86-1.75 (m, 1H, $BnCH_2$), 1.65-1.54 (m, 1H, $C\underline{H}_2CH_3$), 1.44-1.35 (m, 1H, $CHCH_3$), 1.33-1.22 (m, 1H, $C\underline{H}_2CH_3$), 1.13-1.00 (m, 7H, $CHC\underline{H}_3$ and $CHC\underline{H}_3$ and cyclopropyl CH_2), 1.00-0.89 (m, 1H, cyclopropyl CH), 0.86 (dd, *J* = 7.4, 7.4 Hz, 3H, $CH_2C\underline{H}_3$), 0.24-0.16 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.3, 169.8, 167.7, 141.5, 134.8, 132.5, 130.9, 129.0, 128.6, 128.3, 128.3, 127.7, 126.7, 126.1, 123.6, 70.5, 62.2, 55.1, 36.6, 33.3, 33.3, 32.0, 27.7, 27.6, 26.6, 20.8, 16.1, 15.5, 12.2, 11.0; LRMS (ESI) *m/z* 549.27 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{38}N_2O_4Na$: 549.2724 [$(M+Na)^+$], found: 549.2720.

Synthetic procedure for 2-5a and 2-5b

Scheme S2-1. Synthesis of **2-5a** and **2-5b**



General procedure for the preparation of **2-5a** and **2-5b**

To a solution of the substrate (1.0 equiv) in DCM (0.1 M) was added an equivalent amount of TFA and Pd/C (20 mg) at 0 °C. The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred at 0 °C under an atmosphere of hydrogen until the starting material disappeared on TLC. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to yield the corresponding target compound.

Target compound **2-5a**

Target compound **2-5a** (9.00 mg, 0.0166 mmol, quant.) was obtained from **2-2a** as a colorless viscous oil. $[\alpha]^{22}_{D}$ -36.09 (*c* 0.38, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.61 (br, 3H, NH_3^+), 8.29 (d, *J* = 9.2 Hz, 1H, amide NH), 7.93 (d, *J* = 4.6 Hz, 1H, amide NH), 7.27-7.15 (m, 4H, aromatic), 7.15-7.07 (m, 1H, aromatic), 4.66 (d, *J* = 4.6 Hz, 1H, OCH), 4.36-4.25 (m, 1H, Ala CH), 4.13-4.02 (m, 1H, NCH), 3.61-3.53 (m, 1H, COCH), 2.88-2.59 (m, 2H, $PhCH_2$ and cyclopropyl CH), 2.59-2.45 (m, 1H, $PhCH_2$), 1.97-1.57 (m, 4H, $PhCH_2C\underline{H}_2$ (2H), *sec*-butyl CH_2 (1H) and *sec*-butyl CH), 1.54 (d, *J* = 6.3 Hz, 3H, Ala CH_3), 1.33-1.22 (m, 1H, *sec*-butyl CH_2), 1.22-1.13 (m, 1H, NCHC$\underline{H}$), 1.00 (d, *J* = 6.9 Hz, 3H, *sec*-butyl CHC$\underline{H}_3$), 0.97-0.85 (m, 7H, CHC$\underline{H}_3$, *sec*-butyl $CH_2C\underline{H}_3$ and cyclopropyl C$\underline{H}_2$), 0.85-0.77 (m, 1H, cyclopropyl CH), 0.68-0.61 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, THF-d8) δ 171.2, 170.9, 170.0, 143.3, 129.4, 129.2, 126.7, 71.5, 63.3, 54.4, 50.4, 38.4, 35.3, 34.7, 34.1, 28.2, 27.7, 22.1, 18.4, 16.7, 15.5, 11.6, 10.5; LRMS (ESI) *m/z* 466.27 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{25}H_{37}N_3O_4Na$: 466.2676 [$(M+Na)^+$], found: 466.2679; Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 4.0TFA \cdot 5.0H_2O$: C, 38.68; H, 4.73; N, 3.87. Found: C, 38.85; H, 4.58; N, 4.12.

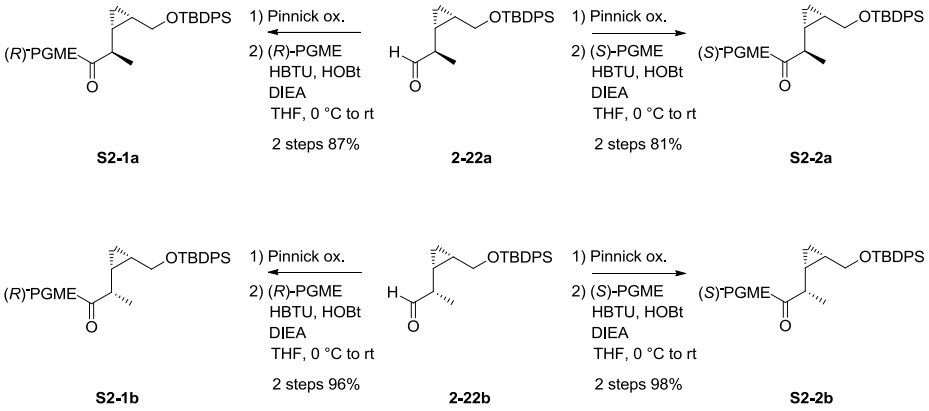
Target compound **2-5b**

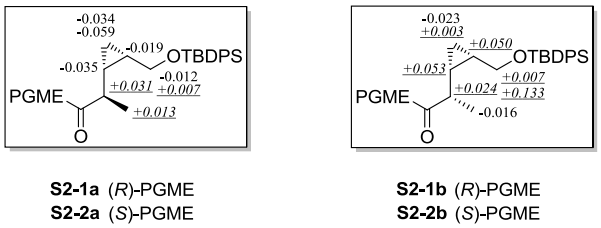
Target compound **2-5b** (14.8 mg, 0.0274 mmol, quant.) was obtained from **2-2b** as a colorless viscous oil. $[\alpha]^{23}_{D}$ -11.08 (*c* 0.74, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.59 (br, 3H, NH_3^+), 8.54 (d, *J* = 7.4 Hz, 1H, amide NH), 8.23 (br, 1H, amide NH), 7.27-7.14 (m, 4H, aromatic), 7.14-7.04 (m, 1H, aromatic), 4.80 (d, *J* = 7.4 Hz, 1H, OCH), 4.30-4.12 (m, 1H, Ala CH), 3.90-3.77 (m, 1H, NCH), 3.75 (dd, *J* = 7.4, 4.0 Hz, 1H, COCH), 3.00 (br, 1H, cyclopropyl CH), 2.72-2.60 (m, 1H, $PhCH_2$), 2.56-2.44 (m, 1H, $PhCH_2$), 2.00-1.85 (m, 2H, $PhCH_2C\underline{H}_2$ and *sec*-butyl CH), 1.69-1.58 (m, 2H, $PhCH_2C\underline{H}_2$ and *sec*-butyl CH_2), 1.55 (br, 3H, Ala CH_3), 1.38-1.19 (m, 2H, *sec*-butyl CH_2 and NCHC$\underline{H}$), 1.02 (d, *J* = 6.9 Hz, 3H, *sec*-butyl CHC$\underline{H}_3$), 1.01 (d, *J* = 6.9 Hz, 3H, CHC$\underline{H}_3$), 0.96-0.81 (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.89 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl $CH_2C\underline{H}_3$), 0.36-0.26 (m, 1H, cyclopropyl CH_2);

^{13}C-NMR (125 MHz, THF-d8) δ 171.7, 171.2, 170.5, 143.3, 129.4, 129.2, 126.6, 71.7, 63.2, 55.4, 50.3, 36.7, 34.7, 33.9, 29.7, 27.7, 23.1, 18.4, 17.5, 16.8, 11.6, 10.0; LRMS (ESI) *m/z* 444.29 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{25}H_{38}N_3O_4$: 444.2857 [$(M+H)^+$], found: 444.2853; Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 2.5TFA \cdot 4.5H_2O$: C, 42.39; H, 5.39; N, 4.63. Found: C, 42.48; H, 5.74; N, 4.64.

Determination of the absolute configuration of 2-22a and 2-22b by the PGME method

Scheme S2-2. Synthesis of PGME amide **S2-1a** – **S2-2a** and **S2-1b** – **S2-2b**





S2-1a (*R*)-PGME **S2-1b** (*R*)-PGME
S2-2a (*S*)-PGME **S2-2b** (*S*)-PGME

Figure S2-1. Determination of the absolute configuration of **2-22a** and **2-22b** by the PGME method ($\Delta\delta = \delta_S - \delta_R$ (ppm; $CDCl_3$))

PGME amide **S2-1a**

To a solution of aldehyde **2-22a** (25.4 mg, 0.0692 mmol, dr 97:3) in THF (1.5 ml) was added 80% aqueous *t*-BuOH (2.3 ml), NaH_2PO_4-$2H_2O$ (16.2 mg, 0.104 mmol, 1.5 equiv), 2-methyl-2-butene (58.6 µl, 0.554 mmol, 8.0 equiv) and $NaClO_2$ (53.2 mg, 0.558 mmol, 4.0 equiv). After 1 h at rt, the reaction mixture was diluted with DCM, washed with 1 M HCl, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1-0:1) to yield the corresponding carboxylic acid as a colorless viscous oil.

To a solution of the oil in THF (3.0 ml) was added (*R*)-PGME·HCl (21.1 mg, 0.105 mmol, 1.5 equiv), HBTU (39.7 mg, 0.105 mmol, 1.5 equiv) and HOBt·H_2O (16.0 mg, 0.105 mmol, 1.5 equiv) and cooled to 0 °C. DIEA (69 µl, 0.489 mmol, 7.0 equiv) was added at 0 °C. After 10 min, the reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was concentrated *in vacuo* to remove THF and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 7:1) to yield PGME amide **S2-1a** (31.9

mg, 0.0602 mmol, 2 steps 87%) as a white solid. $[\alpha]^{26}_{D}$ -90.41 (*c* 0.68, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.737-7.611 (7.674) (m, 4H, aromatic), 7.450-7.293 (7.372) (m, 11H, aromatic), 6.730 (d, *J* = 6.9 Hz, 1H, amide NH), 5.582 (d, *J* = 6.9 Hz, 1H, PGME α-H), 3.838 (dd, *J* = 11.5, 5.2 Hz, 1H, CH_2O), 3.725 (s, 3H, CH_3O), 3.506 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 1.898-1.799 (1.848) (m, 1H, C<u>H</u>CH_3), 1.354 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.314-1.214 (1.265) (m, 1H, cyclopropyl CH), 1.127-0.991 (1.063) (m, 1H, cyclopropyl CH), 1.050 (s, 9H, CCH_3), 0.833-0.750 (0.792) (m, 1H, cyclopropyl CH_2), 0.160-0.086 (0.121) (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.3, 171.5, 136.7, 135.6, 135.5, 133.7, 133.6, 129.6, 128.9, 128.4, 127.7, 127.6, 127.1, 63.4, 56.2, 52.8, 40.4, 26.8, 20.1, 19.2, 17.8, 17.8, 8.2; LRMS (ESI) *m/z* 552.26 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{39}NO_4SiNa$: 552.2541 [$(M+Na)^+$], found: 552.2538.

PGME amide **S2-2a**

PGME amide **S2-2a** (33.4 mg, 0.0631 mmol, 2 steps 81%, a white solid) was prepared from aldehyde **2-22a** (28.6 mg, 0.0779 mmol) as described for PGME amide **S2-1a** using (*S*)-PGME instead of (*R*)-PGME. $[\alpha]^{26}_{D}$ 30.82 (*c* 0.69, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.732-7.634 (7.683) (m, 4H, aromatic), 7.472-7.285 (7.379) (m, 11H, aromatic), 6.709 (d, *J* = 6.9 Hz, 1H, amide NH), 5.581 (d, *J* = 6.9 Hz, 1H, PGME α-H), 3.826 (dd, *J* = 11.5, 5.2 Hz, 1H, CH_2O), 3.726 (s, 3H, CH_3O), 3.513 (dd, *J* = 11.5, 8.6 Hz, 1H, CH_2O), 1.931-1.828 (1.879) (m, 1H, C<u>H</u>CH_3), 1.367 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.302-1.188 (1.246) (m, 1H, cyclopropyl CH), 1.109-0.976 (1.055 and 1.028) (m, 10H, cyclopropyl CH and CCH_3), 0.778-0.695 (0.733) (m, 1H, cyclopropyl CH_2), 0.127-0.044 (0.087) (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.4, 171.5, 136.6, 135.6, 135.5, 133.7, 133.6, 129.7, 129.6, 128.9, 128.4, 127.7, 127.6, 127.1, 63.4, 56.2, 52.8, 40.4, 26.8, 20.1, 19.2, 17.9, 17.9, 8.2; LRMS (ESI) *m/z* 552.25 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{39}NO_4SiNa$: 552.2541 [$(M+Na)^+$], found: 552.2536.

PGME amide **S2-1b**

PGME amide **S2-1b** (24.4 mg, 0.0461 mmol, 2 steps 96%, a colorless viscous oil) was prepared from aldehyde **2-22b** (17.6 mg, 0.0480 mmol) as described for PGME amide **S2-1a**. $[\alpha]^{26}_{D}$ -17.84 (*c* 1.24, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.669-7.604 (7.637) (m, 4H, aromatic), 7.453-7.324 (7.389) (m, 6H, aromatic), 7.289-7.196 (7.243) (m, 5H, aromatic), 6.702 (d, *J* = 6.9 Hz, 1H, amide NH), 5.506 (d, *J* = 6.9 Hz, 1H, PGME α-H), 3.869 (dd, *J* = 10.9, 5.7 Hz, 1H, CH_2O), 3.647 (s, 3H, CH_3O), 3.597 (dd, *J* = 10.9, 6.3 Hz, 1H, CH_2O), 1.992-1.898 (1.946) (m, 1H, C<u>H</u>CH_3), 1.304-1.154 (1.243 and 1.200) (m, 4H, CHC<u>H</u>$_3$ and cyclopropyl CH), 1.098-0.972 (1.043 and 1.020) (m, 10H, CC<u>H</u>$_3$ and cyclopropyl CH), 0.795-0.712 (0.752) (m, 1H, cyclopropyl CH_2), 0.150-0.087 (0.115) (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.3, 171.2, 136.5, 135.6, 135.6, 133.8, 133.6, 129.6, 128.9, 128.3, 127.6, 127.6, 127.1, 63.3, 56.3, 52.6, 40.2, 26.9, 19.8, 19.1, 18.5, 18.3, 8.2; LRMS (ESI) *m/z* 552.25 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{39}NO_4SiNa$: 552.2541 [$(M+Na)^+$], found: 552.2533.

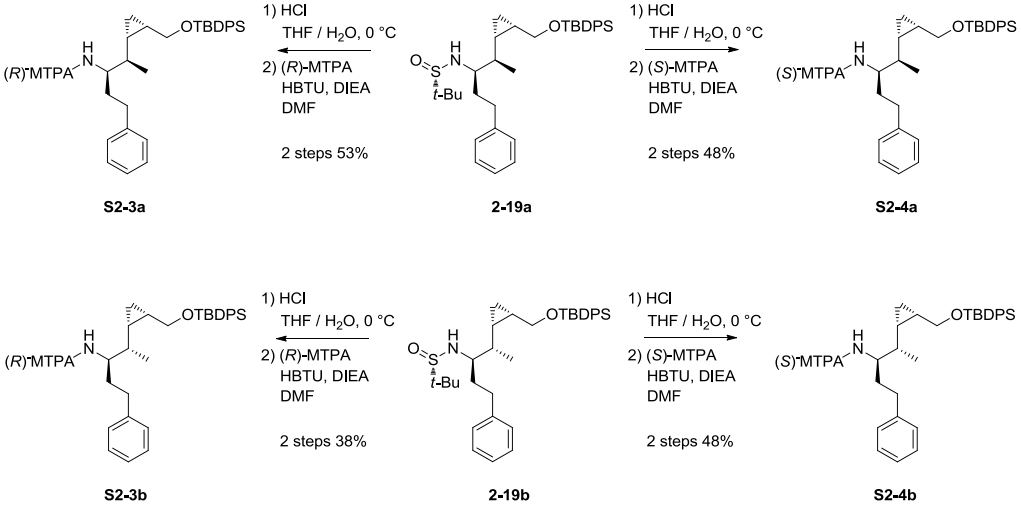
PGME amide **S2-2b**

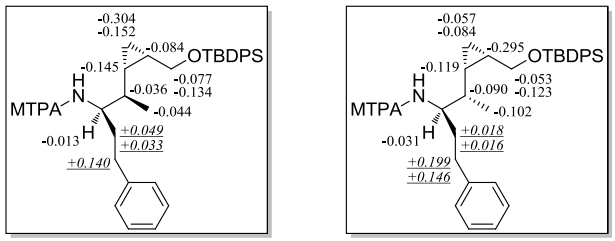
PGME amide **S2-2b** (29.4 mg, 0.0555 mmol, 2 steps 98%, a colorless viscous oil) was prepared from aldehyde **2-22b** (20.8 mg, 0.0566 mmol) as described for PGME amide **S2-1a** using (*S*)-PGME instead of (*R*)-PGME. $[\alpha]^{26}_{D}$ 101.46 (*c* 0.60, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.681-7.604 (7.643) (m, 4H, aromatic), 7.451-7.317 (7.384) (m, 6H, aromatic), 7.288-7.202 (7.245) (m, 5H, aromatic), 6.800 (d, *J* = 6.9 Hz, 1H, amide NH), 5.489 (d, *J* = 6.9 Hz, 1H, PGME α-H), 3.876 (dd, *J* = 11.5, 6.3 Hz, 1H, CH_2O), 3.730 (dd, *J* = 11.5, 6.3 Hz, 1H, CH_2O), 3.670 (s, 3H, CH_3O),

2.017-1.930 (1.970) (m, 1H, C<u>H</u>CH$_3$), 1.303-1.177 (1.250 and 1.227) (m, 4H, cyclopropyl CH and CHC<u>H</u>$_3$), 1.148-1.044 (1.096) (m, 1H, cyclopropyl CH), 0.992 (s, 9H, CCH$_3$), 0.794-0.717 (0.755) (m, 1H, cyclopropyl CH$_2$), 0.131-0.055 (0.092) (m, 1H, cyclopropyl CH$_2$); ^{13}C-NMR (125 MHz, CDCl$_3$) δ 175.2, 171.3, 136.5, 135.6, 133.7, 133.6, 129.6, 128.9, 128.3, 127.6, 127.6, 127.2, 63.5, 56.5, 52.6, 39.8, 26.9, 19.9, 19.1, 18.6, 18.0, 8.1; LRMS (ESI) *m/z* 552.25 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{32}H_{39}NO_4SiNa$: 552.2541 [(M+Na)$^+$], found: 552.2534.

Determination of the absolute configuration of 2-19a and 2-19b by the modified Mosher's method

Scheme S2-3. Synthesis of Mosher amide **S2-3a** – **S2-4b** and **S2-3b** – **S2-4b**





S2-3a (*R*)-MTPA
S2-4a (*S*)-MTPA

S2-3b (*R*)-MTPA
S2-4b (*S*)-MTPA

Figure S2-2. Determination of the absolute configuration of **2-19a** and **2-19b** by the modified Mosher's method (Δδ = δ_S - δ_R (ppm; CDCl$_3$))

<u>Mosher amide **S2-3a**</u>

To a solution of sulfinamide **2-19a** (8.06 mg, 0.0140 mmol) in THF (1.0 ml) was added 12 M HCl (0.10 ml) at 0 °C. After 2 h at 0 °C, the reaction mixture was neutralized with sat. NaHCO$_3$ and the resulting mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure to give the corresponding amine as a colorless viscous oil.

To a solution of (*R*)-MTPA (9.83 mg, 0.0420 mmol, 3.0 equiv) in DMF (1.0 ml) was added HBTU (14.3 mg, 0.0378 mmol, 2.7 equiv) and DIEA (5.9 µl, 0.0420 mmol, 3.0 equiv). After 5 min at rt, a solution of the aforementioned amine in DMF (1.0 ml) was added and the resulting mixture was stirred for 50 h. The reaction mixture was diluted with

AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 10:1) to yield Mosher amide **S2-3a** (5.10 mg, 0.00741 mmol, 2 steps 53%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.748-7.047 (7.398) (m, 20H, aromatic), 6.792 (d, *J* = 10.0 Hz, 1H, amide NH), 4.154-4.048 (4.102) (m, 1H, NCH), 3.773 (dd, *J* = 10.9, 5.4 Hz, 1H, CH_2O), 3.492 (dd, *J* = 10.9, 8.6 Hz, 1H, CH_2O), 3.433 (s, 3H, OCH_3), 2.566-2.438 (2.502) (m, 2H, benzyl CH_2), 1.932-1.801 (1.864) (m, 1H, $BnCH_2$), 1.761-1.616 (1.680) (m, 1H, $BnCH_2$), 1.326-1.184 (1.234) (m, 1H, $CHCH_3$), 1.185-0.982 (1.146, 1.108 and 1.046) (m, 13H, CCH_3, $CHCH_3$ and cyclopropyl CH), 0.801-0.618 (0.731 and 0.688) (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), 0.000 (br, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 710.32 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{41}H_{48}NO_3F_3SiNa$: 710.3248 [$(M+Na)^+$], found: 710.3246.

Mosher amide **S2-4a**

Mosher amide **S2-4a** (5.90 mg, 0.00858 mmol, 2 steps 48%, a colorless oil) was prepared from sulfinamide **2-19a** (10.3 mg, 0.0179 mmol) as described for Mosher amide **S2-3a** using (*S*)-MTPA instead of (*R*)-MTPA. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.748-7.623 (7.686) (m, 4H, aromatic), 7.515 (d, *J* = 7.7 Hz, 2H, aromatic), 7.470-7.347 (7.409) (m, 6H, aromatic), 7.347-7.233 (7.290) (m, 5H, aromatic), 7.233-7.146 (7.190) (m, 3H, aromatic), 6.736 (d, *J* = 10.0 Hz, 1H, amide NH), 4.157-4.034 (4.089) (m, 1H, NCH), 3.696 (dd, *J* = 11.3, 5.4 Hz, 1H, CH_2O), 3.471 (s, 3H, OCH_3), 3.358 (dd, *J* = 11.3, 8.6 Hz, 1H, CH_2O), 2.752-2.542 (2.642) (m, 2H, benzyl CH_2), 1.989-1.838 (1.913) (m, 1H, $BnCH_2$), 1.792-1.642 (1.713) (m, 1H, $BnCH_2$), 1.254-1.133 (1.198) (m, 1H, $CHCH_3$), 1.133-0.998 (1.064, 1.062 and 1.037) (m, 13H, $CHCH_3$, CCH_3, cyclopropyl CH), 0.654-0.473 (0.586 and 0.536) (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), -0.243--0.362 (-0.304) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 710.32 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{41}H_{48}NO_3F_3SiNa$: 710.3248 [$(M+Na)^+$], found: 710.3246.

Mosher amide **S2-3b**

Mosher amide **S2-3b** (4.4 mg, 0.00640 mmol, 2 steps 38%, a colorless oil) was prepared from sulfinamide **2-19b** (9.70 mg, 0.0168 mmol) as described for Mosher amide **S2-3a**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.772-7.667 (7.714) (m, 4H, aromatic), 7.533 (br, 2H, aromatic), 7.445-7.310 (7.378) (m, 9H, aromatic), 7.226 (dd, *J* = 7.4, 7.4 Hz, 2H, aromatic), 7.190-7.125 (7.155) (m, 1H, aromatic), 7.037 (d, *J* = 7.4 Hz, 2H, aromatic), 6.856 (d, *J* = 8.6 Hz, 1H, amide NH), 4.177-4.087 (4.131) (m, 1H, NCH), 3.826-3.727 (3.778) (m, 2H, CH_2O), 3.288 (s, 3H, OCH_3), 2.541-2.441 (2.495) (m, 2H, benzyl CH_2), 2.001-1.893 (1.944) (m, 1H, $BnCH_2$), 1.742-1.626 (1.681) (m, 1H, $BnCH_2$), 1.514-1.422 (1.474) (m, 1H, $CHCH_3$), 1.231-1.126 (1.180) (m, 1H, cyclopropyl CH), 1.049 (s, 9H, CCH_3), 0.991 (d, *J* = 6.9 Hz, 3H, $CHCH_3$), 0.741-0.601 (0.684 and 0.649) (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), -0.028--0.107 (-0.058) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 710.33 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{41}H_{48}NO_3F_3SiNa$: 710.3248 [$(M+Na)^+$], found: 710.3247.

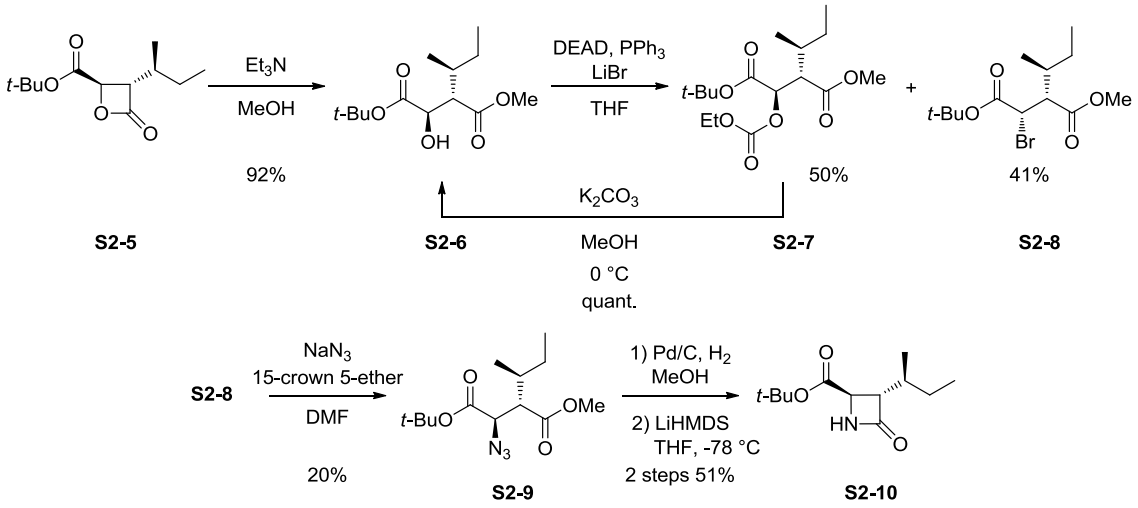
Mosher amide **S2-4b**

Mosher amide **S2-4b** (5.30 mg, 0.00770 mmol, 2 steps 48%, a colorless oil) was prepared from sulfinamide **2-19b** (9.24 mg, 0.0160 mmol) as described for Mosher amide **S2-3a** using (*S*)-MTPA instead of (*R*)-MTPA. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.751-7.649 (7.695) (m, 4H, aromatic), 7.585-7.490 (7.542) (m, 2H, aromatic), 7.451-7.321 (7.386) (m, 9H, aromatic), 7.296-7.225 (7.259) (m, 2H, aromatic), 7.211-7.111 (7.161) (m, 3H, aromatic), 6.776 (d, *J* = 9.2 Hz, 1H, amide NH), 4.151-4.054 (4.100) (m, 1H, NCH), 3.725 (dd, *J* = 11.5, 7.4 Hz, 1H, CH_2O), 3.655 (dd, *J* = 11.5, 6.3 Hz,

1H, CH_2O), 3.341 (s, 3H, OCH_3), 2.751-2.561 (2.694 and 2.641) (m, 2H, benzyl CH_2), 2.011-1.913 (1.962) (m, 1H, $BnCH_2$), 1.738-1.618 (1.697) (m, 1H, $BnCH_2$), 1.440-1.330 (1.384) (m, 1H, $CHCH_3$), 1.059 (s, 9H, CCH_3), 0.951-0.818 (0.885) (m, 1H, cyclopropyl CH), 0.889 (d, J = 6.9 Hz, 3H, $CHCH_3$), 0.617-0.497 (0.565) (m, 2H, cyclopropyl CH_2 and cyclopropyl CH), -0.071--0.160 (-0.115) (m, 1H, cyclopropyl CH_2); LRMS (ESI) *m/z* 710.32 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{41}H_{48}NO_3F_3SiNa$: 710.3248 [$(M+Na)^+$], found: 710.3248.

Synthetic procedure for β-lactam congener 2-1

Scheme S2-4. Synthesis of β-lactam unit **S2-10**



Alcohol **S2-6**

To a solution of β-lactone **S2-5**[41] (726 mg, 3.18 mmol) in MeOH (32 ml) was added triethylamine (1.77 ml, 12.7mmol, 4.0 equiv). After 12 h at rt, the reaction mixture was concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 7:1) to yield alcohol **S2-6** (759 mg, 2.91 mmol, 92%) as a yellow oil. $[\alpha]^{21}_D$ 9.25 (*c* 0.73, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.24 (dd, J = 8.6, 2.9 Hz, 1H, C**H**OH), 3.66 (s, 3H, OCH_3), 3.21 (d, J = 9.2 Hz, 1H, OH), 2.63 (dd, J = 8.6, 2.9 Hz, 1H, COCH), 2.11-2.00 (m, 1H, C**H**CH_3), 1.51-1.38 (m, 10H, CCH_3 and C**H**$_2CH_3$ (1H)), 1.26-1.14 (m, 1H, C**H**$_2CH_3$), 1.03 (d, J = 6.9 Hz, 3H, $CHCH_3$), 0.92 (dd, J = 7.4, 7.4 Hz, 3H, CH_2CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.5, 172.9, 82.5, 69.5, 53.6, 51.4, 33.5, 27.8, 27.5, 16.1, 11.2; LRMS (ESI) *m/z* 283.2 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{13}H_{24}O_5Na$: 283.1521 [$(M+Na)^+$], found: 283.1530.

Bromide **S2-8**

To a solution of PPh_3 (1.70 g, 6.48 mmol, 5.0 equiv) in THF (7.0 ml) was added DEAD (3.25 ml, 6.48 mmol, 40% in toluene). After 20 min at rt, a solution of alcohol **S2-6** (337 mg, 1.30 mmol) and LiBr (1.13 g, 13.0 mmol, 10 equiv) in THF (8.0 ml) was added and the resulting mixture was stirred for 2 h. The reaction mixture was concentrated and the residue was dissolved in Et_2O, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 22:1) to yield carbonate **S2-7** (215 mg, 0.647 mmol, 50%, a colorless oil) and bromide **S2-8** (172 mg, 0.533 mmol, 41%, a color less

oil). Carbonate **S2-7**: $[\alpha]^{20}_D$ 16.44 (*c* 0.90, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.09 (d, *J* = 4.9 Hz, 1H, OCH), 4.29-4.15 (m, 2H, $OCOCH_2$), 3.69 (s, 3H, $COOCH_3$), 2.89 (dd, *J* = 7.2, 4.9 Hz, 1H, OCHC<u>H</u>), 2.03-1.89 (m, 1H, *sec*-butyl CH), 1.58-1.45 (m, 1H, *sec*-butyl CH_2), 1.48 (s, 9H, CCH_3), 1.32 (t, *J* = 7.2 Hz, 3H, $OCOCH_2C\underline{H}_3$), 1.24-1.10 (m, 1H, *sec*-butyl CH_2), 0.96 (d, *J* = 7.2 Hz, 3H, $CHC\underline{H}_3$), 0.92 (t, *J* = 7.2 Hz, 3H, $CH_2C\underline{H}_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.2, 167.9, 154.4, 82.7, 73.7, 64.5, 51.6, 51.5, 33.0, 27.8, 26.8, 16.4, 14.1, 11.5; LRMS (ESI) *m/z* 355.17 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{16}H_{28}O_7Na$: 355.1727 $[(M+Na)^+]$, found: 355.1730. Bromide **S2-8**: $[\alpha]^{19}_D$ -45.25 (*c* 0.41, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.38 (d, *J* = 10.9 Hz, 1H, CHBr), 3.67 (s, 3H, OCH_3), 2.96 (dd, *J* = 10.9, 2.9 Hz, 1H, COCH), 2.11-2.01 (m, 1H, $C\underline{H}CH_3$), 1.53-1.48 (m, 1H, $C\underline{H}_2CH_3$), 1.46 (s, 9H, CCH_3), 1.09 (d, *J* = 6.9 Hz, 3H, $CHC\underline{H}_3$), 0.97-0.87 (m, 4H, $C\underline{H}_2CH_3$ and $CH_2C\underline{H}_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.8, 168.4, 82.4, 54.5, 51.5, 46.3, 34.6, 27.6, 24.1, 18.0, 12.2; LRMS (ESI) *m/z* 345.07 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{13}H_{23}O_4BrNa$: 345.0672 $[(M+Na)^+]$, found: 345.0675.

<u>Alcohol **S2-6**</u>

To a solution of carbonate **S2-7** (500 mg, 1.50 mmol) in MeOH (15 ml) was added K_2CO_3 (1.04 g, 7.52 mmol, 5.0 equiv) at 0 °C. After 30 min at 0 °C, AcOH (516 µl, 9.02 mmol, 6.0 equiv) was added and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield alcohol **S2-6** (400 mg, 1.54 mmol, quant.) as a yellow oil.

<u>Azide **S2-9**</u>

To a solution of bromide **S2-8** (158 mg, 0.488 mmol) in DMF (5.0 ml) was added 15-crown-5 ether (194 µl, 0.975 mmol, 2.0 equiv) and NaN_3 (63.4 mg, 0.975 mmol, 2.0 equiv). After 5 h at rt, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 49:1) to yield azide **S2-9** (28.0 mg, 0.0981 mmol, 20%) as a colorless oil. $[\alpha]^{20}_D$ 35.04 (*c* 0.75, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.99 (d, *J* = 6.9 Hz, 1H, CHN_3), 3.71 (s, 3H, OCH_3), 2.83 (dd, *J* = 14.3 Hz, 1H, COCH), 1.90-1.79 (m, 1H, $CHCH_3$), 1.57-1.45 (m, 10H, $C\underline{H}_2CH_3$ and CCH_3), 1.21-1.09 (m, 1H, $C\underline{H}_2CH_3$), 0.99 (d, *J* = 6.9 Hz, 3H, $CHCH_3$), 0.91 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.0, 168.1, 83.4, 61.7, 52.2, 51.7, 34.2, 27.9, 26.2, 16.8, 11.6; LRMS (ESI) *m/z* 308.16 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{13}H_{23}N_3O_4Na$: 308.1581 $[(M+Na)^+]$, found: 308.1584.

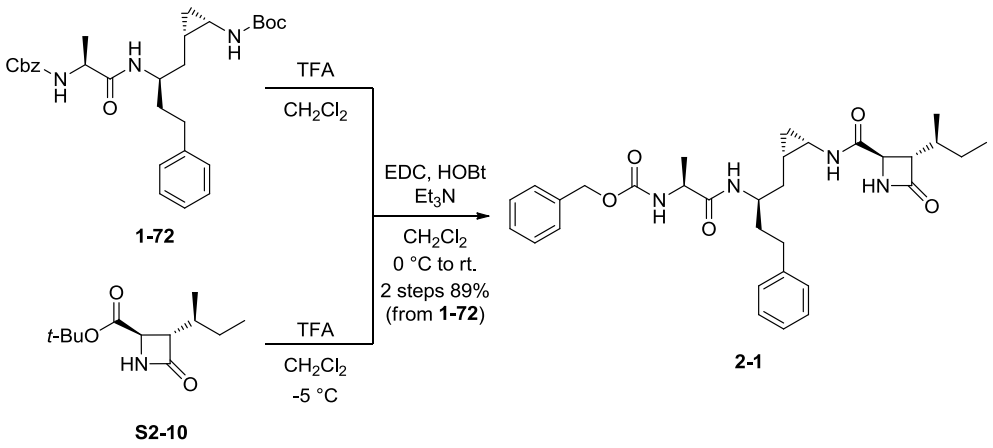
<u>β-lactam **S2-10**</u>

To a solution of azide **S2-9** (28.0 mg, 0.0981 mmol) in MeOH (1.0 ml) was added Pd/C (28.0 mg). The flask was purged with hydrogen and the reaction mixture was stirred under an atmosphere of hydrogen for 30 min. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding amine as a colorless oil.

To a solution of the oil in THF (10 ml) was added LiHMDS (0.123 ml, 0.196 mmol, 2.0 equiv) at -78 °C. After 1 h at -78 °C, the reaction was quenched with sat. NH_4Cl, diluted with water and concentrated *in vacuo* to remove THF. The residual aqueous solution was extracted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 5:1-3:1-0:1) to yield β-lactam **S2-10** (11.3 mg, 0.0497 mmol, 2 steps 51%) as a white solid. $[\alpha]^{19}_D$ -1.21 (*c* 0.88, $CHCl_3$); mp 78-79 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.02 (br, 1H, lactam NH), 3.84

(d, *J* = 2.9 Hz, 1H, lactam CH), 3.21-3.16 (m, 1H, lactam CH), 1.95-1.86 (m, 1H, C<u>H</u>CH$_3$), 1.69-1.58 (m, 1H, C<u>H$_2$</u>CH$_3$), 1.48 (s, 9H, CCH$_3$), 1.39-1.27 (m, 1H, C<u>H$_2$</u>CH$_3$), 1.01 (d, *J* = 6.9 Hz, 3H, CHCH$_3$), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, CH$_2$C<u>H$_3$</u>); ^{13}C-NMR (500 MHz, CDCl$_3$) δ 170.5, 169.5, 82.3, 62.5, 51.5, 33.7, 27.9, 27.1, 16.1, 11.3; LRMS (ESI) *m/z* 250.14 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{12}H_{21}NO_3Na$: 250.1414 [(M+Na)$^+$], found: 250.1417.

Scheme S2-5. Synthesis of β-lactam congener **2-1**



<u>β-Lactam congener **2-1**</u>

To a solution of carbamate **1-72**[37] (14.9 mg, 0.0292 mmol) in DCM (300 µl) was added TFA (300 µl). After 15 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow oil.

To a solution of β-lactam **S2-10** (8.30 mg, 0.0365 mmol, 1.3 equiv) in DCM (400 µl) was added TFA (400 µl) at -5 °C. After 20 h at -5 °C, the reaction mixture was concentrated *in vacuo* to give the corresponding carboxylic acid as a white solid.

To a solution of the solid in DCM (1.0 ml) was added HOBt·H$_2$O (4.8 mg, 0.0317 mmol, 1.3 equiv) and EDC·HCl (6.1 mg, 0.0317 mmol, 1.3 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was used as a solution of the corresponding HOBt ester in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (12 µl, 0.0877 mmol, 3.0 equiv) and a solution of the HOBt ester in DCM (1.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. NaHCO$_3$ and brine, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:5-0:1) to yield β-lactam congener **2-1** (14.6 mg, 0.0260 mmol, 2 steps 89%) as a colorless viscous oil. $[\alpha]^{20}_D$ 41.24 (*c* 0.35, CHCl$_3$); ^{1}H-NMR (400 MHz, CDCl$_3$) δ 7.46 (br, 1H, amide NH), 7.40-7.24 (m, 7H, aromatic), 7.24-7.10 (m, 3H, aromatic), 6.98 (br, 1H, lactam NH), 6.67 (br, 1H, amide NH), 5.48 (d, *J* = 7.2 Hz, 1H, carbamate NH), 5.13 (d, *J* = 12.6 Hz, 1H, benzyl CH$_2$), 5.08 (d, *J* = 12.6 Hz, 1H, benzyl CH$_2$), 4.18 (dq, *J* = 7.2, 7.2 Hz, 1H, Ala CH$_3$), 3.87 (d, *J* = 2.7 Hz, 1H, NCOCH), 3.77-3.62 (m, 1H, NCH), 3.21 (dd, *J* = 7.2, 2.7 Hz, 1H, NCOCHC<u>H</u>), 2.79-2.51 (m, 3H, benzyl CH$_2$ and cyclopropyl CH), 2.00 (br, 1H, BnCH$_2$), 1.93-1.77 (m, 2H, NCHC<u>H$_2$</u> (1H) and *sec*-butyl CH), 1.77-1.57 (m, 3H, *sec*-butyl CH$_2$ (1H) and BnCH$_2$), 1.45-1.21 (m, 4H, *sec*-butyl CH$_2$ (1H) and Ala CH$_3$), 1.21-1.08 (m, 1H, NCHC<u>H$_2$</u>), 1.08-0.96 (m, 1H, cyclopropyl CH), 1.00 (d, *J* = 6.7 Hz, 3H, *sec*-butyl CHC<u>H$_3$</u>), 0.96-0.77 (m, 4H, cyclopropyl CH$_2$ (1H), CH$_2$C<u>H$_3$</u>), 0.30-0.17 (m, 1H, cyclopropyl CH$_2$); ^{13}C-NMR (100 MHz, CDCl$_3$) δ 173.2, 173.0, 170.7, 156.0, 141.1, 136.0, 128.6, 128.6, 128.3, 128.2, 128.1, 126.2, 67.1, 62.9, 52.7,

50.8, 50.5, 35.5, 33.8, 33.7, 32.6, 27.0, 26.7, 18.4, 16.2, 13.7, 12.7, 11.3; LRMS (ESI) *m/z* 563.33 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{32}H_{43}N_4O_5$: 563.3233 [$(M+H)^+$], found: 563.3229.

Stability testing of 1-79, 2-5a and 2-5b

0.1 M TEAA buffer

Solutions of **1-79**, **2-5a** and **2-5b** in DMSO (50 µl, 7.5 mM) were diluted with 0.1 M TEAA buffer (950 µl, pH 7.4) and the resulting mixtures were incubated at 37 °C. The time courses were analyzed by RP-HPLC (Mightysil RP-18 GP 250-4.6 (5 µm), MeOH/ H_2O/ TFA 60:40:0.1, 0.8 ml/ min, rt, 210 nm, retention times: **1-79,** 15.1 min; **2-5a**, 17.5 min; **2-5b,** 20.9 min) at various time points from 0 to 32 h.

Human AB serum

Solutions of **1-79**, **2-5a** and **2-5b** in DMSO (50 µl, 23 mM) were diluted with human AB serum (950 µl, Sigma-Aldrich) and the resulting mixtures were incubated at 37 °C. At various time points from 0 to 60 min, the reaction mixtures (100 µl) were sampled, which were immediately quenched with CH_3CN (300 µl). The resulting mixtures were centrifuged (10,000 rpm) for 2 min at 4 °C and the supernatants were analyzed by RP-HPLC (Mightysil RP-18 GP 250-4.6 (5 µm), MeOH/ H_2O/ TFA 60:40:0.1, 0.8 ml/ min, rt, 210 nm, retention times: **1-79**, 15.1 min; **2-5a**, 17.5 min; **2-5b**, 20.9 min).

Proteasome assay

Inhibitory activity of compounds **2-1**, **2-2a** – **2-4a** and **2-2b** – **2-4b** on the ChT-L activity of human 20S proteasome was measured as described previously.[37]

Cell proliferation assay

Inhibitory activity of compounds **2-2a** and **2-2b** on cell growth of Hs-Sultan and KB cells were measured as described by Tabata *et al.*[100], and of HCT116 cells was measured as described previously.[37]

Conformational analysis of 1-18, 1-24, 1-25, 2-2a – 2-4a and 2-2b – 2-4b by MM-calculations

Conformational analysis of **1-18**, **1-24**, **1-25**, **2-2a** – **2-4a** and **2-2b** – **2-4b** was performed by MacroModel 9.9 (Schrödinger LLC) using MMFFs as a force field. Initial 3D structures of all compounds were constructed by "Paste Special As 3D" of Maestro 9.2 (Schrödinger LLC). First, "Energy Minimization" of these compounds was performed. Second, "Conformational Search" of those minimized structures was performed with "Mixed torsional/Low-mode sampling" method (Maximum iterations, 5000; Maximum number of steps, 10000; Energy window for saving structures, 42.0 kJ/mol). Finally, "Multiple Minimization" of generated conformers was performed (Maximum iterations, 50000). Default values were used for all other parameters.

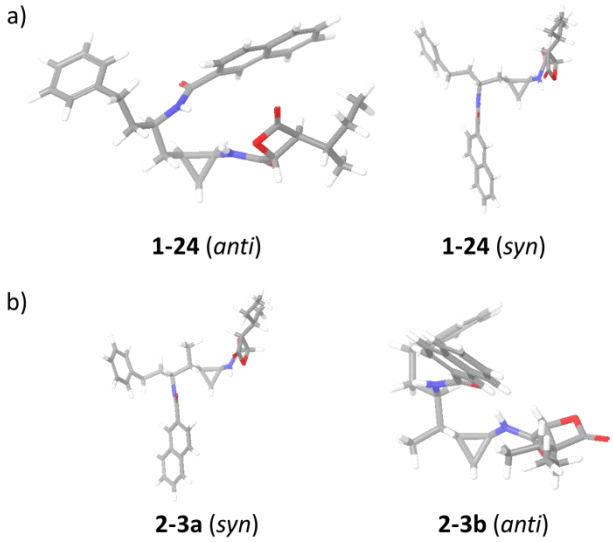


Figure S2-3. Stable conformations of **1-24** and its conformationally restricted analogs **2-3a** and **2-3b** obtained by MacroModel

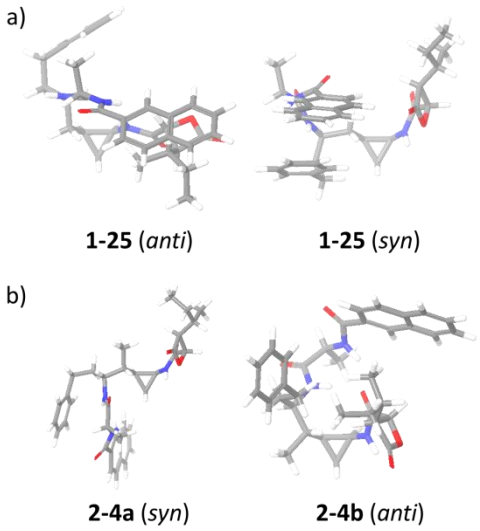


Figure S2-4. Stable conformations of **1-25** and its conformationally restricted analogs **2-4a** and **2-4b** obtained by MacroModel

Table S2-1. Stable conformation of **1-24**, **1-25**, **2-3a** – **2-4a** and **2-3b** – **2-4b** and energy difference between *syn*- and *anti*-conformer

compound number	preferred conformation	energy difference between *syn*- and *anti*-conformer [kcal/mol]
1-24	*anti*	2.3
2-3a	*syn*	3.5
2-3b	*anti*	6.1
1-25	*anti*	2.8
2-4a	*syn*	3.8

2-4b	*anti*	7.8

Docking simulation

Ligand preparation

All initial 3D structures of docked compounds were constructed by "Paste Special As 3D", followed by energy minimization was performed by "LigPrep" using OPLS-2005 as a force field. The resulting structures were used in docking simulations. Default values were used for all other parameters.

Grid generation for docking simulation of belactosin derivatives

The grids used for docking simulation were generated by "Receptor Grid Generation" of Glide 5.7 (Schrödinger LLC). The X-ray crystal structure of proteasome complexed with fluorosalinosporamide A (PDB code; 3GPT) was refined for docking simulations using the Protein Preparation Wizard Script within Maestro 9.2. After that, the structure of fluorosalinosporamide A was deleted and the resulting ligand free side chain of Thr residue was trimmed. To specify the center of the grid, X-ray crystal structure of proteasome complexed with homobelactosin C derivative (PDB code; 3E47) was superimposed, and the ligand structure was merged, whose centroid was used as the center of generating grid. Default values were used for all other parameters.

Grid generation for docking simulation of salinosporamide A derivatives

As a center of the grid, centroid of the fluorosalinosporamide A in 3GPT was used instead of homobelactosin C derivative.

Preparation of the core structure used in docking simulation

The covalent bond between fluorosalinosporamide A and proteasome Thr residue in the aforementioned refined structure was deleted. Next, β-lactone ring of the fluorosalinosporamide A was re-constructed manually by ligating the hydroxyl oxygen atom and carbonyl carbon atom. The resulting structure was minimized by "Clean up geometry" and its β-lactone structure consisting of five atoms was used as a core for docking simulations.

Ligand docking

The docking simulations were performed by "Ligand Docking" of Glide 5.7 (Schrödinger LLC), using the aforementioned core structure (tolerance, 1.0 Å) for restricting the position of the β-lactone ring in docked ligands (Write out at most 25 poses per ligand; Number of poses ligand to include 25). The docking results shown in this paper were representative of top 3 ranked poses. Default values were used for all other parameters.

Rescoring by Prime MM-GBSA

The obtained docking poses of salinosporamide A and its derivatives were rescored by "Prime MM-GBSA" of Prime 3.0 (Schrödinger LLC) using default values for all parameters.

Table S2-2. IC_{50} values for CT-L activity of yeast 20S proteasome in literature and calculated Prime MM-GBSA ΔG bind

compound number	Prime MM-GBSA ΔG bind	clogP[a]	IC50 [nM]	pIC50
salinosporamide A	-83.3	0.97	1.9	8.72
2-6	-77.8	0.55	2.2	8.66
2-7	-77.3	1.29	27.5	7.56
2-8	-77.3	0.87	9.3	8.03
2-9	-69.2	0.45	93.4	7.03
2-10	-74.9	0.97	132	6.88
2-11	-65.4	0.53	101	7.00
2-12	-67.9	0.55	245	6.61
2-13	-68.5	1.04	1029	5.99
2-14	-79.4	1.85	20.8	7.68
2-15	-78.4	0.94	16	7.80

[a]Calculated by ChemBioDraw Ultra 12.0.

Combustion analysis data for the target compounds

Table S2-3. Table listing combustion analysis data for the target compounds

2-2a	Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 1.6H_2O$: C, 65.35; H, 7.68; N, 6.93.	Found: C, 65.04; H, 7.28; N, 6.70.
2-3a	Anal. calcd for $C_{33}H_{38}N_2O_4 \cdot 0.4H_2O$: C, 74.24; H, 7.33; N, 5.25.	Found: C, 74.46; H, 7.34; N, 5.18.
2-4a	Anal. calcd for $C_{36}H_{43}N_3O_5 \cdot 0.55H_2O$: C, 71.16; H, 7.31; N, 6.92.	Found: C, 71.55; H, 7.46; N, 6.52.
2-2b	Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.4H_2O$: C, 67.76; H, 7.55; N, 7.18.	Found: C, 67.84; H, 7.56; N, 7.02.
2-3b	Anal. calcd for $C_{33}H_{38}N_2O_4 \cdot 0.5H_2O$: C, 73.99; H, 7.34; N, 5.23.	Found: C, 73.87; H, 7.25; N, 5.09.
2-4b	Anal. calcd for $C_{36}H_{43}N_3O_5 \cdot 0.4H_2O$: C, 71.48; H, 7.30; N, 6.95.	Found: C, 71.74; H, 7.43; N, 6.62.
2-1	Anal. calcd for $C_{32}H_{42}N_4O_5 \cdot 0.5H_2O$: C, 67.23; H, 7.58; N, 9.80.	Found: C, 67.21; H, 7.63; N, 10.10

Synthetic procedures for 3-4 – 3-7

General procedure for the preparation of **3-14** – **3-17**

To a solution of the hydrochloride salt of a primary amine (1.0 equiv) in MeOH (0.1 M) was added aldehyde (5.0 equiv). After 30 min at rt, $NaBH_3CN$ (4.5 equiv) was added and the resulting mixture was stirred for 28 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in $CHCl_3$, washed with sat. $NaHCO_3$, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography to yield the corresponding tertiary amine.

benzyl (2-(diphenethylamino)ethyl)carbamate **3-14**

Amine **3-14** (860.2 mg, 2.14 mmol, 96%) was obtained as a colorless oil by the reaction of amine **3-12** (515 mg, 2.23 mmol) and phenylacetaldehyde. Purification was conducted by silica gel column chromatography (*n*-hexane/ AcOEt 10:3-3:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.43-7.02 (m, 15 H, aromatic), 5.06 (br, 2H, Cbz CH_2), 4.82 (br, 1H, carbamate NH), 3.10 (td, *J* = 11.2, 5.4 Hz, 2H, C$\underline{H}_2$NH), 2.78-2.63 (m, 8H, benzyl CH_2 and BnCH_2), 2.58 (t, *J* = 5.4 Hz, 2H, C$\underline{H}_2$CH_2NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.3, 140.4, 136.7, 128.7, 128.4, 128.4, 128.0, 127.9, 126.0, 66.4, 55.5, 52.6, 38.4, 33.6; LRMS (ESI) *m/z* 403.24 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{26}H_{31}N_2O_2$: 403.2380 [$(M+Na)^+$], found: 403.2379.

benzyl (2-(bis(3-phenylpropyl)amino)ethyl)carbamate **3-15**

Amine **3-15** (258 mg, 0.599 mmol, quant.) was obtained as a colorless oil by the reaction of amine **3-12** (135 mg, 0.587 mmol) and 3-phenylpropionaldehyde. Purification was conducted by silica gel column chromatography (*n*-hexane/ AcOEt 3:2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39-7.09 (m, 15H, aromatic), 5.25 (br, 1H, carbamate NH), 5.08 (br, 2H, Cbz CH_2), 3.20 (dd, *J* = 11.3, 5.4 Hz, 2H, C$\underline{H}_2$NH), 2.56 (t, *J* = 7.7 Hz, 4H, benzyl CH_2 or BnCH_2C$\underline{H}_2$N), 2.51 (t, *J* = 5.9 Hz, 2H, C$\underline{H}_2$CH_2NH), 2.44 (t, *J* = 7.2 Hz, 4H, BnCH_2C$\underline{H}_2$N), 1.71 (tt, *J* = 7.7, 7.2 Hz, 4H, Bn$C\underline{H}_2$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.3, 142.0, 136.6, 128.4, 128.3, 128.0, 128.0, 125.7, 66.5, 53.2, 53.0, 38.5, 33.6, 28.7; LRMS (ESI) *m/z* 431.27 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{28}H_{35}N_2O_2$: 431.2693 [$(M+H)^+$], found: 431.2689.

benzyl (3-(diphenethylamino)propyl)carbamate **3-16**

Amine **3-16** (762 mg, 1.83 mmol, 86%) was obtained as a colorless oil by the reaction of amine **3-13** (518 mg, 2.12 mmol) and phenylacetaldehyde. Purification was conducted by silica gel column chromatography (*n*-hexane/ AcOEt 3:2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39-7.07 (m, 15H, aromatic), 5.64 (br, 1H, carbamate NH), 5.08 (s, 2H, Cbz CH_2), 3.19 (td, *J* = 5.9, 5.9 Hz, 2H, C$\underline{H}_2$NH), 2.79-2.64 (m, 8H, benzyl CH_2 and BnCH_2), 2.58 (t, *J* = 6.3 Hz, 2H, C$\underline{H}_2$CH_2CH_2NH), 1.61 (tt, *J* = 6.3, 5.9 Hz, 2H, C$\underline{H}_2$CH_2NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.3, 140.3, 136.8, 128.7, 128.4, 128.3, 128.0, 127.9, 126.0, 66.3, 55.7, 52.5, 40.4, 33.5, 26.6; LRMS (ESI) *m/z* 417.25 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{27}H_{33}N_2O_2$: 417.2537 [$(M+H)^+$], found: 417.2533.

benzyl (3-(bis(3-phenylpropyl)amino)propyl)carbamate **3-17**

Amine **3-17** (819 mg, 1.84 mmol, 90%) was obtained as a colorless oil by the reaction of amine **3-13** (503 mg, 2.06 mmol) and 3-phenylpropionaldehyde. Purification was conducted by silica gel column chromatography (*n*-hexane/

AcOEt 5:1-2:1-1:2). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.37-7.09 (m, 15H, aromatic), 6.16 (br, 1H, carbamate NH), 5.07 (s, 2H, Cbz CH_2), 3.27 (dd, *J* = 11.5, 5.7 Hz, 2H, C<u>H</u>$_2$NH), 2.58 (t, *J* = 7.4 Hz, 4H, benzyl CH_2 or BnCH_2C<u>H</u>$_2$N), 2.47 (t, *J* = 6.3 Hz, 2H, C<u>H</u>$_2$CH_2CH_2NH), 2.41 (t, *J* = 7.4 Hz, 4H, benzyl CH_2 or BnCH_2C<u>H</u>$_2$N), 1.73 (tt, *J* = 7.4, 7.4 Hz, 4H, BnCH_2), 1.65-1.53 (m, 2H, C<u>H</u>$_2$CH_2NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 156.3, 142.0, 136.8, 128.3, 128.2, 127.9, 127.8, 125.7, 66.3, 53.4, 53.3, 40.9, 33.6, 28.5, 26.1; LRMS (ESI) *m/z* 445.28 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{29}H_{37}N_2O_2$: 445.2850 [$(M+H)^+$], found: 445.2847.

<u>General procedure for the preparation of target compounds **3-4** – **3-7**</u>

To a solution of the Cbz-protected amine (1.0 equiv) in MeOH (0.1 M) was added $Pd(OH)_2$/C (50% w/w of the substrate). The flask was purged with hydrogen and the reaction mixture was stirred under an atmosphere of hydrogen (balloon pressure) for 6 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding primary amine.

To a solution of carboxylic acid **1-35**[101] (2.5 equiv) in DCM (0.1 M) was added triethylamine (2.5 equiv) and PivCl (2.3 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (0.1 M) was added triethylamine (1.5 equiv) and a solution of the acid anhydride in DCM at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 18 h. The reaction mixture was diluted with DCM, washed with sat. $NaHCO_3$, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography to yield the corresponding target compound.

<u>Target compound **3-4**</u>

The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 2:1) to yield target compound **3-4** (77.8 mg, 0.184 mmol, 2 steps 90%) as a colorless oil. $[\alpha]^{25}_D$ -3.30 (*c* 1.31, CH_2Cl_2) δ 7.34-7.11 (m, 10H, aromatic), 6.46 (br, 1H, amide NH), 4.47 (d, *J* = 4.6 Hz, 1H, NHCOC<u>H</u>), 3.52 (dd, *J* = 8.0, 4.6 Hz, 1H, OCOCH), 3.30-3.20 (m, 1H, C<u>H</u>$_2$NH), 3.18-3.08 (m, 1H, C<u>H</u>$_2$NH), 2.77 (t, *J* = 7.4 Hz, 4H, benzyl CH_2 or BnCH_2), 2.70 (t, *J* = 7.4 Hz, 4H, benzyl CH_2 or BnCH_2), 2.62 (t, *J* = 5.7 Hz, 2H, C<u>H</u>$_2$CH_2NH), 2.02-1.91 (m, 1H, *sec*-butyl CH), 1.71-1.60 (m, 1H, *sec*-butyl CH_2), 1.37-1.23 (m, 1H, *sec*-butyl CH_2), 1.06 (d, *J* = 6.9 Hz, 3H, *sec*-butyl CH_3), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.3, 167.6, 140.2, 128.7, 128.5, 126.1, 70.6, 62.6, 55.7, 52.4, 36.8, 33.8, 33.7, 26.6, 16.3, 11.0; LRMS (ESI) *m/z* 423.26 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{26}H_{35}N_2O_3$: 423.2642 [$(M+H)^+$], found: 423.2642.

<u>Target compound **3-5**</u>

The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:2) to yield target compound **3-5** (59.6 mg, 0.132 mmol, 2 steps quant.) as a colorless oil. $[\alpha]^{25}_D$ -5.95 (*c* 0.46, CH_2Cl_2); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.31-7.25 (m, 4H, aromatic), 7.21-7.14 (m, 6H, aromatic), 6.94 (br, 1H, amide NH), 4.57 (d, *J* = 4.6 Hz, 1H, NHCOC<u>H</u>), 3.55 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 3.37-3.21 (m, 2H, C<u>H</u>$_2$NH), 2.60 (t, *J* = 7.4 Hz, 4H, benzyl CH_2 or BnCH_2C<u>H</u>$_2$N), 2.56 (dd, *J* = 6.3, 5.7 Hz, 2H, C<u>H</u>$_2$CH_2NH), 2.47 (t, *J* = 7.4 Hz, 4H, benzyl CH_2 or BnCH_2C<u>H</u>$_2$N), 2.02-1.91 (m, 1H, *sec*-butyl CH), 1.73 (tt, *J* = 7.4, 7.4 Hz, 4H, BnCH_2), 1.70-1.59 (m, 1H, *sec*-butyl

CH_2), 1.35-1.25 (m, 1H, *sec*-butyl CH_2), 1.06 (d, *J* = 6.9 Hz, 3H, *sec*-butyl CH_3), 0.93 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.2, 167.8, 141.9, 128.3, 128.3, 125.8, 70.7, 62.9, 53.2, 52.3, 36.7, 33.8, 33.5, 28.6, 26.6, 16.3, 11.0; LRMS (ESI) *m/z* 451.30 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{28}H_{39}N_2O_3$: 451.2955 [$(M+H)^+$], found: 451.2957.

Target compound **3-6**

The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:2) to yield target compound **3-6** (51.8 mg, 0.119 mmol, 2 steps 83%) as a colorless oil. $[\alpha]^{25}_D$ -5.00 (*c* 0.56, CH_2Cl_2); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.00 (br, 1H, amide NH), 7.33-7.15 (m, 10H, aromatic), 4.53 (d, *J* = 4.6 Hz, 1H, NHCOC<u>H</u>), 3.55 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 3.45-3.35 (m, 1H, C<u>H</u>$_2$NH), 3.30-3.21 (m, 1H, C<u>H</u>$_2$NH), 2.85-2.73 (m, 8H, benzyl CH_2 and $BnCH_2$), 2.73-2.62 (m, 2H, C<u>H</u>$_2$$CH_2CH_2$NH), 2.02-1.92 (m, 1H, *sec*-butyl CH), 1.72-1.60 (m, 3H, C<u>H</u>$_2$$CH_2$NH and *sec*-butyl CH_2 (1H)), 1.37-1.24 (m, 1H, *sec*-butyl CH_2), 1.07 (d, *J* = 6.3 Hz, 3H, *sec*-butyl CH_3), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.4, 167.8, 140.2, 128.7, 128.4, 126.1, 70.7, 62.8, 55.7, 53.0, 39.6, 33.8, 33.1, 26.6, 25.4, 16.3, 11.0; LRMS (ESI) *m/z* 437.28 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{27}H_{37}N_2O_3$: 437.2799 [$(M+H)^+$], found: 437.2800.

Target compound **3-7**

The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 1:2) to yield target compound **3-7** (47.9 mg, 0.103 mmol, 2 steps 67%) as a colorless oil. $[\alpha]^{25}_D$ -7.17 (*c* 0.51, CH_2Cl_2); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.25 (br, 1H, amide NH), 7.32-7.14 (m, 10H, aromatic), 4.54 (d, *J* = 4.6 Hz, 1H, NHCOC<u>H</u>), 3.52 (dd, *J* = 8.0, 4.6 Hz, 1H, OCOCH), 3.48-3.38 (m, 1H, C<u>H</u>$_2$NH), 3.38-3.28 (m, 1H, C<u>H</u>$_2$NH), 2.64-2.39 (m, 10H, benzyl CH_2, $BnCH_2C$<u>H</u>$_2$ and C<u>H</u>$_2$$CH_2CH_2$NH), 2.02-1.91 (m, 1H, *sec*-butyl CH), 1.77 (tt, *J* = 8.0, 7.4 Hz, 4H, $BnCH_2$), 1.74-1.54 (m, 3H, C<u>H</u>$_2$$CH_2$NH and *sec*-butyl CH_2 (1H)), 1.37-1.21 (m, 1H, *sec*-butyl CH_2), 1.07 (d, *J* = 6.9 Hz, 3H, *sec*-butyl CH_3), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.4, 167.8, 142.0, 128.3, 125.8, 70.8, 62.8, 53.5, 39.9, 33.8, 33.7, 28.1, 26.6, 25.1, 16.3, 11.0; LRMS (ESI) *m/z* 465.31 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{29}H_{41}N_2O_3$: 465.3112 [$(M+H)^+$], found: 465.3115.

Synthetic procedures for 3-8 and 3-10

(9*H*-fluoren-9-yl)methyl (3,3-bis(benzyloxy)propyl)carbamate **3-19**

To a solution of carbamate **3-18** (594 mg, 2.00 mmol) in DCM (20 ml) was added DMP (1.27 g, 3.00 mmol, 1.5 equiv). After 30 min at rt, the reaction was quenched with a solution of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (1:3), extracted with $CHCl_3$, the organic layer was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield the corresponding aldehyde as a white solid.

To a solution of the solid in benzene (20 ml) was added BnOH (827 µl, 7.99 mmol, 4.0 equiv) and *p*-TsOH·H_2O (3.80 mg, 0.0200 mmol, 0.01 equiv) and the resulting mixture was refluxed in a flask equipped with a Dean-Stark trap. After 19 h, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in $CHCl_3$, washed with sat. $NaHCO_3$, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 15:1-5:1) to yield benzyl acetal **3-19** (560 mg, 1.13 mmol, 2 steps 57%) as a white solid. mp 93-94 °C; ^{1}H-NMR (400 MHz, $CDCl_3$, 328 K) δ 7.73 (d, *J* = 7.6 Hz, 2H, aromatic), 7.54 (d,

J = 7.2 Hz, 2H, aromatic), 7.40-7.21 (m, 14H, aromatic), 5.02 (br, 1H, carbamate NH), 4.78 (t, *J* = 4.9 Hz, 1H, OCHO), 4.67 (d, *J* = 11.7 Hz, 2H, benzyl CH_2), 4.56 (d, *J* = 11.7 Hz, 2H, benzyl CH_2), 4.36 (d, *J* = 6.7 Hz, 2H, Fmoc CH_2), 4.17 (t, *J* = 6.7 Hz, 1H, Fmoc CH), 3.31 (br, 2H, C$\underline{H}_2$NH), 1.94 (br, 2H, C$\underline{H}_2$$CH_2$NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.3, 143.9, 141.2, 137.7, 128.5, 127.8, 127.6, 127.0, 125.0, 119.9, 101.0, 67.9, 66.5, 47.2, 37.0, 33.1; LRMS (ESI) *m/z* 516.22 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{32}H_{31}NO_4Na$: 516.2145 [$(M+Na)^+$], found: 516.2142.

3,3-bis(benzyloxy)propan-1-amine **3-20**

To a solution of benzyl acetal **3-19** (117 mg, 0.236 mmol) in MeOH (25 ml) was added K_2CO_3 (326 mg, 2.36 mmol, 10 equiv). After 23 h at rt, the reaction mixture was concentrated *in vacuo*. The crude product was purified by NH-silica gel column chromatography (*n*-hexane/ $CHCl_3$/ MeOH 1:0:0-0:4:1) to yield amine **3-20** (62.1 mg, 0.229 mmol, 97%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.44-7.25 (m, 10H, aromatic), 4.85 (t, *J* = 5.8 Hz, 1H, OCHO), 4.67 (d, *J* = 11.7 Hz, 2H, benzyl CH_2), 4.58 (d, *J* = 11.7 Hz, 2H, benzyl CH_2), 2.82 (t, *J* = 6.7 Hz, 2H, C$\underline{H}_2$NH), 1.92 (td, *J* = 6.7, 5.8 Hz, 2H, C$\underline{H}_2$$CH_2$$NH_2$), 1.44 (br, 2H, NH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.1, 128.5, 128.4, 127.9, 127.8, 127.8, 127.7, 127.6, 100.8, 67.4, 38.1, 37.0; LRMS (APCI) *m/z* 272.16 [$(M+H)^+$]; HRMS (APCI) calcd for $C_{17}H_{22}NO_2$: 272.1645 [$(M+H)^+$], found: 272.1648.

Target compound **3-8**

To a solution of carboxylic acid **1-35**[101] (54.9 mg, 0.319 mmol, 2.5 equiv) in DCM (3.0 ml) was added triethylamine (44.4 µl, 0.319 mmol, 2.5 equiv) and PivCl (36.1 µl, 0.293 mmol, 2.3 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of amine **3-20** (34.6 mg, 0.128 mmol, 1.0 equiv) in DCM (1.5 ml) was added triethylamine (26.6 µl, 0.191 mmol, 1.5 equiv) and a solution of the acid anhydride in DCM at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 19 h. The reaction mixture was diluted with AcOEt, washed with 1M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield target compound **3-8** (51.7 mg, 0.122 mmol, 95%) as a colorless oil. $[\alpha]^{23}_D$ 4.44 (*c* 0.94, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42-7.27 (m, 10H, aromatic), 6.84 (br, 1H, amide NH), 4.80 (dd, *J* = 5.4, 4.9 Hz, 1H, OCHO), 4.70 (d, *J* = 11.7 Hz, 1H, benzyl CH_2), 4.69 (d, *J* = 11.7 Hz, 1H, benzyl CH_2), 4.57 (d, *J* = 11.7 Hz, 1H, benzyl CH_2), 4.56 (d, *J* = 11.7 Hz, 1H, benzyl CH_2), 4.51 (d, *J* = 4.5 Hz, 1H, NHCOC$\underline{H}$), 3.53 (dd, *J* = 7.6, 4.5 Hz, 1H, OCOCH), 3.53-3.34 (m, 2H, C$\underline{H}_2$NH), 2.03-1.88 (m, 3H, C$\underline{H}_2$$CH_2$NH and *sec*-butyl CH), 1.72-1.58 (m, 1H, *sec*-butyl CH_2), 1.38-1.21 (m, 1H, *sec*-butyl CH_2), 1.05 (d, *J* = 6.7 Hz, 3H, *sec*-butyl CH_3), 0.94 (dd, *J* = 7.6, 7.2 Hz, 3H, *sec*-butyl CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.1, 167.7, 137.5, 128.6, 127.9, 127.9, 100.9, 70.6, 68.3, 68.2, 62.8, 35.0, 33.8, 32.6, 26.6, 16.3, 11.0; LRMS (ESI) *m/z* 448.21 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{25}H_{31}NO_5Na$: 448.2094 [$(M+Na)^+$], found: 448.2098.

3,3-bis(benzyloxy)propan-1-ol **3-22**

To a solution of ethyl 3,3-diethoxypropanoate **3-21** (118 µl, 0.607 mmol) in benzene (10 ml) was added BnOH (251 µl, 2.43 mmol, 4.0 equiv) and *p*-TsOH·H_2O (1.15 mg, 0.00607 mmol, 0.01 equiv). The reaction mixture was refluxed in a flask equipped with a Dean-Stark trap for 30 h. The reaction mixture was diluted with AcOEt, washed with sat.

$NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to give the corresponding benzyl acetal as a colorless liquid.

To a solution of $LiAlH_4$ (69.1 mg, 1.82 mmol, 3.0 equiv) in Et_2O (2.0 ml) was added the aforementioned liquid in Et_2O (1.0 ml) *via cannula* at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 2 h. To the reaction mixture was added water (69.1 µl), 15% NaOH (69.1 µl) and water (207 µl) at 0 °C and the resulting mixture was vigorously stirred at rt for 1 h. The mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield alcohol **3-22** (99.2 mg, 0.364 mmol, 2 steps 60%) as a colorless liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40-7.26 (m, 10H, aromatic), 4.92 (t, *J* = 5.4 Hz, 1H, OCHO), 4.71 (d, *J* = 11.8 Hz, 2H, benzyl CH_2), 4.59 (d, *J* = 11.8 Hz, 2H, benzyl CH_2), 3.75 (t, *J* = 5.4 Hz, 2H, C<u>H</u>$_2$OH), 2.36 (br, 1H, OH), 2.01 (td, *J* = 5.4, 5.4 Hz, 2H, C<u>H</u>$_2$$CH_2$OH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 137.7, 128.5, 127.8, 101.4, 68.0, 59.0, 35.6; LRMS (ESI) *m/z* 295.13 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{17}H_{20}O_3Na$: 295.1305 [$(M+Na)^+$], found: 295.1305. ^{1}H-NMR and ^{13}C-NMR are in agreement with that reported by Colson.[102]

<u>4,4-bis(benzyloxy)butanenitrile **3-23**</u>

To a solution of alcohol **3-22** (99.2 mg, 0.364 mmol) in DCM (5.0 ml) was added triethylamine (76.1 µl, 0.547 mmol, 1.5 equiv) and MsCl (33.8 µl, 0.437 mmol, 1.2 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding mesylated product as a colorless oil.

To a solution of the oil in DMSO (410 µl) was added NaCN (107 mg, 2.19 mmol, 6.0 equiv). After 15 h at 60 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield nitrile **3-23** (97.1 mg, 0.345 mmol, 2 steps 95%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41-7.26 (m, 10H, aromatic), 4.81 (t, *J* = 5.4 Hz, 1H, OCHO), 4.69 (d, *J* = 11.7 Hz, 2H, benzyl CH_2), 4.56 (d, *J* = 11.7 Hz, 2H, benzyl CH_2), 2.42 (t, *J* = 7.2 Hz, 2H, CH_2CN), 2.04 (m, 2H, C<u>H</u>$_2$$CH_2$CN); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 137.4, 128.5, 127.9, 127.8, 119.3, 100.0, 68.4, 29.4, 12.4; LRMS (ESI) *m/z* 304.13 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{18}H_{19}NO_2Na$: 304.1308 [$(M+Na)^+$], found: 304.1307.

<u>Target compound **3-10**</u>

To a solution of nitrile **3-23** (28.7 mg, 0.102 mmol) in Et_2O (1.0 ml) was added $LiAlH_4$ (15.5 mg, 0.408 mmol, 4.0 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 12 h. To the reaction mixture was added water (28.7 µl), 15% NaOH (28.7 µl) and water (86.1 µl) at 0 °C and the resulting mixture was vigorously stirred at rt for 1 h. The mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding amine as a colorless oil.

To a solution of carboxylic acid **1-35**[101] (43.9 mg, 0.255 mmol, 2.5 equiv) in DCM (2.5 ml) was added triethylamine (35.5 µl, 0.255 mmol, 2.5 equiv) and PivCl (28.3 µl, 0.230 mmol, 2.3 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (21.4 µl, 0.154 mmol, 1.5 equiv) and a solution of the acid anhydride in DCM at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt

and stirred for 19 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield target compound **3-10** (45.9 mg, 0.104 mmol, 2 steps quant.) as a colorless oil. $[\alpha]^{24}_D$ 12.22 (*c* 0.62, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40-7.27 (m, 10H, aromatic), 6.52 (br, 1H, amide NH), 4.74 (t, *J* = 5.7 Hz, 1H, OCHO), 4.68 (d, *J* = 11.5 Hz, 1H, benzyl CH_2), 4.67 (d, *J* = 11.5 Hz, 1H, benzyl CH_2), 4.57 (d, *J* = 4.6 Hz, 1H, NHCOC<u>H</u>), 4.56 (d, *J* = 11.5 Hz, 2H, benzyl CH_2), 3.55 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 3.39-3.24 (m, 2H, C<u>H</u>$_2$NH), 2.03-1.92 (m, 1H, *sec*-butyl CH), 1.82-1.73 (m, 2H, C<u>H</u>$_2$CH$_2$CH$_2$NH), 1.71-1.59 (m, 3H, C<u>H</u>$_2$CH$_2$NH and *sec*-butyl CH_2 (1H)), 1.37-1.25 (m, 1H, *sec*-butyl CH_2), 1.07 (d, *J* = 6.9 Hz, 3H, *sec*-butyl CH_3), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.2, 167.8, 137.9, 128.5, 127.8, 127.8, 127.7, 101.5, 70.7, 67.7, 67.7, 62.9, 38.9, 33.8, 30.7, 26.6, 24.3, 16.3, 11.0; LRMS (ESI) *m/z* 462.23 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{26}H_{33}NO_5Na$: 462.2251 [$(M+Na)^+$], found: 462.2253.

Synthetic procedures for 3-9 and 3-11

<u>diethyl 2-(2,2-dimethoxyethyl)malonate **3-25**</u>

To a solution of sodium ethoxide (9.06 g, 133 mmol, 1.1 equiv) in EtOH (90 ml) was added diethyl malonate (18.3 ml, 121 mmol, 1.0 equiv). After 1 h at rt, bromoacetaldehyde dimethyl acetal (19.9 ml, 169 mmol, 1.4 equiv) was added and the resulting mixture was refluxed for 23 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in DCM, washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 20:1) to yield compound **3-25** (17.8 g, 71.7 mmol, 59%) as a colorless liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.43 (t, *J* = 5.8 Hz, 1H, OCHO), 4.21 (q, *J* = 7.2 Hz, 2H, OCH_2), 4.20 (q, *J* = 7.2 Hz, 2H, OCH_2), 3.49 (t, *J* = 7.2 Hz, 1H, COCHCO), 3.33 (s, 6H, OCH_3), 2.22 (dd, *J* = 7.2, 5.8 Hz, 2H, CHC<u>H</u>$_2$CH), 1.27 (t, *J* = 7.2 Hz, 6H, OCH_2C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.2, 102.5, 61.4, 53.4, 47.8, 31.7, 14.0; LRMS (ESI) *m/z* 271.11 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{11}H_{20}O_6Na$: 271.1152 [$(M+Na)^+$], found: 271.1152. ^{1}H-NMR and ^{13}C-NMR are in agreement with that reported by Vignola.[103]

<u>(((2-(2,2-dimethoxyethyl)propane-1,3-diyl)bis(oxy))bis(methylene))dibenzene **3-26**</u>

To a solution of $LiAlH_4$ (3.64 g, 96.0 mmol, 3.0 equiv) in Et_2O (100 ml) was added compound **3-25** (7.94 g, 32.0 mmol) in Et_2O (45 ml) *via cannula* at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 2 h. To the reaction mixture was added water (3.64 ml), 15% NaOH (3.64 ml) and water (10.9 ml) at 0 °C, and the resulting mixture was vigorously stirred at rt for 1 h. The mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding diol as a colorless liquid.

To a solution of the liquid in DMF (40 ml) was added NaH (3.84 g, 95.9 mmol, 3.0 equiv, 60% oil suspension) at 0 °C. After 30 min at 0 °C, BnBr (9.49 ml, 79.9 mmol, 2.5 equiv) was added and the resulting mixture was warmed to rt. After 35 h at rt, the reaction was quenched with sat. NH_4Cl (70 ml) at 0 °C. The resulting mixture was extracted with AcOEt and the organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield compound **3-26** (7.00 g, 20.3 mmol, 2 steps 64%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38-7.22 (m, 10H, aromatic), 4.49 (s, 4H, benzyl CH_2), 4.49 (t, *J* = 6.3 Hz, 1H, OCHO), 3.55-3.45 (m, 4H, OC<u>H</u>$_2$CH), 3.28 (s, 6H, OCH_3), 2.15-2.03 (m, 1H, CH_2C<u>H</u>CH_2), 1.71 (dd, *J* = 6.3, 6.3 Hz, 2H, CHC<u>H</u>$_2$CH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ

138.6, 128.3, 127.5, 127.4, 103.1, 73.0, 70.8, 52.6, 35.6, 31.6; LRMS (ESI) *m/z* 367.19 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{21}H_{28}O_4Na$: 367.1880 [$(M+Na)^+$], found: 367.1877.

4-(benzyloxy)-3-((benzyloxy)methyl)butan-1-ol **3-27**

To an emulsion of compound **3-26** (2.02 g, 5.87 mmol) in water (6.0 ml) was added acetic acid (6.0 ml). The resulting mixture was vigorously stirred for 5 h. The reaction mixture was diluted with AcOEt, washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure to give the corresponding aldehyde as a colorless oil.

To a solution of the oil in MeOH (60 ml) was added $NaBH_4$ (111 mg, 2.94 mmol, 0.5 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt. After 4 min at rt, acetone (60 ml) was added. After 1 h at rt, the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 4:1) to yield alcohol **3-27** (1.71 g, 5.70 mmol, 2 steps 97%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38-7.25 (m, 10H, aromatic), 4.50 (s, 4H, benzyl CH_2), 3.66 (t, *J* = 6.3 Hz, 2H, C$\underline{H}_2$OH), 3.53 (dd, *J* = 9.0, 6.3 Hz, 2H, OC$\underline{H}_2$CH), 3.44 (dd, *J* = 9.0, 5.8 Hz, 2H, OC$\underline{H}_2$CH), 3.01 (s, 1H, OH), 2.13 (ttt, *J* = 6.3, 6.3, 5.8 Hz, 1H, $CH_2C\underline{H}CH_2$), 1.67 (td, *J* = 6.3, 6.3 Hz, 2H, C$\underline{H}_2CH_2$OH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.0, 128.4, 127.6, 73.2, 71.7, 61.0, 37.7, 33.6; LRMS (ESI) *m/z* 323.16 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{19}H_{24}O_3Na$: 323.1618 [$(M+Na)^+$], found: 323.1616.

(((2-(2-azidoethyl)propane-1,3-diyl)bis(oxy))bis(methylene))dibenzene **3-28**

To a solution of alcohol **3-27** (224 mg, 0.746 mmol) in DCM (3.0 ml) was added triethylamine (156 μl, 1.12 mmol, 1.5 equiv) and MsCl (69.3 μl, 0.895 mmol, 1.2 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding mesylated product as a colorless oil.

To a solution of the oil in DMF (2.5 ml) was added NaN_3 (146 mg, 2.24 mmol, 3.0 equiv). After 3 h at 70 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 15:1) to yield azide **3-28** (223 mg, 0.685 mmol, 2 steps 91%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.37-7.24 (m, 10H, aromatic), 4.48 (s, 4H, benzyl CH_2), 3.50 (dd, *J* = 9.4, 5.4 Hz, 2H, OC$\underline{H}_2$CH), 3.46 (dd, *J* = 9.4, 5.4 Hz, 2H, OC$\underline{H}_2$CH), 3.32 (t, *J* = 7.2 Hz, 2H, C$\underline{H}_2N_3$), 2.05 (m, 1H, $CH_2C\underline{H}CH_2$), 1.71 (td, *J* = 7.2, 7.2 Hz, 2H, C$\underline{H}_2CH_2N_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.3, 128.3, 127.5, 73.1, 70.5, 49.5, 37.0, 28.3; LRMS (ESI) *m/z* 348.17 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{19}H_{23}N_3O_2Na$: 348.1683 [$(M+Na)^+$], found: 348.1681.

4-(benzyloxy)-3-((benzyloxy)methyl)butan-1-amine **3-29**

To a solution of azide **3-28** (71.1 mg, 0.219 mmol) in THF (2.2 ml) was added PPh_3 (63.0 mg, 0.240 mmol, 1.1 equiv) and water (220 μl). After 9 h at rt, the reaction mixture was refluxed for 22 h. The resulting mixture was concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt/ $CHCl_3$/ MeOH 1:2:0:0-0:0:1:1-0:0:0:1) to yield amine **3-29** (68.5 mg, 0.229 mmol, quant.) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.37-7.23 (m, 10H, aromatic), 4.48 (s, 4H, benzyl CH_2), 3.50 (dd, *J* = 9.4, 5.8 Hz, 2H, OC$\underline{H}_2$CH), 3.45 (dd, *J* = 9.4, 5.8 Hz, 2H, OC$\underline{H}_2$CH), 2.74 (t, *J* = 7.2 Hz, 2H, C$\underline{H}_2NH_2$), 2.09-1.92 (m, 1H, $CH_2C\underline{H}CH_2$), 1.99 (br,

2H, NH_2), 1.56 (td, *J* = 7.2, 7.2 Hz, 2H, C<u>H</u>$_2$CH_2NH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.5, 128.3, 127.5, 127.5, 73.1, 71.0, 39.9, 37.3, 33.0; LRMS (ESI) *m/z* 300.20 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{19}H_{26}NO_2$: 300.1958 [$(M+H)^+$], found: 300.1957.

Target compound **3-9**

To a solution of carboxylic acid **1-35**[101] (46.1 mg, 0.268 mmol, 2.5 equiv) in DCM (3.0 ml) was added triethylamine (37.3 µl, 0.268 mmol, 1.0 equiv) and PivCl (29.7 µl, 0.241 mmol, 2.3 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of amine **3-29** (32.1 mg, 0.107 mmol, 1.0 equiv) in DCM (1.5 ml) was added triethylamine (22.4 µl, 0.161 mmol, 1.5 equiv) and a solution of the acid anhydride in DCM at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 19 h. The reaction mixture was diluted with AcOEt, washed with 1M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield target compound **3-9** (50.3 mg, 0.111 mmol, quant.) as a colorless oil. $[\alpha]^{24}_D$ 9.28 (*c* 0.74, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.24 (m, 10H, aromatic), 6.95 (br, 1H, amide NH), 4.53 (d, *J* = 4.6 Hz, 1H, NHCOC<u>H</u>), 4.49 (s, 4H, benzyl CH_2), 3.56 (dd, *J* = 7.4, 4.6 Hz, 1H, OCOCH), 3.52-3.46 (m, 2H, OC<u>H</u>$_2$CH), 3.45-3.29 (m, 4H, OC<u>H</u>$_2$CH and C<u>H</u>$_2$NH), 2.03-1.91 (m, 2H, CH_2C<u>H</u>CH_2 and *sec*-butyl CH), 1.72-1.61 (m, 3H, C<u>H</u>$_2$CH_2NH and *sec*-butyl CH_2 (1H)), 1.36-1.24 (m, 1H, *sec*-butyl CH_2), 1.06 (d, *J* = 6.9 Hz, 3H, *sec*-butyl CH_3), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_3); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 169.3, 167.8, 138.1, 128.4, 128.4, 127.7, 127.6, 127.6, 73.2, 71.1, 71.0, 70.7, 62.8, 37.8, 37.6, 33.8, 29.1, 26.6, 16.3, 11.0; LRMS (ESI) *m/z* 454.26 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{27}H_{36}NO_5$: 454.2588 [$(M+H)^+$], found: 454.2595.

5-(benzyloxy)-4-((benzyloxy)methyl)pentanenitrile **3-30**

To a solution of alcohol **3-27** (279 mg, 0.929 mmol) in DCM (3.7 ml) was added triethylamine (193 µl, 1.39 mmol, 1.5 equiv) and MsCl (86.3 µl, 1.11 mmol, 1.2 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding mesylated product as a colorless oil.

To a solution of the oil in DMSO (1.0 ml) was added NaCN (273 mg, 5.57 mmol, 6.0 equiv). After 3 h at 60 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 7:1) to yield nitrile **3-30** (282 mg, 0.910 mmol, 2 steps 98%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38-7.25 (m, 10H, aromatic), 4.47 (s, 4H, benzyl CH_2), 3.50 (dd, *J* = 9.4, 5.8 Hz, 2H, OCH_2CH), 3.45 (dd, *J* = 9.4, 5.4 Hz, 2H, OCH_2CH), 2.38 (t, *J* = 7.2 Hz, 2H, CH_2CN), 2.06 (m, 1H, CH_2C<u>H</u>CH_2), 1.80 (td, *J* = 7.2, 7.2 Hz, 2H, C<u>H</u>$_2$$CH_2$CN); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.1, 128.4, 127.6, 127.5, 119.9, 73.1, 70.2, 38.4, 25.2, 15.1; LRMS (ESI) *m/z* 332.16 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{20}H_{23}NO_2Na$: 332.1620 [$(M+Na)^+$], found: 332.1620.

Target compound **3-11**

To a solution of $LiAlH_4$ (20.7 mg, 0.546 mmol, 4.4 equiv) in Et_2O (1.0 ml) was added nitrile **3-30** (38.4 mg, 0.124 mmol) in Et_2O (1.0 ml) *via cannula* at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 5 h. To the reaction mixture was added water (20.7 µl), 15% NaOH (20.7 µl) and water (62.1 µl) at 0 °C and the resulting

mixture was vigorously stirred at rt for 1 h. The mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding amine as a colorless oil.

To a solution of carboxylic acid **1-35**[101] (53.4 mg, 0.310 mmol, 2.5 equiv) in DCM (3.0 ml) was added triethylamine (43.1 µl, 0.310 mmol, 2.5 equiv) and PivCl (34.4 µl, 0.279 mmol, 2.3 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.2 ml) was added triethylamine (25.9 µl, 0.186 mmol, 1.5 equiv) and a solution of the acid anhydride in DCM at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 19 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield target compound **3-11** (58.5 mg, 0.125 mmol, 2 steps quant.) as a colorless oil. $[\alpha]^{24}_D$ 13.20 (*c* 1.05, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.37-7.25 (m, 10H, aromatic), 6.41 (br, 1H, amide NH), 4.56 (d, *J* = 4.6 Hz, 1H, NHCOC<u>H</u>), 4.48 (s, 4H, benzyl CH_2), 3.56 (dd, *J* = 8.0, 4.6 Hz, 1H, OCOCH), 3.49 (dd, *J* = 9.2, 5.7 Hz, 2H, OCH_2CH), 3.44 (dd, *J* = 9.2, 5.7 Hz, 2H, OCH_2CH), 3.37-3.20 (m, 2H, CH_2NH), 2.02-1.88 (m, 2H, NHCOC<u>H</u> and $CH_2C\underline{H}CH_2$), 1.71-1.61 (m, 1H, *sec*-butyl CH), 1.59-1.50 (m, 2H, $C\underline{H}_2CH_2NH$), 1.47-1.39 (m, 2H, $C\underline{H}_2CH_2CH_2NH$), 1.37-1.26 (m, 1H, *sec*-butyl CH_2), 1.07 (d, *J* = 6.3 Hz, 3H, *sec*-butyl CH_3), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.2, 167.8, 138.5, 128.3, 127.6, 127.5, 73.1, 70.7, 62.9, 39.4, 39.0, 33.8, 26.8, 26.6, 25.9, 16.3, 11.0; LRMS (ESI) *m/z* 468.28 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{28}H_{38}NO_5$: 468.2745 [$(M+H)^+$], found: 468.2754.

Flexible alignment of compounds 3-4 – 3-11

Flexible alignment of compounds **3-4** – **3-11** onto previously analyzed non-covalent binding conformation of compound **1-18**[49] and covalent binding conformation of homobelactosin C derivatives analyzed by Meijere *et al.*[33] was performed by using Molecular Operating Environment (MOE) 2012.10. Prior to the calculation, β-lactone ring of compounds **3-4** – **3-11** was superimposed with that of **1-18** and the position was fixed during the calculation. Default values were used for all parameters and the top ranked pose of each compounds were shown in this paper.

Proteasomes assay

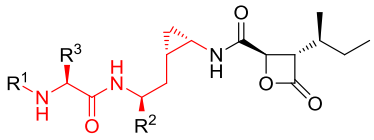
Inhibitory activity of the target compounds **3-4** – **3-11** on ChT-L activity of the human 20S proteasome was measured as described previously.[37]

Cell proliferation assay

Inhibitory activity of the target compounds **3-4** – **3-11** on HCT116 cell growth was measured as described previously.[37]

BEI and SEI values of analogs of 1-18 with same scaffold

Table S3-1. BEI and SEI values of analogs of **1-18** with same scaffold



compound number	R^1	R^2	R^3	BEI	SEI
0-10	Cbz	vinyl	Me	35	14
1-10	H	vinyl	Me	43	14
1-6	Cbz	vinyl	epi-Me	34	14
1-5	Cbz	vinyl	H	36	14
1-7	Cbz	vinyl	isobutyl	32	14
1-8	Cbz	vinyl	Bn	31	14
1-9	Cbz	vinyl	3-indolylmethyl	29	13
1-11	Ac	vinyl	Me	39	13
1-12	Bz	vinyl	Me	36	14
1-13	2-naphthoyl	vinyl	Me	35	15
1-14	Boc	vinyl	Me	36	13
1-15	Cbz	Et	Me	35	14
1-16	Cbz	Ph	Me	30	13
1-17	Cbz	Bn	Me	33	15
1-19	Cbz	phenylpropyl	Me	30	14
1-20	Cbz	1-naphthylmethyl	Me	26	13
1-21	Cbz	2-naphthylmethyl	Me	26	13
1-22	Cbz	1-naphthylethyl	Me	27	13
1-23	Cbz	2-naphthylethyl	Me	28	14
1-25	2-naphthoyl	phenethyl	Me	32	16

Combustion analysis data for the target compounds

Table S3-2. Table listing combustion analysis data for the target compounds

3-4	Anal. calcd for $C_{26}H_{34}N_2O_3$: C, 73.90; H, 8.11; N, 6.63.	Found: C, 73.71; H, 8.21; N, 6.64.
3-5	Anal. calcd for $C_{28}H_{38}N_2O_3$: C, 74.63; H, 8.50; N, 6.22.	Found: C, 74.34; H, 8.61; N, 6.15.
3-6	Anal. calcd for $C_{27}H_{36}N_2O_3 \cdot 0.1H_2O$: C, 73.97; H, 8.32; N, 6.39.	Found: C, 73.80; H, 8.44; N, 6.29.
3-7	Anal. calcd for $C_{29}H_{40}N_2O_3 \cdot 0.2H_2O$: C, 74.39; H, 8.70; N, 5.98.	Found: C, 74.38; H, 8.77; N, 5.91.
3-8	Anal. calcd for $C_{25}H_{31}NO_5$: C, 70.57; H, 7.34; N, 3.29.	Found: C, 70.37; H, 7.41; N, 3.32.
3-9	Anal. calcd for $C_{27}H_{35}NO_5$: C, 71.50; H, 7.78; N, 3.09.	Found: C, 71.26; H, 7.86; N, 3.11.
3-10	Anal. calcd for $C_{26}H_{33}NO_5$: C, 71.05; H, 7.57; N, 3.19.	Found: C, 70.86; H, 7.61; N, 3.19.
3-11	Anal. calcd for $C_{28}H_{37}NO_5$: C, 71.92; H, 7.98; N, 3.00.	Found: C, 71.66; H, 7.94; N, 3.06.

Synthesis of 4-1 – 4-8

(4*R*)-4-Benzyl-3-(4-methylpentanoyl)oxazolidin-2-one **4-10**

The title compound **4-10** (11.1 g, 40.3 mmol, quant., a colorless oil) was prepared from carboxylic acid **4-9** (6.54 ml, 52.0 mmol, 1.3 equiv) and (4*R*)-4-benzyl-2-oxazolidinone (7.09 g, 40.0 mmol, 1.0 equiv) as described for imide **4-15**. $[\alpha]^{23}_{D}$ -54.95 (*c* 1.55, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39-7.24 (m, 3H, aromatic), 7.24-7.17 (m, 2H, aromatic), 4.74-4.61 (m, 1H, NCH), 4.25-4.11 (m, 2H, OCH_2), 3.30 (dd, *J* = 13.5, 3.1 Hz, 1H, benzyl CH_2), 3.05-2.84 (m, 2H, $COCH_2$), 2.76 (dd, *J* = 13.5, 9.9 Hz, 1H, benzyl CH_2), 1.72-1.52 (m, 3H, $CHCH_2$), 0.94 (d, *J* = 6.3 Hz, 6H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.6, 153.4, 135.3, 129.4, 128.9, 127.3, 66.1, 55.1, 37.9, 33.6, 33.1, 27.6, 22.3; LRMS (ESI) *m/z* 298.14 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{16}H_{21}NO_3Na$: 298.1414 [$(M+Na)^+$], found: 298.1411.

Imide **4-11**

Imide **4-11** (11.9 g, 30.6 mmol, 76%, a white solid) was prepared as a single isomer from imide **4-10** (11.1 g, 40.3 mmol) as described for imide **4-16**. $[\alpha]^{23}_{D}$ -32.93 (*c* 0.73, $CHCl_3$); mp 89-90 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39-7.31 (m, 2H, aromatic), 7.31-7.22 (m, 3H, aromatic), 4.73-4.59 (m, 1H, NCH), 4.32-4.19 (m, 1H, COCH), 4.20-4.09 (m, 2H, OCH_2), 3.35 (dd, *J* = 13.5, 3.1 Hz, 1H, benzyl CH_2), 2.83-2.67 (m, 2H, $COCH_2$ and benzyl CH_2), 2.49 (dd, *J* = 16.6, 4.5 Hz, 1H, $COCH_2$), 1.69-1.48 (m, 2H, $CHCH_2$), 1.43 (s, 9H, CCH_3), 1.38-1.29 (m, 1H, $CHCH_2$), 0.94 (d, *J* = 6.7 Hz, 3H, CH_3), 0.92 (d, *J* = 6.7 Hz, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 176.4, 171.3, 152.9, 135.8, 129.5, 128.9, 127.2, 80.7, 65.8, 55.6, 40.9, 37.6, 37.5, 37.2, 28.0, 25.7, 23.3, 21.7; LRMS (ESI) *m/z* 412.21 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{22}H_{31}NO_5Na$: 412.2094 [$(M+Na)^+$], found: 412.2089.

2-Isobutylsuccinic acid 4-*t*-butyl ester **4-12**

The title compound **4-12** (7.39 g, 32.1 mmol, quant., a colorless oil) was prepared from imide **4-11** (11.9 g, 30.6 mmol) as described for carboxylic acid **4-17**. $[\alpha]^{23}_{D}$ -15.24 (*c* 0.49, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.90-2.81 (m, 1H, COCH), 2.59 (dd, *J* = 16.6, 9.2 Hz, 1H, $COCH_2$), 2.37 (dd, *J* = 16.6, 5.2 Hz, 1H, $COCH_2$), 1.71-1.54 (m, 2H, $CHCH_2$), 1.44 (s, 9H, CCH_3), 1.35-1.26 (m, 1H, $CHCH_2$), 0.94 (d, *J* = 6.3 Hz, 3H, CH_3), 0.91 (d, *J* = 6.3 Hz, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 181.3, 171.0, 81.0, 40.9, 39.5, 37.6, 28.0, 25.7, 22.5, 22.2; LRMS (ESI) *m/z* 253.14 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{12}H_{22}O_4Na$: 253.1410 [$(M+Na)^+$], found: 253.1410.

(2*R*,3*S*)-3-Isobutyl-4-oxooxetane-2-carboxylic acid *t*-butyl ester **4-13**

The title compound **4-13** (3.95 g, 17.3 mmol, 2 steps 57%, a brown liquid) was prepared as a single isomer from carboxylic acid **4-12** (7.04 g, 30.6 mmol) as described for β-lactone **4-18**. $[\alpha]^{23}_{D}$ -9.34 (*c* 0.77, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.48 (d, *J* = 4.0 Hz, 1H, OCH), 3.71 (ddd, *J* = 9.9, 9.9, 4.0 Hz, 1H, COCH), 1.89-1.70 (m, 3H, $CHCH_2$), 1.52 (s, 9H, CCH_3), 0.98 (d, *J* = 6.3 Hz, 3H, $CHCH_3$), 0.94 (d, *J* = 5.8 Hz, 3H, $CHCH_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.7, 167.1, 83.6, 72.5, 55.6, 36.7, 27.9, 26.4, 22.4, 21.7; LRMS (ESI) *m/z* 251.13 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{12}H_{20}O_4Na$: 251.1254 [$(M+Na)^+$], found: 251.1255.

(4*R*)-4-Benzyl-3-propionyloxazolidin-2-one **4-15**

To a solution of propionic acid **4-14** (5.84 ml, 78.0 mmol, 1.3 equiv) in THF (300 ml) was added triethylamine (20.9

ml, 150 mmol, 2.5 equiv) and PivCl (8.87 ml, 72.0 mmol, 1.2 equiv) at 0 °C. After 1 h at 0 °C, LiCl (2.80 g, 66.0 mmol, 1.1 equiv) and (4*R*)-4-benzyl-2-oxazolidinone (10.6 g, 60.0 mmol, 1.0 equiv) were added and the resulting mixture was warmed to rt. After 38 h at rt, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield the title compound **4-15** (12.3 g, 52.7 mmol, 88%) as a colorless oil [lit. (for *ent*-**4-15**) a white solid. mp 43-46 °C].[104] $[\alpha]^{22}_D$ -59.79 (*c* 1.42, $CHCl_3$) [lit. (for *ent*-**4-15**) $[\alpha]^{rt}_D$ 55.6 (*c* 1.27, $CHCl_3$)][104]; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.34 (dd, *J* = 7.4, 6.9 Hz, 2H, aromatic), 7.28 (t, *J* = 7.4 Hz, 1H, aromatic), 7.21 (d, *J* = 6.9 Hz, 2H, aromatic), 4.73-4.62 (m, 1H, NCH), 4.24-4.14 (m, 2H, OCH_2), 3.31 (dd, *J* = 13.2, 2.9 Hz, 1H, benzyl CH_2), 3.04-2.87 (m, 2H, $COCH_2$), 2.77 (dd, *J* = 13.2, 9.7 Hz, 1H, benzyl CH_2), 1.21 (dd, *J* = 7.4, 7.4 Hz, 3H, $CH_2C\underline{H}_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 174.0, 153.5, 135.3, 129.4, 128.9, 127.3, 66.2, 55.1, 37.9, 29.2, 8.2; LRMS (ESI) *m/z* 256.09 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{13}H_{15}NO_3Na$: 256.0944 [$(M+Na)^+$], found: 256.0941. ^{1}H-NMR, ^{13}C-NMR, $[\alpha]^{22}_D$ data are in agreement with those reported for *ent*-**4-15** by May.[104]

Imide **4-16**

To a solution of imide **4-15** (12.3 g, 52.7 mmol) in THF (500 ml) was added NaHMDS (42.0 ml, 79.1 mmol, 1.5 equiv, 1.9 M in THF) at -78 °C. After 30 min at -78 °C, $BrCH_2CO_2t$-Bu (15.5 ml, 106 mmol, 2.0 equiv) was added. After 25 h at -78 °C, the reaction was quenched with AcOH (6.04 ml, 106 mmol, 2.0 equiv) and the resulting mixture was concentrated *in vacuo* to remove THF. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was separated by silica gel column chromatography (*n*-hexane/ AcOEt 5:1) and purified by recrystallization from *n*-hexane to yield imide **4-16** (12.0 g, 34.5 mmol, 66%, single isomer) as a colorless needle. $[\alpha]^{24}_D$ -32.02 (*c* 1.22, $CHCl_3$); mp 103-104 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.30 (m, 2H, aromatic), 7.30-7.23 (m, 3H, aromatic), 4.75-4.61 (m, 1H, NCH), 4.27-4.09 (m, 3H, OCH_2 and COCH), 3.33 (dd, *J* = 13.7, 2.9 Hz, 1H, benzyl CH_2), 2.85 (dd, *J* = 16.6, 9.7 Hz, 1H, $COCH_2$), 2.76 (dd, *J* = 13.7, 9.7 Hz, 1H, benzyl CH_2), 2.39 (dd, *J* = 16.6, 4.6 Hz, 1H, $COCH_2$), 1.43 (s, 9H, CCH_3), 1.20 (d, *J* = 6.9 Hz, 3H, $CHC\underline{H}_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 176.3, 171.1, 152.9, 135.6, 129.4, 128.8, 127.1, 80.6, 65.9, 55.3, 38.9, 37.5, 34.5, 28.0, 17.0; LRMS (ESI) *m/z* 370.16 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{19}H_{25}NO_5Na$: 370.1625 [$(M+Na)^+$], found: 370.1618. ^{1}H-NMR and ^{13}C-NMR data are in agreement with those reporeted for *ent*-**4-16** by Stončius.[105]

(2*S*)-2-Methylsuccinic acid 4-*t*-butyl ester **4-17**

To a solution of imide **4-16** (3.40 g, 9.79 mmol) in 75% aqueous THF (100 ml) was added hydrogen peroxide (4.76 g, 48.9 mmol, 5.0 equiv, 35%) and $LiOH \cdot H_2O$ (821 mg, 19.6 mmol, 2.0 equiv) at 0 °C. After 5 min at 0 °C, the reaction mxiture was warmed to rt and stirred for 1 h. The reaction mixture was cooled to 0 °C, quenched with sat. $Na_2S_2O_3$ and concentrated *in vacuo* to remove THF. The residual aqueous solution was diluted with 2 M NaOH, extracted with DCM to remove (4*R*)-4-benzyl-2-oxazolidinone. The aqueous layer was acidified with citric acid and was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt) to yield the title compound **4-17** (1.60 g, 8.50 mmol, 87%) as a white solid. $[\alpha]^{23}_D$ -6.93 (*c* 1.04, $CHCl_3$) [lit. $[\alpha]^{23}_D$ -7.0 (*c* 0.86, $CHCl_3$)][106]; mp 56-57 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.95-2.85 (m, 1H, COCH), 2.64 (dd, *J* = 16.6, 8.0 Hz, 1H, $COCH_2$), 2.37 (dd, *J* = 16.6, 5.7 Hz, 1H, $COCH_2$), 1.44 (s, 9H, CCH_3), 1.24 (d, *J* = 7.4 Hz, 3H, $CHC\underline{H}_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 181.6, 171.0, 81.0,

38.7, 35.8, 28.0, 16.7; LRMS (ESI) *m/z* 211.09 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_9H_{16}O_4Na$: 211.0941 [(M+Na)$^+$], found: 211.0938. ^{1}H-NMR, ^{13}C-NMR and $[\alpha]^{23}_D$ data are in agreement with those reported by Davies.[106]

(2*R*,3*S*)-3-Methyl-4-oxooxetane-2-carboxylic acid *t*-butyl ester **4-18**

To a solution of carboxylic acid **4-17** (3.20 g, 17.0 mmol) in THF (170 ml) was added LiHMDS (23.4 ml, 37.4 mmol, 2.2 equiv, 1.6 M in THF) at -78 °C. After 45 min at -78 °C, CCl_4 (1.80 ml, 18.7 mmol, 1.1 equiv) was added. After 1 h at -78 °C, the reaction was quenched with AcOH (2.43 ml, 42.5 mmol, 2.5 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with 1 M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt) to yield the corresponding chlorinated product as a brown oil.

To a solution of the oil in Et_2O (100 ml) was added 5% $NaHCO_3$ (100 ml). After 21 h at rt, the reaction mixture was diluted with AcOEt, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column dhromatography (*n*-hexane/ AcOEt 10:1) to yield the title compound **4-18** (1.33 g, 7.16 mmol, 2 steps 42%, single isomer) as a white solid. $[\alpha]^{23}_D$ -31.12 (*c* 0.85, $CHCl_3$); mp 42-43 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.45 (d, *J* = 4.0 Hz, 1H, lactone CH), 3.73 (qd, *J* = 7.4, 4.0 Hz, 1H, lactone CH), 1.52 (s, 9H, CCH_3), 1.50 (d, *J* = 7.4 Hz, 3H, $CHCH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.9, 167.0, 83.5, 73.1, 51.6, 27.8, 12.5; LRMS (ESI) *m/z* 209.1 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_9H_{14}O_4Na$: 209.0790 [(M+Na)$^+$], found: 209.0789.

Alcohol **4-19**

To a solution of β-lactone **4-18** (1.32 g, 7.08 mmol) in MeOH (71 ml) was added triethylamine (3.94 ml, 28.3 mmol, 4.0 equiv). After 3 h at rt, the reaction mixture was concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 5:1) to yield alcohol **4-19** (1.55 g, 7.11 mmol, quant.) as a pale yellow liquid. $[\alpha]^{23}_D$ 8.96 (*c* 0.48, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.25 (dd, *J* = 5.7, 3.4 Hz, 1H, C<u>H</u>OH), 3.69 (s, 3H, CO_2CH_3), 3.14 (d, *J* = 5.7 Hz, 1H, OH), 2.98 (qd, *J* = 7.4, 3.4 Hz, 1H, C<u>H</u>CH_3), 1.49 (s, 9H, CCH_3), 1.25 (d, *J* = 7.4 Hz, 3H, $CHC\underline{H}_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.1, 172.3, 83.0, 72.2, 51.7, 43.2, 27.8, 12.1; LRMS (ESI) *m/z* 241.10 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{10}H_{18}O_5Na$: 241.1046 [(M+Na)$^+$], found: 241.1048.

Alcohol **4-20**

To a solution of alcohol **4-19** (31.5 mg, 0.144 mmol) in THF (2.0 ml) was added LiHMDS (361 µl, 0.577 mmol, 4.0 equiv) at -78 °C. After 5 min at -78 °C, 3-bromo-2-methylpropene (291 µl, 2.89 mmol, 20 equiv) was added and the resulting solution was warmed to 0 °C. After 100 min at 0 °C, the reaction was quenched with AcOH and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 15:1) to yield alcohol **4-20** (16.9 mg, 0.0621 mmol, 43%, single isomer) as a colorless oil. $[\alpha]^{23}_D$ -15.63 (*c* 0.93, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.86 (br, 1H, alkenyl CH_2), 4.72 (br, 1H, alkenyl CH_2), 4.12 (d, *J* = 7.6 Hz, 1H, C<u>H</u>OH), 3.72 (s, 3H, CO_2CH_3), 3.40 (d, *J* = 7.6 Hz, 1H, OH), 2.67 (d, *J* = 13.5 Hz, 1H, CCH_2), 2.35 (d, *J* = 13.5 Hz, 1H, CCH_2), 1.69 (s, 3H, allyl CH_3), 1.51 (s, 9H, CCH_3), 1.15 (s, 3H, CCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.0, 171.8, 141.3, 115.4, 83.3, 76.2, 52.0, 50.1, 43.0, 28.0, 23.7, 16.9; LRMS (ESI) *m/z* 295.15 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{14}H_{24}O_5Na$: 295.1516 [(M+Na)$^+$], found: 295.1520.

Alcohol **4-21**

To a solution of alcohol **4-20** (165 mg, 0.604 mmol) in MeOH (10 ml) was added Pd/C (160 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 15 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to yield alcohol **4-21** (174 mg, 0.634 mmol, quant.) as a colorless oil. $[\alpha]^{23}_{D}$ 1.18 (*c* 0.16, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.10 (br, 1H, C<u>H</u>OH), 3.71 (s, 3H, CO_2CH_3), 3.33 (br, 1H, OH), 1.79 (dd, *J* = 13.9, 7.2 Hz, 1H, CH_2), 1.74-1.59 (m, 1H, C<u>H</u>CH_3), 1.58-1.49 (m, 1H, CH_2), 1.49 (s, 9H, CCH_3), 1.17 (s, 3H, CCH_3), 0.92 (d, *J* = 6.3 Hz, 3H, CHC<u>H</u>$_3$), 0.84 (d, *J* = 6.3 Hz, 3H, CHC<u>H</u>$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.6, 171.9, 83.1, 76.6, 51.8, 50.1, 43.6, 28.0, 24.9, 24.5, 23.0, 16.8; LRMS (ESI) *m/z* 297.17 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{14}H_{26}O_5Na$: 297.1673 [$(M+Na)^+$], found: 297.1668.

(2*R*,3*S*)-3-Isobutyl-3-methyl-4-oxooxetane-2-carboxylic acid *t*-butyl ester **4-22**

To a solution of alcohol **4-21** (132 mg, 0.482 mmol) in 50% aqueous THF (10 ml) was added $LiOH \cdot H_2O$ (202 mg, 4.82 mmol, 10 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt. After 18 h at rt, the reaction mixture was diluted with 33% aqueous THF (15 ml). After 47 h at rt, the reaction mixture was concentrated *in vacuo* to remove THF and the residual aqueous solution was diluted with 0.1 M NaOH, extracted with DCM. The aqueous layer was acidified with 1 M HCl and was extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a colorless viscous oil.

To a solution of the viscous oil in DCM (10 ml) was added triethylamine (277 μl, 1.99 mmol, 5.0 equiv) and PyBOP (311 mg, 0.598 mmol, 1.5 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 75 min. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 49:1) to yield the title compound **4-22** (79.3 mg, 0.327 mmol, 2 steps 68%) as a colorless oil. $[\alpha]^{24}_{D}$ -24.89 (*c* 0.58, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.65 (s, 1H, COCH), 1.90-1.78 (m, 2H, C<u>H</u>$_2$ and C<u>H</u>CH_3), 1.71-1.62 (m, 1H, CH_2), 1.52 (s, 9H, CCH_3), 1.31 (s, 3H, CCH_3), 1.00 (d, *J* = 6.3 Hz, 6H, CHC<u>H</u>$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.3, 166.5, 83.8, 60.7, 43.8, 28.1, 24.5, 24.0, 22.1, 15.0; LRMS (ESI) *m/z* 265.14 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{13}H_{22}O_4Na$: 265.1410 [$(M+Na)^+$], found: 265.1410.

(*S*)-4-benzyl-3-((*S*)-3-methylpentanoyl)oxazolidin-2-one **4-25**

To a solution of NaOH (40.0 g, 1.00 mol) in H_2O (250 ml) was added L-isoleucine **4-23** (20.0 g, 153 mmol) at 0 °C. After 10 min at 0 °C, H_2NOSO_3H (20.0 g, 177 mmol, 1.2 equiv) was added. After 30 min at 0 °C, 2.5 M NaOH (150 ml) and H_2NOSO_3H (20.0 g, 177 mmol, 1.2 equiv) was added and the resulting mixture was warmed to rt. After 25 h at rt, the reaction mixture was refluxed for 3 h. The reaction mixture was cooled to 0 °C, H_2SO_4 (64 ml) was added at a rate to keep the internal temperature below 20 °C. The resulting mixture was extracted with DCM, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid **4-24** as a colorless oil.

To a solution of the oil in THF (590 ml) was added triethylamine (50.0 ml, 359 mmol, 3.1 equiv) and PivCl (17.3 ml, 141 mmol, 1.2 equiv) at 0 °C. After 1 h at 0 °C, LiCl (5.47 g, 129 mmol, 1.1 equiv) and (4*S*)-4-benzyl-2-oxazolidinone (20.4 g, 115 mmol, 1.0 equiv) were added and the resulting mixture was warmed to rt. After 13 h at rt, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and

brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield imide **4-25** (23.0 g, 83.6 mmol, 2 steps 74%) as a yellow oil. $[\alpha]^{21}_D$ 57.70 (*c* 1.28, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40-7.18 (m, 5H, aromatic), 4.74-4.65 (m, 1H, BnCH), 4.23-4.12 (m, 2H, OCH_2), 3.32 (dd, *J* = 13.0, 3.6 Hz, 1H, benzyl CH_2), 2.99 (dd, *J* = 16.2, 5.8 Hz, 1H, $COCH_2$), 2.78-2.68 (m, 2H, $COCH_2$ and benzyl CH_2), 2.09-1.95 (m, 1H, C<u>H</u>CH_3), 1.52-1.39 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.36-1.21 (m, 1H, C<u>H</u>$_2$$CH_3$), 0.98 (d, *J* = 6.7 Hz, 3H, CHC<u>H</u>$_3$), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.9, 153.4, 135.3, 129.4, 128.9, 127.3, 66.0, 55.1, 42.1, 37.9, 31.1, 29.4, 19.2, 11.3; LRMS (ESI) *m/z* 298.14 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{16}H_{21}NO_3Na$: 298.1414 [$(M+Na)^+$], found: 298.1419.

<u>(2*S*,3*R*)-*tert*-butyl 3-((*S*)-*sec*-butyl)-4-oxooxetane-2-carboxylate **4-26**</u>

To a solution of imide **4-25** (16.4 g, 59.5 mmol) in THF (800 ml) was added NaHMDS (47.0 ml, 89.3 mmol, 1.5 equiv, 1.9 M in THF) at -78 °C. After 30 min at -78 °C, $BrCH_2CO_2t$-Bu (17.5 ml, 119 mmol, 2.0 equiv) was added. After 30 min at -78 °C, the reaction was quenched with AcOH (6.98 ml, 119 mmol, 2.0 equiv) and the resulting mixture was concentrated *in vacuo* to remove THF. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1-5:1) to give the corresponding imide as a white solid.

To a solution of the solid in THF (380 ml) was added H_2O (120 ml), hydrogen peroxide (22.9 ml, 235 mmol, 5.0 equiv, 35%) and $LiOH\cdot H_2O$ (3.95 g, 94.1 mmol, 2.0 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was cooled to 0 °C, quenched with sat. $Na_2S_2O_3$ and concentrated *in vacuo* to remove THF. The residual aqueous solution was diluted with 0.1 M NaOH, extracted with DCM to remove (4*S*)-4-benzyl-2-oxazolidinone. The aqueous layer was acidified with citric acid and was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a colorless oil.

To a solution of the oil in THF (500 ml) was added LiHMDS (63.1 ml, 101 mmol, 2.2 equiv, 1.6 M in THF) at -78 °C. After 35 min at -78 °C, CCl_4 (4.87 ml, 50.5 mmol, 1.1 equiv) was added. After 1 h at -78 °C, the reaction was quenched with AcOH (12.0 ml, 200 mmol, 3.0 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with 1 M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding chlorinated product as a brown oil.

To a solution of the oil in ether (250 ml) was added 5% $NaHCO_3$ (250 ml). After 43 h at rt, the reaction mixture was diluted with AcOEt, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield β-lactone **4-26** (9.93 g, 43.5 mmol, 4 steps 73%, single isomer) as a pale yellow solid. $[\alpha]^{20}_D$ 5.39 (*c* 0.76, $CHCl_3$); mp 55-56 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.54 (d, *J* = 4.5 Hz, 1H, OCH), 3.51 (dd, *J* = 9.0, 4.5 Hz, 1H, COCH), 2.03-1.91 (m, 1H, C<u>H</u>CH_3), 1.63-1.44 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.51 (s, 9H, CCH_3), 1.37-1.23 (m, 1H, C<u>H</u>$_2$$CH_3$), 1.10 (d, *J* = 6.7 Hz, 3H, CHC<u>H</u>$_3$), 0.95 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.0, 167.4, 83.5, 70.6, 62.7, 34.0, 27.8, 27.1, 16.0, 10.6; LRMS (ESI) *m/z* 251.73 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{12}H_{20}O_4Na$: 251.1259 [$(M+Na)^+$], found: 251.1258.

<u>(2*S*, 3*R*)-1-*tert*-butyl 4-methyl 3-((*S*)-*sec*-butyl)-2-hydroxysuccinate **4-27**</u>

To a solution of β-lactone **4-26** (1.01 g, 4.44 mmol) in MeOH (45.0 ml) was added triethylamine (2.47 ml, 17.8

mmol, 4.0 equiv). After 10 h at rt, the reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield alcohol **25** (1.09 g, 4.19 mmol, 94%) as a pale yellow oil. $[\alpha]^{20}_{D}$ -11.93 (*c* 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.26 (dd, *J* = 9.2, 3.4 Hz, 1H, C<u>H</u>OH), 3.67 (s, 3H, OCH_3), 3.21 (d, *J* = 9.2 Hz, 1H, OH), 2.65 (dd, *J* = 8.6, 3.4 Hz, 1H, COCH), 2.07-1.97 (m, 1H, C<u>H</u>CH_3), 1.68-1.56 (m, 1H, C<u>H$_2$</u>CH_3), 1.48 (s, 9H, CCH_3), 1.35-1.23 (m, 1H, C<u>H$_2$</u>CH_3), 0.96 (d, *J* = 6.9 Hz, 3H, CHC<u>H$_3$</u>), 0.92 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.4, 172.8, 82.5, 69.8, 53.6, 51.4, 33.3, 27.9, 26.2, 16.9, 10.7; LRMS (ESI) *m/z* 283.16 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{13}H_{24}O_5Na$: 283.1521 $[(M+Na)^+]$, found: 283.1530.

<u>(2*S*,3*R*)-3-((*S*)-*sec*-butyl)-4-oxooxetane-2-carboxylic acid **4-29**</u>

To a solution of β-lactone **4-26** (557 mg, 2.44 mmol) in DCM (10.0 ml) was added TFA (10.0 ml) at -5 °C. After 21 h at -5 °C, the reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 4:1-0:1) to yield β-lactone **4-29** (399 mg, 2.32 mmol, 95%) as a colorless oil. $[\alpha]^{24}_{D}$ 1.74 (*c* 0.69, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.37 (br, 1H, COOH), 4.72 (d, *J* = 4.5 Hz, 1H, OCH), 3.69 (dd, *J* = 9.0, 4.5 Hz, 1H, COCH), 2.07-1.94 (m, 1H, C<u>H</u>CH_3), 1.66-1.52 (m, 1H, C<u>H$_2$</u>CH_3), 1.39-1.24 (m, 1H, C<u>H$_2$</u>CH_3), 1.12 (d, *J* = 6.7 Hz, 3H, CHC<u>H$_3$</u>), 0.97 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.0, 168.2, 69.5, 63.5, 34.2, 27.2, 16.0, 10.8; LRMS (ESI) *m/z* 171.07 $[(M-H)^-]$; HRMS (ESI) calcd for $C_8H_{11}O_4$: 171.0663 $[(M-H)^-]$, found: 171.0660.

<u>(2*S*,3*S*)-4-*tert*-butyl 1-methyl 2-((*S*)-*sec*-butyl)-3-hydroxy-2-methylsuccinate **4-28**</u>

To a solution of diisopropylamine (8.78 ml, 62.2 mmol, 5.1 equiv) in THF (80 ml) was added *n*-BuLi (22.9 ml, 61.0 mmol, 5.0 equiv, 2.66 M in Hexane) at 0 °C. After 30 min at 0 °C, the reaction mixture was cooled to -78 °C and β-lactone **4-29** (2.10 g, 12.2 mmol) in THF (40 ml) was added *via cannula*. After 30 min at -78 °C, CH_3I (11.4 ml, 183 mmol, 15 equiv) was added and the reaction mixture was warmed to -40 °C. After 5 min at -40 °C, the reaction was quenched with AcOH (4.29 ml, 74.9 mmol, 6.1 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with 1M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding methylated product **4-30** (dr 3:1) as a yellow oil.

To a solution of the oil in DCM (120 ml) was added THF (10 ml), MS 4 Å (2.10 g) and Ag_2CO_3 (15.1 g, 54.9 mmol, 4.5 equiv). The resulting mixture was cooled to 0 °C and *t*-butyl bromide (8.22 ml, 73.2 mmol, 6.0 equiv) was added. After 5 min at 0 °C, the reaction mixture was warmed to rt and was stirred for 56 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo*. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 99:1) to give the corresponding *t*-butyl ester (dr 3:1) as a colorless oil.

To a solution of the oil in MeOH (31 ml) was added NaOMe (617 µl, 3.10 mmol, 1.0 equiv, 28% in MeOH) at 0 °C. After 1 h at 0 °C, the reaction was quenched with AcOH (273 µl, 4.77 mmol, 1.5 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 99:1) to yield alcohol **4-28** (502 mg, 1.83 mmol, 3 steps 15%, single isomer) as a white solid. $[\alpha]^{25}_{D}$ 19.25 (*c* 0.30, $CHCl_3$); mp 52-54 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.53 (d, *J* = 4.7 Hz, 1H, C<u>H</u>OH), 3.67 (s, 3H, OCH_3), 3.09 (d, *J* = 4.7 Hz, 1H, OH), 1.78-1.65 (m, 2H, C<u>H</u>CH_3 and C<u>H$_2$</u>CH_3), 1.47 (s, 9H, *t*-butyl CH_3), 1.29-1.13 (m, 1H, C<u>H$_2$</u>CH_3), 1.07 (s, 3H, CCH_3), 0.90 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>), 0.86 (d, *J* = 7.2 Hz, 3H, CHC<u>H$_3$</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.1, 173.5, 83.4, 72.7, 54.0, 51.2, 40.3, 27.9, 24.3, 14.9, 13.6, 12.8; LRMS (ESI) *m/z*

297.17 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{14}H_{26}O_5Na$: 297.1673 [(M+Na)$^+$], found: 297.1668.

(2*S*,3*R*)-4-*tert*-butyl 1-methyl 2-((*S*)-*sec*-butyl)-3-hydroxy-2-methylsuccinate **4-31**

To a solution of alcohol **4-28** (20.6 mg, 0.0751 mmol) in DCM (1.0 ml) was added DMP (41.4 mg, 0.0977 mmol, 1.3 equiv) and the reaction mixture was stirred for 3 h. The reaction was quenched with a solution of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (1:3), extracted with $CHCl_3$, the organic layer was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding glyoxylate ester as a colorless oil.

To a solution of the oil and (*R*)-2-methyl-CBS-1,3,2-oxazaborolidine (23.2 mg, 0.0837 mmol, 1.1 equiv) in THF (840 µl) was added BH_3-THF (441 µl, 0.419 mmol, 5.0 equiv, 0.95 M in THF) at -78 °C. After 1 h at -78 °C, the reaction was quenched with MeOH and the resulting mixture was warmed to -40 °C. After 30 min at -40 °C, the solvent was removed under reduced pressure and the residue was dissolved in AcOEt, washed with 1 M HCl, 1M NaOH, sat. NH_4Cl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product (dr 99:1) was purified by flash column chromatography (*n*-hexane/ AcOEt 98:2-15:1) to yield alcohol **4-31** (20.2 mg, 0.0736 mmol, 2 steps 98%, single isomer) as a colorless oil. $[\alpha]^{25}_D$ 4.07 (*c* 1.01, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.04 (d, *J* = 10.8 Hz, 1H, C<u>H</u>OH), 3.71 (s, 3H, OCH_3), 3.62 (d, *J* = 10.8 Hz, 1H, OH), 2.19-2.07 (m, 1H, C<u>H</u>CH_3), 1.47 (s, 9H, *t*-butyl CH_3), 1.24-1.01 (m, 2H, C<u>H$_2$</u>CH_3), 1.06 (s, 3H, CCH_3), 0.94-0.87 (m, 6H, CH_2C<u>H$_3$</u> and CHC<u>H$_3$</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.8, 172.1, 82.5, 75.0, 53,2, 51.8, 37.9, 27.9, 25.0, 13.3, 12.6, 12.5; LRMS (ESI) *m/z* 297.17 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{14}H_{26}O_5Na$: 297.1673 [(M+Na)$^+$], found: 297.1668.

(2*S*,3*S*)-2-((*R*)-2-(benzyloxy)-1-hydroxy-2-oxoethyl)-2,3-dimethylpentanoic acid **4-33**

To a solution of alcohol **4-31** (144 mg, 0.530 mmol) in 1,4-dioxane (5.0 ml) was added 1 M NaOH (5.0 ml) and the reaction mixture was warmed to 80 °C. After 17 h at 80 °C, the reaction mixture was neutralized with weak acid resin (DIAION WK10) and the solvent was removed under reduced pressure to give the corresponding dicarboxylic acid as a colorless viscous oil.

The oil was dissolved in TFAA (2.0 ml) at 0 °C and the reaction mixture was stirred for 2 h at 0 °C. The solvent was removed under reduced pressure at 0 °C to give the corresponding acid anhydride as a white solid.

A solution of the solid in BnOH (1.0 ml) was stirred for 13 h at rt and then the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt/ $CHCl_3$/ MeOH 5:1:0:0-0:0:100:5) to yield carboxylic acid **4-33** (128 mg, 0.436 mmol, 3 steps 82%) as a colorless viscous oil. $[\alpha]^{24}_D$ 4.55 (*c* 0.24, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ 7.43-7.29 (m, 5H, aromatic), 5.19 (d, *J* = 12.0 Hz, 1H, benzyl CH_2), 5.14 (d, *J* = 12.0 Hz, 1H, benzyl CH_2), 4.41 (s, 1H, OCH), 1.90-1.81 (m, 1H, C<u>H</u>CH_3), 1.58-1.46 (m, 1H, C<u>H$_2$</u>CH_3), 1.12-1.04 (m, 1H, C<u>H$_2$</u>CH_3), 1.04 (s, 3H, CCH_3), 0.87 (d, *J* = 6.9 Hz, 3H, CHC<u>H$_3$</u>), 0.85 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>); ^{13}C-NMR (125 MHz, CD_3OD) δ 178.3, 173.9, 137.0, 129.7, 129.6, 129.4, 75.3, 67.9, 54.4, 39.7, 26.0, 14.7, 13.4, 13.0; LRMS (ESI) *m/z* 293.14 [(M-H)$^-$]; HRMS (ESI) calcd for $C_{16}H_{21}O_5$: 293.1395 [(M-H)$^-$], found: 293.1391.

(2*R*,3*S*)-benzyl 3-((*S*)-*sec*-butyl)-3-methyl-4-oxooxetane-2-carboxylate **4-34**

To a solution of carboxylic acid **4-33** (124 mg, 0.420 mmol) in DCM (42 ml) was added triethylamine (175 µl, 1.26 mmol, 3.0 equiv) and PyBOP (328 mg, 0.629 mmol, 1.5 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and was stirred for 25 min. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat.

$NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 15:1) to yield β-lactone **4-34** (87.3 mg, 0.316 mmol, 75%) as a white solid. $[\alpha]^{24}_D$ 0.86 (*c* 1.17, $CHCl_3$); mp 38-39 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41-7.33 (m, 5H, aromatic), 5.29 (d, *J* = 11.9 Hz, 1H, benzyl CH_2), 5.25 (d, *J* = 11.9 Hz, 1H, benzyl CH_2), 4.70 (s, 1H, OCH), 1.89-1.75 (m, 1H, C<u>H</u>CH_3), 1.77-1.66 (m, 1H, C<u>H</u>$_2CH_3$), 1.12 (s, 3H, CCH_3), 1.12-1.04 (m, 1H, C<u>H</u>$_2CH_3$), 1.01 (d, *J* = 6.7 Hz, 3H, CHC<u>H</u>$_3$), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.3, 167.5, 134.7, 128.8, 128.7, 128.7, 74.7, 67.4, 66.1, 39.1, 24.0, 14.2, 11.9, 11.8; LRMS (ESI) *m/z* 299.13 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{16}H_{20}O_4Na$: 299.1254 [$(M+Na)^+$], found: 299.1250.

<u>(2*R*,3*S*)-3-((*S*)-*sec*-butyl)-3-methyl-4-oxooxetane-2-carboxylic acid **4-37**</u>

To a solution of β-lactone **4-34** (23.3 mg, 0.0843 mmol) in THF (1.5 ml) was added Pd/C (23 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 2 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to yield carboxylic acid **4-37** (16.3 mg, 0.0875 mmol, quant) as a white solid. $[\alpha]^{24}_D$ -4.42 (*c* 0.82, $CHCl_3$); mp 85-87 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.81 (br, 1H, COOH), 4.74 (s, 1H, OCH), 1.93-1.83 (m, 1H, C<u>H</u>CH_3), 1.82-1.72 (m, 1H, C<u>H</u>$_2CH_3$), 1.33 (s, 3H, CCH_3), 1.20-1.09 (m, 1H, C<u>H</u>$_2CH_3$), 1.06 (d, *J* = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 0.97 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H</u>$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.9, 172.0, 74.4, 66.4, 39.1, 24.0, 14.2, 12.2, 11.8; LRMS (ESI) *m/z* 185.08 [$(M-H)^-$]; HRMS (ESI) calcd for $C_9H_{13}O_4$: 185.0819 [$(M-H)^-$], found: 185.0814.

<u>Target compound **4-1**</u>

To a solution of carbamate **1-72** (61.3 mg, 0.120 mmol) in DCM (600 µl) was added TFA (600 µl). After 15 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow oil.

To a solution of β-lactone **4-13** (41.3 mg, 0.181 mmol, 1.5 equiv) in DCM (1.0 ml) was added TFA (1.0 ml) at -5 °C. After 20 h at -5 °C, the reaction mixture was concentrated *in vacuo* to give the corresponding carboxylic acid **4-35** as a brown oil.

To a solution of the oil in DCM (2.0 ml) was added triethylamine (25 µl, 0.181 mmol, 1.5 equiv) and PivCl (22 µl, 0.181 mmol, 1.5 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.5 ml) was added triethylamine (50 µl, 0.361 mmol, 3.0 equiv) and a solution of the acid anhydride in DCM (2.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 2:1-3:2) to yield target compound **4-1** (42.0 mg, 0.0745 mmol, 2 steps 62%) as a white amorphous solid. $[\alpha]^{20}_D$ -2.32 (*c* 0.34, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41-7.23 (m, 7H, aromatic), 7.23-7.11 (m, 3H, aromatic), 7.08 (br, 1H, amide NH), 6.67 (d, *J* = 8.1 Hz, 1H, amide NH), 5.61 (d, *J* = 6.3 Hz, 1H, carbamate NH), 5.10 (s, 2H, benzyl CH_2), 4.58 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.33-4.19 (m, 1H, Ala CH), 4.00-3.87 (m, 1H, NCH), 3.81-3.69 (m, 1H, NCOCHC<u>H</u>), 2.77 (br, 1H, cyclopropyl CH), 2.63 (br, 2H, benzyl CH_2), 1.97-1.58 (m, 6H, BnCH_2, NCHC<u>H</u>$_2$ (1H), isobutyl CH_2 and isobutyl CH), 1.39 (d, *J* = 7.2 Hz, 3H, Ala CH_3), 1.24-1.10 (m, 1H, NCHC<u>H</u>$_2$), 1.07-0.84 (m, 8H, cyclopropyl CH_2 (1H), cyclopropyl CH, isobutyl CH_3), 0.27-0.18 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.7, 170.0, 155.9, 141.3, 136.4, 128.5, 128.5, 128.2, 128.1, 127.9, 126.1, 73.7,

66.8, 56.2, 50.6, 49.8, 37.0, 36.9, 32.5, 32.4, 26.9, 26.4, 22.3, 21.9, 19.0, 14.5, 11.6; LRMS (ESI) *m/z* 564.29 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{32}H_{42}N_3O_6$: 564.3074 [$(M+H)^+$], found: 564.3065; Anal. calcd for $C_{32}H_{41}N_3O_6 \cdot 0.1H_2O$: C, 67.97; H, 7.34; N, 7.43. Found: C, 67.82; H, 7.46; N, 7.14.

Target compound **4-2**

To a solution of carbamate **1-72** (40.2 mg, 0.0789 mmol) in DCM (800 µl) was added TFA (800 µl). After 15 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow oil.

To a solution of β-lactone **4-22** (28.7 mg, 0.118 mmol, 1.5 equiv) in DCM (1.0 ml) was added TFA (1.0 ml) at -5 °C. After 20 h at -5 °C, the reaction mixture was concentrated *in vacuo* to give the corresponding carboxylic acid **4-36** as a colorless oil.

To a solution of the oil in DCM (1.0 ml) was added triethylamine (16 µl, 0.118 mmol, 1.5 equiv) and PivCl (15 µl, 0.118 mmol, 1.5 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (33 µl, 0.237 mmol, 3.0 equiv) and a solution of the acid anhydride in DCM (1.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 2:1) to yield target compound **4-2** (45.6 mg, 0.0789 mmol, 2 steps 100%) as a colorless viscous oil. $[\alpha]^{20}_D$ -15.09 (*c* 0.41, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.22 (m, 7H, aromatic), 7.22-7.09 (m, 3H, aromatic), 6.79 (br, 1H, amide NH), 6.77 (br, 1H, amide NH), 5.77 (br, 1H, carbamate NH), 5.13 (d, *J* = 12.6 Hz, 1H, benzyl CH_2), 5.07 (d, *J* = 12.6 Hz, 1H, benzyl CH_2), 4.73 (s, 1H, NCOCH), 4.35 (dq, *J* = 6.9, 6.9 Hz, 1H, Ala CH), 4.11-3.97 (m, 1H, NCH), 2.75 (br, 1H, cyclopropyl CH), 2.61 (br, 2H, benzyl CH_2), 1.92-1.56 (m, 5H, NCHC<u>H</u>$_2$ (1H), $BnCH_2$, isobutyl CH_2 (1H) and isobutyl CH), 1.43 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.36-1.08 (m, 5H, isobutyl CH_2 (1H), NCHC<u>H</u>$_2$ (1H) and CCH_3), 1.08-0.78 (m, 8H, cyclopropyl CH_2 (1H), cyclopropyl CH and isobutyl CH_3), 0.28-0.18 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.2, 172.8, 169.2, 155.9, 141.5, 136.5, 128.5, 128.3, 128.1, 128.0, 126.0, 77.5, 66.7, 60.4, 50.8, 49.5, 43.7, 37.6, 32.3, 32.1, 27.6, 24.5, 24.0, 22.2, 19.1, 15.9, 15.1, 10.8; LRMS (ESI) *m/z* 578.32 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{33}H_{44}N_3O_6$: 578.3230 [$(M+H)^+$], found: 578.3224; Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.1H_2O$: C, 68.39; H, 7.51; N, 7.25. Found: C, 68.45; H, 7.60; N, 7.00.

Target compound **4-3**

To a solution of carbamate **1-72** (35.7 mg, 0.0700 mmol) in DCM (350 µl) was added TFA (350 µl). After 15 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a white solid.

To a solution of carboxylic acid **4-37** (16.3 mg, 0.0875 mmol, 1.3 equiv) in DCM (1.0 ml) was added EDC·HCl (14.6 mg, 0.0761 mmol, 1.1 equiv) and HOAt (10.4 mg, 0.0761 mmol, 1.1 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was used as a solution of the corresponding HOAt ester in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (29.2 µl, 0.210 mmol, 3.0 equiv) and a solution of the HOAt ester in DCM (1.0 ml) at 0 °C. After 2 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:1) to yield target compound **4-3** (41.2 mg, 0.0713 mmol, 2 steps quant.) as a colorless viscous oil. $[\alpha]^{24}_D$ -8.24 (*c* 0.53, $CHCl_3$);

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.39-7.22 (m, 7H, aromatic), 7.22-7.10 (m, 3H, aromatic), 6.82 (br, 2H, amide NH), 5.85 (d, *J* = 7.4 Hz, 1H, carbamate NH), 5.14 (d, *J* = 12.3 Hz, 1H, benzyl CH_2), 5.06 (d, *J* = 12.3 Hz, 1H, benzyl CH_2), 4.60 (s, 1H, OCH), 4.37 (dq, *J* = 7.4, 6.9 Hz, 1H, Ala CH), 4.09-3.99 (m, 1H, NCH), 2.75 (br, 1H, cyclopropyl CH), 2.67-2.54 (m, 2H, PhC$\underline{H}_2$CH$_2$), 1.83-1.63 (m, 5H, NCHC$\underline{H}_2$ (1H), *sec*-butyl CH_2 (1H), $PhCH_2C\underline{H}_2$ (2H) and *sec*-butyl CH), 1.43 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.28-1.15 (m, 1H, NCHC$\underline{H}_2$), 1.20 (s, 3H, CCH_3), 1.14-0.86 (m, 9H, *sec*-butyl CH_2 (1H), cyclopropyl CH, cyclopropyl CH_2 (1H) and *sec*-butyl CH_3), 0.29-0.18 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 172.7, 169.2, 155.8, 141.5, 136.4, 128.4, 128.2, 128.0, 128.0, 126.0, 76.2, 66.7, 65.2, 50.8, 49.4, 39.1, 37.6, 32.3, 32.1, 27.6, 23.9, 19.1, 15.0, 14.1, 12.6, 11.8, 10.7; LRMS (ESI) *m/z* 600.30 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{43}N_3O_6Na$: 600.3044 [$(M+Na)^+$], found: 600.3046; Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.2H_2O$: C, 68.18; H, 7.52; N, 7.23. Found: C, 68.20; H, 7.61; N, 7.10.

Target compound **4-4**

To a solution of carbamate **2-24a** (44.3 mg, 0.0846 mmol) in DCM (500 μl) was added TFA (500 μl). After 1 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a colorless viscous oil.

To a solution of carboxylic acid **4-37** (24.6 mg, 0.132 mmol, 1.6 equiv) in DCM (1.0 ml) was added HOAt (15.6 mg, 0.115 mmol, 1.4 equiv) and EDC·HCl (22.0 mg, 0.115 mmol, 1.4 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was used as a solution of the corresponding HOAt ester in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (35.3 μl, 0.254 mmol, 3.0 equiv) and a solution of the HOAt ester in DCM (1.0 ml) at 0 °C. After 1 h at rt, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:1) to yield target compound **4-4** (45.4 mg, 0.0767 mmol, 2 steps 91%) as a colorless viscous oil. $[\alpha]^{24}_D$ -63.77 (*c* 0.81, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40-7.24 (m, 7H, aromatic), 7.23-7.11 (m, 3H, aromatic), 6.31 (d, *J* = 3.6 Hz, 1H, amide NH), 6.07 (d, *J* = 8.1 Hz, 1H, amide NH), 5.33 (d, *J* = 6.7 Hz, 1H, carbamate NH), 5.11 (s, 2H, benzyl CH_2), 4.59 (s, 1H, OCH), 4.17 (dq, *J* = 7.2, 6.7 Hz, 1H, Ala CH), 4.14-4.04 (m, 1H, NCH), 2.95-2.83 (m, 1H, cyclopropyl CH), 2.70-2.51 (m, 2H, PhC$\underline{H}_2$CH$_2$), 1.90-1.60 (m, 4H, $PhCH_2C\underline{H}_2$ (2H), *sec*-butyl CH_2 (1H) and *sec*-butyl CH), 1.39 (d, *J* = 7.2 Hz, 3H, Ala CH_3), 1.23 (s, 3H, CCH_3), 1.17-1.05 (m, 2H, *sec*-butyl CH_2 and NCHC$\underline{H}$), 1.04 (d, *J* = 6.7 Hz, 3H, *sec*-butyl CHC$\underline{H}_3$), 1.01-0.88 (m, 7H, cyclopropyl CH_2 (1H), NCHCHC$\underline{H}_3$ and *sec*-butyl $CH_2C\underline{H}_3$), 0.81-0.67 (m, 1H, cyclopropyl CH), 0.49-0.36 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 171.8, 168.3, 156.1, 141.5, 136.0, 128.6, 128.5, 128.3, 128.3, 128.1, 126.0, 76.1, 67.1, 65.3, 53.1, 50.8, 39.0, 36.6, 34.6, 32.9, 26.8, 24.0, 21.5, 18.1, 15.3, 14.3, 12.5, 11.8, 11.0; LRMS (ESI) *m/z* 614.32 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{34}H_{45}N_3O_6Na$: 614.3201 [$(M+Na)^+$], found: 614.3203; Anal. calcd for $C_{34}H_{45}N_3O_6 \cdot 0.4H_2O$: C, 68.18; H, 7.71; N, 7.02. Found: C, 68.17; H, 7.73; N, 6.93.

General procedure for the preparation of the target compounds **4-5** – **4-8**

To a solution of the carbamate (1.0 equiv) in DCM (0.1 M) was added an equivalent amount of TFA and Pd/C (20 mg) at 0 °C. The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred at 0 °C under an atmosphere of hydrogen until the starting material disappeared on TLC. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to yield the corresponding target compound.

Target compound **4-5**

Target compound **4-5** (9.60 mg, 0.0182 mmol, quant.) was obtained from **4-1** as a colorless oil. $[\alpha]^{22}_{D}$ -14.02 (*c* 0.39, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.55 (br, 3H, NH_3^+), 8.48 (d, *J* = 8.6 Hz, 1H, amide NH), 8.25 (br, 1H, amide NH), 7.26-7.13 (m, 4H, aromatic), 7.13-7.03 (m, 1H, aromatic), 4.70 (d, *J* = 4.0 Hz, 1H, OCH), 4.30-4.13 (m, 1H, Ala CH), 4.08-4.39 (m, 1H, NCH), 3.84-3.74 (m, 1H, COCH), 2.79 (br, 1H, cyclopropyl CH), 2.72-2.60 (m, 1H, $PhCH_2$), 2.60-2.46 (m, 1H, $PhCH_2$), 1.85-1.62 (m, 5H, $PhCH_2C\underline{H}_2$, isobutyl CH and isobutyl CH_2), 1.54 (d, *J* = 6.3 Hz, 3H, Ala CH_3), 1.53-1.40 (m, 2H, $NCHC\underline{H}_2$), 1.15-1.04 (m, 1H, cyclopropyl CH), 0.95 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.91 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.91-0.79 (m, 1H, cyclopropyl CH_2), 0.41-0.31 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (500 MHz, THF-d8) δ 171.8, 170.9, 170.7, 143.3, 129.4, 129.2, 126.6, 74.4, 57.0, 50.6, 50.5, 38.5, 37.9, 34.5, 33.7, 28.5, 27.5, 23.1, 22.5, 18.3, 16.0, 11.0; LRMS (ESI) *m/z* 430.27 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{24}H_{36}N_3O_4$: 430.2700 $[(M+H)^+]$, found: 430.2705; Anal. calcd for $C_{26}H_{35}F_3N_3O_5 \cdot 2.5TFA \cdot 4.0H_2O$: C, 42.13; H, 5.19; N, 4.76. Found: C, 42.22; H, 4.96; N, 4.64.

Target compound **4-6**

Target compound **4-6** (14.1 mg, 0.0261 mmol, quant.) was obtained from **4-2** as a colorless oil. $[\alpha]^{23}_{D}$ -32.25 (*c* 0.48, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.61 (d, *J* = 8.6 Hz, 1H, amide NH), 8.60 (br, 3H, NH_3^+), 8.19 (d, *J* = 2.9 Hz, 1H, amide NH), 7.24-7.13 (m, 4H, aromatic), 7.13-7.06 (m, 1H, aromatic), 4.86 (s, 1H, OCH), 4.34-4.25 (m, 1H, Ala CH), 4.12-4.01 (m, 1H, NCH), 2.77-2.70 (m, 1H, cyclopropyl CH), 2.70-2.62 (m, 1H, $PhCH_2$), 2.57-2.48 (m, 1H, $PhCH_2$), 1.90-1.62 (m, 5H, $PhCH_2C\underline{H}_2$, isobutyl CH_2 and isobutyl CH), 1.62-1.52 (m, 1H, $NCHC\underline{H}_2$), 1.56 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.37-1.14 (m, 2H, $NCHC\underline{H}_2$ and cyclopropyl CH), 1.26 (s, 3H, CCH_3), 1.02 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.97 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.89-0.79 (m, 1H, cyclopropyl CH_2), 0.46-0.38 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, THF-d8) δ 173.9, 171.3, 170.8, 143.4, 129.5, 129.1, 126.6, 78.6, 61.3, 50.4, 44.7, 39.0, 35.8, 33.7, 28.9, 24.6, 22.8, 18.3, 16.2, 16.1, 11.2; LRMS (ESI) *m/z* 444.29 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{25}H_{38}N_3O_4$: 444.2857 $[(M+H)^+]$, found: 444.2860; Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 2.0TFA \cdot 3.0H_2O$: C, 45.26; H, 5.51; N, 5.11. Found: C, 45.49; H, 5.43; N, 5.20.

Target compound **4-7**

Target compound **4-7** (20.3 mg, 0.0375 mmol, quant.) was obtained from **4-3** as a colorless oil. $[\alpha]^{23}_{D}$ -36.31 (*c* 0.77, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.67 (d, *J* = 8.6 Hz, 1H, amide NH), 8.58 (br, 3H, NH_3^+), 8.19 (br, 1H, amide NH), 7.26-7.13 (m, 4H, aromatic), 7.13-7.05 (m, 1H, aromatic), 4.77 (s, 1H, OCH), 4.38-4.26 (m, 1H, Ala CH), 4.14-4.01 (m, 1H, NCH), 2.77-2.61 (m, 2H, $PhCH_2$ and cyclopropyl CH), 2.58-2.46 (m, 1H, $PhCH_2$), 1.89-1.63 (m, 5H, *sec*-butyl CH_2, $PhCH_2C\underline{H}_2$ and *sec*-butyl CH), 1.63-1.52 (m, 1H, $NCHC\underline{H}_2$), 1.57 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.27-1.08 (m, 2H, $NCHCH_2$ and cyclopropyl CH), 1.21 (s, 3H, CCH_3), 1.01 (d, *J* = 6.9 Hz, 3H, *sec*-butyl $CHC\underline{H}_3$), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl $CH_2C\underline{H}_3$), 0.89-0.78 (m, 1H, cyclopropyl CH_2), 0.48-0.38 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, THF-d8) δ 173.3, 171.5, 170.8, 143.4, 129.5, 129.1, 126.6, 77.1, 66.1, 50.5, 50.4, 40.3, 39.0, 36.1, 33.7, 28.9, 18.2, 16.1, 14.6, 13.1, 12.4, 11.2; LRMS (ESI) *m/z* 444.29 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{25}H_{38}N_3O_4$: 444.2857 $[(M+H)^+]$, found: 444.2869; Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 1.5TFA \cdot 1.5H_2O$: C, 48.78; H, 5.66; N, 5.69. Found: C, 48.56; H, 5.81; N, 5.57.

Target compound **4-8**

Target compound **4-8** (22.0 mg, 0.0396 mmol, quant.) was obtained from **4-4** as a colorless oil. $[\alpha]^{23}_{D}$ -51.07 (*c* 0.97, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.58 (br, 3H, NH_3^+), 8.37 (d, *J* = 9.2 Hz, 1H, amide NH), 7.71 (d, *J* = 3.4 Hz, 1H, amide NH), 7.27-7.14 (m, 4H, aromatic), 7.14-7.06 (m, 1H, aromatic), 4.67 (s, 1H, OCH), 4.44-4.31 (m, 1H, Ala CH), 4.22-4.09 (m, 1H, NCH), 2.84-2.73 (m, 1H, cyclopropyl CH), 2.73-2.61 (m, 1H, $PhCH_2$), 2.58-2.44 (m, 1H, $PhCH_2$), 1.91-1.65 (m, 4H, $PhCH_2C\underline{H}_2$ (2H), *sec*-butyl CH_2 (1H) and *sec*-butyl CH), 1.55 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.19 (s, 3H, CCH_3), 1.16-1.05 (m, 2H, *sec*-butyl CH_2 and NCHC$\underline{H}$), 1.05-0.92 (m, 1H, cyclopropyl CH), 1.01 (d, *J* = 6.9 Hz, 3H, *sec*-butyl $CHC\underline{H}_3$), 0.98 (d, *J* = 6.3 Hz, 3H, $CHCH_3$), 0.93 (dd, *J* = 7.4, 7.4 Hz, 3H, *sec*-butyl $CH_2C\underline{H}_3$), 0.84-0.75 (m, 1H, cyclopropyl CH_2), 0.65-0.56 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, THF-d8) δ 173.5, 170.8, 170.3, 143.3, 129.5, 129.2, 126.6, 77.2, 66.0, 54.0, 50.4, 40.2, 38.4, 36.1, 34.1, 29.1, 23.1, 18.4, 15.3, 14.6, 13.0, 12.4, 10.5; LRMS (ESI) *m/z* 458.30 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{26}H_{40}N_3O_4$: 458.3013 [$(M+H)^+$], found: 458.3026; Anal. calcd for $C_{28}H_{39}F_3N_3O_5 \cdot 1.0TFA \cdot 1.8H_2O$: C, 51.40; H, 6.27; N, 5.99. Found: C, 51.44; H, 6.19; N, 5.87.

Stability testing of 4-5 – 4-8

0.1 M TEAA buffer

Solutions of **4-5** – **4-8** in DMSO (50 µl, 7.5 mM) were diluted with 0.1 M TEAA buffer (950 µl, pH 7.4) and the resulting mixtures were incubated at 37 °C. The time courses were analyzed by RP-HPLC (Mightysil RP-18 GP 250-4.6 (5 µm), 0.8 ml/min, rt, 210 nm, eluents: **4-5**, MeOH/H_2O/TFA 60:40:0.1; **4-6** – **4-8**, MeOH/H_2O/TFA 65:35:0.1. retention times: **4-5**, 15.3 min; **4-6,** 14.2 min; **4-7**, 13.9 min; **4-8**, 16.4 min) at various time points from 0 to 32 h.

Human AB serum

Solutions of **4-5** – **4-8** in DMSO (50 µl, 23 mM) were diluted with human AB serum (950 µl, Sigma-Aldrich) and the resulting mixtures were incubated at 37 °C. At various time points from 0 to 60 min, the reaction mixtures (100 µl) were sampled, which were immediately quenched with CH_3CN (300 µl). The resulting mixtures were centrifuged (10,000 rpm) for 2 min at 4 °C and the supernatants were analyzed by RP-HPLC (Mightysil RP-18 GP 250-4.6 (5 µm), 0.8 ml/min, rt, 210 nm, eluents: **4-5**, MeOH/H_2O/TFA 60:40:0.1; **4-6** – **4-8**, MeOH/H_2O/TFA 65:35:0.1. retention times: **4-5**, 15.3 min; **4-6,** 14.2 min; **4-7**, 13.9 min; **4-8**, 16.4 min).

Proteasome assay

Inhibitory activity of the compound **4-1** – **4-4** on the ChT-L activity of human 20S proteasome was measured as described previously.[37]

Cell proliferation assay

Inhibitory activity of the compound **4-1** – **4-4** on the cell growth of HCT116 cells was measured as described previously.[37]

Combustion analysis data for 4-1 – 4-8

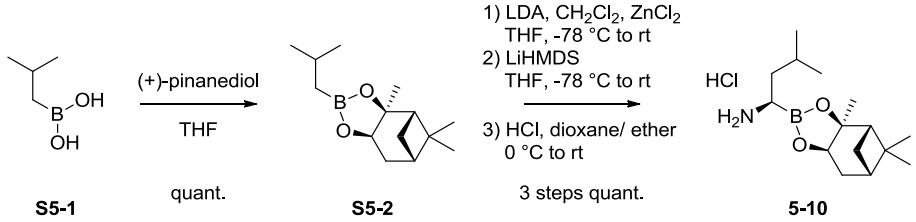
Table S4-1. Table listing combustion analysis data for **4-1** – **4-8**

4-1	Anal. calcd for $C_{32}H_{41}N_3O_6 \cdot 0.1H_2O$: C, 67.97; H, 7.34; N, 7.43.	Found: C, 67.82; H, 7.46; N, 7.14.
4-2	Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.1H_2O$: C, 68.39; H, 7.51; N, 7.25.	Found: C, 68.45; H, 7.60; N, 7.00.
4-3	Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.2H_2O$: C, 68.18; H, 7.52; N, 7.23.	Found: C, 68.20; H, 7.61; N, 7.10.
4-4	Anal. calcd for $C_{34}H_{45}N_3O_6 \cdot 0.4H_2O$: C, 68.18; H, 7.71; N, 7.02.	Found: C, 68.17; H, 7.73; N, 6.93.
4-5	Anal. calcd for $C_{26}H_{35}F_3N_3O_5 \cdot 2.5TFA \cdot 4.0H_2O$: C, 42.13; H, 5.19; N, 4.76.	Found: C, 42.22; H, 4.96; N, 4.64.
4-6	Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 2.0TFA \cdot 3.0H_2O$: C, 45.26; H, 5.51; N, 5.11.	Found: C, 45.49; H, 5.43; N, 5.20.
4-7	Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 1.5TFA \cdot 1.5H_2O$: C, 48.78; H, 5.66; N, 5.69.	Found: C, 48.56; H, 5.81; N, 5.57.
4-8	Anal. calcd for $C_{28}H_{39}F_3N_3O_5 \cdot 1.0TFA \cdot 1.8H_2O$: C, 51.40; H, 6.27; N, 5.99.	Found: C, 51.44; H, 6.19; N, 5.87.

第五章　ペプチドボロン酸とのハイブリッド化合物の創製

Synthetic procedures for 5-10, 5-13 and S5-6

Scheme S5-1. Synthesis of **5-10**



(3a*S*,4*S*,6*S*,7a*R*)-2-isobutyl-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborole **S5-2**

To a solution of isobutylboronic acid **S5-1** (16.0 g, 157 mmol, 1.1 equiv) in THF (180 ml) was added (+)-pinanediol (25.2 g, 148 mmol, 1.0 equiv). After 26 h, the reaction mixture was concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 50:1) to yield pinanediol ester **S5-2** (35.5 g, 150 mmol, quant.) as a colorless liquid. $[\alpha]^{21}_{D}$ 30.76 (*c* 1.01, $CHCl_3$) [lit. $[\alpha]^{25}_{D}$ 26.9 (*c* 1.75, $CHCl_3$)][88]; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.26 (dd, *J* = 8.6, 1.7 Hz, 1H, OCH), 2.38-2.30 (m, 1H, OCHC$\underline{H}_2$), 2.25-2.18 (m, 1H, CCHC$\underline{H}_2$), 2.05 (dd, *J* = 5.7, 5.2 Hz, 1H, CCH), 1.94-1.80 (m, 3H, OCHC$\underline{H}_2$ (1H), OCHCH$_2$C$\underline{H}$ and isobutyl CH), 1.38 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.14 (d, *J* = 10.9 Hz, 1H, CCHC$\underline{H}_2$), 0.95 (d, *J* = 6.9 Hz, 6H, isobutyl CH_3), 0.85 (s, 3H, CH_3), 0.81-0.76 (m, 2H, isobutyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 85.2, 77.4, 51.2, 39.5, 38.1, 35.6, 28.7, 27.1, 26.5, 25.3 25.2, 24.9, 24.0, 21.2; LRMS (EI) *m/z* 236.2 (M^+); HRMS (EI) calcd for $C_{14}H_{25}O_2{}^{10}B$: 235.1984 (M^+), found: 235.1976. ^{1}H-NMR, ^{13}C-NMR and $[\alpha]_D$ data are in agreement with those reported by Inglis.[88]

(*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butan-1-amine hydrochloride **5-10**

To a solution of diisopropylamine (16.9 ml, 120 mmol, 1.2 equiv) in THF (100 ml) was added *n*-BuLi (44.6 ml, 120 mmol, 1.2 equiv, 2.69 M in *n*-hexane) at -78 °C. After 5 min at -78 °C, the reaction mixture was warmed to 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of LDA in THF.

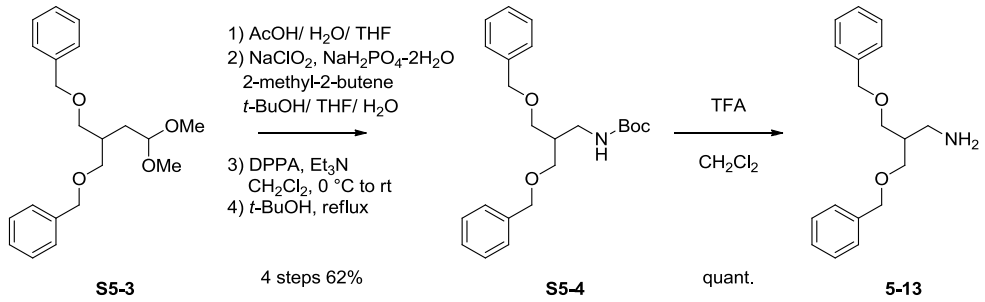
To a solution of pinanediol ester **S5-2** (23.6 g, 100 mmol) in THF (800 ml) was added DCM (64.0 ml, 1.00 mol, 10 equiv) and was cooled to -78 °C. The freshly prepared LDA solution was added and the resulting mixture was stirred for 30 min at -78 °C. $ZnCl_2$ (190 ml, 190 mmol, 1.9 equiv, 1.0 M in diethyl ether) was added and the resulting mixture was warmed to rt. After 14 h at rt, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in hexane and was washed with sat. NH_4Cl, water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a brown liquid.

To a solution of the brown liquid in THF (450 ml) was added LiHMDS (87.1 ml, 113 mmol, 1.1 equiv) at -78 °C. After 5 min at -78 °C, the reaction mixture was warmed to rt and stirred for 7 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in hexane. The resulting mixture was stirred for 1 h and was filtered through a Celite pad. The filtrate was concentrated *in vacuo* to give a brown oil.

To a solution of the oil in dioxane (300 ml) was added diethyl ether (100 ml) and was cooled to 0 °C. 2 M HCl/ diethyl ether (222 ml) was added. After 5 min at 0 °C, the reaction mixture was warmed to rt. After 4 h at rt, the

reaction mixture was concentrated *in vacuo* and the residue was dissolved in hexane (400 ml). The resulting mixture was stirred for 30 min and concentrated *in vacuo* to yield amine hydrochloride **5-10** (31.7 g, 105 mmol, 3 steps quant.) as a brown amorphous solid. $[\alpha]^{21}_{D}$ 10.31 (*c* 1.91, MeOH) [lit. $[\alpha]^{25}_{D}$ 12.7 (*c* 1.02, MeOH)][88]; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.29 (br, 3H, ammonium NH_3), 4.43-4.35 (m, 1H, OCH), 3.03-2.91 (m, 1H, NCH), 2.36-2.19 (m, 2H, OCHC<u>H</u>$_2$ and CCHC<u>H</u>$_2$), 2.06 (dd, *J* = 5.7, 5.2 Hz, 1H, CCH), 2.02-1.87 (m, 3H, OCHC<u>H</u>$_2$ (1H), OCHCH$_2$C<u>H</u> and isobutyl CH), 1.85-1.76 (m, 1H, isobutyl CH_2), 1.68-1.59 (m, 1H, isobutyl CH_2), 1.42 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.18 (d, *J* = 11.5 Hz, 1H, CCHC<u>H</u>$_2$), 0.95 (d, *J* = 6.3 Hz, 6H, isobutyl CH_3), 0.82 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 87.4, 78.6, 67.0, 51.0, 39.3, 38.5, 38.0, 35.5, 35.0, 28.4, 26.9, 26.4, 24.8, 23.9, 22.5, 22.2; LRMS (ESI) *m/z* 266.2 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{15}H_{29}NO_2{}^{10}B$: 265.2322 [$(M+H)^+$], found: 265.2326. $[\alpha]_D$ data is in agreement with that reported by Inglis.[88]

Scheme S5-2. Synthesis of **5-13**



<u>*tert*-butyl (3-(benzyloxy)-2-((benzyloxy)methyl)propyl)carbamate **S5-4**</u>

To a solution of acetal **S5-3**[60] (252 mg, 0.731 mmol) in AcOH (700 µl) was added H_2O (700 µl). The resulting mixture was vigorously stirred for 18 h. The reaction mixture was diluted with AcOEt, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding aldehyde as a colorless oil.

To a solution of the oil in THF (7.0 ml) was added *t*-BuOH (7.0 ml), H_2O (3.5 ml), $NaH_2PO_4 \cdot 2H_2O$ (171 mg, 1.10 mmol, 1.5 equiv), 2-methyl-2-butene (620 µl, 5.85 mmol, 8.0 equiv) and $NaClO_2$ (264 mg, 2.92 mmol, 4.0 equiv). The resulting mixture was stirred for 40 min and was diluted with $CHCl_3$, washed with 1 M HCl, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a pale yellow oil.

To a solution of the oil in DCM (7.0 ml) was added triethylamine (153 µl, 1.10 mmol, 1.5 equiv) and DPPA (189 µl, 0.877 mmol, 1.2 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding acyl azide as a pale yellow oil.

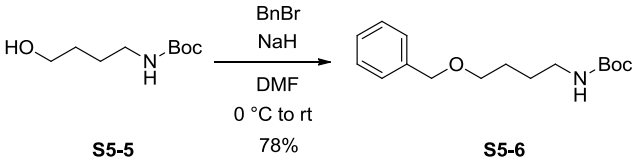
A solution of the oil in *t*-BuOH (10 ml) was refluxed for 34 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield carbamate **S5-4** (175 mg, 0.453 mmol, 4 steps 62%) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.39-7.22 (m, 10H, aromatic), 5.10 (br, 1H, carbamate NH), 4.48 (s, 4H, benzyl CH_2), 3.60-3.45 (m, 4H, OCH_2), 3.27 (dd, *J* = 5.7, 5.7 Hz, 2H, NCH_2), 2.20-2.10 (m, 1H, OCH$_2$C<u>H</u>), 1.43 (s, 9H, CCH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 156.0, 138.2, 128.3, 127.5, 127.5, 78.8, 73.2, 70.0, 41.3, 39.6, 28.4; LRMS (ESI) *m/z* 408.2 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{23}H_{31}NO_4Na$: 408.2145

[(M+Na)$^+$], found: 408.2144.

3-(benzyloxy)-2-((benzyloxy)methyl)propan-1-amine **5-13**

To a solution of carbamate **S5-4** (173 mg, 0.450 mmol) in DCM (2.0 ml) was added TFA (2.0 ml). The resulting mixture was stirred for 10 h and was concentrated *in vacuo*. The crude product was neutralized with amberlyst A21 to yield amine **5-13** (129 mg, 0.453 mmol, quant.) as a pale yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.36-7.22 (m, 10H, aromatic), 4.47 (s, 4H, benzyl CH_2), 3.58-3.46 (m, 4H, OCH_2), 3.39 (br, 2H, NH_2), 2.87 (d, J = 5.7 Hz, 2H, NCH_2), 2.13-2.03 (m, 1H, OCH_2CH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 138.1, 128.3, 127.6, 127.5, 73.2, 69.8, 41.8, 41.3; LRMS (ESI) *m/z* 286.2 [(M+H)$^+$]; HRMS (ESI) calcd for $C_{18}H_{24}NO_2$: 286.1802 [(M+H)$^+$], found: 286.1799.

Scheme S5-3. Synthesis of **S5-6**



S5-5 **S5-6**

tert-butyl (4-(benzyloxy)butyl)carbamate **S5-6**

To a solution of alcohol **S5-5** (2.00 g, 10.6 mmol) in DMF (11 ml) was added NaH (845 mg, 21.1 mmol, 2.0 equiv, 60% oil suspension) at 0 °C. After 30 min at 0 °C, BnBr (1.88 ml, 15.9 mmol, 1.5 equiv) was added and the reaction mixture was warmed to rt and stirred for 10 h. The reaction was quenched with AcOH (1.82 ml, 31.7 mmol, 3.0 equiv) at 0 °C, and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield benzyl ether **S5-6** (2.31 g, 8.26 mmol, 78%) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.30 (m, 4H, aromatic), 7.30-7.24 (m, 1H, aromatic), 4.67 (br, 1H, carbamate NH), 4.49 (s, 2H, benzyl CH_2), 3.48 (t, J = 6.3 Hz, 2H, OCH_2), 3.19-3.07 (m, 2H, NCH_2), 1.69-1.52 (m, 4H, OCH_2CH_2 and NCH_2CH_2), 1.44 (s, 9H, CCH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 155.9, 138.4, 128.4, 128.3, 127.6, 127.5, 78.9, 72.9, 69.9, 40.3, 28.4, 27.0, 26.8; LRMS (ESI) *m/z* 302.2 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{16}H_{25}NO_3Na$: 302.1727 [(M+Na)$^+$], found: 302.1725.

Synthetic procedures for 5-1a – 5-9a and 5-1b – 5-3b

(*S*)-benzyl 3-acetamido-4-(((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)amino)-4-oxobutanoate **5-12a**

To a solution of Boc-Asp(OBn)-OH (3.84 g, 11.9 mmol, 2.0 equiv) in DCM (120 ml) was added triethylamine (1.66 ml, 11.9 mmol, 2.0 equiv) and PivCl (1.47 ml, 11.9 mmol, 2.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield the corresponding acid anhydride as a colorless oil.

To a solution of amine hydrochloride **5-10**[88] (1.79 g, 5.95 mmol) in DCM (150 ml) was added triethylamine (2.48

ml, 17.9 mmol, 3.0 equiv) and a solution of the aforementioned acid anhydride in DCM (30 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt. After 14 h at rt, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield compound **5-11a** as a white amorphous solid.

To a solution of the amorphous solid in DCM (60 ml) was added TFA (12 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt. After 15 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a brown viscous oil.

To a solution of the viscous oil in DCM (60 ml) was added triethylamine (2.48 ml, 17.9 mmol, 3.0 equiv) and Ac_2O (1.12 ml, 11.9 mmol, 2.0 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt. After 20 min at rt, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 1:1-1:2) to yield compound **5-12a** (2.07 g, 4.04 mmol, 3 steps 68%) as a white amorphous solid. $[\alpha]^{21}_D$ -17.65 (*c* 0.97, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5H, aromatic), 6.83 (d, *J* = 7.4 Hz, 1H, amide NH), 6.68 (d, *J* = 5.7 Hz, 1H, amide NH), 5.15 (d, *J* = 12.3 Hz, 1H, benzyl CH_2), 5.12 (d, *J* = 12.3 Hz, 1H, benzyl CH_2), 4.87-4.78 (m, 1H, NCHCO), 4.32-4.23 (m, 1H, OCH), 3.28-3.1 (m, 1H, NCHB), 2.97 (dd, *J* = 17.2, 4.6 Hz, 1H, NCHC$\underline{H}_2$), 2.66 (dd, *J* = 17.2, 6.9 Hz, 1H, NCHC$\underline{H}_2$), 2.35-2.26 (m, 1H, OCHC$\underline{H}_2$), 2.21-2.13 (m, 1H, CCHC$\underline{H}_2$), 2.07-1.96 (m, 1H, CCH), 1.99 (s, 3H, acetyl CH_3), 1.91-1.85 (m, 1H, OCHCH$_2$C$\underline{H}$), 1.85-1.78 (m, 1H, OCHC$\underline{H}_2$), 1.67-1.56 (m, 1H, isobutyl CH), 1.51-1.38 (m, 2H, isobutyl CH_2), 1.36 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.21 (d, *J* = 10.9 Hz, 1H, CCHC$\underline{H}_2$), 0.90 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.89 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.83 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.8, 170.4, 170.0, 135.3, 128.6, 128.3, 128.2, 85.8, 77.8, 66.8, 51.2, 48.6, 39.8, 39.4, 38.1, 36.0, 35.7, 35.4, 28.5, 27.0, 26.2, 25.4, 24.0, 23.1,, 23.0, 21.9; LRMS (ESI) *m/z* 535.3 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{28}H_{41}N_2O_6{}^{10}BNa$: 534.2986 [(M+Na)$^+$], found: 534.2991.

(*S*)-benzyl 4-acetamido-5-(((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)amino)-5-oxopentanoate **5-12b**

Compound **5-12b** (1.84 g, 3.49 mmol, 3 steps 84%, a white amorphous solid) was prepared from amine hydrochloride **5-10**[88] (1.25 g, 4.15 mmol) as described for compound **5-12b** using Boc-Glu(OBn)-OH instead of Boc-Asp(OBn)-OH. $[\alpha]^{19}_D$ -17.75 (*c* 0.83, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40-7.29 (m, 5H, aromatic), 6.60 (d, *J* = 5.2 Hz, 1H, amide NH), 6.57 (d, *J* = 8.0 Hz, 1H, amide NH), 5.13 (d, *J* = 12.3 Hz, 1H, benzyl CH_2), 5.10 (d, *J* = 12.3 Hz, 1H, benzyl CH_2), 4.58-4.49 (m, 1H, NCHCO), 4.27 (dd, *J* = 8.9, 2.0 Hz, 1H, OCH), 3.28-3.17 (m, 1H, NCHB), 2.61-2.49 (m, 1H, COCH), 2.499-2.39 (m, 1H, COCH), 2.35-2.25 (m, 1H, OCHC$\underline{H}_2$), 2.21-2.06 (m, 2H, COCH$_2$C$\underline{H}_2$), 2.06-1.92 (m, 2H, CCHC$\underline{H}_2$ and CCH), 1.95 (s, 3H, acetyl CH_3), 1.91-1.85 (m, 1H, OCHCH$_2$C$\underline{H}$), 1.85-1.78 (m, 1H, OCHC$\underline{H}_2$), 1.70-1.59 (m, 1H, isobutyl CH), 1.52-1.39 (m, 2H, isobutyl CH_2), 1.36 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.22 (d, *J* = 10.9 Hz, 1H, CCHC$\underline{H}_2$), 0.91 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.89 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.82 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.1, 171.2, 170.0, 135.7, 128.5, 128.2, 128.2, 85.8, 77.8, 66.4, 51.5, 51.2, 39.8, 39.5, 38.1, 35.8, 35.4, 30.1, 28.5, 27.9, 27.0, 26.2, 25.4, 24.0, 23.1, 23.0, 21.9; LRMS (ESI) *m/z* 549.3 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{29}H_{43}N_2O_6{}^{10}BNa$: 548.3143 [(M+Na)$^+$], found: 548.3142.

(*S*)-2-acetamido-N^4-(3-(benzyloxy)-2-((benzyloxy)methyl)propyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimeth

ylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)succinamide **5-1a**

To a solution of compound **5-12a** (77.5 mg, 0.151 mmol, 1.1 equiv) in THF (6.0 ml) was added Pd/C (50 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 3 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding carboxylic acid as a white amorphous solid.

To a solution of amine **5-13** (39.1 mg, 0.137 mmol, 1.0 equiv) in DCM (2.0 ml) was added a solution of the aforementioned amorphous solid in DCM (1.5 ml), HOAt (20.5 mg, 0.151 mmol, 1.1 equiv), DIEA (25.6 µl, 0.151 mmol, 1.1 equiv) and EDC·HCl (28.9 mg, 0.151 mmol, 1.1 equiv) at -18 °C. After 10 min at -18 °C, the reaction mixture was warmed to 0 °C. After 2 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by reverse-phase HPLC (Mightysil RP-18 250-20 (5 µm), acetonitrile/ H_2O 70:30) to yield compound **5-1a** (64.5 mg, 0.0935 mmol, 68% from **5-13**) as a colorless viscous oil. $[\alpha]^{22}_D$ -3.01 (*c* 1.24, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.44-7.21 (m, 12H, amide NH and aromatic), 6.53 (t, *J* = 4.9 Hz, 1H, amide NH), 4.73-4.63 (m, 1H, NCHCO), 4.48 (s, 4H, benzyl CH_2), 4.32-4.23 (m, 1H, OCH), 3.62-3.45 (m, 4H, OCH_2), 3.45-3.29 (m, 2H, NCH_2), 3.22-3.10 (m, 1H, NCHB), 2.65 (dd, *J* = 15.3, 3.6 Hz, 1H, $COCH_2$), 2.34 (dd, *J* = 15.3, 7.2 Hz, 1H, $COCH_2$), 2.35-2.24 (m, 1H, OCHC$\underline{H}_2$), 2.23-2.11 (m, 2H, $OCH_2C\underline{H}$ and CCHC$\underline{H}_2$), 2.04-1.97 (m, 1H, CCH), 2.00 (s, 3H, acetyl CH_3), 1.92-1.76 (m, 2H, OCHC$\underline{H}_2$ and $OCHCH_2C\underline{H}$), 1.70-1.56 (m, 1H, isobutyl CH), 1.54-1.32 (m, 2H, isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.23 (d, *J* = 10.8 Hz, 1H, CCHC$\underline{H}_2$), 0.90 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.88 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.82 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.1, 171.0, 170.1, 138.0, 128.4, 127.7, 127.6, 85.6, 77.6, 73.2, 73.2, 70.1, 70.0, 51.3, 49.1, 40.5, 39.9, 39.5, 39.0, 38.1, 37.2, 35.7, 35.5, 28.5, 27.0, 26.3, 25.4, 24.0, 23.2 23.1, 21.9; LRMS (ESI) *m/z* 690.4 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{39}H_{57}N_3O_7B$: 690.4290 [$(M+H)^+$], found: 690.4286.

(*S*)-2-acetamido-N^4-(4-(benzyloxy)-3-((benzyloxy)methyl)butyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)succinamide **5-2a**

Compound **5-2a** (93.9 mg, 0.133 mmol, 77%, a colorless viscous oil) was prepared from amine **3-29**[60] (51.7 mg, 0.173 mmol) as described for compound **5-1a**. $[\alpha]^{23}_D$ -5.51 (*c* 1.10, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.25 (m, 12H, amide NH and aromatic), 6.47 (t, *J* = 5.2 Hz, 1H, amide NH), 4.66-4.59 (m, 1H, NCHCO), 4.48 (s, 4H, benzyl CH_2), 4.28 (dd, *J* = 8.6, 1.7 Hz, 1H, OCH), 3.57-3.48 (m, 2H, OCH_2), 3.48-3.39 (m, 2H, OCH_2), 3.34-3.19 (m, 2H, NCH_2), 3.18-3.10 (m, 1H, NCHB), 2.53 (dd, *J* = 15.5, 3.4 Hz, 1H, $COCH_2$), 2.35-2.27 (m, 1H, OCHC$\underline{H}_2$), 2.23 (dd, *J* = 15.5, 7.4 Hz, 1H, $COCH_2$), 2.21-2.13 (m, 1H, CCHC$\underline{H}_2$), 2.07-1.94 (m, 2H, $OCH_2C\underline{H}$ and CCH), 2.00 (s, 3H, acetyl CH_3), 1.91-1.85 (m, 1H, $OCHCH_2C\underline{H}$), 1.85-1.78 (m, 1H, OCHC$\underline{H}_2$), 1.69-1.56 (m, 3H, $NCH_2C\underline{H}_2$ and isobutyl CH), 1.51-1.33 (m, 2H, isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.23 (d, *J* = 10.9 Hz, 1H, CCHC$\underline{H}_2$), 0.89 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.88 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.82 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.0, 170.1, 138.1, 128.4, 127.6, 85.6, 77.6, 73.2, 71.2, 71.2, 51.3, 49.1, 39.9, 39.5, 38.1, 37.9, 37.8, 37.0, 35.7, 35.5, 28.9, 28.5, 27.1, 26.3, 25.4, 24.0, 23.2, 23.1, 21.9; LRMS (ESI) *m/z* 704.4 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{40}H_{59}N_3O_7{}^{10}B$: 703.4477 [$(M+H)^+$], found: 703.4482.

(*S*)-2-acetamido-N^4-(5-(benzyloxy)-4-((benzyloxy)methyl)pentyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)succinamide **5-3a**

Compound **5-3a** (98.6 mg, 0.137 mmol, 71%, a colorless viscous oil) was prepared from amine **3-31**[60] (61.0 mg, 0.195 mmol) as described for compound **5-1a**. $[\alpha]^{23}_{D}$ -12.33 (*c* 1.40, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.39-7.24 (m, 12H, amide NH and aromatic), 6.24 (t, *J* = 5.2 Hz, 1H, amide NH), 4.69-4.62 (m, 1H, NCHCO), 4.47 (s, 4H, benzyl CH_2), 4.28 (dd, *J* = 8.9, 2.0 Hz, 1H, OCH), 3.54-3.40 (m, 4H, OCH_2), 3.30-3.19 (m, 1H, NCHB), 3.19-3.08 (m, 2H, NCH_2), 2.68 (dd, *J* = 14.9, 3.4 Hz, 1H, $COCH_2$), 2.38 (dd, *J* = 14.9, 6.9 Hz, 1H, $COCH_2$), 2.35-2.26 (m, 1H, $OCHCH_2$), 2.23-2.12 (m, 1H, $CCHCH_2$), 2.05-1.97 (m, 1H, CCH), 2.00 (s, 3H, acetyl CH_3), 1.97-1.85 (m, 2H, OCH_2CH and $OCHCH_2CH$), 1.85-1.78 (m, 1H, $OCHCH_2$), 1.68-1.57 (m, 1H, isobutyl CH), 1.57-1.37 (m, 6H, NCH_2CH_2, $NCH_2CH_2CH_2$ and isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.23 (d, *J* = 10.9 Hz, 1H, $CCHCH_2$), 0.90 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.88 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.83 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.0, 170.3, 138.4, 128.3, 127.5, 127.5, 85.6, 77.7, 73.1, 70.8, 51.3, 49.4, 39.9, 39.7, 39.5, 39.0, 38.1, 37.3, 35.7, 35.5, 28.5, 27.0, 26.7, 26.3, 26.0, 25.4, 24.0, 23.2, 23.0, 21.9; LRMS (ESI) *m/z* 718.5 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{41}H_{61}N_3O_7{}^{10}B$: 717.4633 $[(M+H)^+]$, found: 717.4642.

(*S*)-2-acetamido-N^5-(3-(benzyloxy)-2-((benzyloxy)methyl)propyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)pentanediamide **5-1b**

Compound **5-1b** (71.1 mg, 0.101 mmol, 78%, a colorless viscous oil) was prepared from amine **5-13** (36.8 mg, 0.129 mmol) as described for compound **5-1a** using compound **5-12b** instead of compound **5-12a**. $[\alpha]^{23}_{D}$ -17.19 (*c* 0.64, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40-7.22 (m, 10H, aromatic), 7.17 (d, *J* = 6.9 Hz, 1H, amide NH), 7.12 (d, *J* = 4.6 Hz, 1H, amide NH), 6.55 (t, *J* = 5.7 Hz, 1H, amide NH), 4.49 (s, 2H, benzyl CH_2), 4.48 (s, 2H, benzyl CH_2), 4.45-4.37 (m, 1H, NCHCO), 4.28 (dd, *J* = 8.9, 2.0 Hz, 1H, OCH), 3.61-3.47 (m, 4H, OCH_2), 3.39 (dd, *J* = 5.7, 5.7 Hz, 2H, NCH_2), 3.18-3.09 (m, 1H, NCHB), 2.36-2.11 (m, 5H, $OCHCH_2$, $CCHCH_2$, OCH_2CH and $COCH_2$), 2.04-1.84 (m, 4H, CCH, $COCH_2CH_2$ and $OCHCH_2CH$), 1.98 (s, 3H, acetyl CH_3), 1.84-1.79 (m, 1H, $OCHCH_2$), 1.71-1.60 (m, 1H, isobutyl CH), 1.52-1.37 (m, 2H, isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.25 (d, *J* = 10.9 Hz, 1H, $CCHCH_2$), 0.90 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.89 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.83 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.9, 171.8, 170.4, 138.1, 128.4, 127.7, 127.6, 127.6, 85.4, 77.6, 73.3, 73.2, 70.2, 51.7, 51.4, 40.7, 39.9, 39.6, 39.1, 38.1, 36.2, 35.6, 32.5, 28.6, 28.2, 27.1, 26.3, 25.4, 24.0, 23.2, 23.0, 22.0; LRMS (ESI) *m/z* 704.5 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{40}H_{59}N_3O_7{}^{10}B$: 703.4477 $[(M+H)^+]$, found: 703.4483.

(*S*)-2-acetamido-N^5-(4-(benzyloxy)-3-((benzyloxy)methyl)butyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)pentanediamide **5-2b**

Compound **5-2b** (101 mg, 0.141 mmol, 84%, a colorless viscous oil) was prepared from amine **3-29**[60] (50.2 mg, 0.168 mmol) as described for compound **5-1a** using compound **5-12b** instead of compound **5-12a**. $[\alpha]^{24}_{D}$ -19.84 (*c* 1.42, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.39-7.25 (m, 10H, aromatic), 7.15 (d, *J* = 4.6 Hz, 1H, amide NH), 7.12 (d, *J* = 7.4 Hz, 1H, amide NH), 6.59 (t, *J* = 5.2 Hz, 1H, amide NH), 4.48 (s, 4H, benzyl CH_2), 4.44-4.36 (m, 1H, NCHCO), 4.27 (dd, *J* = 8.9, 2.0 Hz, 1H, OCH), 3.57-3.49 (m, 2H, OCH_2), 3.49-3.40 (m, 2H, OCH_2), 3.34-3.22 (m, 2H, NCH_2), 3.16-3.07 (m, 1H, NCHB), 2.37-2.27 (m, 1H, $OCHCH_2$), 2.20-2.04 (m, 3H, $CCHCH_2$ (1H) and $COCH_2$), 2.04-1.91 (m, 3H, OCH_2CH, $COCH_2CH_2$ (1H) and CCH), 1.97 (s, 3H, acetyl CH_3), 1.91-1.86 (m, 2H, $OCHCH_2CH$ and $COCH_2CH_2$), 1.86-1.78 (m, 1H, $OCHCH_2$), 1.70-1.59 (m, 3H, NCH_2CH_2 and isobutyl CH), 1.51-1.37 (m, 2H, isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.26 (d, *J* = 10.9 Hz, 1H, $CCHCH_2$), 0.90 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.89 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.83 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 171.8, 170.4, 138.1, 138.1, 128.4,

127.6, 127.6, 85.4, 77.5, 73.2, 73.2, 71.3, 71.2, 51.6, 51.4, 39.9, 39.6, 38.1, 37.8, 37.7, 36.3, 35.6, 32.3, 29.0, 28.6, 28.4, 27.1, 26.3, 25.4, 24.0, 23.2, 23.0, 22.0; LRMS (ESI) *m/z* 718.5 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{41}H_{61}N_3O_7B$: 718.4603 [$(M+H)^+$], found: 718.4601.

(*S*)-2-acetamido-N^5-(5-(benzyloxy)-4-((benzyloxy)methyl)pentyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)pentanediamide **5-3b**

Compound **5-3b** (107 mg, 0.146 mmol, 75%, a colorless viscous oil) was prepared form amine **3-31**[60] (61.0 mg, 0.195 mmol) as described for compound **5-1a** using compound **5-12b** instead of compound **5-12a**. $[\alpha]^{24}_D$ -19.70 (*c* 1.83, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.23 (m, 10H, aromatic), 7.12 (d, *J* = 4.6 Hz, 1H, amide NH), 7.05 (d, *J* = 6.9 Hz, 1H, amide NH), 6.35 (t, *J* = 5.7 Hz, 1H, amide NH), 4.47-4.40 (m, 1H, NCHCO), 4.47 (s, 4H, benzyl CH_2), 4.28 (dd, *J* = 8.9, 2.0 Hz, 1H, OCH), 3.55-3.40 (m, 4H, OCH_2), 3.26-3.17 (m, 2H, NCH_2), 3.16-3.09 (m, 1H, NCHB), 2.37-2.12 (m, 4H, $OCHCH_2$ (1H), $CCHCH_2$ (1H) and $COCH_2$), 2.06-1.86 (m, 5H, CCH, $COCH_2CH_2$, OCH_2CH, $OCHCH_2CH$), 1.97 (s, 3H, acetyl CH_3), 1.86-1.79 (m, 1H, $OCHCH_2$), 1.71-1.61 (m, 1H, isobutyl CH), 1.58-1.40 (m, 6H, NCH_2CH_2, $NCH_2CH_2CH_2$ and isobutyl CH_2), 1.38 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.26 (d, *J* = 10.9 Hz, 1H, $CCHCH_2$), 0.91 (d, *J* = 6.9 Hz, 3H, isobutyl CH_3), 0.89 (d, *J* = 6.9 Hz, 3H, isobutyl CH_3), 0.83 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.7, 171.7, 170.4, 138.4, 128.3, 127.5, 127.5, 85.5, 77.6, 73.1, 70.8, 51.7, 51.4, 39.8, 39.7, 39.5, 39.0, 38.1, 36.2, 35.6, 32.5, 28.7, 28.6, 27.1, 26.8, 26.2, 26.0, 25.4, 24.0, 23.1, 23.0, 22.0; LRMS (ESI) *m/z* 732.5 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{42}H_{63}N_3O_7{}^{10}B$: 731.4790 [$(M+H)^+$], found: 731.4794.

(*S*)-N^4-(4-(benzyloxy)-3-((benzyloxy)methyl)butyl)-2-(2,5-dichlorobenzamido)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)succinamide **5-4a**

To a solution of Boc-Asp(OBn)-OH (25.6 mg, 0.0792 mmol, 1.0 equiv) in DCM (1.0 ml) was added triethylamine (11.0 μl, 0.0792 mmol, 1.0 equiv) and PivCl (9.75 μl, 0.0792 mmol, 1.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding acid anhydride as a colorless oil.

To a solution of **5-10**[88] (23.9 mg, 0.0792 mmol) in DCM (1.0 ml) was added triethylamine (27.6 μl, 0.198 mmol, 2.5 equiv) and a solution of the aforementioned acid anhydride in DCM (1.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 30 min. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give **5-11a** as a pale yellow viscous oil.

To a solution of the viscous oil in THF (1.0 ml) was added Pd/C (45 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 90 min. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding carboxylic acid as a white amorphous solid.

To a solution of the amorphous solid in DCM (1.0 ml) was added amine **3-29**[60] (23.7 mg, 0.0792 mmol, 1.0 equiv), HOAt (10.8 mg, 0.0792 mmol, 1.0 equiv), DIEA (13.5 μl, 0.0792 mmol, 1.0 equiv) and EDC·HCl (15.2 mg, 0.0792 mmol, 1.0 equiv) at -18 °C. After 5 min at -18 °C, the reaction mixture was warmed to rt and stirred for 16 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give **5-14a** as a

colorless viscous oil.

To a solution of the oil in DCM (500 µl) was added TFA (500 µl) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was concentrated *in vacuo* to give the corresponding amine as a brown viscous oil.

To a solution of the oil in DCM (1.0 ml) was added triethylamine (22.0 µl, 0.158 mmol, 2.0 equiv) and 2,5-dichlorobenzoyl chloride (16.6 mg, 0.0792 mmol, 1.0 equiv) at 0 °C. After 3 h at 0 °C, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by reverse-phase HPLC (Mightysil RP-18 250-20 (5 µm), acetonitrile/ H_2O 70:30) to yield target compound **5-4a** (33.7 mg, 0.0404 mmol, 5 steps 49%) as a colorless viscous oil. $[\alpha]^{24}_D$ 8.94 (*c* 0.89, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.08 (d, *J* = 6.9 Hz, 1H, amide NH), 7.59 (s, 1H, aromatic), 7.39-7.23 (m, 13H, amide NH and aromatic), 6.49 (t, *J* = 4.6 Hz, 1H, amide NH), 4.89-4.80 (m, 1H, NCHCO), 4.48 (s, 4H, benzyl CH_2), 4.33-4.25 (m, 1H, OCH), 3.57-3.47 (m, 2H, OCH_2), 3.47-3.39 (m, 2H, OCH_2), 3.32-3.20 (m, 3H, NCHB and NCH_2), 2.68 (dd, *J* = 15.5, 2.9 Hz, 1H, $COCH_2$), 2.37-2.26 (m, 2H, OCHC<u>H</u>$_2$ and COC<u>H</u>$_2$), 2.22-2.14 (m, 1H, CCHC<u>H</u>$_2$), 2.06-1.96 (m, 2H, CCH and OCH_2C<u>H</u>), 1.91-1.85 (m, 1H, OCHCH_2C<u>H</u>), 1.85-1.78 (m, 1H, OCHC<u>H</u>$_2$), 1.69-1.58 (m, 3H, NCH_2C<u>H</u>$_2$ and isobutyl CH), 1.54-1.33 (m, 2H, isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.23 (d, *J* = 10.9 Hz, 1H, CCHC<u>H</u>$_2$), 0.89 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.88 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.82 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.9, 170.2, 164.9, 138.1, 135.9, 133.1, 131.4, 131.4, 129.8, 129.1, 128.4, 127.7, 127.6, 85.8, 77.8, 73.2, 71.3, 71.3, 51.3, 49.9, 40.0, 39.5, 38.1, 38.0, 37.9, 36.8, 35.6, 35.5, 29.0, 28.5, 27.0, 26.3, 25.3, 24.0, 23.1, 21.9; LRMS (ESI) *m/z* 834.4 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{45}H_{59}N_3O_7{}^{10}BCl_2$: 833.3854 [$(M+H)^+$], found: 833.3857.

<u>(*S*)-2-benzamido-N^4-(4-(benzyloxy)-3-((benzyloxy)methyl)butyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)succinamide</u> **5-5a**

Target compound **5-5a** (33.9 mg, 0.0443 mmol, 5 steps 53%, a colorless viscous oil) was prepared from **5-10**[88] (25.2 mg, 0.0835 mmol) as described for **5-4a** using benzoyl chloride instead of 2,5-dichlorobenzoyl chloride. $[\alpha]^{23}_D$ 7.10 (*c* 0.88, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.36 (d, *J* = 6.9 Hz, 1H, amide NH), 7.84 (d, *J* = 7.4 Hz, 1H, aromatic), 7.55-7.47 (m, 1H, aromatic), 7.47-3.38 (m, 3H, amide NH and aromatic), 7.38-7.23 (m, 10H, aromatic), 6.56 (t, *J* = 5.2 Hz, 1H, amide NH), 4.87-4.78 (m, 1H, NCHCO), 4.49 (s, 4H, benzyl CH_2), 4.32-4.24 (m, 1H, OCH), 3.57-3.48 (m, 2H, OCH_2), 3.48-3.39 (m, 2H, OCH_2), 3.37-3.23 (m, 2H, NCH_2), 3.21-3.12 (m, 1H, NCHB), 2.62 (dd, *J* = 15.5, 3.4 Hz, 1H, $COCH_2$), 2.34-2.28 (m, 1H, OCHC<u>H</u>$_2$), 2.31 (dd, *J* = 15.5, 6.9 Hz, 1H, $COCH_2$), 2.21-2.12 (m, 1H, CCHC<u>H</u>$_2$), 2.06-1.98 (m, 2H, CCH and OCH_2C<u>H</u>), 1.94-1.78 (m, 2H, OCHC<u>H</u>$_2$ and OCHCH_2C<u>H</u>), 1.69-1.57 (m, 3H, NCH_2C<u>H</u>$_2$ and isobutyl CH), 1.53-1.33 (m, 2H, isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.24 (d, *J* = 10.9 Hz, 1H, CCHC<u>H</u>$_2$), 0.88 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.86 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.82 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.4, 171.1, 167.0, 138.1, 133.5, 131.8, 128.6, 128.4, 127.6, 127.1, 85.6, 77.7, 73.2, 71.3, 71.2, 51.3, 49.6, 39.9, 39.5, 38.1, 38.0, 37.9, 36.7, 35.8, 35.5, 28.9, 28.5, 27.1, 26.3, 25.4, 24.0, 23.1, 22.0; LRMS (ESI) *m/z* 766.5 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{45}H_{61}N_3O_7{}^{10}B$: 765.4633 [$(M+H)^+$], found: 765.4634.

<u>(*S*)-N^4-(4-(benzyloxy)-3-((benzyloxy)methyl)butyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)-2-(pyrazine-2-carboxamido)succinamide</u> **5-6a**

Target compound **5-6a** (28.4 mg, 0.0370 mmol, 5 steps 47%, a colorless viscous oil) was prepared from **5-10**[88]

(23.7 mg, 0.0787 mmol) as described for **5-4a** using pyrazinecarboxylic acid and PivCl instead of 2,5-dichlorobenzoyl chloride. $[\alpha]^{23}_{D}$ 11.26 (*c* 0.70, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.37 (s, 1H, aromatic), 9.28 (d, *J* = 7.4 Hz, 1H, amide NH), 8.74 (d, *J* = 2.3 Hz, 1H, aromatic), 8.58 (d, *J* = 2.3 Hz, 1H, aromatic), 7.38-7.19 (m, 11H, amide NH and aromatic), 6.50 (t, *J* = 4.6 Hz, 1H, amide NH), 4.92-4.83 (m, 1H, NCHCO), 4.48 (s, 4H, benzyl CH_2), 4.32-4.23 (m, 1H, OCH), 3.52 (dd, *J* = 9.2, 5.7 Hz, 2H, OCH_2), 3.44 (dd, *J* = 9.2, 6.3 Hz, 2H, OCH_2), 3.35-3.24 (m, 2H, NCH_2), 3.24-3.14 (m, 1H, NCHB), 2.63 (dd, *J* = 15.5, 3.4 Hz, 1H, $COCH_2$), 2.35-2.25 (m, 1H, $OCHCH_2$), 2.34 (dd, *J* = 15.5, 7.4 Hz, 1H, $COCH_2$), 2.20-2.12 (m, 1H, $CCHCH_2$), 2.05-1.94 (m, 2H, CCH and OCH_2CH), 1.90-1.84 (m, 1H, $OCHCH_2CH$), 1.84-1.77 (m, 1H, $OCHCH_2$), 1.69-1.56 (m, 3H, NCH_2CH_2 and isobutyl CH), 1.56-1.33 (m, 2H, isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.23 (d, *J* = 10.9 Hz, 1H, $CCHCH_2$), 0.87 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.86 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.82 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.6, 170.5, 163.0, 147.3, 144.2, 144.2, 143.0, 138.1, 128.4, 127.7, 85.7, 77.7, 73.3, 71.4, 71.3, 51.3, 49.2, 39.9, 39.5, 38.1, 38.1, 37.9, 37.2, 35.7, 35.5, 29.0, 28.5, 27.1, 26.3, 25.4, 24.0, 23.1, 22.0; LRMS (ESI) *m/z* 768.5 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{43}H_{59}N_5O_7{}^{10}B$: 767.4538 [$(M+H)^+$], found: 767.4541.

(*S*)-N^4-(4-(benzyloxy)-3-((benzyloxy)methyl)butyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)-2-(6-phenylpicolinamido)succinamide **5-7a**

Target compound **5-7a** (35.0 mg, 0.0415 mmol, 5 steps 54%, a colorless viscous oil) was prepared from **5-10**[88] (23.2 mg, 0.0769 mmol) as described for **5-4a** using 6-phenylpyridine-2-carboxylic acid and PivCl instead of 2,5-dichlorobenzoyl chloride. $[\alpha]^{23}_{D}$ 23.38 (*c* 0.81, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.40 (d, *J* = 7.4 Hz, 1H, amide NH), 8.18-8.06 (m, 3H, aromatic), 7.95-7.86 (m, 2H, aromatic), 7.56-7.40 (m, 4H, amide NH and aromatic), 7.34-7.19 (m, 10H, aromatic), 6.51 (t, *J* = 5.2 Hz, 1H, amide NH), 4.95-4.87 (m, 1H, NCHCO), 4.46 (s, 4H, benzyl CH_2), 4.34-4.26 (m, 1H, OCH), 3.55-3.46 (m, 2H, OCH_2), 3.46-3.38 (m, 2H, OCH_2), 3.35-3.26 (m, 2H, NCH_2), 3.14-3.05 (m, 1H, NCHB), 2.69 (dd, *J* = 15.5, 3.4, 1H, $COCH_2$), 2.39 (dd, *J* = 15.5, 8.0 Hz, 1H, $COCH_2$), 2.34-2.26 (m, 1H, $OCHCH_2$), 2.21-2.12 (m, 1H, $CCHCH_2$), 2.05-1.96 (m, 2H, CCH and OCH_2CH), 1.90-1.78 (m, 2H, $OCHCH_2$ and $OCHCH_2CH$), 1.70-1.54 (m, 3H, NCH_2CH_2 and isobutyl CH), 1.54-1.36 (m, 2H, isobutyl CH_2), 1.40 (s, 3H, CH_3), 1.31 (d, *J* = 10.9 Hz, 1H, $CCHCH_2$), 1.26 (s, 3H, CH_3), 0.87 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.87 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.83 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.3, 170.5, 164.3, 155.9, 149.2, 138.1, 137.9, 129.5, 128.8, 128.4, 127.6, 126.9, 122.8, 120.5, 85.4, 77.5, 73.2, 71.3, 71.3, 51.4, 48.9, 40.0, 39.6, 38.1, 38.0, 38.0, 37.8, 36.1, 35.6, 29.0, 28.5, 27.1, 26.3, 25.4, 24.0, 23.1, 22.0; LRMS (ESI) *m/z* 843.5 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{50}H_{64}N_4O_7{}^{10}B$: 842.4899 [$(M+H)^+$], found: 842.4900.

(*S*)-2-acetamido-N^4-methyl-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)succinamide **5-8a**

To a solution of benzyl ester **5-12a** (93.5 mg, 0.1825 mmol) in THF (2.0 ml) was added Pd/C (90.0 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 2 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding carboxylic acid **5-15a** as a white amorphous solid.

To a solution of the amorphous solid in DCM (2.0 ml) was added CH_3NH_2·HCl (43.9 mg, 0.650 mmol, 3.5 equiv), HOAt (25.2 mg, 0.185 mmol, 1.0 equiv), DIEA (252 μl, 1.48 mmol, 8.0 equiv) and EDC·HCl (39.1 mg, 0.204 mmol, 1.1 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 19 h. The reaction mixture

was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by reverse-phase HPLC (Mightysil RP-18 250-20 (5 μm), acetonitrile/ H_2O 60:40) to yield target compound **5-8a** (14.3 mg, 0.0329 mmol, 2 steps 18%) as a colorless viscous oil. $[\alpha]^{24}_D$ -33.54 (*c* 0.21, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40 (d, *J* = 6.9 Hz, 1H, amide NH), 7.33 (d, *J* = 5.2 Hz, 1H, amide NH), 6.34 (d, *J* = 4.6 Hz, 1H, amide NH), 4.73-4.63 (m, 1H, NCHCO), 4.34-4.24 (m, 1H, OCH), 3.22-3.11 (m, 1H, NCHB), 2.84-2.72 (m, 1H, COCH), 2.78 (d, *J* = 4.6 Hz, 3H, NCH_3), 2.47 (dd, *J* = 14.9, 6.9 Hz, 1H, $COCH_2$), 2.37-2.27 (m, 1H, OCHC$\underline{H}_2$), 2.23-2.14 (m, 1H, CCHC$\underline{H}_2$), 2.07-1.98 (m, 1H, CCH), 2.03 (s, 3H, acetyl CH_3), 1.98-1.86 (m, 1H, $OCHCH_2C\underline{H}$), 1.86-1.78 (m, 1H, OCHC$\underline{H}_2$), 1.69-1.57 (m, 1H, isobutyl CH), 1.52-1.38 (m, 2H, isobutyl CH_2), 1.38 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.23 (d, *J* = 10.9 Hz, 1H, CCHC$\underline{H}_2$), 0.91 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.89 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.84 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.8, 171.1, 170.5, 85.7, 77.7, 51.3, 49.5, 39.9, 39.5, 38.2, 37.3, 35.8, 35.5, 28.5, 27.1, 26.3, 26.3, 25.4, 24.0, 23.2, 23.1, 21.9; LRMS (ESI) *m/z* 436.3 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{22}H_{39}N_3O_5{}^{10}B$: 435.3014 [$(M+H)^+$], found: 435.3014.

(*S*)-2-acetamido-N^4-(4-(benzyloxy)butyl)-N^1-((*R*)-3-methyl-1-((3a*S*,4*S*,6*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)butyl)succinamide **5-9a**

To a solution of benzyl ester **5-12a** (89.2 mg, 0.174 mmol) in THF (2.0 ml) was added Pd/C (90.0 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 2 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding carboxylic acid **5-15a** as a white amorphous solid.

S5-6 (48.6 mg, 0.174 mmol, 1.0 equiv) was dissolved in 4 M HCl in AcOEt (2.0 ml) and was stirred at rt for 1 h. The reaction mixture was concentrated *in vacuo* to give the corresponding amine as a colorless viscous oil.

To a solution of **5-15a** in DCM (2.0 ml) was added above mentioned amine, DIEA (88.8 μl, 0.522 mmol, 3.0 equiv), HOAt (23.7 mg, 0.174 mmol, 1.0 equiv) and EDC·HCl (33.4 mg, 0.174 mmol, 1.0 equiv) at – 18 °C. After 10 min at – 18 °C, the reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by reverse-phase HPLC (Mightysil RP-18 250-20 (5 μm), acetonitrile/ H_2O 65:35) to yield target compound **5-9a** (77.2 mg, 0.132 mmol, 2 steps 76%) as a colorless viscous oil. $[\alpha]^{23}_D$ -12.39 (*c* 0.54, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40-7.25 (m, 7H, amide NH and aromatic), 6.34 (t, *J* = 5.2 Hz, 1H, amide NH), 4.65 (m, 1H, NCHCO), 4.50 (s, 2H, benzyl CH_2), 4.28 (dd, *J* = 8.6, 1.7 Hz, 1H, OCH), 3.49 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.31-3.11 (m, 3H, NCH_2 and NCHB), 2.68 (dd, *J* = 14.9, 3.4 Hz, 1H, $COCH_2$), 2.38 (dd, *J* = 14.9, 7.4 Hz, 1H, $COCH_2$), 2.35-2.27 (m, 1H, OCHC$\underline{H}_2$), 2.22-2.13 (m, 1H, CCHC$\underline{H}_2$), 2.05-1.99 (m, 1H, CCH), 2.02 (s, 3H, acetyl CH_3), 1.92-1.86 (m, 1H, $OCHCH_2C\underline{H}$), 1.86-1.78 (m, 1H, OCHC$\underline{H}_2$), 1.69-1.56 (m, 5H, $OCH_2C\underline{H}_2$, $OCH_2CH_2C\underline{H}_2$ and isobutyl CH), 1.52-1.35 (m, 2H, isobutyl CH_2), 1.37 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.23 (d, *J* = 10.9 Hz, 1H, CCHC$\underline{H}_2$), 0.90 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.88 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.83 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.2, 171.0, 170.3, 138.2, 128.4, 127.7, 127.6, 85.7, 77.7, 73.0, 69.9, 51.3, 49.4, 39.9, 39.5, 39.5, 38.1, 37.3, 35.8, 35.5, 28.5, 27.2, 27.1, 26.3, 26.2, 25.4, 24.0, 23.2, 23.1, 21.9; LRMS (ESI) *m/z* 584.4 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{32}H_{51}N_3O_6{}^{10}B$: 583.3902 [$(M+H)^+$], found: 583.3907.

Docking simulation

The docking simulations of **5-1a** – **5-3a** and **5-1b** – **5-3b** were performed based on the concept described previously by us.[49]

Ligand preparation

Initial 3D structure of **5-1a** – **5-3a** and **5-1b** – **5-3b** (without pinanediol ester) was constructed by "Paste Special As 3D", followed by energy minimization was performed by "LigPrep" using OPLS-2005 as a force field. The resulting structures were used in docking simulation. Default values were used for all other parameters.

Grid generation for docking simulation of **5-1a** – **5-3a** and **5-1b** – **5-3b**

The grids used for docking simulation were generated by "Receptor Grid Generation" of Glide 5.7 (Schrödinger LLC). The X-ray crystal structure of proteasome in complex with bortezomib (PDB code; 2F16) was refined for docking simulations using the Protein Preparation Wizard Script within Maestro 9.2. After that, the covalent bond between bortezomib and proteasome was deleted and the resulting ligand free side chain of Thr residue was trimmed. To specify the center of the grid, X-ray crystal structure of proteasome in complex with homobelactosin C derivative (PDB code; 3E47) was superimposed, and the ligand structure was merged. The centroid of the two ligands, bortezomib and homobelactosin C derivative, was used as the center of generating grid. Default values were used for all other parameters.

Preparation of the core structure used in docking simulation

The X-ray crystal structure of proteasome in complex with bortezomib (PDB code; 3MG0) was superimposed with the structure used for grid generation. The boronic acid moiety consisting of four heavy atoms was used as a core for docking simulation.

Ligand docking

The docking simulation was performed by "Ligand Docking" of Glide 5.7 (Schrödinger LLC), using the aforementioned core structure (tolerance, 1.0 Å) for restricting the position of the boronic acid moiety in **5-1a** – **5-3a** and **5-1b** – **5-3b** (Write out at most 25 poses per ligand; Number of poses ligand to include 25). The docking results shown in the manuscript are the top ranked poses. Default values were used for all other parameters.

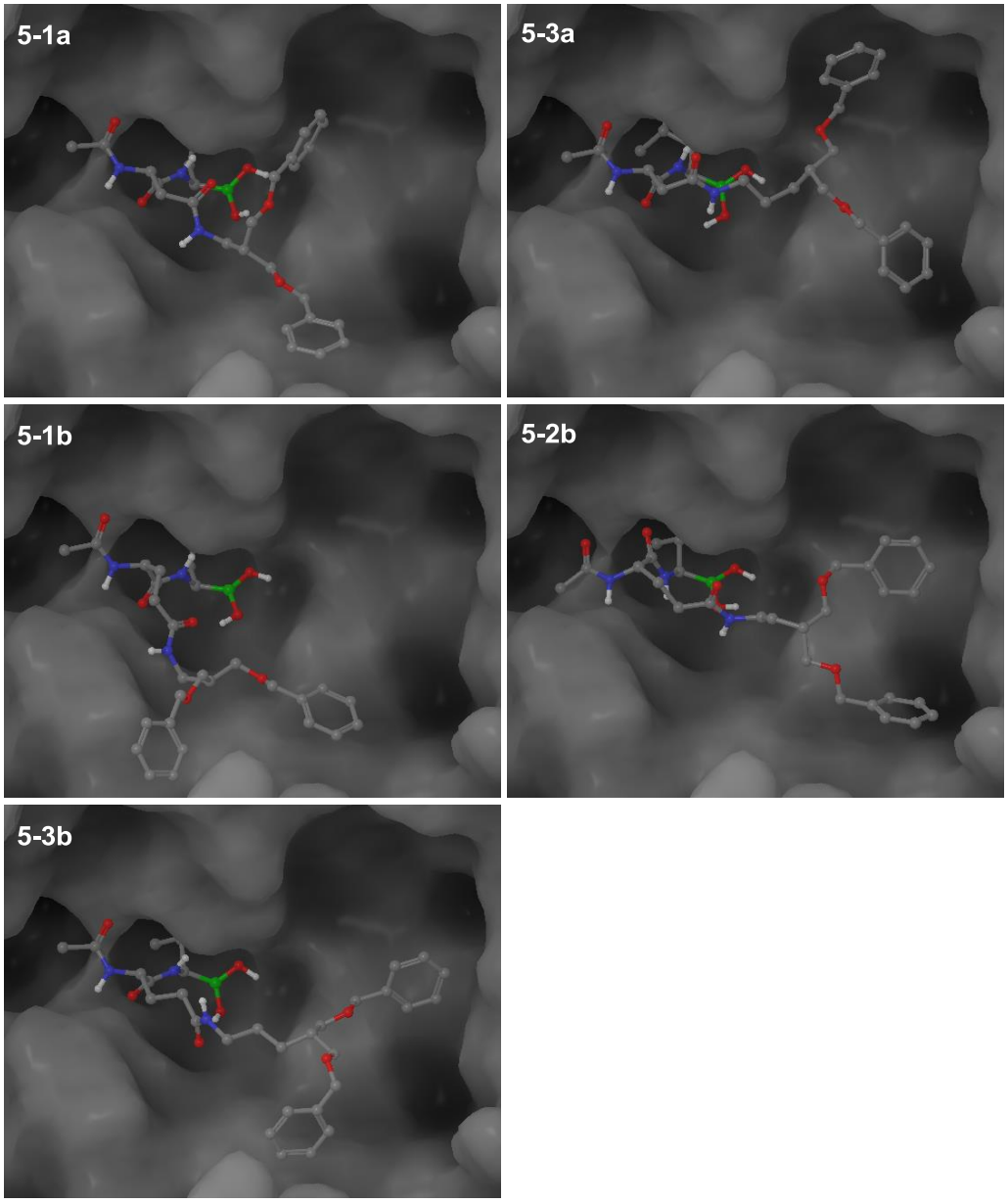


Figure S5-1. Docking results for hybrid compounds **5-1a**, **5-3a** and **5-1b** – **5-3b**

Proteasome assay

Inhibitory activity of compounds **5-1a** – **5-9a** and **5-1b** – **5-3b** on the ChT-L, C-L and T-L activities of human 20S proteasome were measured as described previously.[37]

Cell proliferation assay

Inhibitory activity of compounds **5-1a** – **5-9a** and **5-1b** – **5-3b** on HCT116 cell growth was measured as described previously.[37]

Proteasome active site enzyme-linked immunosorbent assay

Inhibitory activity of compounds **5-2a** and **5-9a** on β5 and β5i subunits were measured as described previously.[37]

Substrate-based assays of cathepsins

In substrate-based assays, each reaction containing a certain protease was initiated by the addition of an appropriate substrate, and the resultant fluorescence signal was detected with a plate-based spectrofluorometer SpectraMax M5SK (Molecular Devices) as described previously by Arastu-Kapur, S. *et al.*[44]

Assays for the sensitivity of cathepsin A protease activity to belactosin A derivatives were performed in a reaction buffer [50 mM MOPS (pH 5.5)] containing 22 ng/mL of recombinant cathepsin A (R&D systems) and 40 μM substrate, Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(Dnp)-OH (R&D systems) in 96-well half-area plates (Corning). Each chemical compound in DMSO at different concentrations was diluted 3-fold with the reaction buffer. After preincubation of 15 μL of 13 ng/mL protease solution with 5 μL of each chemical diluent at 25 °C for 5 minutes, the reaction was initiated by the addition of 5 μL of the 200 μM substrate solution, followed by incubation at 25 °C for a further 2 hours. The protease activity in each reaction was measured as fluorescence intensity. The excitation wavelength was 320 nm and the emission wavelength was 405 nm. At least three experiments were performed per condition, and the averages and standard deviations of inhibition rates in each condition were evaluated to determine IC_{50} values using GraphPad Prism4.

Protease activities of cathepsin B and cathepsin G were examined by a similar method as described above. The protease activity of 40 ng/mL of recombinant cathepsin B (R&D systems) was measured using 10 μM Z-Leu-Arg-AMC (R&D systems) in assay buffer consisting of 25 mM MES, pH 5.0, at 25 °C for 30 minutes. The excitation wavelength was 380 nm and the emission wavelength was 460 nm. 8.0 μU/mL of recombinant cathepsin G (R&D systems) with 200 μM Suc-Ala-Ala-Pro-Phe-AMC (Bachem) was used in assay buffer consisting of 50 mM Tris-HCl, pH 7.5, and 150 mM NaCl at 25 °C for an hour. The excitation and emission wavelengths were 380 nm and 460 nm, respectively.

Cell cycle analysis

The cell cycle distribution of HCT116 cells treated with compounds **5-2a** and **5-9a** was measured as described previously.[37]

Immunoblot analysis

Immunoblot analysis of p53 and cPARP in HCT116 cells treated with **5-2a** and **5-9a** was performed as described previously.[37]

Reversibility assay

Reversibility of the proteasome inhibitory effect of **5-2a** and **5-9a** was measured as described previously.[37]

Combustion analysis data for the target compounds

Table S5-1. Table listing combustion analysis data for the target compounds

5-1a	Anal. calcd for $C_{39}H_{56}N_3O_7B$: C, 67.92; H, 8.18; N, 6.09.	Found: C, 67.73; H, 8.33; N, 6.01.
5-2a	Anal. calcd for $C_{40}H_{58}N_3O_7B \cdot 0.1H_2O$: C, 68.10; H, 8.31; N, 5.96.	Found: C, 67.90; H, 8.51; N, 5.85.
5-3a	Anal. calcd for $C_{41}H_{60}N_3O_7B \cdot 1.8H_2O$: C, 65.64; H, 8.55; N, 5.60.	Found: C, 65.86; H, 8.85; N, 5.36.
5-1b	Anal. calcd for $C_{40}H_{58}N_3O_7B$: C, 68.27; H, 8.31; N, 5.97.	Found: C, 67.97; H, 8.48; N, 5.90.
5-2b	Anal. calcd for $C_{41}H_{60}N_3O_7B$: C, 68.61; H, 8.43; N, 5.85.	Found: C, 68.94; H, 8.82; N, 5.76.
5-3b	Anal. calcd for $C_{42}H_{62}N_3O_7B \cdot 0.3H_2O$: C, 68.43; H, 8.56; N, 5.70.	Found: C, 68.30; H, 8.83; N, 5.60.
5-4a	Anal. calcd for $C_{45}H_{58}N_3O_7BCl_2$: C, 64.75; H, 7.00; N, 5.03.	Found: C, 64.65; H, 7.20; N, 4.77.
5-5a	Anal. calcd for $C_{45}H_{60}N_3O_7B \cdot 0.2H_2O$: C, 70.25; H, 7.91; N, 5.46.	Found: C, 70.03; H, 8.15; N, 5.33.
5-6a	Anal. calcd for $C_{43}H_{58}N_5O_7B \cdot 0.5H_2O$: C, 66.49; H, 7.66; N, 9.02.	Found: C, 66.56; H, 8.01; N, 8.78.
5-7a	Anal. calcd for $C_{50}H_{63}N_4O_7B \cdot 0.5H_2O$: C, 70.50; H, 7.57; N, 6.58.	Found: C, 70.61; H, 7.97; N, 6.34.
5-8a	Anal. calcd for $C_{22}H_{38}N_3O_5B \cdot 0.2H_2O$: C, 60.20; H, 8.82; N, 9.57.	Found: C, 60.25; H, 9.02; N, 9.27.
5-9a	Anal. calcd for $C_{32}H_{50}N_3O_6B$: C, 65.86; H, 8.64; N, 7.20.	Found: C, 65.61; H, 8.75; N, 7.01.

Synthetic procedures for target compounds 6-1 and 6-2

Target compound **6-1**

To a solution of Boc-Asp-OBn **6-3** (50.0 mg, 0.155 mmol) in DCM (1.5 ml) was added triethylamine (21.6 μl, 0.155 mmol, 1.0 equiv) and PivCl (19.1 μl, 0.155 mmol, 1.0 equiv) at 0 °C. After 1 h at 0 °C, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding acid anhydride as a colorless oil.

To a solution of amine **3-29**[60] (46.4 mg, 0.155 mmol, 1.0 equiv) in DCM (1.5 ml) was added triethylamine (21.6 μl, 0.155 mmol, 1.0 equiv) and the aforementioned acid anhydride in DCM (1.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding coupling product as a colorless viscous oil.

To a solution of the oil in DCM (1.0 ml) was added TFA (1.0 ml). After 2 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a pale yellow oil.

To a solution of the oil in DCM (2.0 ml) was added triethylamine (47.5 μl, 0.341 mmol, 2.2 equiv) and Ac_2O (14.7 μl, 0.155 mmol, 1.0 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the acetylated product as a colorless viscous oil.

To a solution of the oil in EtOH (2.0 ml) was added Pd/C (80 mg) and 1,4-cyclohexadiene (145 μl, 1.55 mmol, 10 equiv). After 140 min at rt, the reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to give the corresponding carboxylic acid as a colorless viscous oil.

To a solution of epoxyketone unit **6-8**[107] (42.1 mg, 0.155 mmol, 1.0 equiv) in DCM (750 μl) was added TFA (750 μl). After 20 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a white solid.

To a solution of the solid and the aforementioned carboxylic acid in THF (5.0 ml) was added HOBt (31.4 mg, 0.233 mmol, 1.5 equiv), HBTU (88.4 mg, 0.233 mmol, 1.5 equiv) and DIEA (109 μl, 0.775 mmol, 5.0 equiv) at -5 °C. After 18 h at -5 °C, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by HPLC (Mightysil Si 60 250-20 (5 μm), *n*-hexane/ AcOEt 1:5) to yield target compound **6-1** (56.6 mg, 0.0928 mmol, 6 steps 60%) as a colorless viscous oil. $[\alpha]^{29}_{D}$ 55.40 (*c* 0.50, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.4 Hz, 1H, amide NH), 7.38-7.23 (m, 11H, amide NH and aromatic), 6.52 (t, *J* = 5.2 Hz, 1H, amide NH), 4.68-4.60 (m, 1H, NCH), 4.48 (s, 4H, benzyl CH_2), 4.48-4.41 (m, 1H, NCH), 3.56-3.48 (m, 2H, OCH_2), 3.48-3.40 (m, 2H, OCH_2), 3.35-3.22 (m, 2H, NCH_2), 3.28 (d, *J* = 5.2 Hz, 1H, epoxide CH_2), 2.84 (d, *J* = 5.2 Hz, 1H, epoxide CH_2), 2.45 (dd, *J* = 15.5, 3.4 Hz, 1H, $COCH_2$), 2.29 (dd, *J* = 15.5, 7.4 Hz, 1H, $COCH_2$), 2.06-1.96 (m, 1H, $OCH_2C\underline{H}$), 1.99 (s, 3H, acetyl CH_3), 1.73-1.58 (m, 3H, $NCH_2C\underline{H}_2$ and isobutyl CH), 1.56-1.45 (m, 1H, isobutyl CH_2), 1.48 (s, 3H, CH_3), 1.35-1.23 (m, 1H, isobutyl CH_2), 0.93 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.91 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 208.1, 171.1, 171.1, 170.0, 138.1, 128.3, 127.6, 73.2, 71.2, 71.2, 59.1, 52.3, 50.9, 49.3, 39.3, 37.9, 37.7, 37.1, 28.9, 25.2, 23.3, 23.1, 21.1, 16.7; LRMS (ESI) *m/z* 610.4 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{34}H_{48}N_3O_7$: 610.3487 [$(M+H)^+$], found: 610.3483.

Target compound **6-2**

Target compound **6-2** (70.3 mg, 0.144 mmol, 6 steps 60%, colorless viscous oil) was prepared from Boc-Asp-OBn **6-3** as described for target compound **6-1** using amine **5-16** instead of **3-29**. $[\alpha]^{29}_{D}$ 65.21 (*c* 0.56, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 7.4 Hz, 1H, amide NH), 7.38-7.24 (m, 6H, amide NH and aromatic), 6.56 (t, *J* = 5.2 Hz, 1H, amide NH), 4.72-4.65 (m, 1H, NCH), 4.52-4.42 (m, 1H, NCH), 4.49 (s, 2H, benzyl CH_2), 3.49 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.32-3.17 (m, 2H, NCH_2), 3.28 (d, *J* = 5.2 Hz, 1H, epoxide CH_2), 2.86 (d, *J* = 5.2 Hz, 1H, epoxide CH_2), 2.60 (dd, *J*= 15.5, 4.0 Hz, 1H, $COCH_2$), 2.45 (dd, *J* = 15.5, 7.4 Hz, 1H, $COCH_2$), 2.00 (s, 3H, acetyl CH_3), 1.74-1.56 (m, 5H, $NCH_2C\underline{H}_2$, $OCH_2C\underline{H}_2$ and isobutyl CH), 1.56-1.46 (m, 1H, isobutyl CH_2), 1.49 (s, 3H, CH_3), 1.35-1.24 (m, 1H, isobutyl CH_2), 0.93 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.92 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 208.2, 171.1, 171.1, 170.2, 138.2, 128.3, 127.6, 127.5, 72.9, 69.8, 59.1, 52.3, 50.9, 49.5, 39.4, 39.2, 37.4, 27.0, 26.1, 25.1, 23.2, 23.0, 21.1, 16.7; LRMS (ESI) *m/z* 490.3 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{26}H_{40}N_3O_6$: 490.2912 $[(M+H)^+]$, found: 490.2908.

Computational simulations

The binding mode of target compounds **6-1** and **6-2** in the proteasome binding pocket was computationally simulated by MacroModel 9.9 (Schrödinger LLC) using OPLS-2005 as a force field. The X-ray crystal structure of PR-957 in complex with proteasome (PDB code: 3UN4)[108] was refined for simulations using the Protein Preparation Wizard Script within Maestro 9.2 (Schrödinger LLC). After that, the structure of the PR-957 was manually converted into the target compounds **6-1** or **6-2**. Then, "Minimization" of the complex was performed, in which the ligand structure was selected as freely moving atoms and, around that, a shell with a radius of 15 Å was constructed and the atoms in the shell were frozen. This setting was also applied in the following "Dynamics" and "Multiple Minimization". Next, using the obtained complex structure, "Dynamics" was performed (Number of structures to sample, 100; Simulation time, 100 ps). Finally, using the sampled structures, "Multiple Minimization" was performed and the top-ranked pose was shown in the manuscript. Default values were used for all other parameters.

Proteasomes assay

Inhibitory activity of target compounds **6-1** and **6-2** on ChT-L activity of the human 20S proteasome was investigated as described previously.[37]

Cell proliferation assay

Inhibitory activity of target compounds **6-1** and **6-2** on HCT116 cell growth was investigated as described previously.[37]

Combustion analysis data for the target compounds.

Table S1. Table listing combustion analysis data for the target compounds.

6-1	Anal. calcd for $C_{34}H_{47}N_3O_7 \cdot 0.4H_2O$: C, 66.19; H, 7.81; N, 6.81.	Found: C, 66.55; H, 7.92; N, 6.42.
6-2	Anal. calcd for $C_{26}H_{39}N_3O_6 \cdot 0.2H_2O$: C, 63.32; H, 8.05; N, 8.52.	Found: C, 63.45; H, 8.05; N, 8.13.

参考文献

1. (a) Orlowski, M. The multicatalytic proteinase complex, a major extralysosomal proteolytic system. *Biochemistry* **1990**, *29*, 10289-10297; (b) Ciechanover, A. The ubiquitin-proteasome proteolytic pathway. *Cell* **1994**, *79*, 13-21.

2. (a) Boya, P.; Reggiori, F.; Codogno, P. Emerging regulation and functions of autophagy. *Nat Cell Biol* **2013**, *15*, 713-720; (b) Mariño, G.; López-Otín, C. Autophagy: molecular mechanisms, physiological functions and relevance in human pathology. *CMLS, Cell. Mol. Life Sci.* **2004**, *61*, 1439-1454.

3. (a) Fuchs, S. Y. The role of ubiquitin-proteasome pathway in oncogenic signaling. *Cancer Biol. Ther.* **2002**, *1*, 337-341; (b) Chen, J. J.; Lin, F.; Qin, Z. H. The roles of the proteasome pathway in signal transduction and neurodegenerative diseases. *Neurosci. Bull.* **2008**, *24*, 183-194.

4. (a) Muchamuel, T.; Basler, M.; Aujay, M. A.; Suzuki, E.; Kalim, K. W.; Lauer, C.; Sylvain, C.; Ring, E. R.; Shields, J.; Jiang, J.; Shwonek, P.; Parlati, F.; Demo, S. D.; Bennett, M. K.; Kirk, C. J.; Groettrup, M. A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. *Nat. Med.* **2009**, *15*, 781-787; (b) Vembar, S. S.; Brodsky, J. L. One step at a time: endoplasmic reticulum-associated degradation. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 944-957; (c) Ron, D.; Walter, P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 519-529; (d) Hiller, M. M.; Finger, A.; Schweiger, M.; Wolf, D. H. ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. *Science* **1996**, *273*, 1725-1728.

5. King, R. W.; Deshaies, R. J.; Peters, J. M.; Kirschner, M. W. How proteolysis drives the cell cycle. *Science* **1996**, *274*, 1652-1659.

6. Murata, S.; Yashiroda, H.; Tanaka, K. Molecular mechanisms of proteasome assembly. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 104-115.

7. DeMartino, G. N.; Slaughter, C. A. The Proteasome, a Novel Protease Regulated by Multiple Mechanisms. *J. Biol. Chem.* **1999**, *274*, 22123-22126.

8. (a) Coux, O.; Tanaka, K.; Goldberg, A. L. Structure and functions of the 20S and 26S proteasomes. *Annu. Rev. Biochem.* **1996**, *65*, 801-847; (b) Baumeister, W.; Walz, J.; Zuhl, F.; Seemuller, E. The proteasome: paradigm of a self-compartmentalizing protease. *Cell* **1998**, *92*, 367-380.

9. Kloetzel, P. M. Antigen processing by the proteasome. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 179-187.

10. Griffin, T.; Nandi, D.; Cruz, M.; Fehling, H. J.; Van Kaer, L. Immunoproteasome assembly: cooperative incorporation of interferon gamma (IFN-gamma)-inducible subunits. *The Journal of experimental medicine* **1998**, *187*, 97-104.

11. Murata, S.; Sasaki, K.; Kishimoto, T.; Niwa, S.-i.; Hayashi, H.; Takahama, Y.; Tanaka, K. Regulation of CD8+ T Cell Development by Thymus-Specific Proteasomes. *Science* **2007**, *316*, 1349-1353.

12. (a) Glynne, R.; Powis, S. H.; Beck, S.; Kelly, A.; Kerr, L.-A.; Trowsdale, J. A proteasome-related gene between the two ABC transporter loci in the class II region of the human MHC. *Nature* **1991**, *353*, 357-360; (b) Martinez, C. K.; Monaco, J. J. Homology of proteasome subunits to a major histocompatibility complex-linked LMP gene. *Nature* **1991**, *353*, 664-667; (c) Nandi, D.; Jiang, H.; Monaco, J. J. Identification of MECL-1 (LMP-10) as the third IFN-gamma-inducible proteasome subunit. *The Journal of Immunology* **1996**, *156*, 2361-2364.

13. Kloetzel, P.-M.; Ossendorp, F. Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. *Curr. Opin. Immunol.* **2004**, *16*, 76-81.

14. Murata, S.; Takahama, Y.; Tanaka, K. Thymoproteasome: probable role in generating positively selecting peptides.

Curr. Opin. Immunol. **2008**, *20*, 192-196.

15. Kloetzel, P. M. Generation of major histocompatibility complex class I antigens: functional interplay between proteasomes and TPPII. *Nat. Immunol.* **2004**, *5*, 661-669.

16. Takahama, Y.; Tanaka, K.; Murata, S. Modest cortex and promiscuous medulla for thymic repertoire formation. *Trends Immunol.* **2008**, *29*, 251-255.

17. Karin, M. Nuclear factor-[kappa]B in cancer development and progression. *Nature* **2006**, *441*, 431-436.

18. Vousden, K. H.; Lu, X. Live or let die: the cell's response to p53. *Nat. Rev. Cancer* **2002**, *2*, 594-604.

19. Fujieda, S.; Inuzuka, M.; Tanaka, N.; Sunaga, H.; Fan, G. K.; Ito, T.; Sugimoto, C.; Tsuzuki, H.; Saito, H. Expression of p27 is associated with Bax expression and spontaneous apoptosis in oral and oropharyngeal carcinoma. *Int. J. Cancer* **1999**, *84*, 315-320.

20. Bross, P. F.; Kane, R.; Farrell, A. T.; Abraham, S.; Benson, K.; Brower, M. E.; Bradley, S.; Gobburu, J. V.; Goheer, A.; Lee, S. L.; Leighton, J.; Liang, C. Y.; Lostritto, R. T.; McGuinn, W. D.; Morse, D. E.; Rahman, A.; Rosario, L. A.; Verbois, S. L.; Williams, G.; Wang, Y. C.; Pazdur, R. Approval summary for bortezomib for injection in the treatment of multiple myeloma. *Clin. Cancer Res.* **2004**, *10*, 3954-3964.

21. (a) Shah, J. J.; Orlowski, R. Z. Proteasome inhibitors in the treatment of multiple myeloma. *Leukemia* **2009**, *23*, 1964-1979; (b) Kumar, S.; Rajkumar, S. V. Many facets of bortezomib resistance/susceptibility. *Blood* **2008**, *112*, 2177-2178; (c) Richardson, P. G.; Briemberg, H.; Jagannath, S.; Wen, P. Y.; Barlogie, B.; Berenson, J.; Singhal, S.; Siegel, D. S.; Irwin, D.; Schuster, M.; Srkalovic, G.; Alexanian, R.; Rajkumar, S. V.; Limentani, S.; Alsina, M.; Orlowski, R. Z.; Najarian, K.; Esseltine, D.; Anderson, K. C.; Amato, A. A. Frequency, characteristics, and reversibility of peripheral neuropathy during treatment of advanced multiple myeloma with bortezomib. *J. Clin. Oncol.* **2006**, *24*, 3113-3120; (d) Menashe, J. Managing and avoiding bortezomib toxicity. *Community Oncology* **2007**, *4*, 480-484.

22. Abraham, J. Carfilzomib and bortezomib therapy in patients with multiple myeloma. *Community Oncology* **2012**, *9*, 278-282.

23. Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 355-357.

24. Piva, R.; Ruggeri, B.; Williams, M.; Costa, G.; Tamagno, I.; Ferrero, D.; Giai, V.; Coscia, M.; Peola, S.; Massaia, M.; Pezzoni, G.; Allievi, C.; Pescalli, N.; Cassin, M.; di Giovine, S.; Nicoli, P.; de Feudis, P.; Strepponi, I.; Roato, I.; Ferracini, R.; Bussolati, B.; Camussi, G.; Jones-Bolin, S.; Hunter, K.; Zhao, H.; Neri, A.; Palumbo, A.; Berkers, C.; Ovaa, H.; Bernareggi, A.; Inghirami, G. CEP-18770: A novel, orally active proteasome inhibitor with a tumor-selective pharmacologic profile competitive with bortezomib. *Blood* **2008**, *111*, 2765-2775.

25. (a) Kupperman, E.; Lee, E. C.; Cao, Y.; Bannerman, B.; Fitzgerald, M.; Berger, A.; Yu, J.; Yang, Y.; Hales, P.; Bruzzese, F.; Liu, J.; Blank, J.; Garcia, K.; Tsu, C.; Dick, L.; Fleming, P.; Yu, L.; Manfredi, M.; Rolfe, M.; Bolen, J. Evaluation of the Proteasome Inhibitor MLN9708 in Preclinical Models of Human Cancer. *Cancer Res.* **2010**, *70*, 1970-1980; (b) Chauhan, D.; Tian, Z.; Zhou, B.; Kuhn, D.; Orlowski, R.; Raje, N.; Richardson, P.; Anderson, K. C. In Vitro and In Vivo Selective Antitumor Activity of a Novel Orally Bioavailable Proteasome Inhibitor MLN9708 against Multiple Myeloma Cells. *Clin. Cancer Res.* **2011**, *17*, 5311-5321.

26. Chauhan, D.; Singh, A. V.; Aujay, M.; Kirk, C. J.; Bandi, M.; Ciccarelli, B.; Raje, N.; Richardson, P.; Anderson, K. C. A novel orally active proteasome inhibitor ONX 0912 triggers in vitro and in vivo cytotoxicity in multiple myeloma. *Blood* **2010**, *116*, 4906-4915.

27. Kisselev, A. F.; van der Linden, W. A.; Overkleeft, H. S. Proteasome Inhibitors: An Expanding Army Attacking a Unique Target. *Chem. Biol.* **2012**, *19*, 99-115.

28. Kisselev, A. F.; Callard, A.; Goldberg, A. L. Importance of the Different Proteolytic Sites of the Proteasome and the Efficacy of Inhibitors Varies with the Protein Substrate. *J. Biol. Chem.* **2006**, *281*, 8582-8590.

29. Parlati, F.; Lee, S. J.; Aujay, M.; Suzuki, E.; Levitsky, K.; Lorens, J. B.; Micklem, D. R.; Ruurs, P.; Sylvain, C.; Lu, Y.; Shenk, K. D.; Bennett, M. K. Carfilzomib can induce tumor cell death through selective inhibition of the chymotrypsin-like activity of the proteasome. *Blood* **2009**, *114*, 3439-3447.

30. (a) Chauhan, D.; Singh, A.; Brahmandam, M.; Podar, K.; Hideshirna, T.; Richardson, P.; Munshi, N.; Palladino, M. A.; Anderson, K. C. Combination of proteasome inhibitors bortezomib and NPI-0052 trigger in vivo synergistic cytotoxicity in multiple myeloma. *Blood* **2008**, *111*, 1654-1664; (b) Britton, M.; Lucas, M. M.; Downey, S. L.; Screen, M.; Pletnev, A. A.; Verdoes, M.; Tokhunts, R. A.; Amir, O.; Goddard, A. L.; Pelphrey, P. M.; Wright, D. L.; Overkleeft, H. S.; Kisselev, A. F. Selective Inhibitor of Proteasome's Caspase-like Sites Sensitizes Cells to Specific Inhibition of Chymotrypsin-like Sites. *Chem. Biol.* **2009**, *16*, 1278-1289; (c) Mirabella, Anne C.; Pletnev, Alexandre A.; Downey, Sondra L.; Florea, Bogdan I.; Shabaneh, Tamer B.; Britton, M.; Verdoes, M.; Filippov, Dmitri V.; Overkleeft, Herman S.; Kisselev, Alexei F. Specific Cell-Permeable Inhibitor of Proteasome Trypsin-like Sites Selectively Sensitizes Myeloma Cells to Bortezomib and Carfilzomib. *Chem. Biol.* **2011**, *18*, 608-618.

31. (a) Huber, E. M.; Groll, M. Inhibitors for the Immuno- and Constitutive Proteasome: Current and Future Trends in Drug Development. *Angew Chem Int Edit* **2012**, *51*, 8708-8720; (b) Singh, A. V.; Bandi, M.; Aujay, M. A.; Kirk, C. J.; Hark, D. E.; Raje, N.; Chauhan, D.; Anderson, K. C. PR-924, a selective inhibitor of the immunoproteasome subunit LMP-7, blocks multiple myeloma cell growth both in vitro and in vivo. *Br J Haematol* **2011**, *152*, 155-163; (c) Kuhn, D. J.; Hunsucker, S. A.; Chen, Q.; Voorhees, P. M.; Orlowski, M.; Orlowski, R. Z. Targeted inhibition of the immunoproteasome is a potent strategy against models of multiple myeloma that overcomes resistance to conventional drugs and nonspecific proteasome inhibitors. *Blood* **2009**, *113*, 4667-4676; (d) Ho, Y. K.; Bargagna-Mohan, P.; Wehenkel, M.; Mohan, R.; Kim, K.-B. LMP2-Specific Inhibitors: Chemical Genetic Tools for Proteasome Biology. *Chem. Biol.* **2007**, *14*, 419-430; (e) Demo, S. D.; Kirk, C. J.; Aujay, M. A.; Buchholz, T. J.; Dajee, M.; Ho, M. N.; Jiang, J.; Laidig, G. J.; Lewis, E. R.; Parlati, F.; Shenk, K. D.; Smyth, M. S.; Sun, C. M.; Vallone, M. K.; Woo, T. M.; Molineaux, C. J.; Bennett, M. K. Antitumor Activity of PR-171, a Novel Irreversible Inhibitor of the Proteasome. *Cancer Res.* **2007**, *67*, 6383-6391.

32. (a) Asai, A.; Hasegawa, A.; Ochiai, K.; Yamashita, Y.; Mizukami, T. Belactosin A, a novel antitumor antibiotic acting on cyclin/CDK mediated cell cycle regulation, produced by Streptomyces sp. *J. Antibiot.* **2000**, *53*, 81-83; (b) Asai, A.; Tsujita, T.; Sharma, S. V.; Yamashita, Y.; Akinaga, S.; Funakoshi, M.; Kobayashi, H.; Mizukami, T. A new structural class of proteasome inhibitors identified by microbial screening using yeast-based assay. *Biochem. Pharmacol.* **2004**, *67*, 227-234.

33. (a) Groll, M.; Larionov, O. V.; Huber, R.; De Meijere, A. Inhibitor-binding mode of homobelactosin C to proteasomes: New insights into class I MHC ligand generation. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 4576-4579; (b) Korotkov, V. S.; Ludwig, A.; Larionov, O. V.; Lygin, A. V.; Groll, M.; de Meijere, A. Synthesis and biological activity of optimized belactosin C congeners. *Org. Biomol. Chem.* **2011**, *9*, 7791-7798.

34. (a) Grawert, M. A.; Groll, M. Exploiting nature's rich source of proteasome inhibitors as starting points in drug development. *Chem. Commun.* **2012**, *48*, 1364-1378; (b) Borissenko, L.; Groll, M. 20S proteasome and its inhibitors: crystallographic knowledge for drug development. *Chem. Rev.* **2007**, *107*, 687-717.

35. Yoshida, K.; Yamaguchi, K.; Sone, T.; Unno, Y.; Asai, A.; Yokosawa, H.; Matsuda, A.; Arisawa, M.; Shuto, S. Synthesis of 2,3- and 3,4-methanoamino acid equivalents with stereochemical diversity and their conversion into the

tripeptide proteasome inhibitor belactosin a and its highly potent cis-cyclopropane stereoisomer. *Org. Lett.* **2008**, *10*, 3571-3574.

36. Yoshida, K.; Yamaguchi, K.; Mizuno, A.; Unno, Y.; Asai, A.; Sone, T.; Yokosawa, H.; Matsuda, A.; Arisawa, M.; Shuto, S. Three-dimensional structure-activity relationship study of belactosin A and its stereo- and regioisomers: development of potent proteasome inhibitors by a stereochemical diversity-oriented strategy. *Org. Biomol. Chem.* **2009**, *7*, 1868-1877.

37. Kawamura, S.; Unno, Y.; List, A.; Mizuno, A.; Tanaka, M.; Sasaki, T.; Arisawa, M.; Asai, A.; Groll, M.; Shuto, S. Potent Proteasome Inhibitors Derived from the Unnatural cis-Cyclopropane Isomer of Belactosin A: Synthesis, Biological Activity, and Mode of Action. *J. Med. Chem.* **2013**, *56*, 3689-3700.

38. Kazuta, Y.; Matsuda, A.; Shuto, S. Development of Versatile cis- and trans-Dicarbon-Substituted Chiral Cyclopropane Units: Synthesis of (1S,2R)- and (1R,2R)-2-Aminomethyl-1-(1H-imidazol-4-yl)cyclopropanes and Their Enantiomers as Conformationally Restricted Analogues of Histamine. *J. Org. Chem.* **2002**, *67*, 1669-1677.

39. (a) Kazuta, Y.; Hirano, K.; Natsume, K.; Yamada, S.; Kimura, R.; Matsumoto, S.; Furuichi, K.; Matsuda, A.; Shuto, S. Cyclopropane-based conformational restriction of histamine. (1S,2S)-2-(2-aminoethyl)-1-(1H-imidazol-4-yl)cyclopropane, a highly selective agonist for the histamine H3 receptor, having a cis-cyclopropane structure. *J. Med. Chem.* **2003**, *46*, 1980-1988; (b) Watanabe, M.; Kazuta, Y.; Hayashi, H.; Yamada, S.; Matsuda, A.; Shuto, S. Stereochemical diversity-oriented conformational restriction strategy. Development of potent histamine H3 and/or H4 receptor antagonists with an imidazolylcyclopropane structure. *J. Med. Chem.* **2006**, *49*, 5587-5596; (c) Watanabe, M.; Hirokawa, T.; Kobayashi, T.; Yoshida, A.; Ito, Y.; Yamada, S.; Orimoto, N.; Yamasaki, Y.; Arisawa, M.; Shuto, S. Investigation of the bioactive conformation of histamine H3 receptor antagonists by the cyclopropylic strain-based conformational restriction strategy. *J. Med. Chem.* **2010**, *53*, 3585-3593; (d) Watanabe, M.; Kobayashi, T.; Hirokawa, T.; Yoshida, A.; Ito, Y.; Yamada, S.; Orimoto, N.; Yamasaki, Y.; Arisawa, M.; Shuto, S. Cyclopropane-based stereochemical diversity-oriented conformational restriction strategy: histamine H3 and/or H4 receptor ligands with the 2,3-methanobutane backbone. *Org. Biomol. Chem.* **2012**, *10*, 736-745.

40. Cogan, D. A.; Liu, G.; Ellman, J. Asymmetric synthesis of chiral amines by highly diastereoselective 1, 2-additions of organometallic reagents to N-tert-butanesulfinyl imines. *Tetrahedron* **1999**, *55*, 8883-8904.

41. Armstrong, A.; Scutt, J. N. Total synthesis of (+)-belactosin A. *Chem. Commun.* **2004**, 510-511.

42. (a) Charette, A. B.; Juteau, H. Design of Amphoteric Bifunctional Ligands - Application to the Enantioselective Simmons-Smith Cyclopropanation of Allylic Alcohols. *J Am Chem Soc* **1994**, *116*, 2651-2652; (b) Charette, A. B.; Juteau, H.; Lebel, H.; Molinaro, C. Enantioselective cyclopropanation of allylic alcohols with dioxaborolane ligands: Scope and synthetic applications. *J Am Chem Soc* **1998**, *120*, 11943-11952.

43. (a) Kusumi, T.; Fukushima, T.; Ohtani, I.; Kakisawa, H. Elucidation of the absolute configurations of amino acids and amines by the modified mosher's method. *Tetrahedron Lett.* **1991**, *32*, 2939-2942; (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. *J. Am. Chem. Soc.* **1991**, *113*, 4092-4096.

44. Arastu-Kapur, S.; Anderl, J. L.; Kraus, M.; Parlati, F.; Shenk, K. D.; Lee, S. J.; Muchamuel, T.; Bennett, M. K.; Driessen, C.; Ball, A. J.; Kirk, C. J. Nonproteasomal Targets of the Proteasome Inhibitors Bortezomib and Carfilzomib: a Link to Clinical Adverse Events. *Clin. Cancer Res.* **2011**, *17*, 2734-2743.

45. Kuhn, D. J.; Chen, Q.; Voorhees, P. M.; Strader, J. S.; Shenk, K. D.; Sun, C. M.; Demo, S. D.; Bennett, M. K.; van Leeuwen, F. W.; Chanan-Khan, A. A.; Orlowski, R. Z. Potent activity of carfilzomib, a novel, irreversible inhibitor of the

ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. *Blood* **2007**, *110*, 3281-3290.

46. (a) Honda, R.; Tanaka, H.; Yasuda, H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett.* **1997**, *420*, 25-27; (b) Levine, A. J. p53, the Cellular Gatekeeper for Growth and Division. *Cell* **1997**, *88*, 323-331; (c) Vogelstein, B.; Lane, D.; Levine, A. J. Surfing the p53 network. *Nature* **2000**, *408*, 307-310; (d) Oren, M. Decision making by p53: life, death and cancer. *Cell Death Differ.* **2003**, *10*, 431-442.

47. Williamson, M. J.; Blank, J. L.; Bruzzese, F. J.; Cao, Y.; Daniels, J. S.; Dick, L. R.; Labutti, J.; Mazzola, A. M.; Patil, A. D.; Reimer, C. L.; Solomon, M. S.; Stirling, M.; Tian, Y.; Tsu, C. A.; Weatherhead, G. S.; Zhang, J. X.; Rolfe, M. Comparison of biochemical and biological effects of ML858 (salinosporamide A) and bortezomib. *Mol. Cancer Ther.* **2006**, *5*, 3052-3061.

48. Eustáquio, A. S.; Moore, B. S. Mutasynthesis of Fluorosalinosporamide, a Potent and Reversible Inhibitor of the Proteasome. *Angew. Chem. Int. Ed.* **2008**, *47*, 3936-3938.

49. Kawamura, S.; Unno, Y.; Tanaka, M.; Sasaki, T.; Yamano, A.; Hirokawa, T.; Kameda, T.; Asai, A.; Arisawa, M.; Shuto, S. Investigation of the Non-Covalent Binding Mode of Covalent Proteasome Inhibitors around the Transition State by Combined Use of Cyclopropylic Strain-Based Conformational Restriction and Computational Modeling. *J. Med. Chem.* **2013**, *56*, 5829-5842.

50. (a) Singh, J.; Petter, R. C.; Baillie, T. A.; Whitty, A. The resurgence of covalent drugs. *Nat. Rev. Drug Discovery* **2011**, *10*, 307-317; (b) Smith, A. J.; Zhang, X.; Leach, A. G.; Houk, K. N. Beyond picomolar affinities: quantitative aspects of noncovalent and covalent binding of drugs to proteins. *J. Med. Chem.* **2009**, *52*, 225-233; (c) Potashman, M. H.; Duggan, M. E. Covalent Modifiers: An Orthogonal Approach to Drug Design. *J. Med. Chem.* **2009**, *52*, 1231-1246.

51. (a) Parthasarathy, S.; Murthy, M. Protein thermal stability: insights from atomic displacement parameters (B values). *Protein Eng.* **2000**, *13*, 9-13; (b) Yuan, Z.; Zhao, J.; Wang, Z.-X. Flexibility analysis of enzyme active sites by crystallographic temperature factors. *Protein Eng.* **2003**, *16*, 109-114; (c) Radivojac, P.; Obradovic, Z.; Smith, D. K.; Zhu, G.; Vucetic, S.; Brown, C. J.; Lawson, J. D.; Dunker, A. K. Protein flexibility and intrinsic disorder. *Protein Sci.* **2009**, *13*, 71-80.

52. (a) Kozikowski, A. P. *Drug design for neuroscience*. Raven Press New York: 1993; (b) Silverman, R. B. *The organic chemistry of drug design and drug action*. Academic Press: 2004; (c) Wermuth, C. G. *The practice of medicinal chemistry*. Academic Press: 2008.

53. (a) Shuto, S.; Ono, S.; Hase, Y.; Kamiyama, N.; Takada, H.; Yamasihita, K.; Matsuda, A. Conformational restriction by repulsion between adjacent substituents on a cyclopropane ring: Design and enantioselective synthesis of 1-phenyl-2-(1-aminoalkyl)-N,N-diethylcyclopropanecarboxamide as potent NMDA receptor antagonists. *J. Org. Chem.* **1996**, *61*, 915-923; (b) Shuto, S.; Ono, S.; Hase, Y.; Ueno, Y.; Noguchi, T.; Yoshii, K.; Matsuda, A. Synthesis and biological activity of conformationally restricted analogs of milnacipran: (1S,2R)-1-phenyl-2-[(S)-1-aminopropyl]-N,N-diethylcyclopropanecarboxami de, an efficient noncompetitive N-methyl-D-aspartic acid receptor antagonist. *J. Med. Chem.* **1996**, *39*, 4844-4852; (c) Shuto, S.; Ono, S.; Imoto, H.; Yoshii, K.; Matsuda, A. Synthesis and biological activity of conformationally restricted analogues of milnacipran: (1S, 2R)-1-phenyl-2-[(R)-1-amino-2-propynyl]-N,N- diethylcyclopropanecarboxamide is a novel class of NMDA receptor channel blocker. *J. Med. Chem.* **1998**, *41*, 3507-3514; (d) Ono, S.; Ogawa, K.; Yamashita, K.; Yamamoto, T.; Kazuta, Y.; Matsuda, A.; Shuto, S. Conformational analysis of the NMDA receptor antagonist (1S,2R)-1-phenyl-2-[(S)-1-aminopropyl]-N,N-diethylcyclopropanecarboxamide (PPDC) designed by a novel conformational restriction method based on the structural feature of cyclopropane ring. *Chem. Pharm. Bull.* **2002**, *50*, 966-968; (e)

Yamaguchi, K.; Kazuta, Y.; Hirano, K.; Yamada, S.; Matsuda, A.; Shuto, S. Synthesis of 1-arylpiperazyl-2-phenylcyclopropanes designed as antidopaminergic agents: cyclopropane-based conformationally restricted analogs of haloperidol. *Bioorg. Med. Chem.* **2008**, *16*, 8875-8881.

54. Groll, M.; Huber, R.; Potts, B. C. M. Crystal structures of salinosporamide A (NPI-0052) and B (NPI-0047) in complex with the 20S proteasome reveal important consequences of beta-lactone ring opening and a mechanism for irreversible binding. *J. Am. Chem. Soc.* **2006**, *128*, 5136-5141.

55. Groll, M.; McArthur, K. A.; Macherla, V. R.; Manam, R. R.; Potts, B. C. Snapshots of the fluorosalinosporamide/20S complex offer mechanistic insights for fine tuning proteasome inhibition. *J. Med. Chem.* **2009**, *52*, 5420-5428.

56. (a) Nett, M.; Gulder, T. A. M.; Kale, A. J.; Hughes, C. C.; Moore, B. S. Function-oriented biosynthesis of β-lactone proteasome inhibitors in Salinispora tropica. *J. Med. Chem.* **2009**, *52*, 6163-6167; (b) Manam, R. R.; McArthur, K. A.; Chao, T. H.; Weiss, J.; Ali, J. A.; Palombella, V. J.; Groll, M.; Lloyd, G. K.; Palladino, M. A.; Neuteboom, S. T. C. Leaving groups prolong the duration of 20S proteasome inhibition and enhance the potency of salinosporamides. *J. Med. Chem.* **2008**, *51*, 6711-6724.

57. (a) Guimarães, C. MM-GB/SA Rescoring of Docking Poses. *Methods in molecular biology (Clifton, NJ)* **2012**, *819*, 255; (b) Guimarães, C. R. W.; Cardozo, M. MM-GB/SA Rescoring of Docking Poses in Structure-Based Lead Optimization. *J. Chem. Inf. Model.* **2008**, *48*, 958-970; (c) Lyne, P. D.; Lamb, M. L.; Saeh, J. C. Accurate Prediction of the Relative Potencies of Members of a Series of Kinase Inhibitors Using Molecular Docking and MM-GBSA Scoring. *J. Med. Chem.* **2006**, *49*, 4805-4808.

58. Evans, D. A.; Wu, L. D.; Wiener, J. J. M.; Johnson, J. S.; Ripin, D. H. B.; Tedrow, J. S. A General Method for the Synthesis of Enantiomerically Pure β-Substituted, β-Amino Acids through α-Substituted Succinic Acid Derivatives. *J. Org. Chem.* **1999**, *64*, 6411-6417.

59. Yabuuchi, T.; Kusumi, T. Phenylglycine methyl ester, a useful tool for absolute configuration determination of various chiral carboxylic acids. *J. Org. Chem.* **2000**, *65*, 397-404.

60. Kawamura, S.; Unno, Y.; Hirokawa, T.; Asai, A.; Arisawa, M.; Shuto, S. Rational Hopping of a Peptidic Scaffold into Non-Peptidic Scaffolds: Structurally Novel Potent Proteasome Inhibitors Derived from a Natural Product, Belactosin A. *Chem. Commun.* **2014**, *50*, 2445-2447.

61. (a) Borchardt, R.; Kerns, E.; Hageman, M.; Thakker, D.; Stevens, J. *Optimizing the" drug-like" Properties of Leads in Drug Discovery*. Springer: 2006; Vol. 4; (b) Kerns, E.; Di, L. *Drug-like properties: concepts, structure design and methods: from ADME to toxicity optimization*. Academic Press: 2010.

62. (a) Böhm, H.-J.; Flohr, A.; Stahl, M. Scaffold hopping. *Drug Discovery Today: Technologies* **2004**, *1*, 217-224; (b) Schneider, G.; Schneider, P.; Renner, S. Scaffold-hopping: How far can you jump? *Qsar Comb Sci* **2006**, *25*, 1162-1171; (c) Zhao, H. Scaffold selection and scaffold hopping in lead generation: a medicinal chemistry perspective. *Drug Discovery Today* **2007**, *12*, 149-155; (d) Langdon, S. R.; Ertl, P.; Brown, N. Bioisosteric Replacement and Scaffold Hopping in Lead Generation and Optimization. *Mol Inform* **2010**, *29*, 366-385; (e) Sun, H.; Tawa, G.; Wallqvist, A. Classification of scaffold-hopping approaches. *Drug Discovery Today* **2012**, *17*, 310-324.

63. (a) Harvey, A. L. Natural products in drug discovery. *Drug Discovery Today* **2008**, *13*, 894-901; (b) Adessi, C.; Soto, C. Converting a peptide into a drug: strategies to improve stability and bioavailability. *Curr. Med. Chem.* **2002**, *9*, 963-978.

64. Schneider, G.; Neidhart, W.; Giller, T.; Schmid, G. "Scaffold-Hopping" by Topological Pharmacophore Search: A

Contribution to Virtual Screening. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 2894-2896.

65. Blount, M. A.; Beasley, A.; Zoraghi, R.; Sekhar, K. R.; Bessay, E. P.; Francis, S. H.; Corbin, J. D. Binding of tritiated sildenafil, tadalafil, or vardenafil to the phosphodiesterase-5 catalytic site displays potency, specificity, heterogeneity, and cGMP stimulation. *Mol. Pharmacol.* **2004**, *66*, 144-152.

66. Furet, P.; Bold, G.; Hofmann, F.; Manley, P.; Meyer, T.; Altmann, K.-H. Identification of a new chemical class of potent angiogenesis inhibitors based on conformational considerations and database searching. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2967-2971.

67. Rosenström, U.; Sköld, C.; Lindeberg, G.; Botros, M.; Nyberg, F.; Karlén, A.; Hallberg, A. Design, Synthesis, and Incorporation of a β-Turn Mimetic in Angiotensin II Forming Novel Pseudopeptides with Affinity for AT1 and AT2 Receptors. *J. Med. Chem.* **2006**, *49*, 6133-6137.

68. Claude Cohen, N. Medicine pipeline: Structure-Based Drug Design and the Discovery of Aliskiren (Tekturna®): Perseverance and Creativity to Overcome a R&D Pipeline Challenge‡. *Chemical biology & drug design* **2007**, *70*, 557-565.

69. Abad-Zapatero, C.; Metz, J. T. Ligand efficiency indices as guideposts for drug discovery. *Drug Discovery Today* **2005**, *10*, 464-469.

70. (a) Vieth, M.; Siegel, M. G.; Higgs, R. E.; Watson, I. A.; Robertson, D. H.; Savin, K. A.; Durst, G. L.; Hipskind, P. A. Characteristic Physical Properties and Structural Fragments of Marketed Oral Drugs. *J. Med. Chem.* **2003**, *47*, 224-232; (b) Wenlock, M. C.; Austin, R. P.; Barton, P.; Davis, A. M.; Leeson, P. D. A Comparison of Physiochemical Property Profiles of Development and Marketed Oral Drugs. *J. Med. Chem.* **2003**, *46*, 1250-1256; (c) Egan, W. J.; Merz, K. M.; Baldwin, J. J. Prediction of Drug Absorption Using Multivariate Statistics. *J. Med. Chem.* **2000**, *43*, 3867-3877; (d) Ertl, P.; Rohde, B.; Selzer, P. Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties. *J. Med. Chem.* **2000**, *43*, 3714-3717; (e) Palm, K.; Stenberg, P.; Luthman, K.; Artursson1, P. Polar molecular surface properties predict the intestinal absorption of drugs in humans. *Pharm. Res.* **1997**, *14*, 568-571.

71. Kawamura, S.; Unno, Y.; Asai, A.; Arisawa, M.; Shuto, S. Design and Synthesis of the Stabilized Analogs of Belactosin A with the Unnatural cis-Cyclopropane Structure. *Org. Biomol. Chem.* **2013**, *11*, 6615-6622.

72. (a) Nassar, A. E.; Kamel, A. M.; Clarimont, C. Improving the decision-making process in the structural modification of drug candidates: enhancing metabolic stability. *Drug Discovery Today* **2004**, *9*, 1020-1028; (b) Thompson, T. N. Optimization of metabolic stability as a goal of modern drug design. *Med. Res. Rev.* **2001**, *21*, 412-449.

73. (a) Stepan, A. F.; Walker, D. P.; Bauman, J.; Price, D. A.; Baillie, T. A.; Kalgutkar, A. S.; Aleo, M. D. Structural Alert/Reactive Metabolite Concept as Applied in Medicinal Chemistry to Mitigate the Risk of Idiosyncratic Drug Toxicity: A Perspective Based on the Critical Examination of Trends in the Top 200 Drugs Marketed in the United States. *Chem. Res. Toxicol.* **2011**, *24*, 1345-1410; (b) Shu, Y. Z.; Johnson, B. M.; Yang, T. J. Role of biotransformation studies in minimizing metabolism-related liabilities in drug discovery. *The AAPS journal* **2008**, *10*, 178-192; (c) Evans, D. C.; Watt, A. P.; Nicoll-Griffith, D. A.; Baillie, T. A. Drug−Protein Adducts: An Industry Perspective on Minimizing the Potential for Drug Bioactivation in Drug Discovery and Development. *Chem. Res. Toxicol.* **2003**, *17*, 3-16; (d) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. Biotransformation Reactions of Five-Membered Aromatic Heterocyclic Rings. *Chem. Res. Toxicol.* **2002**, *15*, 269-299.

74. Newman, M. S. *Steric effects in organic chemistry*. J. Wiley: 1956.

75. Ho, G.-J.; Mathre, D. J. Lithium-Initiated Imide Formation. A Simple Method for N-Acylation of 2-Oxazolidinones and Bornane-2,10-Sultam. *J. Org. Chem.* **1995**, *60*, 2271-2273.

76. Beckett, R. P.; Crimmin, M. J.; Davis, M. H.; Spavold, Z. A SHORT DIASTEREOSELECTIVE SYNTHESIS OF 2,3-DISUBSTITUTED SUCCINATES. *Synlett* **1993**, 137-138.

77. Evans, D. A.; Britton, T. C.; Ellman, J. A. Contrasteric carboximide hydrolysis with lithium hydroperoxide. *Tetrahedron Lett.* **1987**, *28*, 6141-6144.

78. Barlaam, B.; Bird, T. G.; Lambert-van der Brempt, C.; Campbell, D.; Foster, S. J.; Maciewicz, R. New α-Substituted Succinate-Based Hydroxamic Acids as TNFα Convertase Inhibitors. *J. Med. Chem.* **1999**, *42*, 4890-4908.

79. Frater, G.; Mueller, U.; Guenther, W. The stereoselective α-alkylation of chiral β-hydroxy esters and some applications thereof. *Tetrahedron* **1984**, *40*, 1269-1277.

80. Valls, N.; Borregán, M.; Bonjoch, J. Synthesis of β-chloro α-amino acids: (2S,3R)- and (2S,3S)-3-chloroleucine. *Tetrahedron Lett.* **2006**, *47*, 3701-3705.

81. Doldouras, G. A.; Kollonitsch, J. A direct, selective, and general method for reductive deamination of primary amines. *J. Am. Chem. Soc.* **1978**, *100*, 341-342.

82. Mulzer, J.; Kerkmann, T. α Deprotonation of β-lactones - an example of a forbidden β elimination. *J. Am. Chem. Soc.* **1980**, *102*, 3620-3622.

83. Corey, E. J.; Helal, C. J. Reduction of Carbonyl Compounds with Chiral Oxazaborolidine Catalysts: A New Paradigm for Enantioselective Catalysis and a Powerful New Synthetic Method. *Angew. Chem. Int. Ed.* **1998**, *37*, 1986-2012.

84. (a) Rajashekhar, B.; Kaiser, E. T. Synthesis of enantiomerically pure γ-amino-β-hydroxybutyric acid using malic acid as the chiral precursor. *J. Org. Chem.* **1985**, *50*, 5480-5484; (b) Liesen, G. P.; Sukenik, C. N. Activated anhydrides of tartaric and malic acids. *J. Org. Chem.* **1987**, *52*, 455-457.

85. Groll, M.; Berkers, C. R.; Ploegh, H. L.; Ovaa, H. Crystal structure of the boronic acid-based proteasome inhibitor bortezomib in complex with the yeast 20S proteasome. *Structure* **2006**, *14*, 451-456.

86. (a) Hideshima, T.; Richardson, P.; Chauhan, D.; Palombella, V. J.; Elliott, P. J.; Adams, J.; Anderson, K. C. The Proteasome Inhibitor PS-341 Inhibits Growth, Induces Apoptosis, and Overcomes Drug Resistance in Human Multiple Myeloma Cells. *Cancer Res.* **2001**, *61*, 3071-3076; (b) Zhu, Y.; Zhu, X.; Wu, G.; Ma, Y.; Li, Y.; Zhao, X.; Yuan, Y.; Yang, J.; Yu, S.; Shao, F.; Li, R.; Ke, Y.; Lu, A.; Liu, Z.; Zhang, L. Synthesis, in Vitro and in Vivo Biological Evaluation, Docking Studies, and Structure−Activity Relationship (SAR) Discussion of Dipeptidyl Boronic Acid Proteasome Inhibitors Composed of β-Amino Acids. *J. Med. Chem.* **2010**, *53*, 1990-1999; (c) Zhu, Y.; Wu, G.; Zhu, X.; Ma, Y.; Zhao, X.; Li, Y.; Yuan, Y.; Yang, J.; Yu, S.; Shao, F.; Lei, M. Synthesis, in Vitro and in Vivo Biological Evaluation, and Comprehensive Understanding of Structure−Activity Relationships of Dipeptidyl Boronic Acid Proteasome Inhibitors Constructed from β-Amino Acids. *J. Med. Chem.* **2010**, *53*, 8619-8626; (d) Zhu, Y.; Zhao, X.; Zhu, X.; Wu, G.; Li, Y.; Ma, Y.; Yuan, Y.; Yang, J.; Hu, Y.; Ai, L.; Gao, Q. Design, Synthesis, Biological Evaluation, and Structure−Activity Relationship (SAR) Discussion of Dipeptidyl Boronate Proteasome Inhibitors, Part I: Comprehensive Understanding of the SAR of α-Amino Acid Boronates. *J. Med. Chem.* **2009**, *52*, 4192-4199; (e) Dorsey, B. D.; Iqbal, M.; Chatterjee, S.; Menta, E.; Bernardini, R.; Bernareggi, A.; Cassara, P. G.; D'Arasmo, G.; Ferretti, E.; De Munari, S.; Oliva, A.; Pezzoni, G.; Allievi, C.; Strepponi, I.; Ruggeri, B.; Ator, M. A.; Williams, M.; MallamoT, J. P. Discovery of a potent, selective, and orally active proteasome inhibitor for the treatment of cancer. *J. Med. Chem.* **2008**, *51*, 1068-1072; (f) Adams, J.; Behnke, M.; Chen, S.; Cruickshank, A. A.; Dick, L. R.; Grenier, L.; Klunder, J. M.; Ma, Y.-T.; Plamondon, L.; Stein, R. L. Potent and selective inhibitors of the proteasome: Dipeptidyl boronic acids. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333-338.

87. (a) Verdoes, M.; Florea, B. I.; Menendez-Benito, V.; Maynard, C. J.; Witte, M. D.; van der Linden, W. A.; van den Nieuwendijk, A. M. C. H.; Hofmann, T.; Berkers, C. R.; van Leeuwen, F. W. B.; Groothuis, T. A.; Leeuwenburgh, M. A.;

Ovaa, H.; Neefjes, J. J.; Filippov, D. V.; van der Marel, G. A.; Dantuma, N. P.; Overkleeft, H. S. A Fluorescent Broad-Spectrum Proteasome Inhibitor for Labeling Proteasomes In Vitro and In Vivo. *Chem. Biol.* **2006**, *13*, 1217-1226; (b) Geurink, P. P.; Liu, N.; Spaans, M. P.; Downey, S. L.; van den Nieuwendijk, A. M. C. H.; van der Marel, G. A.; Kisselev, A. F.; Florea, B. I.; Overkleeft, H. S. Incorporation of Fluorinated Phenylalanine Generates Highly Specific Inhibitor of Proteasome's Chymotrypsin-like Sites. *J. Med. Chem.* **2010**, *53*, 2319-2323.

88. Inglis, S. R.; Woon, E. C. Y.; Thompson, A. L.; Schofield, C. J. Observations on the Deprotection of Pinanediol and Pinacol Boronate Esters via Fluorinated Intermediates. *J. Org. Chem.* **2009**, *75*, 468-471.

89. (a) Copeland, R. A.; Pompliano, D. L.; Meek, T. D. Drug–target residence time and its implications for lead optimization. *Nat. Rev. Drug Discovery* **2006**, *5*, 730-739; (b) Lu, H.; Tonge, P. J. Drug-target residence time: critical information for lead optimization. *Curr. Opin. Chem. Biol.* **2010**, *14*, 467-474; (c) Tummino, P. J.; Copeland, R. A. Residence Time of Receptor− Ligand Complexes and Its Effect on Biological Function. *Biochemistry* **2008**, *47*, 5481-5492.

90. Voorhees, P. M.; Orlowski, R. Z., The proteasome and proteasome inhibitors in cancer therapy. In *Annu. Rev. Pharmacol. Toxicol.*, 2006; Vol. 46, pp 189-213.

91. Groll, M.; Kim, K. B.; Kairies, N.; Huber, R.; Crews, C. M. Crystal structure of epoxomicin : 20S proteasome reveals a molecular basis for selectivity of alpha ',beta '-epoxyketone proteasome inhibitors. *J. Am. Chem. Soc.* **2000**, *122*, 1237-1238.

92. Elofsson, M.; Splittgerber, U.; Myung, J.; Mohan, R.; Crews, C. M. Towards subunit-specific proteasome inhibitors: synthesis and evaluation of peptide alpha ',beta '-epoxyketones. *Chem. Biol.* **1999**, *6*, 811-822.

93. Sin, N.; Kim, K. B.; Elofsson, M.; Meng, L.; Auth, H.; Kwok, B. H.; Crews, C. M. Total synthesis of the potent proteasome inhibitor epoxomicin: a useful tool for understanding proteasome biology. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2283-2288.

94. Kisselev, A. F.; Goldberg, A. L., Monitoring Activity and Inhibition of 26S Proteasomes with Fluorogenic Peptide Substrates. In *Methods Enzymol.*, Raymond, J. D., Ed. Academic Press: 2005; Vol. Volume 398, pp 364-378.

95. Groll, M.; Huber, R. Purification, crystallization, and X-ray analysis of the yeast 20S proteasome. *Methods Enzymol.* **2005**, *398*, 329-336.

96. Kabsch, W. Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J. Appl. Cryst.* **1993**, *26*, 795-800.

97. Brunger, A. T. Assessment of phase accuracy by cross validation: the free R value. Methods and applications. *Acta Crystallogr. D Biol. Crystallogr.* **1993**, *49*, 24-36.

98. Turk, D. Improvement of a programm for molecular graphics and manipulation of electron densities and its application for protein structure determination. *Thesis* **1992**, Technische Universitaet Muenchen.

99. Groll, M.; Ditzel, L.; Lowe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. Structure of 20S proteasome from yeast at 2.4 A resolution. *Nature* **1997**, *386*, 463-471.

100. Tabata, S.; Tanaka, M.; Endo, Y.; Obata, T.; Matsuda, A.; Sasaki, T. Antitumor mechanisms of 3′ -ethynyluridine and 3′ -ethynylcytidine as RNA synthesis inhibitors: development and characterization of 3′ -ethynyluridine- resistant cells. *Cancer Lett.* **1997**, *116*, 225-231.

101. Armstrong, A.; Scutt, J. N. Total synthesis of (+)-belactosin A. *Chem. Commun.* **2004**, 510-511.

102. Colson, P.-J.; Hegedus, L. S. Synthesis of .beta.-Lactones by the Photochemical Reactions of Chromium Alkoxycarbene Complexes with Aldehydes. *J. Org. Chem.* **1994**, *59*, 4972-4976.

103. Vignola, N.; List, B. Catalytic Asymmetric Intramolecular α-Alkylation of Aldehydes. *J. Am. Chem. Soc.* **2003**,

126, 450-451.

104. May, A. E.; Willoughby, P. H.; Hoye, T. R. Decarboxylative Isomerization of N-Acyl-2-oxazolidinones to 2-Oxazolines. *J. Org. Chem.* **2008**, *73*, 3292-3294.

105. Stončius, A.; Nahrwold, M.; Sewald, N. Chiral Succinate: A Precursor for Enantiomerically Pure β2-Amino Acids. *Synthesis* **2005**, *2005*, 1829,1837.

106. G. Davies, S.; J. Dixon, D. Asymmetric syntheses of moiramide B and andrimid. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2635-2644.

107. Witte, M. D.; Florea, B. I.; Verdoes, M.; Adeyanju, O.; Van der Marel, G. A.; Overkleeft, H. S. O-GlcNAc Peptide Epoxyketones Are Recognized by Mammalian Proteasomes. *J. Am. Chem. Soc.* **2009**, *131*, 12064-12065.

108. Huber, E. M.; Basler, M.; Schwab, R.; Heinemeyer, W.; Kirk, C. J.; Groettrup, M.; Groll, M. Immuno-and Constitutive Proteasome Crystal Structures Reveal Differences in Substrate and Inhibitor Specificity. *Cell* **2012**, *148*, 727-738.