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博士論文

プラスバシン A<sub>3</sub> の全合成と作用機序解明に向けた研究

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## 略号表

Ac	acetyl
acac	acetylacetonate
Al	allyl
Ala	alanine
<i>aq.</i>	aqueous
Arg	arginine
Asp	aspartic acid
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
Bz	benzoyl
Cbz	benzyloxycarbonyl
CD	circular dichroism
CDI	1,1'-carbonyldiimidazole
Chol	cholesterol
COSY	correlation spectroscopy
Cp	cyclopentadienyl
Cy	cyclohexyl
DBD	4- <i>N,N</i> -(dimethylaminosulfonyl)-7-hydrazino-2,1,3-benzoxadiazole
DEAD	diethyl azodicarboxylate
DET	diethyl tartrate
DMAP	4- <i>N,N</i> -dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	<i>N,N</i> -dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EPC	egg phosphatidylcholine
ESIMS	electrospray ionization mass spectrometry
Et	ethyl
Fib	fibroin

FITC	fluorescein isothiocyanate
Fmoc	9-fluorenylmethyloxycarbonyl
GlcNAc	<i>N</i> -acetylglucosamine
Glu	glucose
Gly	glycine
HBTU	2-(1 <i>H</i> -benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HFIP	1,1,1,3,3,3-hexafluoroisopropanol
HMDS	1,1,1,3,3,3-hexamethyldisilazane
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxy-1,2,3-benzotriazole
HPLC	high performance liquid chromatography
H-bond	hydrogen bond
<sup>i</sup> Pr	isopropyl
IR	infrared spectroscopy
ITC	isothermal titration calorimetry
JU-3CR	Joullié-Ugi three-component reaction
LC	liquid chromatography
MD	molecular dynamics
Me	methyl
MIC	minimum inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
Ms	methanesulfonyl
MS	mass spectrometry
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
MS4A	molecular sieves 4A
Mur	muramic acid
NHS	<i>N</i> -hydroxysuccinimide
NMM	<i>N</i> -methylmorpholine
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
OPM	orientations of proteins in membranes

Orn	ornithine
PBPs	penicillin binding proteins
PDB	protein data bank
PDR	pan-drug-resistant
Pf	phenylfluorenyl
Ph	phenyl
Pht	phthaloyl
PMB	<i>p</i> -methoxybenzyl
ppm	parts per million
Pro	proline
PSA	polar surface area
PyBOP	(benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate
PyAOP	(7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
QCM	quartz crystal microbalance
<i>r.d.s.</i>	rate determining step
rt	room temperature
<i>sat.</i>	saturated
Ser	serine
TFFH	fluoro- <i>N,N,N',N'</i> -tetramethylformamidinium hexafluorophosphate
TBHP	<i>tert</i> -butyl hydroperoxide
<sup>t</sup> Bu	<i>tert</i> -butyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
THF	tetrahydrofran
Thr	threonine
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
Troc	2,2,2-trichloroethyl
T3P	propylphosphonic anhydride

UDP	uridine-5'-diphosphate
UMP	uridine-5'-monophosphate
U-4CR	Ugi four-component reaction
VRE	vancomycin-resistant Enterococci
VRSA	vancomycin-resistant <i>Staphylococcus aureus</i>
XDR	extensively drug resistant

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## 序論

感染症治療の要となる抗菌薬の開発研究は 20 世紀に精力的に行われた。この結果、数多くの抗菌薬が開発されるに至り、一時人類は感染症を完全に制圧したとさえ考えられるようになった。しかし、近年、薬剤耐性菌が出現し、既存の抗菌薬による治療が困難になってきている。Figure 1 は抗菌薬開発とそれに対する薬剤耐性菌の出現の年表である<sup>1</sup>。1950 年に導入されたテトラサイクリンに対する耐性菌はその 9 年後に報告されているが、最近開発されたセフトロリンに対する耐性は 1 年後に生じている。このように、近年では、病原菌が薬剤耐性獲得までに要する時間が短くなっており、新規抗菌薬を開発するにあたり、薬剤耐性を生じにくい性質を有するリードを選択することが重要であると考えられる。

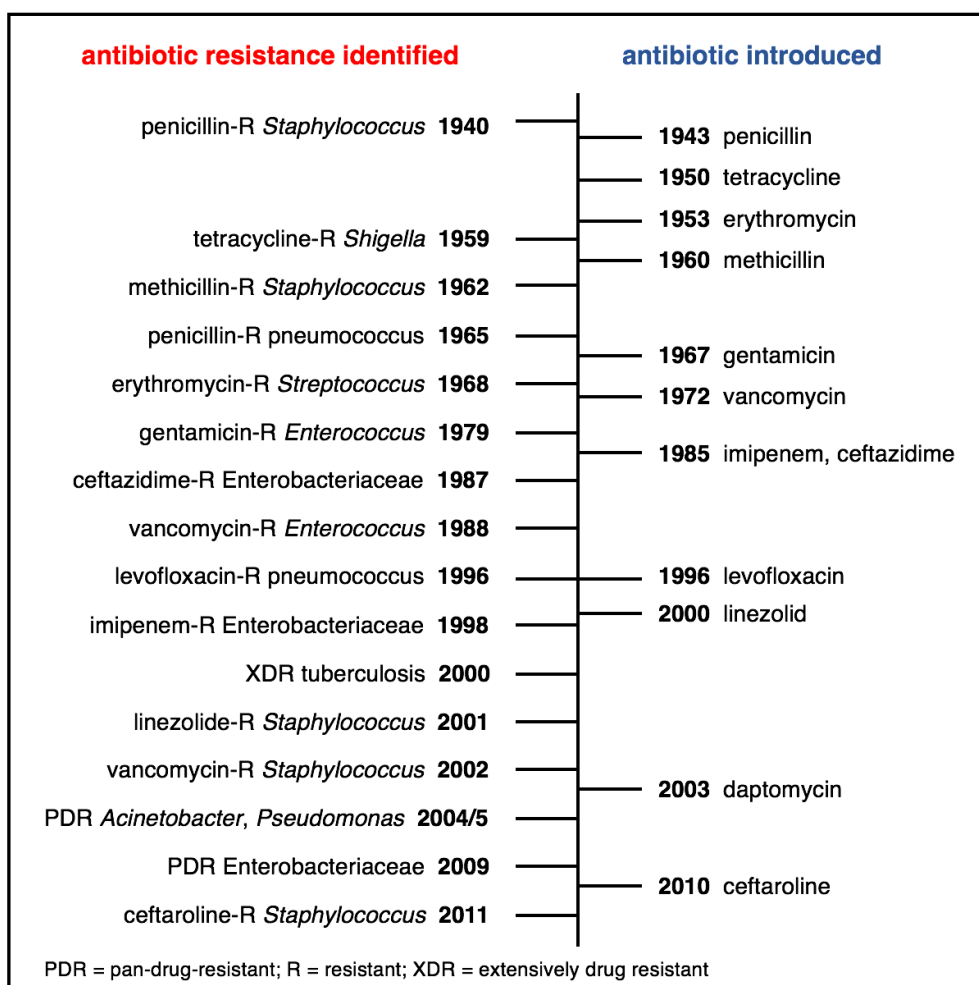


Figure 1. Developing antibacterial resistance

抗菌薬の作用機序として、ペプチドグリカンの生合成を阻害するものが数多く知られている。ペプチドグリカンの生合成経路を Figure 2 に示す。まず、細胞質において、酵素 MurA、MurB により UDP-GlcNAc とホスホエノールピルビン酸から UDP-MurNAc が合成される<sup>2,4</sup>。続いて MurC-F により L-Ala、D-Glu、L-Lys、D-Ala-D-Ala が順次縮合されることで Park nucleotide が合成される<sup>5</sup>。次に、MraY により糖ペプチド部が UMP の脱離を伴いウンデカプレニルリン酸へと転位されることでリポド I が合成される<sup>6,7</sup>。このリポド I は、続く MurG により GlcNAc が付加され、リポド II となる<sup>8</sup>。リポド II は近年見出されたフリッパーゼである MurJ により細胞外側へと移動し<sup>9,10</sup>、これがペニシリン結合タンパク質(PBP)の働きにより重合することでペプチドグリカンが生合成される<sup>11</sup>。この一連の生合成経路の中でも、ペプチドグリカン生合成の最終段階であるリポド II の重合過程は、細胞外で進行するため、化合物の膜透過性を考慮する必要がないことから、抗菌薬の標的として適している。ペニシリン等のβ-ラクタム系抗菌薬は

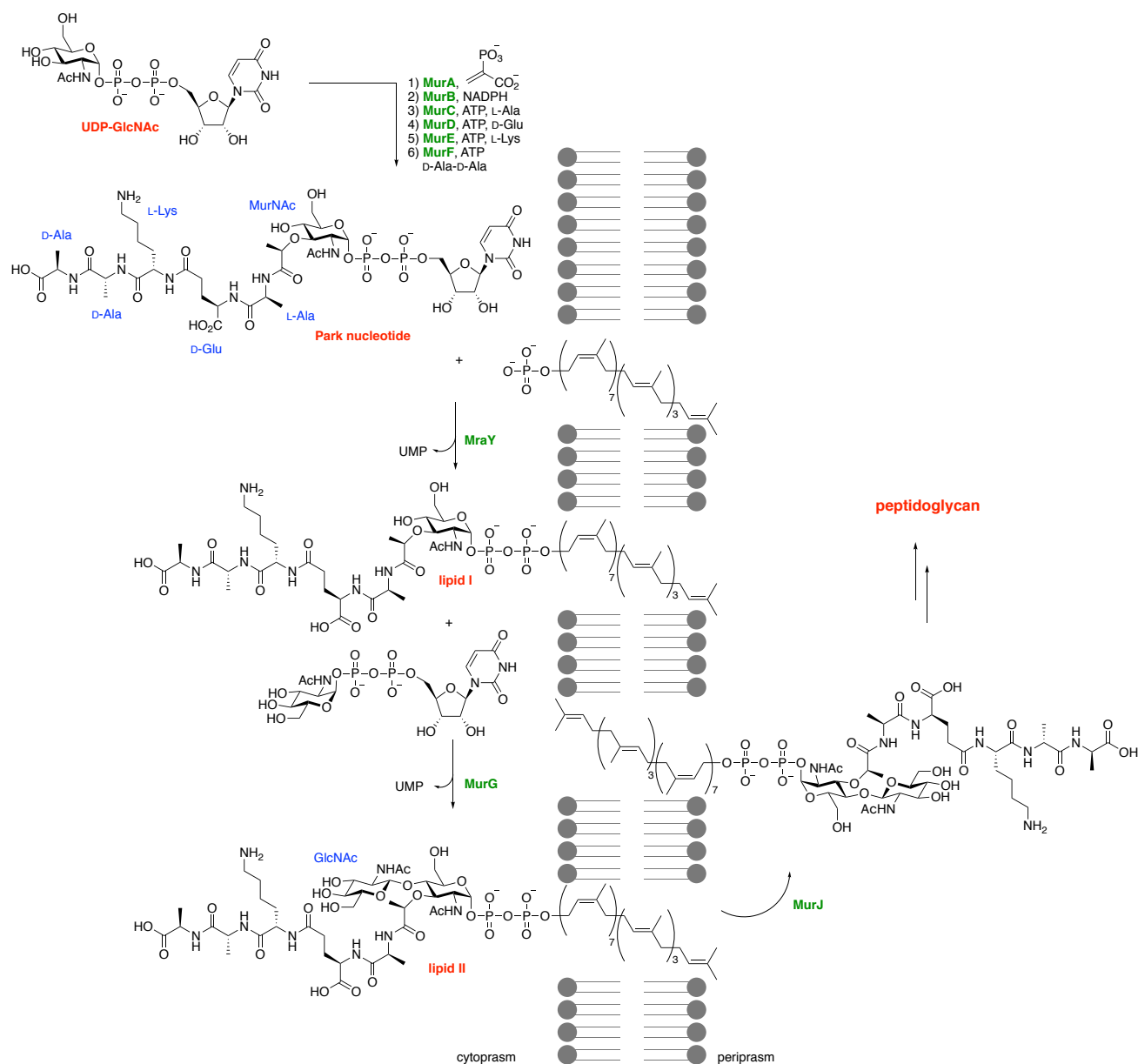
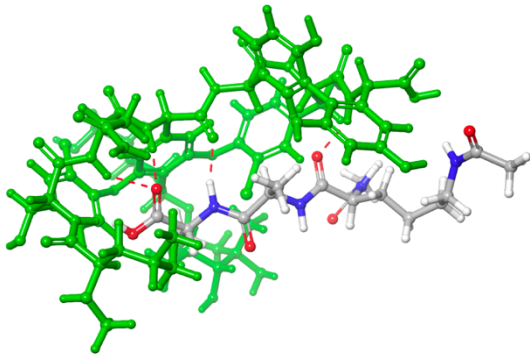


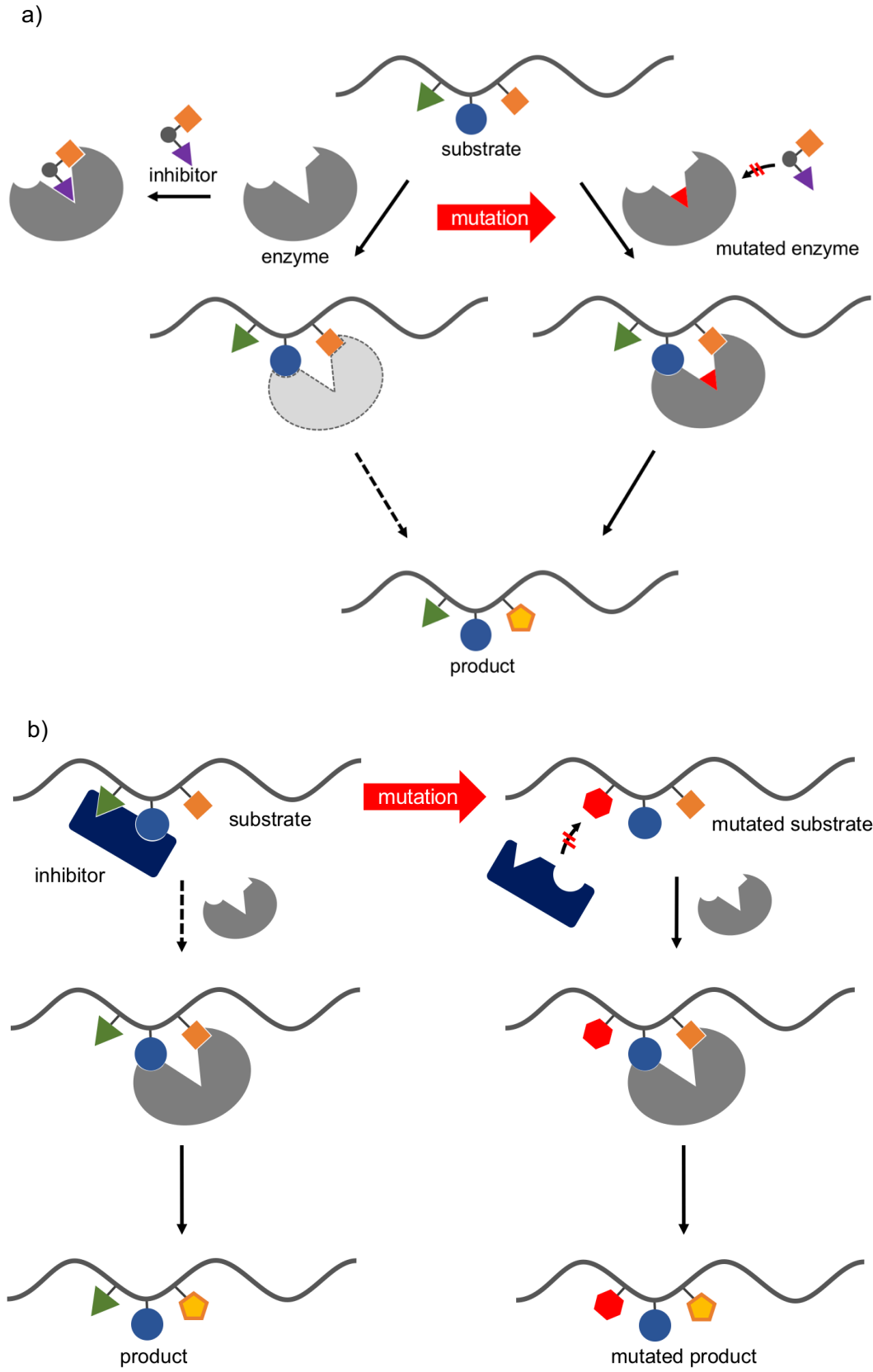
Figure 2. Biosynthesis of peptidoglycan

リポド II の重合を担うペニシリン結合タンパク質(penicillin binding proteins: PBPs)に結合することでリポド II の生合成を阻害するが、これに対しては数多くの耐性菌が報告されている<sup>12</sup>。一方で、現在臨床の場で用いられているバンコマイシンは、酵素の基質となるリポド II の D-Ala-D-Ala 末端に直接結合することでペプチドグリカンへの重合を阻害し<sup>13</sup>、バンコマイシンと Ac-Lys(Ac)-D-Ala-D-Ala ペプチドとの結合に関しては、X 線結晶構造が解かれている(Figure 3, PDB ID: IFVM)<sup>14</sup>。



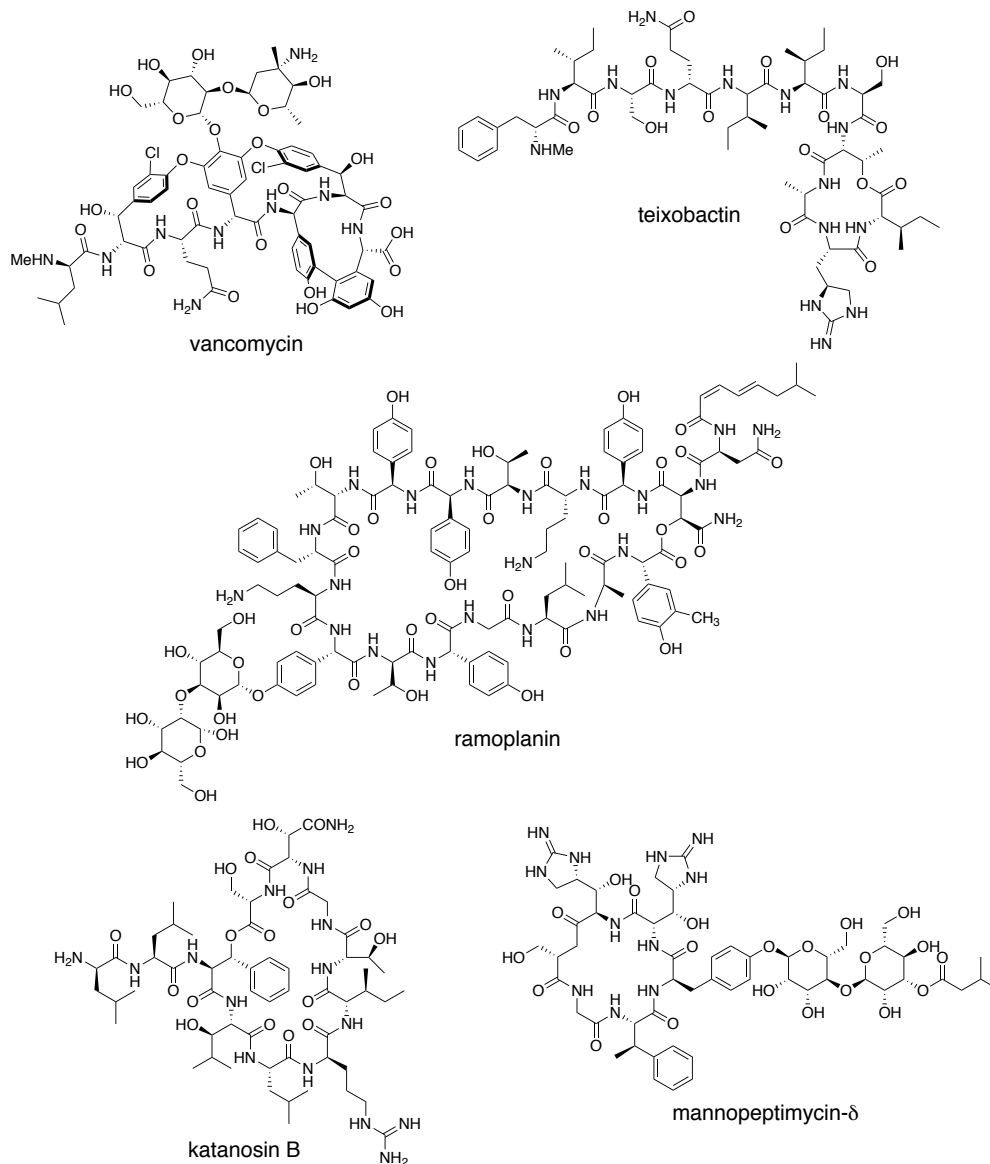
**Figure 3.** X-ray crystal structure of vancomycin-Ac-Lys(Ac)-D-Ala-D-Ala complex (green: vancomycin)

バンコマイシンに対する耐性菌の報告は、その導入から 16 年後であり、他のクラスの抗菌薬と比較して薬剤耐性に高い抵抗性を有する(Figure 1)。薬剤耐性獲得のメカニズムの一つとして、薬剤の標的分子の変異により、薬剤と標的分子の結合親和性が低下するメカニズムが挙げられる。酵素阻害剤に対する耐性の場合、薬剤の結合を阻害するような変異を持つ酵素は、元の酵素と同様に基質を認識することが可能であり、酵素反応の進行、続く変異前と同様の機能を発揮する生成物の産生により、薬剤耐性を獲得に至る(Figure 4a)。一方、バンコマイシンのような基質阻害剤に対する耐性の場合、基質の変異は生成物の変異として引き継がれる(Figure 4b)。この変異生成物が、元の生成物と同等の機能を発揮できなければ、生物は死に至ることとなり、結果的に薬剤耐性を生じない。従って、基質阻害剤は酵素阻害剤と比較して、薬剤耐性を生じにくいものと考えられる。バンコマイシンの例では、バンコマイシンが結合できない変異リポド II から生合成されたペプチドグリカンは、細胞壁としての機能が弱まり、抗菌薬非存在下でさえも生存できないため、薬剤耐性に対して抵抗性を有すると考察できる。

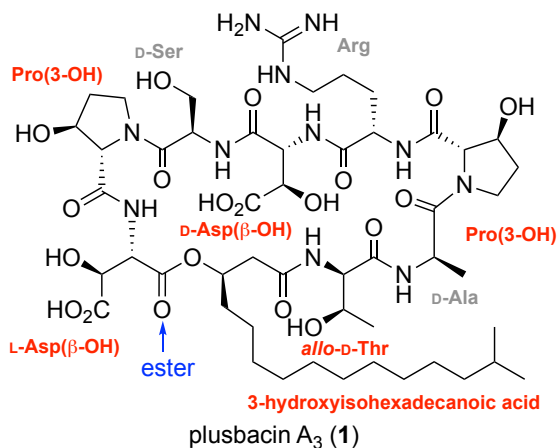


**Figure 4.** Mechanism of drug resistance

リピド II に結合して抗菌作用を示す天然物としては、バンコマイシンの他にテイクソバクチン<sup>15</sup> やラモプラニン<sup>16</sup>、カタノシン B<sup>17, 18</sup>、マンノペプチマイシン- $\delta$ <sup>19, 20</sup> が知られており、特にテイクソバクチンに対しては、in vitro の系において、薬剤耐性菌の出現がほとんど認められないことが報告されている (Figure 5)<sup>15</sup>。以上のように、リピド II に結合する天然物は薬剤耐性が生じにくい性質を有していると考えられ、新規抗菌薬のリード化合物として理想的である。



**Figure 5.** Natural products targeting to lipid II



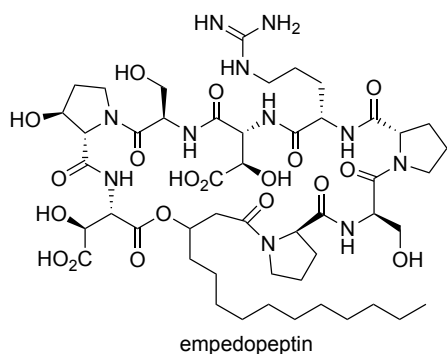
**Figure 6.** Chemical structure of plusbacin A<sub>3</sub>

プラスバシン A<sub>3</sub>(1)は、1992年にグラム陰性菌であるシュードモナス属から単離された28員環ノナデプシペプチドであり、*allo*-D-Thr、*threo*-β-ヒドロキシ-Asp [Asp(β-OH)]の両エナンチオマー、2つの *trans*-3-ヒドロキシ-L-Pro [Pro(3-OH)]の計5個の非天然アミノ酸と3-ヒドロキシイソヘキサデカン酸を含んでいる(Figure 6)<sup>21,22</sup>。本化合物は、広範囲のグラム陽性菌に対し、バンコマイシンと同様にペプチドグリカン生合成を阻害し、抗菌活性を有することが知られている(Table 1)<sup>23</sup>。またバンコマイシン耐性株に対しても同様の阻害作用を有することから、バンコマイシンとは異なる機構でペプチドグリカンの生合成を阻害することが示唆されている。プラスバシン A<sub>3</sub>の抗菌活性は細胞壁成分の添加によって阻害されること、ペプチドグリカンの生合成のみならず、リポド II を含むリポド中間体の生合成も阻害することから、その標的分子は細胞壁生合成酵素ではないと考えられている<sup>23</sup>。また、プラスバシン A<sub>3</sub>類縁体であるエンペドペプチン (Figure 7a)はリポド II に結合することで重合を阻害するが、結合位置はバンコマイシンとは異なり、エンペドペプチンの二箇所のカルボン酸とリポド II のジリン酸が Ca<sup>2+</sup>を介して結合すると考えられている(Figure 7b)<sup>24</sup>。これらの情報より、プラスバシン A<sub>3</sub>の標的分子もリポド II であることが示唆される。

**Table 1.** Antibacterial activity of plusbacin A<sub>3</sub>

Strain	MIC (μg/mL)	
	vancomycin	plusbacin A <sub>3</sub>
<i>S. aureus</i>		
NCTC8325	1.56	0.78
Mu50	12.5	1.56
<i>E. faecalis</i>		
SR1004	1.56	3.13
SR7914	>50	1.56
<i>E. faecium</i>		
SR15941	1.56	3.13
SR7917	>50	3.13

a)

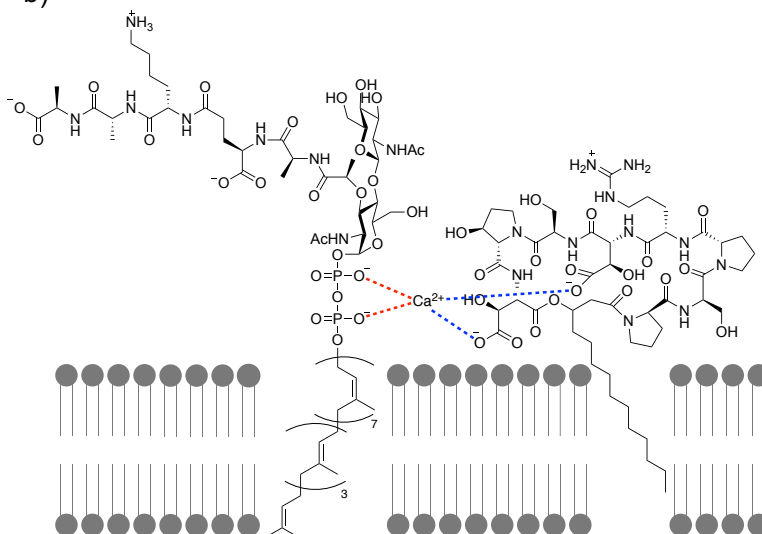


**Figure 7**

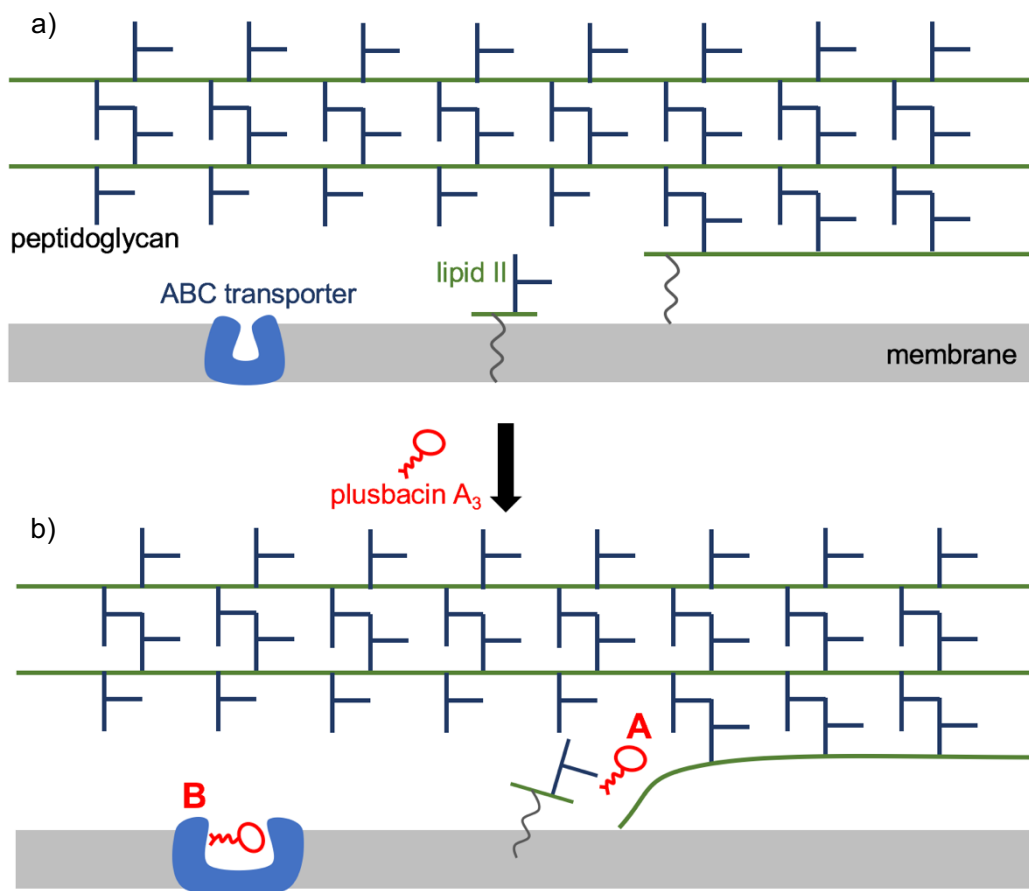
a) Chemical structure of empedopeptin

b) Inhibition mechanism of empedopeptin

b)



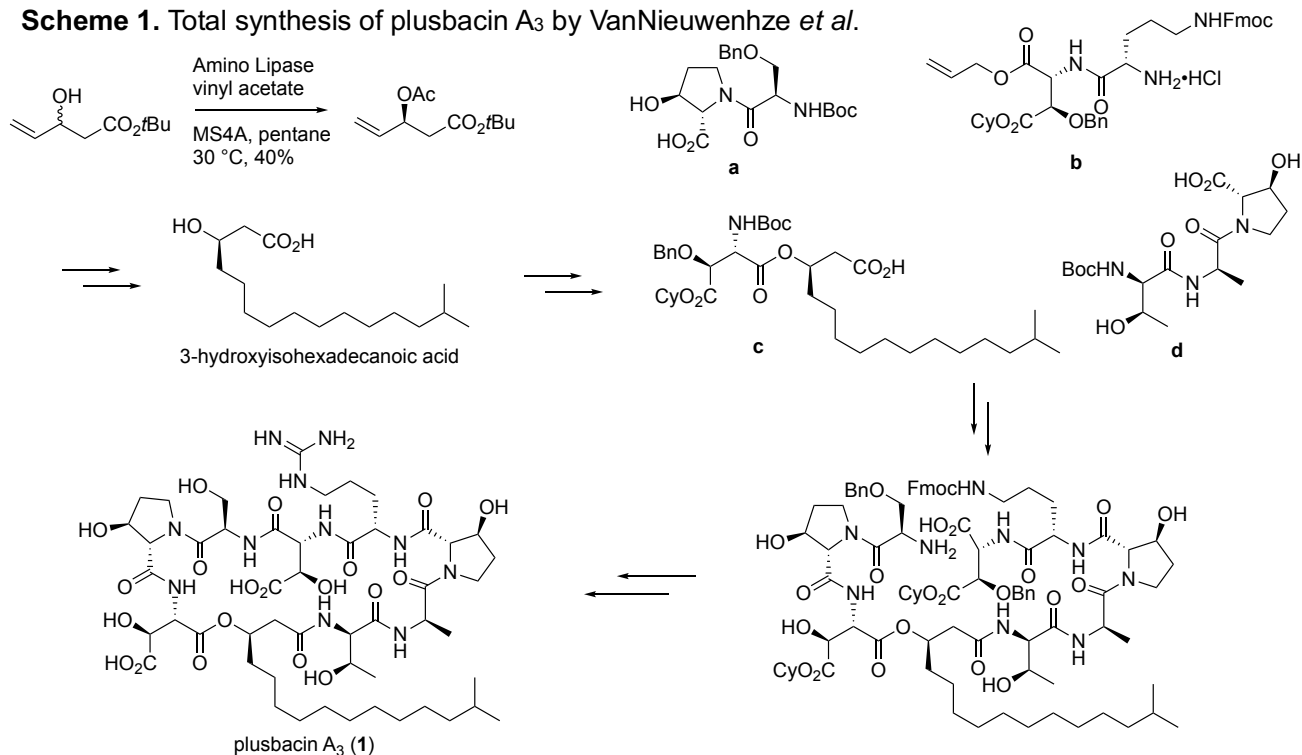
現在問題となっているバンコマイシン耐性菌の耐性メカニズムは、バンコマイシンの結合部位である D-Ala-D-Ala 末端の変異であるが<sup>25</sup>、結合部位の異なるプラスバシン A<sub>3</sub>はこの耐性の影響を受けず、バンコマイシンと同程度の強い抗菌活性を示す。研究開始当初、プラスバシン A<sub>3</sub>の作用機序に関する知見は上述の報告のみであったが、ごく最近、Schaefer らにより固体 NMR を用いたプラスバシン A<sub>3</sub>の作用機序解明に関する報告がなされ、プラスバシン A<sub>3</sub>が2つの作用機序を有する可能性が示唆された(Figure 8)<sup>26</sup>。一つはペプチドグリカンの末端にリピド II が組み込まれる過程を阻害するものであり(Figure 8b, A)、もう一方は細胞膜上の ABC トランスポーターに結合し、その機能を阻害するというものである(Figure 8b, B)。この作用機序はエンペドペプチンでの報告と異なり、プラスバシン A<sub>3</sub>の作用機序に関してはさらなる検討の余地がある。



**Figure 8.** Proposed mechanism of plusbacin A<sub>3</sub> reported by Schaefer *et al.*

筆者はプラスバシン A<sub>3</sub>が、バンコマイシンと同様に薬剤耐性に対して抵抗性を有すると予想し、リピド II に結合するという仮定のもとプラスバシン A<sub>3</sub>の詳細な作用機序の解明を目指し、研究に着手した。

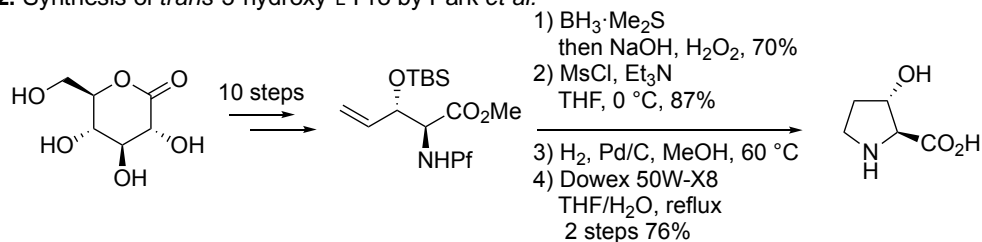
**Scheme 1. Total synthesis of plusbacin A<sub>3</sub> by VanNieuwenhze *et al.***



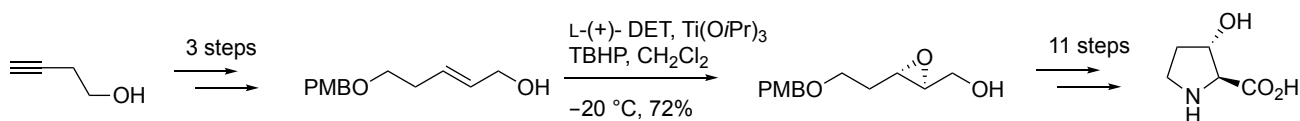
プラスバシン A<sub>3</sub> の全合成は 2007 年に VanNieuwenhze らによって達成されている (Scheme 1)<sup>27</sup>。3-ヒドロキシイソヘキサデカン酸は、ラセミ体のβ-ヒドロキシエステルからアミノリパーゼ PS を用いる速度論的光学分割を経て合成し、ペプチドカップリングによって合成した 4 つのセグメント **a-d** の脱水縮合と D-Ser、D-*threo*-β-ヒドロキシ-Asp 間でのマクロラクタム化により 28 員環を構築することで全合成を達成している。しかし、本合成法において出発物質として用いている *trans*-3-ヒドロキシ-L-Pro の合成は多工程を要し<sup>\*1</sup>、構造活性相関研究を見据えた誘導体合成へと展開するのは困難であるといえる。

<sup>\*1</sup> Park らは D-グルコノラク톤からアリルグリシン誘導体を経由して 14 工程で (Scheme 2)<sup>28</sup>、Chandrasekhar らは 3-ブチン-1-オールから Sharpless 不斉エポキシ化を経て 15 工程で *trans*-3-ヒドロキシ-L-Pro を合成している (Scheme 3)<sup>29</sup>。

**Scheme 2. Synthesis of *trans*-3-hydroxy-L-Pro by Park *et al.***



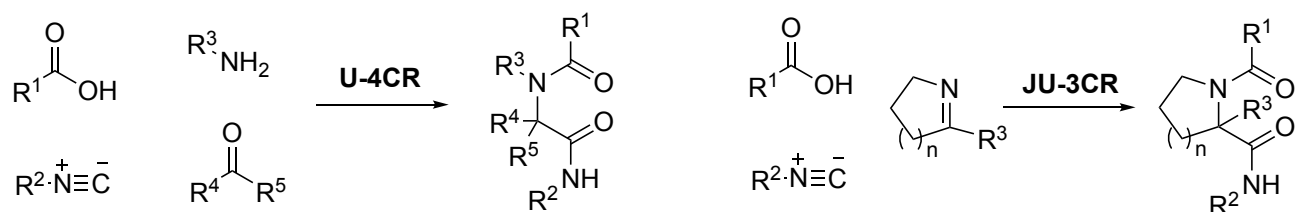
**Scheme 3. Synthesis of *trans*-3-hydroxy-L-Pro by Chandrasekhar *et al.***





筆者は、非天然アミノ酸の合成法として有用である Ugi 反応(U-4CR)<sup>30</sup> 及び Joullié-Ugi 反応(JU-3CR)に着目した<sup>31</sup>。Ugi 反応はカルボン酸、カルボニル化合物、アミン、イソシアニドの 4 成分反応、Joullié-Ugi 反応は Ugi 反応におけるカルボニル化合物とアミンを環状イミンへと変換した 3 成分反応である(Scheme 4)。これらの多成分反応は、基質の種類を変更するのみで多様性に富んだ生成物を容易に与える合成化学上興味深い反応である。

**Scheme 4.** Ugi reaction and Joullié-Ugi reaction



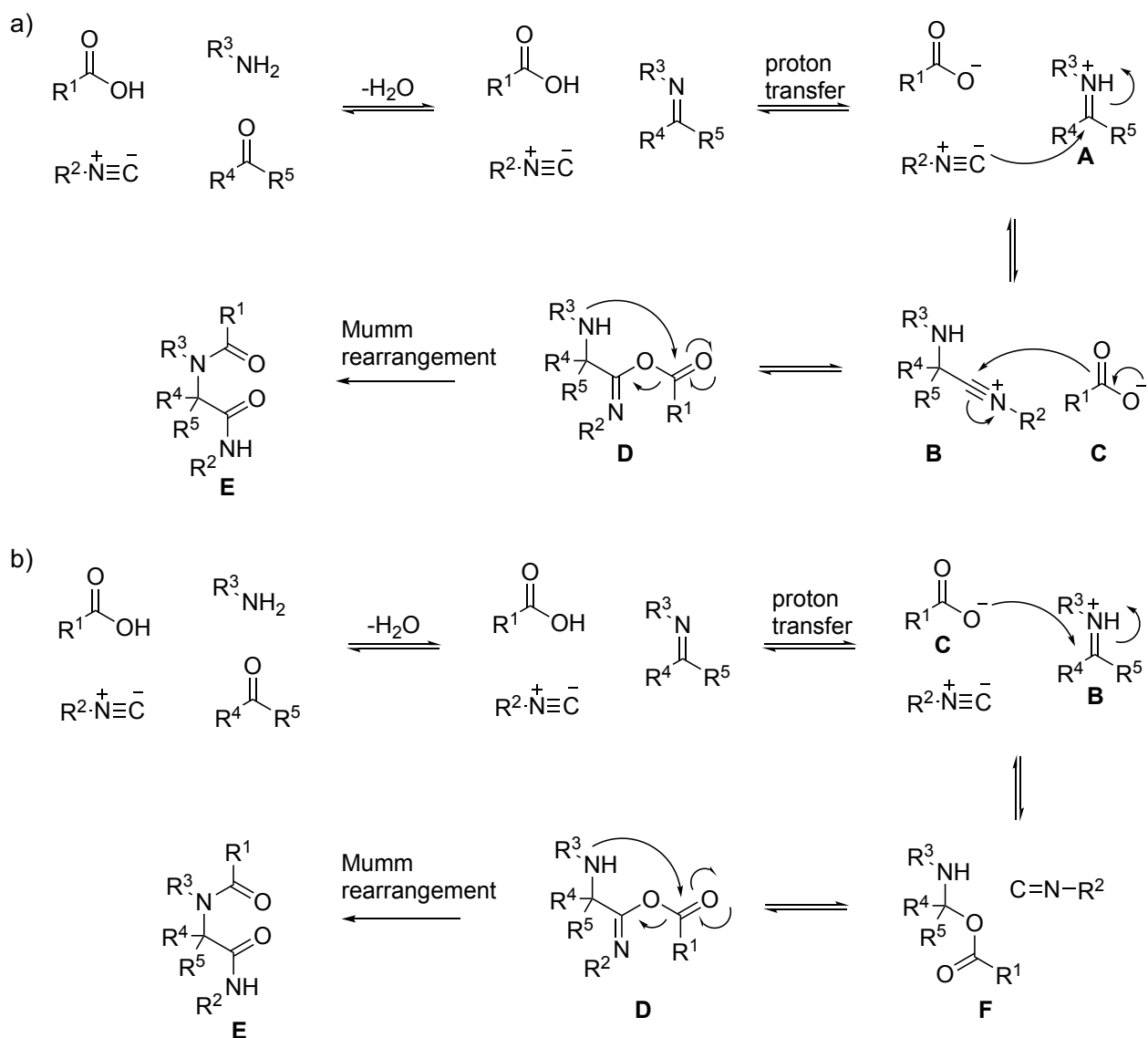
筆者は、プラスバシン A<sub>3</sub> のような中分子ペプチドの合成の際に Joullié-Ugi 反応の利点を最大限利用することを目的として、任意のジアステレオマーを得ることのできるジアステレオ多様な反応への展開を検討した (第一章)。次に、本反応を用いて非天然アミノ酸である Pro(3-OH)を構築することでプラスバシン A<sub>3</sub> の効率的な合成法の開発を行い、プラスバシン A<sub>3</sub> とその誘導体の全合成を行った (第二章)。また、プラスバシン A<sub>3</sub> の詳細な作用機序解明を目的としてリピド II との相互作用を調べ、作用機序に関する考察を行った (第三、四、五章)。以下、その詳細について述べる。

## 本論

### 第一章 ジアステレオ多様の Joullié-Ugi 反応と反応機構解析

不斉炭素を有する化合物の立体選択的な合成は現代有機合成化学の重要なテーマの一つであり、特に不斉触媒を用いたエナンチオ選択的な有機合成化学の進歩は目覚ましいものがある。一方で複数の不斉炭素を有する化合物のジアステレオ選択的な合成は、不斉反応が発展した今日においても未だ難しい課題である。エナンチオ選択的な合成においては、利用する不斉源の立体化学を反転させることで二つのエナンチオマーのうち望みの一方を同様の反応条件下で合成することができる。一方、ジアステレオ選択的な反応については、二つのジアステレオマーのどちらか一方を選択的に得る手法は数多く開発されているが、異なるジアステレオマーを同様の反応条件下で合成することは容易ではない。実際に、不斉炭素を複数有する複雑な化合物の合成においては、望みとしないジアステレオマーからの立体反転や出発物質・合成経路そのものが見直しが散見される。この問題点を解決するべく、最近ではエナンチオ選択的合成と同様に、同じ出発物質から同様の反応条件を利用していずれのジアステレオマーも選択的に合成できる手法が研究されている。このような要件を満たす反応は「ジアステレオ多様な反応」と呼ばれ、出発物質が有する不斉点を利用した合成法その他、不斉触媒を利用したエナンチオ選択的かつジアステレオ多様な反応まで、近年いくつかの報告がなされるようになってきている<sup>32-36</sup>。

JU-3CR は Ugi 反応におけるカルボニル化合物とアミンの代わりに環状イミンを用いる反応である。前者はイミンを出発物質として用い、後者はイミンを反応系中で発生させるという相違点があるものの、両者は共にイミン、カルボン酸、イソシアニド間での反応であるという点で同様の反応機構で進行していると考えるのが妥当である。このように3成分以上の化合物が one-pot で反応するという性質上、これらの反応の反応機構は複雑であり、これまでに Ugi 反応の反応機構については数多くの研究がなされ、長い間議論されてきた。



**Figure 9.** Reaction mechanisms of Ugi reaction proposed by a) Ugi *et al.* (1959) and b) Ugi *et al.* (1997)

Ugi らは 1959 年、イミンとカルボン酸の酸塩基反応により生成したイミニウムイオン **A** に対しイソシアニドが付加し、生じたニトリリウムイオン中間体 **B** に対するカルボキシラートイオン **C** の付加、続くアシルイミデート中間体 **D** からのアシル基の分子内転位(Mumm 転位)により  $\alpha$ -アシルアミノカルボキサミド **E** を与える機構を提唱した(Figure 9a)<sup>37</sup>。また、1997 年に Ugi らはイミニウムイオン **B** に対してカルボキシラートイオン **C** が付加することによって生成するアシルヘミアミナル中間体 **F** からイソシアニドが挿入反応を起こし Mumm 転位前駆体 **D** が生成する機構を提唱した(Figure 9b)<sup>38</sup>。2014 年、Neto らは ESI-MS/MS 測定におけるセグメントイオンを根拠として後者の機構を否定しており<sup>39</sup>、現在では前者の機構が広く受け入れられている。

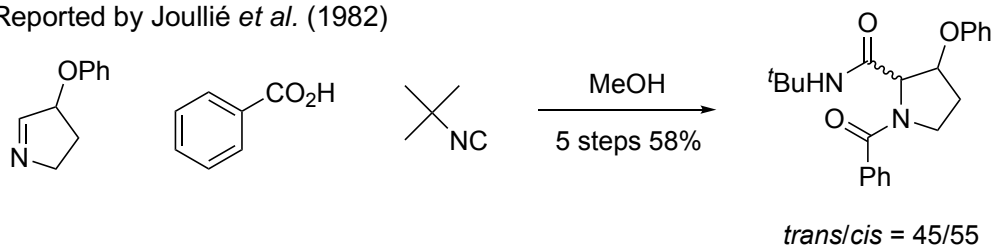
この反応機構において、新たに生じる不斉点の制御に重要となる本反応の律速段階については、Ugi らによって Mumm 転位であると提唱され、そこに至るまでの個々の反応は全て平衡反応であると考えられ

てきた<sup>37</sup>。しかし、近年この仮説を覆すいくつかの研究事例が報告されている。Lessard ら Codée らは、計算化学的手法により本反応の律速段階がイソシアニド付加の段階であり、この段階が不可逆であることを提唱している<sup>40, 41</sup>。また、Furman らは、酸素官能基を有する 6 員環または 5 員環イミンを用いた JU-3CR の立体選択性が、Woerpel らによって提唱されたオキソカルベニウムイオンに対するアリル化反応における立体電子効果<sup>42</sup>と同様に、イソシアニドの付加の段階における立体電子効果で説明できると述べている<sup>43</sup>。このように Ugi 反応、JU-3CR の反応機構については発見から 50 年以上経った現在でも議論がなされており、ここから本反応の反応機構の複雑さを伺い知ることができる。

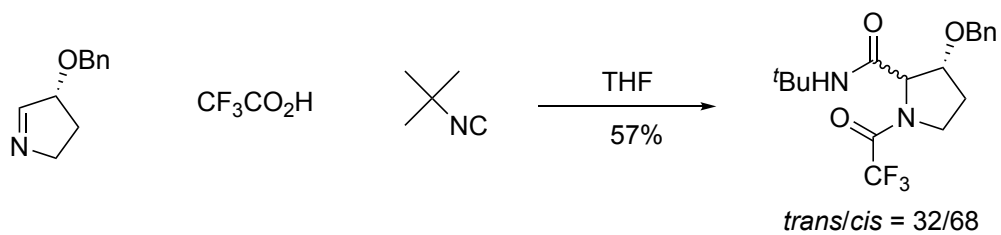
筆者はまず、 $\alpha$ 位に酸素置換基を有する一置換光学活性 5 員環イミンを用いた JU-3CR のジアステレオ選択性に関して検討することを計画した。この反応を用いることで、プラスバシン A<sub>3</sub> の構成要素となっている非天然アミノ酸である 3-hydroxy-Pro を一段階で構築することができる。これまでの報告例では、 $\alpha$ 位にフェノキシ基またはベンジルオキシ基が置換した 5 員環イミンを用いた場合、*cis* 体がやや優先的に生成するものの、立体選択性の発現は困難であることが報告されている(Scheme 5a, b)<sup>31, 43</sup>。一方で、5 員環イミンの $\beta$ 位にベンジルオキシ基が置換した場合、同様に *cis* 体が優先するものの、立体選択性が向上することがわかっている(Scheme 5c)<sup>43</sup>。本章では、JU-3CR において同一の出発物質から両ジアステレオマーをそれぞれ選択的に得る条件を見出したため、これに関して述べた後に、立体選択性の制御に重要となる本反応の律速段階を明らかにし、本反応の反応機構に関する詳細な知見を得たため、これについて詳述する。

**Scheme 5.** Joullié-Ugi reaction of five-membered cyclic imine

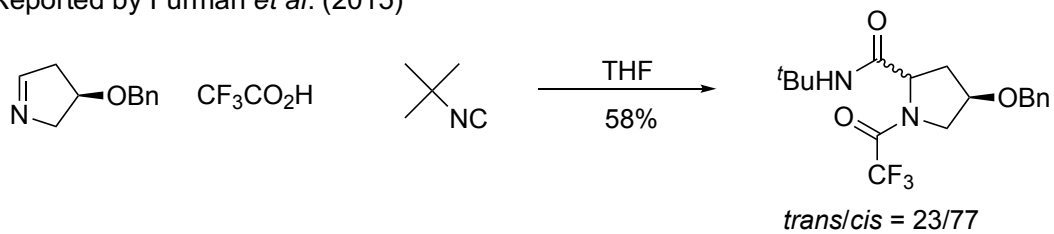
a) Reported by Joullié *et al.* (1982)



b) Reported by Furman *et al.* (2015)



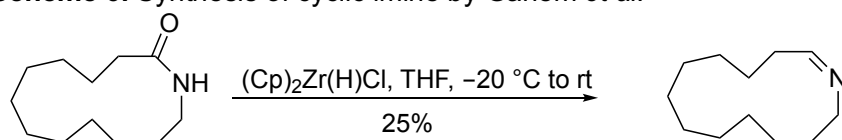
c) Reported by Furman *et al.* (2015)



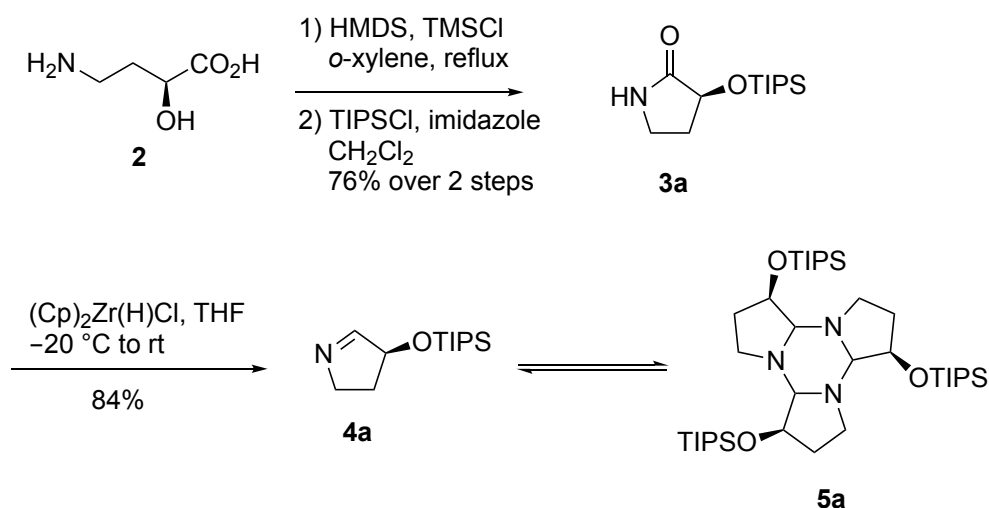
## 第一節 Joullié-Ugi 反応の立体選択性と溶媒効果

先に述べたように、 $\alpha$ 位にフェノキシ基またはベンジルオキシ基が置換した一置換5員環イミンを用いたJU-3CRにおいては、生成物のジアステレオ選択性が低い。筆者は、立体障害の大きいシロキシ基を組み込んだイミンを用いることで立体選択性が向上するものと考え、シリル基の中でも嵩高いTIPS基で置換したイミン **4a** を用いた反応を検討することにした。5員環イミン **4a** は、Schwartz 試薬を用いた Ganem らの方法 (Scheme 6)<sup>44</sup> を参考に、ラクタム **3a** から合成することにした。

**Scheme 6.** Synthesis of cyclic imine by Ganem *et al.*

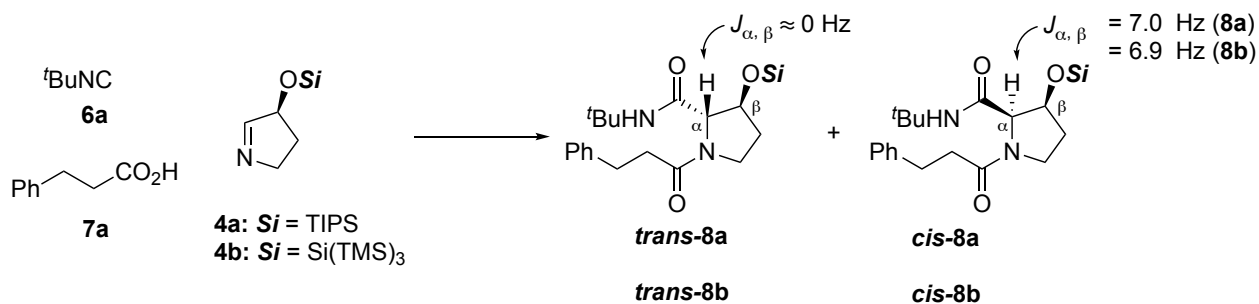


**Scheme 7.** Synthesis of cyclic imine **4a**



市販の $\gamma$ -アミノ酸 **2** を HMDS、*o*-キシレン中で還流することでラクタム化し<sup>45</sup>、水酸基のシリル化を行い TIPS 体 **3a** とした。このラクタムを Schwartz 試薬により還元したところ、良好な収率で5員環イミン **4a** を得た(Scheme 7)。なおイミン **4a** は溶液中で三量体 **5a** との平衡混合物として存在した。望みのイミンを合成できたことから、続いて JU-3CR のジアステレオ選択性に関して検討を行った(Table 2)。

**Table 2.** Optimization of reaction conditions



entry	solvent	temp. (°C)	imine	trans/cis	yield (%)
1	toluene	rt	<b>4a</b>	15/85	63
2	CH <sub>2</sub> Cl <sub>2</sub>	rt	<b>4a</b>	17/83	54
3	MeOH	rt	<b>4a</b>	42/58	57
4	<i>i</i> PrOH	rt	<b>4a</b>	20/80	57
5	TFE	rt	<b>4a</b>	72/28	62
6	HFIP	rt	<b>4a</b>	82/18	60
7	THF	rt	<b>4a</b>	45/55	20
8	MeCN	rt	<b>4a</b>	36/64	47
9	toluene	-78	<b>4a</b>	15/85	45
10	toluene	70	<b>4a</b>	20/80	45
11	HFIP	0	<b>4a</b>	85/15	54
12	HFIP	reflux	<b>4a</b>	71/29	50
13	toluene	rt	<b>4b</b>	27/73	42
14	HFIP	rt	<b>4b</b>	>99/1	52

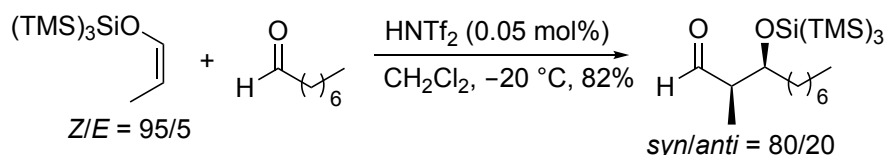
まずモデル基質として 3-フェニルプロピオン酸 (**7a**)、*t*-ブチルイソシアニド (**6a**)を用い、反応条件の検討を行った。カルボン酸 **7a**、5員環イミン **4a**、イソシアニド **6a** それぞれ 1 等量を 0.1 M の濃度でトルエン中、室温下で反応させたところ、Joullié-Ugi 成績体 **trans-8a** と **cis-8b** が、合計収率 63%で得られた (Table 2, entry 1)。両ジアステレオマーはハイフラッシュシリカゲルカラムクロマトグラフィーにより容易に分離可能であり、ジアステレオ比は *trans/cis* = 15/85 であった。なお、各ジアステレオマーの立体配置の決定は Joullié らの報告を参考に、<sup>1</sup>H NMR スペクトルにおける $\alpha$ プロトンの多重度 (*trans* 体は一重線、*cis* 体は二重線) により決定した<sup>31</sup>。次に反応溶媒の検討を行った。トルエンと同様の非極性溶媒である CH<sub>2</sub>Cl<sub>2</sub> を用いた場合には *cis* 体を優先して与えた(entry 2)。一方、プロトン性極性溶媒である *i*PrOH、MeOH、トリフルオロエタノール(TFE)、ヘキサフルオロイソプロパノール(HFIP)を用いた場合にはジアステレオ選択性の逆転がみられ、HFIP を用いた場合は *trans/cis* = 82/18 で *trans* 体を優先して与えた(entries 3-6)。また、非プロトン性極性溶媒である THF やアセトニトリルをもちいた際は選択性、収率ともに低

下した(entries 7, 8)。次にトルエン、または HFIP を溶媒として反応温度の検討を行った(entries 9-12)。いずれの溶媒においても、反応温度を下げてでもジアステレオ選択性に変化が見られないが、高温で反応を行うとジアステレオ選択性が低下することがわかった。以上の結果より、本反応の最適温度は室温とし、次に $\alpha$ -シロキシ基の立体障害の増大によりジアステレオ選択性の向上を狙うこととした。TIPS 基よりも立体障害の大きいシリル基としてトリス(トリメチルシリル)シリル基(以下スーパーシリル基)<sup>\*2</sup>を選択し、イミン **4b**<sup>\*3</sup>を用いてトルエンまたは HFIP を溶媒として反応を検討した(entries 13, 14)。トルエン中ではジアステレオ選択性(*trans/cis* = 27/73)、収率ともに低下したことから、本反応系において、 $\alpha$ シロキシ基の立体障害は *cis* 体の生成には不利であることが示唆された。一方 HFIP 中では若干の収率の低下が見られたものの完全な立体選択性で *trans* 体が得られた。

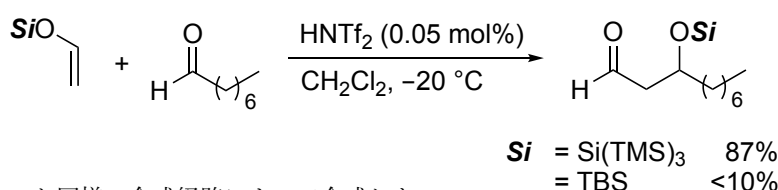
以上の検討結果から、*trans* 体を選択的に得るためには、スーパーシリル基で保護したイミン **4b** を用いて、HFIP 中で反応を行い、*cis* 体を選択的に得るには TIPS 基で保護したイミン **4a** を用いて、トルエン中で反応を行えばよいことが明らかとなった。しかし、同一の出発物質から任意のジアステレオマーを合成する反応という点と収率に着目するのであれば、TIPS 基で保護した $\alpha$ -ヒドロキシイミン **4a** を用いるのが最適であると判断し、次に基質適応範囲の検討を行うこととした。

<sup>\*2</sup>スーパーシリル基が置換した化合物は1964年にSmithらによってはじめて合成された<sup>46</sup>。その後1994年にApeloigらによってその立体的かさ高さが*t*-Bu基と同程度であることが報告された<sup>47</sup>。これはSi-Si結合距離が3.0 Å程度と通常の有機化合物で見られる結合の1.5~2倍であるため中心ケイ素原子付近での立体障害が減少しているためである。2006年、山本らは、スーパーシリル基が置換したシリルエノールエーテルを利用した*syn*選択的アルドール反応を報告している(Scheme 8)<sup>48</sup>。この反応においてスーパーシリル基はその立体障害により選択性を向上させるとともに、生成物において、長いSi-Si結合が遠隔位に存在するカルボニル基を求核付加から守ることで、副反応である多重アルドール反応を防ぐ役割を果たしている(Scheme 9)。すなわち、中心付近は比較的立体障害が小さいのに対し、離れた位置では強力な立体障害を生じるといふ傘のような構造がスーパーシリル基の特徴とみなすことができる。

**Scheme 8.** Aldol reaction by Yamomoto *et. al.*



**Scheme 9.** Effect of supersilyl group for aldol reaction



<sup>\*3</sup>イミン **4b** は **4a** と同様の合成経路によって合成した。



## 第二節 基質適応範囲の検討

続いてシロキシイミン **4a** を用いた JU-3CR におけるカルボン酸について基質適応範囲の検討を行った (Table 3)。トルエン中においてはイソ酪酸(**7b**)を用いると収率が低下し、ピバル酸(**7c**)を用いた場合には反応が進行せず、原料回収となった\*<sup>4</sup>。また、芳香族カルボン酸 **7d** では問題なく反応が進行するものの、 $\alpha, \beta$ -不飽和カルボン酸 **7e** では収率が低下した。 $\alpha$ -アミノ酸もカルボン酸として用いることができ、Val 保護体 **7f** を用いた場合でも選択性と収率の低下は見られなかった。

**Table 3.** Scope of carboxylic acids

R	product	solvent = <b>toluene</b>		solvent = <b>HFIP</b>	
		<i>trans/cis</i>	yield (%)	<i>trans/cis</i>	yield (%)
	<b>7a</b>	15/85	63	82/18	60
	<b>9b</b>	10/90	12	88/12	71
	<b>9c</b>	-	-	85/15	77
	<b>9d</b>	12/88	75	86/14	87
	<b>9e</b>	10/90	36	85/15	90
	<b>9f</b>	11/89	75	71/29	79

\*<sup>4</sup> カルボン酸 $\alpha$ 位のかさ高さに伴う収率の低下は、カルボン酸の立体障害の増大か酸性度の低下によるものと考えられるが、そのどちらが原因であるのついてはこの結果から断定することはできない。

一方で、HFIP 中では **7a-f** のいずれのカルボン酸を用いた場合でも立体選択性を損なうことなく良好な収率で目的化合物を得ることができた。これは酸性度の高いアルコールである HFIP ( $pK_a = 9.3$ ) がイミンの活性化に関与し、イミニウムイオンの反応性が向上したためと考えられる。なお **7c** を用いた際の生成物である **9c** は  $^1\text{H NMR}$  スペクトルにおいてブロードなピークを与え、 $\alpha$ プロトンの多重度による立体決定が行えなかったため、以下に述べるような立体化学の推定を行った。

**9c** 以外のいずれの場合にも、*trans* 体がより低極性の化合物であり、ここから **9c** においても低極性の化合物が *trans* 体であると予想される。また、構造が類似している **9b** の  $^1\text{H NMR}$  スペクトルでは両ジアステレオマー間で明確な違いが見られる (Figure 10)。3-Hydroxy-Pro 部  $\delta$  位の二つのプロトンが *trans* 体では 3.8 ppm 付近に重なって観測されるのに対し、*cis* 体では 3.8 ppm 付近と 3.6 ppm に分かれて観測される。さらに 3-hydroxy-Pro 部  $\gamma$  位のプロトンは *trans* 体では 2.2 ppm 付近と 2.0 ppm 付近に、*cis* 体では 2.3 ppm、2.1 ppm 付近に観測される。**9c** はどちらのジアステレオマーも  $^1\text{H NMR}$  スペクトルにおいて **9b** と類似の化学シフト値を与えることから、低極性化合物が *trans* 体であることが示唆される。さらに  $^t\text{Bu}$  基が置換した窒素原子上の水素原子に対する差 NOE 測定の結果、*trans* 体と推定されるジアステレオマーでは  $\beta$ プロトンとの間にはほとんど相関が見られなかったのに対し、*cis* 体と推定されるジアステレオマーでは  $\beta$ プロトンとの間に相関が見られた (Figure 11)。Macromodel を用いた分子力学計算により得られた最安定配座における NH- $\beta$ 水素間の距離は *trans* 体で 4.40 Å、*cis* 体で 3.86 Å であることから、差 NOE 測定からも上記の推測を支持する結果が得られた (Figure 12)。

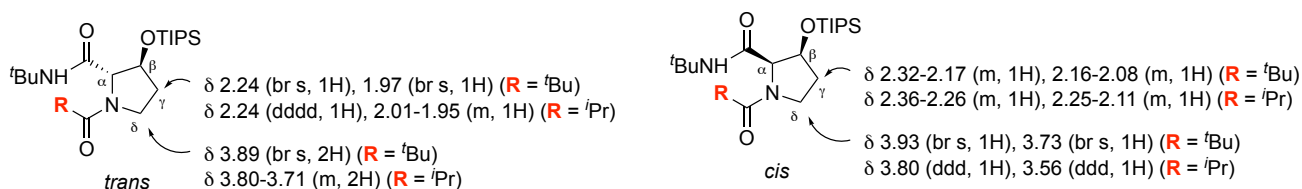


Figure 10. Comparison of  $^1\text{H NMR}$  spectra between **9b** and **9c**

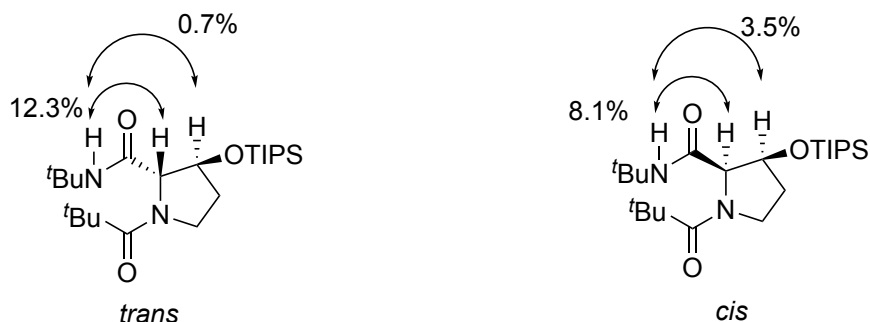
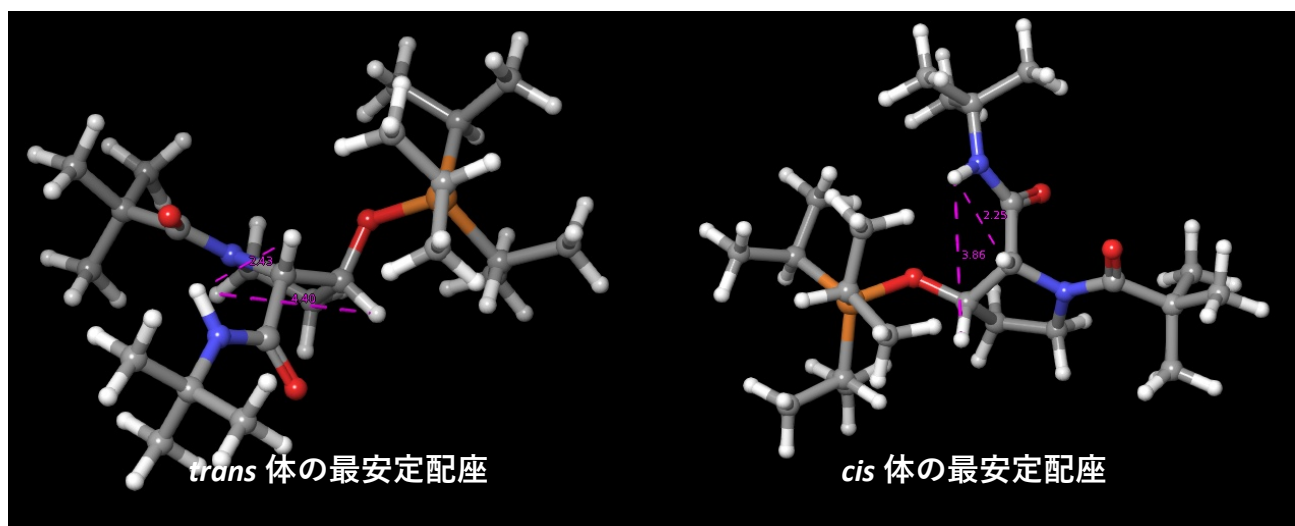


Figure 11. Results of NOE experiment of **9c**



**Figure 12.** Energy-minimized conformation of each diastereomer of **9c** calculated by MacroModel ver 10.9

以上の検討結果より、本反応はトルエン中では立体障害の大きいカルボン酸を用いると反応が進行しないか、収率が低下するという制約があるものの、*cis* 選択的、*trans* 選択的条件いずれにおいても立体選択性に対する顕著な影響は見られないことが明らかとなった。

**Table 4.** Scope of isocyanides

R	product	solvent = <b>toluene</b>		solvent = <b>HFIP</b>	
		<i>trans/cis</i>	yield (%)	<i>trans/cis</i>	yield (%)
	<b>10a</b>	15/85	63	82/18	60
	<b>10b</b>	17/83	42	74/26	81
	<b>10c</b>	11/89	72	46/54	93

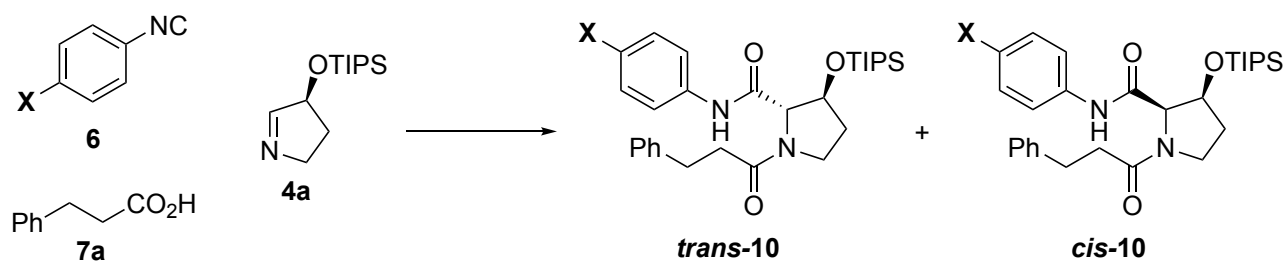
次にイソシアニドについて基質適応範囲の検討を行った(Table 4)。トルエン中では第三級イソシアニド **6a**、第一級イソシアニド **6b**、芳香族イソシアニド **6c** いずれを用いた場合でも立体選択性の大きな変化は見られず *cis* 体を選択的に与え、その収率は第一級イソシアニド **6b** を用いた場合には低下し、芳香族イソシアニド **6c** を用いた場合では向上した。

一方で HFIP 中においては、Table 2、entry 6 と比較すると、カルボン酸の検討の際とは異なりイソシアニドの構造により立体選択性に大きな変化が見られた。第一級イソシアニド **6b**、芳香族イソシアニド **6c** ともに良好な収率で目的化合物を与えるものの、第一級イソシアニド **6b** ではジアステレオ選択性が低下し(*trans/cis* = 74/26)、芳香族イソシアニド **6c** ではジアステレオ選択性はほとんど発現せず *cis* 体が若干優先する結果となった(*trans/cis* = 46/54)。このような立体選択性の変化の要因として立体効果と電子効果が考えられる。まず立体効果に着目すると、一般に  $sp^2$  炭素原子に対する付加反応は、速度論的にも熱力学的にも、立体障害の少ない面から反応した生成物が得られるため、本反応の様なイミンに対する付加反応も同様に考えることができる。第三級イソシアニド **6a**、第一級イソシアニド **6b** を比較するとより立体障害の大きい **6a** において、より高い面選択性が得られたことを説明することができる。しかし、平面性の高い芳香族イソシアニド **6c** とは明確な比較が難しい。次に電子効果に着目すると第三級  $sp^3$  炭素原子、第一級  $sp^3$  炭素原子、 $sp^2$  炭素原子の順に電子不足となっており、より電子豊富なイソシアニドが *trans* 体を優先して与えたと考えられる。そこで立体選択性に関してさらなる知見を得ることを目的としてこのような立体効果を除外し、電子効果のみを検証する反応系で立体選択性を調べることにした。

### 第三節 ジアステレオ選択性に対する電子効果と速度論的解析

JU-3CR の立体選択性に対するイソシアニドの電子効果を調べるために、フェニルイソシアニド **6c** の *p* 位に種々の置換基 **X** を持つイソシアニド **6d–6g** を用いて反応を行った (Table 5)。ジアステレオ選択性にイソシアニドの電子効果が影響するのであれば、置換基 **X** の電子供与性・求引性によりジアステレオ選択性が変化することが予想される。

**Table 5.** Effect of electron density of isocyanide on diastereoselectivity



isocyanide	X	product	solvent = toluene		solvent = HFIP	
			<i>trans/cis</i>	yield (%)	<i>trans/cis</i>	yield (%)
<b>6d</b>	OMe	<b>10d</b>	11/89	77	60/40	88
<b>6e</b>	Me	<b>10e</b>	10/90	81	53/47	94
<b>6c</b>	H	<b>10c</b>	11/89	72	46/54	93
<b>6f</b>	Cl	<b>10f</b>	10/90	67	38/62	93
<b>6g</b>	NO <sub>2</sub>	<b>10g</b>	-	-	17/83	75

検討の結果、トルエン中での反応の場合、これまでの検討結果と同様にいずれのイソシアニドを用いた場合でも約 90:10 のジアステレオ選択性で *cis* 体を優先して与えることが明らかになり、イソシアニドの電子効果はジアステレオ選択性に影響しないことがわかった。また、電子求引性基の影響によりイソシアニドの求核性が低下すると収率が低下する傾向が見られ、最も電子求引性が大きいニトロ基を有する **6g** を用いた場合には反応が進行しなかった。

次に HFIP 中での検討を行った。その結果、予想通り置換基 **X** の電子求引性・供与性とジアステレオ選択性の間には相関が見られることがわかった。すなわち、メトキシ基、メチル基、水素原子、クロル基、ニトロ基と電子供与性が低下していく順 (電子求引性が増大する順) で *trans/cis* 比が 60/40、53/47、46/54、38/62、17/83 と順に変化していき、最も電子供与性の大きいメトキシ基の場合に *trans* 体が、最も電子求引性の大きいニトロ基の場合に *cis* 体がより選択的に得られることが明らかになった。

以上の検討結果から、HFIP 中においては、本反応のジアステレオ選択性は溶媒効果とイソシアニドの電子効果によって大きく影響を受け、イソシアニドの立体効果やカルボン酸の構造はジアステレオ選択性にほとんど影響を与えないことがわかった。そこで次に、イソシアニドの電子効果と反応速度の関係を物理化学的に解析することで本反応の反応機構と律速段階について考察し、ジアステレオ選択性が溶媒によって変化する要因を明らかにすることとした。

反応における電子効果を詳細に解析するために、本反応系において Hammett 則が成立するかを調べることにした<sup>49</sup>。通常 Hammett プロットでは置換基定数 $\sigma$ に対して反応速度定数  $k$  の対数をプロットする。本反応では *trans* 体、*cis* 体二つのジアステレオマーが生成するため、Hammett プロットをとるためにはそれぞれのジアステレオマーが生成する反応を区別し、その反応速度定数  $k_{trans}$ 、 $k_{cis}$  についての解析を行う必要がある。しかし本反応は非常に進行が速く、反応速度定数を算出することができなかった。そこで Anslyn らの報告<sup>50</sup>を参考に、ジアステレオ比の対数( $\log(trans/cis)$ )を用いて Hammett プロットをとることとした。 $\log(trans/cis)$ の値は  $k_{trans}$ 、 $k_{cis}$  の対数の差( $\log k_{trans} - \log k_{cis}$ )と等しいと見なすことができるため、 $\log(trans/cis)$ の値を用いて Hammett 則が成立すれば、反応機構について詳細な考察が可能になると考えた。

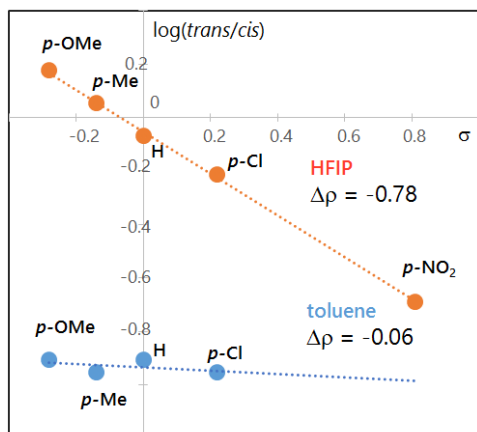


Figure 13. Hammett plot

反応のジアステレオ比に関して Hammett プロットをとった結果、直線関係が得られたことから本反応系では Hammett 則が成立することが明らかとなり、トルエン中と HFIP 中で傾きの異なる直線が得られた(Figure 13)\*<sup>5</sup>。このプロットにより得られる直線の傾きから、*trans* 体と *cis* 体が生成するそれぞれの反応の反応定数の差 $\Delta\rho$  ( $= \rho_{trans} - \rho_{cis}$ )は、トルエン中で $-0.06$ 、HFIP 中で $-0.78$ と求められた。後述するように、Hammett 則では反応定数 $\rho$ を用いることにより律速段階までの電子状態の収支を推測することができる。二つのジアステレオマーが生成する反応において

両ジアステレオマーが同一の反応機構（イミンのどちらの面で反応するかという点のみ異なる）で生成する場合、どちらのジアステレオマーが生成する機構においてもその電子状態は同様に変化すると考えることができる。よって、このような場合は反応定数の差 $\Delta\rho$ は0に近い値となる。以上の様な理由から $\Delta\rho = -0.06$ となるトルエン中において、両ジアステレオマーは同様の機構で生成し、イミンに対する面選択性によってジアステレオ選択性が生じていることが推測される。

一方、HFIP 中では $\Delta\rho = -0.78$ であるため、両ジアステレオマーが生成する反応の反応定数が異なる値を示すことがわかる。この結果は両ジアステレオマーが生成する機構で電子状態の変化の仕方が異なることを意味しており、両ジアステレオマーが異なる機構で生成していることを示唆する。なお、同一反応機構において、律速段階が変化したために立体選択性が変化したという可能性は一本の直線からなるプロット結果により除外することができる。よって HFIP 中では二つの反応機構が競争し、そのどちらを経るかにより異なるジアステレオマーが生成することが示唆された。この結果は、立体選択性の低下の要因が、複数の反応機構が競争しているためであるという仮説を証明するものである。

\*<sup>5</sup>Hammett plot は置換安息香酸の  $pK_a$  を用いて、置換基の電子求引性・供与性を定量的に表現した置換基定数 $\sigma$ （電子供与性は負、電子求引性は正の値）に対し、反応速度定数の対数( $\ln k$ )、または平衡定数の対数( $\ln K$ )をプロットしたものである。プロットが直線となれば、反応速度または平衡定数が置換基の電子求引性・供与性によってある一定の影響を受けることを意味しており、その影響の度合いを表すのが、反応定数 $\rho$ である。この関係は Hammett 式として式①のように表され、反応定数 $\rho$ の符号からは律速段階までの電子密度の変化を知ることができる。

$$\log(k_X/k_H) = \rho\sigma \quad (X \text{ は芳香環上の置換基}) \cdots \textcircled{1}$$

次にそれぞれの具体的な反応機構がどのようなものであるのかを決定するため、それぞれの $\rho_{trans}$ と $\rho_{cis}$ の値を推定することとした。Hammett 則では反応定数 $\rho$ の符号が重要であるが、反応定数の差 $\Delta\rho$ ではそれぞれの符号の情報は失われてしまう。すなわち、HFIP 中での $\Delta\rho = -0.78$ は① $\rho_{trans} < 0$ かつ $\rho_{cis} < 0$ 、② $\rho_{trans} < 0$ かつ $\rho_{cis} > 0$ 、③ $\rho_{trans} > 0$ かつ $\rho_{cis} > 0$ のいずれの場合でも成立しうる。そこで $\rho_{trans}$ と $\rho_{cis}$ の符号を見積もるために競争実験を行うこととした。

**Table 6. Competitive experiments**

entry	solvent	yield(%)			
		<i>trans</i> -10f (Cl)	<i>trans</i> -10d (OMe)	<i>cis</i> -10f (Cl)	<i>cis</i> -10d (OMe)
1	toluene	1	7	15	62
2	HFIP	7	36	15	27

イミン **4a**、カルボン酸 **7a** それぞれ 1 等量に対し、電子密度の異なる二種類のイソシアニド **6d** ( $\sigma = +0.22$ )、**6f** ( $\sigma = -0.28$ )を 1 等量ずつ作用させ、どちらのイソシアニドがより多く消費されるかを調べた (Table 6)。その結果、HFIP 中において *cis* 体ではメトキシ体とクロル体の生成比(OMe/Cl)が 27/15 であり、*trans* 体では OMe/Cl=36/7 と、どちらにおいてもより電子豊富な **6d** からの生成物が得られた。またこの傾向はトルエン中においても同様であることもわかった。

この結果は生成物の立体化学によらず、トルエン中及び HFIP 中のいずれの反応条件においても電子豊富なイソシアニドの方が速く反応することを示している。これは Hammett 則において、どちらの溶媒を用いた場合でも $\rho_{trans} < 0$ かつ $\rho_{cis} < 0$ であることを意味しており、HFIP 中では $\Delta\rho = -0.78$ であることから、 $\rho_{trans} < \rho_{cis} < 0$ 、トルエン中では $\Delta\rho = -0.06$ より $\rho_{trans} \doteq \rho_{cis} < 0$ であることがわかった。

以上の解析結果から、JU-3CR の律速段階と、それぞれの溶媒中における反応機構の相違点について重要な知見を得ることができる。一般的に Hammett 則において、 $\rho < 0$  の反応は律速段階までの過程で芳香環から電子が流出する反応であると見なすことができる。第一章冒頭で述べたように、JU-3CR の律速段階については二通りの主張が存在する。そこでそれぞれの主張に基づいて、律速段階までの反応機構をイソシアニドの関与する段階に着目して Figures 14, 15 に示した。



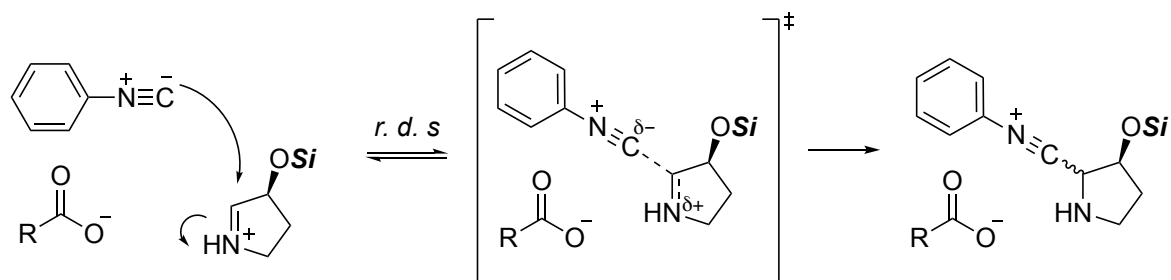


Figure 14. Possible reaction mechanism of JU-3CR (irreversible isocyanide addition)

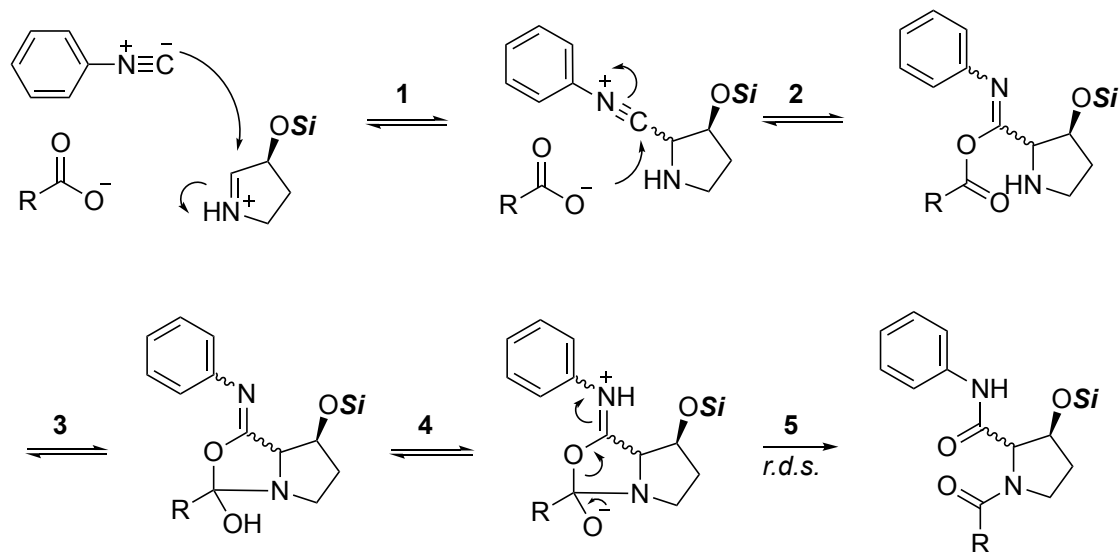
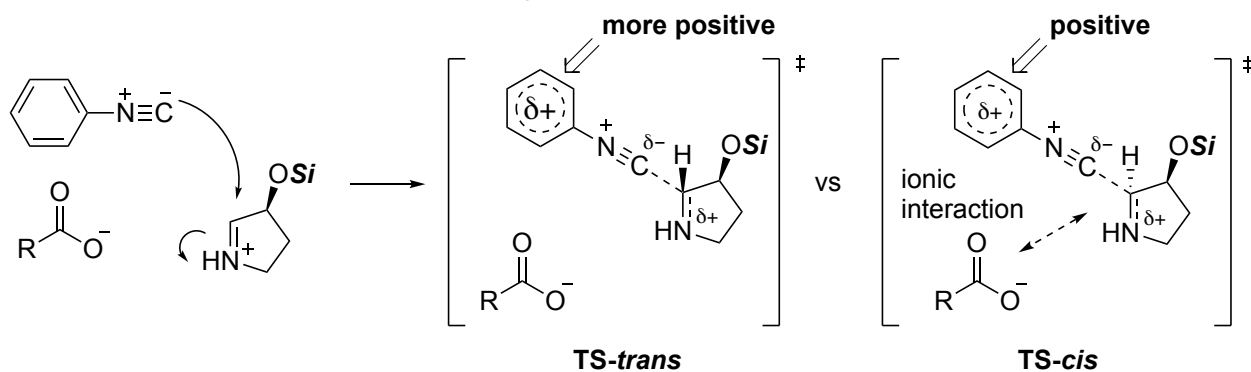


Figure 15. Possible reaction mechanism of JU-3CR (reversible isocyanide addition)

まず、イソシアニドの付加が律速段階の場合、反応は芳香環から電子が流出する遷移状態を経て進行するため、 $\rho < 0$  となることが予想される(Figure 14)。

次に、Mumm 転位が律速段階の場合を考える。この場合は Figure 15, 1-4 に示したような多段階の平衡反応を経て、段階 5 でイミダートが不可逆的に脱離する。多段階反応に対する Hammett 解析は複雑であるが、この場合は芳香環から電子が流出する段階 1、4 と芳香環に電子が流入する段階 2、5 を経るため、それぞれの段階での  $\rho$  が互いに打ち消し合って最終的に  $\rho$  は 0 に近くなると予想される。以上の考察と、 $\rho < 0$  という実験結果を比較すると、JU-3CR は Figure 14 で示したようにイソシアニドの付加を律速段階として進行していることが示唆された。

次に反応機構に対する溶媒効果を考察した。Hammett 則では、前述したようにその符号が大きな意味を持つが、その絶対値も重要である。反応定数 $\rho$ の絶対値は律速段階の遷移状態に至るまでに芳香環から流出する、もしくは芳香環に流入する電子密度の程度を反映している。すなわち $\rho < 0$ の反応では、その絶対値が大きいほど芳香環からの電子の流出が大きいことを示唆し、これは遷移状態において生じる正電荷がより大きいことを意味している。そこで、上記の様に反応定数が遷移状態の電荷分布を反映するのであれば、反応定数の差 $\Delta\rho$ はそれぞれのジアステレオマーが生じる過程の律速段階における電荷分布の差を反映していると考えられる。トルエン中での $\Delta\rho$ は0に近い値をとることから、*cis*体、*trans*体はともに芳香環が同程度の正電荷を帯びた遷移状態を経ると推察できる。一方、HFIP 中では $\Delta\rho = -0.78$ であることから *trans*体が生成する際の遷移状態では *cis*体が生成する際の遷移状態よりも芳香環が大きな正電荷を帯びていることが示唆される(Figure 16)。



**Figure 16.** Transition state of isocyanide addition

このような反応定数の差は、イミニウムイオンの対カチオンであるカルボキシラートイオンの効果を考慮することで説明することができる。カルボキシラートイオンは負電荷を帯びた化学種であるため、正電荷を帯びたイミニウムイオンとの静電相互作用によって遷移状態におけるイミニウムイオンの正電荷を弱める働きがあると考えられる。すなわち、上記のような相互作用が存在せず、イミニウムイオンとイソシアニドが二分子で反応する場合は、遷移状態に至るまでにイソシアニドから多くの電子が流出する必要があるが(Figure 16, **TS-trans**)、イミニウムイオン、カルボキシラートイオン、イソシアニドの三成分からなる遷移状態を経て反応が進行するのであれば、イミニウムイオンの正電荷の一部をカルボキシラートイオンが引き受けることができるため、イソシアニドから流出する電子は相対的に少なくなる(Figure 16, **TS-cis**)。よって *cis*体が生成する際には三分子からなる遷移状態をとり、イミニウムイオンの正電荷をカルボキシラートイオンの負電荷が安定化しながら反応が進行していることが示唆された。

#### 第四節 推定反応機構

以上の実験結果とその解析結果に矛盾しない反応機構を以下のように推定した。本反応はトルエン中と HFIP 中で異なる経路をとるため、まずトルエン中での反応機構を Figure 17 に示す。トルエンのような非極性溶媒は、イオン性化学種を溶媒和により安定化することができないうえ、誘電率が低いことからカチオン-アニオン間の静電相互作用を強化する傾向がある<sup>51</sup>。よって、イミンとカルボン酸の酸塩基反応によってイミニウムイオンとカルボキシラートイオンが生成し、これが互いに相互作用することで接触イオン対(I)が生成する。この接触イオン対(I)ではカルボキシラートイオンがシロキシ基の逆面に位置してその面を遮蔽しているため、イソシアニドの付加は面選択的に進行し、カルボキシラートイオンが位置する面とは逆の面から付加する。この際、S<sub>N</sub>2 反応様の遷移状態(TS1)をとることでイミニウムイオンが帯びる正電荷の一部はカルボキシラートイオンによって安定化され、*cis* 体を与える。この S<sub>N</sub>2 反応様の反応機構は熱力学的に不安定と予想される *cis* 体が非極性溶媒中において優先して得られることを合理的に説明することができる\*<sup>6</sup>。この機構では *trans* 体、*cis* 体のどちらも同じ反応機構で生成することから、Figure 13 に示した Hammett プロットの傾きが小さいこととも矛盾しない。

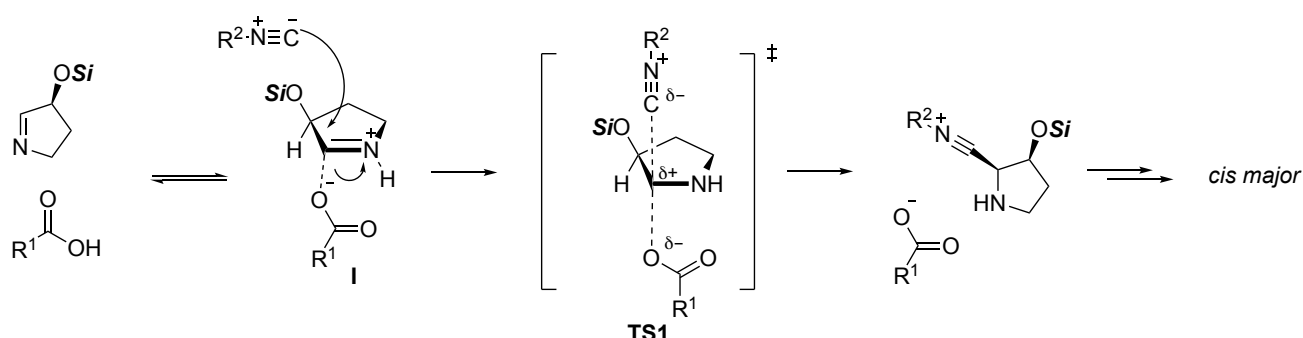
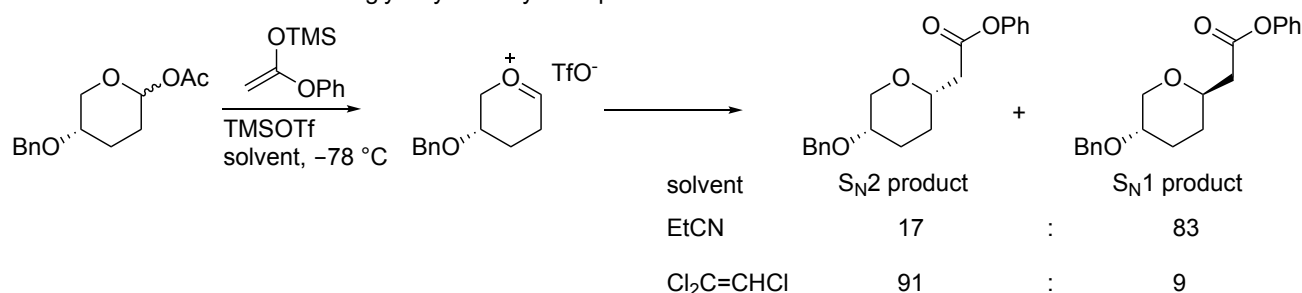


Figure 17. Proposed reaction mechanism in toluene

\*<sup>6</sup>Woerpel らはテトラヒドロピランアセタールの C-及び O-グリコシル化において同様の報告を行っている<sup>52</sup>。TMSOTf を用いてグリコシルドナーの活性化を行うと、イオン対であるグリコシルトリフラートが生成し、この中間体からの反応は非極性溶媒中では S<sub>N</sub>2 反応が優先し、高極性溶媒中では S<sub>N</sub>1 反応が優先する結果が得られている (Scheme10)。

Scheme 10. Solvent effect on glycosylation by Woerpel et. al.



次に HFIP 中での推定反応機構を示す(Figure 18)。HFIP は非常に極性の高い溶媒であり、トルエンとは対照的にカチオン-アニオン間の静電相互作用を弱め、それぞれのイオンを効果的に溶媒和する性質を有している。この性質により、イミニウムイオンとカルボキシレートイオンそれぞれが単独で溶媒和された溶媒介在イオン対(II)が安定化され、系中は I と II の平衡状態となる。この溶媒介在イオン対(II)のイミニウムイオンに対するイソシアニドの付加はシロキシ基の立体障害によりシロキシ基とは逆の面で行われ、TS1 よりもイソシアニドが正電荷を帯びる遷移状態(TS2)を経て *trans* 体が生成する。この遷移状態では TS1 とは異なりカルボキシレートイオンが溶媒によって隔てられており、カルボキシレートイオンの持つ負電荷は遷移状態の安定化に関与しない。

この反応機構で最も重要な点は *cis* 体は TS2 を経て生成しないことである。すなわち、反応系中に共存する接触イオン対(I)からトルエン中と同様の機構で *cis* 体が生成する。このようにそれぞれのジアステレオマーが異なる機構で生成することは Figure 13 に示した Hammett プロットの傾きが大きいことから示唆される。

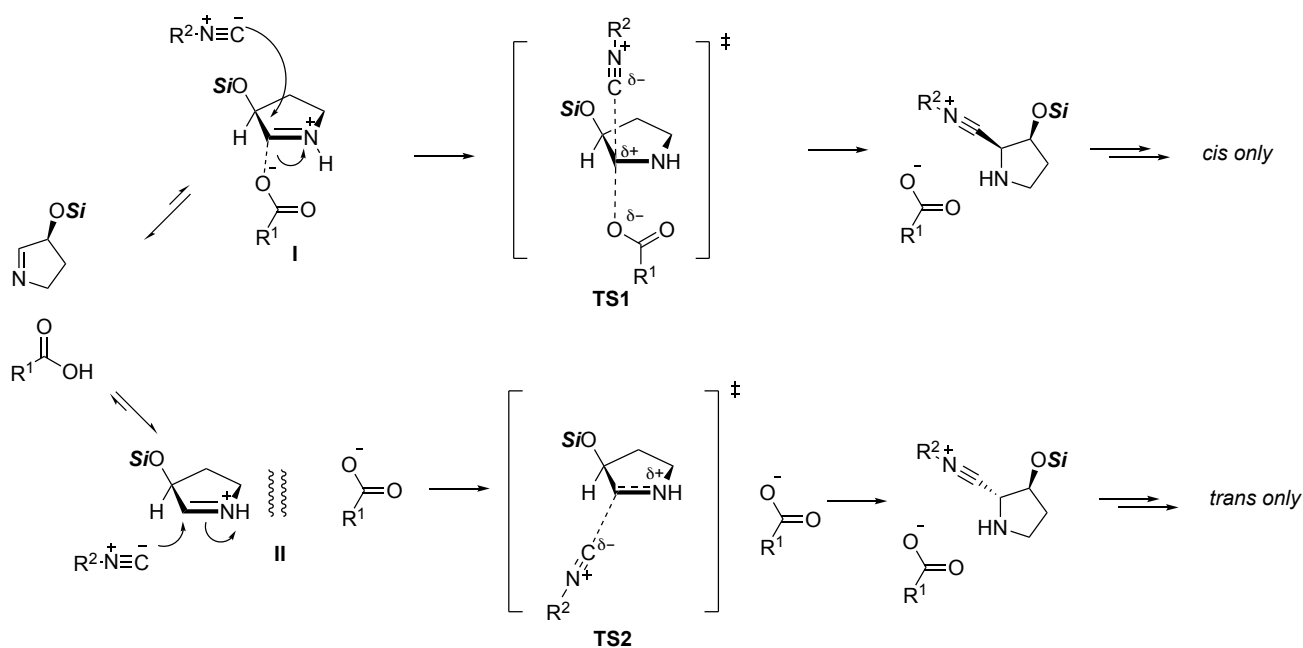


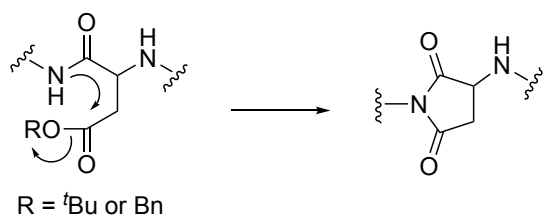
Figure 18. Proposed reaction mechanism in HFIP

以上のように筆者は、 $\alpha$ 位に酸素置換基を有する一置換光学活性 5 員環イミンを用いた JU-3CR をジアステレオ多様な反応へと展開することに成功した。また反応の速度論的解析と Hammett 解析の結果から、本反応の律速段階がイミニウムイオンに対するイソシアニドの付加の段階であることを明らかにした。同様の解析から本反応が二つの異なるイオン対中間体を経て進行していることを証明し、それぞれのイオン対に対する溶媒効果によって立体選択性が変化することも明らかとした。本研究は多成分反応をジアステレオ多様な反応へ展開できたという点で特徴的であり、また、未だ不斉反応が報告されていない Ugi 反応や JU-3CR の不斉化へ向けた知見を提供するものであるといえる。次章では、本反応を利用したプラスバシン A<sub>3</sub> とその誘導体の全合成について詳細を述べる。

## 第二章 プラスバシン A<sub>3</sub> の合成研究

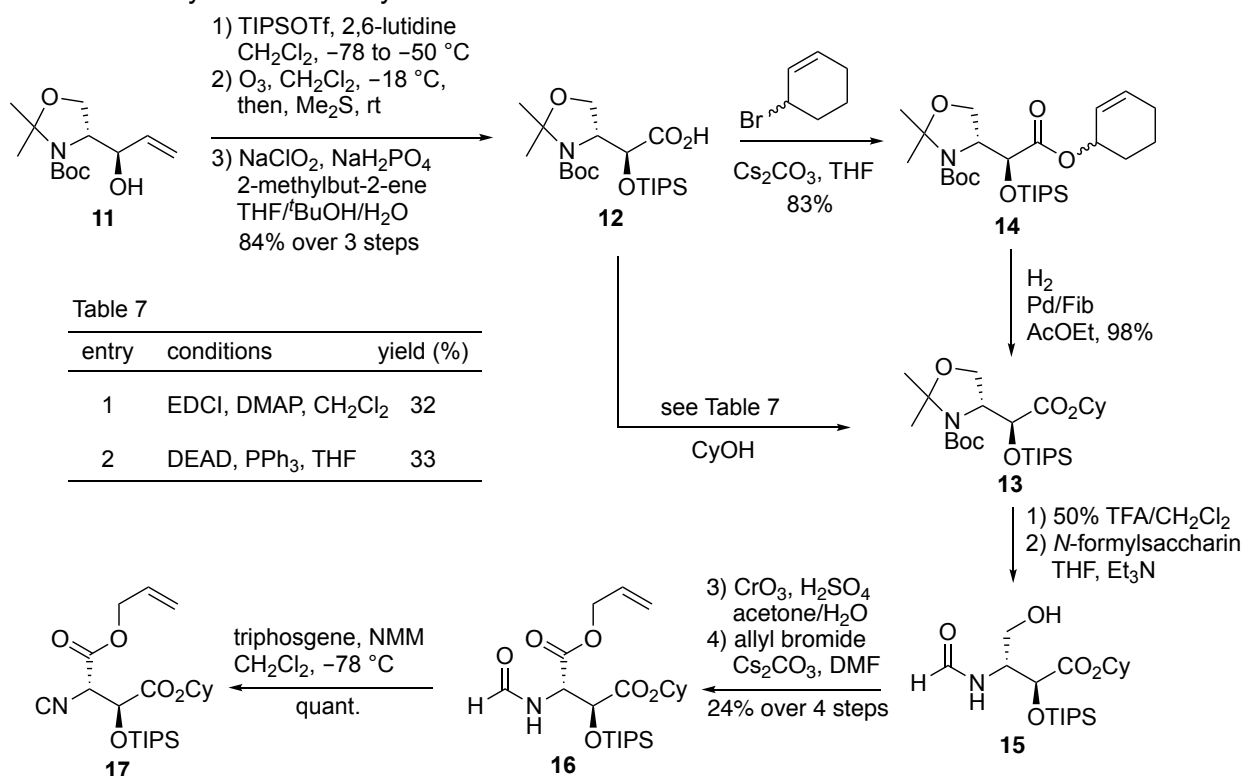
### 第一節 プラスバシン A<sub>3</sub> の逆合成解析 (合成経路 1)

第一章でジアステレオ多様の JU-3CR を開発したので、次に本反応を用いたプラスバシン A<sub>3</sub> の合成研究を行った。まず、プラスバシン A<sub>3</sub> の逆合成解析を行う前に本反応の基質適応範囲について調べることとし、β-ヒドロキシ-Asp 由来のイソシアニドの合成を行った。なお、Asp-β-カルボン酸の保護基としてベンジルエステル、*t*-ブチルエステルを用いると、α-アシルアミノ基との環化反応によりイミドが生成することが知られており (Figure 19)、この副反応を抑制するために嵩高いシクロヘキシルエステルが用いられる<sup>53</sup>。このことから筆者はイソシアニドのカルボン酸の保護基としてシクロヘキシルエステルを用いることとした。シクロヘキシルエステルは TfOH/TFA や、アニソール/無水 HF などの強酸性溶媒を用いることで除去することができる<sup>54</sup>。



**Figure 19.** Aspartimide formation

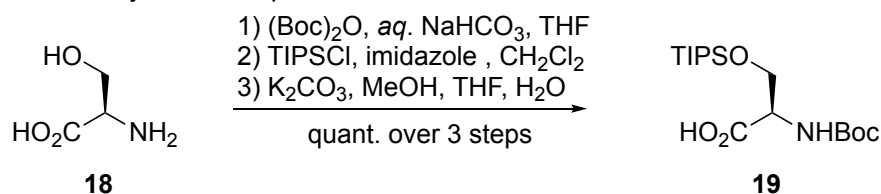
**Scheme 11. Synthesis of isocyanide **17****



検討に用いたイソシアニド **17** の合成を Scheme 11 に示した。D-Ser 由来の Garner アルデヒドから調製した文献既知のアルコール **11**<sup>55</sup> に対し、水酸基の TIPS 化、末端オレフィンのオゾン分解と、生じたアルデヒドの Kraus 酸化を行うことでカルボン酸 **12** を合成した。続いてカルボン酸 **12** からシクロヘキシルエステル **13** への変換を試みた。シクロヘキサノールとの脱水縮合では複雑な混合物を与え、収率が低下した (Table 7, entry 1)。シクロヘキサノールとの光延反応においても収率の改善は見られなかった (entry 2)。そこで 3-ブロモシクロヘキセンとの S<sub>N</sub>2 反応によりシクロヘキセニルエステル **14** へと変換した後に、接触水素還元によりシクロヘキシルエステル **13** へと導いた<sup>56</sup>。この際、シクロヘキセニル基の除去が競争して起こるのを防ぐために、Pd-フィブロイン (Pd/Fib)<sup>57</sup> を触媒として用いた。次に、得られたシクロヘキシルエステル **13** のイソプロピリデン基と Boc 基を TFA により除去し、*N*-ホルミルサッカリン<sup>58</sup> を用いてアミン選択的にホルミル化を行うことでアルコール **15** を得た。このアルコール **15** を Jones 酸化の条件に付し、得られたカルボン酸をアリルエステルとして保護することでホルムアミド **16** を得た。これをトリホスゲンにより脱水することでイソシアニド **17** を合成した。

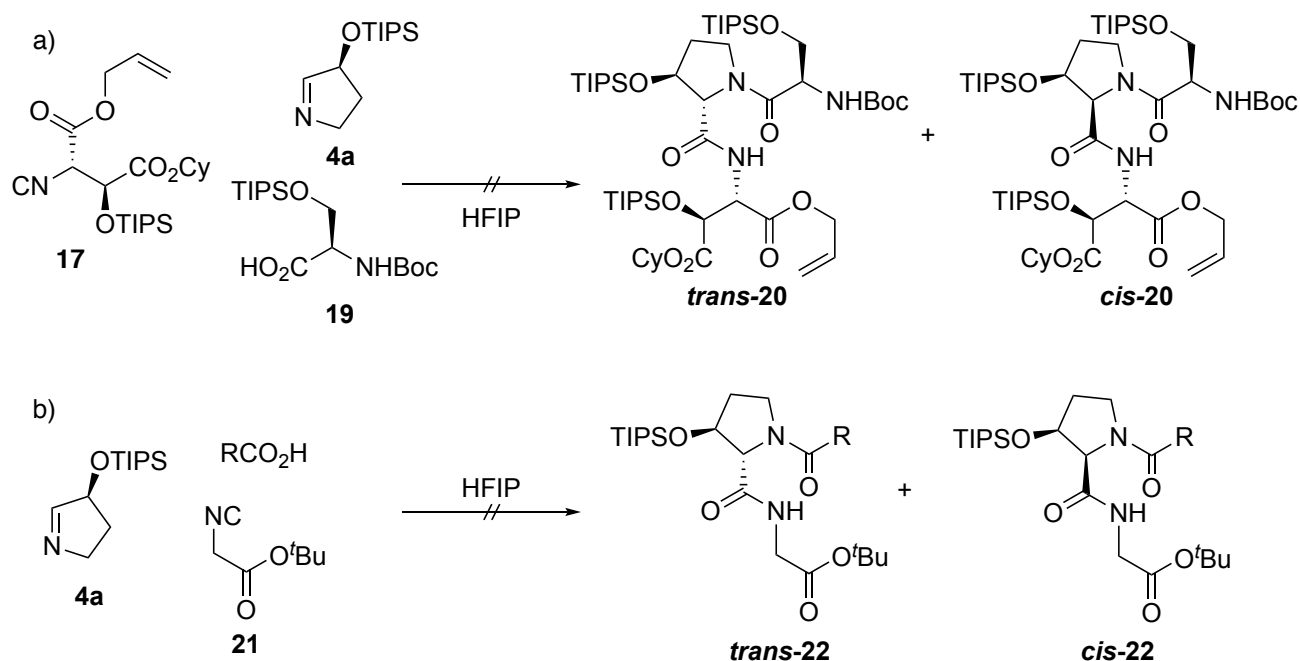
JU-3CR の検討に用いるカルボン酸 **19** は D-Ser (**18**) のアミノ基を Boc 化した後、水酸基とカルボン酸を TIPS 基で保護し、MeOH, H<sub>2</sub>O 存在下で K<sub>2</sub>CO<sub>3</sub> を作用させることにより TIPS エステルをカルボン酸へと変換することで合成した(Scheme 12)。

**Scheme 12. Synthesis of protected D-Ser 19**



合成したイソシアニド **17**、カルボン酸 **19**、イミン **4a** を用いて HFIP 中で JU-3CR を行ったところ、反応は複雑な混合物を与え、望みの JU-3CR 成績体 **20** は全く得られなかった(Scheme 13a)。また、より単純な基質であるイソシアノ酢酸エステル **21** を用いた場合も同様に複雑な混合物を与え、イソシアノ基β位のカルボニル基の存在が本反応の進行の可否に影響することが示唆された(Scheme 13b)。

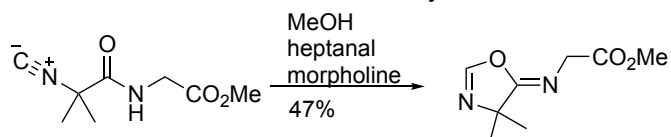
**Scheme 13. JU-3CR using β-isocyanoesters**



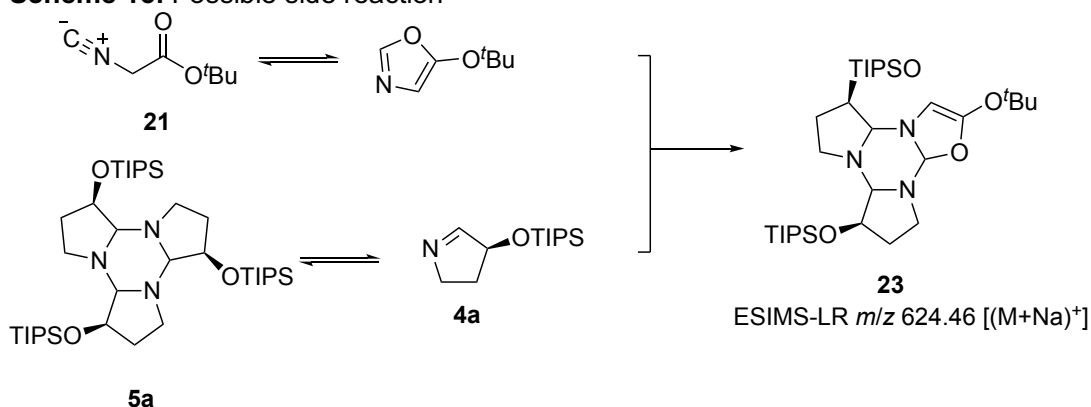


$\alpha$ イソシアノカルボキサミドは分子内環化反応によりオキサゾリンを与えることが Zhu らによって報告されている(Scheme 14)<sup>59</sup>。反応溶液の ESI-MS スペクトルの解析結果からイソシアニド 1 分子とイミン 2 分子が結合した化合物の存在が示唆されたことから、イミンと三量体の平衡にオキサゾリンが加わることによってヘテロ三量体 **23** が生成し、望みとする反応が進行しなかったと考察した(Scheme 15)。

**Scheme 14.** Oxazoline formation by Zhu et al.

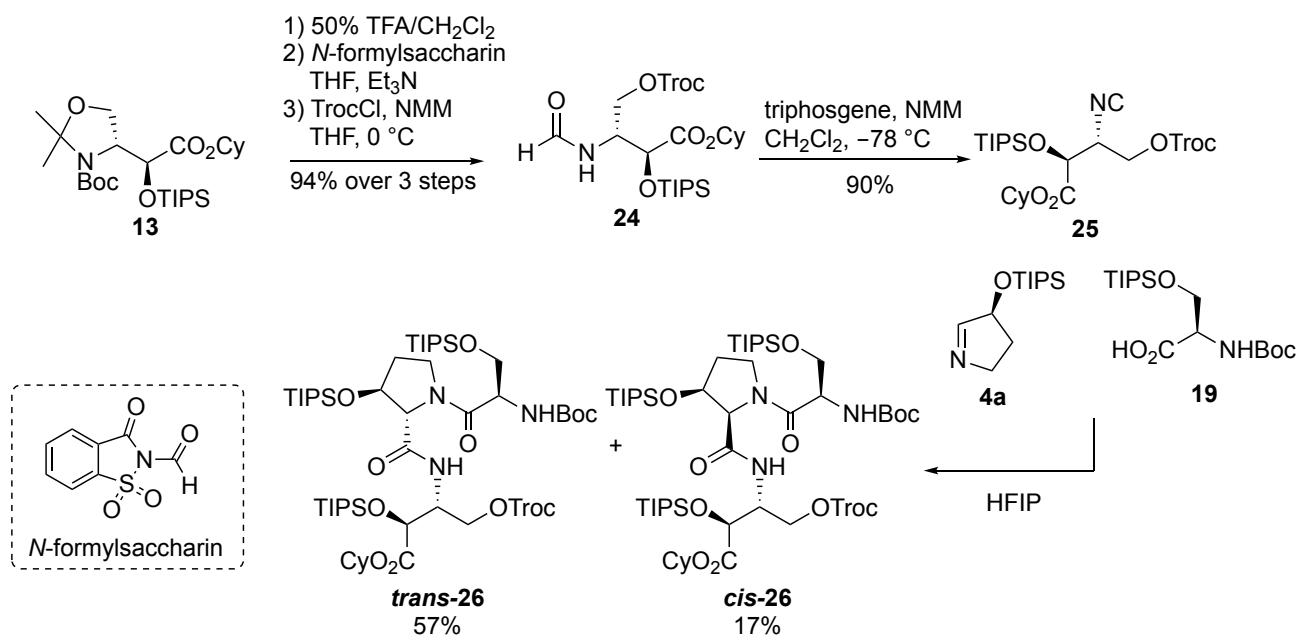


**Scheme 15.** Possible side reaction

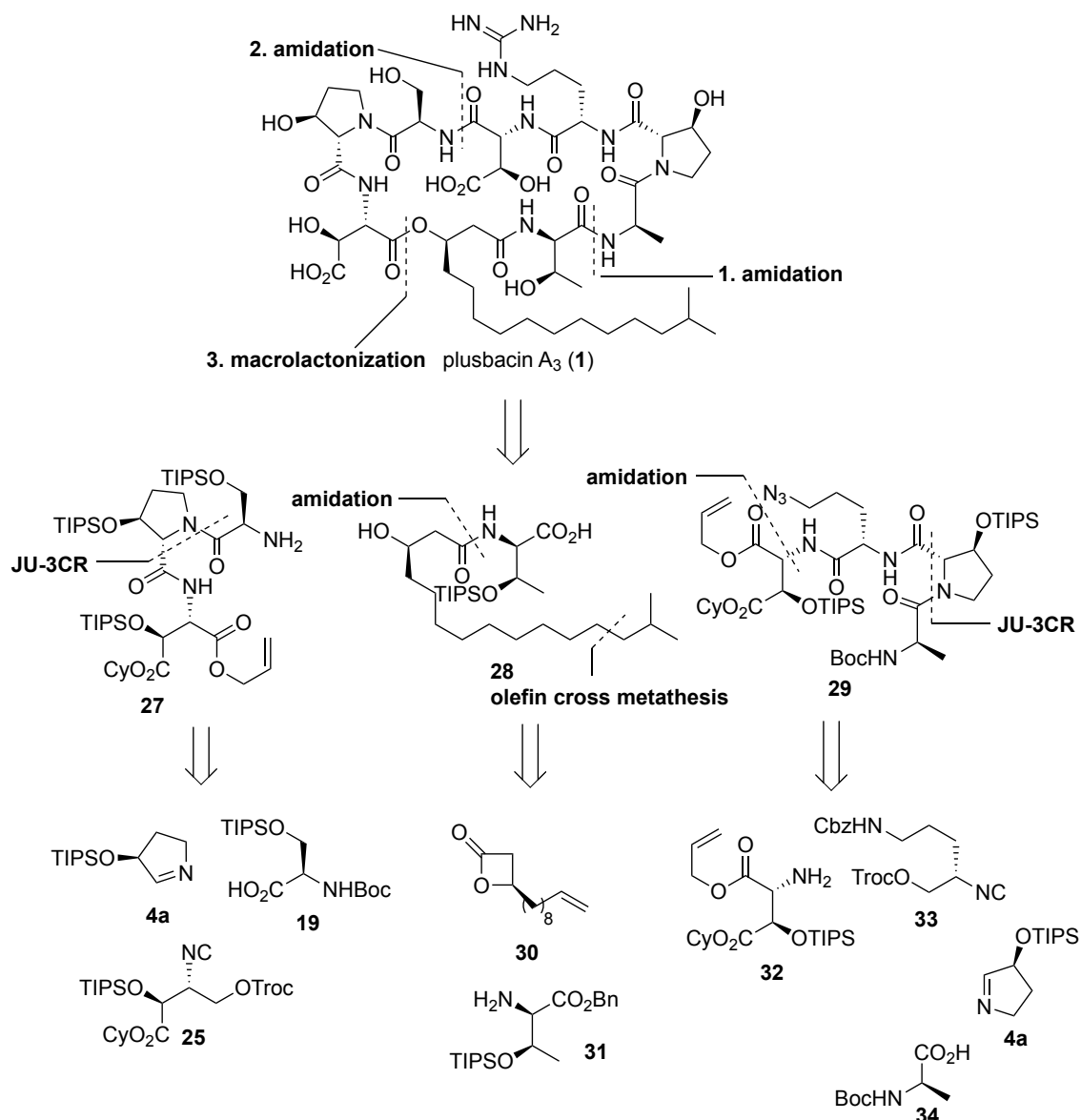


この結果を踏まえ、イソシアノ基 $\beta$ 位のエステルを還元したイソシアニドを用いることで JU-3CR が進行すると考えた。シクロヘキシルエステル **13** から、イソプロピリデン基と Boc 基の除去、*N*-ホルミル化、水酸基の Troc 化を経て合成したホルムアミド **24** をトリホスゲンにより脱水しイソシアニド **25** を合成した。このイソシアニド **25** とカルボン酸 **19**、イミン **4a** を用いて HFIP 中で JU-3CR を行ったところ、望みの *trans*-**26** が収率 57% で得られたため、この基質を用いて全合成を行うこととした(Scheme 16)。

**Scheme 16.** JU-3CR using isocyanide **25**



以上の検討結果を踏まえて筆者が立案したプラスバシン A<sub>3</sub> の逆合成解析を示す (Figure 20)。



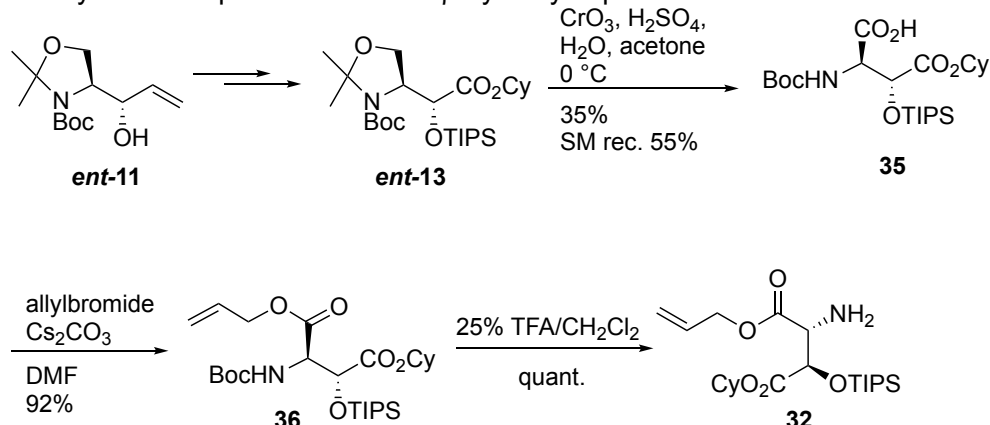
**Figure 20.** Retrosynthetic analysis of plusbacin A<sub>3</sub>: 1<sup>st</sup> approach

プラスバシン A<sub>3</sub> のエステル部位はマクロラクトン化によって合成終盤で構築することとし、環化前駆体は三つのセグメント **27**、**28**、**29** のアミド縮合により合成することとした。トリペプチド **27** はイミン **4a**、カルボン酸 **19**、イソシアニド **25** を用いた JU-3CR によって合成し、テトラペプチド **29** はイミン **4a**、カルボン酸 **34**、イソシアニド **33** を用いた JU-3CR によって合成できるテトラペプチドと *D-threo*-β-ヒドロキシ-Asp 保護体 **32** とのアミド縮合により合成することとした。JU-3CR に用いるイソシアニドはβ-アシロキシイソシアニドとし、JU-3CR の後にカルボン酸への酸化を行うことを計画した。カルボン酸 **28** は 3-ヒドロキシイソヘキサデカン酸と *allo-D-Thr* 保護体 **31** から導くこととし、3-ヒドロキシイソヘキサデカン酸は既知のβ-ラクトン **30** から合成することを計画した。

## 第二節 プラスバシン A<sub>3</sub> の合成研究 (合成経路 1)

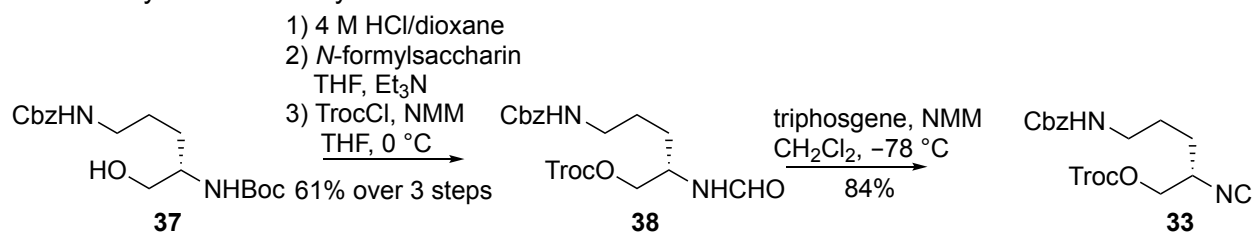
まず合成に必要な各ユニット化合物を合成した。以下にその詳細を述べる。

### Scheme 17. Synthesis of protected *D*-threo-β-hydroxy Asp **32**



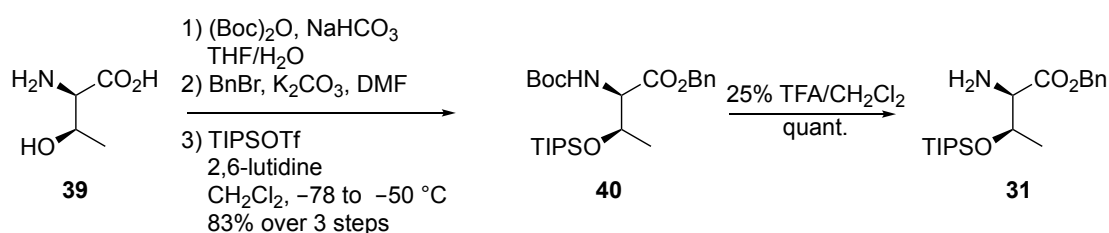
*D*-threo-β-ヒドロキシ-Asp 保護体 **18** は L-Ser からイソシアニド **17**、**19** の合成と同様にして合成したシクロヘキシルエステル **ent-13** から合成した。シクロヘキシルエステル **ent-13** を Jones 酸化することで、イソプロピリデン基の除去と生じた第一級アルコールの酸化を行い、カルボン酸 **35** を得た<sup>27</sup>。得られたカルボン酸 **35** をアリル基で保護し、TFA による Boc 基の除去を経て *D*-threo-β-ヒドロキシ-Asp 保護体 **32** を合成した(Scheme 17)。

### Scheme 18. Synthesis of isocyanide **33**



イソシアニド **33** の合成を Scheme 18 に示す。文献既知のアルコール **37**<sup>60</sup> に対して、酸による Boc 基の除去、*N*-ホルミルサッカリンを用いたアミン選択的なホルミル化、水酸基の Troc 化を行うことでホルムアミド **38** とし、これをトリホスゲンにより脱水することでイソシアニド **33** を合成した。

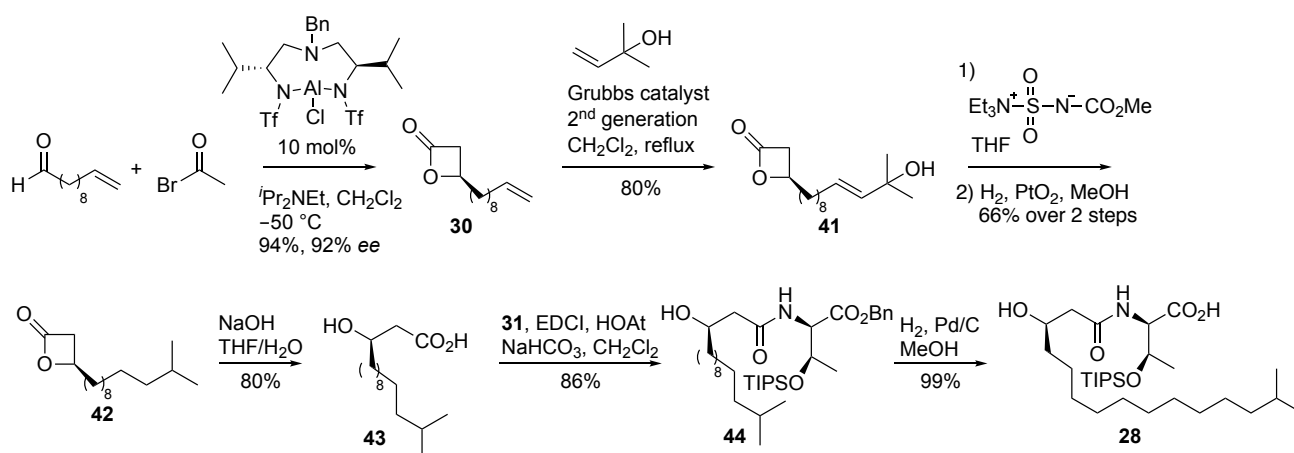
### Scheme 19. Synthesis of protected *allo*-D-Thr **17**



*allo*-D-Thr 保護体 **31** は *allo*-D-Thr (**39**) を適切に保護し、TFA により Boc 基を除去することで合成した(Scheme 19)。

カルボン酸 **28** の合成は以下のように行った(Scheme 15)。Nelson らの報告に従い、10-ウンデセナールとケテンとの触媒的不斉[2+2]付加環化反応によりβ-ラクトン **30**<sup>61</sup> を収率 94%、不斉収率 92% *ee* で合成した。このβ-ラクトン **30** に対し第二世代 Grubbs 触媒存在下、2-メチル-3-ブテン-2-オールを三炭素源としてクロスメタセシス反応を行うことで<sup>62</sup>、脂溶性側鎖を伸張しアリルアルコール **41** を得た<sup>\*7</sup>。これを Burgess 試薬<sup>63</sup> により脱水することでジエンとした後、接触水素還元によりβ-ラクトン **42** へと導いた。続いて、β-ラクトン環を加水分解し、ヘキサンから再結晶することで(*R*)-3-ヒドロキシイソヘキサデカン酸(**43**)を光学純品として収率 80%で得た。次に *allo*-D-Thr 保護体 **31** とのアミド縮合、ベンジル基の除去を経てカルボン酸 **28** を合成した。

**Scheme 20. Synthesis of carboxylic acid 28**



<sup>\*7</sup>クロスメタセシス反応において、三炭素源として3-メチル-1-ブテンを用いると3-メチル-1-ブテンや生成物のオレフィンがより熱力学的に安定な三置換オレフィンへと異性化し、そこからさらにオレフィンメタセシスが進行することで炭素鎖長の異なる化合物からなる混合物が得られた。また生成物と出発物質の分離も困難であった。この異性化を抑制するとともに生成物の分離を容易にするために三炭素源として2-メチル-3-ブテン-2-オールを用いている。

以上のように各ユニットの合成を終えたので、これらの連結を行った。

### Scheme 21. Cleavage of Troc group

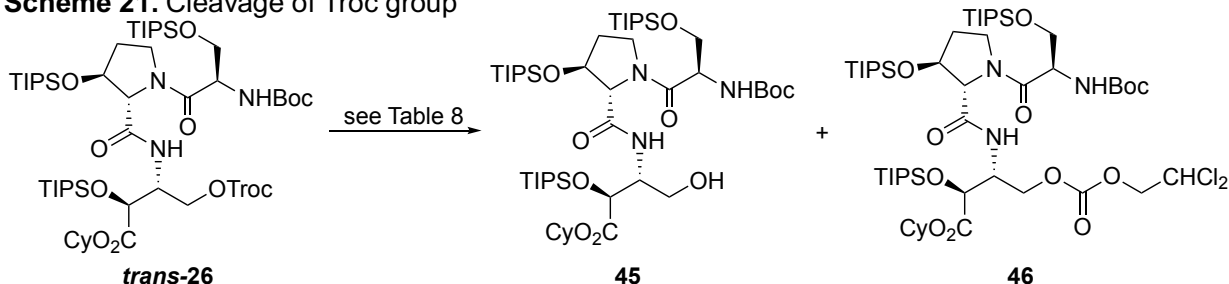
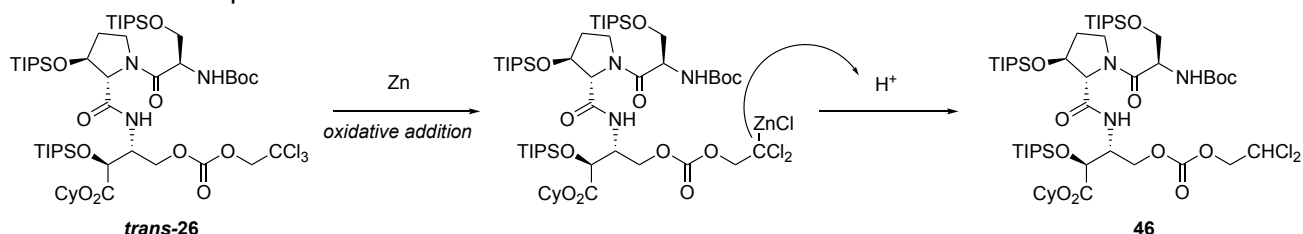


Table 8

entry	condition	yield (%)	
		<b>45</b>	<b>46</b>
1	Zn/Cu, 1 M aq. KH <sub>2</sub> PO <sub>4</sub> , THF	-	-
2	Zn/Cu, NH <sub>4</sub> Cl, MeOH	-	79
3	SmI <sub>2</sub> , THF	50	50
4	Sm, I <sub>2</sub> , MeOH	95	-

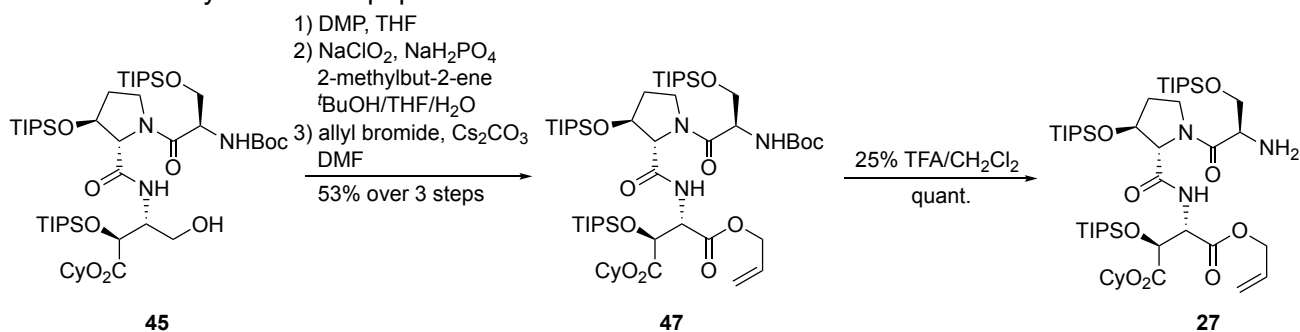
まず、JU-3CR 成績体 **trans-26** に対する Troc 基の除去を検討した(Scheme 21)。亜鉛-銅合金により除去を試みたがリン酸二水素カリウム水溶液を酸として用いた場合は反応が進行せず(Table 8, entry 1)、塩化アンモニウムを用いた場合は Troc 基が還元された脱クロロ体 **46** が中程度の収率で得られた(entry 2)。この脱クロロ体 **46** が生成する機構は次のように考えられる(Scheme 22)。

### Scheme 22. Proposed mechanism of reductive dechlorination



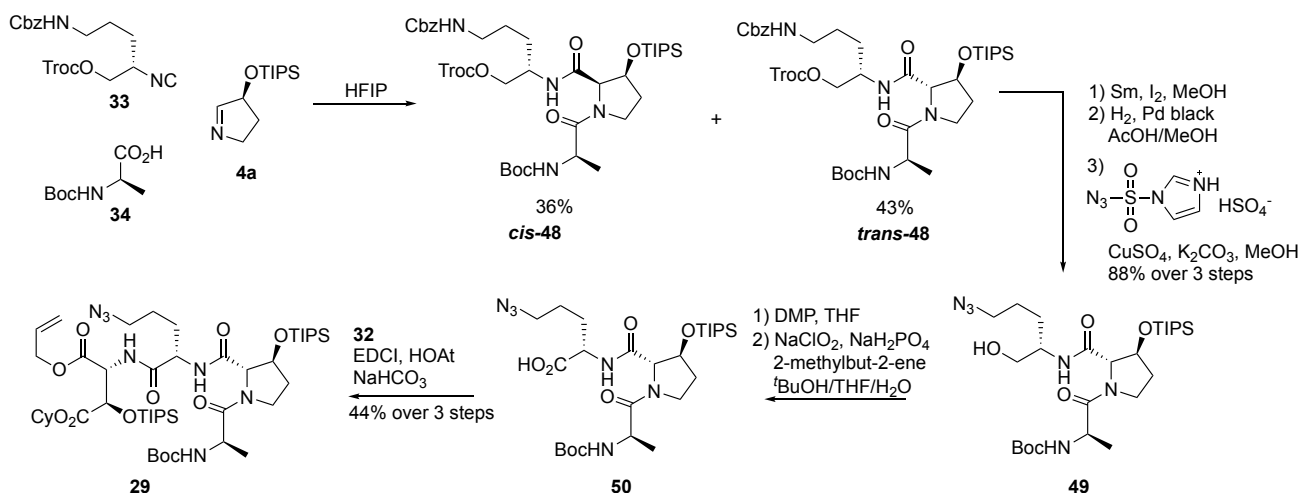
まず **trans-26** の炭素-塩素結合が金属亜鉛に酸化的付加し、これによって生成した有機亜鉛化合物がプロトン化されることにより脱クロロ体 **46** が得られたと考察した。この副反応を抑えるためにプロトン源のない条件下で一電子還元を行えるヨウ化サマリウムを用いたところ、中程度の収率で目的のアルコール **45** を得ることができたが、この条件下においても脱クロロ体 **46** が生成した(entry 3)。そこで、Lewis酸を用いた炭酸エステルの加溶媒分解条件として Troc 体 **trans-26** をメタノール中、金属サマリウムとヨウ素で処理したところ、メタノリシス反応が進行し望みとするアルコール **45** を収率 95%で得ることができた<sup>64</sup> (entry 4)。

### Scheme 23. Synthesis of tripeptide 27



次に得られたアルコール **45** をアミン **27** へと変換した(Scheme 23)。アルコール **45** を Dess-Martin 酸化<sup>65</sup> することでアルデヒドとし、これを Kraus 酸化<sup>66</sup> によってカルボン酸へと変換したのち、カルボキシ基のアリル化と Boc 基の除去を経てアミン **27** を得た。このアミンはシリカゲルカラムクロマトグラフィーによる精製を行わずに次の反応に用いた。

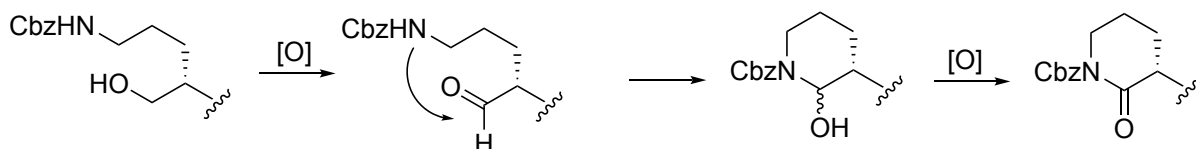
### Scheme 24. Synthesis of tetrapeptide 29



テトラペプチド **29** は JU-3CR を利用して合成した(Scheme 24)。イソシアニド **33**、イミン **4a**、カルボン酸 **34** を HFIP 中で反応させることで *trans/cis* = 54/46 で *trans* 体を若干優先して得た。Scheme 21 と同様にして、MeOH 中で金属サマリウムとヨウ素を用いて *trans*-**48** の Troc 基を除去した後、接触水素還元による Cbz の除去と、生じた第一級アミンに対するジアゾ転位反応<sup>67</sup> によってアジドアルコール **49** を得た。このアルコールを Dess-Martin 酸化、Kraus 酸化の二工程でカルボン酸へと酸化し<sup>\*8</sup>、D-threo-β-ヒドロキシ-Asp 保護体 **32** とアミド縮合することで対応するテトラペプチド **29** を合成した。

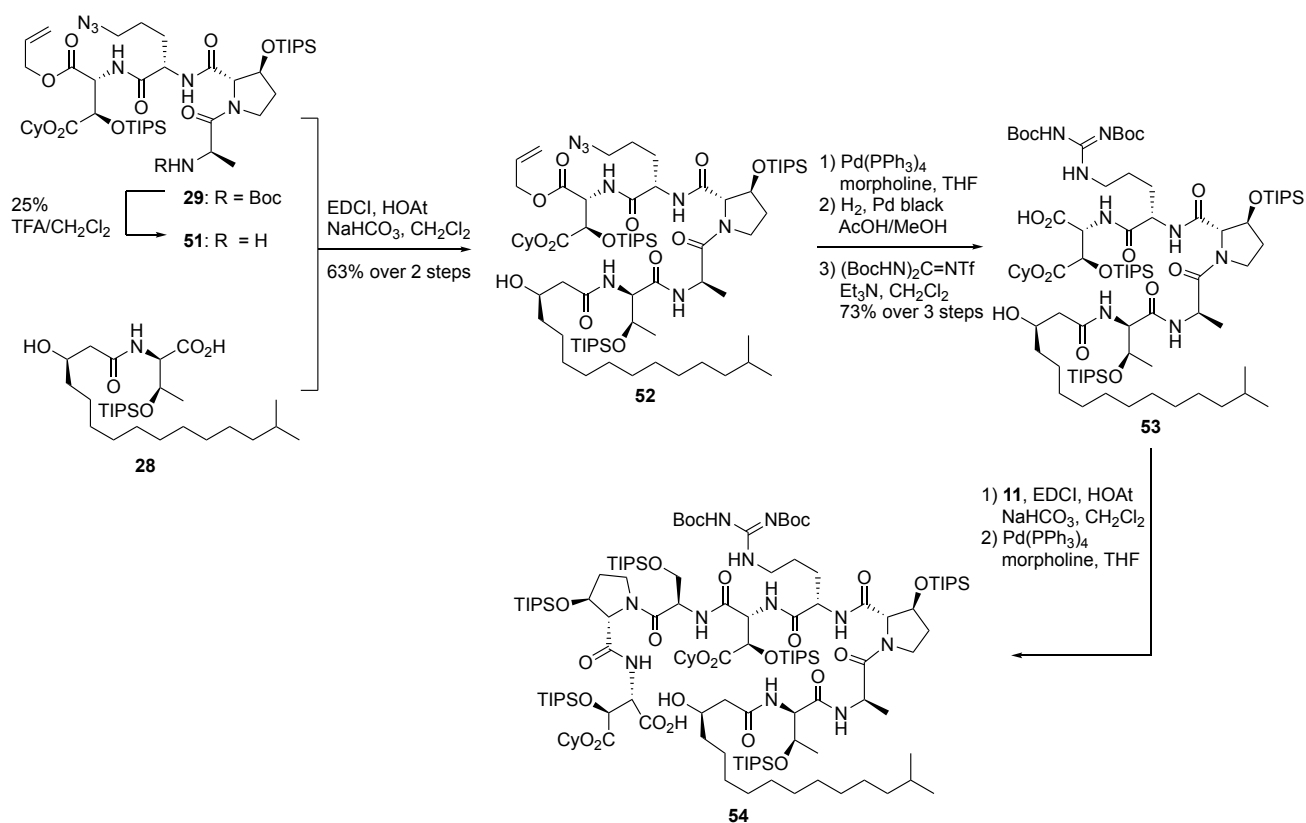
\*8-5-アミノアルコールを酸化すると、中間体のアルデヒドが生成するとともに Cbz 基で保護されたアミンが分子内環化を起こし、生じたヘミアミナル中間体がさらに酸化されることでδ-ラクタムが生成することが明らかになった(Scheme 25)。アルコール **49** では、アミンを解離性プロトンをもたないアジドとして保護することでこの副反応を防いでいる。

### Scheme 25. Lactam formation

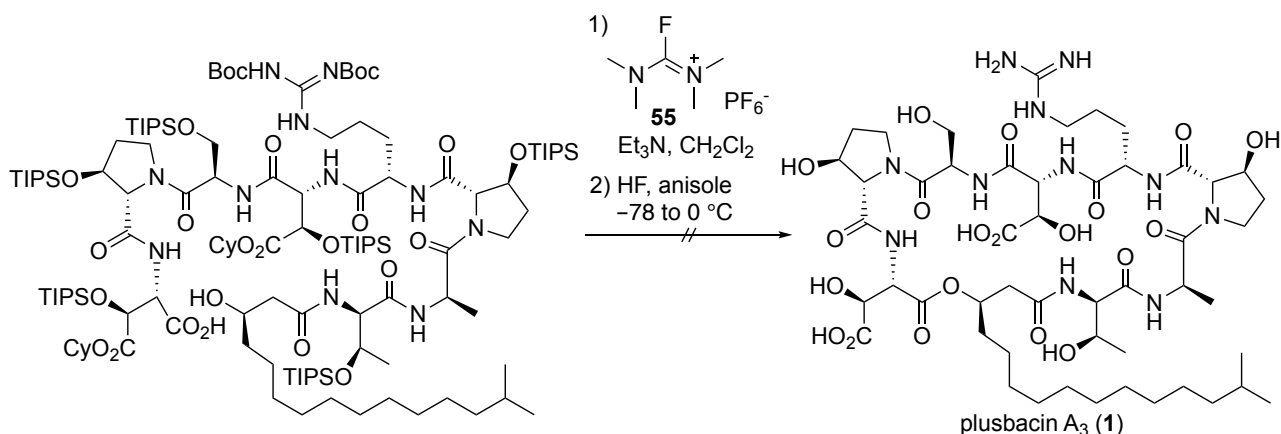


望みのセグメントを合成したので、プラスバシン A<sub>3</sub> の全合成に向けてセグメントの連結と最終段階のマクロラクトン化反応を行った(Scheme 26)。テトラペプチド **29** の N 末端 Boc 基を除去し、カルボン酸 **28** とアミド縮合することでヘキサペプチド **52** を二工程収率 63% で得た。C 末端アシル基とアジド基の還元を行い、生じたアミンを Goodman 試薬<sup>68</sup> によりグアニジル化することで Orn 側鎖を Arg 側鎖へと変換した。このカルボン酸 **53** をアミン **11** と縮合し、C 末端アシル基を除去することでマクロラクトン化前駆体のヒドロキシカルボン酸 **54** を得た。この化合物は不安定であったことからシリカゲルカラムクロマトグラフィーによる精製を行わず、続くマクロラクトン化反応を行った。

**Scheme 26.** Attempt to synthesize plusbacin A<sub>3</sub>



**Scheme 27.** Attempt to synthesize plusbacin A<sub>3</sub>



ヒドロキシカルボン酸 **54** に対するマクロラクトン化条件を検討した。EDCI と DMAP を用いる一般的な脱水縮合条件や光延反応によるエステル化では反応が進行しなかったものの、TFFH (**55**)<sup>69</sup> を用いてカルボン酸を酸フッ化物へと活性化する条件のみ、望みのマクロラクトンと分子量が一致する化合物が得られた。NMR スペクトルでは構造を決定することはできなかったため、脱保護条件に付すこととした (Scheme 27)。VanNieuwenhze らの報告を参考にアニソール存在下、無水フッ化水素を用いる脱保護反応を行い、反応の粗生成物を LC-MS によって分析した。

<sup>69</sup>Fluoro-*N,N,N',N'*-tetramethylformamidiinium hexafluorophosphate (TFFH)はカルボン酸を温和な条件下、酸フッ化物に変換する縮合剤であり 1995 年に Faham らによって報告された<sup>69</sup>。立体障害の大きいアミノ酸の縮合にはしばしば酸塩化物を中間体とした方法が用いられるが、アスパラギン酸から生じる酸塩化物は容易に分子内環化を起こす。一方で酸フッ化物は酸塩化物に比べて安定性が高く、アスパラギン酸から環化反応を起こすことなく酸フッ化物を調製できる<sup>70</sup>。

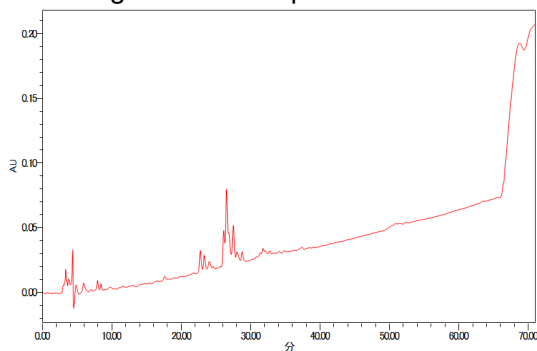


LC-MS 分析の結果を Figure 21 に示す。反応粗生成物(a)では複数のピークが見られ、特に保持時間 27 分から 32 分間にプラスバシン A<sub>3</sub> と同一の分子量及び、その加水分解体の分子量を有するピークが複数得られた(c)。いずれのピークが望みの化合物であるかを確認するため、プラスバシン A<sub>3</sub> の標品との比較を行った。天然物は保持時間 28 分から 29 分間に一つのピークが見られたため(b)、保持時間からは粗生成物(c)におけるピーク A、B、C のいずれかが望みの化合物であると推定された。

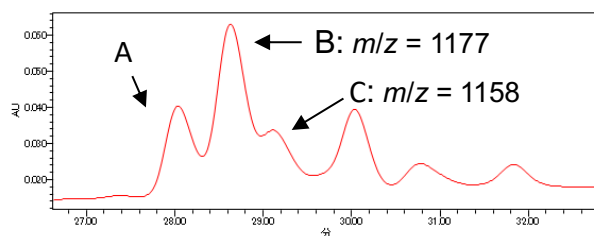
反応粗生成物と標品をダブルインジェクションした LC-MS チャートを Figure 21d に示す。このチャートから天然物はピーク B と一致したが、ピーク B の分子量はプラスバシン A<sub>3</sub> の加水分解体と同じであることから、反応粗生成物には望みの化合物が含まれていないことが明らかになった。

**Figure 21.** LC-MS analysis of the crude product

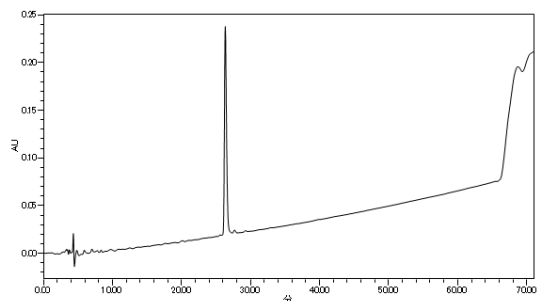
a) chromatogram of crude product



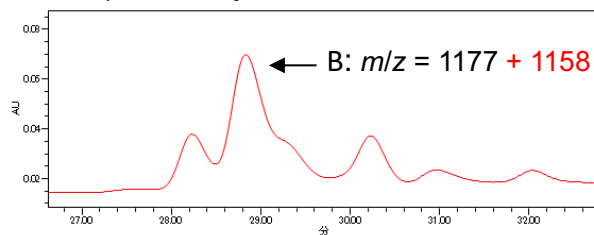
c) chromatogram of crude product



b) Chromatogram of natural plusbacin A<sub>3</sub>

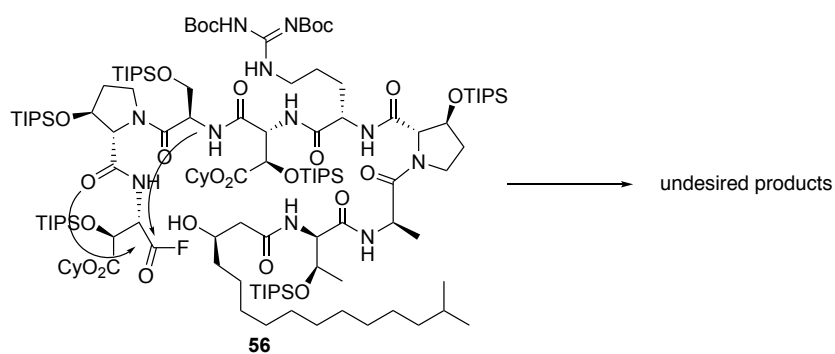


d) double injection of a and b



無水フッ化水素を用いた脱保護反応は VanNieuwenhze らによって報告されていることから、以上の結果はヒドロキシカルボン酸 **54** に対するマクロラクトン化が進行していなかったためであると推察される。カルボン酸と TFFH から生成する酸フッ化物中間体 **56** は反応性が高く、分子内には求核性を有するアミド結合が存在する。よって、酸フッ化物と水酸基が離れた配座をとっていたために、アミド酸素原子や窒素原子が反応することで、望みとしない位置で分子内環化反応が進行したものと考察した(Scheme 28)。

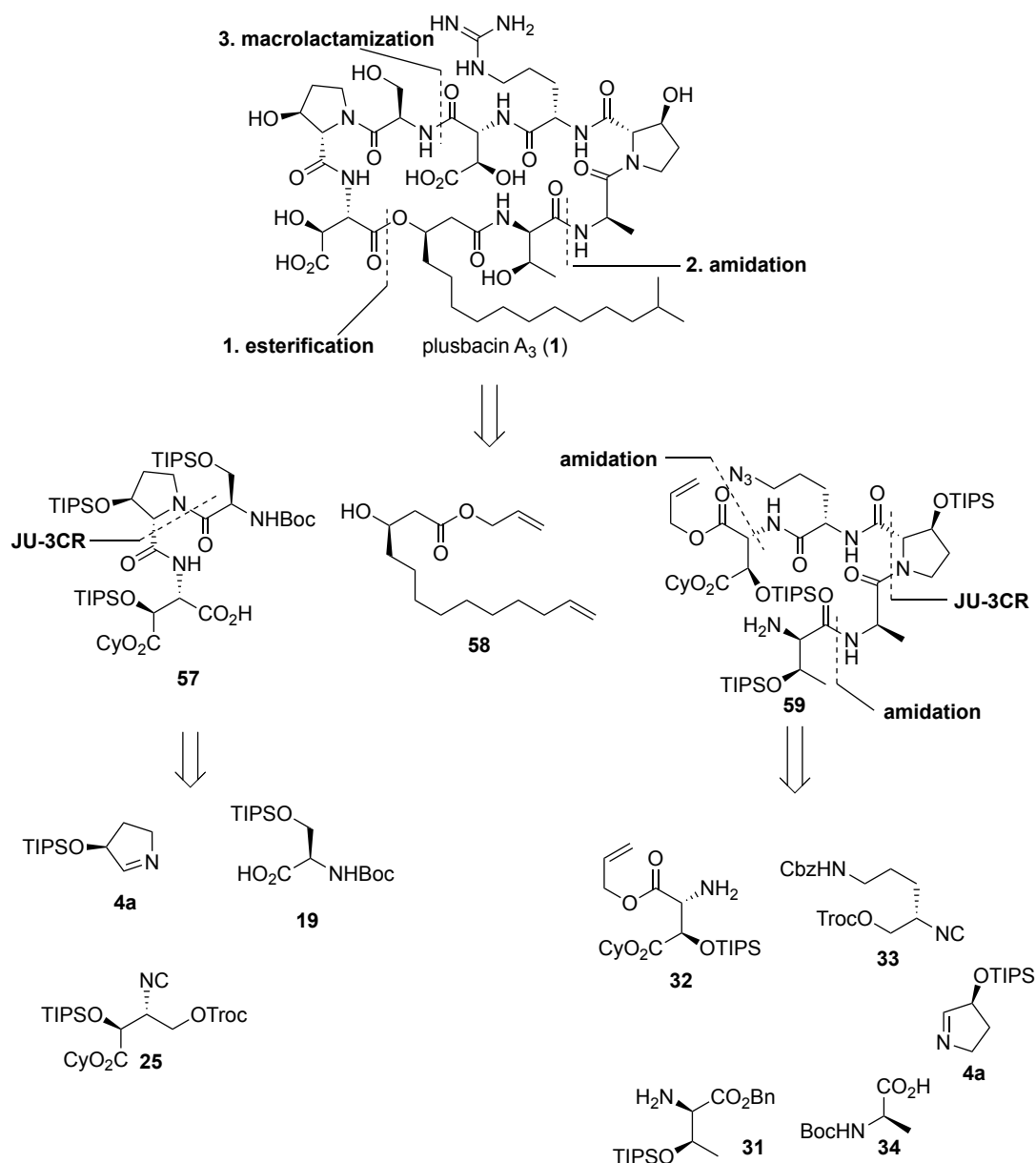
#### Scheme 28. Possible side reaction



ヒドロキシカルボン酸 **54** は望みのエステル結合を構築するのに適していないと考え、次に分子間でのエステル化によりエステル結合を構築する経路で合成研究を行うこととした。

### 第三節 プラスバシン A<sub>3</sub> の逆合成解析 (合成経路 2)

第二節で述べたように、プラスバシン A<sub>3</sub> のエステル部位はマクロラクトン化では構築できなかった。そこで分子間エステル化を経由する新しい合成経路を考案した。以下にその逆合成解析を示す(Figure 22)。



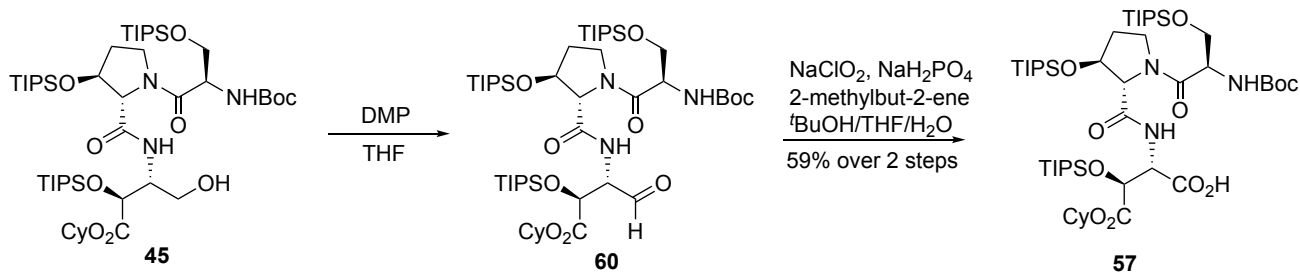
**Figure 22.** Retrosynthetic analysis of plusbacin A<sub>3</sub>: 2<sup>nd</sup> approach

プラスバシン A<sub>3</sub> の 28 員環構造は VanNieuwenhze らと同じ位置でのマクロラクタム化により構築することとし、環化前駆体は第二節で述べたのと同様に JU-3CR を用いて合成できるカルボン酸 **57** と、アルコール **58** とのエステル化、JU-3CR を用いて合成できるペプチド **59** とのアミド化を経て合成することとした。本合成経路では、カルボン酸をより単純な基質とすることで、マクロラクトン化で構築することができなかったエステル部位の構築が容易になると考えられる。

#### 第四節 プラスバシン A<sub>3</sub>の合成研究 (合成経路 2)

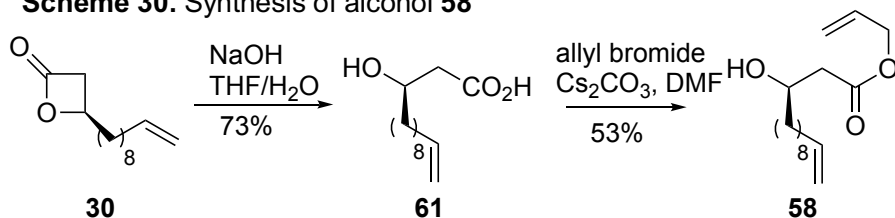
まず、エステル化反応の検討に用いるカルボン酸 **57** とアルコール **58** の合成を行った。

##### Scheme 29. Synthesis of carboxylic acid **57**



カルボン酸 **57** はアルコール **45** を Dess-Martin 酸化、Kraus 酸化の二工程で酸化することによって合成した(Scheme 29)。

##### Scheme 30. Synthesis of alcohol **58**



アルコール **58** は $\beta$ -ラクトン **30** を加水分解することによって得られる $\beta$ -ヒドロキシカルボン酸 **61** のカルボン酸をアリル基で保護することによって合成した(Scheme 30)。

**Scheme 31. Attempt to synthesis of ester 62**

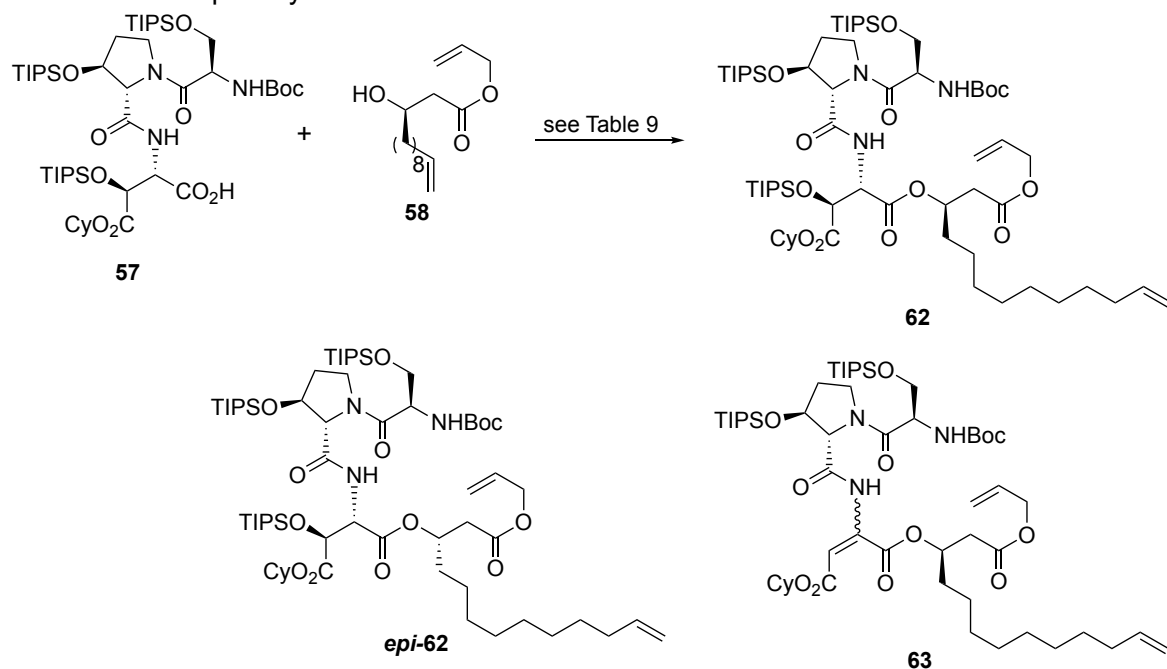
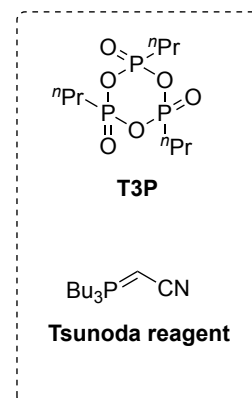


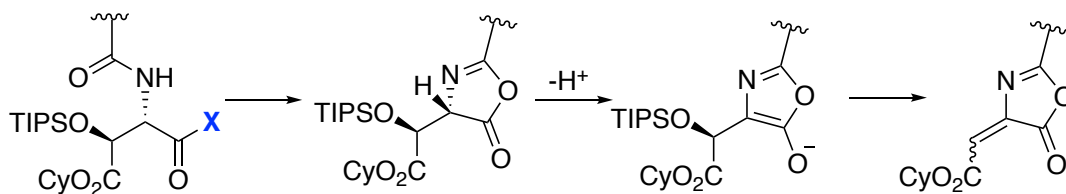
Table 9

entry	conditions	result
1	EDCI, DMAP, CH <sub>2</sub> Cl <sub>2</sub>	<b>63</b> (39%)
2	T3P, NMM, DMF	no reaction
3	TFFH, NMM, CH <sub>2</sub> Cl <sub>2</sub>	decomp.
4	DEAD, PPh <sub>3</sub> , benzene	no reaction
5	Tsunoda reagent, benzene	no reaction
6	Dy(OTf) <sub>3</sub> , toluene, reflux	no reaction



次にカルボン酸 **57** とアルコール **58** を用いるエステル化の検討を行った(Scheme 31)。EDCI、DMAP を用いた一般的なエステル縮合条件下では、エステル結合は形成されるものの、シロキシ基がβ脱離した化合物 **63** が得られた(Table 9, entry 1)。これはカルボン酸の活性化に伴いアズラクトンが生成し、α水素の酸性度が上昇したことが原因と考察した(Scheme 32)。なお、生成物のオレフィンの幾何異性は未決定である。

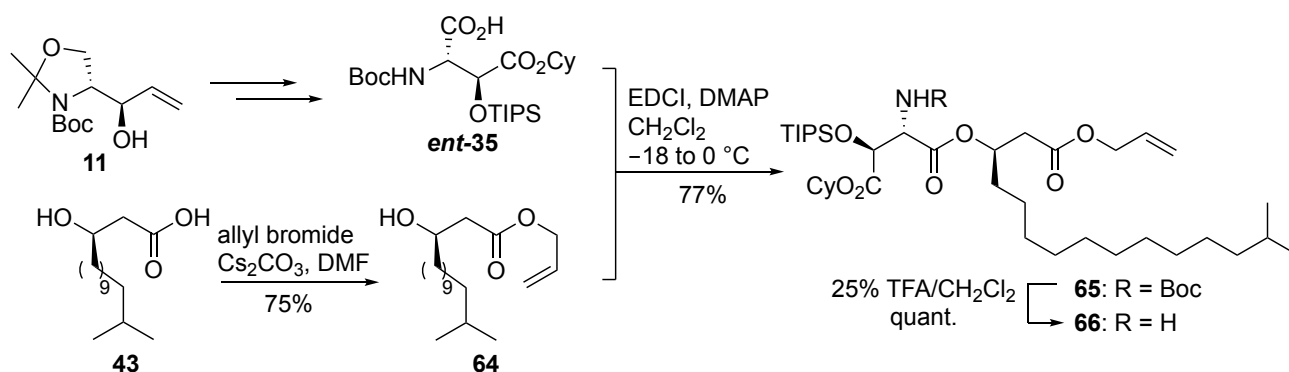
**Scheme 32. Mechanism of the side reaction**



縮合剤として T3P<sup>71</sup> を用いた場合は反応が進行せず(entry 2)、TFFH を用いた場合は基質の分解が進行した(entry 3)。また、DEAD や角田試薬<sup>72</sup> を用いた光延反応条件も試みたが、いずれも反応は進行しない結果となった(entries 4, 5)。最後に Lewis 酸として Dy(OTf)<sub>3</sub> を用いた条件<sup>73</sup> においても望みの化合物 **62** は全く得られなかった(entry 6)。

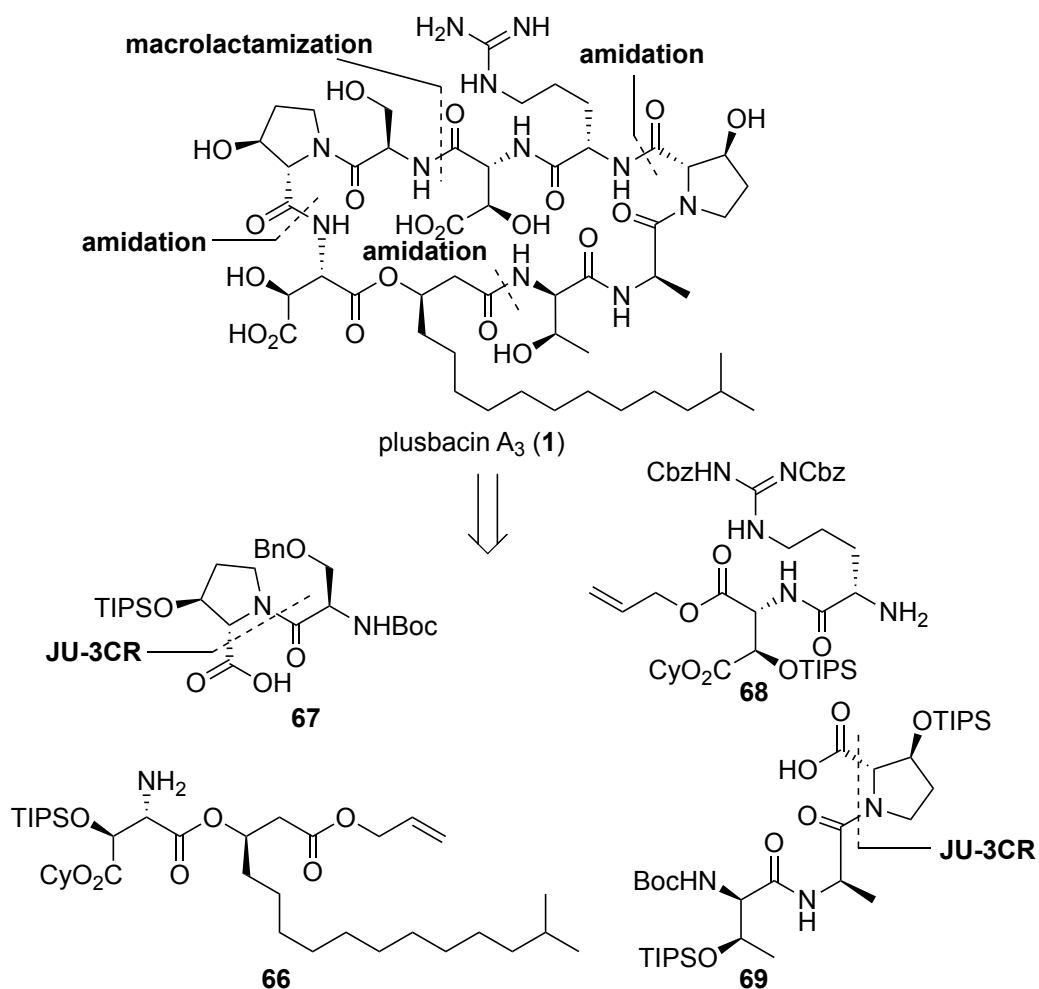
Table 9, entry 1 の結果からアズラクトンの形成を抑制することができれば、副反応のβ脱離が抑制できると考えられたため、このような環化反応を抑制するべく、アミノ基をカーバメートで保護することとした。

**Scheme 33. Ester formation**



化合物 **11** から導いたアミノ基を Boc 基で保護した *threo*-β-ヒドロキシ-Asp 保護体 **ent-35** と、ヒドロキシカルボン酸 **43** のカルボン酸をアリル基で保護したアルコール **64** を用いて脱水縮合を行うと、収率 77% で望みのエステル **65** が得られ、シロキシ基の脱離は見られなかった(Scheme 33)。続く変換を見据え、**65** の Boc 基を酸性条件下で除去することでアミン **66** を得た。このアミンから誘導できるイソシアニドはα-イソシアノカルボニル構造を有するため、第一節で述べたように JU-3CR の基質として適していないと考えられる。そこで、このアミンを C 末端がカルボン酸である Pro(3-OH) と脱水縮合することで合成を進めることとし、再度合成経路の変更を行った。

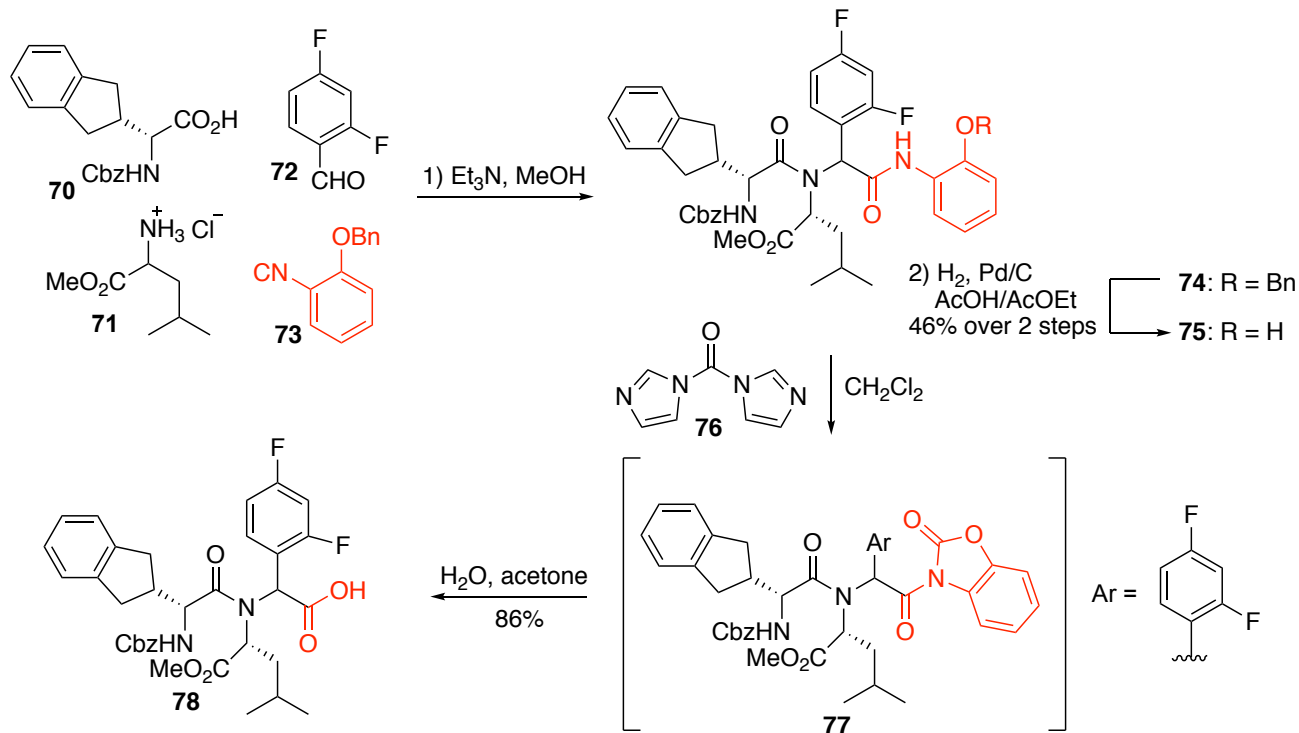
第五節 プラスバシン A<sub>3</sub> の逆合成解析 (合成経路 3)



**Figure 23.** Retrosynthetic analysis of plusbacin A<sub>3</sub>: 3<sup>rd</sup> approach

上図に筆者が再度考案した逆合成解析を示す(Figure 23)。28 員環の構築は VanNieuwenhze らの報告と同じ位置でのマクロラクタム化により行うこととし、環化前駆体は Pro(3-OH)セグメント **67**、**69** とアミン **66**、**68** から合成することとした。C 末端がカルボン酸である Pro(3-OH)セグメント **67**、**69** は JU-3CR により直接合成することができないため、コンバーチブルイソシアニドを用いた JU-3CR により合成することとした。

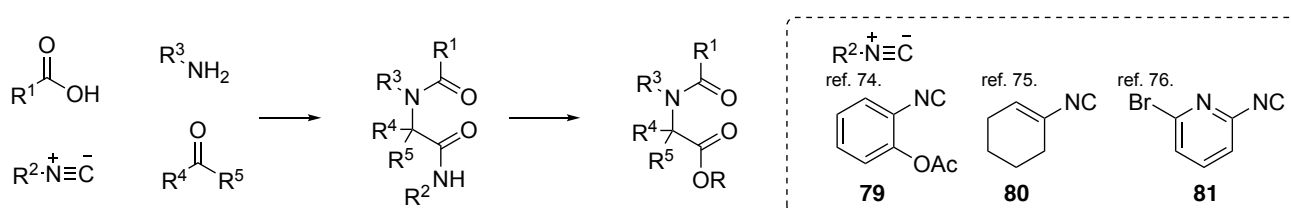
**Scheme 34.** Example of convertible isocyanide by Borthwick *et al.*



Borthwick らは、コンバーチブルイソシアニド **73**<sup>\*10</sup> を用いて合成した Ugi 成績体 **74** のベンジル基を除去して得たフェノール **75** に対して、1,1'-carbonyldiimidazole (CDI, **76**) を作用させることで、カルボニル基を *N*-アシルオキサゾリジノン **77** として活性化し、これを加水分解することでカルボン酸 **78** へと変換している (Scheme 34)<sup>74</sup>。この報告を参考に同様の芳香族イソシアニドを用いて Pro(3-OH)セグメント **67**、**69** の合成を行うこととした。

\*<sup>10</sup>Ugi 反応により、生成物にはイソシアニド由来のアミド結合が構築されるが、これを他の官能基へと変換することができれば、有機合成化学上さらに有用である。このような観点の元、このアミド結合を選択的にカルボン酸やエステルへと変換できるイソシアニド **79-81** が考案されている<sup>75-77</sup> (Scheme 35)。これらはコンバーチブルイソシアニドと呼ばれイソシアニド **73** はその中でも温和な条件下、アミド結合を他の官能基へと変換できる。

**Scheme 35.** Convertible isocyanide

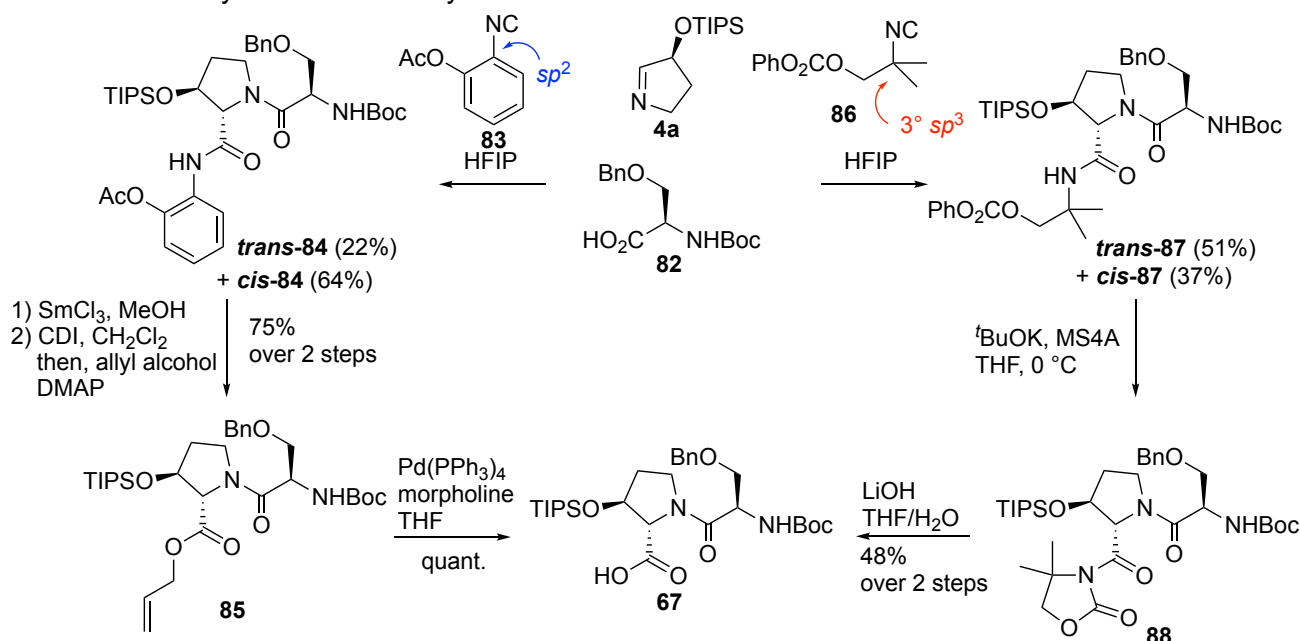




## 第六節 プラスバシン A<sub>3</sub>の全合成 (合成経路3)

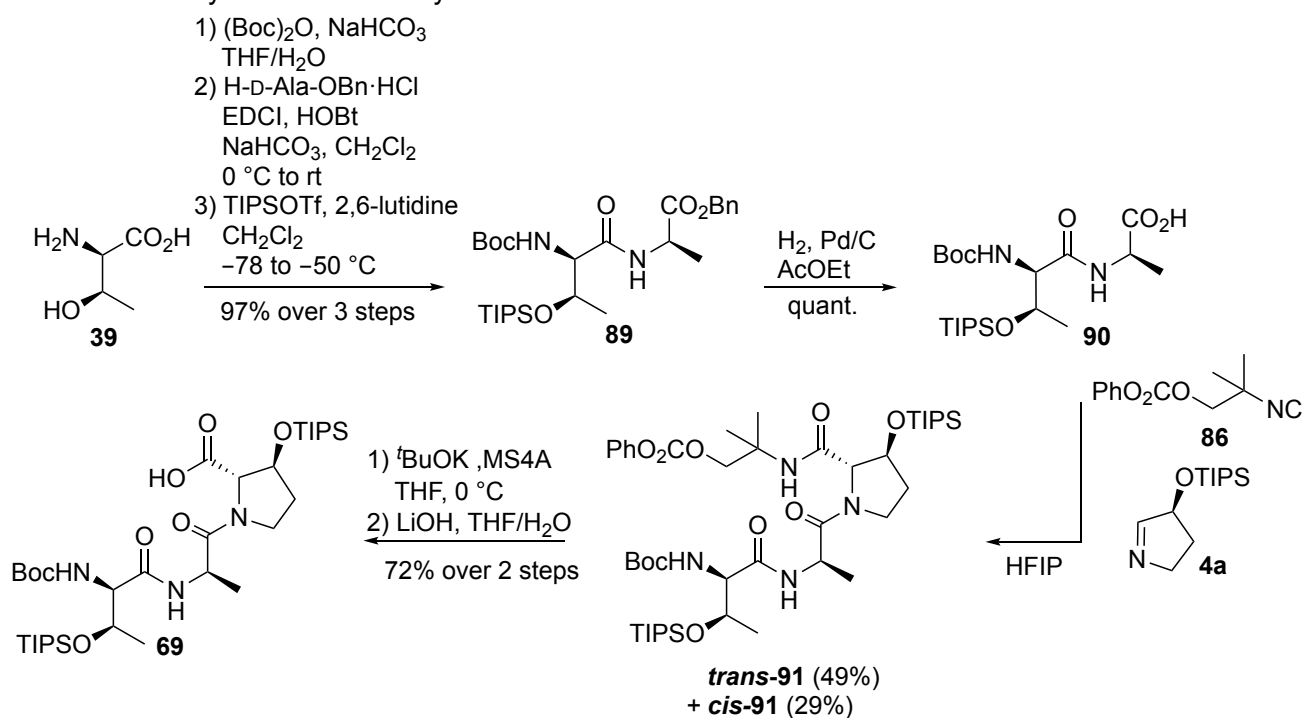
コンバーチブルイソシアニドを用いた JU-3CR により Pro(3-OH)セグメントを合成することとし、まずはセグメント **67** の合成を行った(Scheme 36)。

**Scheme 36.** Synthesis of carboxylic acid **67**



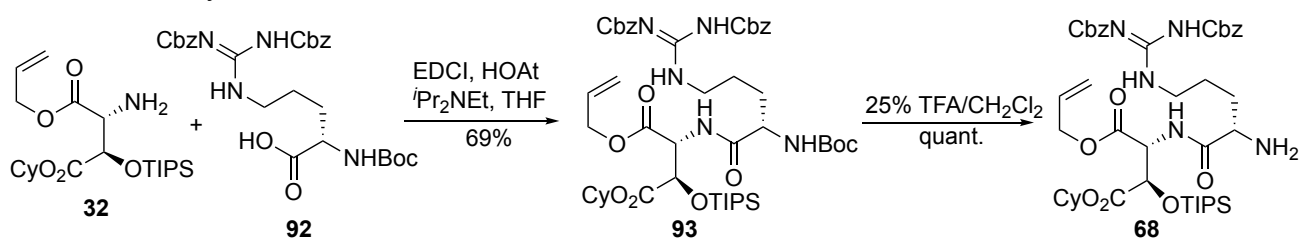
五員環イミン **4a** と D-Ser 保護体 **82**、コンバーチブルイソシアニド **83** を用いて HFIP 中で JU-3CR を行った。その結果 Joullié-Ugi 成績体は得られたものの、望みとしないジアステレオマー **cis-84** が収率 64% で優先して得られ、望みの **trans-84** の収率は 22% であった ( $trans/cis = 74/26$ )。望みのジアステレオマー **trans-84** のアセチル基を三価サマリウムを用いて除去し、*N*-アシルオキサゾリジノンを経てアリルエステル **85** へと変換した。このアリル基を 0 価パラジウム触媒を用いて除去することでセグメント **67** を合成した。このように、JU-3CR のジアステレオ選択性に課題を残すものの、コンバーチブルイソシアニド **83** を用いて Pro(3-OH)セグメントを合成することができた。第一章で述べたように、本反応のジアステレオ選択性はイソシアニドの電子密度に影響を受け、電子豊富なイソシアニドを用いた際に *trans* 選択性は向上する。イソシアニド **83** はイソシアノ基に電子求引性の  $sp^2$  炭素原子が結合しており、この電子求引性誘起効果により、*cis* 体が優先して得られたものと考えられる。そこで福山、菅らにより報告された第三級アルキル基を有するイソシアニド **86**<sup>78</sup> を用いたところ、ジアステレオ選択性は改善し、望みのジアステレオマー **trans-87** が収率 51% で得られた ( $trans/cis = 58/42$ )。この **trans-87** に <sup>t</sup>BuOK を作用させると、フェノールの脱離に伴い分子内環化反応が進行し、*N*-アシルオキサゾリジノン **88** が得られた。これを加水分解することで、セグメント **67** へと導いた。

**Scheme 37. Synthesis of carboxylic acid 69**



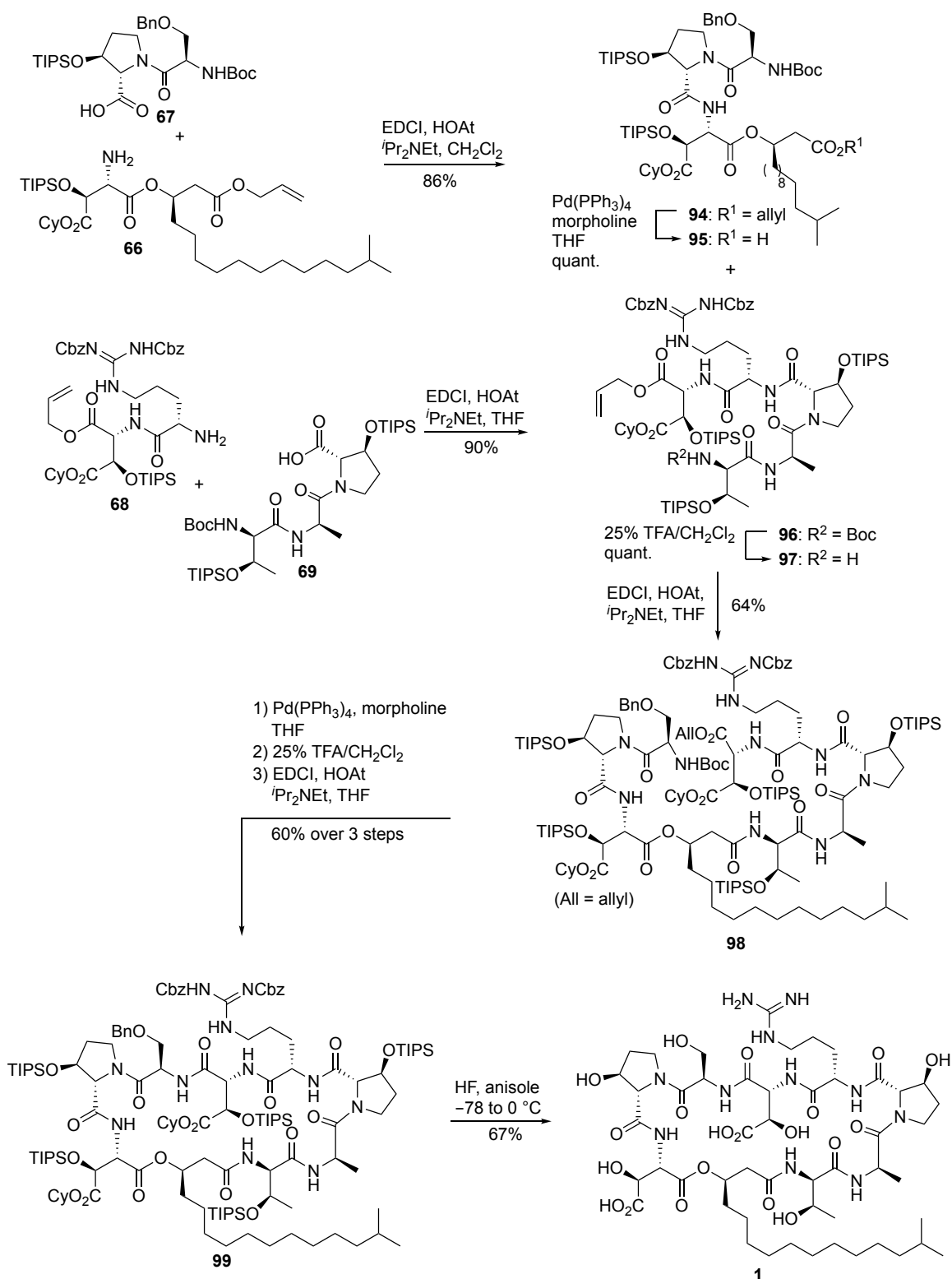
次に同様の合成法を用いてもう一方の Pro(3-OH)セグメント **69** の合成を行った(Scheme 37)。Allo-D-Thr (**39**)のアミンを Boc 化し、D-Ala 保護体との縮合と、第二級水酸基の TIPS 化を経てジペプチド **89** を三工程収率 97%で得た。接触水素還元によりベンジル基を除去することで得られたカルボン酸 **90** と五員環イミン **4a**、コンバーチブルイソシアニド **86** を用いた HFIP 中での JU-3CR により、Joullié-Ugi 成績体 **trans-91** を得た。これを N-アシルオキサゾリジノンを経由してカルボン酸へと変換し、セグメント **69** を合成した。

**Scheme 38. Synthesis of amine 68**



残りのセグメント **68** は D-threo-β-ヒドロキシ-Asp 保護体 **32** と Arg 保護体 **92** の縮合と、Boc 基の除去を経て合成した(Scheme 38)。

**Scheme 39.** Total synthesis of plusbacin A<sub>3</sub>



4つのセグメントの合成を終えたので、プラスバシン A<sub>3</sub>の全合成に向けてセグメントの連結を行った (Scheme 39)。カルボン酸 **67** とアミン **66** の縮合によりテトラデプシペプチド **94** を得た後、C末端のアリル基を除去することでカルボン酸 **95** を合成した。また、カルボン酸 **69** とアミン **68** から脱水縮合と N末端 Boc の除去によりアミン **97** を合成した。合成したアミン **97** とカルボン酸 **95** を縮合し、収率 64%で環

化前駆体を得た。C末端のアリル基とN末端Bocを除去し、EDCIとHOAtを用いてマクロラクタム化を行うと、環化反応が進行し、望みの環化体 **99** が三工程収率 60%で得られた。この環化体 **99** に対してアニソール存在下無水フッ化水素を作用させることですべての保護基を除去し、プラスバシン A<sub>3</sub>の全合成を達成した。合成したプラスバシン A<sub>3</sub>の逆相 HPLC 分析を行ったところ(Figure 24)、保持時間は 10.6 min であり天然物と完全に一致した(Figure 24a, d, e)。

column: COSMOSIL 5C<sub>18</sub>-MS-II; eluent: 47.5% MeCN/H<sub>2</sub>O (0.1% TFA);  
flow 1 mL/min; detection: UV 210 nm

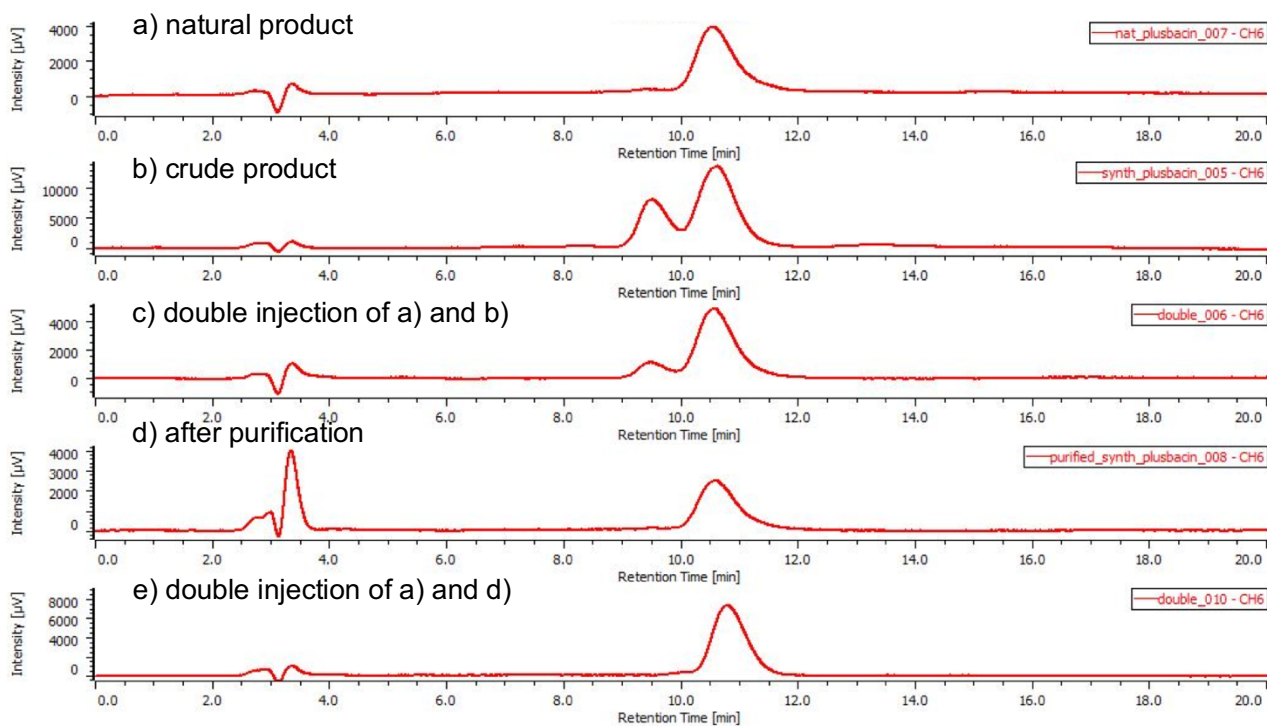
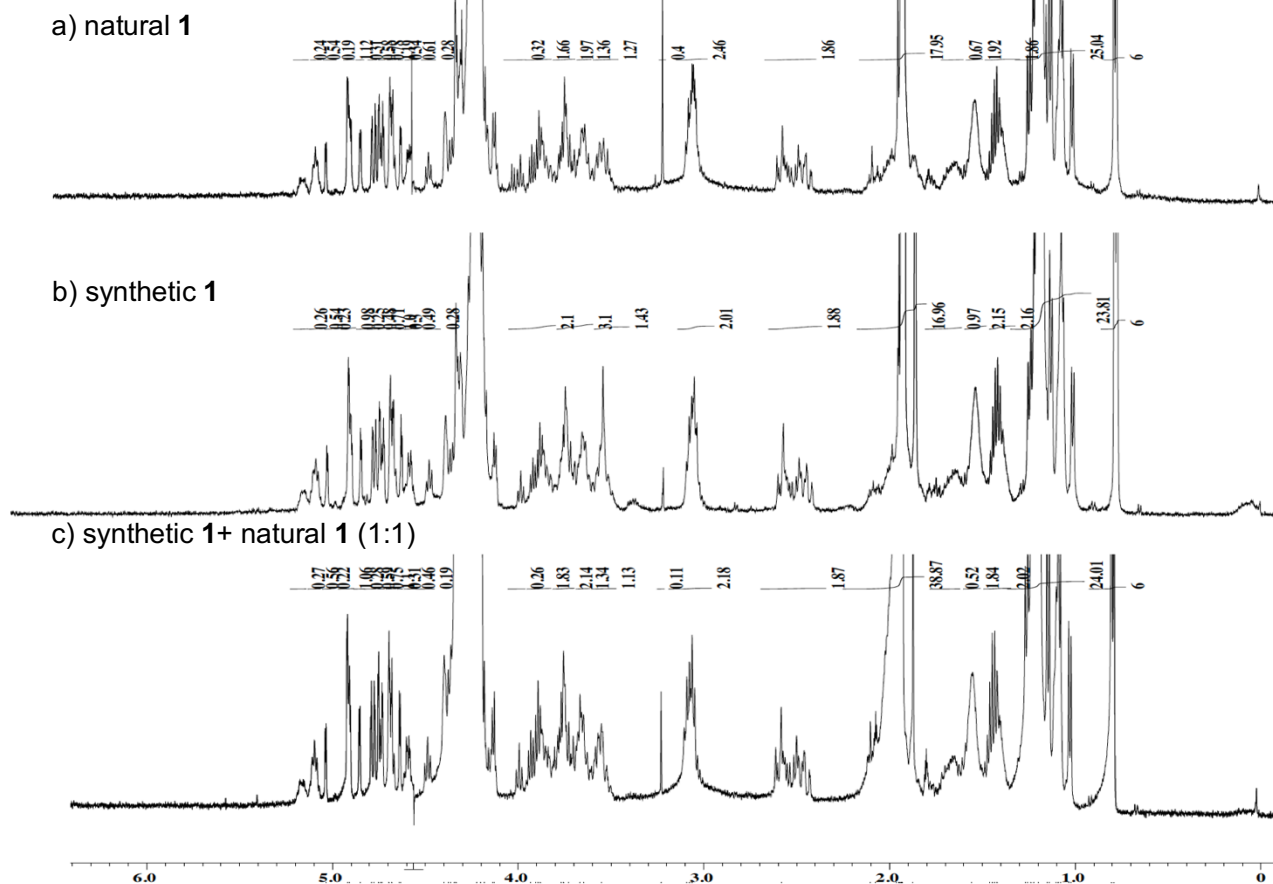


Figure 24. HPLC analysis of synthetic plusbacin A<sub>3</sub>

また、合成品と天然物の  $^1\text{H NMR}$  スペクトルの比較を行った(Figure 25)。両者のスペクトルはほとんどの部分で良い一致を示した(Figure 25a, b)。ペプチドのイオン型による僅かなスペクトルの違いが見られたため、両者を 1:1 で混合し、 $^1\text{H NMR}$  スペクトルを測定したところ、一つのスペクトルに収束した(Figure 23c)。従って、合成品が天然物と確かに一致することが確認できた。

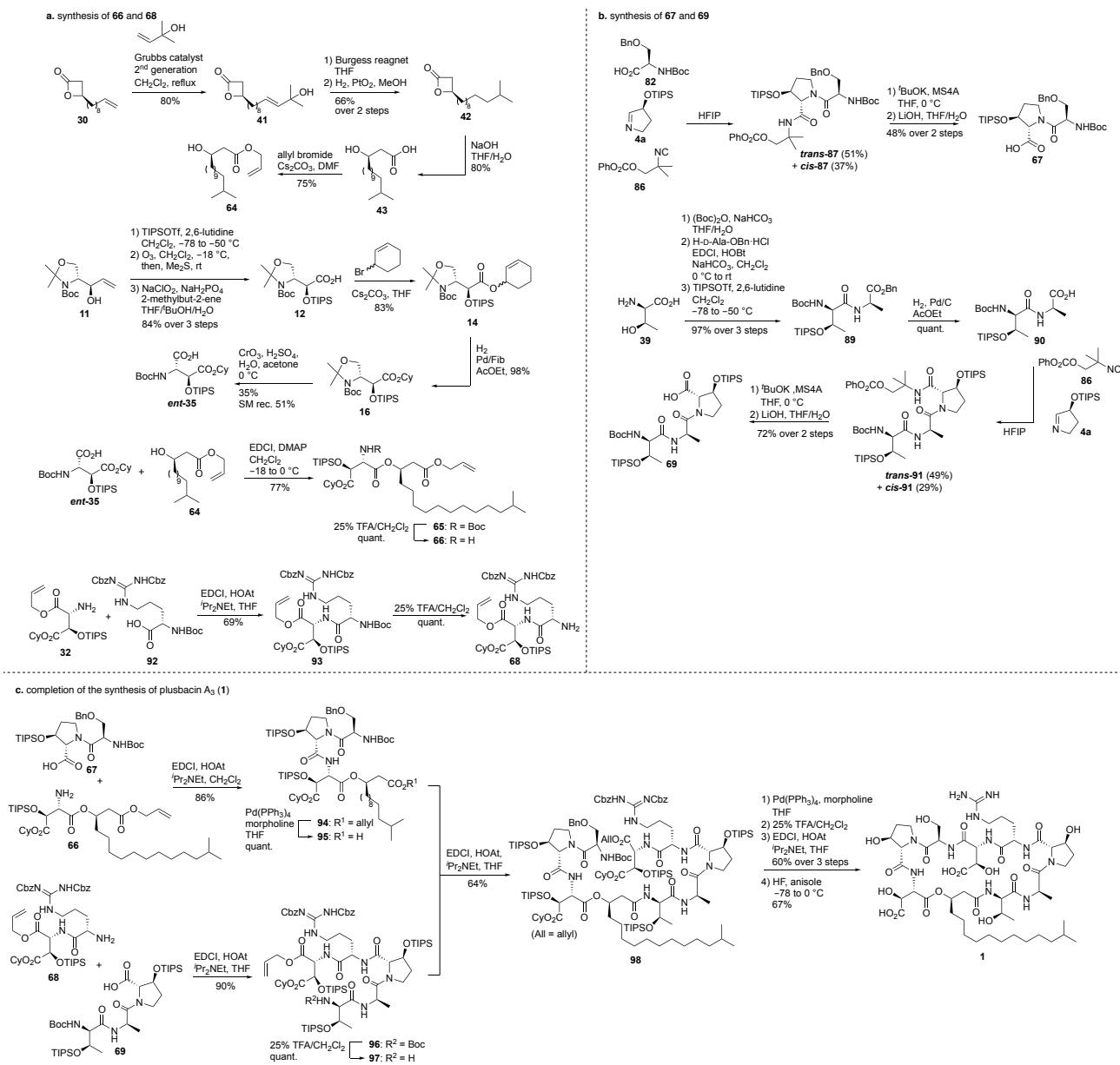
$^1\text{H NMR}$  (500 MHz,  $\text{D}_2\text{O}/\text{CD}_3\text{CN}/\text{TFA} = 500/500/1$ )



**Figure 25.** Comparison of  $^1\text{H NMR}$  spectra of synthetic and natural lplusbacin  $\text{A}_3$

以上より、筆者はプラスバシン A<sub>3</sub> の全合成を達成した(Scheme 40)。

### Scheme 40. Summary of synthesis of plusbacin A<sub>3</sub> (1)



## 第七節 ジデオキシ誘導体の合成

プラスバシン A<sub>3</sub>の全合成を達成したため、次に誘導体の合成に着手した。序論で述べたようにプラスバシン A<sub>3</sub>はその類縁体であるエンペドペプチンと同様の作用機序で抗菌活性を示すと考えられており、この際に Ca<sup>2+</sup>とカルボン酸の結合が重要であると考えられる。そこで、*threo*-β-ヒドロキシ-Aspの水酸基はプラスバシン A<sub>3</sub>の抗菌活性に影響しないと考え、合成に多段階を要すこのアミノ酸残基を Asp に変換したジデオキシ誘導体 **100** を設計した(Figure 26)。

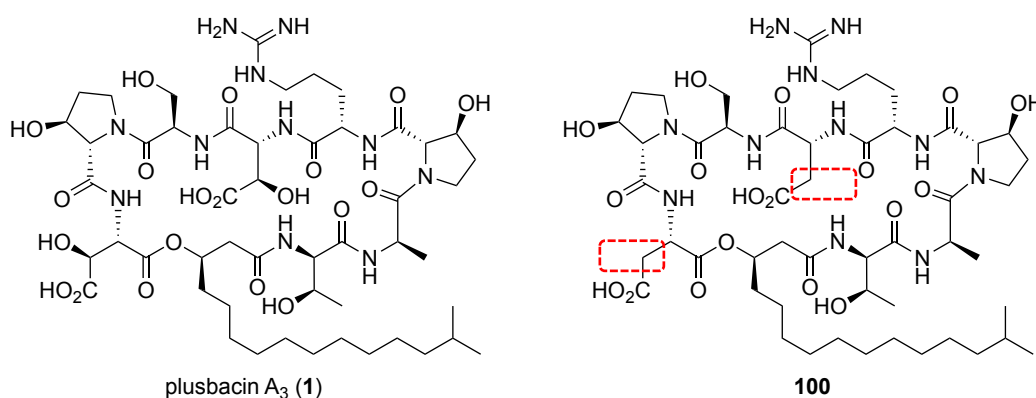
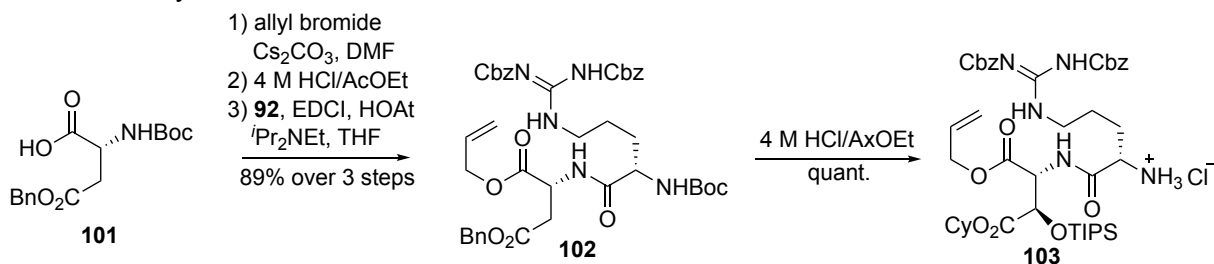


Figure 26. Chemical structure of dideoxy analogue **100**

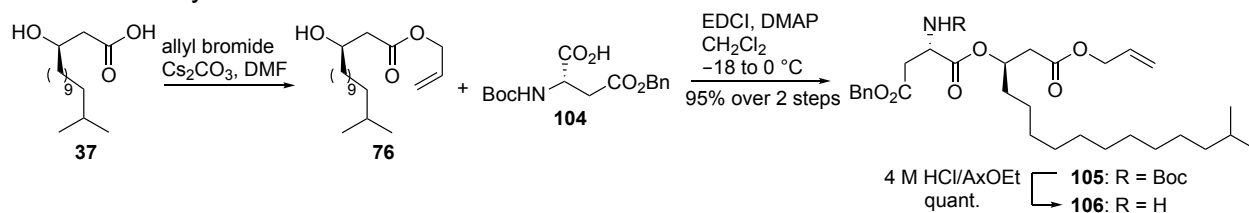
ジデオキシ誘導体 **100** はプラスバシン A<sub>3</sub>の全合成に用いたセグメントの *threo*-β-ヒドロキシ-Asp 残基を Asp 残基に変換したセグメントを用いて同様に合成することができると考え、セグメントの合成を行った。

### Scheme 41. Synthesis of amine **103**



市販の D-Asp 保護体 **101** の保護基を適切に変換し、得られたアミンと Arg 保護体 **92** とのペプチドカップリングによりジペプチド **102** を三工程収率 89%で得た。得られたジペプチド **102** の Boc 基を除去することで望みのアミン **103** を得た(Scheme 41)。

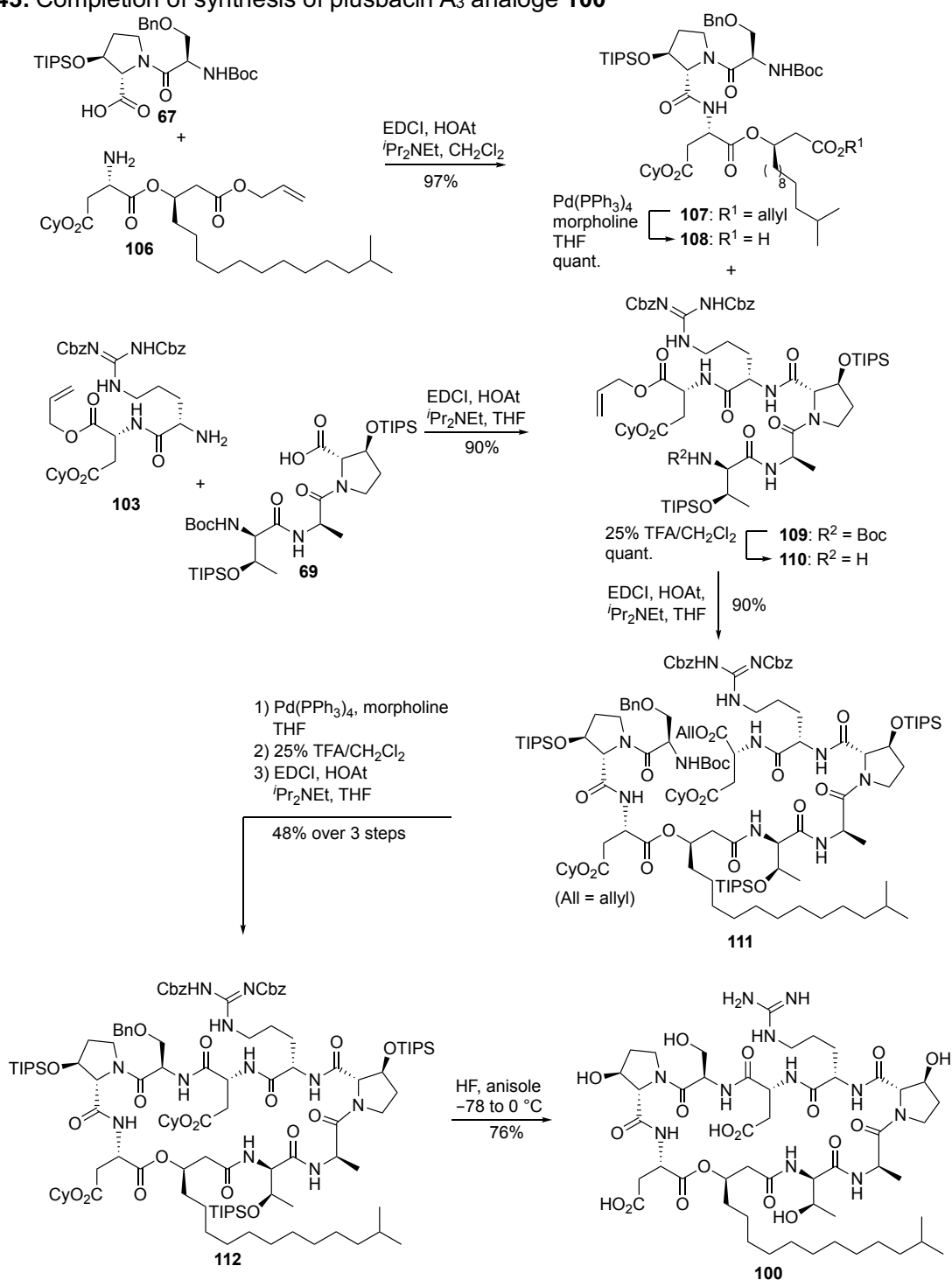
### Scheme 42. Synthesis of amine **106**



3-ヒドロキシイソヘキサデカン酸(**43**)のカルボン酸をアリル基で保護した後、L-Asp 保護体 **104** と脱水縮合することでエステル **105** を得た。このエステルの Boc 基を酸性条件下除去することでアミン **106** を塩酸塩として得た(Scheme 42)。



**Scheme 43.** Completion of synthesis of plusbacin A<sub>3</sub> analoge **100**



望みの二つのセグメントを合成したので、プラスバシン A<sub>3</sub>の全合成と同様、セグメントの連結と環化反応を行った(Scheme 43)。セグメント **67** と **106** の脱水縮合とアリル基の除去により **108** を、**103** と **69** の脱水縮合と Boc 基の除去により **110** をそれぞれ合成した。合成した **108** と **110** をアミド縮合することで鎖状ノナデプシペプチド **111** を収率 90%で得た。次に、C末端アリル基と N末端 Boc 基をそれぞれ除去し、高希釈条件下で EDCI、HOAt を作用させることでマクロラクタム化を行い、三工程収率 48%で環化

体 **112** を合成した。最後に、全ての保護基をアニソール存在下、無水フッ化水素を用いて除去し、逆相 HPLC による精製を経て、ジデオキシ誘導体 **100** を収率 76% で得た。

以上のように筆者は Joulli-Ugi 反応を鍵反応としたプラスバシン A<sub>3</sub>(**1**)の全合成を達成し、本合成経路を利用してプラスバシン A<sub>3</sub>のジデオキシ誘導体 **100** も合成した。続く第三章では合成したプラスバシン A<sub>3</sub>とジデオキシ誘導体 **100** の生物活性について述べることとする。

### 第三章 プラスバシン A<sub>3</sub> 及び誘導体の生物活性

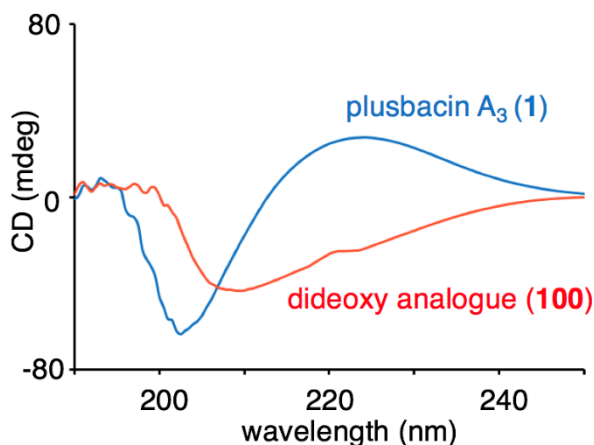
#### 第一節 プラスバシン A<sub>3</sub> 誘導体と天然物の構造と生物活性の比較

**Table 10.** Minimum inhibitory concentrations of **1** and **100**

Strain	MICs <sup>a</sup> (μg/mL)	
	plusbacin A <sub>3</sub> ( <b>1</b> )	dideoxy analogue <b>100</b>
Smith (MSSA)	2	-
MSSA1 (MSSA)	2	>128
MR-6 (MRSA)	1	>128

<sup>a</sup> minimum inhibitory concentration

合成したプラスバシン A<sub>3</sub> とジデオキシ誘導体 **100** の抗菌活性を測定した (Table 10)。プラスバシン A<sub>3</sub> は黄色ブドウ球菌(Smith, MSSA1, MR-6)に対して MIC 値 1-2 μg/mL で抗菌活性を示したのに対し、ジデオキシ誘導体 **100** の MIC 値は MSSA1、MR-6 のいずれに対しても 128 μg/mL 以上であり、抗菌活性は認められなかった。この結果はプラスバシン A<sub>3</sub> (**1**)に含まれる *threo*-β-ヒドロキシ-Asp の水酸基が活性に必須であることを示している。活性消失の理由として、分子内水素結合の様式が変化したことによる化合物配座の変化が考えられたため、CD スペクトルを用いて、化合物の配座の比較を行った。



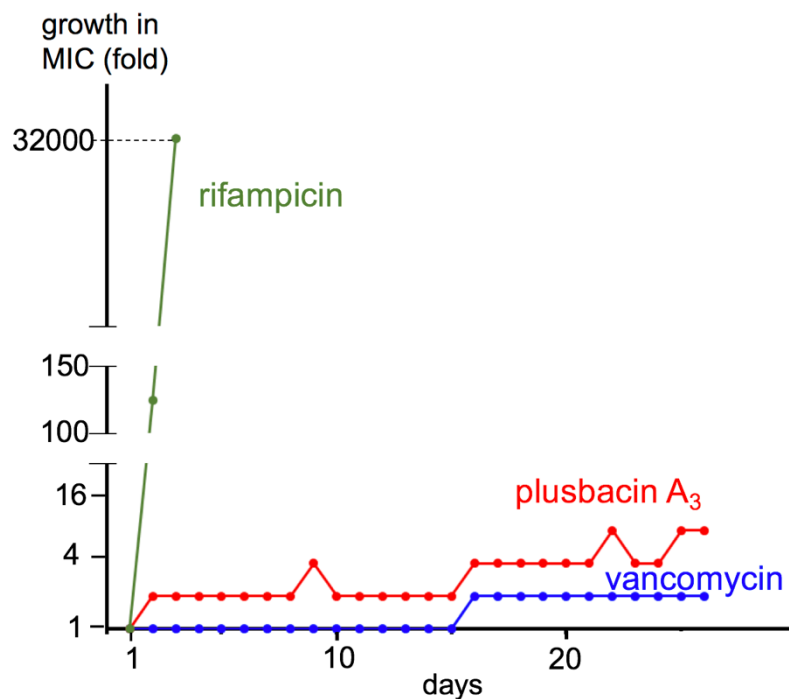
**Figure 27.** CD spectra of **1** and **100**

Figure 27 に **1** と **100** の CD スペクトルを示した。青線で示したプラスバシン A<sub>3</sub> スペクトルは 223 nm 付近に正の、203 nm 付近に負の極大が見られる。これに対し、赤線で示したジデオキシ誘導体のスペクトルには正の極大が見られなかった。このように CD スペクトルから、両者の二次構造が大きく異なることが示唆された。従って、プラスバシン A<sub>3</sub> の *threo*-β-ヒドロキシ-Asp の水酸基は分子内水素結合により環状ペプチドの構造を活性配座に保つ役割を担っていると考えられ、この水素結合については第五章

で詳述する。以上のように、抗菌活性に対する水酸基とプラスバシン A<sub>3</sub> の構造の重要性を示すことができた点は、今後の構造活性相関研究に有用な情報を与えるものといえる。

## 第二節 プラスバシン A<sub>3</sub> に対する薬剤耐性と耐性株に対するゲノムシーケンス解析

序論で述べたようにリピド II に結合する化合物は薬剤耐性に抵抗性示すものが多く、プラスバシン A<sub>3</sub> も同様の性質を示すものと予想される。そこで、プラスバシン A<sub>3</sub> に対する薬剤耐性がどの程度生じるのかを調べることにした。黄色ブドウ球菌 Smith 株に対しプラスバシン A<sub>3</sub> を低濃度で作用させ、時間経過に伴い MIC 値が増大する割合を調べた。また、一般的な抗菌薬の一例としてリファンピシンを、薬剤耐性に抵抗性を示す化合物としてバンコマイシンを用いて同様の実験を行った。その結果、リファンピシンの MIC 値は 3 日後に 32000 倍に増大するのに対し、プラスバシン A<sub>3</sub> の MIC 値は 25 日後においても 8 倍の増大にとどまった(Figure 28)。これはバンコマイシンと比較しても 4 倍の値であり、プラスバシン A<sub>3</sub> が薬剤耐性に抵抗性を有することを明らかにした。



**Figure 28.** Resistance acquisition during serial passaging in the presence of sub-MIC levels of antimicrobials. The x axis is the number of days, and the y axis is the highest concentration of the cells during passaging.

次に、この実験により得られたプラスバシン A<sub>3</sub>耐性株に対するゲノムシーケンス解析を行った。解析の結果、いくつかのタンパク質をコードする遺伝子に変異が見られた。この結果を Figure 29 に示す。Figure 29 に示した変異によりタンパク質の機能がどのように変化したかは不明であるため、詳細な議論はできないものの、VraE に対する変異は、リポド II に結合する抗菌ペプチドであるナイシンに対する耐性株からも同様に報告されており<sup>79</sup>、この結果はプラスバシン A<sub>3</sub> の標的分子がリポド II である可能性を支持する結果であると考えられる。

Protein	Mutation
alpha/beta hydrolase	Arg48His; Ile18Thr
ArcR	Ser27Pro
Bacteriophage	Val2Leu
Capsular polysaccharide synthesis enzyme CapA	Ile50Val
Di-tripeptide ABC transporter	Gly61Val
Hypothetical protein	Tyr154fs
Integrase: 7 total mutations	*402fs; Glu393fs; Glu397*; His383fs; Lys398*; Lys398Asn; Val400del
Lip2	*602del
Phage tail protein	His72Tyr; Ser41Ala
Putative periplasmic-iron-binding protein BitC	Ser88Leu
Putative uncharacterized protein	Leu7Ser
Similar to two-component response regulator	Ser105Leu
Transposase	Asp24Asn; Phe153Leu; Ser151Pro
VraE	Pro325Ala

**Figure 29.** List of proteins associated with plusbacin A<sub>3</sub>-resistant *S. aureus* suggested by the genome sequence analysis

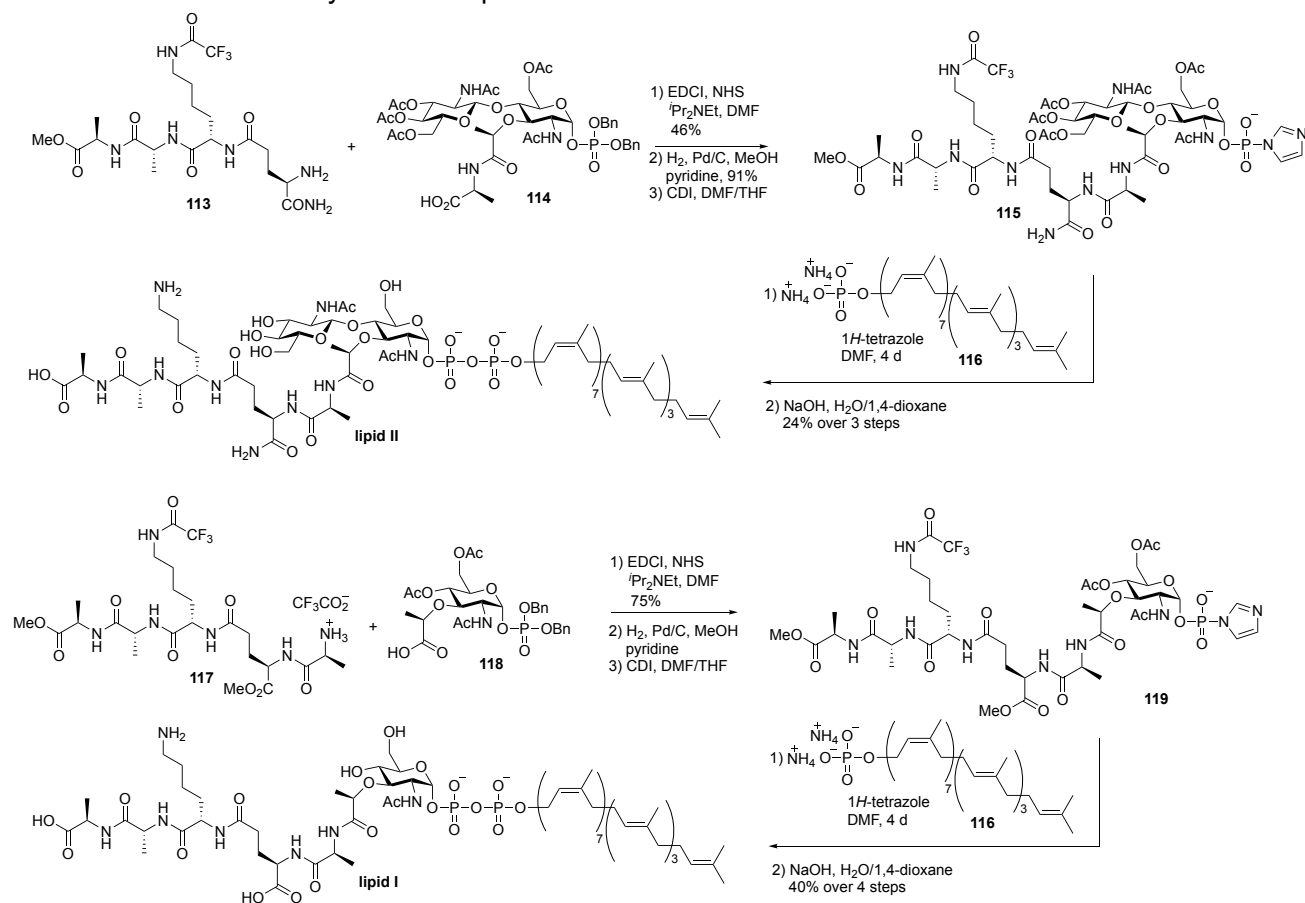
## 第四章 細胞壁合成前駆体の固相全合成

### 第一節 合成計画

これまでに述べたように、プラスバシン A<sub>3</sub> の標的分子はリポド II であることが示唆されるが、どのような結合様式で結合するのかは未解明であった。この結合様式を解明することができれば、プラスバシン A<sub>3</sub> をリードとした新規抗菌薬の設計指針が得られるものと考え、次にプラスバシン A<sub>3</sub> とリポド II の相互作用を調べることを計画した。

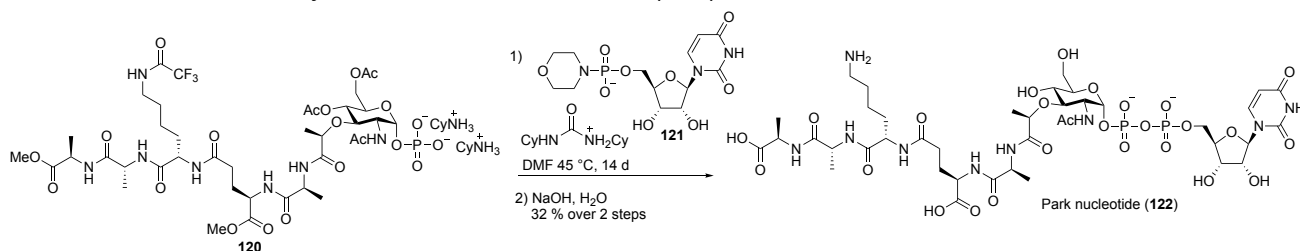
リポド II は細菌の膜成分であるが、その存在量は極めて少なく、グラム陰性細菌の場合には一細胞あたり 2000 分子程度しか存在しない<sup>80</sup>。このような背景から、リポド II やその生合成前駆体のリポド I、Park ヌクレオチドの量的供給を目的として、これまでにこれら細胞壁生合成前駆体及びその誘導体の化学合成が報告されている。Scheme 44 に VanNieuwenhze らにより報告されたリポド I 及びリポド II の初の全合成例を示す<sup>81,82</sup>。いずれも同様の合成戦略により全合成が達成されている。ペプチドセグメント **113** または **117** と糖セグメント **114** もしくは **118** との脱水縮合により糖ペプチドを合成し、このリン酸基をイミダゾリドとして活性化することで **115**、**119** へと導いている。この活性化されたリン酸に対しウンデカプレニルリン酸 **116** を反応させることでリポド I、リポド II の全合成を達成している。

**Scheme 44.** First total synthesis of lipids I and II



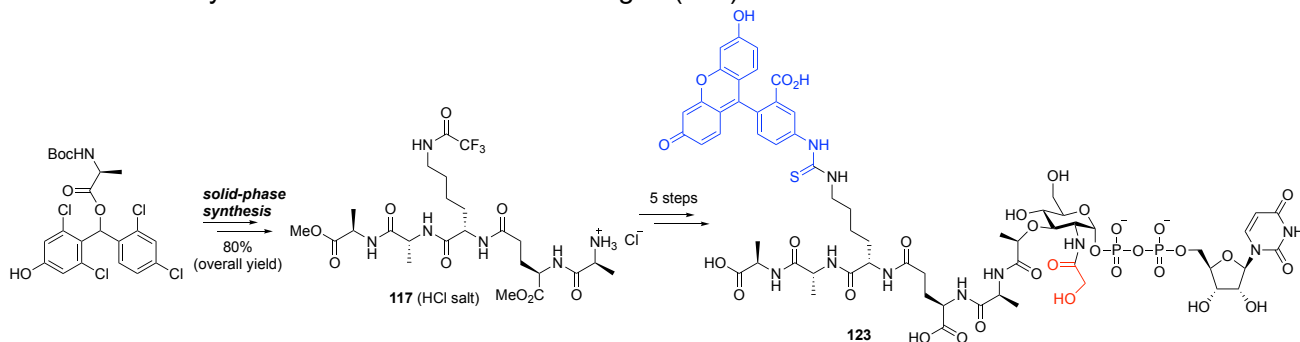
また、Parkヌクレオチド (**122**)の初の全合成は Hitchcock らにより達成されており、ウリジン 5'-モノリン酸から調製したモルホリデート **121** とモノリン酸 **120** を用いたリン酸基間の縮合により全合成が達成されている(Scheme 45)<sup>83</sup>。非対称ジリン酸化の際に活性化するモノリン酸が異なるという違いがあるものの、いずれの例でも、リン酸基間の縮合には 4 日から 2 週間の長時間を要し、その収率も低いことが問題であった。

#### Scheme 45. First total synthesis of Park nucleotide (**122**)



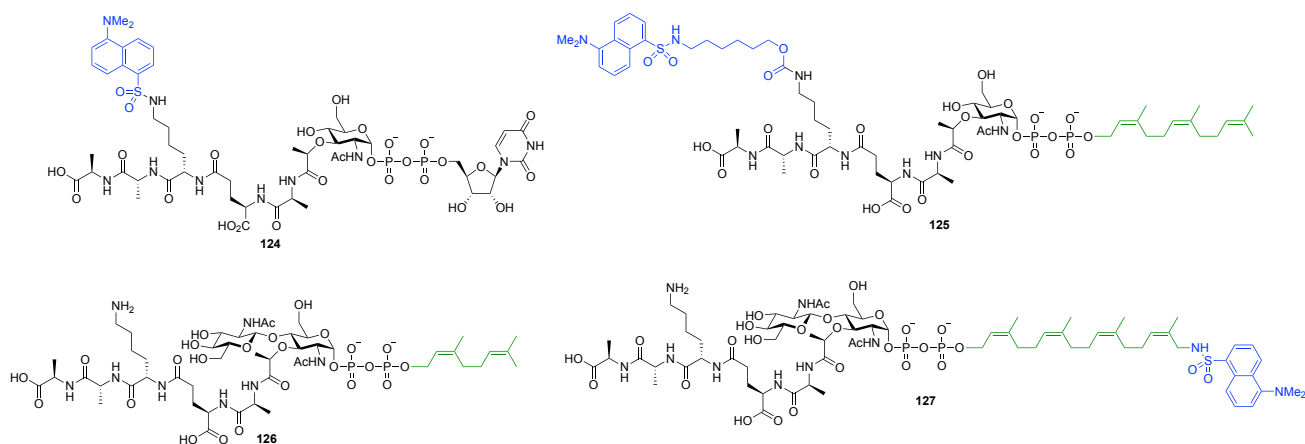
リピド II はペプチド、糖、ジリン酸、脂溶性側鎖という性質の異なる成分を同一分子内に有しており、上記のような液相での合成の場合、化合物の単離・精製操作が煩雑になると考えられる。そこで筆者はリピド II の合成を固相法で行うことを計画した。固相法では全ての中間体を固相樹脂上で扱うため、液相法で問題となる単離・精製操作を簡便に行うことができる。また、固相合成法はペプチド部や糖部の構造を変化させた誘導体を網羅的に合成するのもにも適しており、プラスバシン A<sub>3</sub> の作用機序解明のためにも適した方法であると考えられる。

#### Scheme 46. Synthesis of Park nucleotide analogue (**123**)



黒須らは、結核菌の Parkヌクレオチドの蛍光誘導体 **123** の合成を報告しており、その際にペプチド **117** は Boc 固相合成法により合成し、その後は液相で合成が行われている(Scheme 46)<sup>84</sup>。従って、糖部の導入と低反応性が問題となっているジリン酸化をいかにして固相上で行うかが重要であると予想される。

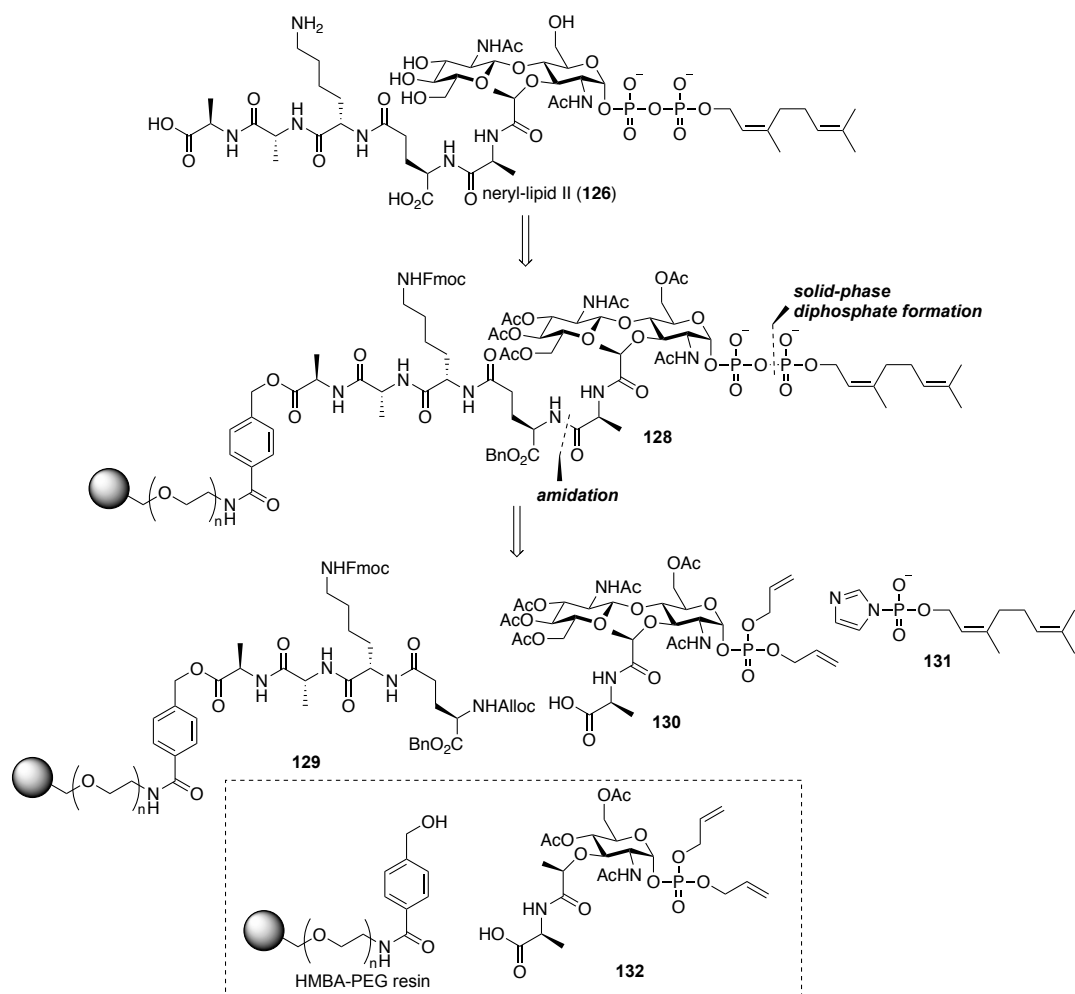




**Figure 30.** Reported analogues

Figure 30 にこれまで合成されたリポド I, II、Parkヌクレオチドの誘導体の例を示した。ダンシル Parkヌクレオチド (**124**)は *MraY* 阻害剤のアッセイの基質として用いられる<sup>85,86</sup>。また、より短い脂溶性側鎖を有する蛍光標識リポド I **125** は酵素反応を用いて合成がなされている<sup>87</sup>。リポド II の誘導体としてはより短い脂溶性側鎖である neryl 基を有するネリルリポド II (**126**)<sup>88</sup> や脂溶性側鎖部位に蛍光標識が施された **127**<sup>89</sup> が報告されている。

以上の知見を踏まえ、プラスバシン A<sub>3</sub> との結合親和性評価を行うために、より単純な neryl 基を有する **126** を合成標的として設定した。また、固相合成の有用性を示すべく、リポド I や Parkヌクレオチド及びその蛍光標識体の合成へも適用可能な合成法を開発することとした。



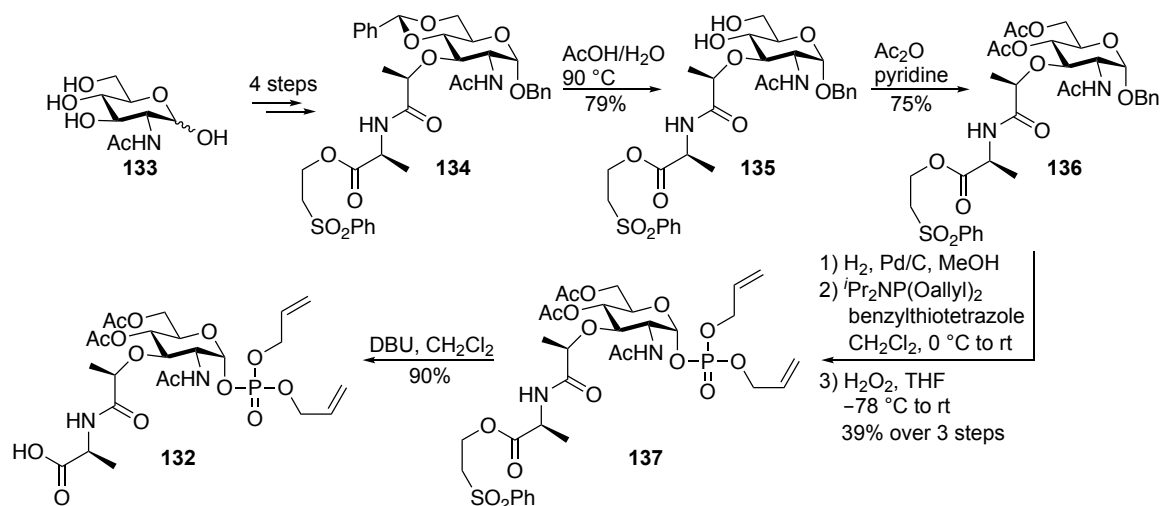
**Figure 31.** Retrosynthetic analysis of neryl-lipid II (126)

ネリルリピド II (126)の逆合成解析を Figure 31 に示す。リピド II のジリン酸結合は酸性条件化で酸素原子がプロトン化を受けることによりリン酸基の脱離能が上昇し、容易に加水分解される。また、ジリン酸が脱離基として働くことで *N*-アセチルムラミン酸 (MurNAc) のアノマー結合の開裂が進行しうる。従って、Fmoc ペプチド固相合成法で一般的に用いられる酸性条件下での脱樹脂反応は利用できないと予想した。そこで、塩基性水溶液中で加水分解することで脱樹脂可能なポリエチレングリコール型ヒドロキシメチル安息香酸(HMBA-PEG)樹脂を固相担体として選択した。リピド II の Lys 残基のアミノ基に蛍光基を導入することを見据え、Lys 残基の保護基は弱塩基性条件下選択的に除去できる Fmoc 基を選択し、カルボン酸と水酸基の保護基はそれぞれ、脱樹脂と同時に除去できるベンジルエステルまたは、アセチル基とした。固相担持ネリルリピド II 128 は固相担持テトラペプチド 129 に対して二糖ユニット 130 を縮合し、続いて neryl リン酸から導けるイミダゾリド 131 との固相上でのジリン酸化を行うことで合成することとし、固相担持テトラペプチド 129 は Fmoc 法と Alloc 法を用いて合成することとした。本合成経路は二糖ユニット 130 を単糖ユニット 132 に変更することでリピド I 誘導体や Park ヌクレオチドの合成へも応用可能である。

## 第二節 ネリルリピド I, II と Park ヌクレオチドおよび蛍光標識体の固相全合成

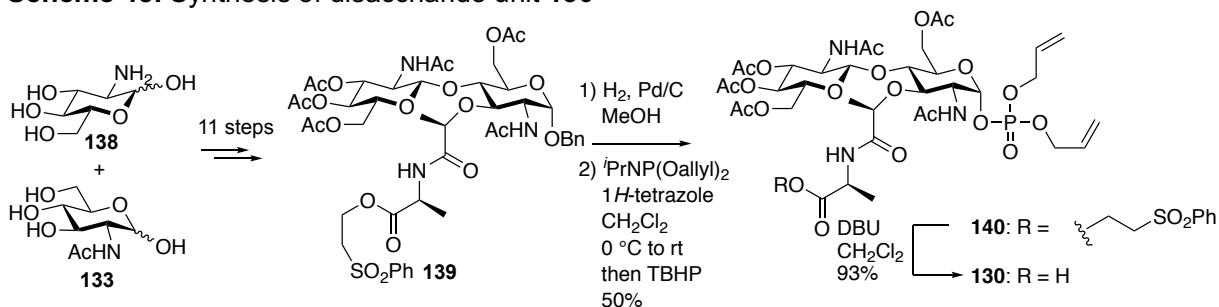
まず、合成に用いるユニット化合物の合成を行った。

**Scheme 47. Synthesis of monosaccharide unit 132**



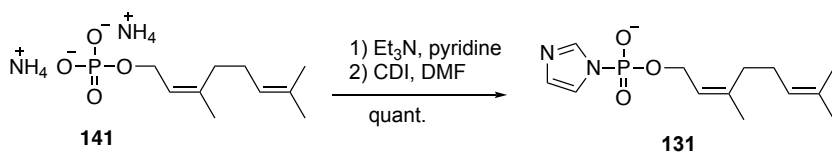
単糖ユニット **132** は D-GlcNAc(**133**)から合成した文献既知のムラミン酸保護体 **134**<sup>90</sup> から合成した (Scheme 47)。ムラミン酸保護体 **134** のベンジリデンアセタールを酸性条件下で除去し、生じた 1,3-ジオールをアセチル基で保護した。ベンジルエーテルを加水素分解し、生じた水酸基に対してホスホロアミダイトを作用させて得た亜リン酸エステルを、過酸化水素を用いて酸化することで糖リン酸 **137** を得た。最後にフェニルスルホニルエチル基を DBU を用いたβ脱離反応により除去し、単糖ユニット **132** を合成した。

**Scheme 48. Synthesis of disaccharide unit 130**



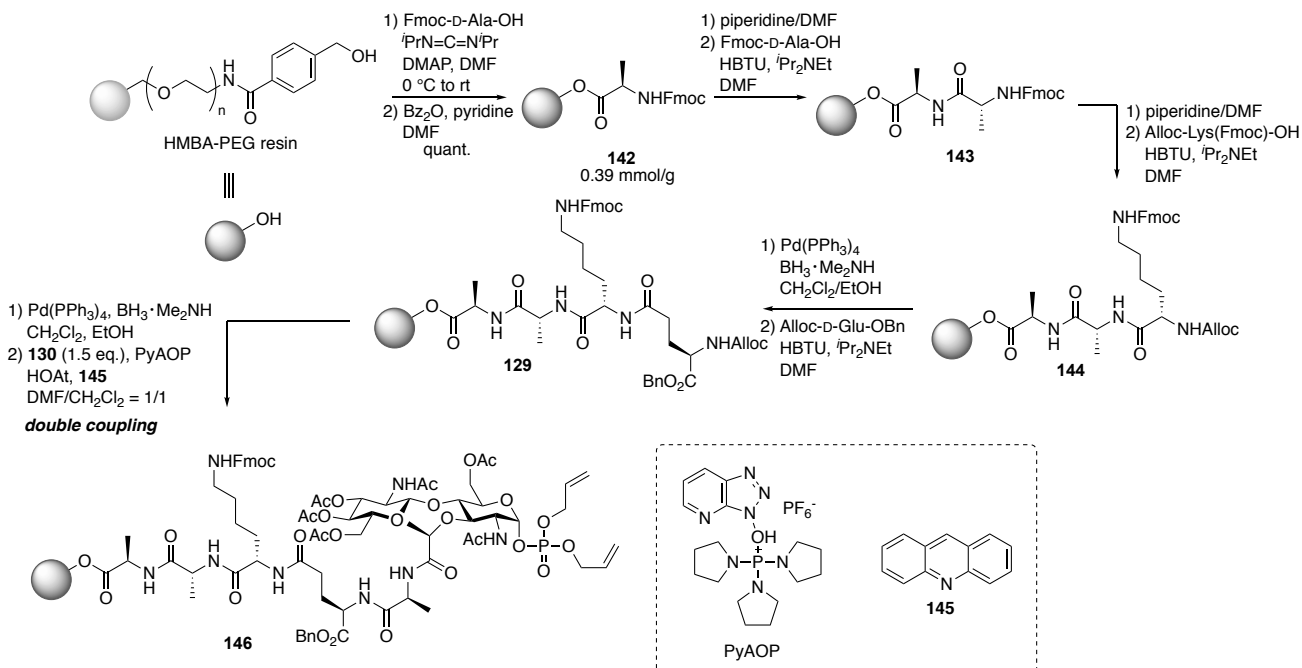
二糖ユニット **130** は D-GlcNAc(**133**)と D-グルコサミン(**138**)から合成した文献既知の二糖 **139**<sup>90</sup> から同様に合成した (Scheme 48)。二糖 **139** のベンジル基を除去して得られたアルコールをリン酸エステル **140** へと変換し、フェニルスルホニルエチル基を除去することで **130** を得た。

### Scheme 49. Synthesis of imidazolide **131**



イミダズリド **131** はネリルリン酸アンモニウム塩(**141**)<sup>91</sup> をトリエチルアミン塩へと変換したのち、CDI と反応させることで合成した(Scheme 49)。このイミダズリド **131** は単離することなく次の反応に用いた。

### Scheme 50. Solid-phase synthesis of glycopeptide **146**



ユニット化合物の合成を終えたので、まずはネリルリピド II (**126**)の合成を行った(Scheme 50)。HMBA-PEG樹脂に担持されたFmoc-D-Ala **142**を出発物質とし、Fmoc法によりFmoc-D-Ala-OH、Alloc-L-Lys(Fmoc)-OHを順次縮合した。ここでα-アミノ基の保護基をAlloc基に変更したため、以降はAlloc法を用いてAlloc-D-Glu-OBnと二糖ユニット **130**を縮合した。二糖ユニット **130**の縮合では **130**のL-Ala残基のエピメリ化が懸念されたが、縮合剤としてPyAOP、塩基として弱塩基であるacridine (**145**)を用い、DMFとCH<sub>2</sub>Cl<sub>2</sub>の混合溶媒<sup>\*11</sup>で反応を行うことでエピメリ化を抑制しつつ縮合を行った。また、二糖ユニット **130**は1.5当量用い、この縮合を二回行うことで反応は定量的に進行した。なお、各アミド縮合の進行はKaiser試験により確認した。

<sup>\*11</sup> アミノ酸の縮合ではアズラクトンの形成に伴い、活性化されたカルボン酸のα位の異性化が進行する。異性化を抑制するためにHOBtのような添加剤が利用されるが、反応溶媒もこの異性化に影響を与え、CH<sub>2</sub>Cl<sub>2</sub>のような非極性溶媒を共溶媒とすることで異性化を抑制できることが報告されている<sup>92</sup>。

### Scheme 51. Optimization of diphosphate formation

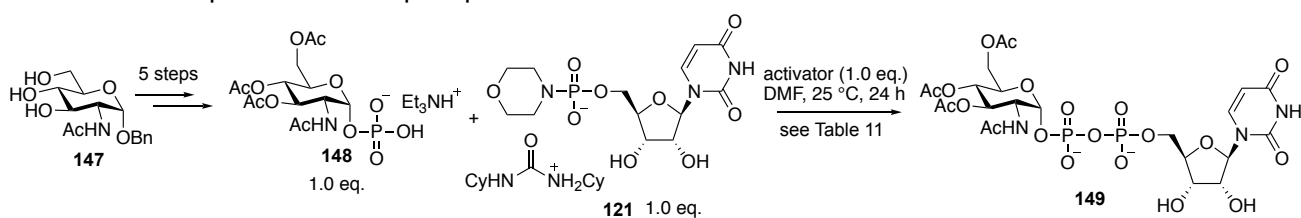


Table 11

entry	activator	yield (%) <sup>a</sup>	entry	activator	yield (%) <sup>a</sup>
1	none	no reaction			
2		18	4		47
	1H-tetrazole ( <b>150</b> )			<b>152</b>	
	<i>pK<sub>a</sub></i> 4.8			<i>pK<sub>a</sub></i> 3.7	
3		48	5		58 (31) <sup>b</sup>
	<b>151</b>			<b>153</b>	
	<i>pK<sub>a</sub></i> 4.2			<i>pK<sub>a</sub></i> 2.9	

<sup>a</sup>Yields were determined by <sup>31</sup>P NMR. <sup>b</sup>Isolated yield.

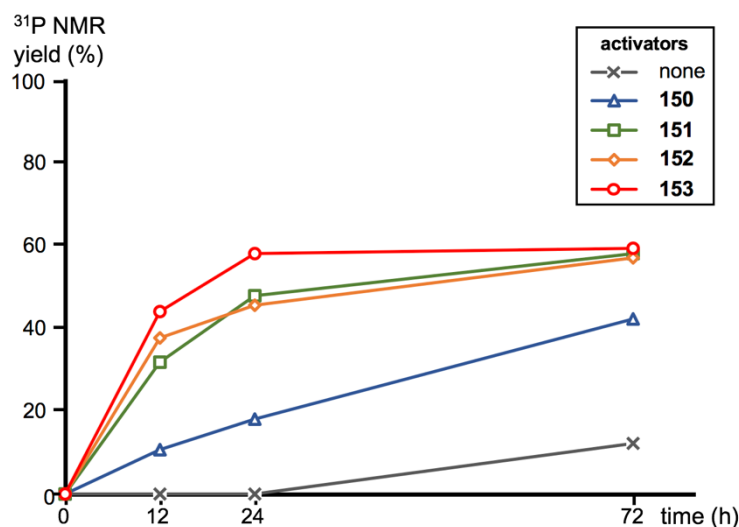


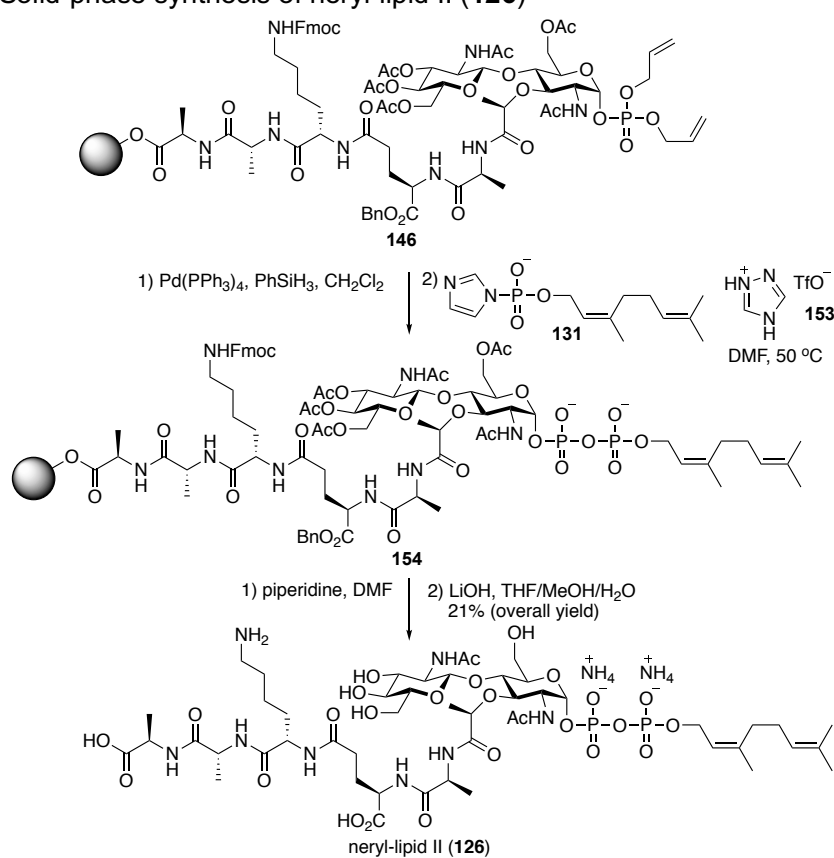
Figure 32. Time course of diphosphate formation

固相上での糖ペプチドの合成を達成したため、次にリン酸基間の縮合を行った。前述のように過去の合成例ではリン酸基間の縮合に長時間を要し、その収率も低いことが問題であった。固相合成では攪拌効率の低下から、反応速度が更に低下することが予想されたため、固相上でのリン酸基間の縮合に先立ち、液相で反応条件の最適化を行うこととした。

N-アセチル-D-グルコサミン保護体 **147** から合成した文献既知の糖リン酸 **148**<sup>93</sup> をモデル基質とし、Park スクレオチドの合成に用いるモルホリデート **121** とのリン酸基間の縮合を検討した(Scheme 51)。反応は DMF 溶媒中 25 °Cで行い、<sup>31</sup>P NMR で追跡した。過去の例を参考に活性化剤として 1H-tetrazole (**150**)を用

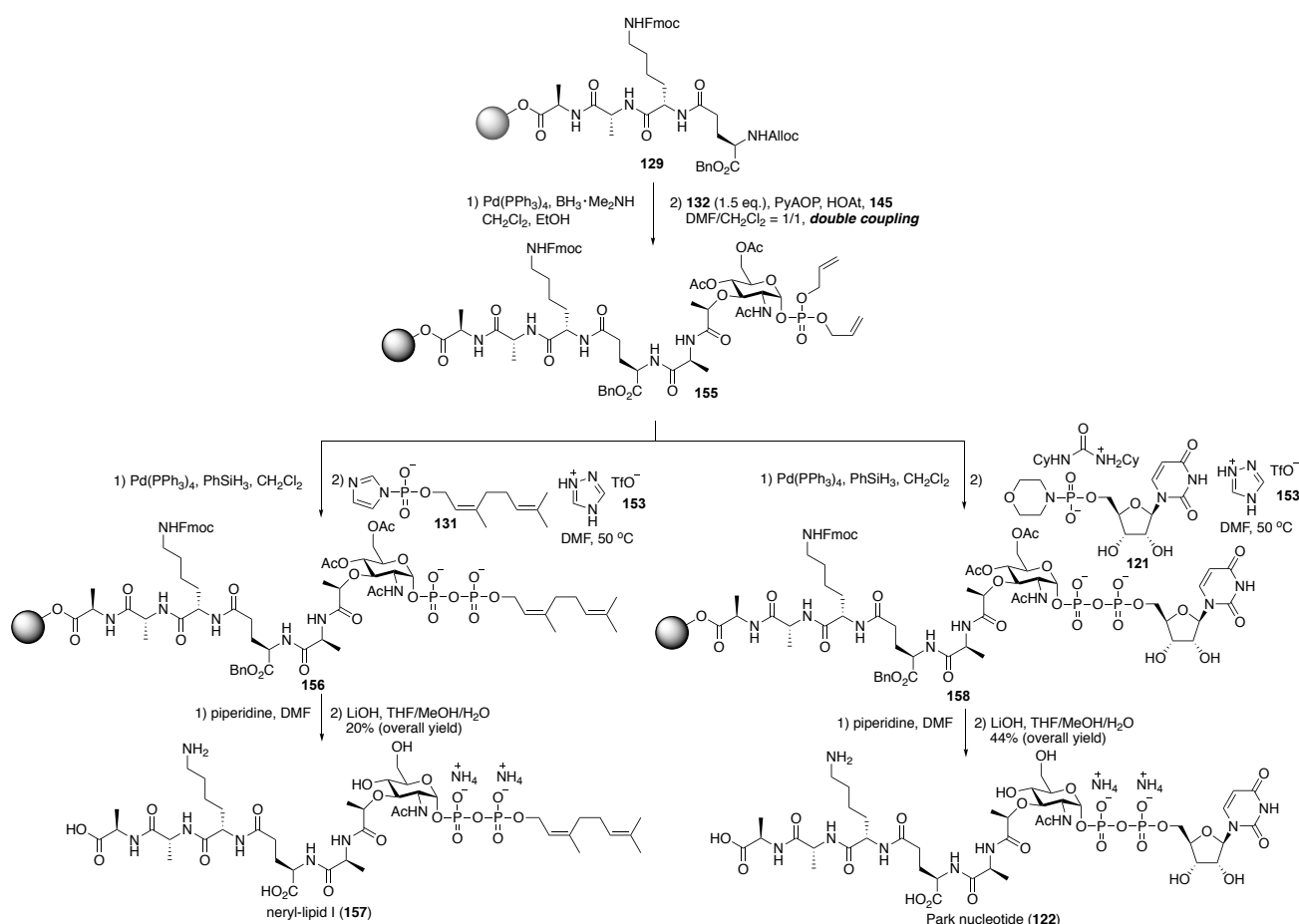
いたところ、24 時間後の収率は 18%であった(Table 11, entry 2)。本反応は活性化剤が **121** のモルホリン窒素原子をプロトン化することで活性化し、反応が進行する。そこで、より酸性度の大きい活性化剤を用いて引き続き検討を行うこととした。 $pK_a$  4.2 の **151** や  $pK_a$  3.7 の **152** を用いると、収率はそれぞれ 48%、47%まで向上した(entries 3,4)。次に関根らにより報告された **153** ( $pK_a$  2.9)<sup>94</sup> を用いたところ、58%というこれまでで最も高い収率が得られた(entry 5)。本反応の時間経過を Figure 32 に示した。狙い通り活性化剤の酸性度の向上に伴い反応速度が増大していることがわかり、**153** を用いた場合は 24 時間で反応が定常状態に達することが明らかになった。以上の検討より、ジリン酸化の活性化剤は **153** が最適であると決定し、次に固相上でのリン酸基間の縮合を行うことでネリルリピド II (**126**)の合成を行った。

### Scheme 52. Solid-phase synthesis of neryl-lipid II (**126**)



固相担持糖ペプチド **146** のアリル基を Pd(PPh<sub>3</sub>)<sub>4</sub> と PhSiH<sub>3</sub> を用いて除去し、イミダゾリド **151** を活性化剤 **153** 存在下で反応させた。固相上での反応性の低下から反応温度を 50 °Cとしたところ、リン酸基間の縮合が進行し、**154** を得た。この Fmoc 基をピペリジンを用いて除去した後、ベンジル基とアセチル基の除去と脱樹脂を LiOH を用いて THF、MeOH、H<sub>2</sub>O 混合溶媒中で行った。得られた粗生成物を逆相 HPLC により精製し、ネリルリピド II (**126**)を得た。合成した **126** のスペクトルデータは文献値<sup>88</sup> と良い一致を示し、ここにネリルリピド II (**126**)の固相全合成を総収率 21%で達成した (Scheme 52)。

**Scheme 53. Solid-phase synthesis of neryl-lipid I (157) and Park nucleotide (122)**

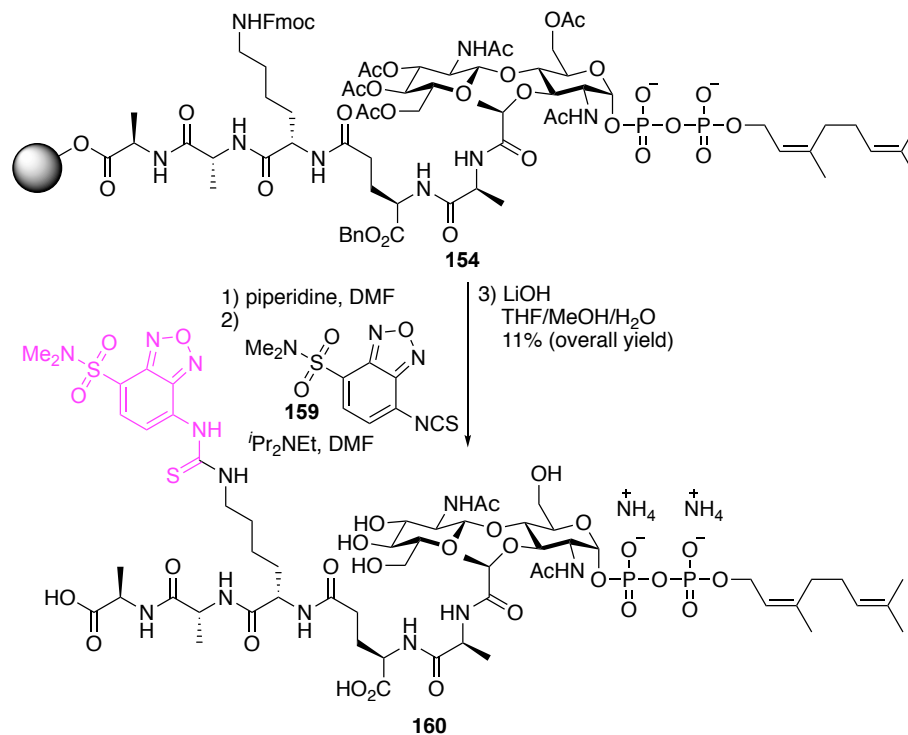


次に確立した合成経路を用いてネリルリピド I (**157**)と Parkヌクレオチド (**122**)の合成も行った(Scheme 53)。固相担持テトラペプチド **129** に対して単糖ユニット **132** を同様の条件下縮合し、**155** を得た。このアシル基を 0 価パラジウム触媒を用いて除去し、ネリルリピド II (**126**)の合成と同様に **131** とのジリン酸基間の縮合と脱保護、脱樹脂を行うことでネリルリピド I (**157**)の固相合成を総収率 20%で達成した。固相担持糖ペプチド **155** からリン酸基間の縮合に用いる **131** をウリジン 5'-モノリン酸から誘導されるモルホリデート **121** へと変更し、同様の変換を行うことで Parkヌクレオチド (**122**)の固相合成も達成した。合成した **122** のスペクトルデータは文献値<sup>95</sup>と良い一致を示した。

以上のように筆者はネリルリピド II (**126**)、ネリルリピド I (**157**)、Parkヌクレオチド (**122**)の固相全合成を達成した。本合成法は用いるユニットを変更することでこれら 3 種類の化合物を迅速に合成することが可能であり、糖ペプチド部の変換も自在に行うことができる効率的なものである。

Lipids I, II 及び Park ヌクレオチドに蛍光基を導入した蛍光標識体は細胞壁合成酵素の機能評価に用いられる<sup>86, 96</sup>。確立した合成経路は Lys 残基のアミノ基に化学修飾の導入が可能な経路となっているため、ネリルリピド II (126)、ネリルリピド I (157)、Park ヌクレオチド (122)の Lys 残基にそれぞれ異なる蛍光基を導入した蛍光標識体の合成を行うこととした。

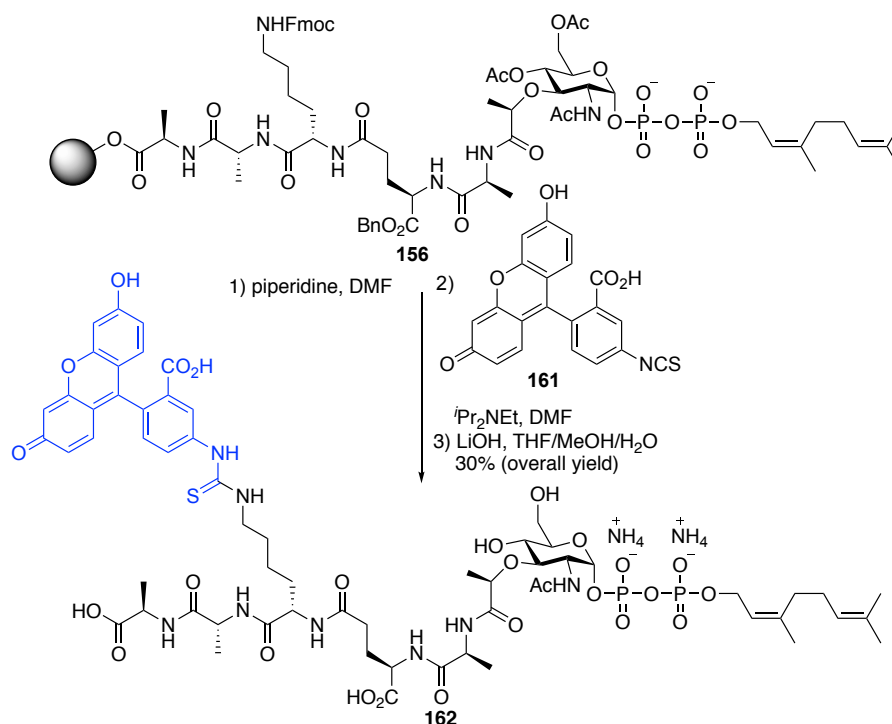
**Scheme 54. Solid-phase synthesis of DBD-neryl-lipid II (159)**



固相担持ネリルリピド II (154)の Fmoc 基をピペリジンを用いて除去し、生じたアミンに対してイソチオシアネート 159 を作用させることで DBD 基をチオウレアを介して導入した。続いて塩基性条件下での脱保護と脱樹脂を行い、逆相 HPLC により精製することで、DBD-ネリルリピド II (160)を合成した(Scheme 54)。

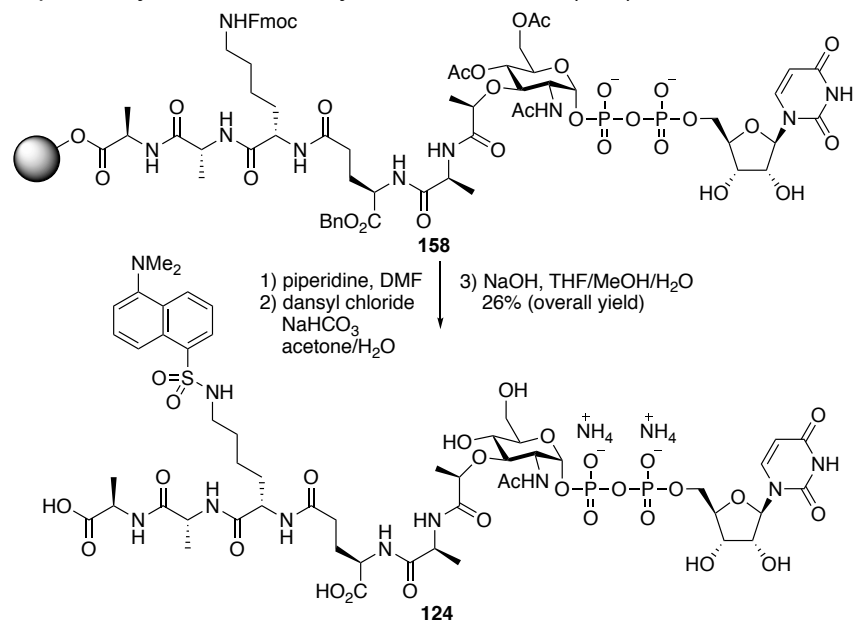


**Scheme 55. Solid-phase synthesis of FITC-neryl-lipid I (162)**



同様の合成経路でフルオレセインを導入した FITC-ネリルリピド I (162)を合成した(Scheme 55)。固相担持 neryl リピド I 156 の Fmoc 基を除去し、得られたアミンと FITC (161)を反応させることでフルオレセインを導入し、脱保護と脱樹脂を行うことで FITC-ネリルリピド I (162)を得た。

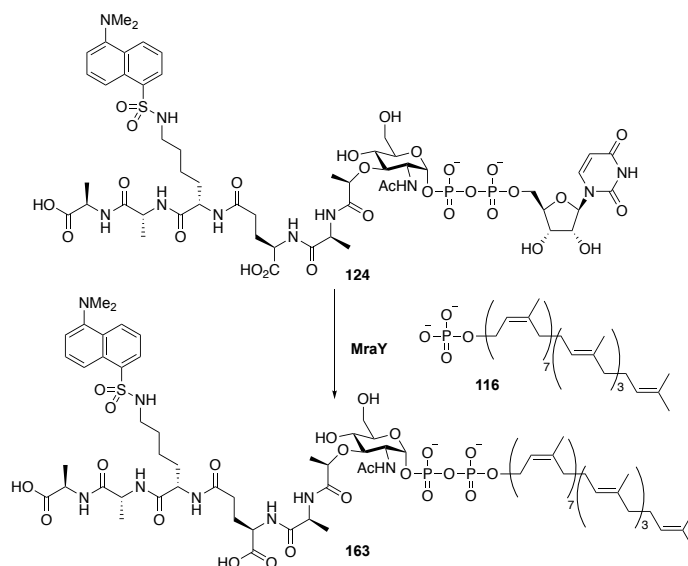
**Scheme 56. Solid-phase synthesis of dansyl-Park nucleotide (124)**



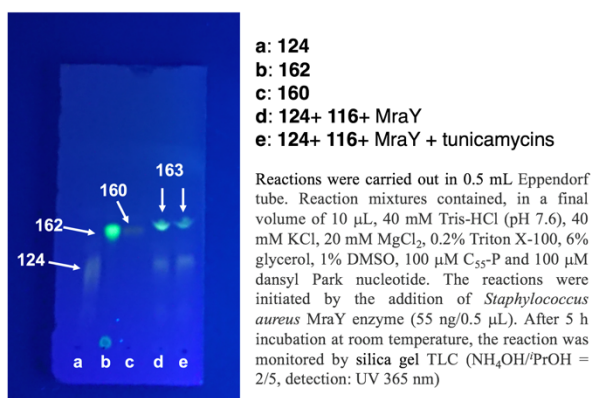
同様にしてダンシル Park ヌクレオチド (124)も合成した(Scheme 56)。固相担持 Park ヌクレオチド 158 の Fmoc 基を除去し、ダンシルクロライドとのスルホニル化を行った。DMF を反応溶媒とした際には反応が進行せず、これは Park ヌクレオチドの極性が高いために、DMF 中では固相樹脂の凝集が起こったた

めであると考察した。そこで含水溶媒中で反応を行ったところスルホニル化は完結し、脱保護と脱樹脂を経て dansyl ダンシル Park ヌクレオチド (**124**)を得た。標品とのスペクトルデータの比較は、合成した **124** を陽イオン交換により Na<sup>+</sup>塩へと変換して行い、得られたスペクトルデータは標品と良い一致を示した。

**Scheme 57. Enzymatic reaction of dansyl Park nucleotide (**124**)**



前述のようにダンシル Park ヌクレオチド (**124**)は酵素 MraY の基質となることで、阻害剤評価に用いられている(Scheme 57)。そこで、合成した **124** が実際に MraY の基質となるのかを確認することとした。ダンシル Park ヌクレオチド (**124**)とウンデカプレニルリン酸(**116**)を黄色ブドウ球菌由来の MraY 存在下インキュベートし、反応の進行を TLC により追跡した(Figure 33)。ダンシル Park ヌクレオチド (**124**)から生成したダンシルリピド I (**163**)のスポットは **160** や **162** と近い位置に新たに現れ(Figure 33, lane a-d)、MraY 阻害剤であるツニカマイシン<sup>97</sup>を加えたところ(Figure 33, lane e)、**163** の生成量の減少が見られたことから、合成した **124** が MraY の基質となり **163** へと変換されたことが示唆された。



**Figure 33. TLC analysis of enzymatic reaction**

以上のように、筆者は lipids I, II 及び Park ヌクレオチドに蛍光基を導入した蛍光標識体 **124**、**160**、**162** の固相合成を達成し、本合成法が細胞壁生合成前駆体の誘導体合成に適していることを示した。続く第五章では合成したネリルリピド II (**126**)とプラスバシン A<sub>3</sub>の相互作用と、プラスバシン A<sub>3</sub>の作用機序に関する研究について述べる。

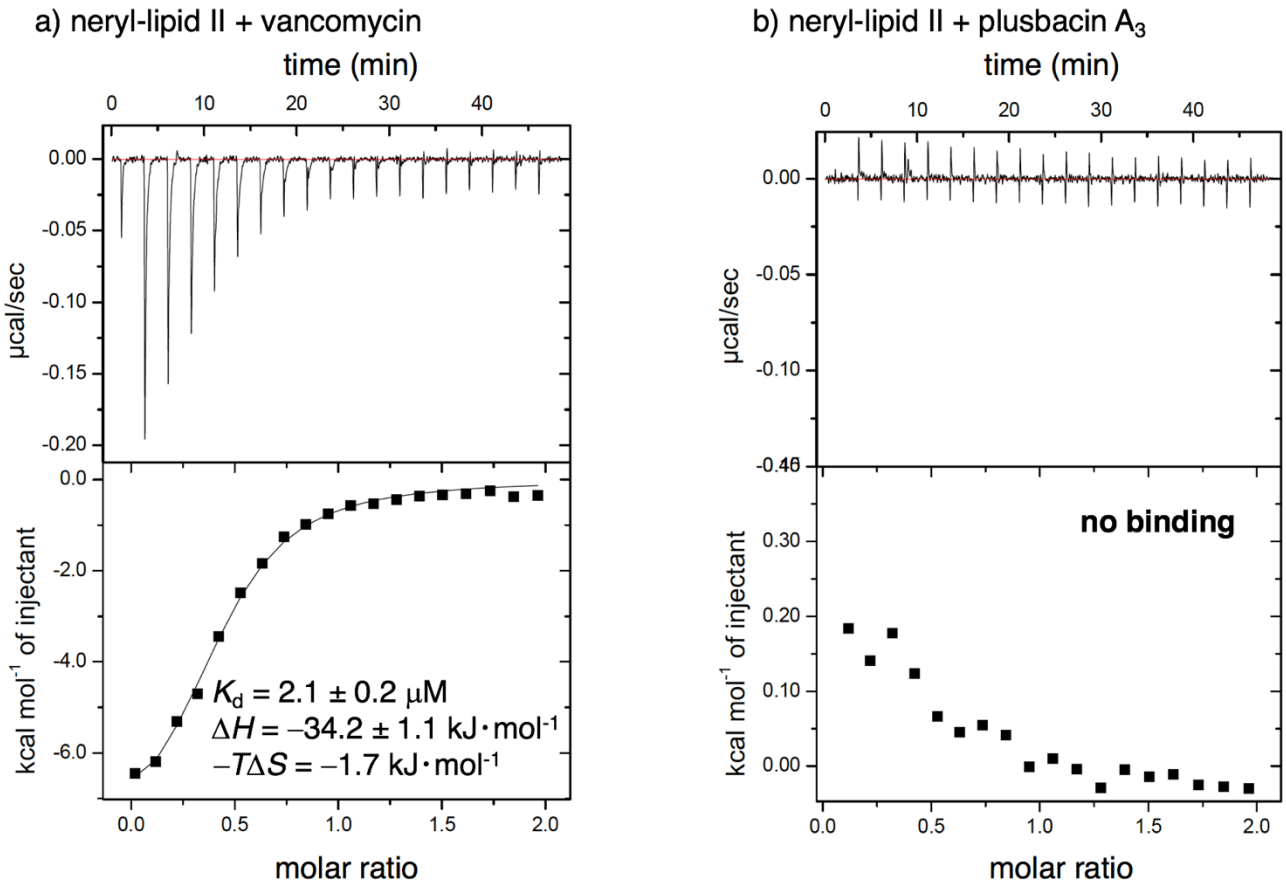
## 第五章 プラスバシン A<sub>3</sub> の作用機序に関する研究

### 第一節 プラスバシン A<sub>3</sub> とネリルリピド II の相互作用解析

プラスバシン A<sub>3</sub> とネリルリピド II の結合親和性を評価するにあたり、リピド II とバンコマイシンの相互作用に関する知見を参考にすることとした。バンコマイシンはリピド II の D-Ala-D-Ala 末端に結合することが知られており、Ac-Lys(Ac)-D-Ala-D-Ala ペプチドとの結合の解離定数と熱力学的パラメーターは、等温滴定カロリメトリー(ITC)により測定されている(Table 12)<sup>98</sup>。ITC 測定は修飾が不要であるうえ、解離定数と各種熱力学的パラメーターを同時に測定できることから、プラスバシン A<sub>3</sub> とリピド II の結合親和性評価にも適していると考えた。そこで ITC を用いて結合親和性を測定することとし、ポジティブコントロールとしてバンコマイシンを用いて同様の測定を行うことを計画した。

**Table 12.** Thermodynamic parameter for complexation of vancomycin with Ac-Lys(Ac)-D-Ala-D-Ala

$K_d$ ( $\mu\text{M}$ )	2.1 $\pm$ 0.3
$\Delta G^\circ$ ( $\text{kJ}\cdot\text{mol}^{-1}$ )	-32.4 $\pm$ 0.4
$\Delta H^\circ$ ( $\text{kJ}\cdot\text{mol}^{-1}$ )	-40.1 $\pm$ 1.0
$T\Delta S^\circ$ ( $\text{kJ}\cdot\text{mol}^{-1}$ )	-7.7 $\pm$ 1.0



**Figure 34.** ITC raw data and binding isotherms for antibiotics interacting with neryl-lipid II.

a) 200  $\mu\text{M}$  vancomycin was titrated into 20  $\mu\text{M}$  neryl-lipid II (+ 2% DMSO).

b) 400  $\mu\text{M}$  plusbacin A<sub>3</sub> was titrated into 40  $\mu\text{M}$  neryl-lipid II (+ 4% DMSO).

buffer: 10 mM sodium phosphate (pH 7.3), 150 mM NaCl, 0.9 mM CaCl<sub>2</sub>

ネリルリピド II (20  $\mu\text{M}$ )に対してバンコマイシン(200  $\mu\text{M}$ )を滴定したところ、バンコマイシンは文献値とよく一致する  $K_d$  値 2.1  $\mu\text{M}$  の結合親和性を示した(Figure 34a)。一方プラスバシン A<sub>3</sub> はバンコマイシンよりも高濃度(400  $\mu\text{M}$ )条件においても結合親和性を示さなかった(Figure 34b)。従って、プラスバシン A<sub>3</sub> の結合部位はバンコマイシンが結合する D-Ala-D-Ala 末端ではなく、本測定条件下ではネリルリピド II と結合しないことが明らかとなった。

細菌のリピド II は細胞膜上に存在しており、プラスバシン A<sub>3</sub> の脂溶性側鎖は細胞膜と相互作用することが予想される。実際、プラスバシン A<sub>3</sub> の脂溶性側鎖は抗菌活性に必須であることが Schaefer らにより報告されている<sup>99</sup>。上記の実験結果から、プラスバシン A<sub>3</sub> とネリルリピド II の結合には細胞膜の存在が必須であると考え、次にプラスバシン A<sub>3</sub> と細胞膜の相互作用について調べることにした。

## 第二節 リポソームとプラスバシン A<sub>3</sub>の相互作用

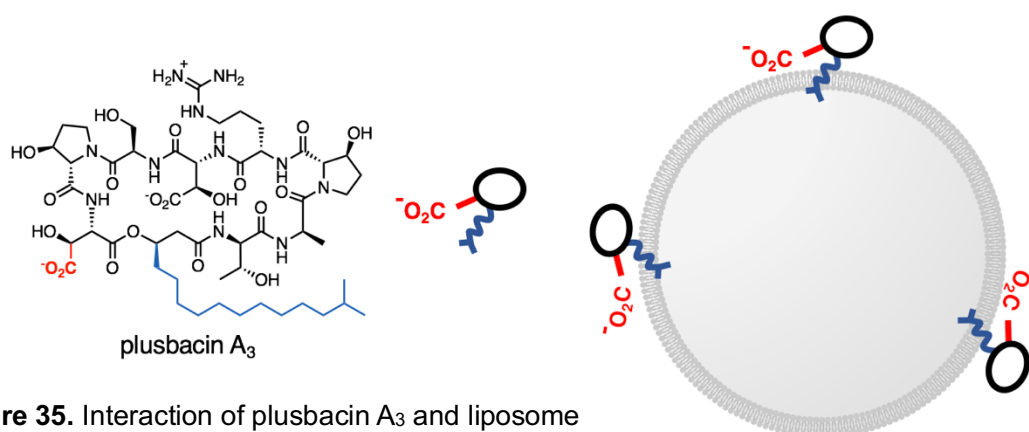
プラスバシン A<sub>3</sub>の類縁体であるエンペドペプチンについては、水晶振動子マイクロバランス(QCM)法を用いて脂質膜との相互作用が調べられている(Table 13)<sup>23</sup>。Table 13 で示したようにエンペドペプチンとリン脂質膜の親和性は  $K_d$  値 0.77  $\mu\text{M}$  を示し、その親和性は  $\text{Ca}^{2+}$ 存在下で更に増大することが報告されている。

**Table 13.** Binding affinity of empedopeptin to phospholipid membrane

	$k_a$ ( $\text{M}^{-1}\cdot\text{s}^{-1}$ )	$k_d$ ( $\text{s}^{-1}$ )	$K_d$ ( $\mu\text{M}$ )
without $\text{Ca}^{2+}$	2835 $\pm$ 507	2.10 $\pm$ 0.86 $\times 10^{-3}$	0.77 $\pm$ 0.41
1.25 mM $\text{Ca}^{2+}$	4736 $\pm$ 1262	1.47 $\pm$ 0.70 $\times 10^{-3}$	0.30 $\pm$ 0.11

association rate ( $k_a$ ), dissociation ( $k_d$ ) rate and overall binding affinity ( $K_d$ ) of empedopeptin.

プラスバシン A<sub>3</sub>と細胞膜の相互作用をより簡便に調べるために、細胞膜を脂質二重膜からなるリポソームで模倣し、プラスバシン A<sub>3</sub>との相互作用を調べた。プラスバシン A<sub>3</sub>は2つのカルボン酸と一つのグアニジンを含んでいるため、中性条件下では一価の負電荷を帯びる。この負電荷により、リポソームの脂質膜上にプラスバシン A<sub>3</sub>が挿入するとリポソームのゼータ電位<sup>\*12</sup>が低下する(Figure 35)。



**Figure 35.** Interaction of plusbacin A<sub>3</sub> and liposome

<sup>\*12</sup> リポソームの表面は電荷を有する極性官能基が存在し、この電荷に起因する表面電荷により、これとは逆の電荷を有する水溶液中のイオンはリポソーム膜の近傍に位置する。このイオンはリポソームとともに運動し、このようなりポソームに伴ってイオンの移動が起こる面をすべり面とよび、無限遠を基準としてすべり面の電位をゼータ電位と定義する。

卵黄ホスファチジルコリン(EPC)とコレステロール(Chol)からなるリポソームに対しプラスバシン A<sub>3</sub>を加えたところ、コントロールの DMSO を添加した時と比べてゼータ電位は低下し、濃度依存性も確認することができた(Table 14, Figure 36)。従ってプラスバシン A<sub>3</sub>は、脂溶性側鎖を脂質二重膜に挿入し、リポソーム上に局在することが明らかとなった。また、抗菌活性が消失したジデオキシ誘導体 **100** についても同様の結果が得られた(Table 14, Figure 36)。この結果からプラスバシン A<sub>3</sub>はその脂溶性側鎖を用いて細胞膜に挿入することが示唆されるが、この過程には *threo*-β-ヒドロキシ-Asp の水酸基は関与しないことが明らかとなった。

**Table 14.** Properties of the liposome treated with **1**

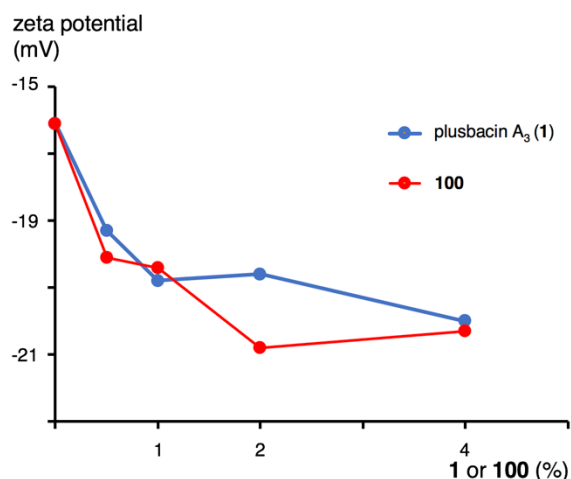
plusbacin A <sub>3</sub> (%)	0	0.5	1	2	4
average size (nm)	144.7	137.9	140.1	136.9	143.6
zeta potential (mV)	-16.1	-19.3	-20.8	-20.6	-22.0

A solution of 0.55 mM liposome (EPC:Chol = 7:3) in 10 mM sodiumphosphate (pH 7.3 + 1% DMSO) was treated with plusbacin A<sub>3</sub>.

**Table 15.** Properties of the liposome treated with **100**

<b>100</b> (%)	0	0.5	1	2	4
average size (nm)	144.7	139.9	137.1	138.0	141.3
zeta potential (mV)	-16.1	-20.1	-20.4	-22.8	-22.3

A solution of 0.55 mM liposome (EPC:Chol = 7:3) in 10 mM sodiumphosphate (pH 7.3 + 1% DMSO) was treated with **100**.



**Figure 36.** Concentration dependence of zeta potential

以上より、プラスバシン A<sub>3</sub>は細胞膜に挿入した後にリポド II と相互作用すると予想される。そこで、細胞膜上での両者の相互作用や、脂質膜の役割に関する知見を得るべく、分子動力学計算を用いた分子シミュレーションを行うこととした。次節ではこの結果について述べる。

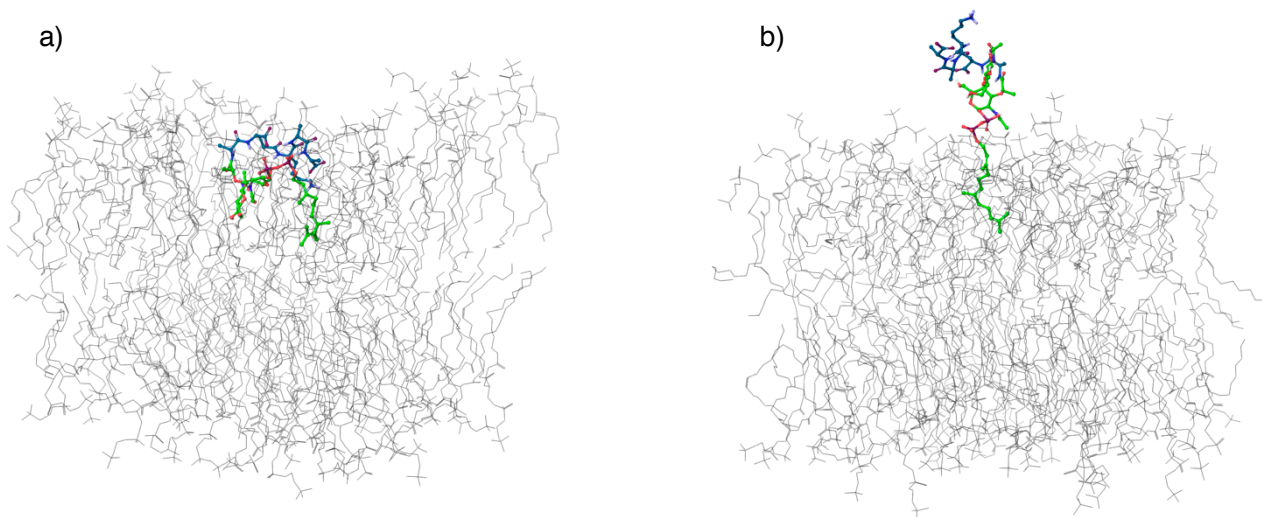
### 第三節 プラスバシン A<sub>3</sub>とリピド II 誘導体の分子動力学シミュレーション

これまで、述べたように、プラスバシン A<sub>3</sub>とリピド II の相互作用を直接観測することはできず、リピド II との結合には、細胞膜との相互作用が重要である可能性が示唆された。メカニズム解明のための研究指針を見出す一助とするべく、計算化学的手法により、プラスバシン A<sub>3</sub>とリピド II の相互作用を調べることを計画した。

創薬化学研究において計算化学の果たす役割は年々大きくなっている。タンパク質と低分子化合物の相互作用に関してはドッキング計算が一般的に用いられる。ドッキング計算は、タンパク質の“くぼみ”を結合ポケットに指定し、低分子化合物の三次元構造が結合ポケットの三次元構造に近づくような結合様式を探索する。結合ポケットを指定するという性質上、ドッキング計算を行う対象は、タンパク質のように安定な高次構造を取っているものに限定される。一方、計算対象となる系の原子と結合を“球”と“バネ”のように捉え、その時間経過を求める分子動力学計算はタンパク質と低分子化合物の複合体の動的性質を調べる目的で利用される。しかし、分子動力学計算によりタンパク質と低分子化合物の結合形成・乖離という一連のイベントを求める場合、計算コストが増大することから、これを達成した例は数例のみである<sup>100,101</sup>。リピド II はタンパク質と比較して遥かに分子量が小さい鎖状化合物であり、最近リン脂質膜上のリピド II の分子動力学シミュレーションが報告され、糖ペプチド部及び、脂溶性側鎖は大きく運動することが示された<sup>102</sup>。このように安定な配座を持たない化合物に対する相互作用を計算化学により求める際には、ドッキング計算よりも分子動力学計算が適していると考えられる。さらに、リピド II を対象とする系は原子数が少ないため、計算コストも低下することから、プラスバシン A<sub>3</sub>との相互作用をシミュレーションすることが十分可能であると考えた。そこで、リピド II 誘導体とプラスバシン A<sub>3</sub>が、脂質二重膜存在下でどのような挙動を示すのかを、分子動力学シミュレーションにより調べることにした。本節ではプラスバシン A<sub>3</sub>とリピド II の相互作用に関して、分子動力学シミュレーション結果から推測されることについて詳述する。

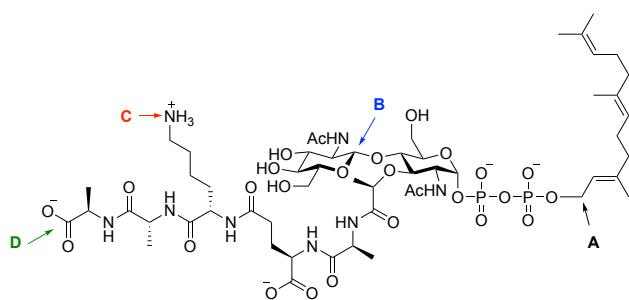


まず、脂質二重膜上にリポドIIを導入した初期構造を作成した。初期構造の作成にあたり、ファルネシルリポドIIとナイシンの複合体のNMR構造(PDB ID: 1WCO)<sup>103</sup>を参考にした。Orientation of Proteins in Membranes (OPM)データベース<sup>\*13</sup>からこのNMR構造をダウンロードし、ナイシンの構造を削除後、1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC)膜を配置した(Figure 37a)。この構造に対してシュレディンガー社の計算プログラムdesmondを用いて50 nsecのMD計算を行い、50 nsec後の構造をFigure 37bに示した。

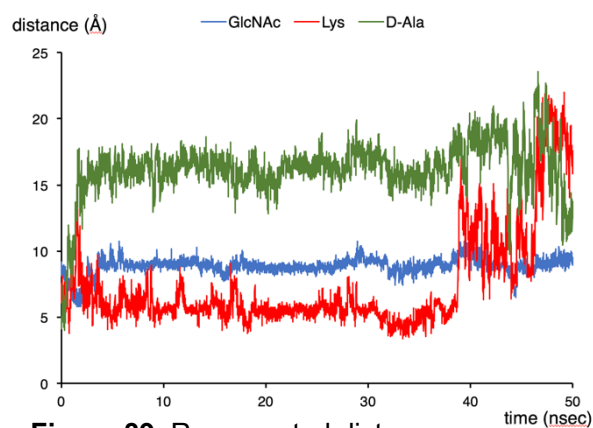


**Figure 37.** MD simulation of lipid II analogue

初期構造ではリポドIIの糖ペプチド部が脂質膜に接しているのに対し、50 nsec後の構造では糖ペプチド部は溶液側に露出し、過去の報告と同様に、リポドIIの配座は大きく変化することがわかった。またこの間のリポドIIの動きを解析するためGlcNAc1位炭素原子(Figure 38, B)、Lys-ε窒素原子(Figure 38, C)、末端D-Ala-α炭素原子(Figure 38, D)とfarnesyl基1位炭素原子(Figure 38, A)間の距離を調べた(Figure 39)。その結果、これらの距離の変化が大きく、リポドIIは柔軟な構造を有することが示唆された。



**Figure 38.** Structure of farnesyl-lipid II



**Figure 39.** Represented distance

\*13 膜タンパク質に対する計算では、タンパク質に対して適した座標に脂質膜を配置する必要がある。Protein Data Banc (PDB) から得たタンパク質の三次元構造に対し、脂質膜を配置する場合、脂質膜の座標の決定が煩雑になる。OPM データベースは PDB データを元にタンパク質と脂質膜の位置関係を計算し、そのデータを加えた構造情報のデータベースである。

以上の結果より、50 nsec 後の構造を新たな初期構造として選択し、この系にプラスバシン A<sub>3</sub>を加えて再度 MD シミュレーションを行うこととした。まず、プラスバシン A<sub>3</sub> の三次元構造を作成した。プラスバシン A<sub>3</sub> の構造情報に関しては Wohlrab が DMSO-*d*<sub>6</sub> 中での NMR 構造を報告しており(Figure 40)<sup>103</sup>、Figure 41 に示すような分子内水素結合の存在が示唆されている。そこでこの報告を参考に、シュレディンガー社の計算プログラム Prime を用いてプラスバシン A<sub>3</sub> の安定配座を探索し、これらの水素結合様式に最も近い構造を選択した(Figure 42)。

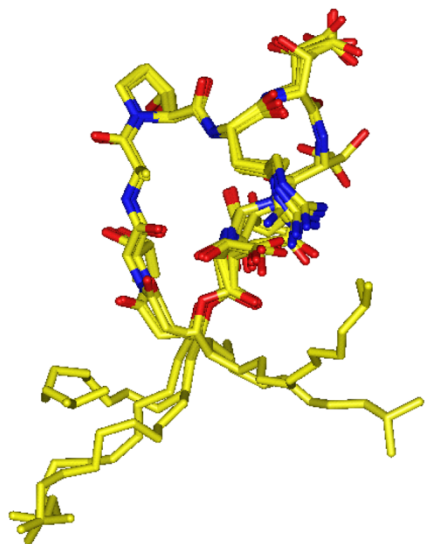


Figure 40. NMR structure of plusbacin A<sub>3</sub><sup>103</sup>

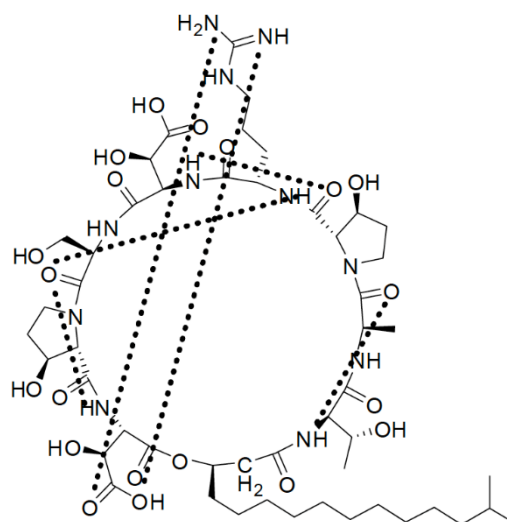


Figure 41. Intramolecular hydrogen bond<sup>103</sup>

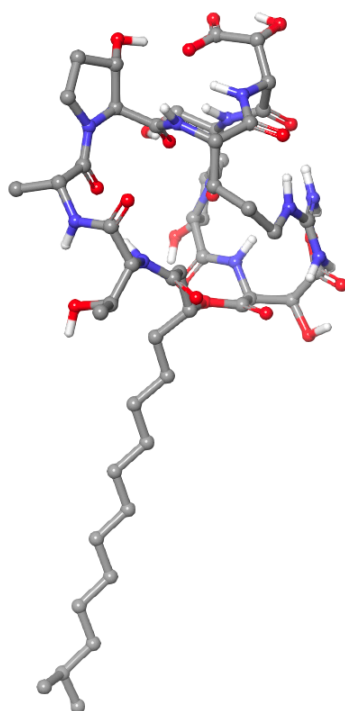
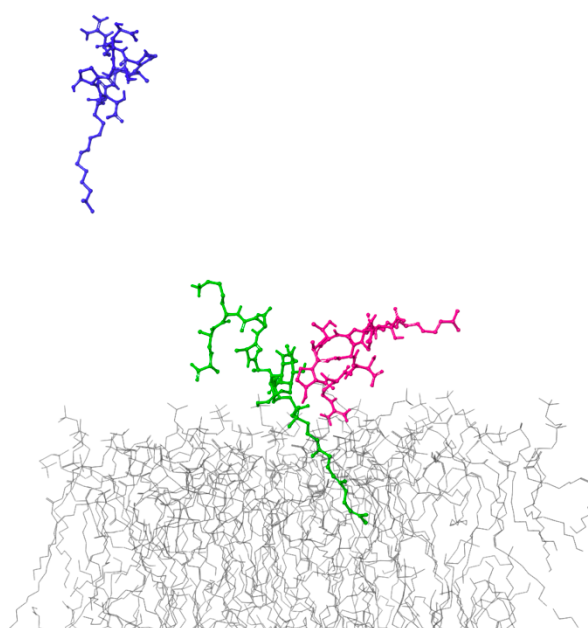
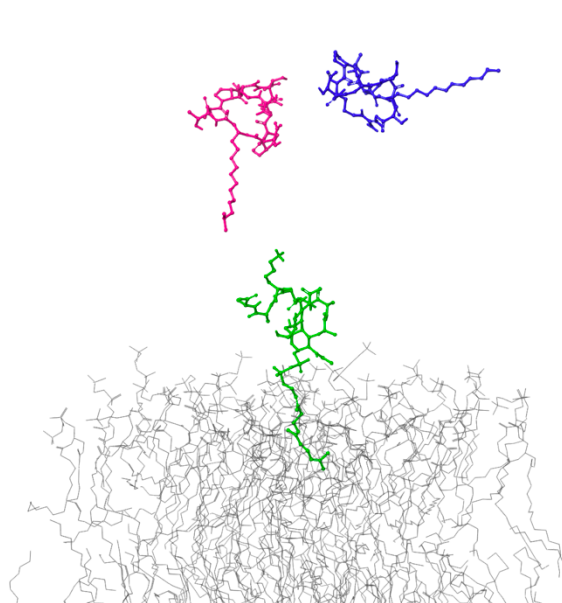


Figure 42. Selected structure of plusbacin A<sub>3</sub>

Figure 37b の構造の溶液中に Figure 42 で示した構造のプラスバシン A<sub>3</sub> を二分子配置し、MD シミュレーションの初期構造を作成した(Figure 43a)。この構造に対し、2 μsec の分子動力学シミュレーションを実施した。溶液中のプラスバシン A<sub>3</sub> はリポド II に接触することはあるものの、強固な結合は形成されなかった(Figure 43b)。その後、一分子のプラスバシン A<sub>3</sub> は脂質二重膜に挿入し(Figure 43c)、最終的にもう一分子のプラスバシン A<sub>3</sub> も脂質二重膜に挿入した(Figure 43d)。

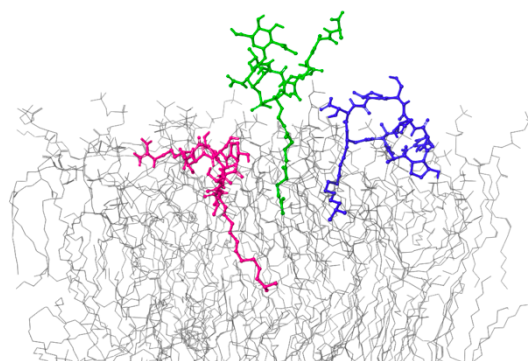
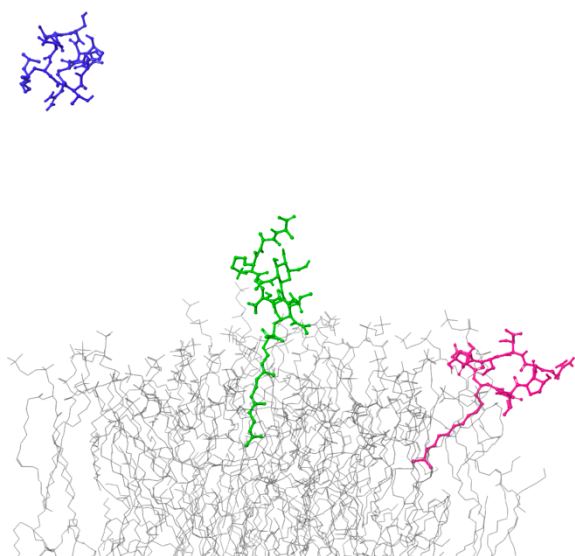
a)  $t = 0$  nsec

b)  $t = 400$  nsec



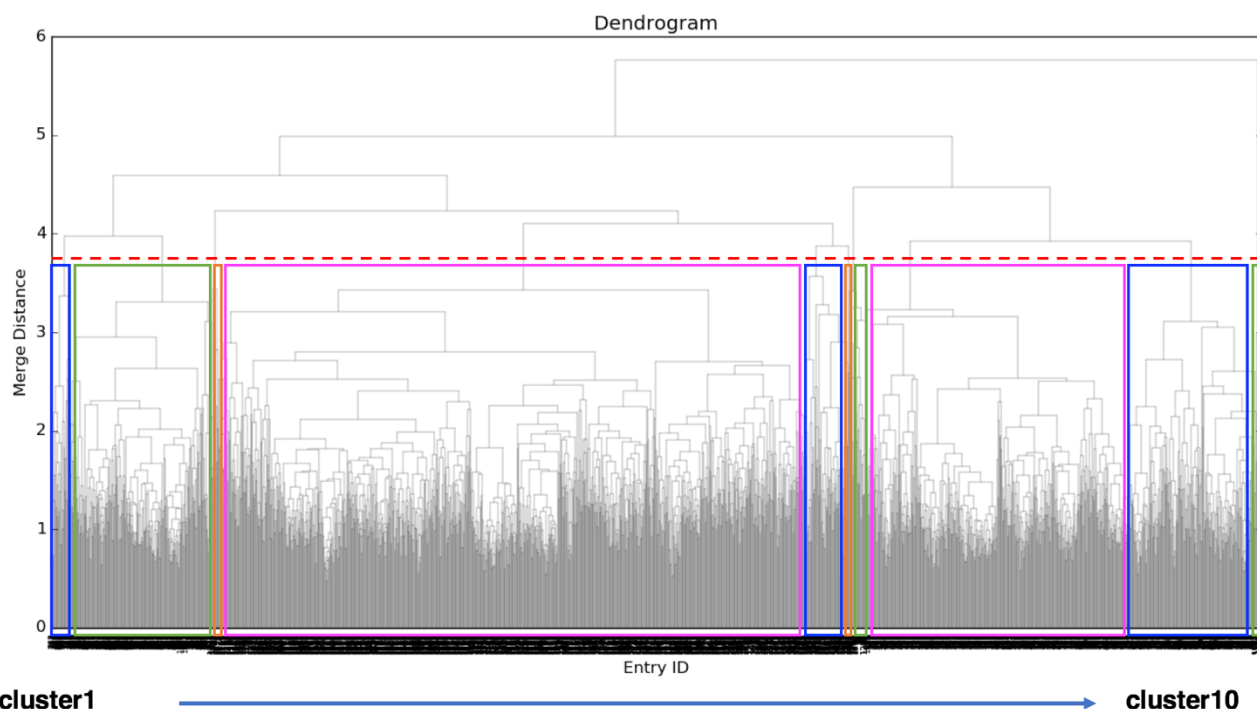
c)  $t = 1.4$  μsec

d)  $t = 2$  μsec



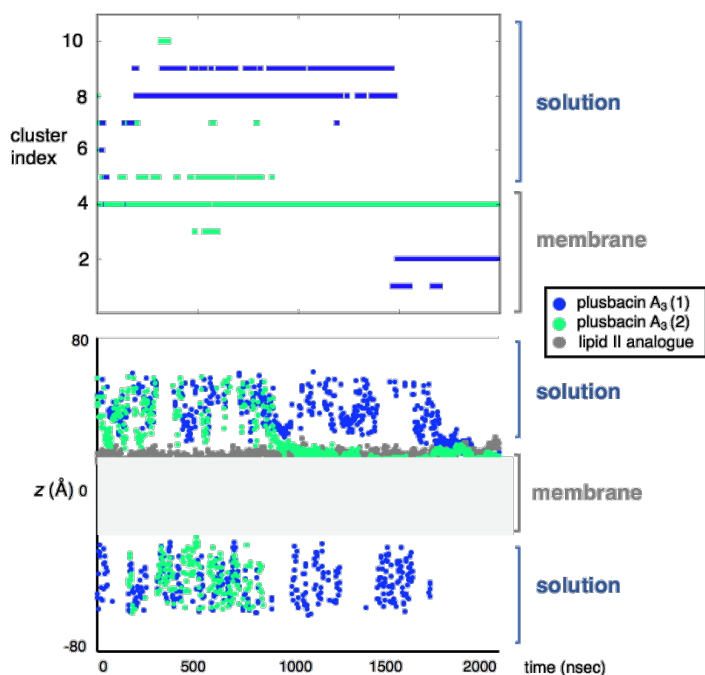
**Figure 43.** Snapshot of MD simulation (blue: plusbacin A<sub>3</sub>-1, pink: plusbacin A<sub>3</sub>-2, green: lipid II analogue, gray: membrane)

第一節で述べたように、脂質二重膜非存在下ではプラスバシン A<sub>3</sub> はリポド II と結合せず、MD 計算の結果から溶液中のプラスバシン A<sub>3</sub> と脂質二重膜中のリポド II の結合は弱いことが示唆された。また、脂質二重膜上でのリポド II とプラスバシン A<sub>3</sub> の直接の相互作用は見られず、このイベントをシミュレーションするには、さらに長時間のシミュレーションが必要であると考えられる。しかし、第二節で述べたリポソームを用いた実験の結果と同様に、プラスバシン A<sub>3</sub> が脂質二重膜に挿入することが計算化学からも支持された。これらの結果より、脂質膜に挿入することでプラスバシン A<sub>3</sub> の配座が変化し、リポド II に結合する可能性があると考えた。そこで、溶液中と脂質膜中での配座を比較するため、MD シミュレーションで得られた構造に対してコンフォメーションのクラスタリングを行うこととした。

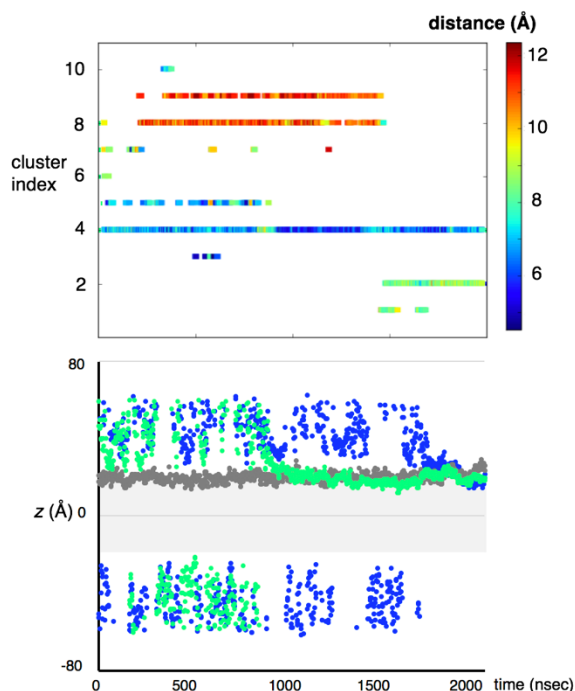


**Figure 44.** Dendrogram of structures of plusbacin A<sub>3</sub>

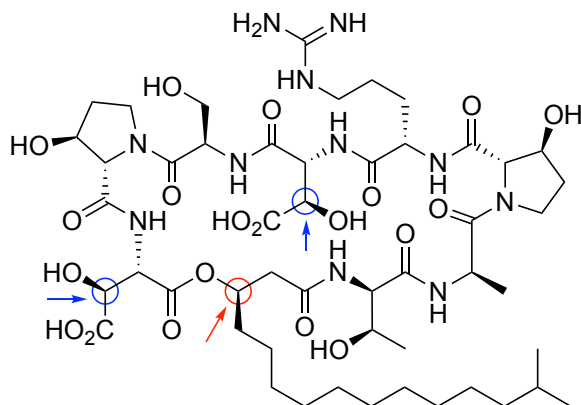
MD シミュレーションにおける 2 nsec ごとの構造を抽出した計 2002 個のプラスバシン A<sub>3</sub> の構造に対してコンフォメーションのクラスタリングを行い、得られたデンドログラムを Figure 44 に示す。このデンドログラムにおいてマージ距離約 3.7 (Figure 44 赤破線) でクラスタリングを行い、クラスター1-10 に分類した。各クラスターはクラスター番号が近いほど似た配座であることを示している。このクラスターを用いて、溶液中と脂質膜中のプラスバシン A<sub>3</sub> の配座を比較した。



**Figure 45.** Time course of distribution of conformation



**Figure 46.** Relationship between conformation and distance between  $\beta$ -hydroxy-Asps

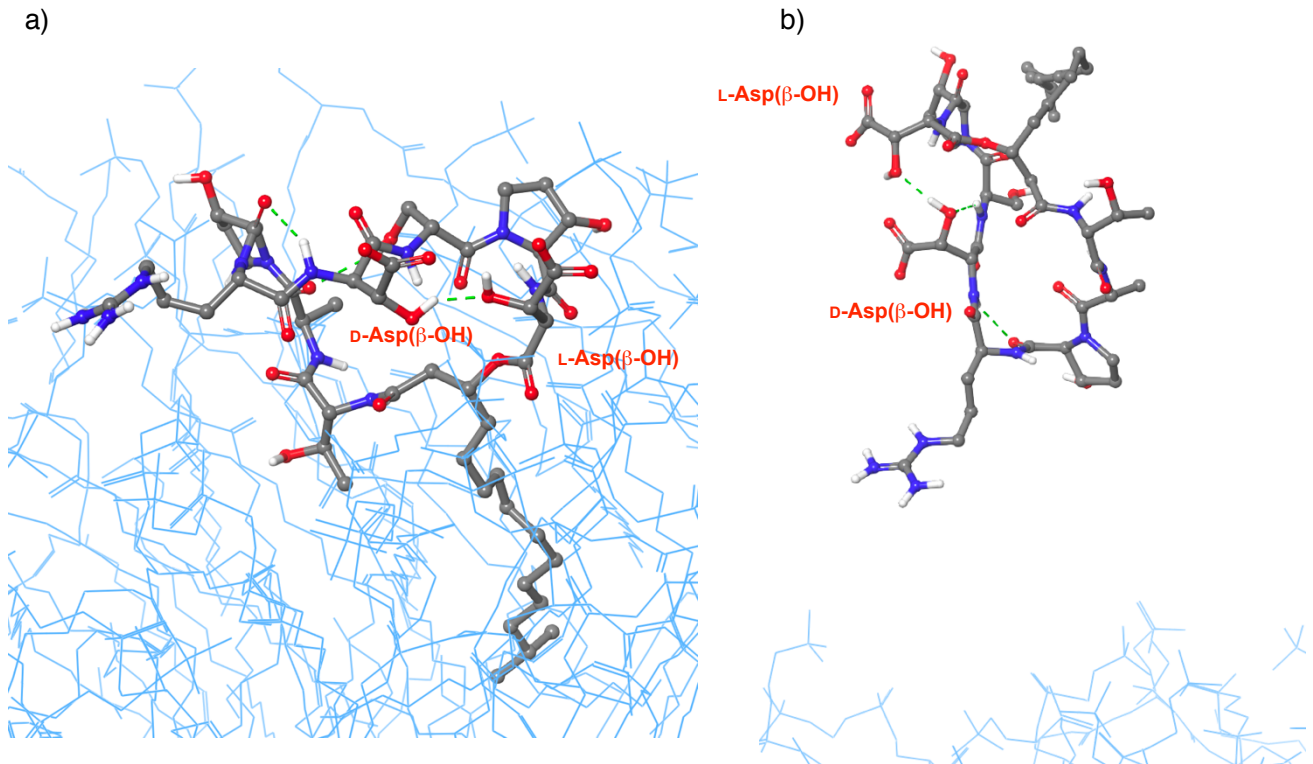


**Figure 47.** Structure of plusbacin A<sub>3</sub>

Figure 45 上段はクラスターの時間分布を示しており、二分子のプラスバシン A<sub>3</sub> はそれぞれ色分けして表記している。下段は、Figure 47 赤矢印で示したプラスバシン A<sub>3</sub> の 3-ヒドロキシイソヘキサデカン酸部 3 位炭素原子の脂質二重膜からの距離の時間経過を示しており、ここから青で示したプラスバシン A<sub>3</sub> 分子は約 1.7  $\mu$ sec、緑で示した分子は約 0.9  $\mu$ sec のシミュレーション時間で脂質膜への挿入を起こしていることがわかる。脂質膜への挿入前後のクラスターの分布を比較すると、その分布が異なる。青色で示した分子では溶液中で主にクラスター 8,9 を脂質膜中で 1,2 を用いており、緑で示した分子は溶液中ではクラスター 4,5 を、脂質膜中ではクラスター 4 を取っている。この結果から、クラスター 1-4 は脂質膜中で優先し、クラスター 5-10 は溶液中で優先する配座であることが示唆される。

次に、クラスター間の構造の差異を調べるため、抗菌活性に必須であった2つの $\beta$ -ヒドロキシ-Asp 残基の $\beta$ 炭素原子(Figure 47 青矢印)間の距離を調べた。Figure 46 は、Figure 45 上段の図を2つの $\beta$ -ヒドロキシ-Asp 残基 $\beta$ 炭素原子間の距離で色分けしたものであり、ここからクラスター1-4はこの距離が短く、一方クラスター5-10はこの距離が長くなっている。ここから、2つの $\beta$ -ヒドロキシ-Asp 残基間の分子内水素結合の存在が示唆され、この水素結合は脂質膜中で形成されやすいと推察される。

2つの $\beta$ -ヒドロキシ-Asp 残基間の水素結合について調べるため、分子動力学シミュレーションにおける0.1 nsecごとの構造を抽出して調べた結果、脂質膜中(Figure 48a)、溶液中(Figure 48b)ともに $\beta$ -ヒドロキシ-Asp 残基間の分子内水素結合が見られる構造が存在した。



**Figure 48.** Intramolecular hydrogen bond of plusbacin A<sub>3</sub>

次に、このような分子内水素結合を有する構造の数を解析した。脂質プラスバシン A<sub>3</sub> の 3-ヒドロキシイソヘキサデカン酸部 3 位炭素原子の脂質二重膜からの距離が 30 Å 以下の構造を脂質膜上に存在する構造とみなすと、Figure 45 青色で示した分子は  $t=881.0$  nsec で、緑で示した分子は  $t=1703.3$  nsec で脂質膜に挿入している(Figure 49)。脂質膜中、溶液中の構造の中で $\beta$ -ヒドロキシ-Asp 残基間の分子内水素結合が見られる構造の数をそれぞれ求め、二分子のプラスバシン A<sub>3</sub> での値を平均すると、脂質二重膜中では 21%、溶液中では 3.7%の構造がこの水素結合を形成していた。プラスバシン A<sub>3</sub> の配座自由度が大きいいため、脂質膜中においても、この水素結合が形成される時間が約 20%であったと考察でき、これは Figure 44 で示したデンドログラムからも示唆されるが、この結果の解釈については、溶液中での NMR 構造の解析等の実験データとの比較が必要である。なお、この水素結合は第三章で述べたジデオキシ誘導体との CD スペクトルの差の要因であると考察でき、脂質膜中ではこの水素結合によりプラスバシン A<sub>3</sub> の配座が溶液中とは異なると考えられる。

molecule	time (nsec)	location	number of structures	
			H-bond	total
plusbacin A <sub>3</sub> (blue)	0-880.9	solution	993	8809
	881.0-2000.0	membrane	2988	11192
plusbacin A <sub>3</sub> (green)	0-1703.2	solution	36	17032
	1703.3-2000.0	membrane	0	2970

**Figure 49.** Structural analysis



#### 第四節 プラスバシン A<sub>3</sub> の推定作用機序

第一節から第三節までの結果を踏まえ、プラスバシン A<sub>3</sub> の推定反応機構を考察した。溶液中のプラスバシン A<sub>3</sub> はリピド II と結合せず、まず細胞膜へ挿入が起こる。つぎに、膜状に移動したプラスバシン A<sub>3</sub> は、 $\beta$ -ヒドロキシ-Asp 残基を介した分子内水素結合が形成されることで配座の変化が起こる。この水素結合によりプラスバシン A<sub>3</sub> は溶液中とは異なる配座をとってリピド II と結合し、その重合を阻害しているものと推察される(Figure 50)。以上の機構は計算のみの実験結果を伴わない、全くの推測ではあるが、今後この機構を実験的に検証するべく、リポソーム存在下でリピド II とプラスバシン A<sub>3</sub> の結合を調べる実験系を構築し、詳細を検討する予定である。

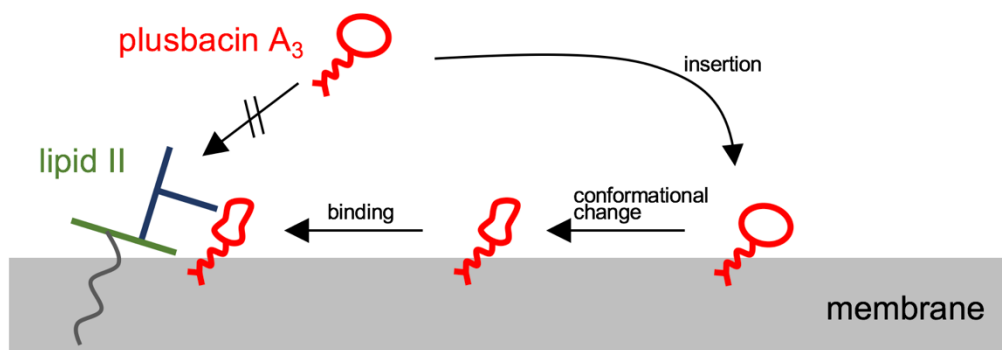


Figure 50. Proposed mechanism of plusbacin A<sub>3</sub>

## 結語

- 1) 五員環イミンを用いる JU-3CR の立体選択性を溶媒により制御し、各ジアステレオマーを同一の出発物質から得るジアステレオ多様の JU-3CR を開発した。
- 2) JU-3CR の反応速度論的解析から二つの異なる反応機構が競争していることを証明し、その結果立体選択性の変化が生じることを明らかにした。
- 3) JU-3CR を用いる プラスバシン A<sub>3</sub> の効率的合成法を確立し、プラスバシン A<sub>3</sub> の全合成及びジデオキシ誘導体の合成を行った。
- 4) プラスバシン A<sub>3</sub> が抗菌活性を示すために必要な配座は *threo*-β-ヒドロキシ-Asp 部位の水酸基によって保たれていることを明らかにした。
- 5) 固相合成法を用いた細胞壁生合成前駆体群の合成法を確立した。
- 6) 脂質膜非存在下ではプラスバシン A<sub>3</sub> はリピド II と結合せず、プラスバシン A<sub>3</sub> はリポソームと相互作用することから、細胞膜脂質二重層に挿入することが示唆された。

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## 実験の部

NMR スペクトルは、JEOL JNM-ECA-500、JEOL JMM-ECS-400、JEOL JNM-ECX-400P を用いて測定した。<sup>1</sup>H NMR および <sup>13</sup>C NMR の化学シフトは、テトラメチルシランを内部標準としたときの $\delta$ 値を ppm で、スピン結合定数  $J$  値を Hz で表示した。

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。またシグナルの帰属は <sup>1</sup>H-<sup>1</sup>H COSY スペクトルに基づいて行った。

質量分析は、Thermo Scientific Exactive を用いて測定した。

IR スペクトルは、日本分光 FT/IR-460 を用いて測定した。

CD スペクトルは日本分光 Jasco J720 を用いて測定した。

反応溶媒として用いた塩化メチレン、アセトニトリルは五酸化二リンより蒸留したものをを用いた。MeOH、トルエンは金属ナトリウムより蒸留したものをを用いた。水は脱イオン水を Millipore Millia-Q<sup>®</sup> Advantage A10<sup>®</sup> 超純水製造装置で精製したものをを用いた。その他の試薬および溶媒については特に記載のない限り市販のものをを用いた。

TLC は Merck silica gel 60 F<sub>254</sub> を用いた。

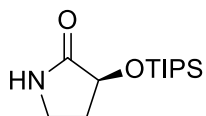
順相シリカゲルカラムクロマトグラフィーは、充填剤として Merck silica gel 60 (0.063-0.200)、Kanto Chemical Silica Gel 60N (spherical, neutral, 63-210  $\mu$ m) を用いた。フラッシュシリカゲルカラムクロマトグラフィーには充填剤として Kanto Chemical Silica Gel 60N (spherical, neutral, 40-50  $\mu$ m) を用いた。

SH シリカゲルは Fuji Silysia Chemical LTD. Scavenger SH silica を用いた。

セライト濾過には nacalai tesque Celite 545 を用いた。

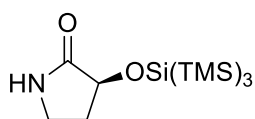
## 第一章

### (S)-3-(Triisopropylsiloxy)-2-pyrrolidinone (3a)



A suspension of (*S*)-4-amino-2-hydroxybutyric acid (**2**, 16.6 g, 139 mmol) and HMDS (210 mL) in *o*-xylene (260 mL) was treated with TMSCl (300  $\mu$ L, 2.37 mmol) at 145 °C for 14 h. The mixture was concentrated *in vacuo*, and the residue in EtOH (270 mL) was treated with 1 M *aq.* HCl (30 mL) for 10 min. The mixture was concentrated *in vacuo* to afford a crude lactam. A suspension of the crude lactam and imidazole (24.9 g, 366 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) was treated with TIPSCl (31.3 mL, 146 mmol) at room temperature for 13 h. The reaction was quenched with MeOH (10 mL), and the mixture was concentrated *in vacuo*. The residue was diluted with AcOEt, and the organic phase was washed with 1 M *aq.* HCl, *sat. aq.* NaHCO<sub>3</sub> and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ( $\phi$ 10 cm  $\times$  20 cm, 1-15% AcOEt/hexane) to afford **3a** (27.3 g, 105 mmol, 76% over 2 steps) as a pale yellow oil. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  7.72 (br s, 1H, NH), 4.26 (dd, 1H,  $\alpha$ -CH,  $J_{\alpha\text{-CH}, \beta\text{-CH}} = J_{\alpha\text{-CH}, \beta\text{-CH}} = 8.0$  Hz), 3.19-3.13 (m, 1H,  $\gamma$ -CH), 3.09 (ddd, 1H,  $\gamma$ -CH,  $J_{\gamma\text{-CH}, \beta\text{-CH}} = J_{\gamma\text{-CH}, \beta\text{-CH}} = J_{\gamma\text{-CH}, \gamma\text{-CH}} = 8.0$  Hz), 2.33 (dddd, 1H,  $\beta$ -CH,  $J_{\beta\text{-CH}, \beta\text{-CH}} = 12.6$ ,  $J_{\beta\text{-CH}, \alpha\text{-CH}} = J_{\beta\text{-CH}, \gamma\text{-CH}} = 8.0$ ,  $J_{\beta\text{-CH}, \gamma\text{-CH}} = 2.9$  Hz), 1.81 (dddd, 1H,  $\beta$ -CH,  $J_{\beta\text{-CH}, \beta\text{-CH}} = 12.6$ ,  $J_{\beta\text{-CH}, \alpha\text{-CH}} = J_{\beta\text{-CH}, \gamma\text{-CH}} = J_{\beta\text{-CH}, \gamma\text{-CH}} = 8.0$  Hz), 1.18-0.95 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  177.2, 70.5, 38.5, 32.1, 17.9, 17.9, 12.5, 12.2; ESIMS-LR *m/z* 280/17 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>13</sub>H<sub>27</sub>O<sub>2</sub>NNaSi 280.1703, found 280.1705; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -30.72 (*c* 0.63, CHCl<sub>3</sub>).

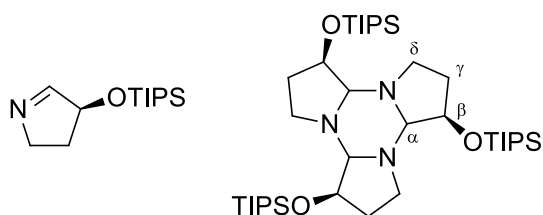
### (S)-3-[Tris(trimethylsilyl)siloxy]-2-pyrrolidinone (3b)



A suspension of (*S*)-4-amino-2-hydroxybutyric acid (**2**, 500 mg, 4.2 mmol) and HMDS (6.0 mL) in *o*-xylene (10 mL) was treated with TMSCl (10  $\mu$ L, 79  $\mu$ mol) at 145 °C for 22.5 h. The mixture was concentrated *in vacuo*, and the residue in EtOH (4 mL) was treated with 1 M *aq.* HCl (1 mL) for 10 min. The mixture was concentrated *in vacuo* to afford a crude lactam. A suspension of the crude lactam and DMAP (616 mg, 5.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was treated with chlorotris(trimethyl)silane (1.20 g, 5.0 mmol) at room temperature for 25.5 h. The reaction was quenched with MeOH (1.0 mL) and the mixture was concentrated *in vacuo*. The residue was diluted with AcOEt, and the organic phase was washed with 1 M *aq.* HCl, *sat. aq.* NaHCO<sub>3</sub> and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (3-40% AcOEt/hexane) to afford **3b** (965 mg, 2.77 mmol, 66% over 2 steps) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  5.38 (br s, 1H, NH), 3.90 (dd, 1H,  $\alpha$ -CH,  $J_{\alpha\text{-CH}, \beta\text{-CH}} = J_{\alpha\text{-CH}, \beta\text{-CH}} = 7.7$  Hz), 3.38-3.31 (m, 1H,  $\gamma$ -CH), 3.22 (ddd, 1H,  $\gamma$ -CH,  $J_{\gamma\text{-CH}, \gamma\text{-CH}} = 9.2$ ,  $J_{\gamma\text{-CH}, \beta\text{-CH}} = 7.7$ ,  $J_{\gamma\text{-CH}, \beta\text{-CH}} = 8.7$  Hz), 2.38-2.31 (m, 1H,  $\beta$ -CH), 1.99-1.90 (m, 1H,  $\beta$ -CH), 0.21 (s, 27H, CH<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  176.6, 73.8, 38.5, 31.8, 0.4; ESIMS-LR *m/z* 370.15 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>13</sub>H<sub>33</sub>O<sub>2</sub>NNaSi<sub>4</sub> 370.1481, found 370.1479; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -45.27 (*c* 0.71, CHCl<sub>3</sub>).

### (3*S*)-3-(Triisopropylsiloxy)-4,5-dihydropyrrole (**4a**)

### (4*S*,9*S*,14*S*)-4,9,14-Tris(triisopropylsiloxy)-1,6,11-triazatetracyclo[10.3.0.0<sup>2,6</sup>.0<sup>7,11</sup>]pentadecane (**5a**)

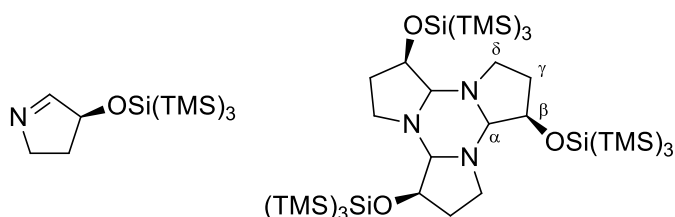


A solution of **3a** (51 mg, 0.20 mmol) in THF (0.5 mL) was added to a suspension of (Cp)<sub>2</sub>Zr(H)Cl (124 mg, 0.48 mmol) in THF (0.5 mL) at -20 °C over 30 min. The whole mixture was warmed to room temperature, and stirred for 6 h. To the mixture, ice cold hexane was added, and insolubles were filtered off through

a Celite pad, and the filtrate was concentrated *in vacuo* to afford equilibrium mixture of **4a** and **5a** (40 mg, 0.17 mmol, 84%) as a yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a equilibrium mixture of **4a** and **5a**). Selected data for **4a**: δ 7.54 (s, 1H, N=CH), 4.90 (m, 1H, α-CH), 4.07-3.99 (m, 1H, γ-CH), 3.77-3.68 (m, 1H, γ-CH), 1.72-1.58 (m, 2H, β-CH), 1.42-0.88 (m, 21H, <sup>1</sup>Pr<sub>3</sub>Si); Selected data for **5a**: δ 4.23 (m, 9H, β-CH), 2.98 (m, 3H, α-CH), 2.92 (m, 9H, δ-CH), 2.58 (m, 9H, δ-CH), 2.25-2.15 (m, 9H, γ-CH), 1.72-1.58 (m, 9H, γ-CH), 1.42-0.88 (m, 63H, <sup>1</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz, a equilibrium mixture of **4a** and **5a**) δ 168.6, 137.7, 132.5, 130.2, 128.4, 89.3, 79.0, 77.4, 73.7, 60.1, 45.0, 32.7, 18.2, 18.1, 18.0, 12.5, 12.2; ESIMS-LR *m/z* 242.19 [(M+H)<sup>+</sup>]; ESIMS-HR calcd for C<sub>13</sub>H<sub>28</sub>ONSi 242.1935, found 242.1935.

### (3*S*)-3-[Tris(trimethylsilyl)siloxy]-4,5-dihydropyrrole (**4b**)

### (4*S*,9*S*,14*S*)-4,9,14-Tris[tris(trimethylsilyl)siloxy]-1,6,11-triazatetracyclo[10.3.0.0<sup>2,6</sup>.0<sup>7,11</sup>]pentadecane (**5b**)



A solution of **3b** (35 mg, 0.10 mmol) in THF (1.0 mL) was treated with (Cp)<sub>2</sub>Zr(H)Cl (77 mg, 0.30 mmol) at 0 °C for 5 min. The mixture was warmed to room temperature and stirred for 50 min. To the mixture, ice cold hexane was added, and insolubles were filtered off through a Celite pad, and the filtrate

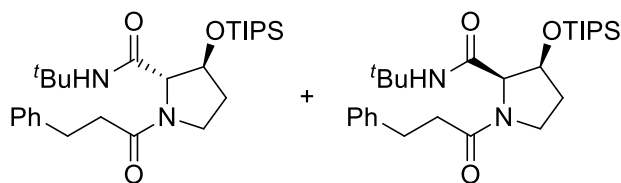
was concentrated *in vacuo*. The residue was purified by short silica gel column chromatography (50% AcOEt/hexane) to afford equilibrium mixture of **4b** and **5b** (10 mg, 0.030 mmol, 30%) as a white foam. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several conformers of a equilibrium mixture of **4b** and **5b**). Selected data for **4b**: δ 7.48-7.343 (m, 1H, N=CH), 4.50 (dd, 1H, α-CH, *J*<sub>α-CH, β-CH</sub> = *J*<sub>α-CH, γ-CH</sub> = 6.9 Hz), 4.03-3.94 (m, 1H, γ-CH), 3.76-3.67 (m, 1H, γ-CH), 1.72-1.48 (m, 2H, β-CH), 0.34-0.02 (m, 27H, CH<sub>3</sub>Si). Selected data for **5b**: δ 3.92-3.80 (m, 3H, β-CH), 2.92 (ddd, 9H, δ-CH, *J*<sub>δ-CH, δ-CH</sub> = *J*<sub>δ-CH, γ-CH</sub> = 8.6, *J*<sub>δ-CH, γ-CH</sub> = 2.9 Hz), 2.63 (d, 3H, α-CH, *J*<sub>α-CH, β-CH</sub> = 5.2 Hz), 2.47 (ddd, 9H, δ-CH, *J*<sub>δ-CH, δ-CH</sub> = *J*<sub>δ-CH, γ-CH</sub> = *J*<sub>δ-CH, γ-CH</sub> = 8.6 Hz), 2.20-2.08 (m, 9H, β-CH), 1.48-1.41 (m, 9H, β-CH), 0.34-0.02 (m, 81H, CH<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz, a equilibrium mixture of **4b** and **5b**) δ 168.3, 114.1, 112.6, 89.5, 83.2, 77.8, 60.3, 45.5, 32.6, 32.5, 0.9, 0.7, 0.6, 0.6, 0.4, 0.1, -0.4; ESIMS-LR *m/z* 332.17 [(M+H)<sup>+</sup>]; ESIMS-HR calcd for C<sub>13</sub>H<sub>34</sub>ONSi<sub>4</sub> 332.1712, found 332.1714.

### General procedure of Joullé-Ugi reaction

A solution of imine, carboxylic acid in solvent was treated with isocyanide at room temperature for the amount of time indicated. The mixture was then concentrated *in vacuo*, and the residue was purified by high-flash silica gel column chromatography to afford the product.

**(2*S*,3*S*)-*N*-*tert*-Butyl-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-8a)**

**(2*R*,3*S*)-*N*-*tert*-Butyl-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-8a)**

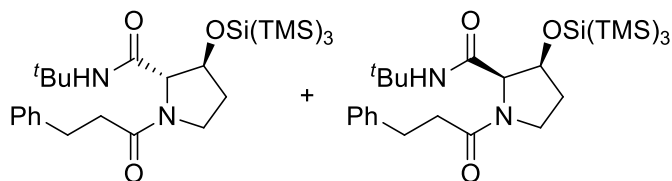


The corresponding compounds were prepared following general procedure using imine **4a** (29.0 mg, 0.12 mmol), 3-phenylpropionic acid (**7a**, 18.0 mg, 0.12 mmol) and *tert*-butyl isocyanide (**6**, 13.6  $\mu$ L, 0.12 mmol) in toluene (1.2 mL). The mixture was stirred at room

temperature for 25 h. Purification by high-flash silica gel column chromatography (10-60% AcOEt/hexane) afforded **trans**-**8a** (4.9 mg, 0.017 mmol, 14%) as a yellow oil and **cis**-**8a** (27.8 mg, 0.059 mmol, 49%) as a yellow oil. Data for **trans**-**8a**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, a mixture of two rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  7.30-7.15 (m, 5H, Ph), 6.87 (s, 1H,  $^t\text{BuNH}$ ), 4.72 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 3.5$  Hz), 4.33 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.66-3.44 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 3.08-2.91 (m, 2H, C(=O)CH<sub>2</sub>), 2.66 (m, 2H, PhCH<sub>2</sub>), 2.21 (ddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 12.4$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 3.5$  Hz), 1.92 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 12.4$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 1.29 (s, 9H,  $^t\text{Bu}$ ), 1.13-0.95 (m, 21H,  $^t\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  173.0, 172.7, 169.2, 168.4, 141.2, 128.7, 128.6, 128.6, 128.5, 126.3, 84.3, 73.1, 71.9, 69.8, 51.6, 51.1, 45.9, 45.1, 36.8, 36.3, 34.5, 32.1, 31.0, 30.9, 28.8, 28.7, 18.1, 12.1; ESIMS-LR  $m/z$  497.32 [(M+Na) $^+$ ]; ESIMS-HR calcd for C<sub>27</sub>H<sub>46</sub>O<sub>3</sub>N<sub>2</sub>NaSi 497.3170, found 497.3175;  $[\alpha]_D^{20} -19.45$  (*c* 3.18, CHCl<sub>3</sub>). Data for **cis**-**8a**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, a mixture of two rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  7.32-7.10 (m, 5H, Ph), 5.51 (s, 1H,  $^t\text{BuNH}$ ), 4.40 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 10.3$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.5$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 7.0$  Hz), 4.28 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 7.0$  Hz), 3.68 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.27 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 7.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 7.3$  Hz), 3.03-2.89 (m, 2H, C(=O)CH<sub>2</sub>), 2.65-2.44 (m, 2H, PhCH<sub>2</sub>), 2.44-2.33 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.13-1.94 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.34 (s, 9H,  $^t\text{Bu}$ ), 1.14-1.00 (m, 21H,  $^t\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  172.4, 171.6, 168.3, 167.6, 141.5, 141.4, 128.6, 128.5, 126.2, 126.2, 73.2, 71.9, 64.8, 63.3, 51.6, 44.6, 43.5, 36.6, 35.7, 32.1, 31.3, 31.3, 30.9, 28.8, 18.2, 18.1, 18.0; ESIMS-LR  $m/z$  497.32 [(M+Na) $^+$ ]; ESIMS-HR calcd for C<sub>27</sub>H<sub>46</sub>O<sub>3</sub>N<sub>2</sub>NaSi 497.3170, found 497.3171;  $[\alpha]_D^{19} +11.33$  (*c* 1.39, CHCl<sub>3</sub>).

**(2*S*,3*S*)-*N*-*tert*-Butyl-1-(3-phenylpropanoyl)-3-[tris(trimethylsilyl)siloxy]pyrrolidine-2-carboxamide (*trans*-8b)**

**(2*R*,3*S*)-*N*-*tert*-Butyl-1-(3-phenylpropanoyl)-3-[tris(trimethylsilyl)siloxy]pyrrolidine-2-carboxamide (*cis*-8b)**



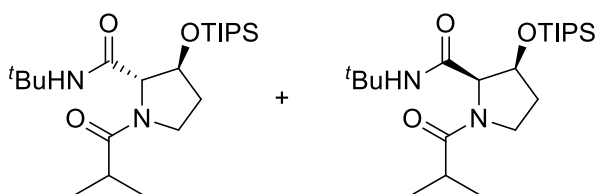
The corresponding compounds were prepared following general procedure using imine **4b** (33.2 mg, 0.10 mmol), 3-phenylpropionic acid (**7a**, 15.0 mg, 0.10 mmol) and *tert*-butyl isocyanide (**6**, 11.3  $\mu$ L, 0.10 mmol) in toluene (1.0 mL). The mixture was stirred at

room temperature for 60 min. Purification by high-flash silica gel column chromatography (10-45% AcOEt/hexane) afforded **trans**-**8b** (6.4 mg, 0.011 mmol, 11%) as a white solid and **cis**-**8b** (17.3 mg, 0.031 mmol, 31%) as a white

solid. Data for **trans-8b**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.31-7.17 (m, 5H, Ph), 6.95 (s, 1H,  $^t\text{BuNH}$ ), 4.30 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 3.5$  Hz), 4.21 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.52-3.41 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 3.06-2.91 (m, 2H,  $\text{C(=O)CH}_2$ ), 2.66-2.61 (m, 2H,  $\text{PhCH}_2$ ), 2.25-2.16 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.82 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.3$  Hz), 1.30 (s, 9H,  $^t\text{Bu}$ ), 0.18 (s, 27H,  $\text{CH}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  173.1, 172.9, 169.1, 169.1, 141.2, 141.1, 128.7, 128.6, 128.5, 128.4, 126.4, 126.3, 77.0, 72.5, 69.5, 69.2, 51.2, 51.1, 45.8, 45.7, 36.4, 36.3, 34.3, 33.1, 31.0, 28.7, 28.7, 28.7, 2.1, 0.3, -0.5, -0.6, -2.2; ESIMS-LR  $m/z$  587.30 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{27}\text{H}_{52}\text{O}_3\text{N}_2\text{NaSi}_4$  587.2947, found 587.2960;  $[\alpha]^{19}_{\text{D}} -37.36$  ( $c$  2.95,  $\text{CHCl}_3$ ). Data for **cis-8b**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.30-7.16 (m, 5H, Ph), 5.40 (s, 1H,  $^t\text{BuNH}$ ), 4.21 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 3.94 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 10.3$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 6.9$  Hz), 3.67-3.61 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.18 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 3.01-2.91 (m, 2H,  $\text{C(=O)CH}_2$ ), 2.64-2.41 (m, 2H,  $\text{PhCH}_2$ ), 2.29-2.19 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.04-1.97 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.34 (s, 9H,  $^t\text{Bu}$ ), 0.20 (s, 27H,  $\text{CH}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  173.5, 170.1, 140.8, 128.7, 128.5, 126.5, 71.6, 59.8, 51.7, 45.3, 35.9, 33.1, 31.1, 28.7, 2.3, 2.1, -0.6, -2.1, -2.2; ESIMS-LR  $m/z$  587.30 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{27}\text{H}_{52}\text{O}_3\text{N}_2\text{NaSi}_4$  587.2947, found 587.2951;  $[\alpha]^{20}_{\text{D}} +42.82$  ( $c$  1.73,  $\text{CHCl}_3$ ).

**(2*S*,3*S*)-*N*-tert-Butyl-1-isobutyl-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-9b)**

**(2*R*,3*S*)-*N*-tert-Butyl-1-isobutyl-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-9b)**



The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol), isobutylic acid (**7b**, 9.3  $\mu\text{L}$ , 0.10 mmol) and *tert*-butyl isocyanide (**6**, 11.3  $\mu\text{L}$ , 0.10 mmol) in HFIP (1.0 mL). The mixture was stirred at room temperature for 60 min.

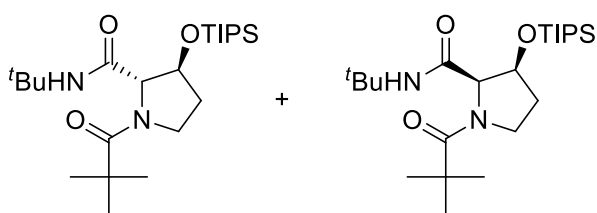
Purification by high-flash silica gel column chromatography (10-40%  $\text{AcOEt}$ /hexane) afforded **trans-9b** (25.5 mg, 0.062 mmol, 62%) as a white solid and **cis-9b** (3.6 mg, 0.009 mmol, 9%) as a white solid. Data for **trans-9b**:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.38 (s, 1H,  $^t\text{BuNH}$ ), 4.57-4.54 (m, 1H, 3-hydroxy-Pro- $\beta$ -CH), 4.23 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.80-3.71 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 2.85 (sept, 1H,  $\text{CH}_3\text{CHC(=O)}$ ,  $J_{\text{CH}_3\text{CHC(=O)}, \text{CH}_3\text{CHC(=O)}} = 6.9$  Hz), 2.24 (dddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 12.6$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 4.0$  Hz), 2.01-1.95 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.32 (s, 9H,  $^t\text{Bu}$ ), 1.19-1.02 (m, 27H,  $\text{CH}_3\text{CHC(=O)}$ ,  $^t\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  179.3, 179.2, 171.6, 78.5, 75.8, 71.8, 71.2, 52.7, 52.3, 46.6, 46.2, 35.2, 33.6, 33.3, 33.2, 28.8, 19.8, 19.6, 19.3, 19.1, 18.5, 18.5; ESIMS-LR  $m/z$  435.30 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{22}\text{H}_{44}\text{O}_3\text{N}_2\text{NaSi}$  435.3013, found 435.3018;  $[\alpha]^{20}_{\text{D}} -59.34$  ( $c$  2.55,  $\text{CHCl}_3$ ). Data for **cis-9b**:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.23 (s, 1H,  $^t\text{BuNH}$ ), 4.62 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 7.5$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.8$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 4.35 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 7.5$  Hz),



3.80 (ddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.7$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.9$  Hz), 3.56 (ddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 2.77 (sept, 1H,  $\text{CH}_3\text{CHC}(\text{C}=\text{O})$ ,  $J_{\text{CH}_3\text{CHC}(\text{C}=\text{O})}$ ,  $\text{CH}_3\text{CHC}(\text{C}=\text{O}) = 6.3$  Hz), 2.36-2.26 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.25-2.11 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.33 (s, 9H,  $\text{tBu}$ ), 1.18-1.06 (m, 27H,  $\text{CH}_3\text{CHC}(\text{C}=\text{O})$ ,  $\text{tPr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  179.0, 178.6, 171.0, 170.9, 74.7, 73.3, 64.9, 64.8, 52.5, 52.3, 45.7, 45.1, 33.9, 33.4, 32.5, 31.8, 29.0, 19.8, 19.6, 19.2, 19.1, 18.6, 18.5, 13.6; ESIMS-LR  $m/z$  435.30 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{22}\text{H}_{44}\text{O}_3\text{N}_2\text{NaSi}$  435.3013, found 435.3016;  $[\alpha]_D^{20} +4.39$  ( $c$  0.36,  $\text{CHCl}_3$ ).

**(2*S*,3*S*)-*N*-tert-Butyl-1-pivaloyl-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-9c)**

**(2*R*,3*S*)-*N*-tert-Butyl-1-pivaloyl-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-9c)**



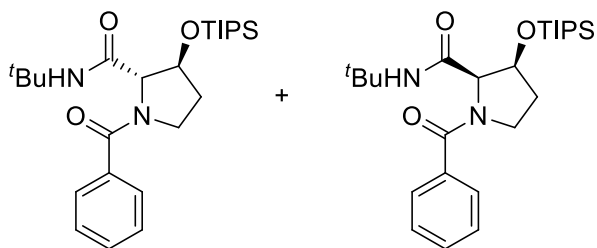
The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol), pivalic acid (**7c**, 10.2 mg, 0.10 mmol) and *tert*-butyl isocyanide (**6**, 11.3  $\mu\text{L}$ , 0.10 mmol) in HFIP (1.0 mL). The mixture was stirred at room temperature for 60 min.

Purification by high-flash silica gel column chromatography

(10-40% AcOEt/hexane) afforded **trans-9c** (27.7 mg, 0.065 mmol, 65%) as a white solid and **cis-9c** (3.6 mg, 0.012 mmol, 12%) as a white solid. Data for **trans-9c**:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  7.24 (s, 1H,  $\text{tBuNH}$ ), 4.46 (br s, 1H, 3-hydroxy-Pro- $\beta$ -CH), 4.24 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.89 (br s, 2H, 3-hydroxy-Pro- $\delta$ -CH), 2.24 (br s, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.97 (br s, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.32 (s, 9H,  $\text{tBuNH}$ ), 1.28 (s, 9H,  $\text{tBuC}(\text{=O})$ ), 1.19-1.02 (m, 21H,  $\text{tPr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  179.5, 172.1, 74.7, 73.3, 52.3, 47.8, 40.1, 36.2, 28.8, 27.8, 18.5, 18.5, 13.6, 13.4, 13.2; ESIMS-LR  $m/z$  449.32 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{23}\text{H}_{46}\text{O}_3\text{N}_2\text{NaSi}$  449.3170, found 449.3171;  $[\alpha]_D^{20} -36.35$  ( $c$  2.77,  $\text{CHCl}_3$ ). Data for **cis-9c**:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  6.98 (s, 1H,  $\text{tBuNH}$ ), 4.59 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 4.45 (br s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.93 (br s, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.73 (br s, 1H, 3-hydroxy-Pro- $\delta$ -CH), 2.32-2.17 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.16-2.08 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.33 (s, 9H,  $\text{tBuNH}$ ), 1.26 (s, 9H,  $\text{tBuC}(\text{=O})$ ), 1.19-1.06 (m, 21H,  $\text{tPr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  179.2, 170.9, 72.4, 67.6, 52.3, 47.4, 35.0, 35.0, 29.0, 27.9, 27.7, 18.7, 18.6, 13.6, 13.4; ESIMS-LR  $m/z$  449.32 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{23}\text{H}_{46}\text{O}_3\text{N}_2\text{NaSi}$  449.3170, found 449.3177;  $[\alpha]_D^{20} +1.86$  ( $c$  0.50,  $\text{CHCl}_3$ ).

**(2*S*,3*S*)-1-Benzoyl-*N*-tert-butyl-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-9d)**

**(2*R*,3*S*)-1-Benzoyl-*N*-tert-butyl-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-9d)**



The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol), benzoic acid (**7d**, 12.2 mg, 0.10 mmol) and *tert*-butyl isocyanide (**6**, 11.3  $\mu\text{L}$ , 0.10 mmol) in HFIP (1.0 mL). The mixture was stirred at room temperature for 60 min.

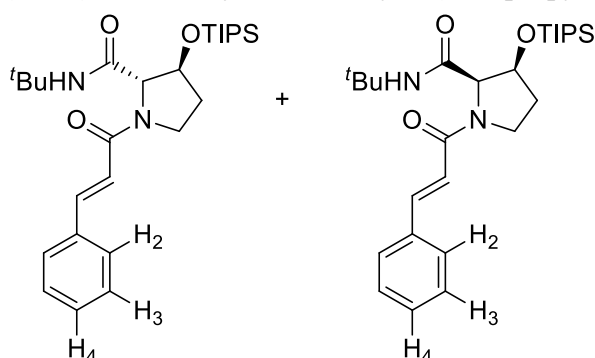
Purification by high-flash silica gel column chromatography

(10-40% AcOEt/hexane) afforded **trans-9d** (33.5 mg, 0.075 mmol, 75%) as a white solid and **cis-9d** (5.3 mg, 0.012

mmol, 12%) as a white solid. Data for **trans-9d**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.50-7.34 (m, 5H, Ph), 6.77 (s, 1H,  $^t\text{BuNH}$ ), 4.85 (br s, 1H, 3-hydroxy-Pro- $\beta$ -CH), 4.52 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.61-3.52 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 2.28-2.20 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.93-1.86 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.35 (s, 9H,  $^t\text{Bu}$ ), 1.16-0.94 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  172.5, 171.5, 169.1, 168.7, 136.4, 130.3, 130.2, 128.5, 126.9, 126.8, 76.0, 73.6, 73.3, 69.7, 51.6, 51.3, 48.3, 45.2, 34.8, 32.1, 28.8, 18.1, 18.0, 12.2, 12.1; ESIMS-LR  $m/z$  469.29 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{25}\text{H}_{42}\text{O}_3\text{N}_2\text{NaSi}$  469.2857, found 469.2869;  $[\alpha]_D^{21}$  -71.52 ( $c$  3.35,  $\text{CHCl}_3$ ). Data for **cis-9d**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.61-7.32 (m, 1H,  $\text{PhCH}=\text{CH}$ ), 5.62 (s, 1H,  $^t\text{BuNH}$ ), 4.70-4.62 (m, 1H, 3-hydroxy-Pro- $\beta$ -CH), 4.51 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.3$  Hz), 3.93-3.69 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.42 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 7.5$  Hz), 2.28-2.14 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.04-1.96 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.36 (s, 9H,  $^t\text{Bu}$ ), 1.19-1.00 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  171.0, 170.7, 167.9, 167.6, 137.0, 136.0, 133.3, 130.3, 130.1, 129.9, 128.4, 128.4, 127.4, 127.0, 73.2, 72.1, 66.2, 65.3, 51.5, 47.9, 47.8, 44.1, 33.4, 30.6, 28.8, 28.6, 18.2, 18.1, 12.4, 12.2; ESIMS-LR  $m/z$  469.29 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{25}\text{H}_{42}\text{O}_3\text{N}_2\text{NaSi}$  469.2857, found 469.2864;  $[\alpha]_D^{20}$  +12.82 ( $c$  2.93,  $\text{CHCl}_3$ ).

**(2*S*,3*S*)-*N*-*tert*-Butyl-1-cinnamoyl-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-9e)**

**(2*R*,3*S*)-*N*-*tert*-Butyl-1-cinnamoyl-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-9e)**



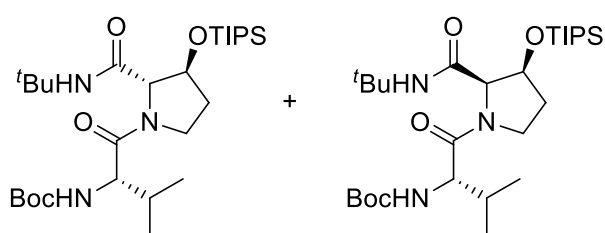
The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol), *trans*-cinnamic acid (**7e**, 14.8 mg, 0.10 mmol) and *tert*-butyl isocyanide (**6**, 11.3  $\mu\text{L}$ , 0.10 mmol) in HFIP (1.0 mL). The mixture was stirred at room temperature for 60 min. Purification by high-flash silica gel column chromatography (10-40% AcOEt/hexane) afforded **trans-9e** (36.1 mg, 0.076 mmol, 76%) as a white solid and **cis-9e** (6.5 mg, 0.012 mmol,

14%) as a white solid. Data for **trans-9e**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.74 (d, 1H,  $\text{PhCH}=\text{CH}$ ,  $J_{\text{PhCH}=\text{CH}, \text{PhCH}=\text{CH}} = 15.8$  Hz), 7.56 (dd, 2H, H-2,  $J_{\text{H-2}, \text{H-3}} = 7.5$ ,  $J_{\text{H-2}, \text{H-4}} = 1.7$  Hz), 7.42-7.34 (m, 3H, H-3, H-4), 7.03 (s, 1H,  $^t\text{BuNH}$ ), 6.81 (d, 1H,  $\text{PhCH}=\text{CH}$ ,  $J_{\text{PhCH}=\text{CH}, \text{PhCH}=\text{CH}} = 15.8$  Hz), 4.81 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 3.4$  Hz), 4.48 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.89-3.75 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 2.35-2.26 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.05-1.99 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.33 (s, 9H,  $^t\text{Bu}$ ), 1.15-1.02 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  169.2, 168.7, 166.6, 143.9, 143.0, 135.1, 134.8, 130.2, 130.0, 129.0, 128.9, 128.1, 118.0, 117.3, 73.0, 72.1, 70.0, 51.7, 51.1, 45.9, 45.3, 34.4, 32.2, 28.8, 18.1, 12.4, 12.1; ESIMS-LR  $m/z$  495.30 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{27}\text{H}_{44}\text{O}_3\text{N}_2\text{NaSi}$  495.3013, found 495.3021;  $[\alpha]_D^{19}$  -95.60 ( $c$  3.61,  $\text{CHCl}_3$ ). Data for **cis-9e**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.69 (d, 1H,  $\text{PhCH}=\text{CH}$ ,  $J_{\text{PhCH}=\text{CH}, \text{PhCH}=\text{CH}} = 15.5$  Hz), 7.54-7.47 (m, 2H, H-2), 7.39-7.32 (m, 3H, H-3, H-4), 6.72 (d, 1H,  $\text{PhCH}=\text{CH}$ ,  $J_{\text{PhCH}=\text{CH}, \text{PhCH}=\text{CH}} = 15.5$  Hz), 5.55 (s, 1H,  $^t\text{BuNH}$ ), 4.53 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 10.3$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.9$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 6.3$  Hz), 4.42 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}}$

$\text{CH} = 6.9 \text{ Hz}$ ), 3.98 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.7$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.3 \text{ Hz}$ ), 3.63 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.7 \text{ Hz}$ ), 2.54-2.44 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.12-1.98 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.36 (s, 9H,  $^t\text{Bu}$ ), 1.18-1.06 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  168.1, 166.5, 165.4, 143.2, 142.8, 135.3, 134.9, 130.0, 129.8, 128.9, 128.2, 128.1, 117.9, 117.6, 73.6, 71.9, 66.6, 63.8, 51.7, 51.6, 44.8, 44.2, 32.4, 32.3, 28.9, 28.8, 18.2, 18.1, 18.1, 12.5, 12.3; ESIMS-LR  $m/z$  495.30 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{27}\text{H}_{44}\text{O}_3\text{N}_2\text{NaSi}$  495.3013, found 495.3012;  $[\alpha]^{19}_{\text{D}} +23.46$  ( $c$  1.52,  $\text{CHCl}_3$ ).

**(2*S*,3*S*)-1-[(*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylbutylyl]-*N*-*tert*-butyl-3-(triisopropylsiloxy)-pyrrolidine-2-carboxamide (*trans*-9f)**

**(2*R*,3*S*)-1-[(*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylbutylyl]-*N*-*tert*-butyl-3-(triisopropylsiloxy)-pyrrolidine-2-carboxamide (*cis*-9f)**



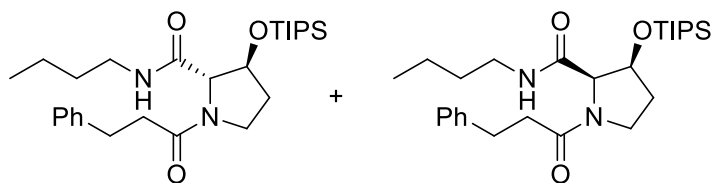
The corresponding compounds were prepared following general procedure using imine **4a** (12.0 mg, 0.05 mmol), Boc-Val-OH (**7f**, 11.0 mg, 0.05 mmol) and *tert*-butyl isocyanide (**6**, 5.7  $\mu\text{L}$ , 0.05 mmol) in HFIP (500  $\mu\text{L}$ ). The mixture was stirred at room temperature for 60 min.

Purification by high-flash silica gel column chromatography

(10-40% AcOEt/hexane) afforded **trans**-9f (15.2 mg, 0.028 mmol, 56%) as a pale yellow oil and **cis**-9f (6.2 mg, 0.011 mmol, 23%) as a pale yellow oil. Data for **trans**-9f:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, a mixture of two rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  6.71 (s, 1H,  $^t\text{BuNH}$ ), 5.15 (d, 1H, Val-NH,  $J_{\text{Val-NH}, \text{Val-}\alpha\text{-CH}} = 9.2 \text{ Hz}$ ), 4.76 (br s, 1H, 3-hydroxy-Pro- $\beta$ -CH), 4.35 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.33 (dd, 1H, Val- $\alpha$ -CH,  $J_{\text{Val-}\alpha\text{-CH}, \text{Val-NH}} = 9.2$ ,  $J_{\text{Val-}\alpha\text{-CH}, \text{Val-}\beta\text{-CH}} = 8.6 \text{ Hz}$ ), 3.91 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2 \text{ Hz}$ ), 3.62 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.3 \text{ Hz}$ ), 2.29-2.21 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.02-1.95 (m, 2H, 3-hydroxy-Pro- $\gamma$ -CH, Val- $\beta$ -CH), 1.42 (s, 9H, Boc- $^t\text{Bu}$ ), 1.29 (s, 9H,  $^t\text{BuNH}$ ), 1.13-1.01 (m, 21H,  $^i\text{Pr}_3\text{Si}$ ), 0.99 (d, 3H, Val- $\gamma$ -CH,  $J_{\text{Val-}\gamma\text{-CH}, \text{Val-}\beta\text{-CH}} = 6.9 \text{ Hz}$ ), 0.94 (d, 3H, Val- $\gamma$ -CH,  $J_{\text{Val-}\gamma\text{-CH}, \text{Val-}\beta\text{-CH}} = 6.3 \text{ Hz}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  173.1, 168.9, 155.8, 79.7, 72.8, 69.5, 56.8, 51.2, 46.1, 34.6, 31.8, 28.8, 28.4, 19.5, 18.1, 17.6, 12.1; ESIMS-LR  $m/z$  564.38 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{28}\text{H}_{55}\text{O}_5\text{N}_3\text{NaSi}$  564.3803, found 564.3809;  $[\alpha]^{21}_{\text{D}} -51.78$  ( $c$  1.52,  $\text{CHCl}_3$ ). Data for **cis**-9f:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, a mixture of two rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  6.00 (s, 1H,  $^t\text{BuNH}$ ), 4.97 (d, 1H, Val-NH,  $J_{\text{Val-NH}, \text{Val-}\alpha\text{-CH}} = 10.3 \text{ Hz}$ ), 4.53-4.41 (m, 1H, 3-hydroxy-Pro- $\beta$ -CH, Val- $\alpha$ -CH), 4.17 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 7.3 \text{ Hz}$ ), 4.00-3.93 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.53-3.41 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 2.35-2.24 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.15-1.97 (m, 2H, 3-hydroxy-Pro- $\gamma$ -CH, Val- $\beta$ -CH), 1.43 (s, 9H, Boc- $^t\text{Bu}$ ), 1.37 (s, 9H,  $^t\text{BuNH}$ ), 1.16-1.02 (m, 21H,  $^i\text{Pr}_3\text{Si}$ ), 0.93-0.86 (m, 6H, Val- $\gamma$ -CH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  171.5, 171.2, 168.3, 167.5, 156.2, 155.8, 79.9, 79.6, 73.2, 72.0, 64.2, 63.3, 57.7, 56.2, 51.6, 51.5, 44.7, 43.7, 32.4, 31.6, 30.6, 30.2, 28.8, 28.8, 28.5, 28.4, 19.9, 19.8, 18.2, 18.1, 17.9, 17.5, 12.3; ESIMS-LR  $m/z$  564.38 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{28}\text{H}_{55}\text{O}_5\text{N}_3\text{NaSi}$  564.3803, found 564.3809;  $[\alpha]^{20}_{\text{D}} -8.21$  ( $c$  1.81,  $\text{CHCl}_3$ ).

**(2*S*,3*S*)-*N*-Butyl-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-10b)**

**(2*R*,3*S*)-*N*-Butyl-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-10b)**



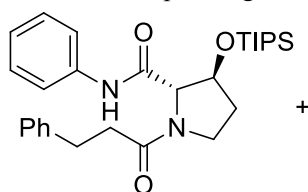
The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol), 3-phenylpropionic acid (**7a**, 15.0 mg, 0.10 mmol) and butyl isocyanide (**6b**, 10.5  $\mu$ L, 0.10 mmol) in HFIP (1.0 mL). The mixture was

stirred at room temperature for 60 min. Purification by high-flash silica gel column chromatography (3-20-40% AcOEt/hexane) afforded **trans**-10b (28.5 mg, 0.060 mmol, 60%) as a pale yellow oil and **cis**-10b (9.8 mg, 0.021 mmol, 21%) as a pale yellow oil. Data for **trans**-10b:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  7.32-7.16 (m, 5H, Ph), 7.00 (br s, 1H, BuNH), 4.79 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 3.5$  Hz), 4.43 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.66 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 11.5$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 3.48 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 3.25-3.06 (m, 2H, H-1), 3.04-2.93 (m, 2H, C(=O)CH<sub>2</sub>), 2.76-2.63 (m, 2H, PhCH<sub>2</sub>), 2.21 (dddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 11.5$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 3.5$  Hz), 1.94 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.9$  Hz), 1.48-1.41 (m, 2H, H-2), 1.39-1.23 (m, 2H, H-3), 1.14-0.96 (m, 21H,  $^i\text{Pr}_3\text{Si}$ ), 0.91 (t, 3H, H-4,  $J_{\text{H-4}, \text{H-3}} = 7.5$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  176.2, 173.2, 169.9, 168.8, 141.1, 140.1, 140.6, 128.6, 128.5, 128.4, 128.4, 126.3, 76.6, 73.0, 71.6, 69.1, 45.9, 45.2, 39.4, 39.2, 36.7, 36.3, 35.6, 34.4, 32.0, 31.6, 31.5, 31.0, 30.9, 20.2, 18.1, 13.9, 13.8, 12.1; ESIMS-LR  $m/z$  497.32 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>27</sub>H<sub>46</sub>O<sub>3</sub>N<sub>2</sub>NaSi 497.3170, found 497.3179; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -38.51 (*c* 2.85, CHCl<sub>3</sub>). Data for **cis**-10b:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  7.31-7.16 (m, 5H, Ph), 5.61 (dd, 1H, BuNH,  $J_{\text{NH}, \text{H-1}} = J_{\text{NH}, \text{H-1}} = 4.6$  Hz), 4.48 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 6.9$  Hz), 4.37 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 3.65 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 3.30-3.14 (m, 3H, 3-hydroxy-Pro- $\delta$ -CH, H-1), 2.99-2.92 (m, 2H, C(=O)CH<sub>2</sub>), 2.67-2.51 (m, 2H, PhCH<sub>2</sub>), 2.09-2.02 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.98-1.88 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.51-1.39 (m, 2H, H-2), 1.39-1.24 (m, 2H, H-3), 1.13-0.99 (m, 21H,  $^i\text{Pr}_3\text{Si}$ ), 0.91 (t, 3H, H-4,  $J_{\text{H-4}, \text{H-3}} = 7.5$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  172.7, 171.8, 168.8, 168.5, 141.4, 141.3, 128.6, 128.6, 126.3, 73.5, 71.9, 65.3, 63.4, 44.8, 43.9, 39.6, 39.5, 36.4, 35.8, 32.6, 32.1, 31.7, 31.7, 31.3, 30.9, 20.3, 18.1, 18.1, 18.0, 13.9, 13.8, 12.3, 12.3; ESIMS-LR  $m/z$  497.32 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>27</sub>H<sub>46</sub>O<sub>3</sub>N<sub>2</sub>NaSi 497.3170, found 497.3173; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +8.51 (*c* 1.65, CHCl<sub>3</sub>).

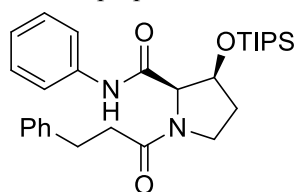
**(2*S*,3*S*)-*N*-Phenyl-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-10c)**

**(2*R*,3*S*)-*N*-Phenyl-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-10c)**

The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol),



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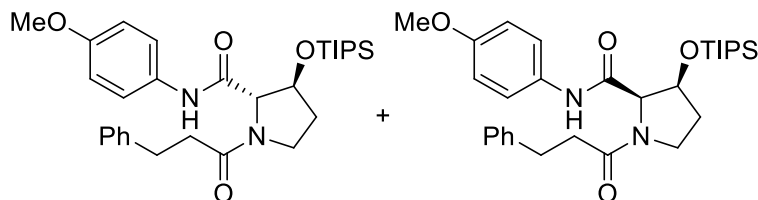


3-phenylpropionic acid (**7a**, 15.0 mg, 0.10 mmol) and phenyl isocyanide (**6c**, 10.3 mg, 0.10 mmol) in HFIP (1.0 mL). The mixture was stirred at room temperature for 60 min. Purification by high-flash silica gel column chromatography (3-15-35% AcOEt/hexane) afforded

***trans*-10c** (21.2 mg, 0.043 mmol, 43%) as a pale yellow solid and ***cis*-10c** (24.9 mg, 0.050 mmol, 50%) as a pale yellow solid. Data for ***trans*-10c**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer) δ 9.59 (s, 1H, PhNH), 7.49 (d, 2H, H-2,  $J_{H-2, H-3} = 7.5$  Hz), 7.30 (dd, 2H, H-3,  $J_{3,2} = J_{3,4} = 7.5$  Hz), 7.28-7.16 (m, 5H, Ph), 7.08 (t, 1H, H-4,  $J_{4,3} = 7.5$ ), 4.95 (d, 1H, 3-hydroxy-Pro-β-CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.9$  Hz), 4.66 (s, 1H, 3-hydroxy-Pro-α-CH), 3.65 (ddd, 1H, 3-hydroxy-Pro-δ-CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 11.5$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 3.47 (dd, 1H, 3-hydroxy-Pro-δ-CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 8.6$  Hz), 3.09-2.97 (m, 2H, C(=O)CH<sub>2</sub>), 2.77-2.65 (m, 2H, PhCH<sub>2</sub>), 2.24 (dddd, 1H, 3-hydroxy-Pro-γ-CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 12.6$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 11.5$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 2.9$  Hz), 2.01 (dd, 1H, 3-hydroxy-Pro-γ-CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 12.6$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.9$  Hz), 1.16-1.02 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 174.2, 168.1, 140.9, 138.2, 129.0, 128.7, 128.4, 126.4, 124.2, 119.9, 72.3, 69.5, 46.1, 36.4, 34.4, 31.0, 18.1, 12.1; ESIMS-LR *m/z* 517.29 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>29</sub>H<sub>42</sub>O<sub>3</sub>N<sub>2</sub>NaSi 517.2857, found 517.2866; [α]<sub>D</sub><sup>19</sup> -47.16 (*c* 2.12, CHCl<sub>3</sub>). Data for ***cis*-10c**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer) δ 7.47 (d, 2H, H-2,  $J_{2,3} = 8.6$  Hz), 7.46 (s, 1H, PhNH), 7.33-7.12 (m, 7H, H-3, Ph), 7.06 (t, 1H, H-4,  $J_{4,3} = 8.6$  Hz), 4.60 (ddd, 1H, 3-hydroxy-Pro-β-CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 6.9$  Hz), 4.55 (d, 1H, 3-hydroxy-Pro-α-CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 3.78-3.70 (m, 1H, 3-hydroxy-Pro-δ-CH), 3.38 (ddd, 1H, 3-hydroxy-Pro-δ-CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 3.01-2.93 (m, 2H, C(=O)CH<sub>2</sub>), 2.71-2.56 (m, 2H, PhCH<sub>2</sub>), 2.16-2.09 (m, 1H, 3-hydroxy-Pro-γ-CH), 2.01-1.95 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.11-0.98 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 173.1, 172.0, 167.5, 166.9, 141.3, 141.1, 138.1, 137.4, 129.1, 128.6, 128.6, 128.6, 128.5, 128.4, 126.3, 124.6, 123.7, 119.9, 119.7, 73.9, 72.2, 66.2, 64.1, 45.0, 44.2, 36.4, 35.9, 32.7, 32.2, 31.4, 30.9, 18.4, 18.3, 18.1, 18.0, 12.9, 12.5, 12.3, 12.3; ESIMS-LR *m/z* 517.29 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>29</sub>H<sub>42</sub>O<sub>3</sub>N<sub>2</sub>NaSi 517.2857, found 517.2872; [α]<sub>D</sub><sup>19</sup> +15.36 (*c* 2.49, CHCl<sub>3</sub>).

**(2*S*,3*S*)-*N*-(4-Methoxyphenyl)-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide**  
**(*trans*-10d)**

**(2*R*,3*S*)-*N*-(4-Methoxyphenyl)-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide** (*cis*-**10d**)

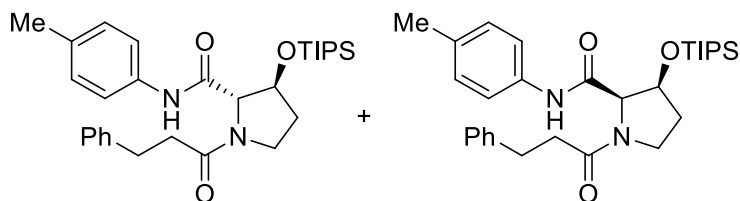


The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol), 3-phenylpropionic acid (**7a**, 15.0 mg, 0.10 mmol) and *p*-methoxyphenyl isocyanide (**6d**,

13.3 mg, 0.10 mmol) in HFIP (1.0 mL). The mixture was stirred at room temperature for 60 min. Purification by high-flash silica gel column chromatography (3-15-35% AcOEt/hexane) afforded **trans-10d** (27.4 mg, 0.052 mmol, 52%) as a pale yellow solid and **cis-10d** (18.6 mg, 0.035 mmol, 35%) as a pale yellow solid. Data for **trans-10d**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  9.41 (s, 1H, ArNH), 7.40 (d, 2H, H-2,  $J_{2,3} = 8.6$  Hz), 7.28-7.15 (m, 5H, Ph), 6.83 (d, 1H, H-3,  $J_{3,2} = 8.6$  Hz), 4.94 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.9$  Hz), 4.63 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.78 (s, 3H, OCH<sub>3</sub>), 3.65 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 10.9$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.3$  Hz), 3.47 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 8.6$  Hz), 3.09-2.97 (m, 2H, C(=O)CH<sub>2</sub>), 2.77-2.65 (m, 2H, PhCH<sub>2</sub>), 2.24 (dddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 10.9$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 2.9$  Hz), 2.00 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.3$  Hz), 1.15-1.02 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  174.0, 167.8, 156.3, 140.9, 131.4, 128.7, 128.4, 126.4, 121.4, 114.1, 72.4, 69.4, 55.6, 46.1, 36.3, 34.4, 31.0, 18.1, 12.1; ESIMS-LR  $m/z$  547.30 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>30</sub>H<sub>44</sub>O<sub>4</sub>N<sub>2</sub>NaSi 547.2963, found 547.2967;  $[\alpha]_D^{21} -37.55$  ( $c$  2.74, CHCl<sub>3</sub>). Data for **cis-10d**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.46 (s, 1H, ArNH), 7.38 (d, 2H, H-2,  $J_{2,3} = 8.6$  Hz), 7.32-7.13 (m, 7H, H-3, Ph), 6.80 (d, 1H, H-3,  $J_{3,2} = 8.6$  Hz), 4.59 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 6.9$  Hz), 4.55 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 3.82-3.67 (m, 4H, OCH<sub>3</sub>, 3-hydroxy-Pro- $\delta$ -CH), 3.38 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 2.98 (dd, 2H, C(=O)CH<sub>2</sub>,  $J_{\text{C(=O)CH}_2, \text{PhCH}} = J_{\text{C(=O)CH}_2, \text{PhCH}} = 8.1$  Hz), 2.71-2.48 (m, 2H, PhCH<sub>2</sub>), 2.16-2.07 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.98 (ddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.9$  Hz), 1.13-0.94 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  173.2, 172.0, 167.2, 166.6, 156.6, 156.1, 141.4, 141.2, 140.7, 131.5, 130.7, 128.7, 128.6, 128.4, 126.4, 126.3, 121.6, 121.5, 114.3, 113.9, 73.9, 72.2, 66.2, 64.0, 55.6, 45.0, 44.3, 36.5, 35.9, 35.6, 32.7, 32.3, 31.4, 31.0, 18.1, 18.1, 12.4, 12.3; ESIMS-LR  $m/z$  547.30 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>30</sub>H<sub>44</sub>O<sub>4</sub>N<sub>2</sub>NaSi 547.2963, found 547.2972;  $[\alpha]_D^{21} +12.74$  ( $c$  1.86, CHCl<sub>3</sub>).

**(2*S*,3*S*)-1-(3-Phenylpropanoyl)-*N*-(*p*-tolyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-10e)**

**(2*R*,3*S*)-1-(3-Phenylpropanoyl)-*N*-(*p*-tolyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-10e)**

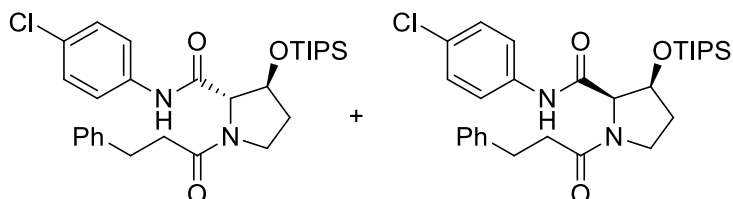


The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol), 3-phenylpropionic acid (**7a**, 15.0 mg, 0.10 mmol) and *p*-tolyl isocyanide (**6e**, 11.8 mg, 0.10 mmol) in HFIP (1.0 mL). The mixture was stirred at

room temperature for 60 min. Purification by high-flash silica gel column chromatography (3-15-35% AcOEt/hexane) afforded **trans**-10e (25.6 mg, 0.050 mmol, 50%) as a pale yellow solid and **cis**-10e (22.3 mg, 0.044 mmol, 44%) as a pale yellow solid. Data for **trans**-10e:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  9.47 (s, 1H, ArNH), 7.37 (d, 2H, H-2,  $J_{2,3} = 8.6$  Hz), 7.27-7.16 (m, 5H, Ph), 7.10 (d, 1H, H-3,  $J_{3,2} = 8.6$  Hz), 4.94 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.9$  Hz), 4.64 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.64 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.3$  Hz), 3.46 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 3.08-2.97 (m, 2H, C(=O)CH<sub>2</sub>), 2.77-2.64 (m, 2H, PhCH<sub>2</sub>), 2.30 (s, 3H, CH<sub>3</sub>), 2.24 (dddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 13.8$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 2.9$  Hz), 1.99 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.3$  Hz), 1.15-1.02 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  174.1, 167.9, 140.9, 135.6, 133.7, 129.5, 128.7, 128.4, 126.4, 119.9, 72.3, 69.5, 46.1, 36.4, 34.4, 31.0, 21.0, 18.1, 12.1; ESIMS-LR  $m/z$  531.10 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>30</sub>H<sub>44</sub>O<sub>3</sub>N<sub>2</sub>NaSi 531.3013, found 531.3022;  $[\alpha]_D^{20}$  -42.41 (*c* 2.56, CHCl<sub>3</sub>). Data for **cis**-10e:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.40 (s, 1H, ArNH), 7.35 (d, 2H, H-2,  $J_{2,3} = 8.0$  Hz), 7.30-7.13 (m, 5H, Ph), 7.07 (d, 1H, H-3,  $J_{3,2} = 8.0$  Hz), 4.58 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.9$  Hz), 4.55 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 3.78-3.67 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.38 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 8.6$  Hz), 3.01-2.93 (m, 2H, C(=O)CH<sub>2</sub>), 2.70-2.56 (m, 2H, PhCH<sub>2</sub>), 2.29 (s, 3H, CH<sub>3</sub>), 2.15-2.08 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.98 (ddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 7.5$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 8.6$  Hz), 1.13-0.94 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.9, 173.1, 172.0, 167.2, 166.7, 141.3, 141.1, 140.9, 135.6, 134.9, 134.1, 133.3, 129.6, 129.2, 129.0, 128.6, 128.5, 128.4, 126.9, 126.3, 119.9, 119.8, 74.9, 72.2, 66.1, 64.0, 44.9, 44.2, 36.4, 35.9, 32.7, 32.2, 31.4, 31.1, 30.9, 21.0, 18.3, 18.1, 18.0, 12.3, 12.3; ESIMS-LR  $m/z$  531.10 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>30</sub>H<sub>44</sub>O<sub>3</sub>N<sub>2</sub>NaSi 531.3013, found 531.3023;  $[\alpha]_D^{21}$  +16.13 (*c* 2.23, CHCl<sub>3</sub>).

**(2*S*,3*S*)-*N*-(4-Chlorophenyl)-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-**10f**)**

**(2*R*,3*S*)-*N*-(4-Chlorophenyl)-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-**10f**)**



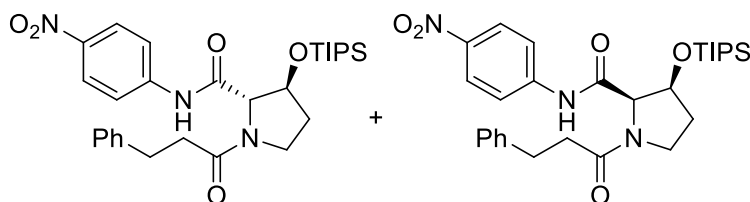
The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol) 3-phenylpropionic acid (**7a**, 15.0 mg, 0.10 mmol) and *p*-chlorophenyl isocyanide (**6f**, 13.8 mg, 0.10 mmol) in HFIP (1.0 mL). The mixture was

stirred at room temperature for 60 min. Purification by high-flash silica gel column chromatography (3-15-35% AcOEt/hexane) afforded *trans*-**10f** (18.5 mg, 0.035 mmol, 35%) as a white solid and *cis*-**9f** (30.7 mg, 0.058 mmol, 58%) as a white solid. Data for *trans*-**10f**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  9.71 (s, 1H, ArNH), 7.43 (d, 2H, H-2,  $J_{2,3} = 9.2$  Hz), 7.27-7.16 (m, 7H, Ph, H-3), 4.94 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.9$  Hz), 4.63 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.65 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 11.5$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 3.47 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 8.6$  Hz), 3.09-2.97 (m, 2H, C(=O)CH<sub>2</sub>), 2.78-2.66 (m, 2H, PhCH<sub>2</sub>), 2.21 (dddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 11.5$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 2.9$  Hz), 2.01 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.9$  Hz), 1.16-1.01 (m, 21H,  $\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  174.3, 168.2, 140.8, 136.8, 129.0, 129.0, 128.7, 128.4, 126.5, 121.1, 72.2, 69.5, 46.2, 36.3, 34.4, 30.1, 18.1, 12.1; ESIMS-LR  $m/z$  551.25 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>29</sub>H<sub>41</sub>O<sub>3</sub>N<sub>2</sub>ClNaSi 551.2467, found 551.2474;  $[\alpha]_D^{21} -32.97$  ( $c$  1.85, CHCl<sub>3</sub>). Data for *cis*-**10f**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.80 (s, 1H, ArNH), 7.37 (d, 2H, H-2,  $J_{2,3} = 8.6$  Hz), 7.30-7.13 (m, 7H, H-3, Ph), 4.61 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 8.6$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 6.9$  Hz), 4.58 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 3.76 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 3.5$  Hz), 3.40 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 3.01-2.94 (m, 2H, C(=O)CH<sub>2</sub>), 2.72-2.64 (m, 2H, PhCH<sub>2</sub>), 2.16-2.09 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.00-1.94 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.10-0.93 (m, 21H,  $\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  172.0, 167.8, 141.1, 137.1, 129.1, 128.7, 128.6, 128.4, 128.3, 128.1, 126.4, 126.3, 121.1, 120.5, 72.3, 64.1, 45.2, 44.3, 35.9, 32.8, 31.1, 31.0, 18.1, 18.0, 12.3, 12.1; ESIMS-LR  $m/z$  551.25 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>29</sub>H<sub>41</sub>O<sub>3</sub>N<sub>2</sub>ClNaSi 551.2467, found 551.2470;  $[\alpha]_D^{21} -12.90$  ( $c$  3.07, CHCl<sub>3</sub>).



**(2*S*,3*S*)-*N*-(4-Nitrophenyl)-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*trans*-10g)**

**(2*R*,3*S*)-*N*-(4-Nitrophenyl)-1-(3-phenylpropanoyl)-3-(triisopropylsiloxy)pyrrolidine-2-carboxamide (*cis*-10g)**



The corresponding compounds were prepared following general procedure using imine **4a** (24.1 mg, 0.10 mmol), 3-phenylpropionic acid (**7a**, 15.0 mg, 0.1 mmol) and *p*-nitrophenyl isocyanide (**6g**, 14.8 mg, 0.10 mmol) in HFIP (1.0 mL). The mixture

was stirred at room temperature for 60 min. Purification by high-flash silica gel column chromatography (3-15-35% AcOEt/hexane) afforded **trans-10g** (6.9 mg, 0.013 mmol, 13%) as a yellow solid and **cis-10g** (33.7 mg, 0.062 mmol, 62%) as a yellow solid. Data for **trans-10g**:  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  10.29 (s, 1H, ArNH), 8.17 (d, 2H, H-3,  $J_{3,2} = 9.2$  Hz), 7.62 (d, 2H, H-2,  $J_{2,3} = 9.2$  Hz), 7.27-7.16 (m, 5H, Ph), 4.96 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.9$  Hz), 4.66 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.68 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 11.5$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 6.9$  Hz), 3.50 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 3.12-2.97 (m, 2H, C(=O)CH<sub>2</sub>), 2.80-2.69 (m, 2H, PhCH<sub>2</sub>), 2.20 (dddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 11.5$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 2.9$  Hz), 2.04 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.9$  Hz), 1.16-0.99 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  174.8, 168.8, 144.1, 143.5, 140.7, 128.7, 128.4, 126.5, 125.1, 119.4, 71.9, 69.7, 46.3, 36.2, 34.4, 30.9, 18.1, 12.1; ESIMS-LR  $m/z$  562.27 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>29</sub>H<sub>41</sub>O<sub>5</sub>N<sub>3</sub>NaSi 562.2708, found 562.2712;  $[\alpha]_D^{20} -6.22$  (*c* 0.68, CHCl<sub>3</sub>). Data for **cis-10g**:  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 500 MHz, a mixture of two rotamers at 20 °C, selected data for the major rotamer)  $\delta$  9.15 (s, 1H, ArNH), 7.92 (d, 2H, H-3,  $J_{3,2} = 9.2$  Hz), 7.41 (d, 2H, H-2,  $J_{\text{H-2}, \text{H-3}} = 9.2$  Hz), 7.34-7.18 (m, 5H, H-3, Ph), 4.76 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 4.68 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\alpha\text{-CH}} = 6.9$  Hz), 3.83 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.5$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 2.9$  Hz), 3.44 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.5$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.7$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 3.07-2.95 (m, 2H, C(=O)CH<sub>2</sub>), 2.76 (dd, 1H, PhCH<sub>2</sub>,  $J_{\text{PhCH}_2, \text{PhCH}} = 15.5$ ,  $J_{\text{PhCH}_2, \text{C(=O)CH}} = 9.2$ ,  $J_{\text{PhCH}_2, \text{C(=O)CH}} = 6.9$  Hz), 2.63 (dd, 1H, PhCH<sub>2</sub>,  $J_{\text{PhCH}_2, \text{PhCH}} = 15.5$ ,  $J_{\text{PhCH}_2, \text{C(=O)CH}} = 9.2$ ,  $J_{\text{PhCH}_2, \text{C(=O)CH}} = 6.3$  Hz), 2.40 (dddd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 12.0$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.5$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 9.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 9.2$  Hz), 2.20-2.13 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.10-0.91 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  172.3, 168.8, 144.4, 142.6, 140.7, 128.8, 128.3, 126.5, 124.3, 118.5, 72.4, 64.3, 45.4, 35.7, 33.0, 31.0, 18.0, 17.0, 12.3; ESIMS-LR  $m/z$  562.27 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>29</sub>H<sub>41</sub>O<sub>5</sub>N<sub>3</sub>NaSi 562.2708, found 562.2712;  $[\alpha]_D^{20} -70.67$  (*c* 3.37, CHCl<sub>3</sub>).

## Competition experiment

### entry 1

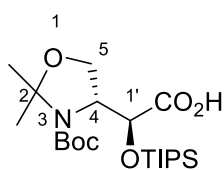
A solution of imine **4a** (24.1 mg, 0.10 mmol) and 3-phenylpropionic acid (**7a**, 15.0 mg, 0.1 mmol) in toluene (500  $\mu$ L) was treated with a solution of *p*-methoxyphenyl isocyanide (**6d**, 13.3 mg, 0.10 mmol) and *p*-chlorophenyl isocyanide (**6f**, 13.8 mg, 0.10 mmol) in toluene (500  $\mu$ L) at room temperature for 4.5 h. The mixture was concentrated *in vacuo*, and the residue was purified by high-flash silica gel column chromatography (3-15-35% AcOEt/hexane) to afford a mixture of *trans*-**10** and a mixture of *cis*-**10**. The mixture of *trans*-**10** was further purified by high-flash silica gel column chromatography (0-10-20% AcOEt/hexane) to afford *trans*-**10d** (3.6 mg, 0.0067 mmol, 7%) as a pale yellow solid and *trans*-**10f** (0.3 mg, 0.0006 mmol, 1%) as a white solid. The mixture of *cis*-**10** was further purified by high-flash silica gel column chromatography (0-25-45% AcOEt/hexane) to afford *cis*-**10d** (30.4 mg, 0.058 mmol, 58%) as a pale yellow solid and *cis*-**10f** (8.0 mg, 0.015 mmol, 15%) as a white solid.

### entry 2

A solution of imine **4a** (24.1 mg, 0.10 mmol) and 3-phenylpropionic acid (**7a**, 15.0 mg, 0.1 mmol) in HFIP (500  $\mu$ L) was treated with a solution of *p*-methoxyphenyl isocyanide (**6d**, 13.3 mg, 0.10 mmol) and *p*-chlorophenyl isocyanide (**6f**, 13.8 mg, 0.10 mmol) in HFIP (500  $\mu$ L) at room temperature and stirred for 4.5 h. The mixture was concentrated *in vacuo*, and the residue was purified by high-flash silica gel column chromatography (3-15-35% AcOEt/hexane) to afford a mixture of *trans*-**10** and a mixture of *cis*-**10**. The mixture of *trans*-**10** was further purified by high-flash silica gel column chromatography (0-10-20% AcOEt/hexane) to afford *trans*-**10d** (18.8 mg, 0.036 mmol, 36%) as a pale yellow solid and *trans*-**10f** (3.9 mg, 0.0074 mmol, 7%) as a white solid. The mixture of *cis*-**10** was further purified by high-flash silica gel column chromatography (0-25-45% AcOEt/hexane) to afford *cis*-**10d** (14.2 mg, 0.027 mmol, 27%) as a pale yellow solid and *cis*-**10f** (7.7 mg, 0.015 mmol, 15%) as a white solid.

## 第二章

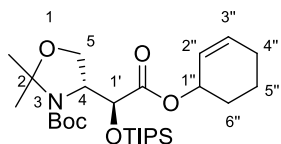
### (S)-2-[(R)-3-(tert-Butoxycarbonyl)-2,2-dimethyloxazolidin-4-yl]-2-(triisopropylsiloxy)acetic acid (**12**)



A mixture of **11** (412 mg, 1.60 mmol) and 2,6-lutidine (373  $\mu\text{L}$ , 3.20 mmol) in  $\text{CH}_2\text{Cl}_2$  (16 mL) was treated with TIPSOTf (473  $\mu\text{L}$ , 1.76 mmol) at  $-78\text{ }^\circ\text{C}$  for 5 min. The mixture was warmed to  $-50\text{ }^\circ\text{C}$  and stirred for 80 min. The reaction was quenched with MeOH, and the mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.*  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$ , and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. A mixture of the residue in  $\text{CH}_2\text{Cl}_2$  (16 mL) was bubbled with  $\text{O}_3$  at  $-18\text{ }^\circ\text{C}$  for 30 min. Dimethylsulfide (4.2 mL, 47 mmol) was added to the mixture, and the whole mixture was stirred for 1 h. The mixture was concentrated *in vacuo*, and the residue and 2-methylbut-2-ene (1 mL, 21.5 mmol) in THF/*t*BuOH (9 mL/9 mL) was treated with  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (998 mg, 6.40 mmol) and  $\text{NaClO}_2$  (289 mg, 3.20 mmol) in  $\text{H}_2\text{O}$  (3 mL) at room temperature for 3 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and  $\text{H}_2\text{O}$ . The organic phase was washed with  $\text{H}_2\text{O}$  and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ( $\phi$  2.5 cm  $\times$  6 cm, 2% MeOH/ $\text{CHCl}_3$ ) to afford **12** (578 mg, 1.34 mmol, 84% over 3 steps) as yellow oil.

$^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 500 MHz)  $\delta$  12.7 (br s, 1H,  $\text{CO}_2\text{H}$ ), 4.60-4.54 (m, 1H, H-1'), 4.29-4.13 (m, 1H, H-5), 4.09-3.90 (m, 2H, H-4, H-5), 1.42 (m, 15H, Me, *t*Bu), 1.08-0.90 (m, 21H, *i*Pr<sub>3</sub>Si);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.1, 156.1, 81.1, 80.5, 71.6, 68.1, 55.0, 42.8, 31.3, 28.4, 18.0, 17.8, 17.5, 17.5, 13.6, 13.1, 12.4, 12.0; ESIMS-LR  $m/z$  430.26 [(M+H)<sup>-</sup>]; ESIMS-HR calcd for  $\text{C}_{21}\text{H}_{40}\text{O}_6\text{NSi}$  430.2630, found 430.2633;  $[\alpha]_D^{20}$  +19.08 (*c* 0.46,  $\text{CHCl}_3$ ).

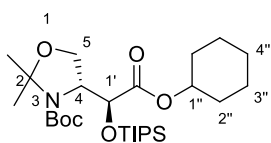
### Cyclohex-2-enyl (S)-2-[(R)-3-(tert-butoxycarbonyl)-2,2-dimethyloxazolidin-4-yl]-2-(triisopropylsiloxy)acetate (**14**)



A mixture of **12** (17.4 mg, 0.040 mmol) and  $\text{Cs}_2\text{CO}_3$  (26 mg, 0.080 mmol) in THF (400  $\mu\text{L}$ ) was treated with 3-bromocyclohexene (6.4  $\mu\text{L}$ , 0.056 mmol) at room temperature for 11 h. 3-Bromocyclohexene (1.4  $\mu\text{L}$ , 0.012 mmol) was added to the mixture, which was stirred for 20 min. The mixture was diluted with AcOEt, and partitioned between AcOEt and  $\text{H}_2\text{O}$ . The organic phase was washed with  $\text{H}_2\text{O}$  and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography ( $\phi$  1 cm  $\times$  2.5 cm, 10%  $\text{Et}_2\text{O}$ /hexane) to afford **14** (16.9 mg, 0.033 mmol, 83%) as a colorless oil.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, a mixture of several rotamers of two diastereomers at  $20\text{ }^\circ\text{C}$ , selected data for the major rotamer):  $\delta$  5.94-5.88 (m, 1H, H-2''), 5.79-5.66 (m, 1H, H-3''), 5.31-5.24 (m, 1H, H-1''), 4.78-4.75 (m, 1H, H-1'), 4.51-4.46 (m, 1H, H-5), 4.44-4.39 (m, 1H, H-5), 4.03-3.97 (m, 1H, H-4), 2.11-1.93 (m, 2H, H-4''), 1.92-1.57 (m, 4H, H-5'', H-6''), 1.57-1.38 (m, 15H, Me, *t*Bu), 1.15-0.97 (m, 21H, *i*Pr<sub>3</sub>Si);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  171.2, 171.2, 170.9, 170.9, 152.6, 152.1, 152.1, 132.7, 132.6, 132.3, 132.1, 126.2, 126.0, 125.8, 125.7, 95.2, 95.2, 94.4, 80.4, 80.3, 70.9, 70.4, 70.3, 69.1, 69.0, 68.8, 68.8, 63.2, 63.1, 62.9, 62.8, 61.2, 60.3, 28.7, 28.7, 28.6, 28.5, 28.3, 28.3, 26.7, 25.9, 25.8, 25.0, 24.9, 24.3, 23.0, 19.2, 19.2, 18.9, 18.2, 18.1, 18.0, 18.0, 18.0, 12.1; ESIMS-LR  $m/z$  534.32 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for  $\text{C}_{27}\text{H}_{49}\text{O}_6\text{NNaSi}$  534.3221, found 534.3324.

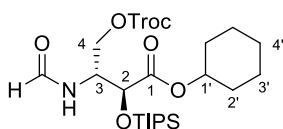
### Cyclohexyl (*S*)-2-[(*R*)-3-(tert-butoxycarbonyl)-2,2-dimethyloxazolidin-4-yl]-2-(triisopropylsiloxy)acetate (**13**)



A mixture of **14** (15.2 mg, 0.030 mmol) and Pd-Fib (2.5% Pd) (7.5 mg) in AcOEt (6 mL) was vigorously stirred at room temperature for 3 h under H<sub>2</sub> atmosphere. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography ( $\phi$  1.5 cm  $\times$  1 cm, CHCl<sub>3</sub>) to afford **13** (15.0 mg, 0.029 mmol, 98%) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer)  $\delta$  4.79-4.73 (m, 1H, H-1''), 4.73 (d, 1H, H-1',  $J_{1',4} = 5.2$  Hz), 4.48 (dd, 1H, H-5,  $J_{H-5, H-5} = 9.2$ ,  $J_{5,4} = 2.3$  Hz), 3.99 (ddd, 1H, H-4,  $J_{4,1'} = 5.2$ ,  $J_{4,5} = 5.8$ ,  $J_{4,5} = 2.3$  Hz), 3.93 (dd, 1H, H-5,  $J_{5,4} = 5.8$ ,  $J_{5,5} = 9.2$  Hz), 1.98-1.81 (m, 2H, H-2''), 1.77-1.67 (m, 2H, H-2'), 1.52-1.30 (m, 21H, H-3'', H-4'', <sup>t</sup>Bu, Me), 1.13-1.01 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  171.0, 170.7, 152.6, 152.1, 95.2, 94.4, 80.4, 80.3, 73.8, 73.3, 70.9, 70.3, 63.1, 62.8, 61.2, 60.3, 35.6, 31.8, 31.7, 31.7, 31.6, 26.7, 25.8, 25.5, 25.4, 25.4, 24.2, 24.1, 24.0, 23.9, 23.9, 23.0, 18.1, 18.0, 18.0, 17.9, 17.8, 12.5, 12.4, 12.4, 12.1; ESIMS-LR  $m/z$  536.34 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>27</sub>H<sub>51</sub>O<sub>6</sub>NNaSi 536.3378, found 536.3387; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +33.94 ( $c$  1.21, CHCl<sub>3</sub>).

### Cyclohexyl (2*S*, 3*R*)-3-(formylamino)-4-(2,2,2-trichloroethoxycarbonyloxy)-2-(triisopropylsiloxy)butanoate (**24**)

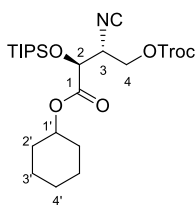


A mixture of **13** (103 mg, 0.200 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was treated with TFA (1 mL) at 0 °C for 30 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was

washed with H<sub>2</sub>O ( $\times$ 2) and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude aminoalcohol. A mixture of the crude aminoalcohol in THF (2 mL) was treated with *N*-formylsaccharin (66 mg, 0.30 mmol) for 30 min. *N*-Formylsaccharin (44 mg, 0.20 mmol) was added to the mixture, and the whole mixture was stirred for 15 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude formamide **15**. A mixture of the crude formamide **15** and NMM (66  $\mu$ L, 0.60 mmol) in THF (2 mL) was treated with TrocCl (41  $\mu$ L, 0.30 mmol) at 0 °C for 25 min. The mixture was warmed to room temperature, and stirred for 45 min. NMM (66  $\mu$ L, 0.60 mmol) and TrocCl (41  $\mu$ L, 0.30 mmol) was added to the mixture, which was stirred for 35 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O ( $\times$ 2) and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ( $\phi$  1 $\times$ 4 cm, 40% AcOEt/hexane) to afford compound **24** (109 mg, 0.19 mmol, 94% over 3 steps) as a pale yellow oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  8.20 (s, 1H, CHO), 6.08 (d, 1H, NHCHO,  $J_{NHCHO,3} = 8.6$  Hz), 4.85-4.67 (m, 5H, CH<sub>2</sub>CCl<sub>3</sub>, H-1', H-3), 4.60 (d, 1H, H-2,  $J_{2,3} = 2.3$  Hz), 4.38 (dd, 1H, H-4,  $J_{4,3} = 5.7$ ,  $J_{4,4} = 10.6$  Hz), 4.27 (dd, 1H, H-4,  $J_{4,3} = 7.5$ ,  $J_{4,4} = 10.6$  Hz), 1.91-1.82 (m, 2H, H-2'), 1.78-1.69 (m, 2H, H-2''), 1.45-1.11 (m, 6H, H-3', H-4'), 1.10-1.02 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  170.7, 170.0, 163.5, 153.6, 94.3, 77.1, 75.1, 74.7, 72.1, 70.5, 67.5, 65.8, 54.3, 49.7, 31.8, 31.7, 31.5, 25.3, 24.0, 23.9, 18.1, 18.0, 12.6, 12.5; ESIMS-LR  $m/z$  600.15 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>23</sub>H<sub>40</sub>O<sub>7</sub>NCl<sub>3</sub>NaSi 598.1539, found 598.1532; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +5.18 ( $c$  0.54, CHCl<sub>3</sub>).

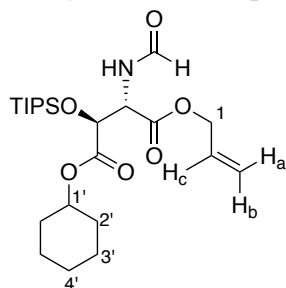
### Cyclohexyl (2*S*, 3*R*)-4-(2,2,2-trichloroethoxycarbonyloxy)-3-isocyano-2-(triisopropylsiloxy)butanoate (**25**)



A mixture of compound **24** (11.5 mg, 0.020 mmol) and NMM (13.4  $\mu$ L, 0.12 mmol) in  $\text{CH}_2\text{Cl}_2$  (400  $\mu$ L) was treated with triphosgene (4.7 mg, 0.016 mmol) at  $-78^\circ\text{C}$  for 45 min. The reaction was quenched with *sat. aq.*  $\text{NaHCO}_3$  (1 mL), and the mixture was partitioned between AcOEt and *sat. aq.*  $\text{NaHCO}_3$ . The organic phase was washed with  $\text{H}_2\text{O}$  ( $\times 2$ ) and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ( $\phi$  0.5  $\times$  2 cm, 10% AcOEt/hexane) to afford compound **25** (9.8 mg, 0.018 mmol, 90%) as a colorless oil.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  4.96 (dddd, 1H, H-1',  $J_{1',2'} = J_{1',2} = 9.2$ ,  $J_{1',2'} = J_{1',2} = 4.0$  Hz), 4.80 (s, 2H,  $\text{CH}_2\text{CCl}_3$ ), 4.57 (d, 1H, H-2,  $J_{2,3} = 3.4$  Hz), 4.51 (dd, 1H, H-4,  $J_{4,3} = 6.9$ ,  $J_{4,4} = 10.9$  Hz), 4.46 (dd, 1H, H-4,  $J_{4,3} = 5.7$ ,  $J_{4,4} = 10.9$  Hz), 4.23 (ddd, 1H, H-3,  $J_{3,2} = 3.4$  Hz,  $J_{3,4} = 5.7$ ,  $J_{3,4} = 6.9$  Hz), 1.97-1.87 (m, 2H, H-2'), 1.78-1.72 (m, 2H, H-2'), 1.51-1.12 (m, 6H, H-3', H-4'), 1.11-1.03 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  168.9, 161.6, 153.4, 94.1, 75.2, 71.2, 70.0, 56.3, 31.7, 31.6, 25.3, 23.9, 18.0, 12.6; IR (neat)  $\nu$  2138.67  $\text{cm}^{-1}$ ; ESIMS-LR  $m/z$  580.14 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{23}\text{H}_{38}\text{O}_6\text{NCl}_3\text{NaSi}$  580.1426, found 580.1426;  $[\alpha]_D^{22} +1.98$  ( $c$  1.00,  $\text{CHCl}_3$ ).

### Formyl-(3*S*)-3-(triisopropylsiloxy)-Asp(O-cyclohexyl)-Oallyl (**16**)

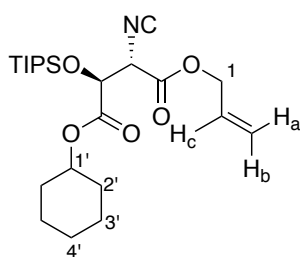


A solution of the crude alcohol **15** (45.2 mg, 0.11 mmol) in acetone (2 mL) was treated with 2.5 M Jones reagent (132  $\mu$ L, 0.33 mmol) at  $0^\circ\text{C}$  for 4 h. The reaction was quenched with  $^i\text{PrOH}$  (1.0 mL), and the mixture was partitioned between  $\text{Et}_2\text{O}$  and 1 M *aq.* HCl. The organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to afford a crude carboxylic acid. A mixture of the crude carboxylic acid and  $\text{Cs}_2\text{CO}_3$  (53.8 mg, 0.165 mmol) in DMF (1 mL) was treated with allyl bromide (14.3  $\mu$ L, 0.165 mmol) at room temperature for 14 h. The mixture was

partitioned between AcOEt and  $\text{H}_2\text{O}$ . The organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (3-10-20% AcOEt/hexane) to afford **xx** (12.1 mg, 0.027 mmol, 24% over 4 steps) as a colorless oil.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  8.25 (s, 1H,  $\text{CHO}$ ), 6.29 (d, 1H,  $\beta$ -hydroxy-Asp-NH,  $J_{\beta\text{-hydroxy-Asp-NH}, \beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 9.7$  Hz), 5.92 (dddd, 1H,  $\text{H}_c$ ,  $J_{\text{H}_c, \text{H}_a} = 17.2$ ,  $J_{\text{H}_c, \text{H}_b} = 10.3$ ,  $J_{\text{H}_c, \text{H-1}} = J_{\text{H}_c, 1} = 6.3$  Hz), 5.35 (dd, 1H,  $\text{H}_a$ ,  $J_{\text{H}_a, \text{H}_c} = 17.2$ ,  $J_{\text{H}_a, \text{H}} = 1.8$  Hz), 5.28 (dd, 1H,  $\text{H}_b$ ,  $J_{\text{H}_b, \text{H}_c} = 10.3$ ,  $J_{\text{H}_b, \text{H-1}} = 1.8$  Hz), 5.16 (dd, 1H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-}\beta\text{-CH}} = 1.7$ ,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \text{hydroxy-Asp-NH}} = 9.7$  Hz), 4.98 (d, 1H,  $\beta$ -hydroxy-Asp- $\beta$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\beta\text{-CH}, \beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 1.7$  Hz), 4.81-4.74 (m, 1H, H-1'), 4.69 (dd, 1H, H-1,  $J_{1, \text{H}_c} = 6.3$ ,  $J_{1, 1} = 13.2$  Hz), 4.59 (dd, 1H, H-1,  $J_{1, \text{H}_c} = 6.3$ ,  $J_{1, 1} = 13.2$  Hz), 1.90-1.81 (m, 2H, H-2'), 1.76-1.69 (m, 2H, H-2'), 1.58-1.51 (m, 1H, H-4'), 1.45-1.18 (m, 5H, H-3', H-4'), 1.16-1.01 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  169.9, 168.7, 160.8, 131.3, 119.7, 74.9, 72.8, 66.9, 54.4, 31.7, 31.5, 25.3, 24.0, 24.0, 18.0, 18.0, 12.6; ESIMS-LR  $m/z$  478.26 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{23}\text{H}_{41}\text{O}_6\text{NNaSi}$  478.2595, found 478.2601;  $[\alpha]_D^{22} +7.94$  ( $c$  0.40,  $\text{CHCl}_3$ ).

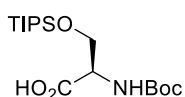
### Allyl cyclohexyl (2*S*, 3*S*)-2-isocyano-3-(triisopropylsiloxy)butandioate (**17**)



A mixture of compound **16** (4.3 mg, 0.016 mmol) and NMM (15.8  $\mu$ L, 0.14 mmol) in  $\text{CH}_2\text{Cl}_2$  (300  $\mu$ L) was treated with triphosgene (5.7 mg, 0.019 mol) at  $-78^\circ\text{C}$  for 1.5 h. The reaction was quenched with *sat. aq.*  $\text{NaHCO}_3$  (1 mL), and the mixture was partitioned between AcOEt and *sat. aq.*  $\text{NaHCO}_3$ . The organic phase was washed with  $\text{H}_2\text{O}$  ( $\times 2$ ) and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to afford a crude isocyanide **15** as a pale yellow oil. This compound was directly used to the next

reaction without further purification.

### Boc-D-Ser(O-triisopropylsilyl)-OH (**19**)

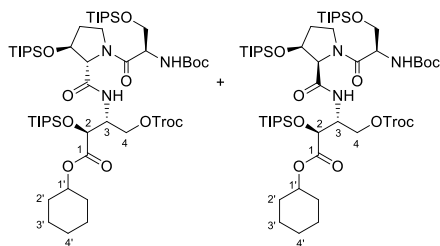


A suspension of **18** (1.05 g, 10.0 mmol) and *sat. aq.*  $\text{NaHCO}_3$  (40 mL) in THF (80 mL) was treated with  $(\text{Boc})_2\text{O}$  (3.22 mL, 14.0 mmol) at  $0^\circ\text{C}$  for 10 min. The mixture was warmed to room temperature, and stirred for 13 h. Di-*tert*-butyl dicarbonate (0.92 mL, 4.0 mmol) was added to the mixture, which was stirred for 2 h. The mixture was partitioned between hexane and  $\text{H}_2\text{O}$ , and the aqueous phase was saturated with  $\text{Na}_2\text{SO}_4$ . The aqueous phase was acidified with 1 M *aq.* HCl, and extracted with AcOEt. The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to afford a crude alcohol. A mixture of the crude alcohol and imidazole (4.77 g, 70 mmol) in  $\text{CH}_2\text{Cl}_2$  (100 mL) was treated with TIPSCl (7.46 mL, 35 mmol) at  $0^\circ\text{C}$  for 10 min. The mixture was warmed to room temperature, and stirred for 4 h. Imidazole (0.68 g, 10 mmol) and TIPSCl (1.07 mL, 5 mmol) was added to the mixture, which was stirred for 2 h. The mixture was concentrated *in vacuo*, and the residue was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.*  $\text{NaHCO}_3$  and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to afford a crude silyl ester. A mixture of the crude silyl ester in THF/MeOH (60 mL/30 mL) was treated with  $\text{K}_2\text{CO}_3$  (1.93 g, 14 mmol) at room temperature for 30 min. Water (20 mL) was added to the mixture, which was stirred for 1.5 h. The mixture was partitioned between hexane and  $\text{H}_2\text{O}$ . The aqueous phase was extracted with  $\text{CHCl}_3/\text{MeOH} = 9/1$ , and the organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was diluted with AcOEt, which was washed with 0.5 M *aq.* HCl/brine = 1/1 and 1 M *aq.* HCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* afford **19** (3.46 g, 9.96 mmol, quant. over 3 steps) as a white solid.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  5.36 (d, 1H, D-Ser-NH,  $J_{\text{D-Ser-NH, D-Ser-}\alpha\text{-CH}} = 9.6$  Hz), 4.39 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{\text{D-Ser-}\beta\text{-CH, D-Ser-}\beta\text{-CH}} = 10.3$ ,  $J_{\text{D-Ser-}\beta\text{-CH, D-Ser-}\alpha\text{-CH}} = 9.6$  Hz), 4.19 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{\text{D-Ser-}\beta\text{-CH, D-Ser-}\beta\text{-CH}} = 10.3$ ,  $J_{\text{D-Ser-}\beta\text{-CH, D-Ser-}\alpha\text{-CH}} = 4.0$  Hz), 3.91 (dd, 1H D-Ser- $\alpha$ -CH,  $J_{\text{D-Ser-}\alpha\text{-CH, D-Ser-}\beta\text{-CH}} = 9.6$ ,  $J_{\text{D-Ser-}\alpha\text{-CH, D-Ser-}\beta\text{-CH}} = 4.0$  Hz), 1.45 (s, 9H,  $^t\text{Bu}$ ), 1.18-0.89 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  176.0, 155.7, 80.3, 64.0, 55.5, 28.4, 17.9, 11.9; ESIMS-LR  $m/z$  360.41 [(M-H) $^-$ ]; ESIMS-HR calcd for  $\text{C}_{17}\text{H}_{35}\text{O}_5\text{NNaSi}$  384.2177, found 384.2199;  $[\alpha]_{\text{D}}^{20} -33.51$  ( $c$  0.88,  $\text{CHCl}_3$ ).

**Cyclohexyl (2*S*,3*R*)-3-[[Boc-D-Ser(O-triisopropylsilyl)-(3*S*)-3-(triisopropylsiloxy)-Pro]amino]-4-[(2,2,2-trichloroethoxycarbonyl)oxy]-2-triisopropylsiloxybutanoate (*trans*-26)**

**Cyclohexyl (2*S*,3*R*)-3-[[Boc-D-Ser(O-triisopropylsilyl)-(3*S*)-3-(triisopropylsiloxy)-D-Pro]amino]-4-[(2,2,2-trichloroethoxycarbonyl)oxy]-2-triisopropylsiloxybutanoate (*cis*-26)**

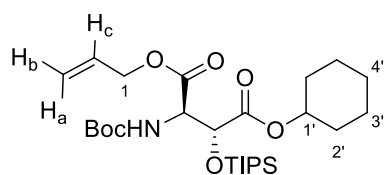


A solution of **4a** (52 mg, 0.24 mmol) and **19** (289 mg, 0.80 mmol) in HFIP (1 mL) was treated with a solution of **25** (88 mg, 0.24 mmol) in HFIP (2 mL) at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-20% acetone/hexane) to afford *trans*-**26** (105 mg, 0.0904 mmol, 57%) as a colorless solid and *cis*-**26** (32 mg, 0.028 mmol, 17%) as a colorless solid.

Data for *trans*-**26**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.17 (d, 1H, C-3-NH,  $J_{C-3-NH, H-3} = 9.2$  Hz), 5.58 (d, 1H, D-Ser-NH,  $J_{D-Ser-NH, D-Ser-α-CH} = 8.1$  Hz), 4.78-4.69 (m, 1H, H-1'), 4.77 (s, 2H, CH<sub>2</sub>CCl<sub>3</sub>), 4.67 (br d, 1H, 3-hydroxy-Pro-β-CH,  $J_{3-hydroxy-Pro-β-CH, 3-hydroxy-Pro-γ-CH} = 3.4$  Hz), 4.62-4.53 (m, 4H, D-Ser-β-CH, H-3, H-2), 4.35 (s, 1H, 3-hydroxy-Pro-α-CH), 4.25 (d, 2H, H-4,  $J_{H-4, H-3} = 6.9$  Hz), 3.95 (dd, 1H, D-Ser-α-CH,  $J_{D-Ser-α-CH, D-Ser-β-CH} = 4.6$ ,  $J_{D-Ser-α-CH, D-Ser-β-CH} = 9.8$  Hz), 3.82-3.75 (m, 2H, 3-hydroxy-Pro-δ-CH), 2.21-2.13 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.94 (dd, 1H, 3-hydroxy-Pro-γ-CH,  $J_{3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-δ-CH} = 5.7$ ,  $J_{3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-γ-CH} = 13.2$  Hz), 1.86-1.48 (m, 4H, H-2'), 1.43 (s, 9H, 'Bu), 1.38-1.20 (m, 6H, H-3', H-4'), 1.19-0.95 (m, 63H, <sup>1</sup>Pf<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 170.8, 170.4, 169.5, 155.4, 153.5, 94.5, 79.4, 74.1, 73.6, 70.6, 69.6, 65.9, 63.7, 54.3, 50.7, 45.6, 34.1, 31.6, 31.3, 28.5, 25.3, 23.9, 23.8, 18.1, 12.5, 12.1, 12.0; ESIMS-LR *m/z* 1184.55 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>53</sub>H<sub>100</sub>O<sub>12</sub>N<sub>3</sub>Cl<sub>3</sub>NaSi<sub>3</sub> 1182.5573, found 1182.5568; [α]<sub>D</sub><sup>20</sup> -6.85 (*c* 0.59, CHCl<sub>3</sub>).

Data for *cis*-**26**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 6.60 (d, 1H, C-3-NH,  $J_{C-3-NH, H-3} = 7.6$  Hz), 5.09 (d, 1H, D-Ser-NH,  $J_{D-Ser-NH, D-Ser-α-CH} = 8.6$  Hz), 4.85-4.69 (m, 4H, CH<sub>2</sub>CCl<sub>3</sub>, H-2, H-1'), 4.62-4.52 (m, 2H, 3-hydroxy-Pro-β-CH, H-3), 4.49-4.28 (m, 2H, D-Ser-β-CH), 4.37 (d, 1H, 3-hydroxy-Pro-α-CH,  $J_{3-hydroxy-Pro-α-CH, 3-hydroxy-Pro-β-CH} = 6.9$  Hz), 4.25 (d, 1H, H-4,  $J_{H-4, H-3} = 6.9$  Hz), 4.26 (dd, 1H, H-4,  $J_{H-4, H-3} = 5.8$ ,  $J_{D-Ser-β-CH, D-Ser-β-CH} = 10.3$  Hz), 3.99 (dd, 1H, D-Ser-α-CH,  $J_{D-Ser-α-CH, D-Ser-β-CH} = 5.3$ ,  $J_{D-Ser-α-CH, D-Ser-β-CH} = 10.3$  Hz), 3.90-3.81 (m, 1H, 3-hydroxy-Pro-δ-CH), 3.81-3.68 (m, 1H, H-4, 3-hydroxy-Pro-δ-CH), 2.37-2.27 (m, 1H, 3-hydroxy-Pro-γ-CH), 2.19-2.11 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.96-1.87 (m, 2H, H-2'), 1.78-1.67 (m, 2H, H-2'), 1.41 (s, 9H, 'Bu), 1.49-1.20 (m, 6H, H-3', H-4'), 1.18-0.97 (m, 63H, <sup>1</sup>Pf<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 171.2, 171.1, 170.9, 168.6, 168.1, 167.9, 155.5, 154.6, 153.6, 153.4, 94.5, 89.3, 79.6, 79.2, 79.0, 77.1, 75.2, 74.8, 74.6, 73.7, 73.2, 71.6, 70.3, 70.2, 65.9, 65.3, 64.2, 63.8, 63.1, 60.1, 53.9, 53.5, 51.7, 51.1, 45.0, 44.7, 43.8, 33.3, 32.7, 31.8, 31.8, 31.6, 30.5, 28.5, 28.4, 25.4, 24.2, 24.2, 24.1, 18.2, 18.1, 18.0, 12.5, 12.4, 12.3, 12.2, 12.0, 12.0; ESIMS-LR *m/z* 1184.55 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>53</sub>H<sub>100</sub>O<sub>12</sub>N<sub>3</sub>Cl<sub>3</sub>NaSi<sub>3</sub> 1182.5573, found 1182.5570; [α]<sub>D</sub><sup>20</sup> +8.48 (*c* 0.59, CHCl<sub>3</sub>).

### Boc-(3*R*)-3-(triisopropylsiloxy)-D-Asp(O-cyclohexyl)-Oallyl (**36**)

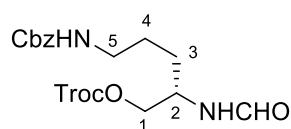


A mixture of *ent*-**13** (179 mg, 0.348 mmol) in acetone (23 mL) was treated with 2.5 M Jones reagent (557  $\mu$ L, 1.39 mmol) at 0 °C for 4.5 h. The reaction was quenched with *i*PrOH (3.0 mL), and the mixture was partitioned between Et<sub>2</sub>O and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by silica

gel column chromatography ( $\phi$  2  $\times$  4 cm, 2% MeOH/CHCl<sub>3</sub>) to afford **35** (60 mg, 0.123 mmol, 35 %, 79% based on 45% conversion) as a yellow oil, and the unreacted starting material (99 mg, 0.193 mmol, 55%) was recovered. A mixture of **35** (9.8 mg, 0.020 mmol) and Cs<sub>2</sub>CO<sub>3</sub> (13 mg, 0.040 mmol) in DMF (0.2 mL) was treated with allylbromide (2.6  $\mu$ L, 0.030 mmol) at room temperature for 11 h. The mixture was partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ( $\phi$  0.5  $\times$  2 cm, CHCl<sub>3</sub>) to afford **36** (9.7 mg, 0.018 mmol, 92%) as a yellow oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  5.92 (dddd, 1H, H<sub>c</sub>,  $J_{H_c, H_a} = 17.2$ ,  $J_{H_c, H_b} = 10.3$ ,  $J_{H_c, H-1} = J_{H_c, 1} = 5.8$  Hz), 5.35 (dd, 1H, H<sub>a</sub>,  $J_{H_a, H_c} = 17.2$ ,  $J_{H_a, H} = 1.8$  Hz), 5.26 (dd, 1H, H<sub>b</sub>,  $J_{H_b, H_c} = 10.3$ ,  $J_{H_b, H-1} = 1.8$  Hz), 5.24 (d, 1H, D- $\beta$ -hydroxy-Asp-NH,  $J_{D-\beta\text{-hydroxy-Asp-NH}, D-\beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 10.4$  Hz), 4.95 (d, 1H, D- $\beta$ -hydroxy-Asp- $\beta$ -CH,  $J_{D-\beta\text{-hydroxy-Asp-}\beta\text{-CH}, D-\beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 1.7$  Hz), 4.83-4.75 (m, 1H, H-1'), 4.78 (dd, 1H, D- $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $J_{D-\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, D-\beta\text{-hydroxy-Asp-}\beta\text{-CH}} = 1.7$ ,  $J_{D-\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, D-\beta\text{-hydroxy-Asp-NH}} = 10.4$  Hz), 4.66 (dd, 1H, H-1,  $J_{1, H_c} = 5.8$ ,  $J_{1, 1} = 13.2$  Hz), 4.59 (dd, 1H, H-1,  $J_{1, H_c} = 5.8$ ,  $J_{1, 1} = 13.2$  Hz), 1.88-1.80 (m, 2H, H-2'), 1.78-1.66 (m, 2H, H-2'), 1.61-1.20 (m, 6H, H-3', H-4'), 1.42 (s, 9H, <sup>t</sup>Bu), 1.14 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  170.1, 169.8, 155.5, 131.6, 119.2, 80.1, 74.5, 73.2, 66.5, 57.7, 31.7, 31.4, 28.4, 25.4, 23.9, 23.8, 18.1, 18.0, 12.6; ESIMS-LR  $m/z$  550.32 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>27</sub>H<sub>49</sub>O<sub>7</sub>NNaSi 550.3171, found 550.3185; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +5.29 (*c* 1.15, CHCl<sub>3</sub>).

### (*S*)-5-[(Benzyloxycarbonyl)amino]-2-(formylamino)pentyl 2,2,2-trichloroethyl carbonate (**38**)



Alcohol **37** (2.11 g, 6.00 mmol) was treated with 4 M HCl/dioxane (30 mL) at room temperature for 15 min. The mixture was concentrated *in vacuo* to afford a crude amine hydrochloride salt. A solution of the crude amine hydrochloride salt and Et<sub>3</sub>N (904  $\mu$ L, 6.00 mmol) in THF (30 mL) was treated with *N*-formylsaccharin (1.39 g, 6.60 mmol) at

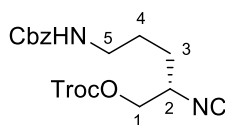
room temperature for 40 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude formamide. A mixture of the crude formamide and NMM (1.45 mL) in THF (30 mL) was treated with TrocCl (909  $\mu$ L, 6.60 mmol) at 0 °C for 10 min. The mixture was partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with 1 M *aq.* HCl, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-1-2% MeOH/CHCl<sub>3</sub>) to afford **38** (1.67 g, 3.66 mmol, 61% over 3 steps) as a yellow oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  8.19 (s, 1H, CHO), 7.41-7.29 (m, 5H, Ph), 5.97 (d, 1H, NHCHO,  $J_{NHCHO, H-2} = 7.7$  Hz), 5.09 (s, 2H, PhCH<sub>2</sub>), 4.98-4.89 (m, 1H, C-5-NH), 4.77 (s, 2H, CH<sub>2</sub>CCl<sub>3</sub>), 4.38-4.19 (m, 3H, H-1, H-2), 3.28-3.13 (m, 2H, H-5), 1.68-1.48 (m, 4H, H-3, H-4); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  164.7, 161.5, 156.7, 153.8, 153.7, 136.5, 128.5, 128.1, 127.9, 94.3, 94.2, 76.8, 70.6, 70.0, 66.5, 51.4, 46.7, 40.4, 40.2, 28.2, 27.9, 26.3, 26.1; ESIMS-



LR  $m/z$  455.95 [(M+H)<sup>+</sup>]; ESIMS-HR calcd for C<sub>17</sub>H<sub>22</sub>O<sub>6</sub>N<sub>2</sub>Cl<sub>3</sub> 455.0538, found 455.0542; [ $\alpha$ ]<sup>20</sup><sub>D</sub> -11.19 (*c* 0.95, CHCl<sub>3</sub>).

### (S)-5-[(Benzyloxycarbonyl)amino]-2-isocyanopentyl 2,2,2-trichloroethyl carbonate (33)

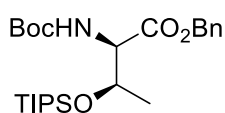


A solution of compound **38** (547 mg, 1.20 mmol) and NMM (793  $\mu$ L, 7.20 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) was treated with triphosgene (285 mg, 0.96 mmol) at -78 °C and stirred for 30 min. The mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated

*in vacuo*. The residue was purified by silica gel column chromatography ( $\phi$  2 cm  $\times$  6 cm, 35% AcOEt/hexane) to afford **33** (442 mg, 1.01 mmol, 84%) as a pale yellow oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.49-7.22 (m, 5H, Ph), 5.10 (s, 2H, PhCH<sub>2</sub>), 4.86-4.72 (m, 3H, CH<sub>2</sub>CCl<sub>3</sub>, C-5-NH), 4.28 (d, 2H, H-1,  $J_{1,2} = 5.5$  Hz), 3.99-3.88 (m, 1H, H-2), 3.33-3.19 (m, 2H, H-5), 1.82-1.64 (m, 4H, H-3, H-4); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  158.8, 156.6, 153.6, 136.5, 128.7, 128.3, 128.2, 94.1, 68.9, 66.9, 53.1, 40.0, 28.3, 26.3; IR (neat)  $\nu$  2140.60 cm<sup>-1</sup>; ESIMS-LR  $m/z$  469.07 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>17</sub>H<sub>20</sub>O<sub>5</sub>N<sub>2</sub>Cl<sub>3</sub> 437.0432, found 437.0429; [ $\alpha$ ]<sup>22</sup><sub>D</sub> -0.88 (*c* 1.00, CHCl<sub>3</sub>).

### Boc-*allo*-D-Thr(O-triisopropylsilyl)-OBn (40)

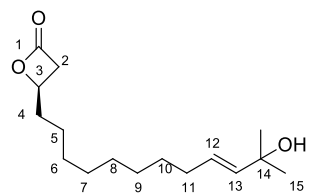


A suspension of **39** (595 mg, 5.0 mmol) and *sat. aq.* NaHCO<sub>3</sub> (7.5 mL) in THF (15 mL) was treated with (Boc)<sub>2</sub>O (2.07 mL, 9.0 mmol) at room temperature for 4 d. The mixture was partitioned between hexane and H<sub>2</sub>O, and the aqueous phase was saturated with Na<sub>2</sub>SO<sub>4</sub>. The

aqueous phase was acidified with 1 M *aq.* HCl, and extracted with AcOEt. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude carboxylic acid. A solution of the crude carboxylic acid and K<sub>2</sub>CO<sub>3</sub> (828 mg, 6.0 mmol) in DMF (25 mL) was treated with BnBr (713  $\mu$ L, 6.0 mmol) at room temperature for 24 h. The mixture was partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude alcohol. A solution of the crude alcohol and 2,6-lutidine (1.16 mL, 10.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) was treated with TIPSOTf (1.48 mL, 5.5 mmol) at -78 °C. Then the mixture was warmed to -50 °C, and stirred for 190 min. The reaction was quenched with MeOH (2 mL), and partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with 1 M *aq.* HCl, *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (3-20% AcOEt/hexane) to afford **40** (1.93 g, 4.14 mmol, 83% over 3 steps) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  7.38-7.29 (m, 5H, Ph), 5.37 (d, 1H, *allo*-D-Thr-NH,  $J_{\text{allo-D-Thr-NH, allo-D-Thr-}\alpha\text{-CH}} = 8.0$  Hz), 5.23 (d, 1H, PhCH,  $J_{\text{PhCH, PhCH}} = 12.1$  Hz), 5.15 (d, 1H, PhCH,  $J_{\text{PhCH, PhCH}} = 12.1$  Hz), 4.36-4.28 (m, 2H, *allo*-D-Thr- $\alpha$ -CH, *allo*-D-Thr- $\beta$ -CH), 1.43 (s, 9H, <sup>t</sup>Bu), 1.25 (d, 3H, *allo*-D-Thr- $\gamma$ -CH,  $J_{\text{allo-D-Thr-}\gamma\text{-CH, allo-D-Thr-}\gamma\text{-CH}} = 6.3$  Hz), 1.08-1.01 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  170.2, 155.4, 135.6, 128.6, 128.4, 79.8, 70.3, 67.0, 60.1, 28.4, 20.7, 18.1, 18.1, 12.6; ESIMS-LR  $m/z$  466.40 [(M+H)<sup>+</sup>]; ESIMS-HR calcd for C<sub>25</sub>H<sub>44</sub>O<sub>5</sub>NSi 466.2983, found 466.3006; [ $\alpha$ ]<sup>20</sup><sub>D</sub> -20.10 (*c* 0.75, CHCl<sub>3</sub>).

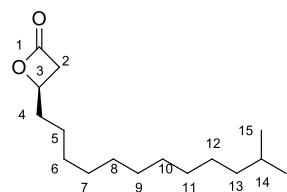
### (R)-4-(11-Hydroxy-11-methyldodec-9-enyl)oxetan-2-one (41)



A solution of **30** (1.84 g, 8.75 mmol) and 2-methylbut-3-en-2-ol (7.35 mL, 70.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (80 mL) was treated with 2<sup>nd</sup> generation Grubbs catalyst (371 mg, 0.44 mmol) under reflux for 3.5 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (40% AcOEt/hexane) to afford **41** (1.87 g, 6.97 mmol, 80%) as a black oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.63-5.56 (m, 2H, H-12, H-13), 4.55-4.46 (m, 1H, H-3), 3.51 (dd, 1H, H-2,  $J_{2,2} = 16.5$ ,  $J_{2,3} = 6.0$  Hz), 3.06 (dd, 1H, H-2,  $J_{2,2} = 16.5$ ,  $J_{2,3} = 4.6$  Hz), 2.06-1.93 (m, 2H, H-11), 1.92-1.81 (m, 1H, H-4), 1.80-1.68 (m, 1H, H-4), 1.50-1.13 (m, 20H, H-5, H-6, H-7, H-8, H-9, H-10, H-15); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 168.5, 137.9, 127.2, 71.4, 70.7, 42.9, 34.7, 32.2, 29.9, 29.4, 29.3, 29.2, 29.1, 24.9; ESIMS-LR  $m/z$  291.19 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>16</sub>H<sub>28</sub>O<sub>3</sub>Na 291.1931, found 291.1929;  $[\alpha]_D^{20} +12.82$  ( $c$  0.16, CHCl<sub>3</sub>).

### (R)-4-(11-Methyldodecyl)oxetan-2-one (42)

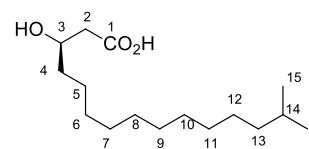


A solution of **41** (685 mg, 2.55 mol) in THF (20 mL) was treated with Burgess reagent (463 mg, 1.94 mmol) at room temperature for 10 min. Burgess reagent (231 mg, 0.97 mmol) was added to the mixture and stirred for 15 min. Hexane (20 mL) was added to the mixture, the precipitate was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo*. A mixture of the residue and PtO<sub>2</sub> (104 mg, 0.46 mmol) in MeOH

(15 mL) was vigorously stirred at room temperature for 20 min under H<sub>2</sub> atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-4% AcOEt/hexane) to afford **42** (431 mg, 1.69 mmol, 66% over 2 steps) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 4.55-4.47 (m, 1H, H-3), 3.41 (dd, 1H, H-2,  $J_{2,2} = 16.5$ ,  $J_{2,3} = 5.5$  Hz), 3.06 (dd, 1H, H-2,  $J_{2,2} = 16.5$ ,  $J_{2,3} = 4.6$  Hz), 1.92-1.67 (m, 2H, H-4), 1.58-1.08 (m, 19H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14), 0.86 (d, 6H, H-15,  $J_{15,14} = 6.9$  Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 168.5, 71.5, 43.2, 39.2, 34.8, 30.1, 29.8, 29.7, 29.6, 29.5, 29.3, 28.1, 27.5, 25.0, 22.8; ESIMS-LR  $m/z$  477.21 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>16</sub>H<sub>30</sub>O<sub>2</sub>Na 477.2138, found 477.2134;  $[\alpha]_D^{20} +22.19$  ( $c$  0.30, CHCl<sub>3</sub>).

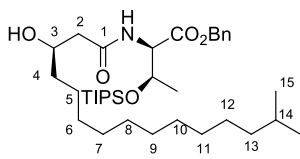
### (R)-3-Hydroxy-14-methylpentadecanoic acid (43)



A mixture of **42** (346 mg, 1.36 mmol) in THF (8 mL) was treated with a solution of NaOH (82 mg, 2.04 mmol) in H<sub>2</sub>O (8 mL) at room temperature for 1 h. The mixture was diluted with AcOEt, and partitioned between 1 M *aq.* HCl and AcOEt. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was recrystallized from hexane to afford **43** (296 mg, 1.09 mmol, 80%) as white crystal.

mp 66-67 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 4.08-4.00 (m, 1H, H-3), 2.59 (dd, 1H, H-2,  $J_{2,2} = 17.0$ ,  $J_{2,3} = 3.2$  Hz), 2.48 (dd, 1H, H-2,  $J_{2,2} = 17.0$ ,  $J_{2,3} = 8.7$  Hz), 1.63-1.40 (m, 5H, H-4, H-5, H-14), 1.39-1.20 (m, 16H, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 0.86 (d, 6H, H-15,  $J_{15,14} = 6.4$  Hz);  $[\alpha]_D^{20} -15.40$  ( $c$  0.50, CHCl<sub>3</sub>). This is a known compound reported in ref. 26.

**(R)-(3-Hydroxy-14-methylpentadecanyl)-allo-D-Thr(O-triisopropylsilyl)-OBn (44)**

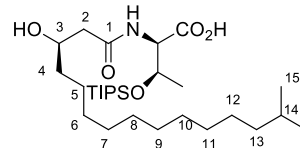


Compound **40** (373 mg, 0.80 mmol) was treated with 25% TFA/CH<sub>2</sub>Cl<sub>2</sub> (8 mL) at room temperature for 10 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude amine

**31**. A solution of the crude amine **31**, **43** (218 mg, 0.80 mmol), NaHCO<sub>3</sub> (202 mg, 2.40 mmol) and HOAt (327 mg, 2.40 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8.0 mL) was treated with EDCI (460 mg, 2.40 mmol) at room temperature for 60 min. The mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>, and the organic phase was washed with 1 M *aq.* HCl, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (30% AcOEt/hexane) to afford **44** (425 mg, 0.69 mmol, 86%) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.37-7.31 (m, 5H, Ph), 6.57 (d, 1H, *allo*-D-Thr-NH, *J*<sub>*allo*-D-Thr-NH, *allo*-D-Thr-α-CH</sub> = 7.7 Hz), 5.24 (d, 1H, PhCH, *J*<sub>PhCH, PhCH</sub> = 12.0 Hz), 5.15 (d, 1H, PhCH, *J*<sub>PhCH, PhCH</sub> = 12.0 Hz), 4.62 (dd, 1H, *allo*-D-Thr-α-CH, *J*<sub>*allo*-D-Thr-α-CH, *allo*-D-Thr-NH</sub> = 7.8, *J*<sub>*allo*-D-Thr-α-CH, *allo*-D-Thr-β-CH</sub> = 2.8 Hz), 4.31 (dq, 1H, *allo*-D-Thr-β-CH, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-α-CH</sub> = 2.8, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-γ-CH</sub> = 6.4 Hz), 3.98-3.88 (m, 1H, H-3), 3.50 (d, 1H, OH, *J*<sub>OH, 3</sub> = 3.2 Hz), 2.37 (dd, 1H, H-2, *J*<sub>2, 2</sub> = 15.1, *J*<sub>2, 3</sub> = 2.8 Hz), 2.30 (dd, 1H, H-2, *J*<sub>2, 2</sub> = 15.1, *J*<sub>2, 3</sub> = 8.7 Hz), 1.58-1.46 (m, 1H, H-14), 1.46-1.37 (m, 2H, H-4), 1.34-1.21 (m, 18H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 1.28 (d, 3H, *allo*-D-Thr-γ-CH, *J*<sub>*allo*-D-Thr-γ-CH, *allo*-D-Thr-β-CH</sub> = 6.4 Hz), 1.07-0.98 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si), 0.86 (d, 6H, H-15, *J*<sub>15, 14</sub> = 6.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.3, 169.8, 135.3, 128.7, 128.5, 70.0, 68.8, 67.3, 58.7, 42.9, 39.1, 37.0, 30.0, 29.8, 29.8, 29.7, 29.6, 28.1, 27.5, 25.6, 22.8, 20.7, 18.1, 18.1; ESIMS-LR *m/z* 642.45 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>36</sub>H<sub>65</sub>O<sub>5</sub>NNaSi 642.4524, found 642.4535; [α]<sup>20</sup><sub>D</sub> -24.20 (*c* 0.49, CHCl<sub>3</sub>).

**(R)-(3-Hydroxy-14-methylpentadecanyl)-allo-D-Thr(O-triisopropylsilyl)-OH (28)**

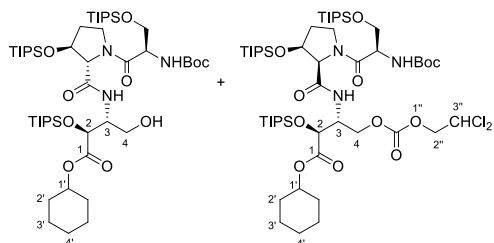


A mixture of **44** (298 mg, 0.48 mmol) and 10% Pd/C (30 mg) in MeOH (8 mL) was vigorously stirred at room temperature for 5 h under H<sub>2</sub> atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to afford **28** (254 mg, 0.48 mmol, 99%) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.87 (d, 1H, *allo*-D-Thr-NH, *J*<sub>*allo*-D-Thr-NH, *allo*-D-Thr-α-CH</sub> = 8.2 Hz), 4.59 (dd, 1H, *allo*-D-Thr-α-CH, *J*<sub>*allo*-D-Thr-α-CH, *allo*-D-Thr-NH</sub> = 7.7, *J*<sub>*allo*-D-Thr-α-CH, *allo*-D-Thr-β-CH</sub> = 3.6 Hz), 4.38 (dq, 1H, *allo*-D-Thr-β-CH, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-α-CH</sub> = 3.6, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-γ-CH</sub> = 6.3 Hz), 4.05-3.96 (m, 1H, H-3), 2.40 (dd, 1H, H-2, *J*<sub>2, 2</sub> = 15.1, *J*<sub>2, 3</sub> = 3.2 Hz), 2.34 (dd, 1H, H-2, *J*<sub>2, 2</sub> = 15.1, *J*<sub>2, 3</sub> = 9.7 Hz), 1.59-1.45 (m, 1H, H-14), 1.28 (d, 3H, *allo*-D-Thr-γ-CH, *J*<sub>*allo*-D-Thr-γ-CH, *allo*-D-Thr-β-CH</sub> = 6.3 Hz), 1.35-1.18 (m, 20H, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 1.09-1.00 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si), 0.85 (d, 6H, H-15, *J*<sub>15, 14</sub> = 6.4 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 173.2, 172.7, 69.1, 69.0, 58.7, 43.4, 39.2, 36.9, 30.1, 29.9, 29.8, 29.8, 29.8, 29.7, 28.1, 27.5, 25.7, 22.8, 20.1, 19.1, 18.1, 18.1, 17.8, 12.5, 12.4; ESIMS-LR *m/z* 552.41 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>29</sub>H<sub>59</sub>O<sub>5</sub>NNaSi 552.4055, found 552.4070; [α]<sup>20</sup><sub>D</sub> -35.94 (*c* 0.45, CHCl<sub>3</sub>).

**Cyclohexyl (2*S*,3*R*)-3-[[Boc-D-Ser(O-triisopropylsilyl)-(3*S*)-3-(triisopropylsiloxy)-Pro]amino]-4-hydroxy-2-triisopropylsiloxybutanoate (45)**

**Cyclohexyl (2*S*,3*R*)-3-[[Boc-D-Ser(O-triisopropylsilyl)-(3*S*)-3-(triisopropylsiloxy)-Pro]amino]-4-[(2,2-dichloroethoxy)oxy]-2-triisopropylsiloxybutanoate (46)**



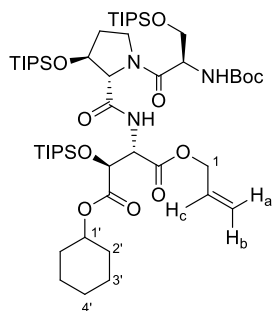
A mixture of *trans*-**26** (7.1 mg, 0.0061 mmol) in THF (50  $\mu$ L) was treated with 0.1 M THF solution of SmI<sub>2</sub> (300  $\mu$ L, 0.03 mmol) at room temperature and stirred for 1 min. The reaction was quenched with air, and the mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with 1 M *aq.* HCl, *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The

residue was purified by silica gel column chromatography ( $\phi$  0.5 cm $\times$ 2 cm, 5-15% AcOEt/hexane) to afford **45** (3.4 mg, 0.0034 mmol, 50 %) as a colorless oil and **46** (3.8 mg, 0.0034 mmol, 50%) as a colorless oil.

Data for **45**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  6.85 (d, 1H, C-3-NH,  $J_{C-3-NH, 3} = 8.7$  Hz), 5.49 (d, 1H, D-Ser-NH,  $J_{D-Ser-NH, D-Ser-\alpha-CH} = 6.4$  Hz), 4.79-4.68 (m, 1H, H-1'), 4.64 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3-hydroxy-Pro-\beta-CH, 3-hydroxy-Pro-\gamma-CH} = 2.8$  Hz), 4.54-4.46 (m, 2H, H-2, D-Ser- $\alpha$ -CH), 4.40 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.31-4.23 (m, 1H, H-3), 3.93 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{D-Ser-\beta-CH, D-Ser-\alpha-CH} = 5.0$ ,  $J_{D-Ser-\beta-CH, D-Ser-\beta-CH} = 10.1$  Hz), 3.85-3.73 (m, 4H, H-4, D-Ser- $\beta$ -CH, 3-hydroxy-Pro- $\delta$ -CH), 3.73-3.64 (m, 1H, H-4), 2.50 (t, 1H, OH,  $J_{OH, 4} = 6.9$  Hz), 2.29-2.15 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.97 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3-hydroxy-Pro-\gamma-CH, 3-hydroxy-Pro-\delta-CH} = 5.8$ ,  $J_{3-hydroxy-Pro-\gamma-CH, 3-hydroxy-Pro-\gamma-CH} = 12.8$  Hz), 1.89-1.65 (m, 2H, H-2'), 1.64-1.19 (m, 6H, H-3', H-4'), 1.42 (s, 9H, <sup>t</sup>Bu), 1.18-0.94 (m, 63H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  171.6, 170.1, 169.1, 155.7, 79.7, 74.1, 74.0, 71.3, 70.3, 63.3, 61.7, 54.9, 54.4, 45.6, 34.0, 31.6, 31.5, 28.5, 25.4, 23.9, 18.1, 12.4, 12.1, 12.0; ESIMS-LR  $m/z$  1008.65 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>50</sub>H<sub>99</sub>O<sub>10</sub>N<sub>3</sub>NaSi<sub>3</sub> 1008.6531, found 1008.6542; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -6.32 (*c* 0.30, CHCl<sub>3</sub>).

Data for **46**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  7.11 (d, 1H, C-3-NH,  $J_{C-3-NH, 3} = 9.2$  Hz), 5.86 (dd, 1H, H-3'',  $J_{3'', 2''} = J_{3'', 3''} = 6.3$  Hz), 5.58 (d, 1H, D-Ser-NH,  $J_{D-Ser-NH, D-Ser-\alpha-CH} = 8.0$  Hz), 4.76-4.69 (m, 1H, H-1'), 4.67 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3-hydroxy-Pro-\beta-CH, 3-hydroxy-Pro-\gamma-CH} = 3.5$  Hz), 4.60-4.52 (m, 4H, D-Ser- $\beta$ -CH, H-2, H-3), 4.50 (dd, 1H, H-2'',  $J_{2'', 3''} = 6.3$ ,  $J_{3'', 3''} = 7.5$  Hz), 4.47 (dd, 1H, H-2'',  $J_{2'', 3''} = 6.3$ ,  $J_{3'', 3''} = 7.5$  Hz), 4.35 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.21 (d, 2H, H-4  $J_{4, 3} = 6.9$  Hz), 3.95 (dd, 1H, D-Ser- $\alpha$ -CH,  $J_{D-Ser-\alpha-CH, D-Ser-\beta-CH} = 4.6$ ,  $J_{D-Ser-\alpha-CH, D-Ser-\beta-CH} = 9.8$  Hz), 3.84-3.75 (m, 2H, H-4, 3-hydroxy-Pro- $\delta$ -CH), 2.21-2.11 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.94 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3-hydroxy-Pro-\gamma-CH, 3-hydroxy-Pro-\delta-CH} = 5.8$ ,  $J_{3-hydroxy-Pro-\gamma-CH, 3-hydroxy-Pro-\gamma-CH} = 12.6$  Hz), 1.89-1.64 (m, 2H, H-2'), 1.52-1.20 (m, 15H, H-3', H-4', <sup>t</sup>Bu), 1.20-0.82 (m, 63H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  170.9, 170.8, 170.4, 169.4, 155.4, 79.4, 74.0, 73.6, 71.4, 70.7, 69.6, 68.0, 65.8, 65.1, 63.7, 55.1, 54.4, 34.1, 31.6, 31.3, 29.8, 28.5, 28.1, 25.3, 23.8, 18.3, 18.1, 12.5, 12.1, 12.0; ESIMS-LR  $m/z$  1148.60 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>53</sub>H<sub>101</sub>O<sub>12</sub>N<sub>3</sub>Cl<sub>2</sub>NaSi<sub>3</sub> 1148.5962, found 1148.5975; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -9.84 (*c* 0.69, CHCl<sub>3</sub>).

**Boc-D-Ser(O-triisopropylsilyl)-(3S)-3-(triisopropylsiloxy)-Pro-(3S)-3-(triisopropylsiloxy)-Asp(O-cyclohexyl)-Oallyl (47)**

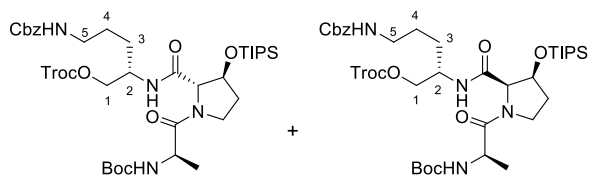


A solution of **45** (151 mg, 0.153 mmol) in THF (2 mL) was treated with Dess-Martin periodinane (1.27 g, 3.0 mmol) at room temperature for 55 min. The reaction was quenched with *sat. aq.* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/*sat. aq.* NaHCO<sub>3</sub> = 1/1, and the mixture was partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude aldehyde. A solution of the crude aldehyde, NaH<sub>2</sub>PO<sub>4</sub> · 2H<sub>2</sub>O (70.2 mg, 0.45 mmol) and 2-methylbut-2-ene (100 μL) in THF/*i*-BuOH/H<sub>2</sub>O (900 μL/900 μL/150 μL) was treated with a solution of NaClO<sub>2</sub> (40.7 mg, 0.45 mmol) in H<sub>2</sub>O (150 μL) at room temperature for 50 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude carboxylic acid. A solution of the crude carboxylic acid and Cs<sub>2</sub>CO<sub>3</sub> (78.2 mg, 0.24 mmol) in DMF (1.5 mL) was treated with allyl bromide (18.2 μL, 0.21 mmol) at room temperature for 16 h. The mixture was partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (50% AcOEt/hexane) to afford **47** (83.5 mg, 0.080 mmol, 53% over 3 steps) as a pale yellow oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 7.33 (d, 1H, β-hydroxy-Asp-NH, *J*<sub>β-hydroxy-Asp-NH, β-hydroxy-Asp-α-CH</sub> = 9.2 Hz), 5.89 (dddd, 1H, H<sub>c</sub>, *J*<sub>H<sub>c</sub>, H<sub>a</sub></sub> = 17.2, *J*<sub>H<sub>c</sub>, H<sub>b</sub></sub> = 10.3, *J*<sub>H<sub>c</sub>, 1</sub> = *J*<sub>H<sub>c</sub>, 1</sub> = 5.7 Hz), 5.53 (d, 1H, D-Ser-NH, *J*<sub>D-Ser-NH, D-Ser-α-CH</sub> = 8.6 Hz), 5.32 (d, 1H, H<sub>a</sub>, *J*<sub>H<sub>a</sub>, H<sub>c</sub></sub> = 17.2 Hz), 5.24 (d, 1H, H<sub>b</sub>, *J*<sub>H<sub>b</sub>, H<sub>c</sub></sub> = 10.3 Hz), 5.00 (br d, 1H, β-hydroxy-Asp-α-CH, *J*<sub>β-hydroxy-Asp-α-CH, β-hydroxy-Asp-NH</sub> = 9.2 Hz), 4.94 (br s, 1H, β-hydroxy-Asp-β-CH), 4.76-4.68 (m, 1H, H-1'), 4.69 (d, 1H, 3-hydroxy-Pro-β-CH, *J*<sub>3-hydroxy-Pro-β-CH, 3-hydroxy-Pro-α-CH</sub> = 2.9 Hz), 4.64-4.54 (m, 3H, D-Ser-α-CH, H-1), 4.41 (s, 1H, 3-hydroxy-Pro-α-CH), 3.94 (dd, 1H, 3-hydroxy-Pro-δ-CH, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-δ-CH</sub> = 9.7, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = 6.3 Hz), 3.84-3.75 (m, 2H, D-Ser-β-CH), 3.69 (dd, 1H, 3-hydroxy-Pro-δ-CH, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-δ-CH</sub> = 9.7, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = 9.2 Hz), 2.21-2.11 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.94 (dd, 1H, 3-hydroxy-Pro-γ-CH, *J*<sub>3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-γ-CH</sub> = 12.6, *J*<sub>3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-δ-CH</sub> = 5.7 Hz), 1.83-1.60 (m, 4H, H-2'), 1.52-1.17 (m, 15H, <sup>t</sup>Bu, H-3', H-4'), 1.13-0.83 (m, 63H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 170.3, 169.8, 169.5, 168.8, 155.2, 131.5, 119.2, 79.2, 74.2, 73.4, 72.7, 69.6, 66.6, 64.1, 55.8, 54.2, 45.6, 34.2, 31.6, 31.2, 28.5, 25.3, 23.8, 23.7, 18.1, 18.0, 18.0, 12.5, 12.1, 12.0; ESIMS-LR *m/z* 1062.67 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>53</sub>H<sub>101</sub>O<sub>11</sub>N<sub>3</sub>NaSi<sub>3</sub> 1062.6636, found 1062.6665; [α]<sub>D</sub><sup>20</sup> -10.20 (*c* 0.68, CHCl<sub>3</sub>).

**(2S)-5-[(Benzyloxycarbonyl)amino]-2-[(Boc-D-Ala-(3S)-3-(triisopropylsiloxy)-Pro)amino]pentyl trichloroethyl carbonate (*trans*-48)** 2,2,2-

**(2S)-5-[(Benzyloxycarbonyl)amino]-2-[(Boc-D-Ala-(3S)-3-(triisopropylsiloxy)-D-Pro)amino]pentyl trichloroethyl carbonate (*cis*-48)** 2,2,2-



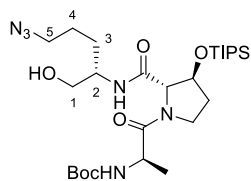
A solution of **4a** (771 mg, 3.19 mmol) and **34** (1.61 mg, 8.52 mmol) in HFIP (25 mL) was treated with a solution of **33** (932 mg, 2.13 mmol) in HFIP (10 mL) at room temperature for 19.5 h. The mixture was concentrated *in vacuo*, and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>.

The organic phase was washed with *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (5-25% acetone/hexane) to afford **trans-48** (794 mg, 0.91 mmol, 43%) as a colorless oil and **cis-48** (666 mg, 0.77 mmol, 36%) as a colorless oil.

Data for **trans-48**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.38-7.28 (m, 5H, Ph), 6.96 (d, 1H, 3-hydroxy-Pro-NH, *J*<sub>3-hydroxy-Pro-NH, H-2</sub> = 8.7 Hz), 5.30 (br s, 1H, C-5-NH), 5.16 (d, 1H, D-Ala-NH, *J*<sub>D-Ala-NH, D-Ala-α-CH</sub> = 6.8 Hz), 5.11 (d, 1H, PhCH, *J*<sub>PhCH, PhCH</sub> = 12.2 Hz), 5.06 (d, 1H, PhCH, *J*<sub>PhCH, PhCH</sub> = 12.2 Hz), 4.79 (d, 1H, Cl<sub>3</sub>CCH, *J*<sub>Cl<sub>3</sub>CCH, Cl<sub>3</sub>CCH</sub> = 11.9 Hz), 4.75 (d, 1H, Cl<sub>3</sub>CCH, *J*<sub>Cl<sub>3</sub>CCH, Cl<sub>3</sub>CCH</sub> = 11.9 Hz), 4.78-4.70 (m, 1H, 3-hydroxy-Pro-β-CH), 4.41 (s, 1H, 3-hydroxy-Pro-α-CH), 4.36 (dq, 1H, D-Ala-α-CH, *J*<sub>D-Ala-α-CH, D-Ala-NH</sub> = 6.8, *J*<sub>D-Ala-α-CH, D-Ala-β-CH</sub> = 6.9 Hz), 4.22-4.12 (m, 2H, H-1), 4.11-3.99 (m, 1H, H-2), 3.81 (dd, 1H, 3-hydroxy-Pro-δ-CH, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-δ-CH</sub> = *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = 8.7 Hz), 3.63 (ddd, 1H, 3-hydroxy-Pro-δ-CH, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-δ-CH</sub> = *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = 8.7 Hz), 3.27-3.12 (m, 2H, H-5), 2.22-2.07 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.97 (dd, 1H, 3-hydroxy-Pro-γ-CH, *J*<sub>3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-γ-CH</sub> = 12.8, *J*<sub>3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-δ-CH</sub> = 8.7 Hz), 1.71-1.45 (m, 4H, H-3, H-4), 1.41 (s, 9H, <sup>t</sup>Bu), 1.24 (d, 3H, D-Ala-β-CH, *J*<sub>D-Ala-β-CH, D-Ala-α-CH</sub> = 6.9 Hz), 1.16-0.94 (m, 21H, <sup>t</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 173.5, 169.5, 156.6, 155.7, 153.9, 136.7, 128.6, 128.3, 128.2, 94.5, 80.2, 77.0, 73.3, 69.9, 69.6, 66.7, 48.3, 48.2, 45.3, 40.6, 33.9, 28.5, 18.0, 16.2, 18.0, 17.1, 12.0; ESIMS-LR *m/z* 889.32 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>38</sub>H<sub>61</sub>O<sub>10</sub>N<sub>4</sub>Cl<sub>3</sub>NaSi 889.3115, found 889.3136; [α]<sub>D</sub><sup>20</sup> -2.53 (*c* 4.57, CHCl<sub>3</sub>).

Data for **cis-48**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.39-7.24 (m, 5H, Ph), 6.02 (d, 1H, 3-hydroxy-Pro-NH, *J*<sub>3-hydroxy-Pro-NH, H-2</sub> = 8.7 Hz), 5.66-5.57 (m, 1H, C-5-NH), 5.40 (d, 1H, D-Ala-NH, *J*<sub>D-Ala-NH, D-Ala-α-CH</sub> = 8.2 Hz), 5.15 (d, 1H, PhCH, *J*<sub>PhCH, PhCH</sub> = 12.4 Hz), 5.05 (d, 1H, PhCH, *J*<sub>PhCH, PhCH</sub> = 12.4 Hz), 4.77 (d, 1H, Cl<sub>3</sub>CCH, *J*<sub>Cl<sub>3</sub>CCH, Cl<sub>3</sub>CCH</sub> = 11.9 Hz), 4.72 (d, 1H, Cl<sub>3</sub>CCH, *J*<sub>Cl<sub>3</sub>CCH, Cl<sub>3</sub>CCH</sub> = 11.9 Hz), 4.73-4.66 (m, 1H, 3-hydroxy-Pro-β-CH), 4.46 (d, 1H, 3-hydroxy-Pro-α-CH, *J*<sub>3-hydroxy-Pro-α-CH, 3-hydroxy-Pro-β-CH</sub> = 5.5 Hz), 4.48-4.42 (m, 1H, D-Ala-α-CH), 4.30 (dd, 1H, H-1, *J*<sub>1,1</sub> = 11.0, *J*<sub>1,2</sub> = 2.8 Hz), 4.25-4.10 (m, 2H, H-1, H-2), 3.84-3.64 (m, 2H, 3-hydroxy-Pro-δ-CH), 3.24-3.13 (m, 2H, H-5), 2.16-2.00 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.70-1.47 (m, 4H, H-3, H-4), 1.41 (s, 9H, <sup>t</sup>Bu), 1.27 (d, 3H, D-Ala-β-CH, *J*<sub>D-Ala-β-CH, D-Ala-α-CH</sub> = 6.9 Hz), 1.13-0.97 (m, 21H, <sup>t</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 173.5, 167.6, 156.6, 155.5, 153.9, 136.8, 128.5, 128.4, 128.1, 128.0, 127.8, 94.3, 80.0, 76.8, 76.3, 71.7, 70.8, 66.6, 65.6, 48.2, 47.8, 45.2, 40.6, 34.1, 29.7, 28.5, 25.5, 18.2, 18.1, 18.0, 12.3, 12.1; ESIMS-LR *m/z* 889.31 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>38</sub>H<sub>61</sub>O<sub>10</sub>N<sub>4</sub>Cl<sub>3</sub>NaSi 889.3115, found 889.3127; [α]<sub>D</sub><sup>20</sup> -19.12 (*c* 0.52, CHCl<sub>3</sub>).

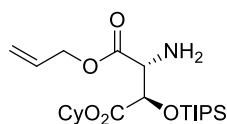
**N-[(S)-4-Azide-1-(hydroxymethyl)butyl]-[(Boc-D-Ala-(3S)-3-(triisopropylsiloxy)-Pro)]amide (49)**



A mixture of compound **trans-48** (495 mg, 0.57 mmol) and metallic Sm (85.7 mg, 0.57 mmol) in MeOH (15 mL) was treated with I<sub>2</sub> (145 mg, 0.57 mmol) at room temperature for 110 min. The reaction was quenched with *sat. aq.* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The mixture was partitioned between Et<sub>2</sub>O and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with 1 M *aq.* HCl and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude alcohol. A mixture of the crude alcohol and Pd black (46 mg) in 5% AcOH/MeOH (10 mL) was vigorously stirred at room temperature for 3.5 h under H<sub>2</sub> atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to afford a crude aminoalcohol. A mixture of the crude aminoalcohol, K<sub>2</sub>CO<sub>3</sub> (394 mg, 2.85 mmol) and CuSO<sub>4</sub>·5H<sub>2</sub>O (1.4 mg, 0.006 mmol) in MeOH (10 mL) was treated with imidazole-1-sulfonyl azide hydrogen sulfate (464 mg, 1.71 mmol) at room temperature for 10.5 h. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (5-25% acetone/hexane) to afford **49** (292 mg, 0.50 mmol, 88% over 3 steps) as a white solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 6.70 (d, 1H, 3-hydroxy-Pro-NH, *J*<sub>3-hydroxy-Pro-NH, H-2</sub> = 8.6 Hz), 5.04 (d, 1H, D-Ala-NH, *J*<sub>D-Ala-NH, D-Ala-α-CH</sub> = 5.7 Hz), 4.76 (br s, 1H, 3-hydroxy-Pro-β-CH), 4.51 (s, 1H, 3-hydroxy-Pro-α-CH), 4.34 (dq, 1H, D-Ala-α-CH, *J*<sub>D-Ala-α-CH, D-Ala-NH</sub> = 5.7, *J*<sub>D-Ala-α-CH, D-Ala-β-CH</sub> = 6.9 Hz), 3.96-3.88 (m, 1H, 3-hydroxy-Pro-δ-CH), 3.88-3.81 (m, 1H, H-2), 3.72 (ddd, 1H, 3-hydroxy-Pro-δ-CH, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-δ-CH</sub> = *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = 8.6 Hz), 3.68-3.61 (m, 1H, H-1), 3.55-3.47 (m, 1H, H-1), 3.34-3.23 (m, 2H, H-5), 3.08 (br s, 1H, OH), 2.07-1.99 (m, 2H, 3-hydroxy-Pro-γ-CH), 1.66-1.54 (m, 4H, H-3, H-4), 1.44 (s, 9H, <sup>t</sup>Bu), 1.31 (d, 3H, D-Ala-β-CH, *J*<sub>D-Ala-β-CH, D-Ala-α-CH</sub> = 6.9 Hz), 1.16-1.02 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 173.4, 169.3, 156.1, 80.4, 73.8, 70.2, 64.6, 51.8, 51.3, 48.5, 45.3, 33.8, 28.5, 28.0, 25.7, 18.0, 16.5, 12.1; ESIMS-LR *m/z* 585.48 [(M+H)<sup>+</sup>]; ESIMS-HR calcd for C<sub>27</sub>H<sub>53</sub>O<sub>6</sub>N<sub>6</sub>Si 585.3790, found 585.3823; [α]<sub>D</sub><sup>20</sup> -13.90 (*c* 2.60, CHCl<sub>3</sub>).

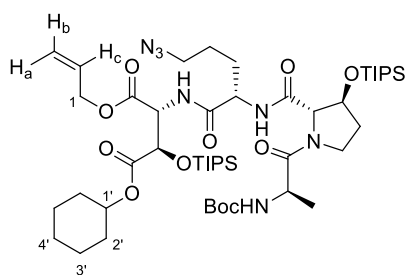
### H-(3S)-3-(triisopropylsiloxy)-Asp(O-cyclohexyl)-Oallyl (32)



A solution of **36** (20.6 mg, 0.039 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (300 μL) was treated with TFA (100 μL) at room temperature for 15 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford **32**.

The amine was directly used to the next reaction without further purification.

### (2S)-5-Azide-2-[Boc-D-Ala-(3S)-3-(triisopropylsiloxy)-Pro]amino-N-[(3R)-3-(triisopropylsiloxy)-D-Asp(O-cyclohexyl)-Oallyl]pentanamide (29)

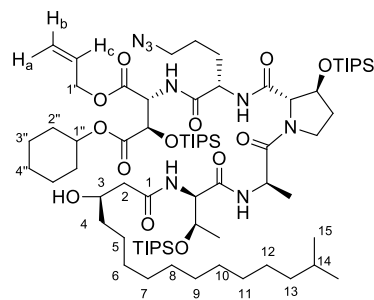


A solution of **49** (23.0 mg, 0.039 mmol) in THF (500 μL) was treated with Dess-Martin periodinane (33.1 mg, 0.078 mmol) at room temperature for 130 min. The reaction was quenched with *sat. aq.* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/*sat. aq.* NaHCO<sub>3</sub> = 1/1, and the mixture was partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude aldehyde. A solution of the crude aldehyde, NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (18.3 mg, 0.12 mmol) and 2-methylbut-2-ene (50 μL) in THF/<sup>t</sup>BuOH/H<sub>2</sub>O (600 μL/600 μL/100 μL) was treated with a solution of NaClO<sub>2</sub> (18.3 mg, 0.12 mmol) in H<sub>2</sub>O (100

$\mu\text{L}$ ) at room temperature for 130 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to afford a crude carboxylic acid. A mixture of the crude carboxylic acid, amine **32** (0.039 mmol),  $\text{NaHCO}_3$  (9.8 mg, 0.12 mmol) and HOAt (15.9 mg, 0.12 mmol) in  $\text{CH}_2\text{Cl}_2$  (400  $\mu\text{L}$ ) was treated with EDCI (22.4 mg, 0.12 mmol) at room temperature for 140 min. The mixture was partitioned between AcOEt and *sat. aq.*  $\text{NaHCO}_3$ . The organic phase was washed with 1 M *aq.* HCl, *sat. aq.*  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$  and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (40% AcOEt/hexane) to afford **29** (17.2 mg, 0.17 mmol, 44% over 3 steps) as a colorless oil.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of several rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  7.32 (d, 1H, Orn- $\alpha$ -NH,  $J_{\text{Orn-}\alpha\text{-NH}, \text{Orn-}\alpha\text{-CH}} = 8.2$  Hz), 6.90 (d, 1H,  $\beta$ -hydroxy-Asp-NH,  $J_{\beta\text{-hydroxy-Asp-NH}, \beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 9.6$  Hz), 5.92 (dddd, 1H,  $\text{H}_c$ ,  $J_{\text{H}_c, \text{H}_a} = 17.9$ ,  $J_{\text{H}_c, \text{H}_b} = 11.0$ ,  $J_{\text{H}_c, \text{H-1}} = J_{\text{H}_c, 1} = 5.9$  Hz), 5.34 (dd, 1H,  $\text{H}_a$ ,  $J_{\text{H}_a, \text{H}_c} = 17.9$ ,  $J_{\text{H}_a, 1} = 1.4$  Hz), 5.36-5.31 (m, 1H, D-Ala-NH), 5.26 (dd, 1H,  $\text{H}_b$ ,  $J_{\text{H}_b, \text{H}_c} = 11.0$ ,  $J_{\text{H}_b, \text{H-1}} = 1.4$  Hz), 5.02 (dd, 1H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-NH}} = 9.6$ ,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-}\beta\text{-CH}} = 1.8$  Hz), 4.93 (d, 1H,  $\beta$ -hydroxy-Asp- $\beta$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\beta\text{-CH}, \beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 1.8$  Hz), 4.78-4.70 (m, 2H, H-1', 3-hydroxy-Pro- $\beta$ -CH), 4.67 (dddd, 1H, H-1,  $J_{1, 1} = 13.3$ ,  $J_{1, \text{H}_c} = 5.9$ ,  $J_{1, \text{H}_b} = J_{1, \text{H}_a} = 1.4$  Hz), 4.56 (dddd, 1H, H-1,  $J_{1, 1} = 13.3$ ,  $J_{1, \text{H}_c} = 5.9$ ,  $J_{1, \text{H}_b} = J_{1, \text{H}_a} = 1.4$  Hz), 4.47 (dq, 1H, D-Ala- $\alpha$ -CH,  $J_{\text{D-Ala-}\alpha\text{-CH}, \text{D-Ala-NH}} = J_{\text{D-Ala-}\alpha\text{-CH}, \text{D-Ala-}\beta\text{-CH}} = 6.9$  Hz), 4.40 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.34 (ddd, Orn- $\alpha$ -CH,  $J_{\text{Orn-}\alpha\text{-CH}, \text{Orn-}\alpha\text{-NH}} = 8.2$ ,  $J_{\text{Orn-}\alpha\text{-CH}, \text{Orn-}\beta\text{-CH}} = J_{\text{Orn-}\alpha\text{-CH}, \text{Orn-}\gamma\text{-CH}} = 9.6$  Hz), 3.80 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.6$  Hz), 3.68 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.29 (ddd, 1H, Orn- $\delta$ -CH,  $J_{\text{Orn-}\delta\text{-CH}, \text{Orn-}\delta\text{-CH}} = 9.2$ ,  $J_{\text{Orn-}\delta\text{-CH}, \text{Orn-}\gamma\text{-CH}} = J_{\text{Orn-}\delta\text{-CH}, \text{Orn-}\gamma\text{-CH}} = 5.0$  Hz), 3.26 (ddd, 1H, Orn- $\delta$ -CH,  $J_{\text{Orn-}\delta\text{-CH}, \text{Orn-}\delta\text{-CH}} = 9.2$ ,  $J_{\text{Orn-}\delta\text{-CH}, \text{Orn-}\gamma\text{-CH}} = J_{\text{Orn-}\delta\text{-CH}, \text{Orn-}\gamma\text{-CH}} = 5.0$  Hz), 2.31-2.16 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.04-1.95 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.95-1.78 (m, 4H, Orn- $\beta$ -CH, H-2'), 1.76-1.62 (m, 4H, Orn- $\gamma$ -CH, H-2'), 1.43 (s, 9H, 'Bu), 1.47-1.18 (m, 6H, H-3', H-4'), 1.30 (d, 3H, D-Ala- $\beta$ -CH,  $J_{\text{D-Ala-}\beta\text{-CH}, \text{D-Ala-}\alpha\text{-CH}} = 6.9$  Hz), 1.17-0.95 (m, 42H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  173.3, 170.6, 169.8, 169.6, 168.7, 155.4, 131.5, 119.3, 79.8, 74.4, 73.1, 72.7, 69.3, 66.6, 55.9, 52.7, 51.0, 48.1, 45.2, 34.0, 31.7, 31.4, 28.8, 28.4, 25.3, 24.8, 23.9, 23.9, 18.0, 18.0, 17.9, 12.5, 12.1; ESIMS-LR  $m/z$  1030.61 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{49}\text{H}_{89}\text{O}_{11}\text{N}_7\text{NaSi}_2$  1030.6051, found 1030.6079;  $[\alpha]_D^{20} -18.03$  ( $c$  1.72,  $\text{CHCl}_3$ ).

**(2S)-5-Azide-2-[(3R)-3-hydroxy-14-methylpentadecanoyl]-allo-D-Thr(O-triisopropylsiloxy)-Boc-D-Ala-(3S)-3-(triisopropylsiloxy)-Pro}amino-N-[(3R)-3-(triisopropylsiloxy)-D-Asp(O-cyclohexyl)-Oallyl]pentanamide (52)**



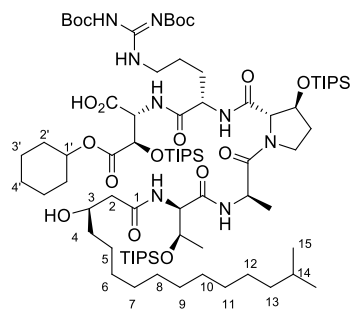
A mixture of **29** (195 mg, 0.193 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.5 mL) was treated with TFA (500  $\mu\text{L}$ ) at room temperature for 35 min. The reaction was quenched with *sat. aq.*  $\text{NaHCO}_3$ , and the mixture was partitioned between AcOEt and *sat. aq.*  $\text{NaHCO}_3$ . The organic phase was washed with  $\text{H}_2\text{O}$  and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to afford a crude amine. A mixture of the crude amine, **28** (112.5 mg, 0.212 mmol),  $\text{NaHCO}_3$  (53.4 mg, 0.636 mmol) and HOAt (86.6 mg, 0.636 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was treated with EDCI (121.9 mg, 0.636 mmol) at room temperature for 20 h. The mixture was partitioned between AcOEt and *sat. aq.*  $\text{NaHCO}_3$ . The organic phase was washed with 1 M *aq.* HCl, *sat. aq.*  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$  and brine, dried



(Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (40% AcOEt/hexane) to afford **52** (173 mg, 0.122 mmol, 63% over 2 steps) as a white solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 7.46 (d, 1H, D-Ala-NH,  $J_{D-Ala-NH, D-Ala-α-CH} = 7.4$  Hz), 7.22 (Orn-α-NH,  $J_{Orn-NH, Orn-α-CH} = 8.2$  Hz), 6.84 (d, 1H, D-β-hydroxy-Asp-NH,  $J_{D-β-hydroxy-Asp-NH, D-β-hydroxy-Asp-α-CH} = 9.6$  Hz), 6.52 (d, 1H, *allo*-D-Thr-NH,  $J_{allo-D-Thr-NH, allo-D-Thr-α-CH} = 6.9$  Hz), 5.92 (dddd, 1H, H<sub>c</sub>,  $J_{H_c, H_a} = 17.4$ ,  $J_{H_c, H_b} = 11.0$ ,  $J_{H_c, 1'} = J_{H_c, 1''} = 6.0$  Hz), 5.33 (d, 1H, H<sub>a</sub>,  $J_{H_a, H_c} = 17.4$  Hz), 5.25 (d, 1H, H<sub>b</sub>,  $J_{H_b, H_c} = 11.0$  Hz), 5.01 (dd, 1H, D-β-hydroxy-Asp-α-CH,  $J_{D-β-hydroxy-Asp-α-CH, D-β-hydroxy-Asp-NH} = 9.6$ ,  $J_{D-β-hydroxy-Asp-α-CH, D-β-hydroxy-Asp-β-CH} = 1.8$  Hz), 4.78-4.61 (m, 4H, *allo*-D-Thr-α-CH, 3-hydroxy-Pro-β-CH, H-1', H-1''), 4.56 (dd, 1H,  $J_{1', 1''} = 13.3$ ,  $J_{1', H_c} = 6.0$  Hz), 4.49-4.40 (m, 2H, D-Ala-α-CH, *allo*-D-Thr-β-CH), 4.35 (ddd, 1H, Orn-α-CH,  $J_{Orn-α-CH, Orn-α-NH} = 8.2$ ,  $J_{Orn-α-CH, Orn-β-CH} = J_{Orn-α-CH, Orn-γ-CH} = 7.3$  Hz), 4.30 (s, 1H, 3-hydroxy-Pro-α-CH), 4.08-3.98 (m, 1H, H-3), 3.86-3.78 (m, 1H, 3-hydroxy-Pro-δ-CH), 3.64 (ddd, 1H, 3-hydroxy-Pro-δ-CH,  $J_{3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-δ-CH} = J_{3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH} = J_{3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH} = 9.6$  Hz), 3.34 (ddd, 1H, Orn-δ-CH,  $J_{Orn-δ-CH, Orn-δ-CH} = 12.8$ ,  $J_{Orn-δ-CH, Orn-γ-CH} = J_{Orn-δ-CH, Orn-γ-CH} = 6.9$  Hz), 3.28 (ddd, 1H, Orn-δ-CH,  $J_{Orn-δ-CH, Orn-δ-CH} = 12.8$ ,  $J_{Orn-δ-CH, Orn-γ-CH} = J_{Orn-δ-CH, Orn-γ-CH} = 6.9$  Hz), 2.38 (dd, 1H, H-2,  $J_{2, 2} = 13.7$ ,  $J_{2, 3} = 2.8$  Hz), 2.28-2.09 (m, 2H, 3-hydroxy-Pro-γ-CH, H-2''), 2.01-1.66 (m, 6H, Orn-β-CH, Orn-γ-CH, H-2''), 1.63-1.18 (m, 38H, D-Ala-β-CH, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-3'', H-4'', <sup>t</sup>Bu), 1.18-0.93 (m, 63H, <sup>i</sup>Pr<sub>3</sub>Si), 0.85 (d, 6H, H-15,  $J_{H-15, H-14} = 6.9$  Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.7, 172.4, 170.9, 169.8, 169.7, 169.5, 168.8, 131.5, 119.4, 74.6, 73.7, 72.8, 69.6, 69.5, 68.4, 66.8, 59.4, 55.9, 52.8, 47.0, 45.4, 43.9, 39.2, 28.1, 34.1, 31.8, 31.6, 30.1, 30.0, 29.9, 29.8, 29.1, 28.1, 27.6, 25.5, 25.4, 24.9, 24.0, 22.8, 19.6, 18.2, 18.2, 18.1, 18.0, 17.3, 12.6, 12.6, 12.1; ESIMS-LR *m/z* 1441.96 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>73</sub>H<sub>138</sub>O<sub>13</sub>N<sub>8</sub>NaSi<sub>3</sub> 1441.9583, found 1441.9631; [α]<sub>D</sub><sup>20</sup> -17.02 (*c* 0.67, CHCl<sub>3</sub>).

**[(3*R*)-3-Hydroxy-14-methylpentadecanoyl]-*allo*-D-Thr(O-triisopropylsiloxy)-Boc-D-Ala-(3*S*)-3-(triisopropylsiloxy)-Pro-Arg(Cbz)<sub>2</sub>-(3*R*)-3-(triisopropylsiloxy)-D-Asp(O-cyclohexyl)-Oallyl (**53**)**

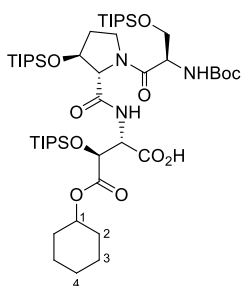


A solution of **52** (143.6 mg, 0.101 mmol) and morpholine (35.2 μL, 0.404 mmol) in THF (2 mL) was treated with Pd(PPh<sub>3</sub>)<sub>4</sub> (3.5 mg, 0.003 mmol) at room temperature for 30 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The catalyst was removed by SH-silica gel column chromatography (φ 1 cm × 4 cm, 1% MeOH/CHCl<sub>3</sub>), and the filtrate was concentrated *in vacuo* to afford a crude carboxylic acid. A mixture of the crude carboxylic acid and Pd black (28 mg) in 50% AcOH/MeOH (2 mL) was vigorously stirred at room temperature for 3.5 h under H<sub>2</sub> atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to afford a crude amine. A suspension of the crude amine and Et<sub>3</sub>N (41.8 μL, 0.30 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was treated with *N,N'*-diBoc-*N''*-triflylguanidine (58.7 mg, 0.15 mmol) at room temperature for 17.5 h. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (1% MeOH/CHCl<sub>3</sub>) to afford **53** (118 mg, 0.074 mmol, 73% over 3 steps) as a white solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 11.47 (br

s, 1H, Arg- $\omega$ -NH), 10.61 (br s, 1H, Arg- $\delta$ -NH), 8.29 (m, 1H, *allo*-D-Thr-NH), 7.91 (br s, 1H, Arg- $\alpha$ -NH), 7.69 (d, 1H, D- $\beta$ -hydroxy-Asp-NH,  $J_{\text{D-}\beta\text{-hydroxy-Asp-NH, D-}\beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 10.1$  Hz), 7.44 (d, 1H, D-Ala-NH,  $J_{\text{D-Ala-NH, D-Ala-}\alpha\text{-CH}} = 8.2$  Hz), 5.22-5.18 (m, 1H, D- $\beta$ -hydroxy-Asp- $\alpha$ -CH), 4.82-4.62 (m, 4H, D- $\beta$ -hydroxy-Asp- $\beta$ -CH, *allo*-D-Thr- $\alpha$ -CH, 3-hydroxy-Pro- $\beta$ -CH, H-1'), 4.58-4.14 (m, 5H, D-Ala- $\alpha$ -CH, *allo*-D-Thr- $\beta$ -CH, Arg- $\alpha$ -CH, 3-hydroxy-Pro- $\alpha$ -CH, C-3-OH), 4.02-3.91 (m, 1H, H-3), 3.79-3.64 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 3.52-3.24 (m, 2H, Arg- $\delta$ -CH), 2.45 (dd, 1H, H-2,  $J_{2,2} = 15.6$ ,  $J_{2,3} = 2.7$  Hz), 2.30 (dd, 1H, H-2,  $J_{2,2} = 15.6$ ,  $J_{2,3} = 9.2$  Hz), 2.13-1.64 (m, 12H, H-4, H-2', 3-hydroxy-Pro- $\gamma$ -CH, Arg- $\beta$ -CH, Arg- $\gamma$ -CH), 1.50 (s, 9H, 'Bu), 1.48 (s, 9H, 'Bu), 1.61-1.20 (m, 39H, *allo*-D-Thr- $\gamma$ -CH, D-Ala- $\beta$ -CH, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-3', H-4'), 1.20-0.95 (m, 63H,  $\text{Pr}_3\text{Si}$ ), 0.85 (d, 6H, H-15,  $J_{15,14} = 6.4$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  173.8, 173.1, 171.4, 170.4, 170.3, 169.1, 163.6, 156.3, 153.3, 132.2, 128.7, 122.3, 118.4, 83.2, 79.4, 74.5, 74.2, 72.4, 70.4, 69.5, 68.7, 59.5, 56.3, 53.0, 47.7, 45.5, 44.1, 40.7, 39.2, 37.7, 33.7, 31.6, 31.4, 30.1, 30.0, 29.9, 29.8, 29.8, 28.4, 28.2, 28.1, 28.0, 27.6, 26.0, 25.6, 25.4, 23.9, 22.8, 19.7, 18.4, 18.2, 18.2, 18.1, 16.1, 13.0, 12.6, 12.5, 12.1; ESIMS-LR  $m/z$  1596.09 [(M+H) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{81}\text{H}_{155}\text{O}_{17}\text{N}_8\text{Si}_3$  1596.0813, found 1596.0860;  $[\alpha]_{\text{D}}^{20} -15.30$  ( $c$  1.55,  $\text{CHCl}_3$ ).

**Boc-D-Ser(O-triisopropylsilyl)-(3S)-3-(triisopropylsiloxy)-Pro-(3S)-3-(triisopropylsiloxy)-Asp(O-cyclohexyl)-OH (57)**

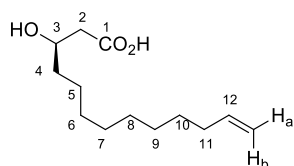


A solution of **45** (87.9 mg, 0.089 mmol) in THF (3 mL) was treated with Dess-Martin periodinane (945 mg, 2.23 mmol) at room temperature for 60 min. The reaction was quenched with *sat. aq.*  $\text{Na}_2\text{S}_2\text{O}_3$ /*sat. aq.*  $\text{NaHCO}_3 = 1/1$ , and the mixture was partitioned between AcOEt and  $\text{H}_2\text{O}$ . The organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to afford a crude aldehyde. A solution of the crude aldehyde,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (41.7 mg, 0.27 mmol) and 2-methylbut-2-ene (50  $\mu\text{L}$ ) in THF/ $\text{tBuOH}/\text{H}_2\text{O}$  (450  $\mu\text{L}/450 \mu\text{L}/100 \mu\text{L}$ ) was treated with a solution of  $\text{NaClO}_2$  (24.1 mg, 0.27 mmol) in  $\text{H}_2\text{O}$

(50  $\mu\text{L}$ ) at room temperature for 50 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-1-2% MeOH/ $\text{CHCl}_3$ ) to afford **57** (52.6 mg, 0.053 mmol, 59% over 2 steps) as a colorless solid.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of several rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  7.18 (d, 1H,  $\beta$ -hydroxy-Asp-NH,  $J_{\beta\text{-hydroxy-Asp-NH, } \beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 6.9$  Hz), 5.55 (d, 1H, D-Ser-NH,  $J_{\text{D-Ser-NH, D-Ser-}\alpha\text{-CH}} = 8.1$  Hz), 4.99-4.90 (m, 2H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $\beta$ -hydroxy-Asp- $\beta$ -CH), 4.78-4.71 (m, 1H, H-1), 4.67-4.58 (m, 2H, D-Ser- $\alpha$ -CH, 3-hydroxy-Pro- $\beta$ -CH), 4.37 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.94 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH, 3-hydroxy-Pro-}\delta\text{-CH}} = 9.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH, 3-hydroxy-Pro-}\gamma\text{-CH}} = 6.3$  Hz), 3.88-3.80 (m, 2H, D-Ser- $\beta$ -CH), 3.77 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH, 3-hydroxy-Pro-}\delta\text{-CH}} = 9.8$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH, 3-hydroxy-Pro-}\gamma\text{-CH}} = 8.6$  Hz), 2.21-2.11 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.98 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH, 3-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH, 3-hydroxy-Pro-}\delta\text{-CH}} = 6.3$  Hz), 1.87-1.63 (m, 4H, H-2), 1.57-1.19 (m, 6H, H-3, H-4), 1.19-0.88 (m, 63H,  $\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  170.9, 169.7, 155.3, 79.6, 74.5, 73.8, 72.3, 63.9, 55.6, 54.4, 45.9, 34.4, 31.5, 31.3, 28.5, 25.3, 23.8, 34.4, 31.5, 31.3, 28.5, 25.3, 23.8, 18.1, 18.0, 12.4, 12.1, 12.0; ESIMS-LR  $m/z$  1022.63 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{50}\text{H}_{97}\text{O}_{11}\text{N}_3\text{NaSi}_3$  1022.6323, found 1022.6338;  $[\alpha]_{\text{D}}^{20} -4.19$  ( $c$  0.43,  $\text{CHCl}_3$ ).

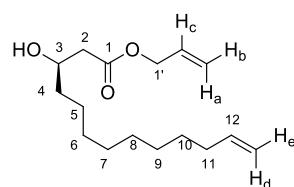
**(R)-3-Hydroxytridec-12-enoic acid (61)**



A mixture of **3** (105 mg, 0.50 mmol) in THF (2 mL) was treated with a solution of NaOH (22 mg, 0.55 mmol) in H<sub>2</sub>O (2 mL) at room temperature for 40 min. The mixture was partitioned between 1 M *aq.* HCl and AcOEt, and the organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was recrystallized from hexane to afford **61** (83 mg, 0.36 mmol, 73%) as a white solid.

mp 55-56 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) 5.81 (dddd, 1H, H-12,  $J_{12, H_b} = 17.4$ ,  $J_{H-12, H_a} = 10.5$ ,  $J_{12, 11} = J_{12, 11} = 6.9$  Hz), 4.99 (d, 1H, H<sub>b</sub>,  $J_{H_b, 12} = 17.4$  Hz), 4.93 (d, 1H, H<sub>a</sub>,  $J_{H_a, 12} = 10.5$  Hz), 4.08-3.98 (m, 1H, H-3), 2.58 (dd, 1H, H-2,  $J_{2, 2} = 16.5$ ,  $J_{2, 3} = 3.2$  Hz), 2.48 (dd, 1H, H-2,  $J_{2, 2} = 16.5$ ,  $J_{2, 3} = 8.7$  Hz), 2.03 (ddd, 2H, H-11,  $J_{11, 10} = J_{11, 10} = J_{11, 12} = 6.9$  Hz), 1.61-1.24 (m, 14H, H-4, H-5, H-6, H-7, H-8, H-9, H-10); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 178.2, 139.3, 114.3, 68.1, 41.2, 36.6, 33.9, 29.6, 29.6, 29.5, 29.2, 29.0, 25.6; ESIMS-LR *m/z* 229.18 [(M+H)<sup>+</sup>]; ESIMS-HR calcd for C<sub>13</sub>H<sub>25</sub>O<sub>3</sub> 229.1798, found 229.1822; [α]<sub>D</sub><sup>20</sup> -14.56 (*c* 0.53, CHCl<sub>3</sub>).

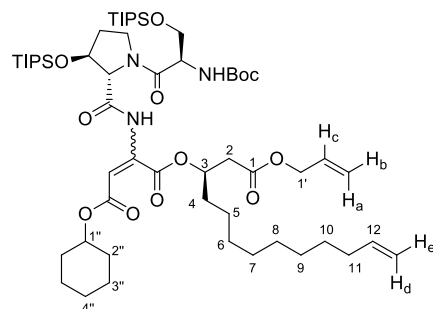
### Allyl (*R*)-3-hydroxytridec-12-enoate (**58**)



A suspension of **61** (114 mg, 0.50 mmol) and Cs<sub>2</sub>CO<sub>3</sub> (195 mg, 0.60 mmol) in DMF (5 mL) was treated with allyl bromide (51.8 μL, 0.60 mmol) at room temperature for 3 h. The mixture was partitioned between AcOEt and H<sub>2</sub>O, and the organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (20% Et<sub>2</sub>O/hexane) to afford **58** (70.5 mg, 0.26 mmol, 53%) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 5.90 (dddd, 1H, H<sub>c</sub>,  $J_{H_c, H_a} = 17.2$ ,  $J_{H_c, H_b} = 10.9$ ,  $J_{H_c, H-1'} = J_{H_c, H-1'} = 5.7$  Hz), 5.79 (dddd, 1H, H-12,  $J_{12, H_d} = 17.4$ ,  $J_{12, H_c} = 10.4$ ,  $J_{12, 11} = J_{12, 11} = 6.3$  Hz), 5.31 (d, 1H, H<sub>a</sub>,  $J_{H_a, H_c} = 17.2$  Hz), 5.24 (d, 1H, H<sub>b</sub>,  $J_{H_b, H_c} = 10.3$  Hz), 4.97 (ddd, 1H, H<sub>d</sub>,  $J_{H_d, 12} = 17.4$ ,  $J_{H_d, 11} = 3.5$ ,  $J_{H_d, 11} = 1.7$  Hz), 4.91 (d, 1H, H<sub>e</sub>,  $J_{H_e, 12} = 10.4$  Hz), 4.60 (d, 2H, H-1',  $J_{1', H_c} = 5.7$  Hz), 4.03-3.96 (m, 1H, H-3), 2.91 (s, 1H, OH), 2.52 (dd, 1H, H-2,  $J_{2, 2} = 16.6$ ,  $J_{2, 3} = 3.5$  Hz), 2.42 (dd, 1H, H-2,  $J_{2, 2} = 16.6$ ,  $J_{2, 3} = 9.2$  Hz), 2.02 (ddd, 2H, H-11,  $J_{11, 12} = J_{11, 10} = 6.3$ ,  $J_{11, 10} = 6.9$  Hz), 1.55-1.47 (m, 2H, H-4), 1.46-1.23 (m, 12H, H-5, H-6, H-7, H-8, H-9, H-10); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 172.8, 139.3, 132.0, 118.7, 114.2, 68.1, 65.4, 41.4, 36.6, 33.9, 29.6, 29.5, 29.2, 29.0, 25.6; ESIMS-LR *m/z* 291.11 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>16</sub>H<sub>29</sub>O<sub>3</sub> 296.2111, found 269.2131; [α]<sub>D</sub><sup>20</sup> -15.40 (*c* 0.50, CHCl<sub>3</sub>).

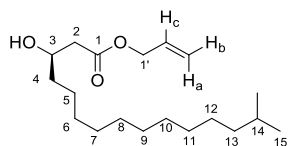
### Cyclohexyl 1-[(allyloxycarbonyl)methyl]undec-10-enyl 2-[Boc-D-Ser(O-triisopropylsilyl)-(3*S*)-3-(triisopropylsiloxy)-Pro]aminobutendioate (**62**)



A solution of **57** (5.0 mg, 0.0050 mmol), **58** (2.0 mg, 0.0075 mmol) and DMAP (0.61 mg, 0.0050 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 μL) was treated with EDCI (1.0 mg, 0.0050 mmol) at room temperature for 130 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (25% Et<sub>2</sub>O/hexane), PTLC (25% Et<sub>2</sub>O/hexane), and flash silica gel column chromatography (φ 0.5 cm×1.5 cm, 25% Et<sub>2</sub>O/hexane) to afford **62** (2.1 mg, 0.0020 mmol, 39%) as a colorless oil.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  5.91 (dddd,  $\text{H}_C$ ,  $J_{\text{H}_C, \text{H}_a} = 17.2$ ,  $J_{\text{H}_C, \text{H}_b} = 10.9$ ,  $J_{\text{H}_C, \text{H-1}'} = J_{\text{H}_C, \text{H-1}''} = 5.8$  Hz), 5.80 (dddd,  $\text{H-12}$ ,  $J_{12, \text{H}_d} = 17.2$ ,  $J_{12, \text{H}_3} = 10.3$ ,  $J_{12, \text{H-11}} = J_{12, \text{H-11}'} = 6.3$  Hz), 5.66 (D-Ser-NH,  $J_{\text{D-Ser-NH}, \text{D-Ser-}\alpha\text{-CH}} = 8.6$  Hz), 5.52 (s, 1H, dehydro-Asp- $\beta$ -CH), 5.38-5.30 (m, 1H, H-3), 5.31 (dd, 1H,  $\text{H}_a$ ,  $J_{\text{H}_a, \text{H}_c} = 17.2$  Hz), 5.24 (d, 1H,  $\text{H}_b$ ,  $J_{\text{H}_b, \text{H}_c} = 10.9$  Hz), 4.99 (dd, 1H,  $\text{H}_d$ ,  $J_{\text{H}_d, 12} = 17.2$  Hz), 4.92 (d, 1H,  $\text{H}_e$ ,  $J_{\text{H}_e, 12} = 10.3$  Hz), 4.88-4.81 (m, 1H, H-1''), 4.72-4.64 (m, 2H, D-Ser- $\alpha$ -CH, 3-hydroxy-Pro- $\beta$ -CH), 4.58 (d, 2H, H-1',  $J_{1', \text{H}_c} = 5.8$  Hz), 4.50 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.98 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{\text{D-Ser-}\beta\text{-CH}, \text{D-Ser-}\alpha\text{-CH}} = 9.8$ ,  $J_{\text{D-Ser-}\beta\text{-CH}, \text{D-Ser-}\alpha\text{-CH}} = 8.0$  Hz), 3.89-3.76 (m, 3H, D-Ser- $\beta$ -CH, 3-hydroxy-Pro- $\delta$ -CH), 2.72 (dd, 1H, H-2,  $J_{2,2} = 15.7$ ,  $J_{2,3} = 6.8$  Hz), 2.59 (dd, 1H, H-2,  $J_{2,2} = 15.7$ ,  $J_{2,3} = 5.7$  Hz), 2.20-2.11 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.07-1.95 (m, 3H, 3-hydroxy-Pro- $\gamma$ -CH, H-11), 1.81 (m, 2H, H-2''), 1.71 (m, 2H, H-2''), 1.50-1.18 (m, 20H, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-3'', H-4''), 1.14-0.94 (m, 42H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  170.1, 155.4, 139.4, 132.1, 118.7, 114.3, 79.6, 73.9, 73.4, 73.4, 73.0, 72.3, 69.9, 65.6, 54.1, 45.5, 39.2, 38.8, 34.4, 34.0, 33.8, 32.1, 31.6, 29.8, 29.6, 29.5, 29.4, 29.2, 29.1, 28.5, 25.4, 25.1, 24.9, 23.7, 22.8, 18.1, 18.1, 14.3, 12.1, 12.0; ESIMS-LR  $m/z$  1098.68 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{57}\text{H}_{101}\text{O}_{12}\text{N}_3\text{NaSi}_2$  1098.6816, found 1098.6824;  $[\alpha]_D^{20} -1.66$  ( $c$  0.18,  $\text{CHCl}_3$ ).

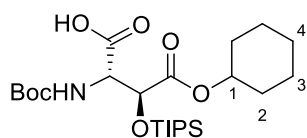
#### Allyl (*R*)-3-hydroxytridec-12-enoate (**64**)



A suspension of **43** (136 mg, 0.50 mmol) and  $\text{Cs}_2\text{CO}_3$  (228 mg, 0.70 mmol) in DMF (5 mL) was treated with allyl bromide (60.5  $\mu\text{L}$ , 0.70 mmol) at room temperature for 10 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and  $\text{H}_2\text{O}$ , and the organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-10-15% AcOEt/hexane) to afford **64** (117 mg, 0.38 mmol, 75%) as a colorless oil.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.89 (dddd, 1H,  $\text{H}_c$ ,  $J_{\text{H}_c, \text{H}_a} = 17.2$ ,  $J_{\text{H}_c, \text{H}_b} = 10.5$ ,  $J_{\text{H}_c, 1'} = J_{\text{H}_c, 1''} = 6.0$  Hz), 5.30 (dd, 1H,  $\text{H}_a$ ,  $J_{\text{H}_a, \text{H}_c} = 17.2$ ,  $J_{\text{H}_a, 1'} = 1.2$  Hz), 5.22 (dd, 1H,  $\text{H}_b$ ,  $J_{\text{H}_b, \text{H}_c} = 10.3$ ,  $J_{\text{H}_b, 1'} = 1.2$  Hz), 4.58 (dd, 2H, H-1',  $J_{1', \text{H}_c} = 5.7$ ,  $J_{1', \text{H}_a} = J_{1', \text{H}_b} = 1.2$ , Hz), 4.03-3.95 (m, 1H, H-3), 2.98 (s, 1H, OH), 2.51 (dd, 1H, H-2,  $J_{2,2} = 16.5$ ,  $J_{2,3} = 3.2$  Hz), 2.41 (dd, 1H, H-2,  $J_{2,2} = 16.5$ ,  $J_{2,3} = 8.7$  Hz), 1.55-1.44 (m, 1H, H-14), 1.44-1.37 (m, 2H, H-4), 1.37-1.08 (18H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 0.83 (d, 6H, H-15,  $J_{15, 14} = 6.9$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.8, 131.9, 118.6, 68.1, 65.4, 41.4, 39.1, 36.6, 30.0, 29.8, 29.7, 29.7, 29.7, 28.0, 27.5, 25.6, 22.8; ESIMS-LR  $m/z$  335.26 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{19}\text{H}_{36}\text{O}_3\text{Na}$  335.2557, found 335.2564;  $[\alpha]_D^{20} -10.09$  ( $c$  0.57,  $\text{CHCl}_3$ ).

#### Boc-(3*S*)-3-(triisopropylsilyloxy)-Asp(O-cyclohexyl)-OH (*ent*-**35**)

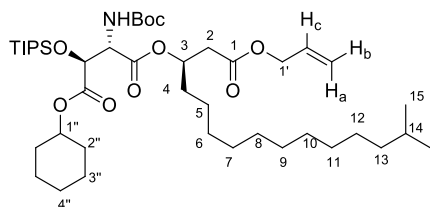


A mixture of **13** (342 mg, 0.67 mmol) in acetone (26 mL) was treated with 2.5 M Jones reagent (910  $\mu\text{L}$ , 2.3 mmol) at 0  $^\circ\text{C}$  for 4.5 h. The reaction was quenched with  $^i\text{PrOH}$  (4.0 mL), and the mixture was partitioned between  $\text{Et}_2\text{O}$  and 1 M *aq.* HCl. The organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ( $\phi$  2  $\times$  4 cm, 2% MeOH/ $\text{CHCl}_3$ ) to afford *ent*-**35** (115 mg, 0.1235 mmol, 35 %, 71% based on 49% conversion) as a yellow oil, and the unreacted starting material (176 mg, 0.34 mmol, 51%) was recovered.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of several rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  5.26 (d, 1H, L- $\beta$ -hydroxy-Asp-NH,  $J_{\text{D-}\beta\text{-hydroxy-Asp-NH}, \text{D-}\beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 9.2$  Hz), 4.95 (br s, 1H, L- $\beta$ -hydroxy-Asp- $\beta$ -CH), 4.83-4.74 (m, 2H, L- $\beta$ -hydroxy-Asp- $\alpha$ -CH, H-1), 1.87-1.81 (m, 2H, H-2), 1.76-1.67 (m, 2H, H-2), 1.55-1.21 (m, 6H, H-3,

H-4), 1.42 (s, 9H, <sup>t</sup>Bu), 1.18-1.01 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 174.8, 169.9, 155.6, 80.5, 74.6, 72.9, 57.3, 31.6, 31.4, 28.3, 25.4, 23.9, 23.8, 18.0, 18.0, 12.8, 12.5; ESIMS-LR *m/z* 510.28 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>24</sub>H<sub>45</sub>O<sub>7</sub>NNaSi 510.2858, found 510.2854; [α]<sub>D</sub><sup>20</sup> -1.20 (*c* 1.77, CHCl<sub>3</sub>).

### Depsiptide 65



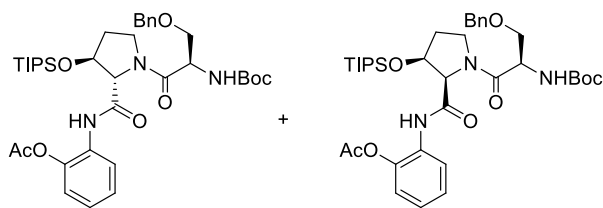
A solution of **64** (23.2 mg, 0.074 mmol), **ent-35** (30.3 mg, 0.062 mmol) and DMAP (7.6 mg, 0.062 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (600 μL) was treated with EDCI (23.8 mg, 0.12 mmol) at -18 °C for 1 min. The mixture was warmed to 0 °C, and stirred for 24 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed

with *sat. aq.* NaHCO<sub>3</sub> and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-3-5% AcOEt/hexane) to afford **65** (37.4 mg, 0.048 mmol, 77%) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 5.90 (dddd, 1H, H<sub>c</sub>, *J*<sub>H<sub>c</sub>, H<sub>a</sub></sub> = 17.2, *J*<sub>H<sub>c</sub>, H<sub>b</sub></sub> = 10.3, *J*<sub>H<sub>c</sub>, 1'</sub> = *J*<sub>H<sub>c</sub>, 1'</sub> = 5.8 Hz), 5.31 (dd, 1H, H<sub>a</sub>, *J*<sub>H<sub>a</sub>, H<sub>c</sub></sub> = 17.2, *J*<sub>H<sub>a</sub>, 1'</sub> = 1.2 Hz), 5.25-5.16 (m, 3H, H<sub>b</sub>, H-3, L-β-hydroxy-Asp-NH), 4.86 (d, 1H, L-β-hydroxy-Asp-β-CH, *J*<sub>L-β-hydroxy-Asp-β-CH, L-β-hydroxy-Asp-α-CH</sub> = 1.8 Hz), 4.82-4.75 (m, 1H, H-1''), 4.69 (dd, L-β-hydroxy-Asp-α-CH, 1H, *J*<sub>L-β-hydroxy-Asp-α-CH, L-β-hydroxy-Asp-NH</sub> = 9.8, *J*<sub>L-β-hydroxy-Asp-α-CH, L-β-hydroxy-Asp-β-CH</sub> = 1.8 Hz), 4.58 (d, 2H, H-1', *J*<sub>1', H<sub>c</sub></sub> = 5.8 Hz), 2.69 (dd, 1H, H-2, *J*<sub>2, 2</sub> = 15.5, *J*<sub>2, 3</sub> = 6.3 Hz), 2.58 (dd, 1H, H-2, *J*<sub>2, 2</sub> = 15.5, *J*<sub>2, 3</sub> = 6.9 Hz), 1.88-1.81 (m, 2H, H-2''), 1.76-1.61 (m, 4H, H-2'', H-3''), 1.56-1.46 (m, 3H, H-4, H-14), 1.41 (s, 9H, <sup>t</sup>Bu), 1.46-1.19 (m, 22H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-3'', H-4''), 1.17-1.01 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si), 0.85 (d, 6H, H-15, *J*<sub>15, 14</sub> = 6.9 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 170.3, 169.9, 169.6, 155.3, 132.1, 118.7, 79.9, 74.4, 43.0, 72.7, 65.5, 57.6, 39.2, 39.0, 33.8, 31.7, 31.4, 30.1, 29.8, 29.8, 29.7, 29.6, 29.6, 28.4, 28.3, 28.1, 27.5, 25.4, 25.1, 24.0, 23.9, 22.8, 18.1, 18.1, 18.0, 12.8, 12.5; ESIMS-LR *m/z* 804.54 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>43</sub>H<sub>79</sub>O<sub>9</sub>NNaSi 804.5416, found 804.5419; [α]<sub>D</sub><sup>20</sup> -7.60 (*c* 0.36, CHCl<sub>3</sub>).

### 2-[Boc-D-Ser(OBn)-(3S)-3-(triisopropylsiloxy)-Pro]aminophenyl acetate (*trans*-84)

### 2-[Boc-D-Ser(OBn)-(3S)-3-(triisopropylsiloxy)-D-Pro]aminophenyl acetate (*cis*-84)



A solution of **4a** (241 mg, 1.00 mmol) and **82** (443 mg, 1.50 mmol) in HFIP (2 mL) was treated with a solution of **83** (242 mg, 1.50 mmol) in HFIP (3 mL) at room temperature for 2 h. The mixture was concentrated *in vacuo*, and the residue was purified by high-flash silica gel column chromatography (0-5-10-40% AcOEt/hexane) to afford

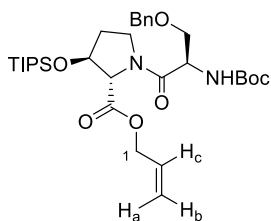
**trans-84** (167 mg, 0.22 mmol, 22%) as a yellow oil and **cis-84** (489 mg, 0.64 mmol, 64%) as a colorless foam.

Data for **trans-84**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 9.44 (s, 1H, Ar-NH), 8.24 (d, 1H, H-6, *J*<sub>6, 5</sub> = 8.1 Hz), 7.38-7.24 (m, 5H, Ph), 7.18 (ddd, 1H, H-5, *J*<sub>5, 6</sub> = 8.1, *J*<sub>5, 4</sub> = 5.7, *J*<sub>5, 3</sub> = 2.9 Hz), 7.10-7.04 (m, 2H, H-3, H-4), 5.23 (d, 1H, D-Ser-NH, *J*<sub>D-Ser-NH, D-Ser-α-CH</sub> = 8.1 Hz), 4.97 (d, 1H, 3-hydroxy-Pro-β-CH, *J*<sub>3-hydroxy-Pro-β-CH, 3-hydroxy-Pro-γ-CH</sub> = 2.9 Hz), 4.75 (ddd, 1H, D-Ser-α-CH, *J*<sub>D-Ser-α-CH, D-Ser-NH</sub> = 8.1, *J*<sub>D-Ser-α-CH, D-Ser-β-CH</sub> = *J*<sub>D-Ser-α-CH, D-Ser-β-CH</sub> = 6.3 Hz), 4.63 (s, 1H, 3-hydroxy-Pro-α-CH), 4.54 (s, 2H, PhCH), 3.89 (ddd, 1H, 3-hydroxy-Pro-δ-CH, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = 10.1, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = 6.9, *J*<sub>3-hydroxy-</sub>

Pro- $\delta$ -CH, 3-hydroxy-Pro- $\gamma$ -CH = 5.8 Hz), 3.83 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 10.1$ ,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 3.67-3.59 (m, 2H, D-Ser- $\beta$ -CH), 2.46 (s, 3H, CH<sub>3</sub>), 2.30-2.21 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.03 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 12.6$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 5.8$  Hz), 1.38 (s, 9H, <sup>t</sup>Bu), 1.13-1.00 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  172.0, 169.3, 167.7, 155.3, 140.1, 137.6, 130.6, 128.6, 127.9, 127.7, 126.3, 124.3, 122.3, 121.9, 80.3, 73.4, 72.5, 69.9, 69.7, 52.3, 45.9, 34.0, 28.3, 21.2, 18.1, 12.1; ESIMS-LR  $m/z$  720.37 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>37</sub>H<sub>55</sub>O<sub>8</sub>N<sub>3</sub>NaSi 720.3651, found 720.3651; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -40.50 (*c* 0.53, CHCl<sub>3</sub>).

Data for **cis-84**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.94 (d, 1H, H-6,  $J_{6,5} = 7.5$  Hz), 7.67 (s, 1H, Ar-NH), 7.31-7.21 (m, 5H, Ph), 7.18-7.05 (m, 3H, H-3, H-4, H-5), 5.31 (d, 1H, D-Ser-NH,  $J_{D\text{-Ser-NH}, D\text{-Ser-}\alpha\text{-CH}} = 8.6$  Hz), 4.72 (m, 2H, 3-hydroxy-Pro- $\beta$ -CH, D-Ser- $\alpha$ -CH), 4.62 (d, 1H, 3-hydroxy-Pro- $\alpha$ -CH,  $J_{3\text{-hydroxy-Pro-}\alpha\text{-CH}, 3\text{-hydroxy-Pro-}\beta\text{-CH}} = 6.9$  Hz), 4.52 (d, 1H, PhCH,  $J_{PhCH, PhCH} = 12.0$  Hz), 4.52 (d, 1H, PhCH,  $J_{PhCH, PhCH} = 12.0$  Hz), 3.97-3.90 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.82-3.72 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.69-3.61 (m, 2H, D-Ser- $\beta$ -CH), 2.34 (s, 3H, CH<sub>3</sub>), 2.37-2.28 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.19-2.11 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.43 (s, 9H, <sup>t</sup>Bu), 1.13-0.94 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  170.4, 168.8, 166.4, 155.4, 141.6, 137.5, 129.6, 128.5, 127.9, 127.8, 127.7, 126.1, 125.0, 123.6, 122.2, 80.1, 73.4, 71.9, 70.4, 65.0, 54.5, 45.3, 33.5, 28.4, 28.1, 21.2, 18.0, 17.9, 12.2; ESIMS-LR  $m/z$  720.37 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>37</sub>H<sub>55</sub>O<sub>8</sub>N<sub>3</sub>NaSi 720.3651, found 720.3655; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +8.98 (*c* 1.07, CHCl<sub>3</sub>).

#### Boc-D-Ser(OBn)-(3*S*)-3-(triisopropylsiloxy)-Pro-Oallyl (**85**)



A solution of **trans-84** (98.6 mg, 0.129 mmol) in MeOH was treated with SmCl<sub>3</sub> · 6H<sub>2</sub>O (94.1 mg, 0.258 mmol) at room temperature for 48 h. The mixture was concentrated *in vacuo*, and the residue was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude phenol. A mixture of the crude phenol in CH<sub>2</sub>Cl<sub>2</sub> (1.3 mL) was treated with 1,1'-

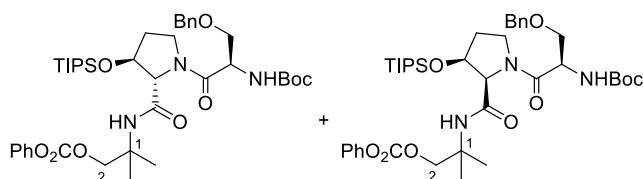
carbonyldiimidazole (105 mg, 0.65 mmol) at room temperature for 40 min. Allyl alcohol (175  $\mu$ L, 2.6 mmol) and DMAP (15.8 mg, 0.129 mmol) was added to the mixture, which was stirred for 250 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (3-13-20% AcOEt/hexane) to afford **85** (58.0 mg, 0.096 mmol, 74% over 2 steps) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  7.34-7.22 (m, 5H, Ph), 5.23 (d, 1H, D-Ser-NH,  $J_{D\text{-Ser-NH}, D\text{-Ser-}\alpha\text{-CH}} = 8.1$  Hz), 5.89 (dddd, 1H, H<sub>c</sub>,  $J_{H_c, H_a} = 17.2$ ,  $J_{H_c, H_b} = 10.9$ ,  $J_{H_c, 1} = J_{H_c, 1} = 5.2$  Hz), 5.40 (d, 1H, D-Ser-NH,  $J_{D\text{-Ser-NH}, D\text{-Ser-}\alpha\text{-CH}} = 8.1$  Hz), 5.35 (dd, 1H, H<sub>a</sub>,  $J_{H_a, H_c} = 17.2$ ,  $J_{H_a, H-1} = 1.8$  Hz), 5.22 (dd, 1H, H<sub>a</sub>,  $J_{H_a, H_c} = 10.9$ ,  $J_{H_a, H-1} = 1.2$  Hz), 4.75 (ddd, 1H, D-Ser- $\alpha$ -CH,  $J_{D\text{-Ser-}\alpha\text{-CH}, D\text{-Ser-NH}} = 8.1$ ,  $J_{D\text{-Ser-}\alpha\text{-CH}, D\text{-Ser-}\beta\text{-CH}} = 5.2$ ,  $J_{D\text{-Ser-}\alpha\text{-CH}, D\text{-Ser-}\beta\text{-CH}} = 6.9$  Hz), 4.68-4.58 (m, 2H, 3-hydroxy-Pro- $\beta$ -CH, H-1), 4.56-4.46 (m, 3H, H-1, PhCH), 4.44 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.92-3.83 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 3.65 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{D\text{-Ser-}\beta\text{-CH}, D\text{-Ser-}\beta\text{-CH}} = 9.2$ ,  $J_{D\text{-Ser-}\beta\text{-CH}, D\text{-Ser-}\alpha\text{-CH}} = 5.2$  Hz), 3.59 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{D\text{-Ser-}\beta\text{-CH}, D\text{-Ser-}\beta\text{-CH}} = 9.2$ ,  $J_{D\text{-Ser-}\beta\text{-CH}, D\text{-Ser-}\alpha\text{-CH}} = 6.9$  Hz), 2.17-2.08 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.98-1.91 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.43 (s, 9H, <sup>t</sup>Bu), 1.15-0.97 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  169.9, 169.7, 155.3, 138.0, 131.7, 128.5, 127.7, 127.7, 118.6, 79.9, 74.3, 73.4, 70.9, 68.9, 66.0, 51.9, 45.5, 34.3, 28.5, 28.4, 18.0, 18.0, 12.1; ESIMS-LR  $m/z$  627.34 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>32</sub>H<sub>52</sub>O<sub>7</sub>N<sub>2</sub>NaSi 627.3436, found 627.3440;

$[\alpha]_D^{20} -0.90$  ( $c$  1.29,  $\text{CHCl}_3$ ).

**Boc-D-Ser(OBn)-(3S)-3-(triisopropylsiloxy)-Pro 2-methyl-1-phenoxy-carbonyloxypropylamide (*trans*-87)**

**Boc-D-Ser(OBn)-(3S)-3-(triisopropylsiloxy)-D-Pro 2-methyl-1-phenoxy-carbonyloxypropylamide (*cis*-87)**



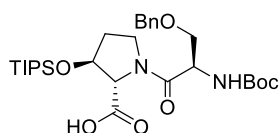
A solution of **4a** (362 mg, 1.5 mmol) and **82** (664 mg, 2.3 mmol) in HFIP (10 mL) was treated with a solution of **86** (493 mg, 2.3 mmol) in HFIP (5 mL) at room temperature for 18 h. The mixture was concentrated *in vacuo*, and the residue was purified by high-flash silica gel column

chromatography (0-5-10-20-35% AcOEt/hexane) to afford *trans*-**87** (575 mg, 0.76 mmol, 51%) as a white foam and *cis*-**87** (421 mg, 0.56 mmol, 37%) as a white foam.

Data for *trans*-**87**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.39-7.16 (m, 10H, Ph), 6.82 (s, 1H, 1-NH), 5.29 (d, 1H, D-Ser-NH,  $J_{\text{D-Ser-NH, D-Ser-}\alpha\text{-CH}} = 6.9$  Hz), 4.75 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH, 3-hydroxy-Pro-}\gamma\text{-CH}} = 2.9$  Hz), 4.62 (ddd, 1H, D-Ser- $\alpha$ -CH,  $J_{\text{D-Ser-}\alpha\text{-CH, D-Ser-NH}} = 6.9$ ,  $J_{\text{D-Ser-}\alpha\text{-CH, D-Ser-}\beta\text{-CH}} = J_{\text{D-Ser-}\alpha\text{-CH, D-Ser-}\beta\text{-CH}} = 6.3$  Hz), 4.51 (s, 2H, Bn), 4.44 (d, 1H, H-2,  $J_{2,2} = 10.3$  Hz), 4.41 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.34 (d, 1H, H-2,  $J_{2,2} = 10.3$  Hz), 3.87-3.73 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 3.62-3.54 (m, 2H, D-Ser- $\beta$ -CH), 2.20-2.11 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.96-1.89 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.41 (s, 9H,  $^t\text{Bu}$ ), 1.38 (s, 3H, 1-Me), 1.36 (s, 3H, 1-Me), 1.11-0.98 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  170.7, 168.9, 155.6, 153.6, 151.2, 129.4, 128.5, 127.9, 127.7, 125.9, 121.0, 79.9, 73.4, 73.3, 72.1, 70.0, 53.1, 52.6, 45.5, 33.6, 28.3, 24.3, 23.8, 17.9, 11.9; ESIMS-LR  $m/z$  778.41 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{40}\text{H}_{61}\text{O}_9\text{N}_3\text{NaSi}$  778.4069, found 778.4061;  $[\alpha]_D^{20} -26.16$  ( $c$  0.57,  $\text{CHCl}_3$ ).

Data for *cis*-**87**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.40-7.16 (m, 10H, Ph), 5.85 (s, 1H, 1-NH), 5.33 (d, 1H, D-Ser-NH,  $J_{\text{D-Ser-NH, D-Ser-}\alpha\text{-CH}} = 8.1$  Hz), 4.68-4.61 (m, 2H, 3-hydroxy-Pro- $\beta$ -CH, D-Ser- $\alpha$ -CH), 4.59 (d, 1H, Bn,  $J_{\text{Bn, Bn}} = 12.3$  Hz), 4.52 (d, 1H, Bn,  $J_{\text{Bn, Bn}} = 12.3$  Hz), 4.41-4.30 (m, 3H, H-2, 3-hydroxy-Pro- $\alpha$ -CH), 3.86-3.80 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.74-3.64 (m, 3H, D-Ser- $\beta$ -CH, 3-hydroxy-Pro- $\delta$ -CH), 2.23-2.15 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.08-2.01 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.41 (s, 9H,  $^t\text{Bu}$ ), 1.40 (s, 3H, 1-Me), 1.38 (s, 3H, 1-Me), 1.15-0.99 (m, 21H,  $^i\text{Pr}_3\text{Si}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  170.8, 170.1, 167.9, 155.4, 154.8, 153.7, 153.6, 151.3, 151.2, 137.9, 137.6, 129.5, 128.5, 128.4, 127.9, 127.7, 127.6, 126.0, 126.0, 121.1, 121.1, 79.8, 79.6, 73.6, 73.5, 73.0, 72.8, 72.5, 71.9, 71.6, 70.5, 65.6, 63.1, 53.7, 53.3, 51.8, 45.2, 43.5, 33.6, 30.4, 28.4, 28.3, 24.1, 23.9, 23.6, 18.1, 18.0, 18.0, 12.5, 12.3, 12.0; ESIMS-LR  $m/z$  778.41 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{40}\text{H}_{61}\text{O}_9\text{N}_3\text{NaSi}$  778.4069, found 778.4063;  $[\alpha]_D^{20} +1.01$  ( $c$  0.92,  $\text{CHCl}_3$ ).

**Boc-D-Ser(OBn)-(3S)-3-(triisopropylsiloxy)-Pro-OH (67)**



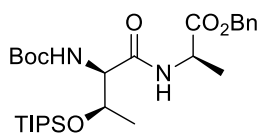
A suspension of *trans*-**87** (55.0 mg, 0.073 mmol) and MS4A (60 mg) in THF (1.5 mL) was treated with 1 M  $^t\text{BuOK}$  in THF (77  $\mu\text{L}$ , 0.077 mmol) at 0 °C for 10 min. 1 M  $^t\text{BuOK}$  in THF (77  $\mu\text{L}$ , 0.077 mmol) was added to the mixture at 0 °C and stirred for 10 min. The reaction was quenched with 1 M *aq.* HCl, and the mixture was partitioned between AcOEt

and 1 M *aq.* HCl. The organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to afford crude oxazolidinone. A solution of the crude oxazolidinone in THF (1.2 mL) was treated with 1 M *aq.* LiOH (99.0  $\mu\text{L}$ , 0.099 mmol) at room temperature for 40 min. The reaction was quenched with 1 M *aq.* HCl, and the mixture

was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-2% MeOH/CHCl<sub>3</sub> + 0.1% AcOH) to afford **67** (19.6 mg, 0.35 mmol, 48% over 2 steps) as a white foam.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 7.36-7.24 (m, 5H, Ph), 5.40 (d, 1H, D-Ser-NH, *J*<sub>D-Ser-NH, D-Ser-α-CH</sub> = 10.3 Hz), 4.82-4.72 (m, 2H, 3-hydroxy-Pro-β-CH, D-Ser-α-CH), 4.51 (s, 2H, Bn), 4.47 (s, 1H, 3-hydroxy-Pro-α-CH), 3.94-3.85 (m, 2H, 3-hydroxy-Pro-δ-CH), 3.68-3.57 (m, 2H, D-Ser-β-CH), 2.19-2.08 (m, 1H, 3-hydroxy-Pro-γ-CH), 2.03-1.94 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.42 (s, 9H, <sup>t</sup>Bu), 1.15-0.95 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.3, 171.6, 155.4, 137.6, 128.6, 127.9, 127.7, 80.4, 73.5, 73.4, 70.1, 69.4, 52.2, 46.1, 34.1, 28.4, 18.0, 12.1; ESIMS-LR *m/z* 563.32 [(M-H)<sup>-</sup>]; ESIMS-HR calcd for C<sub>29</sub>H<sub>47</sub>O<sub>7</sub>N<sub>2</sub>Si 563.3158, found 563.3162; [α]<sup>20</sup><sub>D</sub> -27.39 (*c* 0.33, CHCl<sub>3</sub>).

### Boc-*allo*-D-Thr(O-triisopropylsilyl)-D-Ala-OBn (**89**)



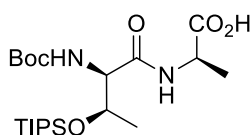
A suspension of **30** (595 mg, 5.0 mmol) in *sat. aq.* NaHCO<sub>3</sub> (7.5 mL) and THF (15 mL) was treated with (Boc)<sub>2</sub>O (2.07 mL, 9.0 mmol) at room temperature for 36 h. The mixture was diluted with H<sub>2</sub>O, and partitioned between hexane and H<sub>2</sub>O, and the aqueous phase was saturated with Na<sub>2</sub>SO<sub>4</sub>. The aqueous phase was acidified with 1 M *aq.* HCl and extracted

with AcOEt. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude carboxylic acid. A solution of the crude carboxylic acid, Boc-D-Ala-OBn·HCl (1.29 g, 6.0 mmol), NaHCO<sub>3</sub> (840 mg, 10.0 mmol) and HOBT·H<sub>2</sub>O (1.35 g, 10.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) was treated with EDCI (1.93 g, 10.0 mmol) at 0 °C for 3.5 h. The mixture was warmed to room temperature, and stirred for 14.5 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with 1 M *aq.* HCl, *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude alcohol. A solution of the crude alcohol and 2,6-lutidine (873 μL, 7.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) was treated with TIPSOTf (1.83 mL, 5.50 mmol) at -78 °C for 15 min. The mixture was warmed to -50 °C, and stirred for 55 min. The reaction was quenched with MeOH (3.0 mL), and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (φ 3 cm × 7 cm, AcOEt) to afford **89** (2.59 g, 4.8 mmol, 97% over 3 steps) as a white solid.

mp 85-86 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 7.39-7.31 (m, 5H, Ph), 6.93 (d, 1H, D-Ala-NH, *J*<sub>D-Ala-NH, D-Ala-α-CH</sub> = 7.5 Hz), 5.17 (s, 2H, PhCH), 5.06 (br s, 1H, *allo*-D-Thr-NH), 4.61 (dq, 1H, D-Ala-α-CH, *J*<sub>D-Ala-α-CH, D-Ala-NH</sub> = *J*<sub>D-Ala-α-CH, D-Ala-β-CH</sub> = 7.5 Hz), 4.38 (dq, 1H, *allo*-D-Thr-β-CH, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-α-CH</sub> = 5.7, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-γ-CH</sub> = 6.3 Hz), 4.11 (dd, 1H, *allo*-D-Thr-α-CH, *J*<sub>*allo*-D-Thr-α-CH, *allo*-D-Thr-β-CH</sub> = *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-NH</sub> = 5.7 Hz), 1.44 (s, 9H, <sup>t</sup>Bu), 1.41 (d, 3H, D-Ala-β-CH, *J*<sub>D-Ala-β-CH, D-Ala-α-CH</sub> = 7.5 Hz), 1.18 (d, 3H, *allo*-D-Thr-γ-CH, *J*<sub>*allo*-D-Thr-γ-CH, *allo*-D-Thr-β-CH</sub> = 6.3 Hz), 1.11-1.02 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.5, 169.6, 135.4, 128.8, 128.6, 128.3, 68.6, 67.3, 48.2, 28.4, 19.4, 18.7, 18.2, 18.1, 12.5; ESIMS-LR *m/z* 559.32 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>28</sub>H<sub>48</sub>O<sub>6</sub>N<sub>2</sub>NaSi 559.3174, found 559.3175; [α]<sup>20</sup><sub>D</sub> -3.78 (*c* 0.56, CHCl<sub>3</sub>).

### Boc-*allo*-D-Thr(O-triisopropylsilyl)-D-Ala-OH (**90**)



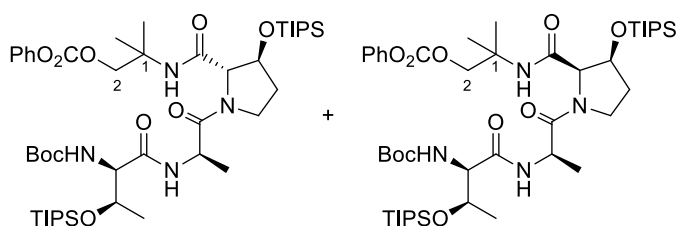


A mixture of **89** (1.88 mg, 3.50 mmol) and 10% Pd/C (188 mg) in AcOEt (30 mL) was vigorously stirred at room temperature for 25 min under H<sub>2</sub> atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to afford **90** (1.56 mg, 3.49 mmol, quant.) as a white solid.

mp 170-172 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 7.07 (d, 1H, D-Ala-NH, *J*<sub>D-Ala-NH, D-Ala-α-CH</sub> = 5.2 Hz), 5.28 (br s, 1H, *allo*-D-Thr-NH), 4.57 (dq, 1H, D-Ala-α-CH, *J*<sub>D-Ala-α-CH, D-Ala-NH</sub> = 5.2, *J*<sub>D-Ala-α-CH, D-Ala-β-CH</sub> = 7.5 Hz), 4.36 (dq, 1H, *allo*-D-Thr-β-CH, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-α-CH</sub> = 4.6, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-γ-CH</sub> = 6.3 Hz), 4.16 (dd, 1H, *allo*-D-Thr-α-CH, *J*<sub>*allo*-D-Thr-α-CH, *allo*-D-Thr-β-CH</sub> = 4.6, *J*<sub>*allo*-D-Thr-β-CH, *allo*-D-Thr-NH</sub> = 6.9 Hz), 1.44 (d, 3H, D-Ala-β-CH, *J*<sub>D-Ala-β-CH, D-Ala-α-CH</sub> = 7.5 Hz), 1.43 (s, 9H, <sup>t</sup>Bu), 1.20 (d, 3H, *allo*-D-Thr-γ-CH, *J*<sub>*allo*-D-Thr-γ-CH, *allo*-D-Thr-β-CH</sub> = 6.3 Hz), 1.09-1.03 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 175.7, 170.1, 68.6, 48.3, 28.3, 18.3, 18.2, 18.1, 12.6; ESIMS-LR *m/z* 469.27 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>21</sub>H<sub>42</sub>O<sub>6</sub>N<sub>2</sub>NaSi 469.2704, found 469.2708; [α]<sub>D</sub><sup>20</sup> -5.67 (*c* 0.52, CHCl<sub>3</sub>).

**Boc-D-*allo*-Thr(O-triisopropylsilyl)-D-Ala-(3S)-3-(triisopropylsiloxy)-Pro 2-methyl-1-phenoxy-carbonyloxy-propylamide (*trans*-91)**

**Boc-D-*allo*-Thr(O-triisopropylsilyl)-D-Ala-(3S)-3-(triisopropylsiloxy)-D-Pro 2-methyl-1-phenoxy-carbonyloxy-propylamide (*cis*-91)**



A solution of **4a** (58.4 mg, 0.24 mmol) and **90** (161 mg, 0.36 mmol) in HFIP (2 mL) was treated with a solution of **86** (79.5 mg, 0.32 mmol) in HFIP (2 mL) at room temperature for 25 h. The mixture was concentrated *in vacuo*, and the residue was purified by high-flash silica gel column chromatography (5-15-

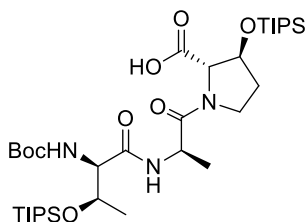
25-35% AcOEt/hexane) to afford **trans**-**91** (106 mg, 0.12 mmol, 49%) as a yellow foam and **cis**-**91** (64.1 mg, 0.071 mmol, 29%) as a white solid.

Data for **trans**-**91**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 7.38 (dd, 2H, *m*-Ph, *J*<sub>*m*-Ph, *o*-Ph</sub> = *J*<sub>*m*-Ph, *p*-Ph</sub> = 7.5 Hz), 7.23 (t, 1H, *p*-Ph, *J*<sub>*p*-Ph, *m*-Ph</sub> = 7.5 Hz), 7.18 (d, 2H, *o*-Ph, *J*<sub>*p*-Ph, *m*-Ph</sub> = 7.5 Hz), 7.14 (br s, 1H, D-Ala-NH), 6.81 (s, 1H, 1-NH), 5.08 (br s, 1H, *allo*-D-Thr-NH), 4.75 (d, 1H, 3-hydroxy-Pro-β-CH, *J*<sub>3-hydroxy-Pro-β-CH, 3-hydroxy-Pro-γ-CH</sub> = 2.3 Hz), 4.67-4.59 (m, 1H, D-Ala-α-CH), 4.45-4.39 (m, 2H, *allo*-D-Thr-β-CH, H-2), 4.38 (s, 1H, 3-hydroxy-Pro-α-CH), 4.34 (d, 1H, H-2, *J*<sub>2,2</sub> = 10.3 Hz), 4.17-4.13 (m, 1H, *allo*-D-Thr-α-CH), 3.80 (dd, 1H, 3-hydroxy-Pro-δ-CH, *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH</sub> = *J*<sub>3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-β-CH</sub> = 8.6 Hz), 3.71-3.63 (m, 1H, 3-hydroxy-Pro-δ-CH), 2.20-2.11 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.96 (dd, 1H, 3-hydroxy-Pro-γ-CH, *J*<sub>3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-δ-CH</sub> = 6.3, *J*<sub>3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-β-CH</sub> = 13.2 Hz), 1.43 (s, 9H, <sup>t</sup>Bu), 1.36 (s, 3H, 1-Me), 1.34 (s, 3H, 1-Me), 1.32 (d, 3H, D-Ala-β-CH, *J*<sub>D-Ala-β-CH, D-Ala-α-CH</sub> = 6.9 Hz), 1.17 (d, 3H, *allo*-D-Thr-γ-CH, *J*<sub>*allo*-D-Thr-γ-CH, *allo*-D-Thr-β-CH</sub> = 6.9 Hz), 1.13-1.00 (m, 42H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.6, 169.9, 168.8, 155.9, 153.7, 151.3, 129.5, 126.1, 121.2, 80.2, 73.4, 72.2, 70.2, 68.5, 60.8, 53.2, 47.3, 45.3, 33.6, 28.4, 24.3, 23.9, 19.3, 18.2, 18.1, 18.0, 17.1, 12.4, 12.0; ESIMS-LR *m/z* 929.55 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>46</sub>H<sub>82</sub>O<sub>10</sub>N<sub>4</sub>NaSi<sub>2</sub> 929.5462, found 929.5451; [α]<sub>D</sub><sup>20</sup> -15.11 (*c* 0.62, CHCl<sub>3</sub>).

Data for **cis**-**91**: mp 100-101 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 7.39 (dd, 2H, *m*-Ph, *J*<sub>*m*-Ph, *o*-Ph</sub> = *J*<sub>*m*-Ph, *p*-Ph</sub> = 7.5 Hz), 7.24 (t, 1H, *p*-Ph, *J*<sub>*p*-Ph, *m*-Ph</sub> = 7.5 Hz),

7.19 (d, 2H, *o*-Ph,  $J_{p-Ph, m-Ph} = 7.5$  Hz), 7.19 (d, 1H, D-Ala-NH,  $J_{D-Ala-NH, D-Ala-\alpha-CH} = 6.9$  Hz), 5.76 (s, 1H, 1-NH), 5.02 (br s, 1H, *allo*-D-Thr-NH), 4.69 (dt, 1H, D-Ala- $\alpha$ -CH,  $J_{D-Ala-\alpha-CH, D-Ala-\beta-CH} = J_{D-Ala-\alpha-CH, D-Ala-NH} = 6.9$  Hz), 4.54 (ddd, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3-hydroxy-Pro-\beta-CH, 3-hydroxy-Pro-\gamma-CH} = 9.2$ ,  $J_{3-hydroxy-Pro-\beta-CH, 3-hydroxy-Pro-\alpha-CH} = J_{3-hydroxy-Pro-\beta-CH, 3-hydroxy-Pro-\gamma-CH} = 9.2$  Hz), 4.41-4.35 (m, 3H, *allo*-D-Thr- $\beta$ -CH, 3-hydroxy-Pro- $\alpha$ -CH, H-2), 4.28 (d, 1H, H-2,  $J_{2, 2} = 10.3$  Hz), 4.15-4.07 (m, 1H, *allo*-D-Thr- $\alpha$ -CH), 3.75-3.69 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.59 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\delta-CH} = J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\gamma-CH} = J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\gamma-CH} = 9.2$  Hz), 2.33-2.24 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.19-2.12 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.43 (s, 9H, <sup>t</sup>Bu), 1.43 (s, 3H, 1-Me), 1.40 (s, 3H, 1-Me), 1.35 (d, 3H, D-Ala- $\beta$ -CH,  $J_{D-Ala-\beta-CH, D-Ala-\alpha-CH} = 6.9$  Hz), 1.18-1.02 (m, 45H, *allo*-D-Thr- $\gamma$ -CH, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  171.5, 169.3, 168.0, 156.1, 153.7, 151.3, 129.6, 126.1, 121.1, 80.3, 73.0, 71.6, 68.6, 63.5, 61.1, 53.5, 48.2, 46.1, 44.5, 32.5, 28.4, 24.1, 23.8, 19.3, 18.5, 18.2, 18.1, 18.1, 12.5, 12.3; ESIMS-LR  $m/z$  929.55 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>46</sub>H<sub>82</sub>O<sub>10</sub>N<sub>4</sub>NaSi<sub>2</sub> 929.5462, found 929.5452; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +5.32 (*c* 0.82, CHCl<sub>3</sub>).

### Boc-D-*allo*-Thr(O-triisopropylsilyl)-D-Ala-(3*S*)-3-(triisopropylsiloxy)-Pro-OH (69)

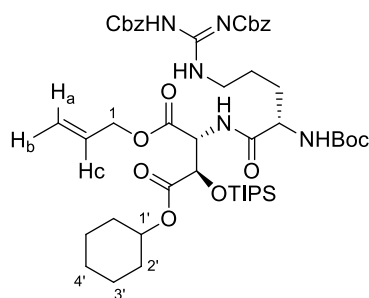


A suspension of **trans-91** (34.6 mg, 0.038 mmol) and MS4A (40 mg) in THF (400  $\mu$ L) was treated with 1 M <sup>t</sup>BuOK in THF (80  $\mu$ L, 0.080 mmol) at 0 °C for 25 min. The reaction was quenched with 1 M *aq.* HCl, and the mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford crude oxazolidinone. A solution of the crude oxazolidinone in THF (400  $\mu$ L) was treated with 1 M *aq.* LiOH (68.0  $\mu$ L, 0.068

mmol) at room temperature for 1 h. The reaction was quenched with 1 M *aq.* HCl, and the mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-2% MeOH/CHCl<sub>3</sub> + 0.1% AcOH) to afford **69** (19.5 mg, 0.027 mmol, 72% over 2 steps) as a white foam.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer)  $\delta$  7.18(d, 1H, D-Ala-NH,  $J_{D-Ala-NH, D-Ala-\alpha-CH} = 8.1$  Hz), 5.23 (d, 1H, *allo*-D-Thr-NH,  $J_{allo-D-Thr-NH, allo-D-Thr-\alpha-CH} = 6.9$  Hz), 4.84 (dq, 1H, D-Ala- $\alpha$ -CH,  $J_{D-Ala-\alpha-CH, D-Ala-NH} = 8.1$ ,  $J_{D-Ala-\alpha-CH, D-Ala-\beta-CH} = 6.9$  Hz), 4.78 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3-hydroxy-Pro-\beta-CH, 3-hydroxy-Pro-\gamma-CH} = 2.3$  Hz), 4.41 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.39-4.32 (m, 1H, *allo*-D-Thr- $\beta$ -CH), 4.14-4.08 (m, 1H, *allo*-D-Thr- $\alpha$ -CH), 3.82 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\delta-CH} = J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\gamma-CH} = 9.2$  Hz), 3.76-3.69 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 2.19-2.10 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.02 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3-hydroxy-Pro-\gamma-CH, 3-hydroxy-Pro-\delta-CH} = 5.7$ ,  $J_{3-hydroxy-Pro-\gamma-CH, 3-hydroxy-Pro-\gamma-CH} = 12.6$  Hz), 1.42 (s, 9H, <sup>t</sup>Bu), 1.34 (d, 3H, D-Ala- $\beta$ -CH,  $J_{D-Ala-\beta-CH, D-Ala-\alpha-CH} = 6.9$  Hz), 1.19 (d, 3H, *allo*-D-Thr- $\gamma$ -CH,  $J_{allo-D-Thr-\gamma-CH, allo-D-Thr-\beta-CH} = 6.3$  Hz), 1.14-1.01 (m, 42H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  173.8, 170.6, 169.5, 156.1, 73.1, 69.5, 68.7, 46.9, 45.7, 34.0, 28.4, 19.6, 18.2, 18.1, 18.0, 17.9, 12.5, 12.1; ESIMS-LR  $m/z$  714.46 [(M-H)<sup>-</sup>]; ESIMS-HR calcd for C<sub>35</sub>H<sub>68</sub>O<sub>8</sub>N<sub>3</sub>Si<sub>2</sub> 714.4550, found 714.4559; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -10.9 (*c* 1.10, CHCl<sub>3</sub>).

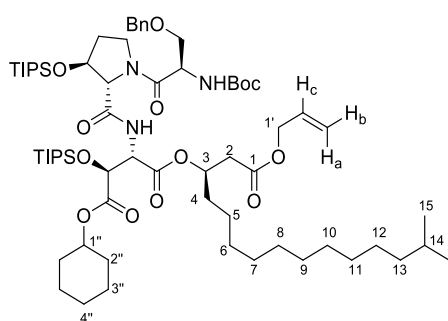
### Boc-Arg(Cbz)<sub>2</sub>-(3*R*)-3-(triisopropylsiloxy)-Asp(O-cyclohexyl)-Oallyl (93)



A solution of **36** (65.8 mg, 0.13 mmol) was treated with 25% TFA/CH<sub>2</sub>Cl<sub>2</sub> (2 mL) at room temperature for 20 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford crude amine. A mixture of the crude amine, Boc-Arg(Cbz)<sub>2</sub>-OH (81.2 mg, 0.15 mmol), <sup>i</sup>Pr<sub>2</sub>NEt (43.5 μL, 0.25 mmol) and HOAt (34.0 mg, 0.25 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.3 mL) was treated with EDCI (47.9 mg, 0.25 mmol) at room temperature for 4.5 h. The mixture was concentrated *in vacuo*, and the residue was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with 1 M *aq.* HCl, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-15-35% AcOEt/hexane) to afford **93** (82.2 mg, 0.086 mmol, 69% over 2 steps) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 9.44 (br s, 1H, Arg-ω-NH), 9.23 (br s, 1H, Arg-δ-NH), 7.41-7.24 (m, 10H, Ph), 6.91 (d, 1H, D-β-hydroxy-Asp-NH, *J*<sub>D-β-hydroxy-Asp-NH, D-β-hydroxy-Asp-α-CH</sub> = 9.2 Hz), 5.87 (dddd, 1H, H<sub>c</sub>, *J*<sub>H<sub>c</sub>, H<sub>a</sub></sub> = 17.2, *J*<sub>H<sub>c</sub>, H<sub>b</sub></sub> = 10.3, *J*<sub>H<sub>c</sub>, H-1</sub> = *J*<sub>H<sub>c</sub>, 1</sub> = 6.3 Hz), 5.31 (dd, 1H, H<sub>a</sub>, *J*<sub>H<sub>a</sub>, H<sub>c</sub></sub> = 17.2, *J*<sub>H<sub>a</sub>, 1</sub> = 1.2 Hz), 5.27-5.19 (m, 3H, Bn, H<sub>b</sub>), 5.14 (s, 2H, Bn), 5.04-4.98 (m, 2H, D-β-hydroxy-Asp-α-CH, Arg-α-NH), 4.93 (d, 1H, D-β-hydroxy-Asp-β-CH, *J*<sub>D-β-hydroxy-Asp-β-CH, D-β-hydroxy-Asp-α-CH</sub> = 2.3 Hz), 4.75-4.69 (m, 1H, H-1'), 4.61 (dd, 1H, H-1, *J*<sub>1, 1'</sub> = 13.2, *J*<sub>1, H<sub>c</sub></sub> = 6.3 Hz), 4.54 (dd, 1H, H-1, *J*<sub>1, 1'</sub> = 13.2, *J*<sub>1, H<sub>c</sub></sub> = 6.3 Hz), 4.20-4.13 (m, 1H, Arg-α-CH), 3.97 (dd, 2H, Arg-δ-CH, *J*<sub>Arg-δ-CH, Arg-γ-CH</sub> = 8.1, *J*<sub>Arg-δ-CH, Arg-γ-CH</sub> = 6.3 Hz), 1.85-1.76 (m, 3H, Arg-β-CH, H-2'), 1.72-1.62 (m, 4H, Arg-γ-CH, H-2'), 1.58-1.15 (m, 7H, Arg-β-CH, H-3', H-4'), 1.41 (s, 9H, <sup>t</sup>Bu), 1.15-0.99 (m, 21H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.0, 169.6, 168.8, 163.9, 160.6, 155.9, 155.6, 137.1, 134.8, 131.4, 128.9, 128.4, 127.8, 127.8, 119.3, 80.0, 74.4, 72.6, 68.9, 67.0, 66.6, 55.9, 54.2, 44.4, 31.6, 31.3, 29.3, 28.3, 25.3, 25.2, 23.8, 23.8, 18.0, 17.9, 12.5; ESIMS-LR *m/z* 974.49 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>49</sub>H<sub>73</sub>O<sub>12</sub>N<sub>5</sub>NaSi 974.4917, found 974.4919; [α]<sub>D</sub><sup>20</sup> -5.84 (*c* 0.41, CHCl<sub>3</sub>).

#### Depsiptide **94**

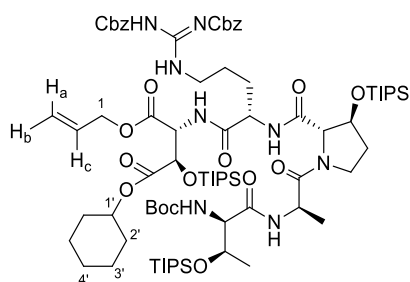


Depsiptide **65** (31.2 mg, 0.040 mmol) was treated with 25% TFA/CH<sub>2</sub>Cl<sub>2</sub> (500 μL) at room temperature for 1 h. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between Et<sub>2</sub>O and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford crude amine **66**. This compound was directly used to the next reaction without further purification. A mixture of the crude amine **66**, **67** (20.9 mg, 0.037 mmol), <sup>i</sup>Pr<sub>2</sub>EtN (12.9 μL, 0.074 mmol) and HOAt (10.1 mg, 0.078 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (400 μL) was treated with EDCI (14.1 mg, 0.074 mmol) at room temperature for 1 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with 1 M *aq.* HCl, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (3-10-15-20% AcOEt/hexane) to afford **94** (38.2 mg, 0.031 mmol, 84% over 2 steps) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 7.37-7.22

(m, 6H, Ph, L-β-hydroxy-Asp-NH), 5.91 (dddd, 1H, H<sub>c</sub>,  $J_{H_c, H_a} = 17.2$ ,  $J_{H_c, H_b} = 10.3$ ,  $J_{H_c, H-1'} = J_{H_c, 1'} = 5.8$  Hz), 5.60 (d, 1H, D-Ser-NH,  $J_{D-Ser-NH, D-Ser-α-CH} = 8.6$  Hz), 5.31 (dd, 1H, H<sub>a</sub>,  $J_{H_a, H_c} = 17.2$ ,  $J_{H_a, 1'} = 1.8$  Hz), 5.23 (dd, 1H, H<sub>b</sub>,  $J_{H_b, H_c} = 10.3$ ,  $J_{H_b, 1'} = 1.2$  Hz), 5.25-5.15 (m, 1H, H-3), 4.95 (dd, L-β-hydroxy-Asp-α-CH, 1H,  $J_{L-β-hydroxy-Asp-α-CH, L-β-hydroxy-Asp-NH} = 9.2$ ,  $J_{L-β-hydroxy-Asp-α-CH, L-β-hydroxy-Asp-β-CH} = 1.8$  Hz), 4.78-4.69 (m, 4H, L-β-hydroxy-Asp-β-CH, 3-hydroxy-Pro-β-CH, D-Ser-α-CH, H-1''), 4.59 (d, 2H, H-1',  $J_{1', H_c} = 5.8$  Hz), 4.56 (d, 1H, Bn,  $J_{Bn, Bn} = 12.6$  Hz), 4.49 (d, 1H, Bn,  $J_{Bn, Bn} = 12.6$  Hz), 4.47 (s, 1H, 3-hydroxy-Pro-α-CH), 3.91-3.84 (m, 1H, 3-hydroxy-Pro-δ-CH), 3.72-3.60 (m, 3H, 3-hydroxy-Pro-δ-CH, D-Ser-β-CH), 2.69 (dd, 1H, H-2,  $J_{2, 2} = 15.5$ ,  $J_{2, 3} = 5.7$  Hz), 2.57 (dd, 1H, H-2,  $J_{2, 2} = 15.5$ ,  $J_{2, 3} = 7.4$  Hz), 2.18-2.09 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.93 (dd, 1H, 3-hydroxy-Pro-γ-CH,  $J_{3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-γ-CH} = 12.6$ ,  $J_{3-hydroxy-Pro-γ-CH, 3-hydroxy-Pro-δ-CH} = 6.3$  Hz), 1.85-1.61 (m, 6H, H-4, H-2''), 1.55-1.47 (m, 1H, H-14), 1.46-1.21 (m, 24H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-3'', H-4''), 1.43 (s, 9H, <sup>t</sup>Bu), 1.17-0.99 (m, 42H, <sup>i</sup>Pr<sub>3</sub>Si), 0.86 (d, 6H, H-15,  $J_{15, 14} = 6.3$  Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 170.3, 169.9, 169.6, 155.3, 132.1, 118.7, 79.9, 74.4, 73.0, 72.7, 65.6, 57.6, 39.2, 38.8, 33.8, 31.7, 31.4, 30.1, 29.8, 29.8, 29.7, 29.6, 29.6, 28.4, 28.3, 28.1, 27.5, 25.4, 25.1, 24.0, 23.9, 22.8, 18.1, 18.1, 18.0, 12.8, 12.5; ESIMS-LR *m/z* 1250.80 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>67</sub>H<sub>117</sub>O<sub>13</sub>N<sub>3</sub>NaSi<sub>2</sub> 1250.8017, found 1250.7998; [α]<sub>D</sub><sup>20</sup> -16.58 (*c* 0.22, CHCl<sub>3</sub>).

**Boc-D-*allo*-Thr(O-triisopropylsilyl)-D-Ala-(3*S*)-3-(triisopropylsiloxy)-Pro-Arg(Cbz)<sub>2</sub>-(3*R*)-3-(triisopropylsiloxy)-Asp(O-cyclohexyl)-Oallyl (96)**



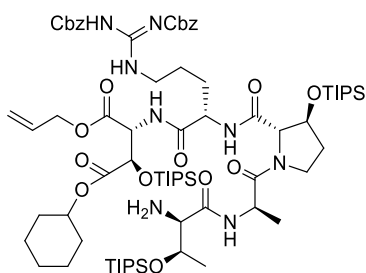
Dipeptide **93** (44.8 mg, 0.047 mmol) was treated with 25% TFA/CH<sub>2</sub>Cl<sub>2</sub> (500 μL) at room temperature for 30 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between Et<sub>2</sub>O and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford crude amine **68**. This compound was directly used to the next reaction without further purification. A mixture of the crude amine **68**, **69** (28.6 mg, 0.040 mmol), <sup>i</sup>Pr<sub>2</sub>N<sup>t</sup>Et (13.9 μL, 0.080

mmol) and HOAt (10.9 mg, 0.080 mmol) in THF (400 μL) was treated with EDCI (15.3 mg, 0.080 mmol) at room temperature for 11 h. The mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with 1 M *aq.* HCl, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (3-15-35% AcOEt/hexane) to afford **96** (55.9 mg, 0.036 mmol, 90% over 2 steps) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer) δ 9.45 (br s, 1H, Arg-ω-NH), 9.22 (br s, 1H, Arg-δ-NH), 7.42-7.24 (m, 11H, Ph, D-Ala-NH), 7.20 (d, 1H, D-β-hydroxy-Asp-NH,  $J_{D-β-hydroxy-Asp-NH, D-β-hydroxy-Asp-α-CH} = 8.6$  Hz), 7.10-7.04 (m, 1H, Arg-α-NH), 5.93 (dddd, 1H, H<sub>c</sub>,  $J_{H_c, H_a} = 17.2$ ,  $J_{H_c, H_b} = 10.9$ ,  $J_{H_c, 1} = J_{H_c, 1} = 5.8$  Hz), 5.44 (br s, 1H, *allo*-D-Thr-NH), 5.33 (dd, 1H, H<sub>a</sub>,  $J_{H_a, H_c} = 17.2$ ,  $J_{H_a, 1} = 1.2$  Hz), 5.29-5.22 (m, 3H, Bn, H<sub>b</sub>), 5.13 (s, 2H, Bn), 5.06 (dd, 1H, D-β-hydroxy-Asp-α-CH,  $J_{D-β-hydroxy-Asp-α-CH, D-β-hydroxy-Asp-β-CH} = 1.8$ ,  $J_{D-β-hydroxy-Asp-α-CH, D-β-hydroxy-Asp-NH} = 8.6$  Hz), 4.90 (d, 1H, D-β-hydroxy-Asp-β-CH,  $J_{D-β-hydroxy-Asp-β-CH, D-β-hydroxy-Asp-α-CH} = 1.8$  Hz), 4.75-4.63 (m, 3H, 3-hydroxy-Pro-β-CH, H-1, H-1'), 4.58-4.48 (m, 2H, D-Ala-α-CH, H-1), 4.37-4.29 (m, 3H, 3-hydroxy-Pro-α-CH, Arg-α-CH, *allo*-D-Thr-β-CH), 4.18-4.11 (m, 1H, *allo*-D-Thr-β-CH), 4.07-3.96 (m, 1H, Arg-δ-CH), 3.95-3.87 (m, 1H, Arg-δ-CH), 3.68 (dd, 1H, 3-hydroxy-Pro-δ-CH,  $J_{3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-δ-CH} = J_{3-hydroxy-Pro-δ-CH, 3-hydroxy-Pro-γ-CH} = 8.6$  Hz), 3.52-3.43 (m, 1H, 3-hydroxy-Pro-δ-CH), 2.13-2.02 (m, 1H, 3-hydroxy-Pro-γ-

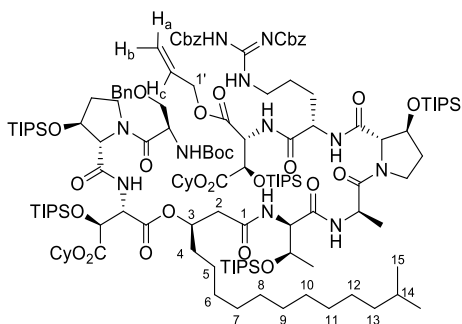
CH), 1.90-1.77 (m, 4H, Arg- $\beta$ -CH, 3-hydroxy-Pro- $\gamma$ -CH, H-2'), 1.72-1.54 (m, 5H, Arg- $\beta$ -CH, Arg- $\gamma$ -CH, H-2'), 1.54-1.23 (m, 6H, H-3', H-4'), 1.38 (s, 9H, <sup>t</sup>Bu), 1.28 (d, 3H, D-Ala- $\beta$ -CH,  $J_{D-Ala-\beta-CH, D-Ala-\alpha-CH} = 6.9$  Hz), 1.18 (d, 3H, *allo*-D-Thr- $\gamma$ -CH,  $J_{allo-D-Thr-\gamma-CH, allo-D-Thr-\beta-CH} = 6.3$  Hz), 1.14-0.99 (m, 63H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  172.2, 172.0, 171.0, 169.9, 169.8, 169.7, 169.1, 169.0, 168.9, 168.8, 164.1, 163.9, 160.8, 160.7, 156.0, 156.0, 155.9, 137.2, 137.0, 135.1, 134.9, 131.7, 131.4, 128.9, 128.8, 128.7, 128.5, 128.4, 128.1, 127.9, 127.8, 119.1, 79.9, 74.3, 73.7, 73.0, 72.7, 69.9, 68.9, 67.0, 66.6, 60.6, 55.9, 54.2, 52.8, 52.5, 47.4, 45.1, 44.5, 33.7, 31.6, 31.6, 31.4, 28.3, 28.0, 25.4, 25.3, 25.2, 23.9, 23.8, 19.7, 18.2, 18.1, 18.0, 18.0, 17.4, 12.5, 12.4, 12.1; ESIMS-LR  $m/z$  1572.89 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>79</sub>H<sub>132</sub>O<sub>17</sub>N<sub>8</sub>NaSi<sub>3</sub> 1571.8910, found 1571.8886;  $[\alpha]_D^{20} -13.48$  ( $c$  0.28, CHCl<sub>3</sub>).

**H-D-*allo*-Thr(O-triisopropylsilyl)-D-Ala-(3S)-3-(triisopropylsiloxy)-Pro-Arg(Cbz)<sub>2</sub>-(3R)-3-(triisopropylsiloxy)-Asp(O-cyclohexyl)-Oallyl (97)**



Pentapeptide **96** (46.5 mg, 0.030 mmol) was treated with 25% TFA/CH<sub>2</sub>Cl<sub>2</sub> (500  $\mu$ L) at room temperature for 1.5 h. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between Et<sub>2</sub>O and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford crude amine **97**. This compound was directly used to the next reaction without further purification.

**Liner depsipeptide 98**



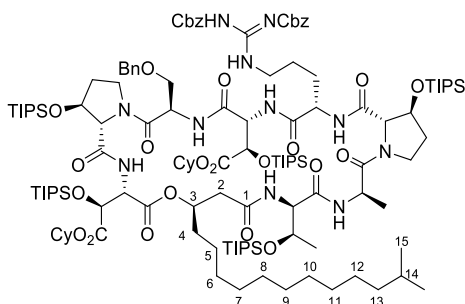
A solution of **94** (34.9 mg, 0.028 mmol) and morpholine (9.8  $\mu$ L, 0.14 mmol) in THF (400  $\mu$ L) was treated with Pd(PPh<sub>3</sub>)<sub>4</sub> (1.6 mg, 0.0014 mmol) at room temperature for 20 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The catalyst was removed by SH-silica gel column chromatography ( $\phi$  2 cm  $\times$  2 cm, 2% MeOH/CHCl<sub>3</sub>), and the filtrate was concentrated *in vacuo* to afford a crude carboxylic acid **95**. A mixture of the crude carboxylic acid **95**, **97**

(0.030 mmol), <sup>i</sup>Pr<sub>2</sub>NEt (9.9  $\mu$ L, 0.057 mmol) and HOAt (7.7 mg, 0.057 mmol) in THF (300  $\mu$ L) was treated with EDCI (10.9 mg, 0.057 mmol) at room temperature for 15 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-10-20-35% AcOEt/hexane) to afford **98** (47.8 mg, 0.018 mmol, 64% over 2 steps) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer)  $\delta$  9.44 (br s, 1H, Arg- $\omega$ -NH), 9.22 (br s, 1H, Arg- $\delta$ -NH), 7.41-7.21 (m, 11H, Ph,  $\beta$ -hydroxy-Asp-NH), 7.10 (d, 1H,  $\beta$ -hydroxy-Asp-NH,  $J_{\beta\text{-hydroxy-Asp-NH}, \beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 9.2$  Hz), 7.04 (d, 1H, D-Ala-NH,  $J_{D-Ala-NH, D-Ala-\alpha-CH} = 6.3$  Hz), 6.89 (d, 1H, Arg- $\alpha$ -NH,  $J_{Arg-\alpha-NH, Arg-\alpha-CH} = 8.1$  Hz), 6.55 (d, 1H, *allo*-D-Thr-NH,  $J_{allo-D-Thr-NH, allo-D-Thr-\alpha-CH} = 8.6$  Hz), 5.92 (dddd, 1H, H<sub>c</sub>,  $J_{H_c, H_a} = 17.2$ ,  $J_{H_c, H_b} = 10.3$ ,  $J_{H_c, H_c'} = J_{H_c, H_c''} = 6.3$  Hz), 5.67 (d, 1H, D-Ser-NH,  $J_{D-Ser-NH, D-Ser-\alpha-CH} = 8.6$  Hz), 5.31 (d, 1H, H<sub>a</sub>,  $J_{H_a, H_c} = 17.2$ ), 5.28-5.21 (m, 3H, Bn, H<sub>b</sub>), 5.18-5.09 (m, 1H, H-3), 5.13 (s, 2H, Bn), 5.02 (d, 1H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-NH}} = 9.2$  Hz), 4.96-4.86 (m, 3H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $\beta$ -hydroxy-

Asp- $\beta$ -CH), 4.78-4.63 (m, 7H, 3-hydroxy-Pro- $\beta$ -CH, D-Ser- $\alpha$ -CH, Cy-1, H-1'), 4.59-4.41 (m, 6H, D-Ala- $\alpha$ -CH, *allo*-D-Thr- $\alpha$ -CH, 3-hydroxy-Pro- $\alpha$ -CH, Bn), 4.35 (ddd, 1H, Arg- $\alpha$ -CH,  $J_{\text{Arg-}\alpha\text{-CH, Arg-}\alpha\text{-NH}} = 8.1$ ,  $J_{\text{Arg-}\alpha\text{-CH, Arg-}\beta\text{-CH}} = J_{\text{Arg-}\alpha\text{-CH, Arg-}\beta\text{-CH}} = 7.5$  Hz), 4.29 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.23 (qd, 1H, *allo*-D-Thr- $\beta$ -CH,  $J_{\text{allo-D-Thr-}\beta\text{-CH, allo-D-Thr-}\alpha\text{-CH}} = J_{\text{allo-D-Thr-}\beta\text{-CH, allo-D-Thr-}\gamma\text{-CH}} = 6.3$  Hz), 4.04-3.97 (m, 1H, Arg- $\delta$ -CH), 3.97-3.83 (m, 2H, Arg- $\delta$ -CH, 3-hydroxy-Pro- $\delta$ -CH), 3.73-3.61 (m, 3H, 3-hydroxy-Pro- $\delta$ -CH, D-Ser- $\beta$ -CH), 3.57 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{\text{3-hydroxy-Pro-}\delta\text{-CH, 3-hydroxy-Pro-}\delta\text{-CH}} = J_{\text{3-hydroxy-Pro-}\delta\text{-CH, 3-hydroxy-Pro-}\gamma\text{-CH}} = 8.6$  Hz), 3.51-3.44 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 2.67 (dd, 1H, H-2,  $J_{\text{H-2, H-2}} = 14.4$ ,  $J_{\text{H-2, H-3}} = 4.0$  Hz), 2.39 (dd, 1H, H-2,  $J_{\text{H-2, H-2}} = 14.4$ ,  $J_{\text{H-2, H-3}} = 8.6$  Hz), 2.20-2.08 (m, 3H, 3-hydroxy-Pro- $\gamma$ -CH), 1.92 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{\text{3-hydroxy-Pro-}\gamma\text{-CH, 3-hydroxy-Pro-}\gamma\text{-CH}} = 12.6$ ,  $J_{\text{3-hydroxy-Pro-}\gamma\text{-CH, 3-hydroxy-Pro-}\delta\text{-CH}} = 6.3$  Hz), 1.88-1.55 (m, 14H, Arg- $\beta$ -CH, Arg- $\gamma$ -CH, H-4, Cy-2), 1.55-1.46 (m, 1H, H-14), 1.46-1.18 (m, 36H, *allo*-D-Thr- $\gamma$ -CH, D-Ala- $\beta$ -CH, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, Cy-3, Cy-4), 1.43 (s, 9H, <sup>t</sup>Bu), 1.18-0.94 (m, 105H, <sup>1</sup>Pr<sub>3</sub>Si), 0.86 (d, 6H, H-15,  $J_{\text{H-15, H-14}} = 6.3$  Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  172.2, 171.0, 170.7, 170.0, 169.9, 169.6, 169.3, 169.1, 168.9, 168.8, 168.6, 164.1, 160.7, 156.1, 155.3, 138.2, 137.2, 135.1, 131.7, 129.0, 128.9, 128.8, 128.5, 128.4, 128.4, 128.4, 128.1, 128.0, 127.9, 127.6, 119.1, 79.5, 74.3, 74.3, 73.8, 73.6, 73.4, 73.2, 72.9, 72.7, 72.6, 70.7, 70.0, 69.7, 69.5, 69.0, 67.1, 66.7, 59.2, 55.9, 52.7, 52.0, 47.8, 45.5, 45.1, 44.6, 40.7, 39.2, 33.9, 33.8, 33.7, 31.7, 31.4, 31.3, 30.1, 29.9, 29.8, 29.8, 28.5, 28.3, 28.1, 27.6, 25.3, 23.9, 22.8, 20.6, 18.2, 18.2, 18.1, 18.0, 17.6, 12.7, 12.7, 12.6, 12.3, 12.1; ESIMS-LR  $m/z$  2643.61 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>138</sub>H<sub>235</sub>O<sub>27</sub>N<sub>11</sub>NaSi<sub>5</sub> 2641.6092, found 2641.6105; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -12.33 (c 0.17, CHCl<sub>3</sub>).

### Fully protected plusbacin A<sub>3</sub> 99



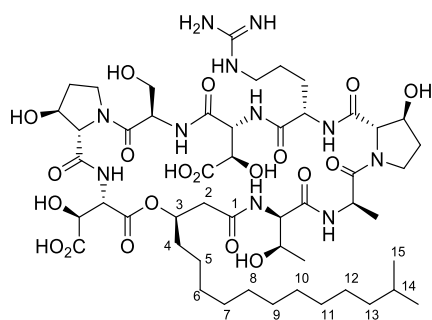
A solution of **98** (32.6 mg, 0.012 mmol) and morpholine (4.3  $\mu$ L, 0.05 mmol) in THF (300  $\mu$ L) was treated with Pd(PPh<sub>3</sub>)<sub>4</sub> (1.4 mg, 0.0012 mmol) at room temperature for 20 min. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The catalyst was removed by SH-silica gel column chromatography ( $\phi$  2 cm  $\times$  2 cm, 2% MeOH/CHCl<sub>3</sub>), and the filtrate was

concentrated *in vacuo* to afford crude carboxylic acid. The carboxylic acid was treated with 25% TFA/CH<sub>2</sub>Cl<sub>2</sub> (300  $\mu$ L) at room temperature for 15 min. The mixture was concentrated *in vacuo* to afford crude amino acid. A mixture of the crude amino acid, <sup>t</sup>Pr<sub>2</sub>NEt (21.6  $\mu$ L, 0.12 mmol) and HOAt (16.9 mg, 0.12 mmol) in THF (12.5 mL) was treated with EDCI (23.8 mg, 0.12 mmol) at room temperature for 13 h. The mixture was concentrated *in vacuo*, and the residue was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-10-25% AcOEt/hexane) to afford **99** (18.3 mg, 0.0074 mmol, 60% over 3 steps) as a pale yellow oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer)  $\delta$  9.43 (br s, 1H, Arg- $\omega$ -NH), 9.24 (br s, 1H, Arg- $\delta$ -NH), 8.09 (d, 1H, D-Ser-NH,  $J_{\text{D-Ser-NH, D-Ser-}\alpha\text{-CH}} = 6.3$  Hz), 7.76 (d, 1H, Arg- $\alpha$ -NH,  $J_{\text{Arg-}\alpha\text{-NH, Arg-}\alpha\text{-CH}} = 8.6$  Hz), 7.61 (d, 1H, D-Ala-NH,  $J_{\text{D-Ala-NH, D-Ala-}\alpha\text{-CH}} = 6.9$  Hz), 7.54-7.19 (m, 11H, Ph, *allo*-D-Thr-NH), 7.00 (d, 1H,  $\beta$ -hydroxy-Asp-NH,  $J_{\beta\text{-hydroxy-Asp-NH, } \beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 10.7$  Hz), 6.69 (d, 1H,  $\beta$ -hydroxy-Asp-NH,  $J_{\beta\text{-hydroxy-Asp-NH, } \beta\text{-hydroxy-Asp-}\alpha\text{-CH}} = 10.1$  Hz), 5.35 (d, 1H, Bn,  $J_{\text{Bn, Bn}} = 12.1$  Hz), 5.29-5.23 (m, 2H,  $\beta$ -hydroxy-Asp-

$\beta$ -CH, Bn), 5.19 (d, 1H, Bn,  $J_{\text{Bn, Bn}} = 12.1$  Hz), 5.18 (dd, 1H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-NH}} = 10.1$ ,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-}\beta\text{-CH}} = 1.7$  Hz), 5.14-5.08 (m, 1H,  $\beta$ -hydroxy-Asp- $\beta$ -CH), 5.05-5.00 (m, 3H,  $\beta$ -hydroxy-Asp- $\beta$ -CH, 3-hydroxy-Pro- $\beta$ -CH, Bn), 4.99 (d, 1H, 3-hydroxy-Pro- $\beta$ -CH,  $J_{3\text{-hydroxy-Pro-}\beta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 1.2$  Hz), 4.95-4.88 (m, 1H, D-Ser- $\alpha$ -CH), 4.83 (br d, 1H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-NH}} = 10.7$ ), 4.77-4.55 (m, 4H, D-Ala- $\alpha$ -CH, H-3, Cy-1), 4.51-4.35 (m, 6H, Arg- $\alpha$ -CH, *allo*-D-Thr- $\alpha$ -CH, *allo*-D-Thr- $\beta$ -CH, 3-hydroxy-Pro- $\alpha$ -CH, Bn), 4.34 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.16-4.10 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 4.05-3.99 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.80 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{\text{D-Ser-}\beta\text{-CH}, \text{D-Ser-}\beta\text{-CH}} = J_{\text{D-Ser-}\beta\text{-CH}, \text{D-Ser-}\alpha\text{-CH}} = 9.2$  Hz), 3.75-3.64 (m, 1H, Arg- $\delta$ -CH), 3.58 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{\text{D-Ser-}\beta\text{-CH}, \text{D-Ser-}\beta\text{-CH}} = 9.2$ ,  $J_{\text{D-Ser-}\beta\text{-CH}, \text{D-Ser-}\alpha\text{-CH}} = 5.8$  Hz), 3.44 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = J_{3\text{-hydroxy-Pro-}\delta\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 9.2$  Hz), 3.08-3.01 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 2.64 (dd, 1H, H-2,  $J_{2,2} = 15.5$ ,  $J_{2,3} = 5.7$  Hz), 2.41-2.32 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 2.23 (dd, 1H, H-2,  $J_{2,2} = 15.5$ ,  $J_{2,3} = 3.0$  Hz), 1.92 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 5.7$  Hz), 1.88-1.55 (m, 16H, 3-hydroxy-Pro- $\gamma$ -CH, Arg- $\beta$ -CH, Arg- $\gamma$ -CH, H-4, Cy-2), 1.56-1.47 (m, 1H, H-14), 1.46-1.12 (m, 36H, *allo*-D-Thr- $\gamma$ -CH, D-Ala- $\beta$ -CH, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, Cy-3, Cy-4), 1.12-0.92 (m, 105H,  $^1\text{Pr}_3\text{Si}$ ), 0.86 (d, 6H, H-15,  $J_{15,14} = 6.3$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  172.6, 172.6, 172.4, 172.0, 171.1, 170.8, 170.1, 169.7, 169.1, 167.1, 164.1, 160.9, 156.1, 138.0, 137.4, 134.9, 129.2, 128.9, 128.7, 128.6, 128.5, 128.2, 127.7, 127.3, 75.7, 74.9, 74.4, 74.3, 73.9, 73.5, 73.3, 72.9, 71.7, 17.5, 69.8, 69.2, 67.4, 67.1, 62.3, 56.7, 54.6, 52.8, 51.7, 47.0, 46.2, 65.5, 44.7, 40.4, 39.2, 34.0, 33.3, 31.8, 31.7, 31.7, 31.6, 31.4, 31.2, 30.1, 29.9, 29.8, 29.8, 29.6, 28.1, 27.6, 25.8, 25.5, 25.4, 25.2, 24.1, 23.9, 22.8, 19.6, 18.3, 18.3, 18.1, 18.0, 18.0, 17.6, 12.6, 12.6, 12.5, 12.3, 12.1, 12.1; ESIMS-LR  $m/z$  2484.52 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{130}\text{H}_{221}\text{O}_{24}\text{N}_{11}\text{NaSi}_5$  2483.5150, found 2483.5200;  $[\alpha]_{\text{D}}^{20} -2.47$  ( $c$  0.35,  $\text{CHCl}_3$ ).

### Plusbacin A<sub>3</sub> (1)



To a solution of **99** (19.2 mg, 7.8  $\mu\text{mol}$ ) in anisole (1 mL) in an HF reaction apparatus, HF gas was distilled at  $-78$   $^{\circ}\text{C}$  to a total volume of approximately 10 mL. The mixture was warmed to  $0$   $^{\circ}\text{C}$  and stirred for 1 h. The mixture was concentrated *in vacuo*, and the crude material was triturated with  $\text{Et}_2\text{O}$  and centrifuged. The crude material was purified by reverse phase HPLC (YMC-Pack R&D ODS D-ODS-5-A,  $250 \times 20$  mm, 0.1% TFA 47.5% MeCN/ $\text{H}_2\text{O}$ ) to afford **1** (6.6 mg, 5.2  $\mu\text{mol}$ , 67%), after

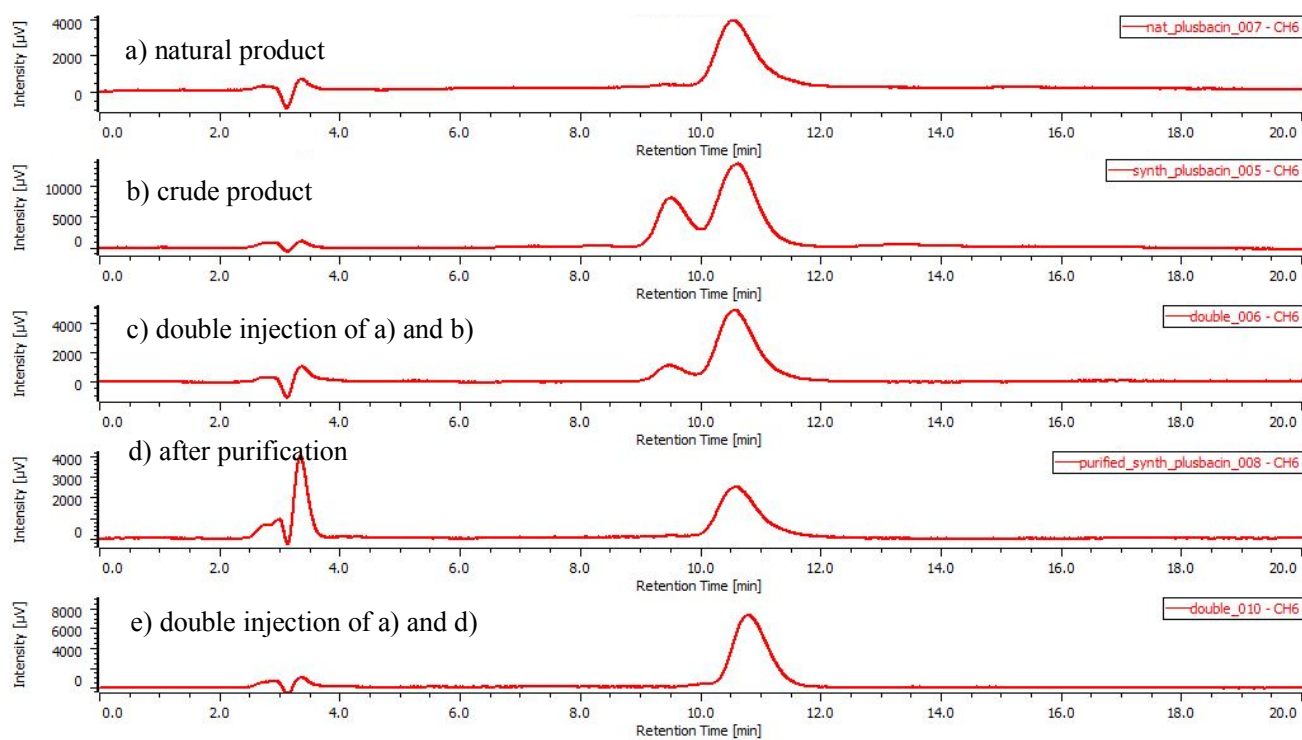
freeze drying, as a white powder.

$^1\text{H}$  NMR ( $\text{CD}_3\text{CN}/\text{D}_2\text{O}/\text{TFA} = 500/500/1$ , 500 MHz, a mixture of several rotamers at  $20$   $^{\circ}\text{C}$ , selected data for the major rotamer)  $\delta$  5.20-5.13 (m, 1H, H-3), 5.13-5.07 (m, 1H, Arg- $\alpha$ -CH), 5.04 (d, 1H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-}\beta\text{-CH}} = 2.9$  Hz), 4.93-4.90 (m, 2H,  $\beta$ -hydroxy-Asp- $\alpha$ -CH,  $\beta$ -hydroxy-Asp- $\beta$ -CH), 4.86 (d, 1H,  $\beta$ -hydroxy-Asp- $\beta$ -CH,  $J_{\beta\text{-hydroxy-Asp-}\alpha\text{-CH}, \beta\text{-hydroxy-Asp-}\beta\text{-CH}} = 2.9$  Hz), 4.81-4.65 (m, 2H, 3-hydroxy-Pro- $\beta$ -CH), 4.63-4.57 (m, 1H, D-Ser- $\alpha$ -CH), 4.42-4.30 (m, 3H, 3-hydroxy-Pro- $\alpha$ -CH, *allo*-D-Thr- $\alpha$ -CH), 4.15-4.12 (m, 1H, *allo*-D-Thr- $\beta$ -CH), 3.96-3.83 (m, 2H, D-Ala- $\alpha$ -CH, 3-hydroxy-Pro- $\delta$ -CH), 3.81-3.71 (m, 2H, D-Ser- $\beta$ -CH), 3.71-3.62 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 3.61-3.51 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 3.12-3.02 (m, 2H, Arg- $\delta$ -CH), 2.63-2.42 (m, 2H, H-2), 2.15-1.74 (m, 4H, 3-hydroxy-Pro- $\gamma$ -CH), 1.62-1.51 (m, 4H, Arg- $\beta$ -CH, H-4), 1.50-1.34 (m, 3H, Arg- $\gamma$ -CH, H-14), 1.28-1.04 (m, 21H, *allo*-D-Thr- $\gamma$ -CH, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 1.03 (d, 3H,  $J_{\text{D-Ala-}\beta\text{-CH}, \text{D-Ala-}}$

$\alpha$ -CH = 6.3 Hz), 0.80 (d, 6H, H-15,  $J_{15, 14} = 6.3$  Hz)

ESIMS-LR  $m/z$  1156.58 [(M-H)<sup>-</sup>]; ESIMS-HR calcd for C<sub>50</sub>H<sub>82</sub>O<sub>20</sub>N<sub>11</sub> 1156.5743, found 1156.5770; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +7.17 ( $c$  0.06, EtOH).

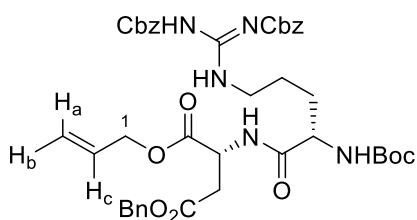
HPLC (column: COSMOSIL 5C<sub>18</sub>-MS-II; eluent: 47.5% MeCN/H<sub>2</sub>O (0.1% TFA); flow 1 mL/min; detection: UV 210 nm) Retention time: natural product (10.6 min), synthetic product (10.6 min), double injection (10.8 min) (for detail in figure S1).



**Figure S1.** Chromatogram of HPLC.



### Boc-Arg(Cbz)<sub>2</sub>-D-Asp(OBn)-Oallyl (**102**)

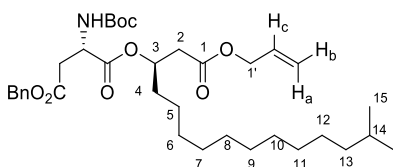


A solution of **101** (162 mg, 0.50 mmol) and Cs<sub>2</sub>CO<sub>3</sub> (326 mg, 1.00 mmol) in DMF (5 mL) was treated with allyl bromide (60.6 μL, 0.70 mmol) at room temperature for 5 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude ester. The crude ester was treated with 4 M HCl/AcOEt (5 mL) at room temperature for

20 min. The mixture was concentrated *in vacuo* to afford a crude amine hydrochloride salt. A mixture of the crude amine hydrochloride salt, Boc-Arg(Cbz)<sub>2</sub>-OH (326 mg, 0.60 mmol), <sup>4</sup>Pr<sub>2</sub>NEt (261 μL, 1.5 mmol) and HOAt (136.1 mg, 1.00 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was treated with EDCI (191.7 mg, 1.00 mmol) at room temperature for 45 min. The mixture was concentrated *in vacuo*, and the residue was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with 1 M *aq.* HCl, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (3-30-60% AcOEt/hexane) to afford **102** (352 mg, 0.45 mmol, 89% over 3 steps) as a white solid.

mp 108-109 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 9.45 (br s, 1H, Arg-ω-NH), 9.29 (br s, 1H, Arg-δ-NH), 7.43-7.24 (m, 15H, Ph), 7.18 (d, 1H, Arg-α-NH, *J*<sub>Arg-α-NH, Arg-α-CH</sub> = 6.3 Hz), 5.78 (dddd, 1H, H<sub>c</sub>, *J*<sub>H<sub>c</sub>, H<sub>a</sub></sub> = 17.2, *J*<sub>H<sub>c</sub>, H<sub>b</sub></sub> = 10.3, *J*<sub>H<sub>c</sub>, 1</sub> = *J*<sub>H<sub>c</sub>, 1</sub> = 4.6 Hz), 5.35 (dd, 1H, H<sub>a</sub>, *J*<sub>H<sub>a</sub>, H<sub>c</sub></sub> = 17.2, *J*<sub>H<sub>a</sub>, 1</sub> = 1.7 Hz), 5.46-5.40 (m, 1H, D-Asp-NH), 5.24 (s, 2H, Bn), 5.18 (d, 1H, Bn, *J*<sub>Bn, Bn</sub> = 13.2 Hz), 5.24 (dd, 1H, H<sub>b</sub>, *J*<sub>H<sub>b</sub>, H<sub>c</sub></sub> = 10.3, *J*<sub>H<sub>b</sub>, H-1</sub> = 1.2 Hz), 5.13 (d, 1H, Bn, *J*<sub>Bn, Bn</sub> = 13.2 Hz), 5.08 (d, 1H, Bn, *J*<sub>Bn, Bn</sub> = 12.1 Hz), 5.04 (d, 1H, Bn, *J*<sub>Bn, Bn</sub> = 12.1 Hz), 4.76 (ddd, 1H, D-Asp-α-CH, *J*<sub>D-Asp-α-CH, D-Asp-NH</sub> = 8.1, *J*<sub>D-Asp-α-CH, D-Asp-β-CH</sub> = *J*<sub>D-Asp-α-CH, D-Asp-β-CH</sub> = 5.2 Hz), 4.58-4.47 (m, 2H, H-1), 4.22-4.14 (m, 1H, Arg-α-CH), 4.03-3.86 (m, 2H, Arg-δ-CH), 2.84 (dd, 1H, D-Asp-β-CH, *J*<sub>D-Asp-β-CH, D-Asp-β-CH</sub> = 17.2, *J*<sub>D-Asp-β-CH, D-Asp-α-CH</sub> = 5.2 Hz), 2.79 (dd, 1H, D-Asp-β-CH, *J*<sub>D-Asp-β-CH, D-Asp-β-CH</sub> = 17.2, *J*<sub>D-Asp-β-CH, D-Asp-α-CH</sub> = 5.2 Hz), 1.82-1.57 (m, 4H, Arg-β-CH, Arg-γ-CH), 1.42 (s, 9H, <sup>t</sup>Bu); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 171.8, 170.5, 169.9, 163.7, 160.6, 155.8, 155.6, 136.9, 135.4, 134.6, 131.4, 128.9, 128.8, 128.6, 128.4, 128.4, 128.3, 127.9, 127.8, 118.6, 79.9, 68.9, 67.0, 66.7, 66.2, 54.0, 48.7, 44.2, 36.0, 28.6, 28.3, 24.8; ESIMS-LR *m/z* 810.33 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>41</sub>H<sub>49</sub>O<sub>11</sub>N<sub>5</sub>Na 810.3321, found 810.3337; [α]<sub>D</sub><sup>20</sup> -3.94 (*c* 0.64, CHCl<sub>3</sub>).

### Depsipeptide **105**

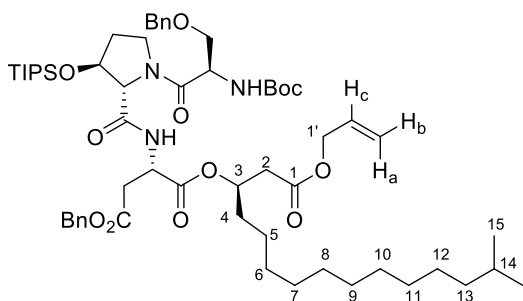


A solution of **37** (60.0 mg, 0.22 mmol) and Cs<sub>2</sub>CO<sub>3</sub> (108 mg, 0.33 mmol) in DMF (2 mL) was treated with allyl bromide (28.5 μL, 0.33 mmol) at room temperature for 4 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and H<sub>2</sub>O. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude alcohol. A

solution of the crude alcohol, Boc-Asp(OBn)-OH (85.4 mg, 0.26 mmol) and DMAP (32.3 mg, 0.26 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was treated with EDCI (101.6 mg, 0.53 mmol) at -18 °C for 1 min. The mixture was warmed to 0 °C, and stirred for 15 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.* NaHCO<sub>3</sub> and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-4-10-20% AcOEt/hexane) to afford **105** (123 mg, 0.21 mmol, 95% over 2 steps) as a colorless oil.

$^1\text{H}$  NMR (500 Hz,  $\text{CDCl}_3$ )  $\delta$  7.38-7.30 (m, 5H, Ph), 5.78 (dddd, 1H,  $\text{H}_c$ ,  $J_{\text{H}_c, \text{H}_a} = 17.2$ ,  $J_{\text{H}_c, \text{H}_b} = 10.3$ ,  $J_{\text{H}_c, 1} = J_{\text{H}_c, 1} = 5.7$  Hz), 5.46 (d, 1H, Asp-NH,  $J_{\text{Asp-NH}, \text{Asp-}\alpha\text{-CH}} = 8.6$  Hz), 5.31 (ddd, 1H,  $\text{H}_a$ ,  $J_{\text{H}_a, \text{H}_c} = 17.2$ ,  $J_{\text{H}_a, 1} = 2.9$ ,  $J_{\text{H}_a, 1} = 1.7$  Hz), 5.28-5.21 (m, 2H, H-3,  $\text{H}_b$ ), 5.13 (d, 1H, Bn,  $J_{\text{Bn}, \text{Bn}} = 12.0$  Hz), 5.10 (d, 1H, Bn,  $J_{\text{Bn}, \text{Bn}} = 12.0$  Hz), 4.58 (d, 2H, H-1',  $J_{1', \text{H}_c} = 5.7$  Hz), 4.54 (ddd, 1H, Asp- $\alpha$ -CH,  $J_{\text{Asp-}\alpha\text{-CH}, \text{Asp-NH}} = 8.6$ ,  $J_{\text{Asp-}\alpha\text{-CH}, \text{Asp-}\beta\text{-CH}} = 5.2$ ,  $J_{\text{Asp-}\alpha\text{-CH}, \text{Asp-}\beta\text{-CH}} = 4.6$  Hz), 3.01 (dd, 1H, Asp- $\beta$ -CH,  $J_{\text{Asp-}\beta\text{-CH}, \text{Asp-}\beta\text{-CH}} = 17.2$ ,  $J_{\text{Asp-}\beta\text{-CH}, \text{Asp-}\alpha\text{-CH}} = 4.6$  Hz), 2.87 (dd, 1H, Asp- $\beta$ -CH,  $J_{\text{Asp-}\beta\text{-CH}, \text{Asp-}\beta\text{-CH}} = 17.2$ ,  $J_{\text{Asp-}\beta\text{-CH}, \text{Asp-}\alpha\text{-CH}} = 5.2$  Hz), 2.63 (dd, 1H, H-2,  $J_{2, 2} = 15.5$ ,  $J_{2, 3} = 6.9$  Hz), 2.56 (dd, 1H, H-2,  $J_{2, 2} = 15.5$ ,  $J_{2, 3} = 5.7$  Hz), 1.63-1.47 (m, 3H, H-4, H-14), 1.44 (s, 9H,  $^t\text{Bu}$ ), 1.32-1.10 (m, 18H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 0.86 (d, 6H, H-15,  $J_{15, 14} = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  170.8, 170.4, 169.9, 155.4, 135.5, 132.1, 128.7, 128.5, 128.4, 118.8, 80.1, 72.3, 66.8, 65.6, 50.2, 39.2, 39.0, 36.9, 33.8, 30.1, 29.8, 29.8, 29.7, 29.6, 29.4, 28.4, 28.1, 27.5, 25.0, 22.8; ESIMS-LR  $m/z$  640.38 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{35}\text{H}_{55}\text{O}_8\text{NNa}$  640.3820, found 640.3823;  $[\alpha]^{20}_{\text{D}} +4.05$  ( $c$  0.39,  $\text{CHCl}_3$ ).

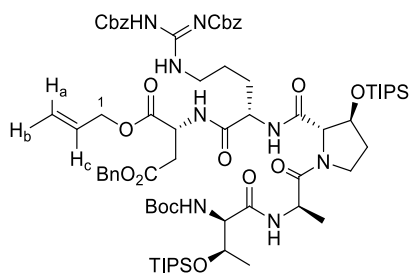
### Depsipeptide 107



Ester **105** (62.3 mg, 0.106 mmol) was treated with 4 M HCl/AcOEt (2 mL) at room temperature for 25 min. The mixture was concentrated *in vacuo* to afford amine **106** as a hydrochloride salt. This compound was directly used to the next reaction without further purification. A solution of the crude amine **106**, **79** (54.2 mg, 0.096 mmol),  $^i\text{Pr}_2\text{NEt}$  (51.9  $\mu\text{L}$ , 0.30 mmol) and HOAt (36.8 mg, 0.19 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was treated with EDCI (36.8 mg, 0.12

mmol) at room temperature for 185 min. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.*  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$  and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (5-15-20% AcOEt/hexane) to afford **107** (96.1 mg, 0.093 mmol, 97% over 2 steps) as a colorless oil.

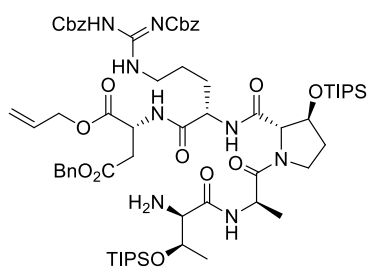
$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.49 (d, 1H, Asp-NH,  $J_{\text{Asp-NH}, \text{Asp-}\alpha\text{-CH}} = 8.0$  Hz), 5.89 (dddd, 1H,  $\text{H}_c$ ,  $J_{\text{H}_c, \text{H}_a} = 17.2$ ,  $J_{\text{H}_c, \text{H}_b} = 10.3$ ,  $J_{\text{H}_c, 1'} = J_{\text{H}_c, 1'} = 4.6$  Hz), 5.39 (d, 1H, D-Ser-NH,  $J_{\text{D-Ser-NH}, \text{D-Ser-}\alpha\text{-CH}} = 6.9$  Hz), 5.30 (d, 1H,  $\text{H}_a$ ,  $J_{\text{H}_a, \text{H}_c} = 17.2$  Hz), 5.25-5.18 (m, 2H,  $\text{H}_b$ , H-3), 5.10 (s, 2H, Bn), 4.74-4.65 (m, 3H, Asp- $\alpha$ -CH, D-Ser- $\alpha$ -CH, 3-hydroxy-Pro- $\beta$ -CH), 4.61-4.48 (m, 4H, Bn, H-1'), 4.43 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 3.84-3.74 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH), 3.61 (d, 2H, D-Ser- $\beta$ -CH,  $J_{\text{D-Ser-}\beta\text{-CH}, \text{D-Ser-}\alpha\text{-CH}} = 6.3$  Hz), 2.95 (dd, 1H, Asp- $\beta$ -CH,  $J_{\text{Asp-}\beta\text{-CH}, \text{Asp-}\beta\text{-CH}} = 17.8$ ,  $J_{\text{Asp-}\beta\text{-CH}, \text{Asp-}\alpha\text{-CH}} = 5.7$  Hz), 2.88 (dd, 1H, Asp- $\beta$ -CH,  $J_{\text{Asp-}\beta\text{-CH}, \text{Asp-}\beta\text{-CH}} = 17.8$ ,  $J_{\text{Asp-}\beta\text{-CH}, \text{Asp-}\alpha\text{-CH}} = 5.2$  Hz), 2.60 (dd, 1H, H-2,  $J_{2, 2} = 15.5$ ,  $J_{2, 3} = 6.9$  Hz), 2.51 (dd, 1H, H-2,  $J_{2, 2} = 15.5$ ,  $J_{2, 3} = 6.3$  Hz), 2.14-2.04 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.93-1.86 (m, 1H, 3-hydroxy-Pro- $\gamma$ -CH), 1.61-1.45 (m, 3H, H-4, H-14), 1.32-1.11 (m, 18H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 1.39 (m, 9H,  $^t\text{Bu}$ ), 1.32-0.96 (m, 21H,  $^i\text{Pr}_3\text{Si}$ ), 0.86 (d, 6H, H-15,  $J_{15, 14} = 7.2$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  170.5, 170.4, 170.0, 169.6, 169.2, 155.6, 137.8, 135.7, 132.1, 128.7, 128.5, 127.9, 127.8, 118.7, 80.1, 73.7, 73.4, 72.3, 70.0, 69.6, 66.8, 52.3, 49.2, 45.6, 39.2, 39.0, 36.2, 33.9, 30.1, 29.9, 29.8, 29.8, 29.6, 29.5, 28.5, 28.1, 27.6, 25.1, 22.8, 18.1, 12.1; ESIMS-LR  $m/z$  1086.64 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{59}\text{H}_{93}\text{O}_{12}\text{N}_3\text{NaSi}$  1086.6421, found 1086.6415;  $[\alpha]^{20}_{\text{D}} -8.38$  ( $c$  1.79,  $\text{CHCl}_3$ ).

**Boc-D-*allo*-Thr(O-triisopropylsilyl)-D-Ala-(3*S*)-3-(triisopropylsiloxy)-Pro-Arg(Cbz)<sub>2</sub>-D-Asp(OBn)-Oallyl****(109)**

Dipeptide **102** (94.5 mg, 0.120 mmol) was treated with 4 M HCl/AcOEt (2 mL) at room temperature for 15 min. The mixture was concentrated *in vacuo* to afford amine **103** as a hydrochloride salt. This compound was directly used to the next reaction without further purification. A solution of the crude amine **103**, **81** (71.6 mg, 0.10 mmol), <sup>i</sup>Pr<sub>2</sub>NEt (55.7 μL, 0.30 mmol) and HOAt (27.2 mg, 0.20 mmol) in THF (1 mL) was treated with EDCI (38.3 mg, 0.20

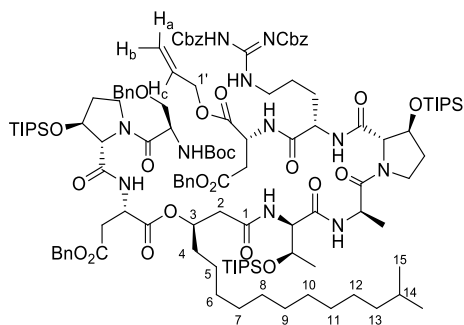
mmol) at room temperature for 4 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-20-35% AcOEt/hexane) to afford **109** (119 mg, 0.086 mmol, 86% over 2 steps) as a colorless oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 9.45 (br s, 1H, Arg-ω-NH), 9.30 (br s, 1H, Arg-δ-NH), 7.64 (br s, 1H, D-Ala-NH), 7.43-7.24 (m, 17H, Ph, Arg-α-NH, D-Asp-NH), 5.93 (br s, 1H, *allo*-D-Thr-NH), 5.80 (dddd, 1H, H<sub>c</sub>, *J*<sub>H<sub>c</sub>, H<sub>a</sub></sub> = 17.2, *J*<sub>H<sub>c</sub>, H<sub>b</sub></sub> = 10.3, *J*<sub>H<sub>c</sub>, 1</sub> = *J*<sub>H<sub>c</sub>, 1</sub> = 5.7 Hz), 5.26 (s, 2H, Bn), 5.24 (dd, 1H, H<sub>a</sub>, *J*<sub>H<sub>a</sub>, H<sub>c</sub></sub> = 17.2, *J*<sub>H<sub>a</sub>, 1</sub> = 1.2 Hz), 5.16 (dd, 1H, H<sub>b</sub>, *J*<sub>H<sub>b</sub>, H<sub>c</sub></sub> = 10.9, *J*<sub>H<sub>a</sub>, 1</sub> = 1.2 Hz), 5.15-5.06 (m, 4H, Bn), 4.88 (ddd, 1H, D-Asp-α-CH, *J*<sub>D-Asp-α-CH, D-Asp-NH</sub> = *J*<sub>D-Asp-α-CH, D-Asp-β-CH</sub> = *J*<sub>D-Asp-α-CH, D-Asp-β-CH</sub> = 5.8 Hz), 4.60 (d, 1H, 3-hydroxy-Pro-β-CH, *J*<sub>3-hydroxy-Pro-β-CH, 3-hydroxy-Pro-γ-CH</sub> = 2.3 Hz), 4.55 (ddd, 1H, H-1, *J*<sub>1, 1</sub> = 13.2, *J*<sub>1, H<sub>c</sub></sub> = *J*<sub>1, H<sub>c</sub></sub> = 5.7, *J*<sub>1, H<sub>b</sub></sub> = 1.2 Hz), 4.52 (ddd, 1H, H-1, *J*<sub>1, 1</sub> = 13.2, *J*<sub>1, H<sub>c</sub></sub> = *J*<sub>1, H<sub>c</sub></sub> = 5.7, *J*<sub>1, H<sub>a</sub></sub> = 1.2 Hz), 4.43-4.31 (m, 3H, D-Ala-α-CH, *allo*-D-Thr-β-CH, 3-hydroxy-Pro-α-CH), 4.26 (ddd, 1H, Arg-α-CH, *J*<sub>Arg-α-CH, Arg-NH</sub> = *J*<sub>Arg-α-CH, Arg-β-CH</sub> = 7.5, *J*<sub>Arg-α-CH, Arg-β-CH</sub> = 4.6 Hz), 4.15-4.10 (m, 1H, *allo*-D-Thr-α-CH), 4.06 (ddd, 1H, Arg-δ-CH, *J*<sub>Arg-δ-CH, Arg-δ-CH</sub> = 14.3, *J*<sub>Arg-δ-CH, Arg-γ-CH</sub> = *J*<sub>Arg-δ-CH, Arg-γ-CH</sub> = 7.5 Hz), 3.92-3.78 (m, 2H, 3-hydroxy-Pro-δ-CH, Arg-δ-CH), 3.50-3.43 (m, 1H, 3-hydroxy-Pro-δ-CH), 2.89 (d, 2H, D-Asp-β-CH, *J*<sub>D-Asp-β-CH, D-Asp-β-CH</sub> = 5.8 Hz), 2.07-1.89 (m, 3H, 3-hydroxy-Pro-γ-CH, Arg-β-CH), 1.79 (m, 1H, 3-hydroxy-Pro-γ-CH), 1.73-1.64 (m, 1H, Arg-γ-CH), 1.60-1.50 (m, 1H, Arg-γ-CH), 1.35 (s, 9H, <sup>t</sup>Bu), 1.30 (d, 3H, D-Ala-β-CH, *J*<sub>D-Ala-β-CH, D-Ala-α-CH</sub> = 6.9 Hz), 1.24 (d, 3H, *allo*-D-Thr-γ-CH, *J*<sub>*allo*-D-Thr-γ-CH, *allo*-D-Thr-β-CH</sub> = 6.3 Hz), 1.18-0.94 (m, 42H, <sup>i</sup>Pr<sub>3</sub>Si); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.7, 171.5, 170.8, 170.6, 170.0, 169.1, 164.1, 160.9, 156.1, 137.1, 136.0, 135.1, 131.8, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 118.5, 80.0, 74.2, 70.7, 69.2, 69.1, 67.1, 66.7, 66.2, 60.5, 53.7, 49.4, 47.6, 45.4, 44.5, 36.7, 33.8, 28.4, 27.6, 25.8, 20.2, 18.3, 18.2, 18.1, 18.0, 16.6, 12.7, 12.1; ESIMS-LR *m/z* 1407.73 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>71</sub>H<sub>108</sub>O<sub>16</sub>N<sub>8</sub>NaSi<sub>2</sub> 1407.7314, found 1407.7311; [α]<sub>D</sub><sup>19</sup> -5.16 (*c* 3.95, CHCl<sub>3</sub>).

**H-D-*allo*-Thr(O-triisopropylsilyl)-D-Ala-(3*S*)-3-(triisopropylsiloxy)-Pro-Arg(Cbz)<sub>2</sub>-D-Asp(OBn)-Oallyl (110)**

Pentapeptide **109** (60.9 mg, 0.044 mmol) was treated with 25% TFA/CH<sub>2</sub>Cl<sub>2</sub> (500 μL) at room temperature for 40 min. The reaction was quenched with *sat. aq.* NaHCO<sub>3</sub>, and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford **110**. The amine was directly used to the next reaction without purification.

## Liner depsipeptide **111**

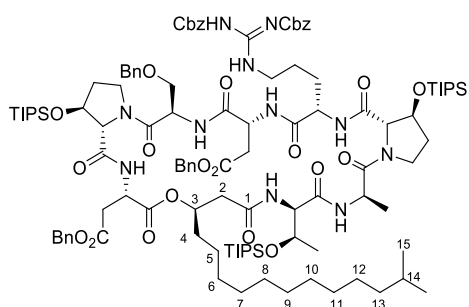


A solution of **107** (41.5 mg, 0.040 mmol) and morpholine (10.3  $\mu\text{L}$ , 0.12 mmol) in THF (500  $\mu\text{L}$ ) was treated with  $\text{Pd}(\text{PPh}_3)_4$  (2.3 mg, 0.002 mmol) at room temperature for 20 min. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The catalyst was removed by SH-silica gel column chromatography ( $\phi$  1 cm  $\times$  3 cm, 1% MeOH/ $\text{CHCl}_3$ ), and the filtrate was concentrated *in vacuo* to afford a crude carboxylic acid **108**. A mixture of

the crude carboxylic acid **108**, **110** (0.044 mmol),  $i\text{Pr}_2\text{NEt}$  (12.9  $\mu\text{L}$ , 0.080 mmol) and HOAt (10.9 mg, 0.080 mmol) in THF (400  $\mu\text{L}$ ) was treated with EDCI (15.3 mg, 0.080 mmol) at room temperature for 23 h. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.*  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$  and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-20-40% AcOEt/hexane) to afford **111** (82.9 mg, 0.036 mmol, 90% over 2 steps) as a pale yellow oil.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz, a mixture of several rotamers at 20  $^\circ\text{C}$ , selected data for the major rotamer)  $\delta$  9.42 (br s, 1H, Arg- $\omega$ -NH), 9.24 (br s, 1H, Arg- $\delta$ -NH), 7.49 (d, 1H, Asp-NH,  $J_{\text{Asp-NH}, \text{Asp-}\alpha\text{-CH}} = 8.0$  Hz), 7.46 (d, 1H, *allo*-D-Thr-NH,  $J_{\text{allo-D-Thr-NH}, \text{allo-D-Thr-}\alpha\text{-CH}} = 5.2$  Hz), 7.44-7.14 (m, 27H, Ph, Asp-NH, Arg- $\alpha$ -NH), 6.87 (d, 1H, D-Ala-NH,  $J_{\text{D-Ala-NH}, \text{D-Ala-}\alpha\text{-CH}} = 8.0$  Hz), 5.80 (dddd, 1H,  $\text{H}_c$ ,  $J_{\text{H}_c, \text{H}_a} = 17.2$ ,  $J_{\text{H}_c, \text{H}_b} = 10.9$ ,  $J_{\text{H}_c, 1'} = J_{\text{H}_c, 1'} = 5.8$  Hz), 5.48 (d, 1H, D-Ser-NH,  $J_{\text{D-Ser-NH}, \text{D-Ser-}\alpha\text{-CH}} = 6.9$  Hz), 5.29-5.05 (m, 11H, Bn,  $\text{H}_a$ ,  $\text{H}_b$ , H-3), 4.77 (ddd, 1H, Asp- $\alpha$ -CH,  $J_{\text{Asp-}\alpha\text{-CH}, \text{Asp-NH}} = J_{\text{Asp-}\alpha\text{-CH}, \text{Asp-}\beta\text{-CH}} = J_{\text{Asp-}\alpha\text{-CH}, \text{Asp-}\gamma\text{-CH}} = 5.7$  Hz), 4.72-4.24 (m, 13H, D-Ser- $\alpha$ -CH, *allo*-D-Thr- $\alpha$ -CH, *allo*-D-Thr- $\beta$ -CH, D-Ala- $\alpha$ -CH, Asp- $\alpha$ -CH, 3-hydroxy-Pro- $\alpha$ -CH, 3-hydroxy-Pro- $\beta$ -CH, Bn, H-1'), 4.07-4.39 (m, 1H, Arg- $\alpha$ -CH), 3.89-3.64 (m, 5H, Arg- $\delta$ -CH, 3-hydroxy-Pro- $\delta$ -CH), 3.61 (d, 2H, D-Ser- $\beta$ -CH,  $J_{\text{D-Ser-}\beta\text{-CH}, \text{D-Ser-}\alpha\text{-CH}} = 6.3$  Hz), 3.46-3.36 (m, 1H, 3-hydroxy-Pro- $\delta$ -CH), 2.98-2.81 (m, 4H, Asp- $\beta$ -CH), 2.46 (dd, 1H, H-2,  $J_{2,2} = 14.9$ ,  $J_{2,3} = 6.3$  Hz), 2.35 (dd, 1H, H-2,  $J_{2,2} = 14.9$ ,  $J_{2,3} = 6.3$  Hz), 2.13-1.83 (m, 9H, Arg- $\beta$ -CH, Arg- $\gamma$ -CH, 3-hydroxy-Pro- $\gamma$ -CH, H-4), 1.79 (dd, 1H, 3-hydroxy-Pro- $\gamma$ -CH,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\gamma\text{-CH}} = 13.2$ ,  $J_{3\text{-hydroxy-Pro-}\gamma\text{-CH}, 3\text{-hydroxy-Pro-}\delta\text{-CH}} = 6.3$  Hz), 1.75-1.44 (m, 3H, H-4, H-14), 1.39 (s, 9H,  $t\text{Bu}$ ), 1.33-0.92 (m, 87H, D-Ala- $\beta$ -CH, *allo*-D-Thr- $\gamma$ -CH, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13,  $i\text{Pr}_3\text{Si}$ ), 0.86 (d, 6H, H-15,  $J_{15,14} = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  172.7, 171.6, 170.7, 170.5, 170.4, 170.2, 170.1, 169.7, 169.1, 164.1, 160.8, 156.1, 155.7, 137.8, 137.1, 135.9, 135.7, 135.0, 131.8, 128.9, 128.8, 128.7, 128.6, 128.5, 158.4, 128.4, 128.3, 128.0, 127.8, 127.7, 118.5, 80.1, 74.1, 73.7, 73.4, 73.4, 70.5, 69.9, 69.6, 69.2, 69.1, 67.1, 66.7, 66.3, 59.3, 53.4, 52.4, 49.4, 49.3, 47.8, 45.6, 45.3, 44.5, 40.9, 36.3, 36.0, 34.1, 33.8, 32.1, 30.1, 29.9, 29.8, 29.8, 29.6, 29.5, 29.4, 28.5, 28.1, 28.0, 27.6, 25.7, 25.3, 22.8, 22.7, 20.3, 19.4, 18.3, 18.3, 18.2, 18.2, 18.1, 16.6, 12.8, 12.6, 12.1; ESIMS-LR  $m/z$  2314.29 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{122}\text{H}_{187}\text{O}_{25}\text{N}_{11}\text{NaSi}_3$  2313.2900, found 2313.2915;  $[\alpha]_D^{20} -5.52$  ( $c$  0.18,  $\text{CHCl}_3$ ).

## Fully protected dideoxy plusbacin A<sub>3</sub> 112



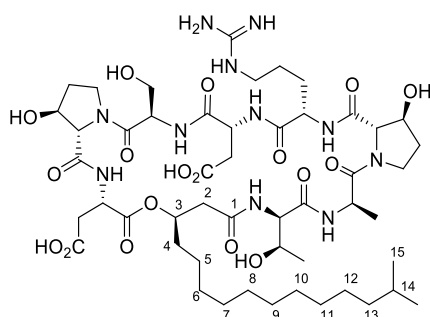
A solution of **111** (34.5 mg, 0.015 mmol) and morpholine (5.2  $\mu$ L, 0.06 mmol) in THF (300  $\mu$ L) was treated with Pd(PPh<sub>3</sub>)<sub>4</sub> (1.7 mg, 0.002 mmol) at room temperature for 25 min. The mixture was diluted with AcOEt, and partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The catalyst was removed by SH-silica gel column chromatography ( $\phi$  1 cm  $\times$  2 cm, 1% MeOH/CHCl<sub>3</sub>), and the filtrate was

concentrated *in vacuo* to afford crude carboxylic acid. A solution of the carboxylic acid in CH<sub>2</sub>Cl<sub>2</sub> (150  $\mu$ L) was treated with 50% TFA/CH<sub>2</sub>Cl<sub>2</sub> (150  $\mu$ L) at room temperature for 15 min. The mixture was concentrated *in vacuo* to afford crude amino acid. A mixture of the crude amino acid, <sup>t</sup>Pr<sub>2</sub>NEt (26.3  $\mu$ L, 0.15 mmol) and HOAt (20.6 mg, 0.15 mmol) in THF (15 mL) was treated with EDCI (28.9 mg, 0.15 mmol) at room temperature for 14 h. The mixture was concentrated *in vacuo*, and the residue was partitioned between AcOEt and 1 M *aq.* HCl. The organic phase was washed with *sat. aq.* NaHCO<sub>3</sub>, H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-20-40% AcOEt/hexane) to afford **112** (15.5 mg, 0.0073 mmol, 48% over 3 steps) as a white foam.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  9.41 (br s, 1H, Arg- $\omega$ -NH), 9.38 (br s, 1H, Arg- $\delta$ -NH), 7.64 (d, 1H, D-Ser-NH,  $J_{D-Ser-NH, D-Ser-\alpha-CH} = 5.2$  Hz), 7.61 (d, 1H, D-Ala-NH,  $J_{D-Ala-NH, D-Ala-\alpha-CH} = 2.9$  Hz), 7.44-7.19 (m, 28H, Ph, Asp-NH, *allo*-D-Thr-NH, Arg- $\alpha$ -NH), 7.08 (d, 1H, Asp-NH,  $J_{Asp-NH, Asp-\alpha-CH} = 9.8$  Hz), 5.37 (d, 1H, Bn,  $J_{Bn, Bn} = 12.1$  Hz), 5.21 (d, 1H, Bn,  $J_{Bn, Bn} = 12.1$  Hz), 5.21-4.98 (m, 8H, Bn, Asp- $\alpha$ -CH, H-3), 4.74 (ddd, 1H, Asp- $\alpha$ -CH,  $J_{Asp-\alpha-CH, Asp-NH} = 9.8$ ,  $J_{Asp-\alpha-CH, Asp-\beta-CH} = 4.6$ ,  $J_{Asp-\alpha-CH, Asp-\gamma-CH} = 4.0$  Hz), 4.60-4.54 (m, 3H, D-Ser- $\alpha$ -CH, 3-hydroxy-Pro- $\beta$ -CH), 4.51 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.43 (s, 2H, Bn), 4.41 (dd, 1H, *allo*-D-Thr- $\alpha$ -CH,  $J_{allo-D-Thr-\alpha-CH, allo-D-Thr-NH} = 8.0$ ,  $J_{allo-D-Thr-\alpha-CH, allo-D-Thr-\beta-CH} = 4.0$  Hz), 4.34 (s, 1H, 3-hydroxy-Pro- $\alpha$ -CH), 4.33 (dq, 1H, *allo*-D-Thr- $\beta$ -CH,  $J_{allo-D-Thr-\beta-CH, allo-D-Thr-\alpha-CH} = 4.0$ ,  $J_{allo-D-Thr-\beta-CH, allo-D-Thr-\gamma-CH} = 6.3$  Hz), 4.28 (dq, 1H, D-Ala- $\alpha$ -CH,  $J_{D-Thr-\alpha-CH, D-Ala-NH} = 2.9$ ,  $J_{D-Ala-\alpha-CH, D-Ala-\beta-CH} = 6.3$  Hz), 4.14 (ddd, Arg- $\alpha$ -CH,  $J = 10.3$ ,  $J = 9.4$ ,  $J = 4.0$  Hz), 4.01-3.93 (m, 3H, Arg- $\delta$ -CH, 3-hydroxy-Pro- $\delta$ -CH), 3.89 (dd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\delta-CH} = J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\gamma-CH} = 9.2$  Hz), 3.85-3.77 (m, 2H, 3-hydroxy-Pro- $\delta$ -CH, D-Ser- $\beta$ -CH), 3.69 (dd, 1H, D-Ser- $\beta$ -CH,  $J_{D-Ser-\beta-CH, D-Ser-\beta-CH} = 9.2$ ,  $J_{D-Ser-\beta-CH, D-Ser-\alpha-CH} = 5.2$  Hz), 3.48 (ddd, 1H, 3-hydroxy-Pro- $\delta$ -CH,  $J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\delta-CH} = J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\gamma-CH} = J_{3-hydroxy-Pro-\delta-CH, 3-hydroxy-Pro-\gamma-CH} = 9.2$  Hz), 3.29 (dd, 1H, Asp- $\beta$ -CH,  $J_{Asp-\beta-CH, Asp-\beta-CH} = 17.8$ ,  $J_{Asp-\beta-CH, Asp-\alpha-CH} = 4.6$  Hz), 3.00 (dd, 1H, Asp- $\beta$ -CH,  $J_{Asp-\beta-CH, Asp-\beta-CH} = 16.6$ ,  $J_{Asp-\beta-CH, Asp-\alpha-CH} = 7.5$  Hz), 2.75 (dd, 1H, Asp- $\beta$ -CH,  $J_{Asp-\beta-CH, Asp-\beta-CH} = 17.8$ ,  $J_{Asp-\beta-CH, Asp-\alpha-CH} = 4.6$  Hz), 2.66 (dd, 1H, Asp- $\beta$ -CH,  $J_{Asp-\beta-CH, Asp-\beta-CH} = 16.6$ ,  $J_{Asp-\beta-CH, Asp-\alpha-CH} = 7.5$  Hz), 2.51 (dd, 1H, H-2,  $J_{2,2} = 16.6$ ,  $J_{2,3} = 4.0$  Hz), 2.51 (dd, 1H, H-2,  $J_{2,2} = 16.6$ ,  $J_{2,3} = 9.2$  Hz), 1.98-1.67 (m, 9H, 3-hydroxy-Pro- $\gamma$ -CH, Arg- $\beta$ -CH, Arg- $\gamma$ -CH, H-4), 1.56-1.46 (m, 3H, H-4, H-5, H-14), 1.46-1.36 (m, 1H, H-5), 1.25 (d, 3H, *allo*-D-Thr- $\gamma$ -CH,  $J_{allo-D-Thr-\gamma-CH, allo-D-Thr-\beta-CH} = 6.3$  Hz), 1.29-0.95 (m, 79H, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, <sup>t</sup>Pr<sub>3</sub>Si), 1.14 (d, 3H, D-Ala- $\beta$ -CH,  $J_{D-Ala-\beta-CH, D-Ala-\alpha-CH} = 6.9$  Hz), 0.86 (d, 6H, H-15,  $J_{15,14} = 6.9$  Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  172.7, 172.6, 171.5, 171.3, 171.2, 170.4, 170.0, 169.8, 169.4, 168.1, 164.2, 161.1, 156.3, 138.0, 137.1, 135.9, 135.8, 134.8, 129.0, 128.8, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.7, 127.4, 74.9, 74.3, 73.4, 73.1, 71.0, 70.4, 69.5, 69.4, 69.0, 67.2, 66.6, 66.5, 59.7, 53.3, 52.9, 49.7, 47.9, 47.4, 45.5, 44.6, 39.8, 39.2,

36.0, 35.8, 33.8, 33.5, 33.3, 30.1, 29.9, 29.8, 29.7, 29.6, 28.1, 27.6, 26.3, 25.6, 25.5, 22.8, 20.5, 18.4, 18.3, 18.1, 15.8, 12.7, 12.2, 12.1; ESIMS-LR  $m/z$  2156.20 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>114</sub>H<sub>173</sub>O<sub>22</sub>N<sub>11</sub>NaSi<sub>3</sub> 2155.1957, found 2155.1961; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -35.86 (*c* 1.55, CHCl<sub>3</sub>).

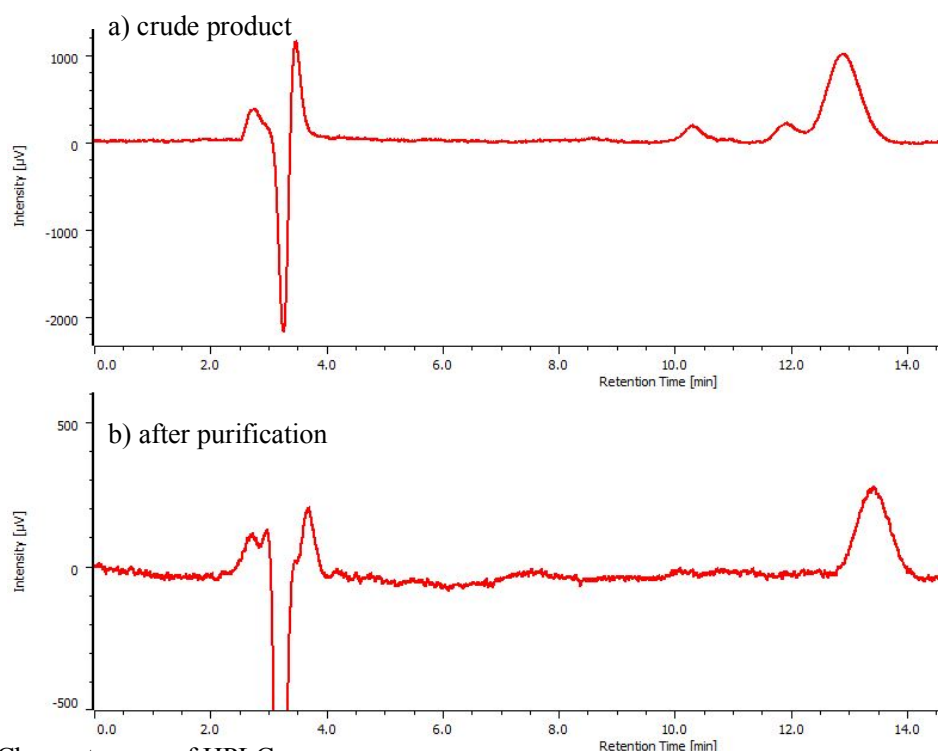
### Dideoxy plusbacin A<sub>3</sub> (**100**)



To a solution of **112** (15.4 mg, 7.2  $\mu$ mol) in anisole (1 mL) in an HF reaction apparatus, HF gas was distilled at -78 °C to a total volume of approximately 10 mL. The mixture was warmed to 0 °C and stirred for 1 h. The mixture was concentrated *in vacuo*, and the crude material was triturated with Et<sub>2</sub>O and centrifuged. The crude material was purified by reverse phase HPLC (YMC-Pack R&D ODS D-ODS-5-A, 250  $\times$  20 mm, 0.1% TFA 45% MeCN/H<sub>2</sub>O) to afford **100** (7.4 mg, 5.5  $\mu$ mol, 76%), after freeze drying, as a white powder.

<sup>1</sup>H NMR (CD<sub>3</sub>CN/D<sub>2</sub>O/TFA = 500/500/1, 500 MHz, a mixture of several rotamers at 20 °C, selected data for the major rotamer)  $\delta$  5.14-5.06 (m, 1H, Arg- $\alpha$ -CH), 5.11-5.00 (m, 1H, H-3), 4.79-4.52 (m, 3H, D-Ser- $\alpha$ -CH, Asp- $\alpha$ -CH), 4.43-4.04 (m, 5H, *allo*-D-Thr- $\beta$ -CH, 3-hydroxy-Pro- $\alpha$ -CH, 3-hydroxy-Pro- $\beta$ -CH), 3.99-3.79 (m, 1H, D-Ser- $\beta$ -CH), 3.77-3.46 (m, 6H, *allo*-D-Thr- $\alpha$ -CH, 3-hydroxy-Pro- $\delta$ -CH, D-Ser- $\beta$ -CH), 3.12-3.01 (m, 2H, Arg- $\delta$ -CH), 2.92-2.62 (m, 4H, Asp- $\beta$ -CH), 2.57-2.38 (m, 2H, H-2), 2.15-1.81 (m, 4H, 3-hydroxy-Pro- $\gamma$ -CH), 1.78-1.59 (m, 2H, Arg- $\beta$ -CH), 1.58-1.37 (m, 4H, Arg- $\gamma$ -CH, H-4, H-14), 1.32-1.02 (m, 25H, Arg- $\gamma$ -CH, D-Ala- $\beta$ -CH, *allo*-D-Thr- $\gamma$ -CH, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 0.80 (d, 6H, H-15,  $J_{15, 14}$  = 6.9 Hz); ESIMS-LR  $m/z$  1126.60 [(M+H)<sup>+</sup>]; ESIMS-HR calcd for C<sub>50</sub>H<sub>84</sub>O<sub>18</sub>N<sub>11</sub> 1126.5990, found 1126.6008; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -10.78 (*c* 0.58, EtOH).

HPLC (column: COSMOSIL 5C<sub>18</sub>-MS-II; eluent: 45% MeCN/H<sub>2</sub>O (0.1% TFA); flow 1 mL/min; detection: UV 210 nm) Retention time: 13.4 min (for detail in Figure S2).



**Figure S2.** Chromatogram of HPLC.

### 第三章

#### **CD measurement**

CD spectra were obtained at 25 °C on a Jasco J720. The solution containing samples (1.7 mg/mL each) in EtOH was prepared, and the spectra were subtracted from EtOH spectrum.

#### **Antimicrobial activity assay**

Antimicrobial susceptibility was determined by broth microdilution assay according to CLSI (Clinical and Laboratory Standards Institute, 2012). Briefly, plusbacin A<sub>3</sub> was serially diluted with cation-adjusted Muller–Hinton Broth (MHB; Becton Dickinson and Company, Franklin Lakes NJ, USA) to obtain a final concentration of 128 µg/mL to 0.125 µg/mL in the wells of a 96-well round-bottomed plate. *Staphylococcus aureus* Smith ATCC13709 was grown overnight in 5 mL tryptic soy broth (TSB; Becton Dickinson and Company) at 37 °C with agitation. The culture was diluted with cation-adjusted MHB to obtain approximately 5 × 10<sup>6</sup> colony forming units (CFU)/mL. 10 µL of the diluted cells were added to each well containing 90 µL of plusbacin A<sub>3</sub>, and mixed well. The plates were incubated at 37 °C and MIC was determined after 20 h as the minimum concentration that did not allow visible growth of bacteria.

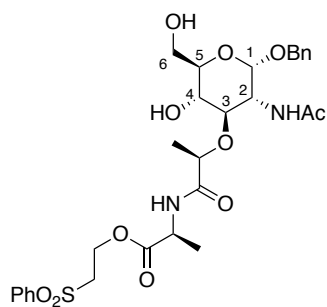
#### **Resistance studies**

*Staphylococcus aureus* Smith ATCC13709 was cultured at 37 °C aerobically in TSB. The full growth was diluted 100-fold with MHB and grown to exponential phase. The exponentially growing cells were further diluted to obtain an OD<sub>600</sub> of 0.01 in 1 mL MHB containing 0.25, 0.5, 1, 2, 4, and 8-fold MIC of plusbacin A<sub>3</sub> or vancomycin or rifampicin. Cells were grown at 37°C with agitation and the cultures from the second highest concentration that allowed visible growth at 24 h were diluted 100-fold with MHB containing different concentrations of respective antibiotics. The sequential culturing was continued for 25 days. After final passage, three independent colonies from plusbacin A<sub>3</sub> treated cells were isolated and their resistance towards plusbacin A<sub>3</sub> was confirmed by MIC determination.

The plusbacin A<sub>3</sub> resistant *S. aureus* Smith strains were cultured at 37 °C aerobically in TSB containing 4 µg/mL plusbacin A<sub>3</sub>. Genomic DNA was isolated using Qiagen DNA-blood Mini Kit (Qiagen, Hilden, Germany) following manufacturer's recommendation using lysostaphin for bacterial lysis. Barcoded library of 400 base-read was prepared after the fragmentation of 100 ng of the DNA using Ion Xpress™ Plus Fragment Library Kit (ThermoFisher Scientific, Waltham, MA USA). The libraries were enriched in an Ion 318™ Chip v2 using Ion Chef (ThermoFischer Scientific), and subsequent sequencing was performed in Ion PGM System (ThermoFischer Scientific). Assembly of the resulting reads and SNP analysis was conducted in the CLC Genomics Workbench ver 9.5.3. (CLC bio, Aarhus, Denmark).

## 第四章

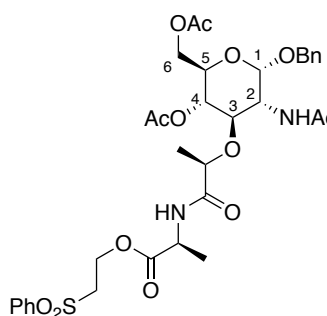
### Benzyl-*N*-acetyl-muramyl-L-alanine phenylsulfonylethyl ester (**135**)



Compound **134** (1.75 g, 2.46 mmol) was treated with 60% AcOH/H<sub>2</sub>O (60 mL) at 90 °C for 1 h. The mixture was concentrated *in vacuo*. The residue was crystallized by CHCl<sub>3</sub>/Et<sub>2</sub>O to afford **135** (1.21 g, 1.94 mmol, 79%) as a white solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.91 (d, 2H, *o*-PhSO<sub>2</sub>,  $J_{o,m} = 7.3$  Hz), 7.69 (t, 1H, *p*-PhSO<sub>2</sub>,  $J_{p,m} = 7.3$  Hz), 7.59 (dd, 2H, *m*-PhSO<sub>2</sub>,  $J_{m,o} = J_{m,p} = 7.3$  Hz), 7.40-7.29 (m, 5H, Ph), 6.92 (d, 1H, Ala-NH,  $J_{\text{Ala-NH, Ala-}\alpha\text{-CH}} = 7.3$  Hz), 5.04 (d, 1H, 2-NH,  $J_{2\text{-NH}, 2} = 9.6$  Hz), 4.91 (d, 1H, H-1,  $J_{1,2} = 3.6$  Hz), 4.71 (d, 1H, Bn,  $J = 11.9$  Hz), 4.47 (d, 1H, Bn,  $J = 11.9$  Hz), 4.50-4.37 (m, 2H, PhSO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 4.29 (dq, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH, Ala-NH}} = J_{\text{Ala-}\alpha\text{-CH, Ala-}\beta\text{-CH}} = 7.3$  Hz), 4.23 (ddd, 1H, H-2,  $J_{2,1} = 3.6$ ,  $J_{2,2\text{-NH}} = 9.6$ ,  $J_{2,3} = 10.1$  Hz), 4.16 (q, 1H, Lac- $\alpha$ -CH,  $J_{\text{Lac-}\alpha\text{-CH, Lac-}\beta\text{-CH}} = 6.9$  Hz), 3.83 (d, 2H, H-6,  $J_{6,5} = 3.2$  Hz), 3.76-3.65 (m, 2H, H-5, H-4), 3.58 (dd, 1H, H-3,  $J_{3,2} = J_{3,4} = 10.1$  Hz), 3.47-3.33 (m, 2H, PhSO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.92 (s, 3H, NAc), 1.42 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH, Lac-}\alpha\text{-CH}} = 6.9$  Hz), 1.33 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 7.3$  Hz). This is a known compound reported in ref 87.

### Benzyl-*N*-acetyl-4,6-diacetylmuramyl-L-alanine phenylsulfonylethyl ester (**136**)

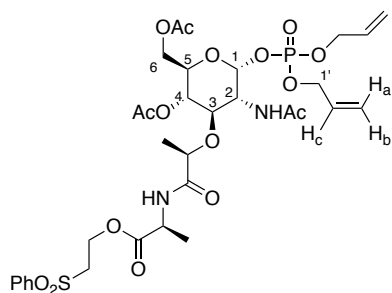


A mixture of **135** (1.12 g, 1.80 mmol) in pyridine (20 mL) was treated with Ac<sub>2</sub>O (406  $\mu$ L, 4.32 mmol) at room temperature for 1 d. Ac<sub>2</sub>O (102  $\mu$ L, 1.08 mmol) was added to the mixture, which was stirred for 24 h. The reaction was quenched with MeOH, then the resulting mixture was concentrated *in vacuo*. The residue was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>, and the organic phase was washed with 1 M *aq.* HCl and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (50-85-100% AcOEt/hexane) to afford **136** (955 mg, 1.35 mmol, 75%) as a colorless foam.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 7.92 (d, 2H, *o*-PhSO<sub>2</sub>,  $J_{o,m} = 6.9$  Hz), 7.68 (t, 1H, *p*-PhSO<sub>2</sub>,  $J_{p,m} = 7.5$  Hz), 7.59 (dd, 2H, *m*-PhSO<sub>2</sub>,  $J_{m,o} = 6.9$ ,  $J_{m,p} = 7.5$  Hz), 7.41-7.56 (m, 5H, Ph), 6.85 (d, 1H, Ala-NH,  $J_{\text{Ala-NH, Ala-}\alpha\text{-CH}} = 6.9$  Hz), 5.81 (d, 1H, 2-NH,  $J_{2\text{-NH}, 2} = 9.2$  Hz), 5.07 (dd, 1H, H-4,  $J_{4,3} = J_{4,5} = 9.8$  Hz), 4.88 (d, 1H, H-1,  $J_{1,2} = 4.0$  Hz), 4.69 (d, 1H, Bn,  $J = 11.5$  Hz), 4.50 (d, 1H, Bn,  $J = 11.5$  Hz), 4.48-4.40 (m, 2H, PhSO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 4.38 (ddd, 1H, H-2,  $J_{2,1} = 4.0$ ,  $J_{2,2\text{-NH}} = 9.2$ ,  $J_{2,3} = 9.8$  Hz), 4.20 (dd, 1H, H-6,  $J_{6,6} = 12.0$ ,  $J_{6,5} = 4.6$  Hz), 4.13 (dq, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH, Ala-NH}} = J_{\text{Ala-}\alpha\text{-CH, Ala-}\beta\text{-CH}} = 6.9$  Hz), 4.03 (dd, 1H, H-6,  $J_{6,6} = 12.0$ ,  $J_{6,5} = 2.3$  Hz), 3.95 (q, 1H, Lac- $\alpha$ -CH,  $J_{\text{Lac-}\alpha\text{-CH, Lac-}\beta\text{-CH}} = 6.3$  Hz), 3.91 (ddd, 1H, H-5,  $J_{5,6} = 2.3$ ,  $J_{5,6} = 4.6$ ,  $J_{5,4} = 9.8$  Hz), 3.64 (dd, 1H, H-3,  $J_{3,2} = J_{3,4} = 9.8$  Hz), 3.50-3.38 (m, 2H, PhSO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.11 (s, 3H, OAc), 2.07 (s, 3H, OAc), 1.88 (s, 3H, NAc), 1.30 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH, Lac-}\alpha\text{-CH}} = 6.3$  Hz), 1.29 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 6.9$  Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.2, 171.8, 170.9, 170.4, 169.5, 139.3, 136.7, 134.2, 129.6, 128.9, 128.7, 128.5, 128.3, 97.2, 78.9, 78.6, 70.4, 69.5, 68.7, 62.3, 58.2, 55.1, 53.1, 48.1, 23.5, 21.0, 20.9, 18.7, 17.0; ESIMS-LR  $m/z$  729.23 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>33</sub>H<sub>42</sub>O<sub>13</sub>N<sub>2</sub>NaS 729.2230, found 729.2299; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +68.36 (*c* 0.23, CHCl<sub>3</sub>).



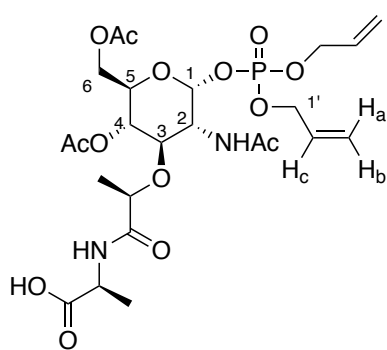
## Glycosylphosphate **137**



A mixture of **136** (424 mg, 0.60 mmol) and 10% Pd/C (600 mg) in MeOH (6 mL) was vigorously stirred under H<sub>2</sub> atmosphere at room temperature for 2.5 h. 10% Pd/C (600 mg) was added to the mixture and vigorously stirred for 8 h under H<sub>2</sub> atmosphere. 10% Pd/C (300 mg) was added to the mixture and vigorously stirred for 12 h under H<sub>2</sub> atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to afford a crude lactol. A mixture of the lactol and 5-(benzylthio)-1*H*-tetrazole (208 mg, 1.08 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was treated with diallyl *N,N*-diisopropylphosphoramidite (238 μL, 0.90 mmol) at 0 °C for 5 min. The mixture was warmed to room temperature and stirred for 1 h. 5-(Benzylthio)-1*H*-tetrazole (138 mg, 0.72 mmol) and diallyl *N,N*-diisopropylphosphoramidite (159 μL, 0.60 mmol) was added to the mixture and stirred for 1 h. The mixture was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and *sat. aq.* NaHCO<sub>3</sub>, and the organic phase was washed with H<sub>2</sub>O and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo* to afford a crude phosphite. A mixture of the phosphite in THF (6 mL) was treated with 30% H<sub>2</sub>O<sub>2</sub> (600 μL) at -78 °C for 5 min. The mixture was warmed to room temperature and stirred for 2.5 h. The reaction was quenched with *sat. aq.* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> at 0 °C, and the mixture was partitioned between AcOEt and *sat. aq.* NaHCO<sub>3</sub>. The organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (50-100% AcOEt/hexane-0-1-2% MeOH/AcOEt) to afford **137** (180 mg, 0.23 mmol, 39% over 3 steps) as a colorless foam.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 7.93 (d, 2H, *o*-PhSO<sub>2</sub>, *J*<sub>*o,m*</sub> = 7.4 Hz), 7.67 (t, 1H, *p*-PhSO<sub>2</sub>, *J*<sub>*p,m*</sub> = 7.5 Hz), 7.60 (dd, 2H, *m*-PhSO<sub>2</sub>, *J*<sub>*m,o*</sub> = 7.4, *J*<sub>*m,p*</sub> = 7.5 Hz), 6.76 (d, 1H, Ala-NH, *J*<sub>Ala-NH, Ala-α-CH</sub> = 6.9 Hz), 6.55 (d, 1H, 2-NH, *J*<sub>2-NH, 2</sub> = 8.6 Hz), 6.01-5.88 (m, 2H, H<sub>c</sub>), 5.70 (dd, 1H, H-1, *J*<sub>1,2</sub> = 2.9, *J*<sub>1,p</sub> = 5.8 Hz), 5.40 (dd, 1H, H<sub>a</sub>, *J*<sub>H<sub>a</sub>, H<sub>c</sub></sub> = 17.2, *J*<sub>H<sub>a</sub>, 1'</sub> = 1.2 Hz), 5.38 (dd, 1H, H<sub>a</sub>, *J*<sub>H<sub>a</sub>, H<sub>c</sub></sub> = 17.2, *J*<sub>H<sub>a</sub>, 1'</sub> = 1.2 Hz), 5.31 (dd, 1H, H<sub>b</sub>, *J*<sub>H<sub>b</sub>, H<sub>c</sub></sub> = 10.3, *J*<sub>H<sub>a</sub>, 1'</sub> = 1.2 Hz), 5.30 (dd, 1H, H<sub>b</sub>, *J*<sub>H<sub>b</sub>, H<sub>c</sub></sub> = 10.3, *J*<sub>H<sub>a</sub>, 1'</sub> = 1.2 Hz), 5.14 (dd, 1H, H-4, *J*<sub>4,3</sub> = 10.3, *J*<sub>4,5</sub> = 9.8 Hz), 4.64-4.55 (m, 4H, H-1'), 4.53-4.44 (m, 2H, PhSO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 4.41-4.35 (m, 1H, H-2), 4.24 (dq, 1H, Ala-α-CH, 7.5, *J*<sub>Ala-α-CH, Ala-NH</sub> = *J*<sub>Ala-α-CH, Ala-β-CH</sub> = 6.9 Hz), 4.20 (dd, 1H, H-6, *J*<sub>6,6</sub> = 12.0, *J*<sub>6,5</sub> = 4.0 Hz), 4.13-4.05 (m 2H, H-5, H-6), 4.03 (q, 1H, Lac-α-CH, *J*<sub>Lac-α-CH, Lac-β-CH</sub> = 6.3 Hz), 3.69 (dd, 1H, H-3, *J*<sub>3,2</sub> = *J*<sub>3,4</sub> = 10.3 Hz), 3.52-3.41 (m, 2H, PhSO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.96 (s, 3H, NAc), 1.34 (d, 3H, Lac-β-CH, *J*<sub>Lac-β-CH, Lac-α-CH</sub> = 6.3 Hz), 1.33 (d, 3H, Ala-β-CH, *J*<sub>Ala-β-CH, Ala-α-CH</sub> = 7.5 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 172.3, 171.6, 170.9, 170.8, 169.3, 139.2, 134.3, 132.2, 132.2, 132.0, 132.0, 129.6, 128.2, 119.4, 119.3, 78.4, 77.0, 70.3, 69.1, 69.0, 69.0, 68.9, 61.8, 58.2, 55.0, 53.3, 53.3, 48.1, 25.1, 23.3, 20.9, 20.9, 18.9, 17.2; <sup>31</sup>P NMR (CDCl<sub>3</sub>, 202 MHz) δ -1.8; ESIMS-LR *m/z* 799.21 [(M+Na)<sup>+</sup>]; ESIMS-HR calcd for C<sub>32</sub>H<sub>45</sub>O<sub>16</sub>N<sub>2</sub>NaPS 799.2120, found 799.2125; [α]<sub>D</sub><sup>20</sup> +49.99 (*c* 1.49, CHCl<sub>3</sub>).

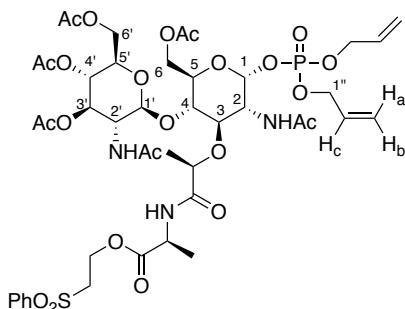
### Glycosylphosphate 132



A mixture of **137** (212 mg, 0.27 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was treated with DBU (44.8  $\mu\text{L}$ , 0.30 mmol) at room temperature for 40 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl, and the aqueous phase was extracted with AcOEt ( $\times 2$ ). Combined organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-1-2% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to afford **132** (149 mg, 0.25 mmol, 90%) as a colorless foam.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.47 (d, 1H, 2-NH,  $J_{2\text{-NH},2} = 8.0$  Hz), 6.86 (d, 1H, Ala-NH,  $J_{\text{Ala-NH}, \text{Ala-}\alpha\text{-CH}} = 6.3$  Hz), 5.98-5.88 (m, 2H, Hc), 5.72 (dd, 1H, H-1,  $J_{1,2} = 3.4$ ,  $J_{1\text{P}} = 6.3$  Hz), 5.39 (dd, 1H,  $H_a$ ,  $J_{H_a, H_c} = 16.6$ ,  $J_{H_a, 1'} = 1.2$  Hz), 5.38 (dd, 1H,  $H_a$ ,  $J_{H_a, H_c} = 17.2$ ,  $J_{H_a, 1'} = 1.2$  Hz), 5.30 (d, 2H,  $H_b$ ,  $J_{H_b, H_c} = 10.3$  Hz), 5.12 (dd, 1H, H-4,  $J_{4,3} = J_{4,5} = 9.2$  Hz), 4.62-4.55 (m, 4H, H-1'), 4.37 (qd, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH}, \text{Ala-}\beta\text{-CH}} = J_{\text{Ala-}\alpha\text{-CH}, \text{Ala-NH}} = 6.9$  Hz), 4.31-4.25 (m, 1H, H-2), 4.22-4.15 (m, 2H, Lac- $\alpha$ -CH, H-6), 4.09-4.04 (m 2H, H-5, H-6), 3.75 (dd, 1H, H-3,  $J_{3,2} = J_{3,4} = 9.7$  Hz), 2.11 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.97 (s, 3H, NAc), 1.47 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.33 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH}, \text{Lac-}\alpha\text{-CH}} = 6.3$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  174.4, 173.4, 171.7, 170.7, 170.8, 169.4, 132.1, 132.0, 131.9, 131.8, 119.5, 119.2, 96.8, 96.8, 78.3, 76.7, 70.4, 69.3, 69.2, 69.0, 69.0, 61.9, 53.5, 53.4, 48.5, 23.0, 20.9, 20.8, 19.2, 17.5;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 202 MHz)  $\delta$  -2.7; ESIMS-LR  $m/z$  631.19 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{24}\text{H}_{37}\text{O}_{14}\text{N}_2\text{NaP}$  631.1875, found 631.1882;  $[\alpha]_D^{20} +63.23$  ( $c$  3.80,  $\text{CHCl}_3$ ).

### Glycosylphosphate 140



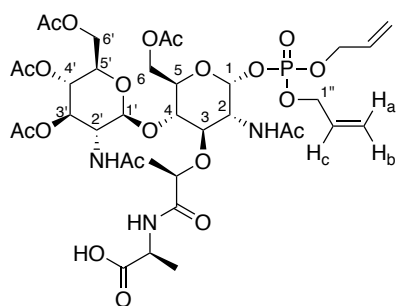
A mixture of **139** (497 mg, 0.50 mmol) and 10% Pd/C (600 mg) in MeOH/EtOH = 1/1 (10 mL) was vigorously stirred under  $\text{H}_2$  atmosphere at room temperature for 24 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to afford a crude lactol. A mixture of the lactol and 1*H*-tetrazole (70 mg, 1.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was treated with diallyl *N,N*-diisopropylphosphoramidite (198  $\mu\text{L}$ , 0.75 mmol) at 0  $^\circ\text{C}$  for 5 min. The mixture was warmed to room temperature and stirred for 1.5 h.

Diallyl *N,N*-diisopropylphosphoramidite (49.5  $\mu\text{L}$ , 0.19 mmol) was added to the mixture and stirred for 10 min. The mixture was cooled to -50  $^\circ\text{C}$ , and treated with 80%  $t\text{BuOOH}$  (1 mL) for 1 h. The reaction was quenched with *sat. aq.*  $\text{Na}_2\text{S}_2\text{O}_3$ , and the mixture was partitioned between AcOEt and *sat. aq.*  $\text{NaHCO}_3$ . The organic phase was washed with  $\text{H}_2\text{O}$  and brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-1-2-5% MeOH/AcOEt) to afford **140** (271 mg, 0.25 mmol, 50% over 2 steps) as a colorless foam.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.93 (d, 2H, *o*-PhSO $_2$ ,  $J_{o,m} = 7.5$  Hz), 7.70 (t, 1H, *p*-PhSO $_2$ ,  $J_{p,m} = 7.5$  Hz), 7.66-7.58 (m, 3H, *m*-PhSO $_2$ , 2-NH), 7.18 (d, 1H, Ala-NH,  $J_{\text{Ala-NH}, \text{Ala-}\alpha\text{-CH}} = 8.1$  Hz), 6.10 (d, 1H, 2'-NH,  $J_{2'\text{-NH}, 2'} = 8.6$  Hz), 5.98-5.87 (m, 3H, Hc, H-1), 5.37 (dd, 1H,  $H_a$ ,  $J_{H_a, H_c} = 17.2$ ,  $J_{H_a, 1'} = 1.2$  Hz), 5.35 (dd, 1H,  $H_a$ ,  $J_{H_a, H_c} = 17.2$ ,  $J_{H_a, 1'} = 1.2$  Hz), 5.26 (d, 1H,  $H_b$ ,  $J_{H_b, H_c} = 10.3$  Hz), 5.24 (d, 1H,  $H_b$ ,  $J_{H_b, H_c} = 10.3$  Hz), 5.20 (dd, 1H, H-3',  $J_{3', 4'} = 9.8$ ,  $J_{3', 2'} = 10.9$  Hz), 5.11 (dd, 1H, H-4',  $J_{4', 3'} = J_{4', 5'} = 10.9$  Hz), 4.64 (q, 1H, Lac- $\alpha$ -CH,  $J_{\text{Lac-}\alpha\text{-CH}, \text{Lac-}\beta\text{-CH}} = 6.3$  Hz), 4.61-4.54 (m, 3H, H-1', H-1''), 4.54-4.48 (m, 4H, PhSO $_2\text{CH}_2\text{CH}_2$ , H-1''), 4.39 (dq, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH}, \text{Ala-NH}} = 8.1$ ,  $J_{\text{Ala-}\alpha\text{-CH}, \text{Ala-}\beta\text{-CH}}$

= 7.5 Hz), 4.35-4.27 (m, 2H, H-6, H-6'), 4.23 (dd, 1H, H-6,  $J_{H-6, H-6'} = 12.6$ ,  $J_{6, 5} = 3.4$  Hz), 4.09 (dd, 1H, H-6',  $J_{6', 6} = 12.6$ ,  $J_{6', 5'} = 2.3$  Hz), 4.02-3.85 (m, 4H, H-2, H-2', H-4, H-5), 3.68-3.63 (m, 1H, H-5'), 3.55 (dd, 1H, H-3,  $J_{3, 4} = J_{H-3, H-2} = 10.1$  Hz), 3.51 (t, 2H,  $\text{PhSO}_2\text{CH}_2\text{CH}_2$ ,  $J_{\text{PhSO}_2\text{CH}_2\text{CH}_2, \text{PhSO}_2\text{CH}_2\text{CH}_2} = 5.7$  Hz), 2.11 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.00 (s, 3H, NAc), 1.99 (s, 3H, NAc), 1.96 (s, 3H, NAc), 1.38 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH}, \text{Lac-}\alpha\text{-CH}} = 6.9$  Hz), 1.35 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 7.5$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  174.8, 171.8, 171.4, 171.3, 171.2, 171.0, 170.7, 169.5, 139.1, 134.3, 132.4, 129.6, 128.3, 118.8, 118.7, 99.8, 95.7, 95.7, 75.0, 74.0, 72.2, 72.2, 71.2, 68.7, 68.6, 68.6, 68.6, 68.4, 62.2, 61.9, 58.4, 55.1, 54.9, 53.9, 53.8, 48.1, 23.4, 23.1, 21.0, 20.8, 20.7, 18.9, 17.3;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 162 MHz)  $\delta$  -2.3; ESIMS-LR  $m/z$  1086.31 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{44}\text{H}_{62}\text{O}_{23}\text{N}_3\text{NaPS}$  1086.3125, found 1086.3120;  $[\alpha]_D^{20} +8.24$  ( $c$  1.24,  $\text{CHCl}_3$ ).

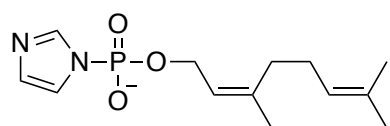
### Glycosylphosphate **130**



A mixture of **140** (127 mg, 0.12 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.5 mL) was treated with DBU (19.4  $\mu\text{L}$ , 0.13 mmol) at room temperature for 40 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl, and the aqueous phase was extracted with AcOEt ( $\times 2$ ). Combined organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-5-10% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to afford **130** (100 mg, 0.11 mmol, 93%) as a colorless foam.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.92 (d, 1H, 2-NH,  $J_{2\text{-NH}, 2} = 4.0$  Hz), 7.73 (d, 1H, Ala-NH,  $J_{\text{Ala-NH}, \text{Ala-}\alpha\text{-CH}} = 7.5$  Hz), 6.49 (d, 1H, 2'-NH,  $J_{2'\text{-NH}, 2'} = 9.7$  Hz), 5.95-5.84 (m, 3H, Hc, H-1), 5.37 (d, 2H, Ha,  $J_{\text{Ha}, \text{Hc}} = 17.2$  Hz), 5.25 (dd, 1H, Hb,  $J_{\text{Hb}, \text{Hc}} = 10.3$ ,  $J_{\text{Ha}, 1'} = 1.2$  Hz), 5.24 (dd, 1H, Hb,  $J_{\text{Hb}, \text{Hc}} = 10.3$ ,  $J_{\text{Hb}, 1'} = 1.2$  Hz), 5.12-5.05 (m, 2H, H-3', H-4'), 4.71 (q, 1H, Lac- $\alpha$ -CH,  $J_{\text{Lac-}\alpha\text{-CH}, \text{Lac-}\beta\text{-CH}} = 6.3$  Hz), 4.59-4.52 (m, 3H, H-1', H-1''), 4.52-4.45 (m, 2H, H-1''), 4.45 (dq, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH}, \text{Ala-NH}} = J_{\text{Ala-}\alpha\text{-CH}, \text{Ala-}\beta\text{-CH}} = 7.5$  Hz), 4.31 (dd, 1H, H-6,  $J_{6, 6} = 12.6$ ,  $J_{6, 5} = 4.6$  Hz), 4.22 (d, 1H, H-6,  $J_{6, 6} = 12.6$ ,  $J_{6, 5} = 3.4$  Hz), 4.17-4.09 (m, 1H, H-2'), 4.08 (dd, 1H, H-6,  $J_{6, 6} = 12.6$ ,  $J_{6, 5} = 2.3$  Hz), 3.97 (dd, 1H, H-4,  $J_{4, 5} = J_{4, 3} = 9.7$  Hz), 3.92-3.86 (m, 1H, H-2), 3.77-3.71 (m, 1H, H-5), 3.66-3.58 (m, 2H, H-5, H-3'), 2.09 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, NAc), 2.00 (s, 3H, NAc), 1.96 (s, 3H, NAc), 1.48 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.35 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH}, \text{Lac-}\alpha\text{-CH}} = 6.3$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.7, 174.3, 171.8, 171.1, 171.1, 170.6, 169.5, 132.1, 132.0, 132.0, 131.9, 119.1, 118.9, 99.3, 95.6, 95.5, 73.7, 73.0, 72.3, 72.0, 71.7, 69.1, 69.0, 68.9, 68.8, 68.5, 62.5, 61.9, 54.7, 53.9, 53.9, 48.8, 23.3, 23.0, 20.9, 20.8, 20.7, 20.7, 18.7, 16.8;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 202 MHz)  $\delta$  -3.2; ESIMS-LR  $m/z$  918.29 [(M+Na) $^+$ ]; ESIMS-HR calcd for  $\text{C}_{36}\text{H}_{54}\text{O}_{21}\text{N}_3\text{NaP}$  918.2880, found 918.2885;  $[\alpha]_D^{20} +7.00$  ( $c$  0.21,  $\text{CHCl}_3$ ).

### Neryl phosphoryl imidazolid (131)



Neryl phosphate ammonium salt (**141**, 2.9 mg, 0.012 mmol) was co-evaporated with  $\text{Et}_3\text{N}$  (20  $\mu\text{L}$ ) in pyridine (1 mL) twice. The residue was co-evaporated with toluene (1 mL) twice to afford neryl phosphate triethylamine salt. A mixture of the phosphate salt in DMF was treated with 1,1'-carbonyldiimidazole (9.5 mg, 0.059 mmol) at room temperature for 3 h. The reaction was quenched with MeOH (100  $\mu\text{L}$ ) and stirred for 30 min. The mixture was concentrated *in vacuo* and co-evaporated with toluene twice to afford **131**. This compound was used without further purification.

$^{31}\text{P}$  NMR (DMSO- $d_6$ , 162 MHz)  $\delta$  -9.6.

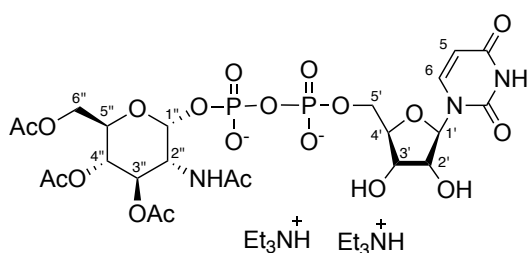
### Procedure for the synthesis of **129**

Each HMBA-PEG resin (150 mg, 0.71 mmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. Each resin was agitated with  $\text{CH}_2\text{Cl}_2$  (1.5 mL, 1 h), After removal of  $\text{CH}_2\text{Cl}_2$ , a solution of Fmoc-D-Ala-OH $\cdot$ H $_2$ O (69 mg, 0.21 mmol) and *N,N'*-dissopropylcarbodiimide (33  $\mu\text{L}$ , 0.21 mmol) in DMF (1 mL) was added at 0 °C. Each mixture was agitated for 40 min. 4-Dimethylaminopyridine (2.5 mg, 0.021 mmol) was added to the mixture at 0 °C, which was warmed to room temperature. After agitation for 1 h at room temperature, solvent and soluble reagents were removed by suction. All resins were subjected to the following washing treatment with DMF (2 mL $\times$ 3), EtOH/ $\text{CH}_2\text{Cl}_2$  = 1/1 (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3) and DMF (2 mL  $\times$ 3). The resins were treated with  $\text{Bz}_2\text{O}$  (48 mg, 0.021 mmol) in 20% pyridine/DMF (1 mL) at room temperature for 1 h, and the resins were washed with DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) to afford **142**. The amount of loading on the resin was determined as follow. Dried **142** (6.0 mg) was agitated with DMF (2 mL) for 30 min, and DBU (40  $\mu\text{L}$ ) was added to the mixture. The mixture was agitated for 30 min. The supernatant was diluted with DMF and MeCN and subjected to UV measurement at 294 nm. The loading rate was determined to be 0.39 mmol/g from the observed absorbance (0.172). The resins **142** were treated with piperidine/DMF (1:4, 5 min, then 1:9, 15 min) to remove the Fmoc group, and the resins were washed with DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3). A solution of Fmoc-D-Ala-OH $\cdot$ H $_2$ O (94 mg, 0.28 mmol), HBTU (105 mg, 0.28 mmol) and  $^i\text{Pr}_2\text{NEt}$  (97  $\mu\text{L}$ , 0.57 mmol) in DMF (750  $\mu\text{L}$ ) was added to the resins, which were agitated for 2 h. All the resins were washed with DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) to afford **143**. Kaiser test indicated the completion of the all coupling reactions. The loading rate was determined as described above. Namely, dried **143** (4.9 mg) was agitated with DMF (2 mL) for 30 min, DBU (40  $\mu\text{L}$ ) was added to the mixture. The mixture was agitated for 30 min. The supernatant was diluted with DMF and MeCN and subjected to UV measurement at 294 nm. The yield was determined to be quantitative from the observed absorbance (0.135). The resins **143** were treated with piperidine/DMF (1:4, 5 min, then 1:9, 15 min) to remove Fmoc group, and the resins were washed with DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3). A solution of Alloc-L-Lys(Fmoc)-OH (96 mg, 0.21 mmol), HBTU (68 mg, 0.21 mmol) and  $^i\text{Pr}_2\text{NEt}$  (72  $\mu\text{L}$ , 0.43 mmol) in DMF (750  $\mu\text{L}$ ) was added to the resins, which were agitated for 2 h. All the resins were washed with DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) to afford **144**. Kaiser test indicated the completion of the all coupling reactions. The amount of loading on the resin was determined as follow. Namely, dried **144** (5.3 mg) was agitated with DMF (2 mL) for 30 min, DBU (40  $\mu\text{L}$ ) was added to the mixture. The mixture was agitated for 30 min. The supernatant was diluted with DMF and MeCN and subjected to UV measurement at 294 nm. The yield was determined to be 87% over 2 steps from the observed absorbance (0.107). The resins **144** were treated with a solution of  $\text{BH}_3\cdot\text{Me}_2\text{NH}$  (25 mg, 0.43 mmol) in EtOH (600  $\mu\text{L}$ ) for 5min, then a solution of  $\text{Pd}(\text{PPh}_3)_4$  (16 mg, 0.014 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was added to the mixture. The mixture was agitated for 15 min. All the resins were washed with 0.5%  $^i\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL $\times$ 8), MeOH (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3). A solution of Alloc-D-Glu-OBn (68 mg, 0.21 mmol), HBTU (68 mg, 0.21 mmol) and  $^i\text{Pr}_2\text{NEt}$  (72  $\mu\text{L}$ , 0.43 mmol) in DMF (750  $\mu\text{L}$ ) was added to the resins, which were agitated for 2 h. All the resins were washed with DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3). Kaiser test indicated the completion of the all coupling reactions. The resin was dried *in vacuo* to afford **129** (194 mg, 0.055 mmol, 0.28 mmol/g, 89% over 2 steps). The yield was calculated by weighing resins.

### Procedure for the synthesis of 146

Resin-bound peptide **129** (100 mg, 0.028 mmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL, 1 h). After removal of CH<sub>2</sub>Cl<sub>2</sub>, the resin was treated with a solution of BH<sub>3</sub>·Me<sub>2</sub>NH (18 mg, 0.33 mmol) in EtOH (600 μL) for 5 min, then a solution of Pd(PPh<sub>3</sub>)<sub>4</sub> (13 mg, 0.011 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was added to the mixture. The mixture was agitated for 15 min. The resin was washed with 0.5% <sup>t</sup>Pr<sub>2</sub>NEt/CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL×8), MeOH (2 mL×3) and DMF/CH<sub>2</sub>Cl<sub>2</sub> = 1/1 (2 mL×3). A solution of **130** (49 mg, 0.055 mmol), PyAOP (38 mg, 0.073 mmol), HOAt (5.0 mg, 0.037 mmol), and **145** (20 mg, 0.11 mmol) in DMF/CH<sub>2</sub>Cl<sub>2</sub> (600 μL) was added to the resin which was agitated for 3 h. The resin was washed with DMF (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3) and DMF/CH<sub>2</sub>Cl<sub>2</sub> = 1/1 (2 mL×3). A solution of **130** (49 mg, 0.055 mmol), PyAOP (38 mg, 0.073 mmol), HOAt (5.0 mg, 0.037 mmol), and **145** (20 mg, 0.11 mmol) in DMF/CH<sub>2</sub>Cl<sub>2</sub> (600 μL) was added to the resin which was agitated for 3 h. The resin was washed with DMF (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3). The resin was treated with a solution of Ac<sub>2</sub>O (10 μL, 0.11 mmol) and <sup>t</sup>Pr<sub>2</sub>NEt (19 μL, 0.11 mmol) in DMF (700 μL) for 30 min. The resin was washed with DMF (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3). Kaiser test indicated the completion of the coupling reaction. The resin was dried *in vacuo* to afford **146** (156 mg, 0.028 mmol, 0.18 mmol/g, quantitative over 2 steps).

### Diphosphate 149

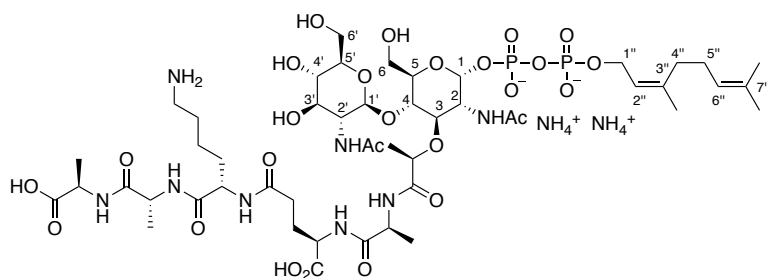


A solution of **121** (16.5 mg, 0.024 mmol) and **148** (12.7 mg, 0.024 mmol) in DMF (250 μL) was treated with **153** (5.3 mg, 0.024 mmol) at 25 °C for 24 h. The mixture was concentrated *in vacuo*. The residue was purified by high-flash ODS column chromatography (0-10% MeCN/25 mM *aq.* AcOH·Et<sub>3</sub>N). The product was further purified by reversed phase HPLC (YMC-Pack

R&D ODS-A, 250 × 20 mm, 8% MeCN/25 mM *aq.* AcOH·Et<sub>3</sub>N) to afford **149** (6.9 mg, 0.0074 mmol, 31%) as a white powder, after freeze drying.

<sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz) δ 9.53 (d, 1H, 2''-NH, *J*<sub>2''-NH, 2''</sub> = 9.2 Hz), 7.93 (d, 1H, H-6, *J*<sub>6, 5</sub> = 6.3 Hz), 5.75 (s, 1H, H-1'), 5.56 (d, 1H, H-5, *J*<sub>5, 6</sub> = 7.5 Hz), 5.37-5.32 (m, 1H, H-1''), 5.08 (dd, 1H, H-3, *J*<sub>3, 2</sub> = 9.2, *J*<sub>3, 4</sub> = 9.7 Hz), 4.93 (dd, 1H, H-4, *J*<sub>4, 3</sub> = *J*<sub>4, 5</sub> = 9.5 Hz), 4.19-3.91 (m, 9H, H-2', H-3', H-4', H-5', H-2'', H-5'', H-6''), 3.11-3.07 (m, 2H, CH<sub>3</sub>CH<sub>2</sub>N), 2.01 (s, 3H, OAc), 1.94 (s 3H, OAc), 1.85 (s, 3H, OAc), 1.83 (s, 3H, NAc), 1.17 (t, 3H CH<sub>3</sub>CH<sub>2</sub>N); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz) δ 170.1, 170.1, 169.6, 169.3, 163.2, 150.8, 140.8, 139.5, 101.7, 93.9, 82.8, 71.6, 67.4, 67.7, 68.4, 67.7, 61.5, 50.9, 47.7, 45.5, 22.3, 20.5, 20.4, 19.0, 11.0, 8.6; <sup>31</sup>P NMR (DMSO-*d*<sub>6</sub>, 202 MHz) δ -11.3 (d, *J*<sub>P, P</sub> = 26.3 Hz), -14.1 (d, *J*<sub>P, P</sub> = 26.3 Hz); ESIMS-LR *m/z* 732.11 [(M-H)<sup>-</sup>]; ESIMS-HR calcd for C<sub>23</sub>H<sub>32</sub>O<sub>20</sub>N<sub>3</sub>P<sub>2</sub> 732.1072, found 732.1060; [α]<sub>D</sub><sup>20</sup> +10.94 (*c* 0.60, DMSO-*d*<sub>6</sub>).

## Neryl-lipid II (126)



Resin-bound peptide **147** (12.4 mg, 2.2  $\mu\text{mol}$ ) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with  $\text{CH}_2\text{Cl}_2$  (1 mL, 1 h). After removal of  $\text{CH}_2\text{Cl}_2$ , the resin was treated with a solution of  $\text{Pd}(\text{PPh}_3)_4$  (2.0 mg, 1.7  $\mu\text{mol}$ ) and  $\text{PhSiH}_3$  (8.9  $\mu\text{L}$ , 73  $\mu\text{mol}$ ) in

$\text{CH}_2\text{Cl}_2$  (300  $\mu\text{L}$ ) for 2 h. The resin was washed with 0.5%  ${}^i\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL $\times$ 8), MeOH (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) and DMF (2 mL $\times$ 3). The resin was treated with a solution of **131** (12  $\mu\text{mol}$ ) and **153** (2.6 mg, 12  $\mu\text{mol}$ ) in DMF (300  $\mu\text{L}$ ) at 50  $^\circ\text{C}$  for 3 d. The resin was washed with DMF (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) to afford **154**. The resin was treated with piperidine/DMF (1:4, 5 min, then 1:9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5%  ${}^i\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), DMF (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) and THF (2 mL  $\times$ 3). The resin was treated with 2 M *aq.* LiOH/MeOH/THF = 1/1/2 (400  $\mu\text{L}$ ) at 0  $^\circ\text{C}$ , then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq.* HCl (220  $\mu\text{L}$ ) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250  $\times$  20 mm, 15% MeCN/50 mM *aq.*  $\text{NH}_4\text{HCO}_3$ ) to afford **126** (0.8 mg, 0.64  $\mu\text{mol}$ , 21% over 12 steps) as a white powder after freeze drying.

${}^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  5.47-5.41 (m, 2H, H-1, H-2''), 5.22-5.17 (m, 1H, H-6''), 4.62 (d, 1H, H-1',  $J_{1', 2'} = 8.6$  Hz), 4.45 (dd, 2H, H-1'',  $J_{1'', 2''} = J_{1'', \text{P}} = 6.9$  Hz), 4.33 (q, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH}, \text{Ala-}\beta\text{-CH}} = 7.5$  Hz), 4.28-4.12 (m, 5H, Ala- $\alpha$ -CH, Lys- $\alpha$ -CH, D-Glu- $\alpha$ -CH, H-2), 4.11 (q, 1H, Lac- $\alpha$ -CH,  $J_{\text{Lac-}\alpha\text{-CH}, \text{Lac-}\beta\text{-CH}} = 7.5$  Hz), 3.97-3.88 (m, 4H, H-4, H-6, H-6'), 3.81 (dd, 1H, H-3,  $J_{3, 4} = J_{3, 2} = 9.7$  Hz), 3.77-3.69 (m, 3H, H-5, H-2', H-6'), 3.55 (dd, 1H, H-3',  $J_{3', 4'} = J_{3', 2'} = 8.6$  Hz), 3.44-3.38 (m, 2H, H-4', H-5'), 3.00 (t, 1H, Lys- $\epsilon$ -CH,  $J_{\text{Lys-}\epsilon\text{-CH}, \text{Lys-}\delta\text{-CH}} = 7.5$  Hz), 2.31 (t, 2H, D-Glu- $\gamma$ -CH,  $J_{\text{D-Glu-}\gamma\text{-CH}, \text{D-Glu-}\beta\text{-CH}} = 8.6$  Hz), 2.20-2.10 (m, 5H, D-Glu- $\beta$ -CH, H-4'', H-5''), 2.05 (s, 3H, NAc), 1.99 (s, 3H, NAc), 1.93-1.85 (m, 1H, D-Glu- $\beta$ -CH), 1.85-1.75 (m, 2H, Lys- $\beta$ -CH), 1.77 (s, 3H, 3''-Me), 1.74-1.65 (m, 2H, Lys- $\delta$ -CH), 1.69 (s, 3H, 7''-Me), 1.62 (s, 3H, 7''-Me), 1.51-1.41 (m, 2H, Lys- $\gamma$ -CH), 1.45 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.44 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 6.9$  Hz), 1.37 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.33 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH}, \text{Lac-}\alpha\text{-CH}} = 6.9$  Hz);  ${}^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ , 202 MHz)  $\delta$  -10.2 (d,  $J_{\text{P}, \text{P}} = 21.4$  Hz), -12.7 (d,  $J_{\text{P}, \text{P}} = 21.4$  Hz); ESIMS-LR  $m/z$  630.24 [(M-2H) $^{2-}$ ]; ESIMS-HR calcd for  $\text{C}_{49}\text{H}_{82}\text{O}_{26}\text{N}_8\text{P}_2$  630.2413, found 630.2425; [ $\alpha$ ] $^{20}_{\text{D}} + 10.00$  ( $c$  0.08, MeOH).

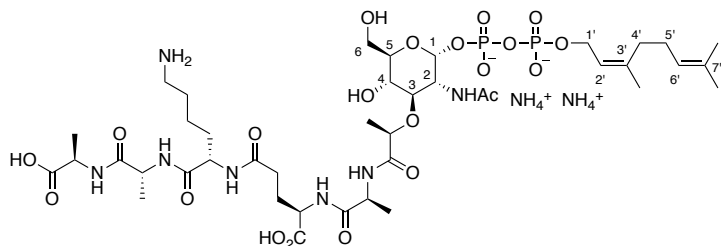
The analytical data for synthetic **126** were in good agreement with the previously reported data.<sup>87</sup>

### Procedure for the synthesis of **155**

Resin-bound peptide **129** (150 mg, 0.042 mmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with  $\text{CH}_2\text{Cl}_2$  (1.5 mL, 1 h). After removal of  $\text{CH}_2\text{Cl}_2$ , the resin was treated with a solution of  $\text{BH}_3 \cdot \text{Me}_2\text{NH}$  (13 mg, 0.22 mmol) in EtOH (600  $\mu\text{L}$ ) for 5 min, then a solution of  $\text{Pd}(\text{PPh}_3)_4$  (8.4 mg, 7.3  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was added to the mixture. The mixture was agitated for 15 min. The resin was washed with 0.5%  ${}^i\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL $\times$ 8), MeOH (2 mL $\times$ 3) and DMF/ $\text{CH}_2\text{Cl}_2$  = 1/1 (2 mL $\times$ 3). A solution of **132** (50 mg, 0.082 mmol), PyAOP (57 mg, 0.11 mmol), HOAt (7.5 mg, 0.055 mmol), and **145** (30 mg, 0.16 mmol) in DMF/ $\text{CH}_2\text{Cl}_2$  (700  $\mu\text{L}$ ) was added to the resin which

was agitated for 3 h. The resin was washed with DMF (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3) and DMF/CH<sub>2</sub>Cl<sub>2</sub> = 1/1 (2 mL×3). A solution of **132** (50 mg, 0.082 mmol), PyAOP (57 mg, 0.11 mmol), HOAt (7.5 mg, 0.055 mmol), and **145** (30 mg, 0.16 mmol) in DMF/CH<sub>2</sub>Cl<sub>2</sub> (700 μL) was added to the resin which was agitated for 3 h. The resin was washed with DMF (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3). The resin was treated with a solution of Ac<sub>2</sub>O (16 μL, 0.16 mmol) and <sup>t</sup>Pr<sub>2</sub>NEt (29 μL, 0.16 mmol) in DMF (800 μL) for 30 min. The resin was washed with DMF (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3). Kaiser test indicated the completion of the coupling reaction. The resin was dried *in vacuo* to afford **155** (189 mg, 0.042 mmol, 0.22 mmol/g, quantitative over 2 steps). The yield was calculated by weighing resin.

### Neryl-lipid I (**157**)

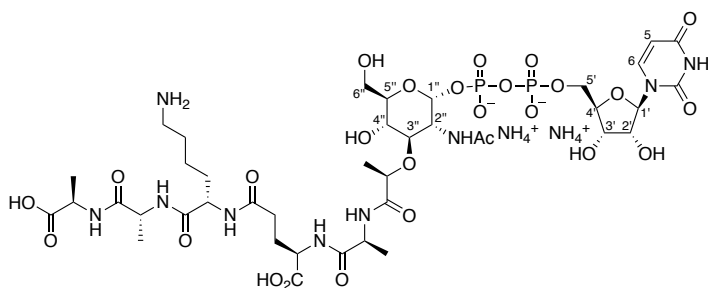


Resin-bound peptide **155** (9.4 mg, 2.1 μmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with CH<sub>2</sub>Cl<sub>2</sub> (1 mL, 1 h). After removal of CH<sub>2</sub>Cl<sub>2</sub>, the resin was treated with a solution of Pd(PPh<sub>3</sub>)<sub>4</sub> (1.9 mg, 1.6 μmol) and PhSiH<sub>3</sub> (8.4 μL,

69 μmol) in CH<sub>2</sub>Cl<sub>2</sub> (400 μL) for 2 h. The resin was washed with 0.5% <sup>t</sup>Pr<sub>2</sub>NEt/CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL×8), MeOH (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3) and DMF (2 mL×3). The resin was treated with a solution of **131** (11 μmol) and **153** (2.4 mg, 11 μmol) in DMF (300 μL) at 50 °C for 2 d. The resin was washed with DMF (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3) to afford **156**. The resin was treated with piperidine/DMF (1:4, 5 min, then 1:9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5% <sup>t</sup>Pr<sub>2</sub>NEt/CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3), DMF (2 mL×3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL×3) and THF (2 mL×3). The resin was treated with 2 M *aq.* LiOH/MeOH/THF = 1/1/2 (400 μL) at 0 °C, then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq.* HCl (210 μL) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250 × 20 mm, 15% MeCN/50 mM *aq.* NH<sub>4</sub>HCO<sub>3</sub>) to afford **157** (0.6 mg, 0.55 μmol, 20% over 12 steps) as a white powder after freeze drying.

<sup>1</sup>H NMR (D<sub>2</sub>O, 500 MHz) δ 5.47-5.42 (m, 2H, H-1, H-2'), 5.20 (t, 1H, H-6', *J*<sub>6',5'</sub> = 6.9 Hz), 4.49-4.43 (m, 2H, H-1'), 4.33 (q, 1H, Ala-α-CH, *J*<sub>Ala-α-CH, Ala-β-CH</sub> = 6.9 Hz), 4.26 (q, 1H, Ala-α-CH, *J*<sub>Ala-α-CH, Ala-β-CH</sub> = 7.5 Hz), 4.23-4.18 (m, 2H, Ala-α-CH, Lys-α-CH), 4.17-4.08 (m, 3H, D-Glu-α-CH, Lac-α-CH, H-2), 3.98-3.93 (m, 1H, H-5), 3.91-3.86 (m, 1H, H-6), 3.84 (dd, 1H, H-6, *J*<sub>6,6</sub> = 12.6, *J*<sub>6,5</sub> = 4.0 Hz), 3.80 (dd, 1H, H-3, *J*<sub>3,4</sub> = *J*<sub>3,2</sub> = 9.7 Hz), 3.64 (dd, 1H, H-4, *J*<sub>4,3</sub> = 9.7, *J*<sub>4,5</sub> = 10.3 Hz), 3.00 (t, 1H, Lys-ε-CH, *J*<sub>Lys-ε-CH, Lys-δ-CH</sub> = 7.5 Hz), 2.31 (t, 2H, D-Glu-γ-CH, *J*<sub>D-Glu-γ-CH, D-Glu-β-CH</sub> = 8.0 Hz), 2.20-2.10 (m, 5H, D-Glu-β-CH, H-4', H-5'), 2.00 (s, 3H, NAc), 1.93-1.85 (m, 1H, D-Glu-β-CH), 1.85-1.75 (m, 2H, Lys-β-CH), 1.77 (s, 3H, 3'-Me), 1.73-1.66 (m, 2H, Lys-δ-CH), 1.69 (s, 3H, 7'-Me), 1.62 (s, 3H, 7'-Me), 1.51-1.42 (m, 2H, Lys-γ-CH), 1.45 (d, 3H, Ala-β-CH, *J*<sub>Ala-β-CH, Ala-α-CH</sub> = 6.9 Hz), 1.41 (d, 3H, Ala-β-CH, *J*<sub>Ala-β-CH, Ala-α-CH</sub> = 6.9 Hz), 1.37 (d, 3H, Ala-β-CH, *J*<sub>Ala-β-CH, Ala-α-CH</sub> = 7.5 Hz), 1.33 (d, 3H, Lac-β-CH, *J*<sub>Lac-β-CH, Lac-α-CH</sub> = 7.5 Hz); <sup>31</sup>P NMR (D<sub>2</sub>O, 202 MHz) δ -10.2 (d, *J*<sub>P,P</sub> = 21.4 Hz), -12.7 (d, *J*<sub>P,P</sub> = 21.4 Hz); ESIMS-LR *m/z* 528.70 [(M-2H)<sup>2-</sup>]; ESIMS-HR calcd for C<sub>41</sub>H<sub>69</sub>O<sub>21</sub>N<sub>7</sub>P<sub>2</sub> 528.7016, found 528.7025; [α]<sup>20</sup><sub>D</sub> +20.42 (*c* 0.06, MeOH).

## Park nucleotide (122)



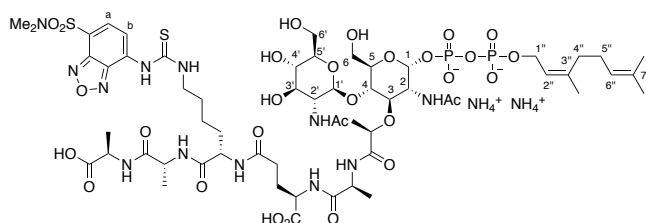
Resin-bound peptide **155** (9.4 mg, 2.1  $\mu\text{mol}$ ) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with  $\text{CH}_2\text{Cl}_2$  (1 mL, 1 h). After removal of  $\text{CH}_2\text{Cl}_2$ , the resin was treated with a solution of  $\text{Pd}(\text{PPh}_3)_4$  (1.9 mg, 1.6  $\mu\text{mol}$ ) and  $\text{PhSiH}_3$  (8.4  $\mu\text{L}$ , 69  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (400  $\mu\text{L}$ ) for 2 h. The resin was

washed with 0.5%  $^t\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL $\times$ 8), MeOH (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) and DMF (2 mL $\times$ 3). The resin was treated with a solution of **121** (7.6 mg, 11  $\mu\text{mol}$ ) and **153** (2.4 mg, 11  $\mu\text{mol}$ ) in DMF (300  $\mu\text{L}$ ) at 50  $^\circ\text{C}$  for 3 d. The resin was washed with DMF (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) to afford **158**. The resin was treated with piperidine/DMF (1:4, 5 min, then 1:9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5%  $^t\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), DMF (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) and THF (2 mL  $\times$ 3). The resin was treated with 2 M *aq.* NaOH/MeOH/THF = 1/1/2 (400  $\mu\text{L}$ ) at 0  $^\circ\text{C}$ , then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq.* HCl (220  $\mu\text{L}$ ) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250  $\times$  20 mm, 1% MeCN/50 mM *aq.*  $\text{NH}_4\text{HCO}_3$ ) to afford **122** (1.4 mg, 1.2  $\mu\text{mol}$ , 44% over 12 steps) as a white powder, after freeze drying.

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  7.96 (d, 1H, H-6,  $J_{6,5} = 8.0$  Hz), 5.99 (d, 1H, H-1',  $J_{1',2'} = 5.2$  Hz), 5.97 (d, 1H, H-5,  $J_{5,6} = 8.0$  Hz), 5.47 (dd, 1H, H-1'',  $J_{1'',2''} = 2.9$ ,  $J_{1'',\text{P}} = 7.5$  Hz), 4.39-4.30 (m, 3H, Ala- $\alpha$ -CH, H-2', H-3'), 4.30-4.09 (m, 9H, H-4', H-5', H-2'', Ala- $\alpha$ -CH, Lac- $\alpha$ -CH, Lys- $\alpha$ -CH, D-Glu- $\alpha$ -CH), 3.98-3.94 (m, 1H, H-5''), 3.88 (dd, 1H, H-6'',  $J_{6'',6''} = 12.6$ ,  $J_{6'',5''} = 2.3$  Hz), 3.84 (dd, 1H, H-6'',  $J_{6'',6''} = 12.6$ ,  $J_{6'',5''} = 4.0$  Hz), 3.80 (dd, 1H, H-3'',  $J_{3'',4''} = J_{3'',2''} = 10.3$  Hz), 3.65 (dd, 1H, H-4'',  $J_{4'',3''} = 10.3$ ,  $J_{4'',5''} = 9.7$  Hz), 3.01 (t, 1H, Lys- $\epsilon$ -CH,  $J_{\text{Lys-}\epsilon\text{-CH}, \text{Lys-}\delta\text{-CH}} = 7.5$  Hz), 2.31 (t, 2H, D-Glu- $\gamma$ -CH,  $J_{\text{D-Glu-}\gamma\text{-CH}, \text{D-Glu-}\beta\text{-CH}} = 8.0$  Hz), 2.20-2.12 (m, 1H, D-Glu- $\beta$ -CH), 2.02 (s, 3H, NAc), 1.92-1.85 (m, 1H, D-Glu- $\beta$ -CH), 1.85-1.74 (m, 2H, Lys- $\beta$ -CH), 1.74-1.65 (m, 2H, Lys- $\delta$ -CH), 1.52-1.41 (m, 2H, Lys- $\gamma$ -CH), 1.45 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.41 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 6.9$  Hz), 1.37 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH}, \text{Ala-}\alpha\text{-CH}} = 6.9$  Hz), 1.34 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH}, \text{Lac-}\alpha\text{-CH}} = 6.9$  Hz);  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ , 162 MHz)  $\delta$  -10.9 (d,  $J_{\text{P},\text{P}} = 21.7$  Hz), -12.6 (d,  $J_{\text{P},\text{P}} = 21.7$  Hz); ESIMS-LR  $m/z$  573.67 [(M-2H) $^{2-}$ ]; ESIMS-HR calcd for  $\text{C}_{40}\text{H}_{63}\text{O}_{26}\text{N}_9\text{P}_2$  573.6685, found 573.6694;  $[\alpha]_{\text{D}}^{20} +13.35$  ( $c$  0.08, MeOH).

The analytical data for synthetic **1** were in good agreement with the previously reported data.<sup>94</sup>

## Neryl-lipid II DBD analogue 160



Resin-bound peptide **147** (12.4 mg, 2.9  $\mu\text{mol}$ ) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with  $\text{CH}_2\text{Cl}_2$  (1 mL, 1 h). After removal of  $\text{CH}_2\text{Cl}_2$ , the resin was treated with a solution of  $\text{Pd}(\text{PPh}_3)_4$  (2.0 mg, 1.7  $\mu\text{mol}$ ) and  $\text{PhSiH}_3$  (8.9  $\mu\text{L}$ , 73  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (300  $\mu\text{L}$ )

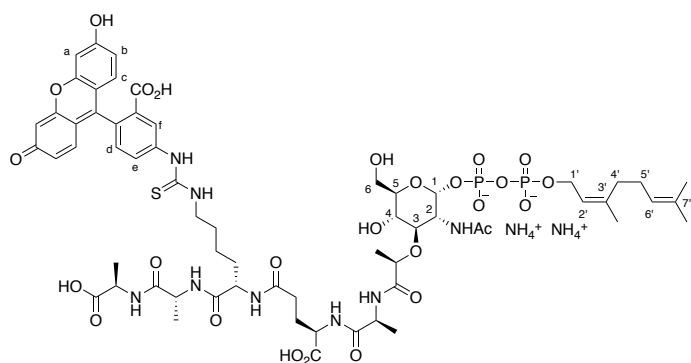
for 2 h. The resin was washed with 0.5%  $^t\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL $\times$ 8), MeOH (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) and DMF (2 mL $\times$ 3). The resin was treated with a solution of **131** (12



$\mu\text{mol}$ ) and **153** (2.6 mg, 12  $\mu\text{mol}$ ) in DMF (300  $\mu\text{L}$ ) at 50  $^{\circ}\text{C}$  for 3 d. The resin was washed with DMF (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) to afford **154**. The resin was treated with piperidine/DMF (1:4, 5min, then 1:9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5%  $^i\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3). The resin was treated with a solution of DBD-NCS (2.5 mg, 8.7  $\mu\text{mol}$ ) and  $^i\text{Pr}_2\text{NEt}$  (1.5  $\mu\text{L}$ , 8.7  $\mu\text{mol}$ ) in DMF (400  $\mu\text{L}$ ) for 2 h. The resin was washed with DMF (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) and THF (2 mL  $\times$ 3). Kaiser test indicated the completion of the coupling reaction. The resin was treated with 2 M *aq.* LiOH/MeOH/THF = 1/1/2 (400  $\mu\text{L}$ ) at 0  $^{\circ}\text{C}$ , then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq.* HCl (220  $\mu\text{L}$ ) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250  $\times$  20 mm, 23% MeCN/50 mM *aq.*  $\text{NH}_4\text{HCO}_3$ ) to afford **160** (0.5 mg, 0.32  $\mu\text{mol}$ , 11% over 13 steps) as a yellow powder, after freeze drying.

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  8.22 (d, 1H, H-a,  $J_{a,b} = 7.5$  Hz), 8.13 (d, 1H,  $J_{b,a} = 7.5$  Hz), 5.45 (dd, 1H, H-1,  $J_{1,2} = 3.4$ ,  $J_{1,P} = 6.3$  Hz), 5.41 (t, 1H, H-2",  $J_{2',1''} = 6.9$  Hz), 5.13 (t, 1H, H-6",  $J_{6'',5''} = 6.9$  Hz), 4.62 (d, 1H, H-1',  $J_{1',2'} = 8.6$  Hz), 4.43 (dd, 2H, H-1",  $J_{1'',2''} = J_{1',P} = 6.3$  Hz), 4.35 (q, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH, Ala-}\beta\text{-CH}} = 6.9$  Hz), 4.26 (q, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH, Ala-}\beta\text{-CH}} = 6.9$  Hz), 4.25-4.12 (m, 4H, Ala- $\alpha$ -CH, Lys- $\alpha$ -CH, D-Glu- $\alpha$ -CH, H-2), 4.11 (q, 1H, Lac- $\alpha$ -CH,  $J_{\text{Lac-}\alpha\text{-CH, Lac-}\beta\text{-CH}} = 6.9$  Hz), 3.98-3.87 (m, 4H, H-4, H-6, H-6'), 3.80 (dd, 1H, H-3,  $J_{3,H-4} = J_{3,2} = 9.7$  Hz), 3.77-3.61 (m, 5H, H-5, H-2', H-6', Lys- $\epsilon$ -CH), 3.55 (dd, 1H, H-3',  $J_{3',4'} = J_{3',2'} = 8.0$  Hz), 3.44-3.37 (m, 2H, H-4', H-5'), 2.86 (s, 3H,  $\text{SO}_2\text{Me}$ ), 2.72 (s 3H,  $\text{SO}_2\text{Me}$ ), 2.38-2.29 (m, 2H, D-Glu- $\gamma$ -CH), 2.20-2.02 (m, 5H, D-Glu- $\beta$ -CH, H-4", H-5"), 2.04 (s, 3H, NAc), 1.98 (s, 3H, NAc), 1.93-1.78 (m, 3H, D-Glu- $\beta$ -CH, Lys- $\beta$ -CH), 1.74 (s, 3H, 3"-Me), 1.76-1.68 (m, 2H, Lys- $\delta$ -CH), 1.65 (s, 3H, 7"-Me), 1.58 (s, 3H, 7"-Me), 1.55-1.40 (m, 2H, Lys- $\gamma$ -CH), 1.44 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.43 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 6.9$  Hz), 1.36 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 6.9$  Hz), 1.32 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH, Lac-}\alpha\text{-CH}} = 6.9$  Hz);  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ , 202 MHz)  $\delta$  -10.2 (d,  $J_{P,P} = 21.4$  Hz), -12.7 (d,  $J_{P,P} = 21.4$  Hz); ESIMS-LR  $m/z$  772.25 [(M-2H) $^{2-}$ ]; ESIMS-HR calcd for  $\text{C}_{58}\text{H}_{90}\text{O}_{29}\text{N}_{12}\text{P}_2\text{S}_2$  772.2432, found 772.2453;  $[\alpha]^{20}_{\text{D}} +28.56$  ( $c$  0.05, MeOH).

### Neryl-lipid I FITC analogue **162**



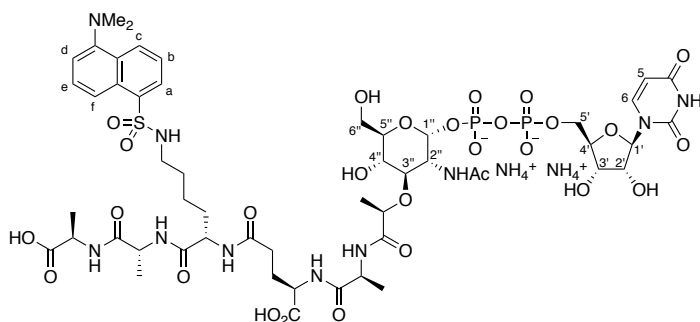
Resin-bound peptide **155** (9.4 mg, 2.1  $\mu\text{mol}$ ) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with  $\text{CH}_2\text{Cl}_2$  (1 mL, 1 h). After removal of  $\text{CH}_2\text{Cl}_2$ , the resin was treated with a solution of  $\text{Pd}(\text{PPh}_3)_4$  (1.9 mg, 1.6  $\mu\text{mol}$ ) and  $\text{PhSiH}_3$  (8.4  $\mu\text{L}$ , 69  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (400  $\mu\text{L}$ ) for 2 h. The resin was washed with 0.5%  $^i\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), 0.5%(w/v) sodium

diethyldithiocarbamate/DMF (1 mL $\times$ 8), MeOH (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) and DMF (2 mL $\times$ 3). The resin was treated with a solution of **131** (11  $\mu\text{mol}$ ) and **153** (2.4 mg, 11  $\mu\text{mol}$ ) in DMF (300  $\mu\text{L}$ ) at 50  $^{\circ}\text{C}$  for 3 d. The resin was washed with DMF (2 mL $\times$ 3),  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3) to afford **156**. The resin was treated with piperidine/DMF (1:4, 5 min, then 1:9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5%  $^i\text{Pr}_2\text{NEt}/\text{CH}_2\text{Cl}_2$  (2 mL $\times$ 3), DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3). The resin was treated with a solution of FITC isomer I (3.2 mg, 8.6  $\mu\text{mol}$ ) and  $^i\text{Pr}_2\text{NEt}$  (2.8  $\mu\text{L}$ , 16  $\mu\text{mol}$ ) in DMF (400  $\mu\text{L}$ ) for 2 h. The resin was washed with DMF (2 mL $\times$ 3) and  $\text{CH}_2\text{Cl}_2$  (2 mL  $\times$ 3). The resin was treated with a solution of FITC isomer I (3.2 mg, 8.6  $\mu\text{mol}$ ) and  $^i\text{Pr}_2\text{NEt}$  (2.8  $\mu\text{L}$ , 16  $\mu\text{mol}$ ) in

DMF (400  $\mu$ L) for 2 h. The resin was washed with DMF (2 mL $\times$ 3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL  $\times$ 3) and THF (2 mL  $\times$ 3). Kaiser test indicated the completion of the coupling reaction. The resin was treated with 2 M *aq.* LiOH/MeOH/THF = 1/1/2 (400  $\mu$ L) at 0  $^{\circ}$ C, then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq.* HCl (210  $\mu$ L) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250  $\times$  20 mm, 15% MeCN/50 mM *aq.* NH<sub>4</sub>HCO<sub>3</sub>) to afford **162** (1.2 mg, 0.81  $\mu$ mol, 30% over 13 steps) as a yellow powder, after freeze drying.

<sup>1</sup>H NMR (D<sub>2</sub>O, 500 MHz)  $\delta$  7.70 (s, 1H, H-f), 7.57 (d, 1H, H-d,  $J_{d,e}$  = 8.0 Hz), 7.37 (d, 1H, H-e,  $J_{e,d}$  = 8.0 Hz), 7.29 (d, 2H, H-c,  $J_{c,b}$  = 8.6 Hz), 6.71-6.67 (m, 4H, H-a, H-b), 5.46-5.39 (m, 2H, H-1, H-2'), 5.17-5.12 (m, 1H, H-6'), 4.47-4.41 (m, 2H, H-1'), 4.35 (q, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH, Ala-}\beta\text{-CH}}$  = 7.5 Hz), 4.28 (q, 1H, Ala- $\alpha$ -CH,  $J_{\text{Ala-}\alpha\text{-CH, Ala-}\beta\text{-CH}}$  = 7.5 Hz), 4.25-4.07 (m, 5H, Ala- $\alpha$ -CH, Lys- $\alpha$ -CH, D-Glu- $\alpha$ -CH, Lac- $\alpha$ -CH, H-2), 3.97-3.92 (m, 1H, H-5), 3.87 (d, 1H, H-6,  $J_{6,6}$  = 12.6 Hz), 3.82 (dd, 1H, H-6,  $J_{6,6}$  = 12.6,  $J_{6,5}$  = 4.0 Hz), 3.77 (dd, 1H, H-3,  $J_{3,4}$  =  $J_{3,2}$  = 9.7 Hz), 3.67-3.69 (m, 3H, H-4, Lys- $\epsilon$ -CH), 2.33-2.27 (m, 2H, D-Glu- $\gamma$ -CH), 2.18-2.04 (m, 5H, D-Glu- $\beta$ -CH, H-4', H-5'), 1.99 (s, 3H, NAc), 1.94-1.74 (m, 3H, D-Glu- $\beta$ -CH, Lys- $\beta$ -CH), 1.73 (s, 3H, 3'-Me), 1.72-1.63 (m, 2H, Lys- $\delta$ -CH), 1.66 (s, 3H, 7'-Me), 1.58 (s, 3H, 7'-Me), 1.51-1.40 (m, 2H, Lys- $\gamma$ -CH), 1.43 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}}$  = 6.9 Hz), 1.39 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}}$  = 6.9 Hz), 1.36 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}}$  = 6.9 Hz), 1.32 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH, Lac-}\alpha\text{-CH}}$  = 7.5 Hz); <sup>31</sup>P NMR (D<sub>2</sub>O, 202 MHz)  $\delta$  -10.2 (d,  $J_{\text{P,P}}$  = 21.4 Hz), -12.7 (d,  $J_{\text{P,P}}$  = 21.4 Hz); ESIMS-LR  $m/z$  723.22 [(M-2H)<sup>2-</sup>]; ESIMS-HR calcd for C<sub>62</sub>H<sub>80</sub>H<sub>26</sub>N<sub>8</sub>P<sub>2</sub>S 723.2195, found 723.2214; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +20.18 (*c* 0.12, MeOH).

#### Dansyl Park nucleotide (**124**)



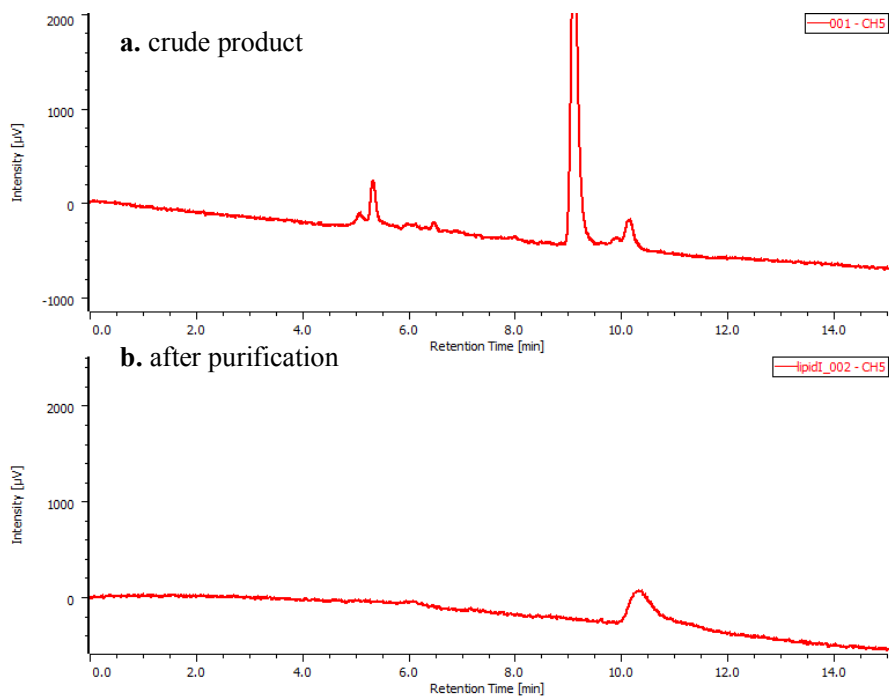
Resin-bound peptide **155** (9.4 mg, 2.1  $\mu$ mol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with CH<sub>2</sub>Cl<sub>2</sub> (1 mL, 1 h). After removal of CH<sub>2</sub>Cl<sub>2</sub>, the resin was treated with a solution of Pd(PPh<sub>3</sub>)<sub>4</sub> (1.9 mg, 1.6  $\mu$ mol) and PhSiH<sub>3</sub> (8.4  $\mu$ L, 69  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (400  $\mu$ L) for 2 h. The resin was washed with 0.5% <sup>i</sup>Pr<sub>2</sub>NEt/CH<sub>2</sub>Cl<sub>2</sub> (2 mL $\times$ 3),

0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL $\times$ 8), MeOH (2 mL $\times$ 3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL  $\times$ 3) and DMF (2 mL $\times$ 3). The resin was treated with a solution of **121** (7.6 mg, 11  $\mu$ mol) and **153** (2.4 mg, 11  $\mu$ mol) in DMF (300  $\mu$ L) at 50  $^{\circ}$ C for 3 d. The resin was washed with DMF (2 mL $\times$ 3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL  $\times$ 3) to afford **158**. The resin was treated with piperidine/DMF (1:4, 5 min, then 1:9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5% <sup>i</sup>Pr<sub>2</sub>NEt/CH<sub>2</sub>Cl<sub>2</sub> (2 mL $\times$ 3), DMF (2 mL $\times$ 3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL  $\times$ 3). The resin was treated with a mixture of dansyl chloride (7.3 mg, 27  $\mu$ mol) in 0.25 M *aq.* NaHCO<sub>3</sub>/acetone = 1/1 (400  $\mu$ L) for 2 h. The resin was washed with MeOH (2 mL $\times$ 3), DMF (2 mL $\times$ 3), CH<sub>2</sub>Cl<sub>2</sub> (2 mL  $\times$ 3) and THF (2 mL  $\times$ 3). Kaiser test indicated the completion of the coupling reaction. The resin was treated with 2 M *aq.* NaOH/MeOH/THF = 1/1/2 (400  $\mu$ L) at 0  $^{\circ}$ C, then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq.* HCl (200  $\mu$ L) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250  $\times$  20 mm, 15% MeCN/50 mM *aq.* NH<sub>4</sub>HCO<sub>3</sub>) and converted into the ammonium salt by ion exchange chromatography (Dowex 50WX4 50-100) to afford **124** (1.0 mg, 0.71  $\mu$ mol, 26% over 13 steps) as a slightly green powder, after freeze drying.

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  8.50 (d, 1H, H-a,  $J_{a,b} = 8.6$  Hz), 8.40 (d, 1H, H-f,  $J_{f,e} = 8.6$  Hz), 8.30 (d, 1H, H-c,  $J_{c,b} = 7.5$  Hz), 7.92 (d, 1H, H-6,  $J_{H-6,H-5} = 8.0$  Hz), 7.76 (dd, 1H, H-e,  $J_{e,f} = 8.6$ ,  $J_{e,d} = 7.5$  Hz), 7.74 (dd, 1H, H-b,  $J_{b,a} = J_{b,c} = 8.6$  Hz), 7.55 (d, H-d,  $J_{d,e} = 7.5$  Hz), 5.95 (d, 1H, H-1',  $J_{1',2'} = 4.6$  Hz), 5.93 (d, 1H, H-5,  $J_{5,6} = 8.0$  Hz), 5.46 (dd, 1H, H-1'',  $J_{1'',2''} = 2.9$ ,  $J_{1'',p} = 7.5$  Hz), 4.35 (dd, 1H, H-2',  $J_{2',1'} = 4.6$ ,  $J_{2',3'} = 8.0$  Hz), 4.29-4.17 (m, 6H, Ala- $\alpha$ -CH, H-3', H-4' H-5'), 4.17-4.07 (m, 4H, H-5', H-2'', Lac- $\alpha$ -CH, D-Glu- $\alpha$ -CH), 3.97-3.92 (m, 1H, H-5''), 3.88-3.71 (m, 3H, H-3'', H-6'', Lys- $\alpha$ -CH), 3.65-3.59 (m, 2H, H-4'', H-6''), 3.00 (s, 6H, dansyl-NMe), 2.95 (t, 1H, Lys- $\epsilon$ -CH,  $J_{\text{Lys-}\epsilon\text{-CH, Lys-}\delta\text{-CH}} = 7.5$  Hz), 2.23 (t, 2H, D-Glu- $\gamma$ -CH,  $J_{\text{D-Glu-}\gamma\text{-CH, D-Glu-}\beta\text{-CH}} = 6.9$  Hz), 2.16-2.06 (m, 1H, D-Glu- $\beta$ -CH), 1.99 (s, 3H, NAc), 1.90-1.80 (m, 1H, D-Glu- $\beta$ -CH), 1.42 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.38 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 6.3$  Hz), 1.31 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH, Lac-}\alpha\text{-CH}} = 6.9$  Hz), 1.30 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.33-1.27 (m, 2H, Lys- $\beta$ -CH), 1.27-1.18 (m, 2H, Lys- $\delta$ -CH), 1.12-1.03 (m, 1H, Lys- $\gamma$ -CH), 1.03-0.93 (m, 1H, Lys- $\gamma$ -CH);  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ , 202 MHz)  $\delta$  -10.8 (d,  $J_{p,p} = 20.4$  Hz), -12.6 (d,  $J_{p,p} = 20.4$  Hz); ESIMS-LR  $m/z$  690.20 [(M-2H) $^{2-}$ ]; ESIMS-HR calcd for  $\text{C}_{52}\text{H}_{74}\text{O}_{28}\text{N}_{10}\text{P}_2\text{S}$  690.1941, found 690.1953; [ $\alpha$ ] $^{20}_{\text{D}}$  +18.56 ( $c$ , MeOH).

For comparison with the reported data, a part of the product was converted into the sodium salt by ion exchange chromatography (Dowex 50WX4 50-100).

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  8.50 (d, 1H, H-a,  $J_{a,b} = 8.6$  Hz), 8.32 (d, 1H, H-f,  $J_{f,e} = 8.6$  Hz), 8.26 (d, 1H, H-c,  $J_{c,b} = 7.5$  Hz), 7.77 (d, 1H, H-6,  $J_{H-6,H-5} = 8.0$  Hz), 7.71 (dd, 1H, H-e,  $J_{e,f} = 8.6$ ,  $J_{e,d} = 7.5$  Hz), 7.69 (dd, 1H, H-b,  $J_{b,a} = 8.6$ ,  $J_{b,c} = 7.5$  Hz), 7.41 (d, H-d,  $J_{d,e} = 7.5$  Hz), 6.01 (d, 1H, H-1',  $J_{1',2'} = 5.2$  Hz), 5.85 (d, 1H, H-5,  $J_{5,6} = 7.5$  Hz), 5.46 (dd, 1H, H-1'',  $J_{1'',2''} = 3.4$ ,  $J_{1'',p} = 6.9$  Hz), 4.35-4.10 (m, 10H, H-2', Ala- $\alpha$ -CH, H-3', H-4' H-5', H-2'', D-Glu- $\alpha$ -CH), 4.07 (q, 1H, Lac- $\alpha$ -CH,  $J_{\text{Lac-}\alpha\text{-CH, Lac-}\beta\text{-CH}} = 7.5$  Hz), 3.97-3.91 (m, 1H, H-5''), 3.88-3.71 (m, 3H, H-3'', H-6'', Lys- $\alpha$ -CH), 3.66-3.58 (m, 2H, H-4'', H-6''), 2.93 (t, 1H, Lys- $\epsilon$ -CH,  $J_{\text{Lys-}\epsilon\text{-CH, Lys-}\delta\text{-CH}} = 6.3$  Hz), 2.88 (s, 6H, dansyl-NMe), 2.23 (t, 2H, D-Glu- $\gamma$ -CH,  $J_{\text{D-Glu-}\gamma\text{-CH, D-Glu-}\beta\text{-CH}} = 7.5$  Hz), 2.15-2.05 (m, 1H, D-Glu- $\beta$ -CH), 1.98 (s, 3H, NAc), 1.90-1.80 (m, 1H, D-Glu- $\beta$ -CH), 1.41 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 6.9$  Hz), 1.38 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 6.3$  Hz), 1.29 (d, 3H, Lac- $\beta$ -CH,  $J_{\text{Lac-}\beta\text{-CH, Lac-}\alpha\text{-CH}} = 7.5$  Hz), 1.29 (d, 3H, Ala- $\beta$ -CH,  $J_{\text{Ala-}\beta\text{-CH, Ala-}\alpha\text{-CH}} = 7.5$  Hz), 1.38-1.15 (m, 4H, Lys- $\beta$ -CH, Lys- $\delta$ -CH), 1.13-1.02 (m, 1H, Lys- $\gamma$ -CH), 1.02-0.93 (m, 1H, Lys- $\gamma$ -CH).

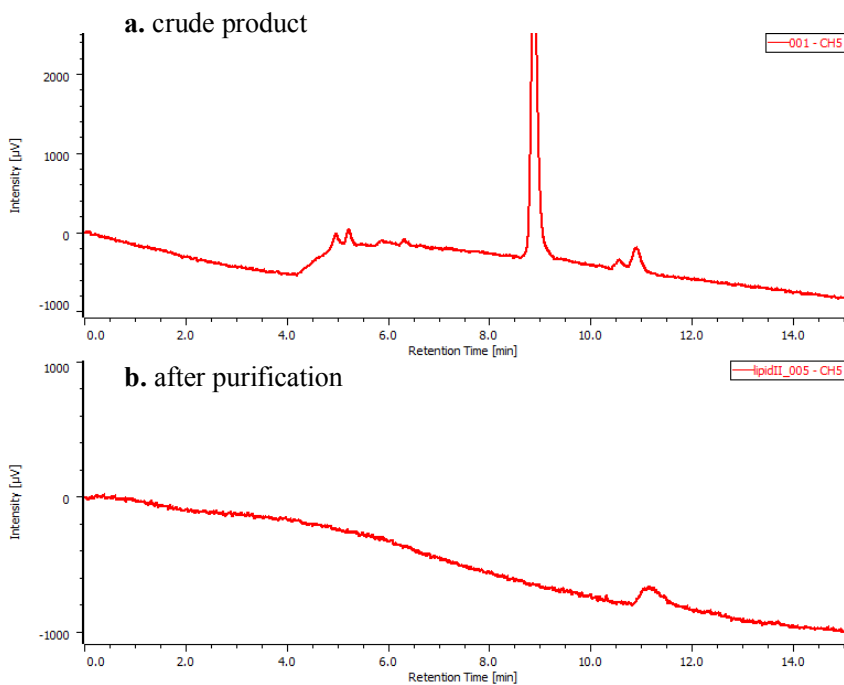


**Conditions**

column: YMC-Pack R&D ODS-A; eluent: 15% MeCN/50 mM *aq.* NH<sub>4</sub>HCO<sub>3</sub>;

flow 8 mL/min; detection: UV 210 nm

**Figure S3.** HPLC analysis of **126**.

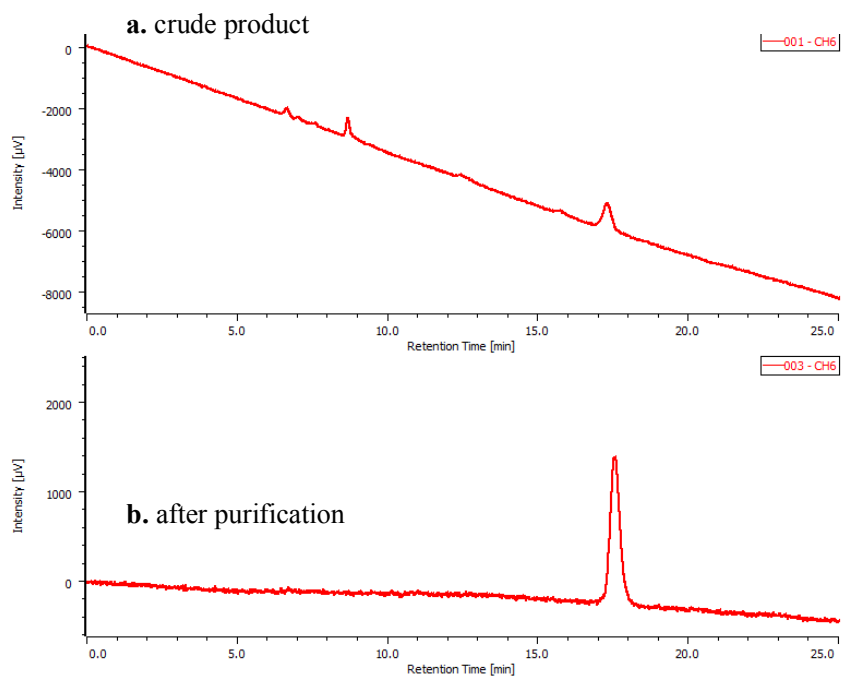


**Conditions**

column: YMC-Pack R&D ODS-A; eluent: 15% MeCN/50 mM *aq.* NH<sub>4</sub>HCO<sub>3</sub>;

flow 8 mL/min; detection: UV 210 nm

**Figure S4.** HPLC analysis of **157**.

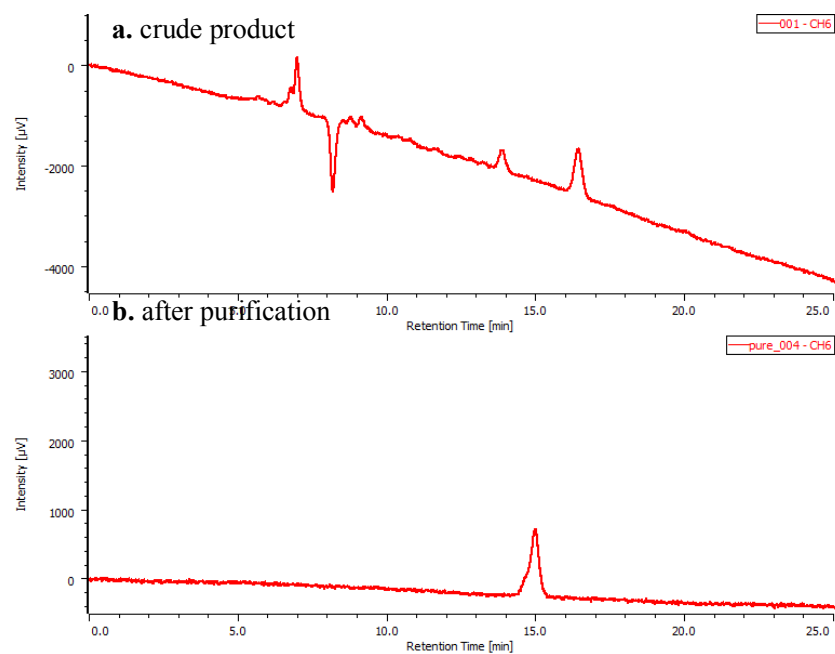


**Conditions**

column: YMC-Pack R&D ODS-A; eluent: 1% MeCN/50 mM *aq.* NH<sub>4</sub>HCO<sub>3</sub>;

flow 8 mL/min; detection: UV 254 nm

**Figure S5.** HPLC analysis of **122**.

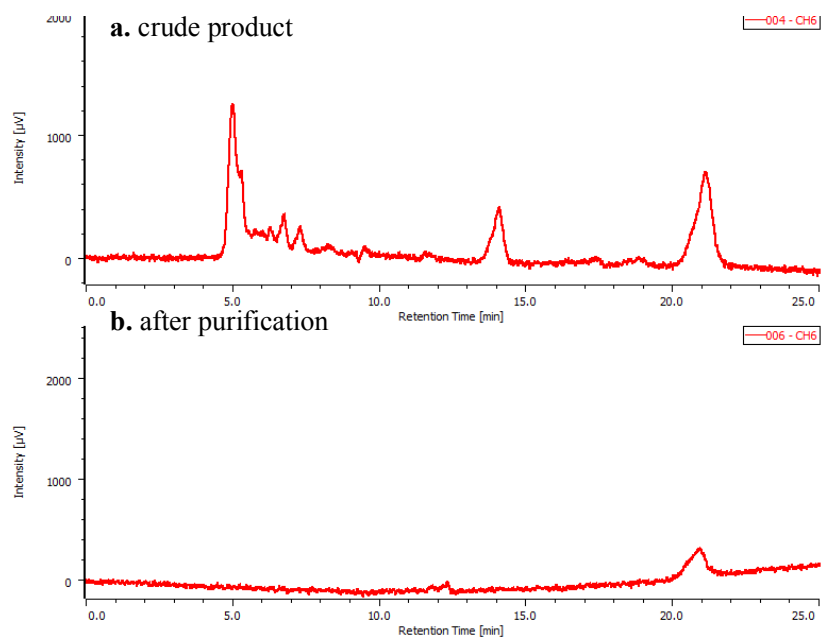


**Conditions**

column: YMC-Pack R&D ODS-A; eluent: 23% MeCN/50 mM *aq.* NH<sub>4</sub>HCO<sub>3</sub>;

flow 8 mL/min; detection: UV 254 nm

**Figure S6.** HPLC analysis of **160**.

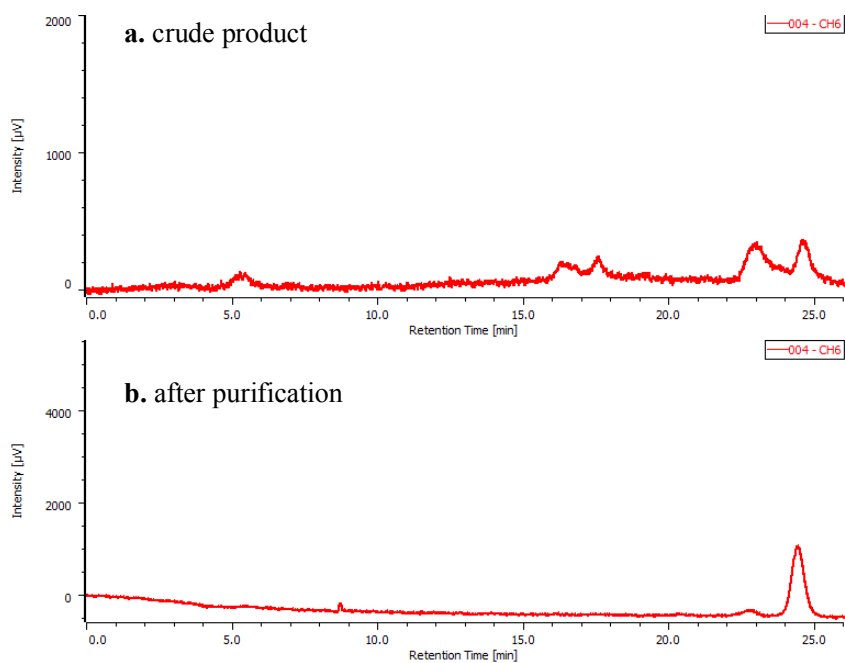


**Conditions**

column: YMC-Pack R&D ODS-A; eluent: 15% MeCN/50 mM *aq.*  $\text{NH}_4\text{HCO}_3$ ;

flow 8 mL/min; detection: UV 254 nm

**Figure S7.** HPLC analysis of **162**.



**Conditions**

column: YMC-Pack R&D ODS-A; eluent: 15% MeCN/50 mM *aq.*  $\text{NH}_4\text{HCO}_3$ ;

flow 8 mL/min; detection: UV 254 nm

**Figure S8.** HPLC analysis of **124**.

**Table S1.** Comparison of <sup>1</sup>H NMR spectra in D<sub>2</sub>O

compound 122		compound 126		compound 124	
Synthetic (500 MHz)	Reported (600 MHz)	Synthetic (500 MHz)	Reported (400 MHz)	Synthetic (500 MHz)	Reported (500 MHz)
7.96	7.94	5.47-5.41	5.45-5.39	8.50	8.48
5.99	5.96-5.94	5.22-5.17	5.20-5.14	8.32	8.27
5.97		4.62	4.60	8.26	8.23
5.47	5.45	4.45	4.42	7.77	7.90
4.39-4.30	4.36-4.03	4.33	4.31	7.71	7.70-7.65
4.30-4.09		4.28-4.12	4.28-4.19	7.69	
3.98-3.94	3.94-3.91	4.11	4.20-4.05	7.41	7.38
3.88	3.87-3.80	3.97-3.88	3.96-3.85	6.01	5.95-5.91
3.84		3.81	3.82-3.76	5.85	
3.80	3.77	3.77-3.69	3.75-3.67	5.46	5.46
3.65	3.62	3.55	3.56-3.49		4.34-4.32
3.01	2.98	3.44-3.38	3.42-3.35	4.35-4.10	4.29-4.23
2.31	2.28		3.18	4.07	4.23-4.10
2.20-2.12	2.14-2.12	3.00	2.98		4.10-4.06
2.02	1.98	2.31	2.30	3.97-3.91	3.96-3.93
1.92-1.85	1.89-1.65	2.20-2.10	2.15-2.09	3.88-3.71	3.85-3.59
1.85-1.74		2.05	2.03	3.66-3.58	
1.74-1.65		1.93-1.85	1.97	2.93	2.90
1.52-1.41	1.44-1.31	1.85-1.75		2.88	2.85
1.45		1.77	1.75	2.23	2.27-2.21
1.41		1.74-1.65		2.15-2.05	2.14-2.05
1.37		1.69	1.67	1.98	1.99
1.34		1.62	1.60	1.90-1.80	1.88-1.81
		1.51-1.41		1.41	1.41
		1.45	1.43	1.38	1.38
		1.44	1.42	1.29	1.30
		1.37	1.35	1.29	
		1.33	1.31	1.33-1.15	1.42-1.39
					1.26-1.15
				1.13-1.02	1.13-1.03
				1.02-0.93	1.03-0.94

## Optimization of the diphosphate formation

### General procedure of diphosphate formation

A solution of **121** (21 mg, 0.030 mmol) and **148** (16 mg, 0.030 mmol) in DMF (300  $\mu$ L) was treated with activator (0.030 mmol) at 25  $^{\circ}$ C. The reactions were monitored by  $^{31}$ P NMR. The signals for the corresponding diphosphate are found at  $\delta$  -11.3 and -14.1 ppm ( $^2J_{P,P} = 26.3$  Hz). The yields were determined by the ratio of the integral value of diphosphate peak to the sum of the integral value of all signals.

### Investigation of the enzyme reaction

Reactions were carried out in 0.5 mL Eppendorf tube. Reaction mixtures contained, in a final volume of 10  $\mu$ L, 40 mM Tris-HCl (pH 7.6), 40 mM KCl, 20 mM MgCl<sub>2</sub>, 0.2% Triton X-100, 6% glycerol, 1% DMSO, 100  $\mu$ M C<sub>55</sub>-P and 100  $\mu$ M **31**. The reactions were initiated by the addition of *Staphylococcus aureus* MraY enzyme (55 ng/0.5  $\mu$ L). After 5 h incubation at room temperature, the reaction was monitored by silica gel TLC (NH<sub>4</sub>OH/PrOH = 2/5, detection: UV 365 nm).



## 第五章

### ITC experiment

ITC experiments were performed using isothermal titration calorimeter VP-ITC (MicroCal, USA). Each experiment consisted of 19 consecutive injections (0.4–2  $\mu\text{L}$ ) of antiabacterial in 10 mM sodium phosphate, 150 mM NaCl, 0.9 mM  $\text{CaCl}_2$  buffer (pH 7.3) (+2–4% DMSO) into the microcalorimetric reaction cell (110  $\mu\text{L}$ ) charged with solution of neryl-lipid II (**126**) in the same buffer solution.

### Preparation of liposome

Egg phosphatidylcholine (EPC), cholesterol (Chol) were obtained from the NOF corporation (Tokyo, Japan). Lipid stock solution prepared in chloroform/MeOH (=1/1) were stored at  $-20\text{ }^\circ\text{C}$  until used. A lipid film was formed on the bottom of a glass tube by evaporating a chloroform/EtOH solution containing 0.55 mM lipids [EPC/Chol (=7/3)]. After the film was formed, 250  $\mu\text{M}$  of 10 mM sodium phosphate (pH 7.3) was added followed by incubation for 15 min at room temperature and sonication for 15 min. Particle diameter and PDI, indicators of particle-size distribution, were measured using a dynamic light scattering (DLS) method (Zetasizer Nano ZS; Malvern Instruments, Worcestershire, UK). Samples were prepared in 10 mM sodium phosphate (pH 7.3) at  $25\text{ }^\circ\text{C}$  and the values of particle diameters are shown in the form of volume distribution. The zeta potential of the samples were also determined in 10 mM sodium phosphate (pH 7.3) at  $25\text{ }^\circ\text{C}$  using a Zetasizer Nano ZS.

### Liposome experiment

A solution of the liposome in 10 mM sodium phosphate (pH 7.3) (0.55 mM, 50  $\mu\text{L}$ ) was treated with 25, 50, 100, 200  $\mu\text{M}$  of **1** or **100** in DMSO/10 mM sodium phosphate (pH 7.3) (=1/9). The mixture was incubated for 10 min at room temperature. Particle diameter, PDI and the zeta potential of the samples were determined at  $25\text{ }^\circ\text{C}$  using a Zetasizer Nano ZS.

### MD simulation

MD simulation was performed with a molecular dynamics software program, Desmond. Conformational search of plusbacin  $\text{A}_3$  was performed with a software program, Prime. Two molecules of plusbacin  $\text{A}_3$  were randomly arranged in the simulation box.

Force field: OPLS3

Solvent box: TIP3P (neutralized by 150 mM NaCl)

Thermostat method: Langevin

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