分岐骨格を有するペプチド性天然物および
その誘導体の合成研究

2022

鈴木 力斗

目次

序論

環状ペプチドは、高い標的親和性、膜透過性、生体内安定性を有することが可能であり、細胞内のタンパク質間相互作用を阻害する薬剤のための創薬スキャフォールドとして期待されている[1-4]。また、医薬品の創製の領域では、治療が困難な疾患に対する治療薬開発のためのシーズとして利用されるだけでなく、低分子医薬品の設計のためのリード化合物[5]、診断薬[6]、キャリア[7]としても応用されている。

これまでに、数多くの環状ペプチド構造からなる天然有機化合物が報告されており、実際に医薬品として利用されている代表的な例として cyclosporin A (**1**) が挙げられる（Figure 1）。真菌から単離された環状ペプチドである cyclosporin A は、細胞内のタンパク質 cyclophilin A と複合体を形成し、calcineurin を阻害する免疫抑制剤として知られている[8]。臨床では経口剤として使われており、優れた受動膜透過性や良好な経口アベイラビリティを示す[9]ことから、cyclosporin A をモデルとした head-to-tail 型の環状ペプチドの膜透過性に関する研究が数多く報告されている[10-16]。

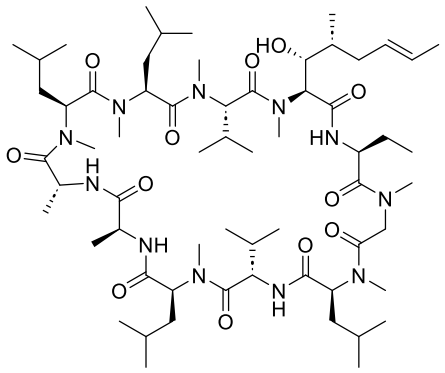


Figure 1. Structure of Cyclosporin A

天然から単離される環状ペプチドには、cyclosporin A のような head-to-tail 型の環状ペプチドだけでなく、分岐骨格を有する環状ペプチドや二環性の環状ペプチドをはじめとする特徴的な骨格からなる環状ペプチドが単離されている。このようなユニークな形状からなる環状ペプチドにも、これまで報告例のない細胞内の標的分子に作用して生物活性を示すものが報告されている[17]。これら分岐型や二環性の環状ペプチドは、cyclosporin A のような head-to-tail 型の環状ペプチドに比べて物理化学的特性を調べられている例が少ないものの[18]、daptomycin (**2**)[19] や romidepsin (**3**)[20] のように医薬品として臨床応用されている環状ペプチドも知られている（Figure 2）。

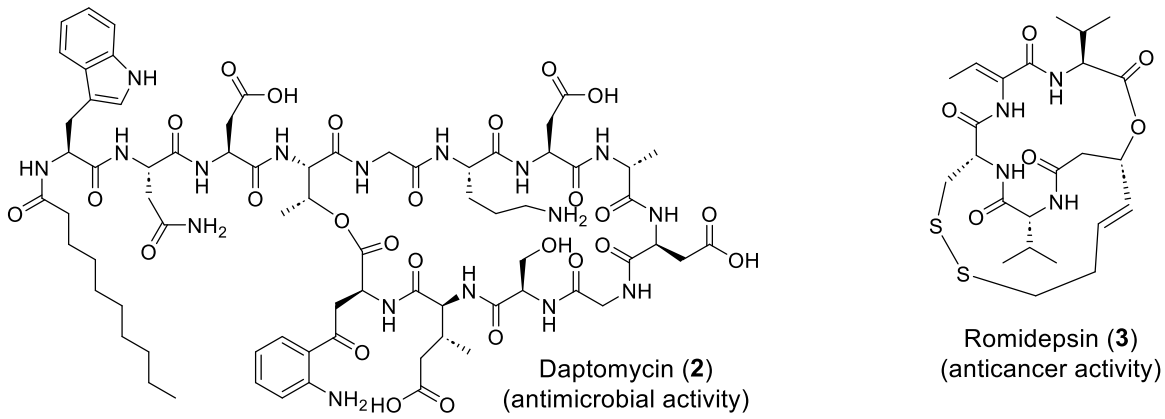


Figure 2. Approved Pharmaceuticals of Branched Cyclic Peptide Natural Products

魅力的な生物活性を示す環状ペプチドの創薬研究の初期段階において必要となるのは、化学構造の決定である。ペプチド性化合物の構造決定には、一般的な天然有機化合物の構造決定で用いられる質量分析や NMR スペクトル解析だけでなく、キラルアミノ酸分析[21]や生合成遺伝子解析などを組み合わせることが可能である。しかしながら、分子サイズが大きいペプチド性化合物の構造決定においては、スペクトルやクロマトグラム上での分離が困難であることなどにより、立体配置を確定できない部分構造が残されている場合や、間違った構造決定がなされている場合がある[22,23]。こうした局面では、天然由来のサンプルのスペクトル解析などにより類推した化学構造の妥当性について、全合成研究により得られた化学合成品との比較により評価することが有効である。

所属研究室では、海洋天然物由来のオーリライド型ペプチド性天然物の一種である odoamide (**4**) の化学合成研究に取り組み、スペクトル解析等では判別できなかったポリケチド部分の立体配置を決定した[24,25]（Figure 3）。この全合成経路の確立に向けた研究においては、合成研究の過程で各種立体異性体や誘導体が得られただけでなく、新たに確立した合成プロセスなどを活用することにより、天然物には見られない修飾を施した類縁体化合物 **5** の創製に展開することができた[26]。

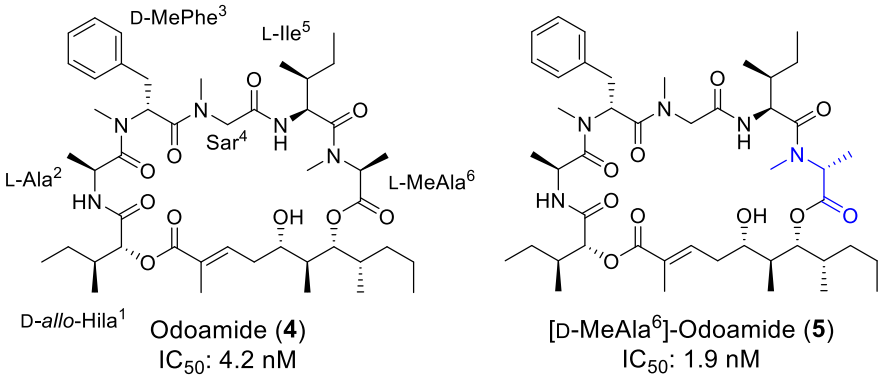


Figure 3. Structures of Odoamide and Its Derivative

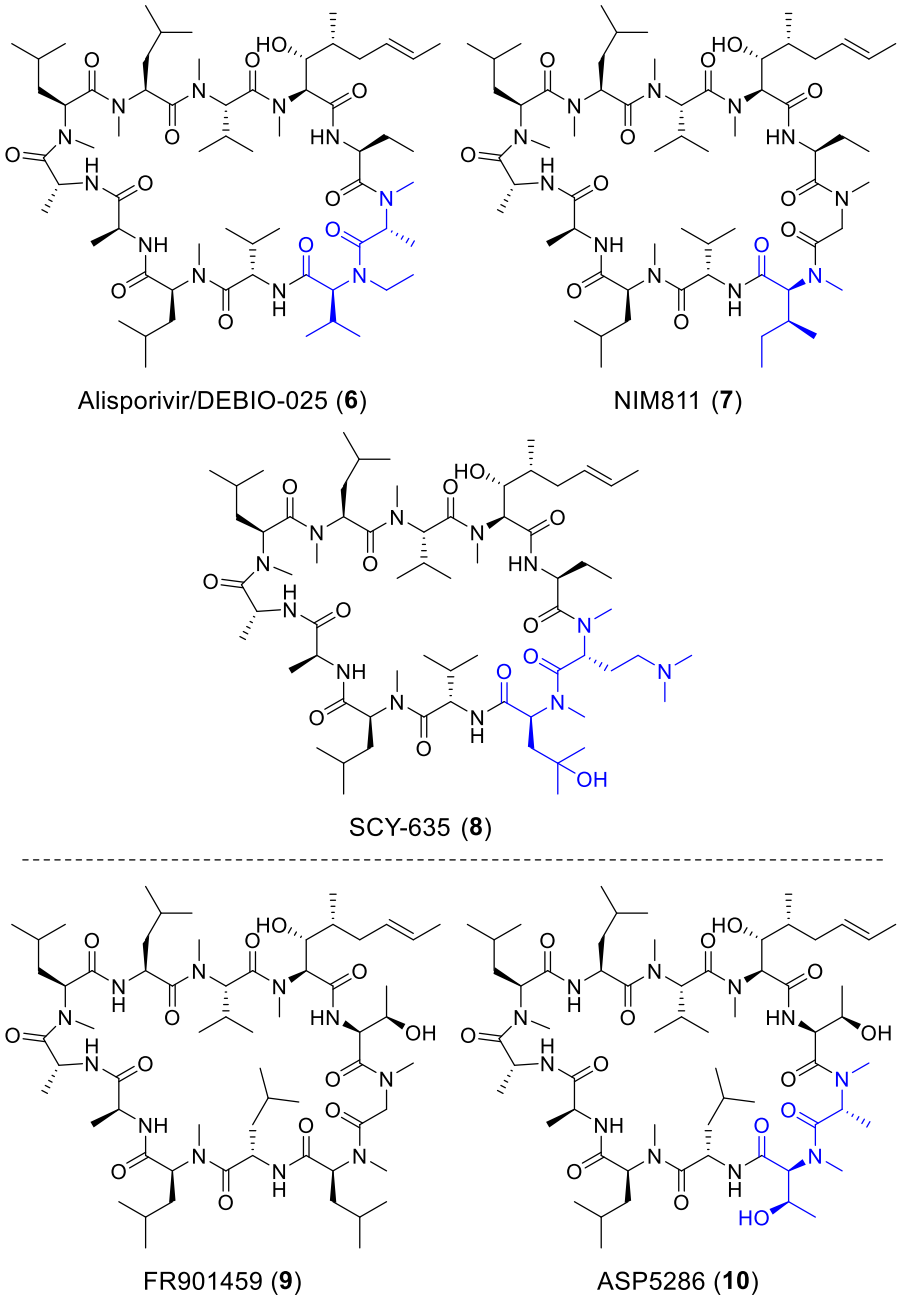


Figure 4. Structures of Non-immunosuppressive Cyclosporin A Derivatives for Anti-HCV Agents

このような環状ペプチド骨格からなる天然物での創薬研究の例として、前述の cyclosporin A の大環状骨格を活かした C 型肝炎ウイルス（HCV）に対する抗ウイルス剤の創製研究が挙げられる[27]。すなわち、cyclosporin A が HCV の複製を抑制するとの知見[28]を端緒として、免疫抑制活性を示さずに HCV に対する抗ウイルス活性のみを示す cyclosporin A 誘導体が設計された（Figure 4）。この構造最適化研究では、cyclosporin A 中の cyclophilin A との相互作用面を維持する一方で、calcineurin との相互作用ができないように環状ペプチドの部分構造を化学修飾することで、活性の分離に成功している。この創薬研究で得られた

alisporivir/DEBIO-025 (**6**)[29]、NIM811 (**7**)[30]、SCY-635 (**8**)[31] は、いずれも C 型肝炎ウイルス感染症治療薬への応用を目指した臨床試験が実施されている。また、アステラス製薬の研究グループは、同様のアプローチにより、*Stachybotrys chartarum* の培養液から単離された cyclosporin A 類似の天然物 FR901459 (**9**)[32] からの構造展開により、優れた生物活性プロファイルを示す抗 HCV 活性ペプチド ASP5286 (**10**) およびその誘導体を見出している [33-35]。

このように、特徴的な化学構造からなる環状ペプチドやその誘導体を化学合成して構造活性相関研究を行うことは、新規医薬品の創製につながる実用的な知見だけでなく、特定の標的分子との相互作用や膜透過性に有効な部分構造の特定をはじめとする基礎的知見を提供することから、創薬科学における重要な研究課題である [36]。分岐骨格をはじめとする複雑な化学構造を有する環状ペプチドの誘導体は、翻訳系を用いた生物学的アプローチにより調製できる場合もあるが、蛍光標識誘導体をはじめとするプローブの創製やこれを用いた標的探索研究へも展開できるという点で、化学合成・修飾を用いたアプローチが有効である。

こうした背景のもと、著者は、細胞内のタンパク質に作用して生物活性を示すペプチド性天然物からの創薬研究の一環として、分岐骨格を有する 2 つの環状ペプチドの合成研究を行った（Figure 5）。

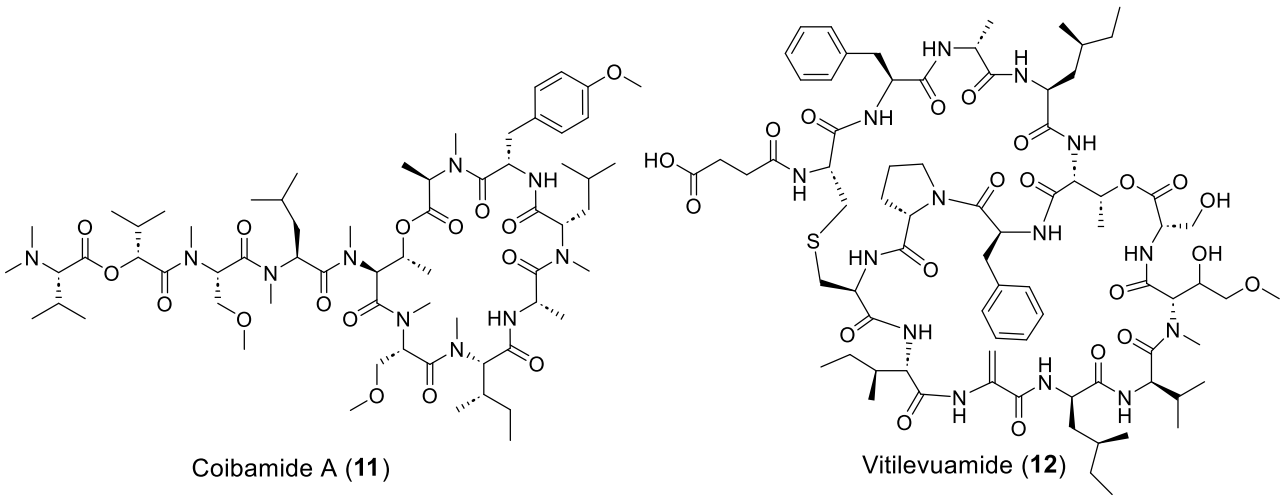


Figure 5. Branched Cyclic Peptide Natural Products Presented in This Thesis

第一章では、投げ縄構造からなる環状デプシペプチド coibamide A (**11**) の構造活性相関研究について論述する。著者は、化学合成が容易な coibamide A の簡易構造誘導体をリード化合物として、複数のアプローチで構造活性相関研究を行った。Coibamide A と同じ標的分子に作用し共通の部分構造を有する天然物 apratoxin A およびその誘導体の構造活性相関情報を参考にし、coibamide A に含まれる芳香族アミノ酸の構造活性相関研究を展開した。また、coibamide A のマクロラクトンを構成するエステル結合周辺の部分構造に関する構造活性相関研究を実施した。これらの検討を通して、coibamide A の芳香族アミノ酸の側鎖をかさ高

い芳香環に置換すると細胞増殖抑制活性が大幅に向上すること、coibamide A のエステル結合をアミド結合や炭素–炭素二重結合に置換しても活性が維持されること、およびこの部分構造の 2 つのメチル基が生物活性に重要な役割を果たしていることを明らかにした。

第二章では、二環性環状ペプチド vitilevuamide (**12**) の合成研究について論述する。著者は、vitilevuamide の化学構造の決定、並びに、構造最適化研究や標的分子・相互作用様式の解明に利用可能なプローブ分子の合成に資する vitilevuamide の化学合成プロセスの確立に向けた検討を行った。Vitilevuamide に含まれる複数の非天然型側鎖を有するアミノ酸を天然アミノ酸に置き換えたモデルペプチドを設計し、vitilevuamide の二環性骨格の効率的な構築を目指した検討を実施した。また、vitilevuamide 中の未決定の立体配置を同定するために必要となる *N*-メチルヒドロキシメトキシニンをはじめとする非天然型側鎖を有するアミノ酸について、固相合成に適用可能な適切な保護基を有するアミノ酸誘導体の合成法を検討した。これらの検討を通して、すべての非天然型アミノ酸の化学合成プロセスを確立するとともに、vitilevuamide の合成において鍵となる二環性骨格の構築とデヒドロアラニンへの変換を含むモデルペプチドの合成プロセスを確立した。

参考文献

1. Naylor, M. R.; Bockus, A. T.; Blanco, M. J.; Lokey, R. S. *Curr. Opin. Chem. Biol.* **2017**, *38*, 141.
2. Gang, D.; Kim, D. W.; Park, H. S. *Genes* **2018**, *9*, 557.
3. Abdalla, M. A.; McGaw, L. J. *Molecules* **2018**, *23*, 2080.
4. Ramadhani, D.; Maharani, R.; Gazzali, A. M.; Muchtaridi, M. *Molecules* **2022**, *27*, 4428.
5. Yoshida, S.; Uehara, S.; Kondo, N.; Takahashi, Y.; Yamamoto, S.; Kameda, A.; Kawagoe, S.; Inoue, N.; Yamada, M.; Yoshimura, N.; Tachibana, Y. *J. Med. Chem.* **2022**, *65*, 10655.
6. Hwang, D.W.; Bahng, N.; Ito, K.; Ha, S.; Kim, M.Y.; Lee, E.; Suga, H.; Lee D.S. *Cancer Lett.* **2017**, *385*, 144.
7. Fu, C.; Yu, L.; Miao, Y.; Liu, X.; Yu, Z.; Wei, M. *Acta Pharm. Sin. B* **2022**, 10.1016/j.apsb.2022.07.020
8. Grevel, J.; Nuesch, E.; Abisch, E.; Kutz, K. *Eur. J. Clin. Pharmacol*. **1986**, *31*, 211.
9. Corbett, K. M.; Ford, L.; Warren, D. B.; Pouton, C. W.; Chalmers, D. K. *J. Med. Chem.* **2021**, *64*, 13131.
10. Rezai, T.; Yu, B.; Millhauser, G. L.; Jacobson, M. P.; Lokey, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 2510.
11. Pye, C. R.; Hewitt, W. M.; Schwochert, J.; Haddad, T. D.; Townsend, C. E.; Etienne, L.; Lao, Y.; Limberakis, C.; Furukawa, A.; Mathiowetz, A. M.; Price, D. A.; Liras, S.; Lokey, R. S. *J. Med. Chem.* **2017**, *60*, 1665.
12. Nielsen, D. S.; Hoang, H. N.; Lohman, R. J.; Hill, T. A.; Lucke, A. J.; Craik, D. J.; Edmonds, D. J.; Griffith, D. A.; Rotter, C. J.; Ruggeri, R. B.; Price, D. A.; Liras, S.; Fairlie, D. P. *Angew. Chem., Int. Ed.* **2014**, *53*, 12059.
13. Wang, C. K.; Northfield, S. E.; Swedberg, J. E.; Colless, B.; Chaousis, S.; Price, D. A.; Liras, S.; Craik, D. J. *Eur. J. Med. Chem.* **2015**, *97*, 202.
14. Nielsen, D. S.; Shepherd, N. E.; Xu, W.; Lucke, A. J.; Stoermer, M. J.; Fairlie, D. P. *Chem. Rev.* **2017**, *117*, 8094.
15. Dougherty, P. G.; Sahni, A.; Pei, D. *Chem. Rev.* **2019**, *119*, 10241.
16. Wang, C. K.; Swedberg, J. E.; Harvey, P. J.; Kaas, Q.; Craik, D. J. *J. Phys. Chem. B* **2018**, *122*, 2261.
17. Eghtedari, M.; Jafari Porzani, S.; Nowruzi, B. *Phytochem. Lett.* **2021**, *42*, 87.
18. Kelly, C. N.; Townsend, C. E.; Jain, A. N.; Naylor, M. R.; Pye, C. R.; Schwochert, J.; Lokey, R. S. *J. Am. Chem. Soc.* **2021**, *143*, 705.
19. Raja, A.; LaBonte, J.; Lebbos, J.; Kirkpatrick, P. *Nat. Rev. Drug Discov.* **2003**, *2*, 943.
20. Grant, C.; Rahman, F.; Piekarz, R.; Peer, C.; Frye, R.; Robey, R. W.; Gardner, E. R.; Figg, W. D.; Bates, S. E. *Expert Rev. Anticancer Ther.* **2010**, *10*, 997.
21. Fernandes, C.; Ribeiro, R.; Pinto, M.; Kijjoa, A. *Molecules* **2023**, *28*, 615.
22. Suyama, T. L.; Gerwick, W. H.; McPhail, K. L. *Bioorg. Med. Chem.* **2011**, *19*, 6675.
23. Ha, M. W.; Kim, J.; Paek, S. M. *Mar. Drugs* **2022**, *20*, 171.

24. Sueyoshi, K.; Kaneda, M.; Sumimoto, S.; Oishi, S.; Fujii, N.; Suenaga, K.; Teruya, T. *Tetrahedron* **2016**, *72*, 5472.
25. Kaneda, M.; Sueyoshi, K.; Teruya, T.; Ohno, H.; Fujii, N.; Oishi, S. *Org. Biomol. Chem.* **2016**, *14*, 9093.
26. Kaneda, M.; Kawaguchi, S.; Fujii, N.; Ohno, H.; Oishi, S. *ACS Med. Chem. Lett.* **2018**, *9*, 365.
27. Han, J.; Lee, M. K.; Jang, Y.; Cho, W. J.; Kim, M. *Drug Discov. Today* **2022**, *27*, 1895.
28. Watashi, K.; Hijikata, M.; Hosaka, M.; Yamaji, M.; Shimotohno, K. *Hepatology* **2003**, *38*, 1282.
29. Ma, S.; Boerner, J. E.; TiongYip, C.; Weidmann, B.; Ryder, N. S.; Cooreman, M. P.; Lin, K. *Antimicrob. Agents Chemother.* **2006**, *50*, 2976.
30. Paeshuyse, J.; Kaul, A.; De Clercq, E.; Rosenwirth, B.; Dumont, J. M.; Scalfaro, P.; Bartenschlager, R.; Neyts, J. *Hepatology* **2006**, *43*, 761.
31. Hopkins, S.; Scorneaux, B.; Huang, Z.; Murray, M. G.; Wring, S.; Smitley, C.; Harris, R.; Erdmann, F.; Fischer, G.; Ribeill, Y. *Antimicrob. Agents Chemother.* **2010**, *54*, 660.
32. Sakamoto, K.; Tsujii, E.; Miyauchi, M.; Nakanishi, T.; Yamashita, M.; Shigematsu, N.; Tada, T.; Izumi, S.; Okuhara, M. *J. Antibiot.* **1993**, *46*, 1788.
33. Makino, T.; Yoshimura, S.; Yamanaka, T.; Sawada, M.; Barrett, D. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127251.
34. Makino, T.; Yoshimura, S.; Neya, M.; Yamanaka, T.; Sawada, M.; Tsujii, E.; Barrett, D. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127308.
35. Makino, T.; Ishida, J.; Yamanaka, T.; Ohki, H.; Uchida, M.; Sawada, M.; Barrett, D. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127423.
36. Jwad, R.; Weissberger, D.; Hunter, L. *Chem. Rev.* **2020**, *120*, 9743.

第一章　Coibamide A の構造活性相関研究

第一節　研究の背景

Coibamide A (**1**) は、海洋シアノバクテリアより単離された 8 個の *N*-メチルアミノ酸を有する 11 残基環状デプシペプチドであり、様々な種類の腫瘍細胞株に対して非常に強力な細胞増殖抑制活性を示す[1]。当初の提唱構造 **2** ではアミノ酸の立体化学はすべて L 体とされていたが、化学合成された提唱構造は天然物とは NMR スペクトルが一致せず、また生物活性も天然物と比較して数千分の 1 に低下していた[2]。所属研究室においても提唱構造 **2** の合成研究に取り組んでおり、この過程で得られた coibamide A のエピ体[L-Hva^2]-coibamide A (**3**) も、天然物 **1** の数分の 1 程度の細胞増殖抑制活性しか示さなかった[3]。2015 年、Yao らは、coibamide A の全合成を達成し、提唱構造の 2 箇所の立体配置を修正した構造が天然物であることを明らかにした（Figure 1）[4]。

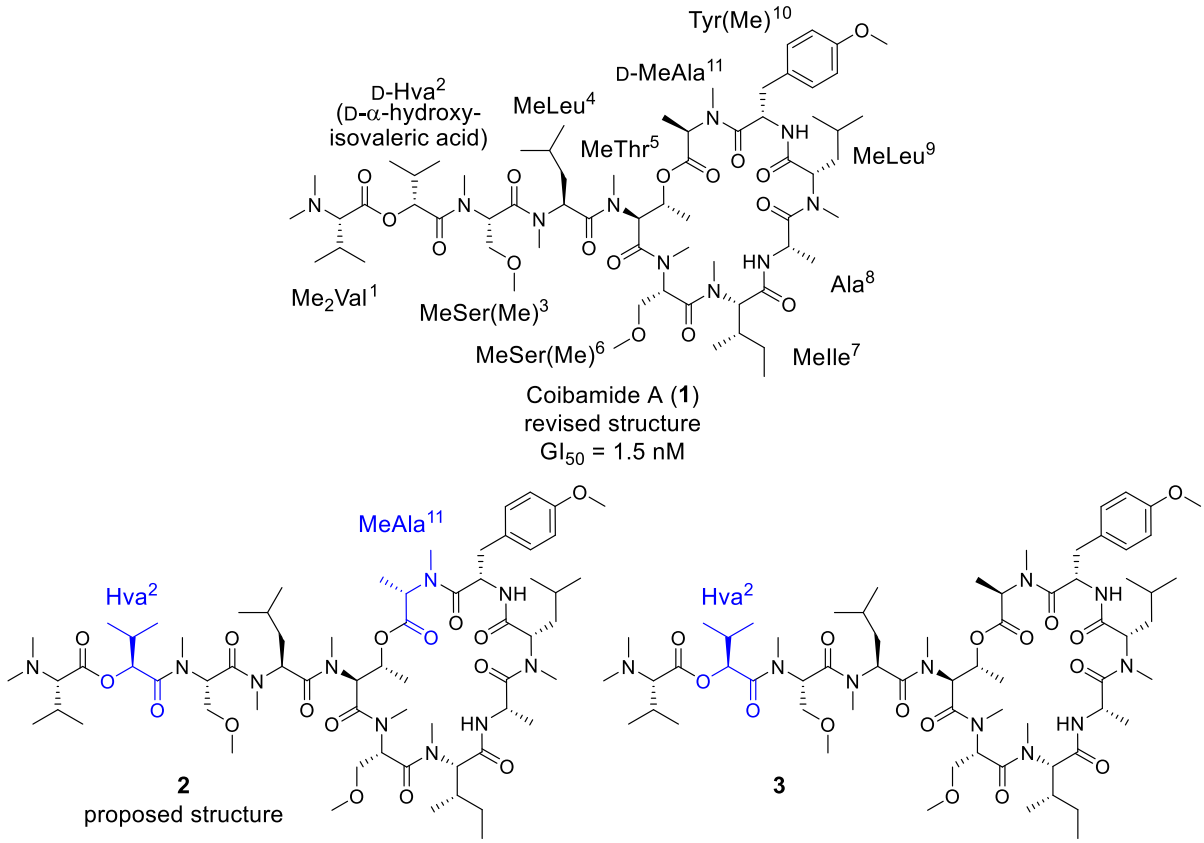


Figure 1. Structures of Coibamide A and Its Stereoisomers. GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

また、Yao らは、coibamide A の構成アミノ酸や環外部分の構造活性相関研究において、構造を簡略化した誘導体の大半は coibamide A と比較して数十分の 1 以下に生物活性が低下したものの、2 箇所の MeSer(Me) を MeAla に置換した誘導体 **4** が生物活性を維持することを

明らかにした（Figure 2）[5]。これら 2 箇所の MeSer(Me) を MeAla に変換した誘導体は、ラセミ化しやすく効率の低い MeSer(Me) の縮合工程を回避して合成できることから、効率的な誘導体合成と構造最適化研究が可能である。

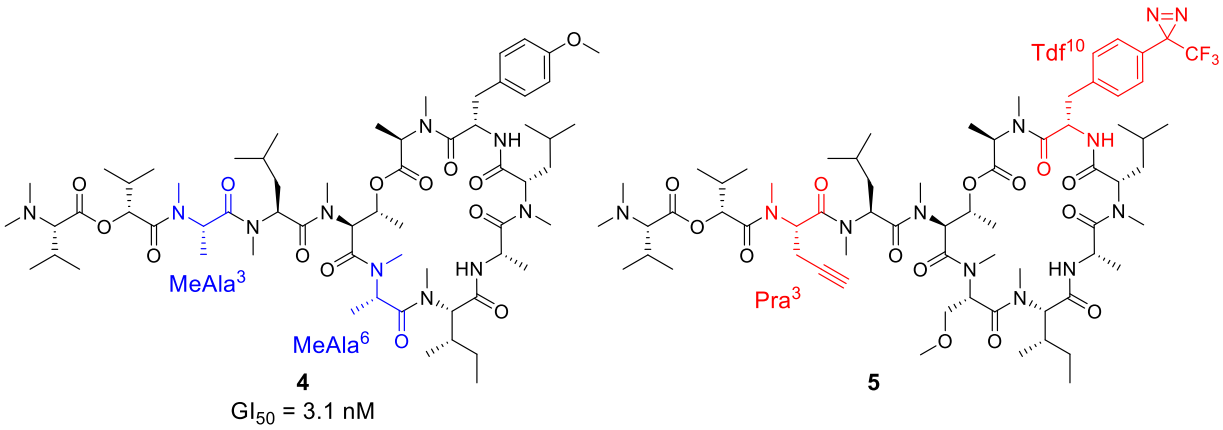


Figure 2. Previously Reported Coibamide A Derivatives. GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

これまでに、複数の coibamide A の細胞レベルでの作用機序解析が行われている。Coibamide A が引き起こすヒト膠芽腫細胞株に対する細胞毒性は mTOR 非依存性のオートファジーを介するものであり [6]、オートファジーの誘導には ATG5 が関与していることが明らかにされている [7]。また、Serrill らは、coibamide A が細胞周期の進行を阻害し、細胞の遊走能力および侵襲能力を低下させることを報告した [8]。この作用は、coibamide A が内在性膜タンパク質である血管内皮細胞増殖因子受容体 2（VEGFR-2）の発現およびそのリガンドである VEGF-A の分泌を強力に阻害したことによると報告されている。さらに、光親和性プローブ **5** を用いた標的探索研究により、coibamide A が小胞体内腔や小胞体膜へのタンパク質の分泌に関与する膜タンパク質複合体 Sec61 の α サブユニットに作用してタンパク質分泌を阻害することが明らかとなった [9]。

Sec61α に作用してタンパク質分泌を阻害する天然物として apratoxin A (**6**)[10,11]、decatransin (**7**)[12]、eeyarestatin I (**8**)[13,14]、cotransin (**9**)[15-17]、ipomoeassin F (**10**)[18]、および mycolactone A/B (**11**)[19] が知られている（Figure 3）。これらの天然物のうち、cotransin は Sec61 由来のタンパク質分泌を基質特異的に阻害するのに対して、apratoxin A、ipomoeassin F および coibamide A は、基質非特異的に阻害すると報告されている。興味深いことに、これらの化合物はいずれも Sec61α に作用するにも関わらず、NCI-60 スクリーニングパネル（60 種類のヒトがん細胞株に対する細胞増殖抑制活性評価）の結果を比較すると、化合物ごとに異なるプロファイルを有することが明らかとなった [9]。この結果は、これらの天然物がそれぞれ異なる様式で Sec61α に作用したり、Sec61α 以外の標的分子にも作用したりすることにより、多様な生物活性を示す可能性を示唆している。このため、基質非特異的に Sec61α を阻害することが想定され

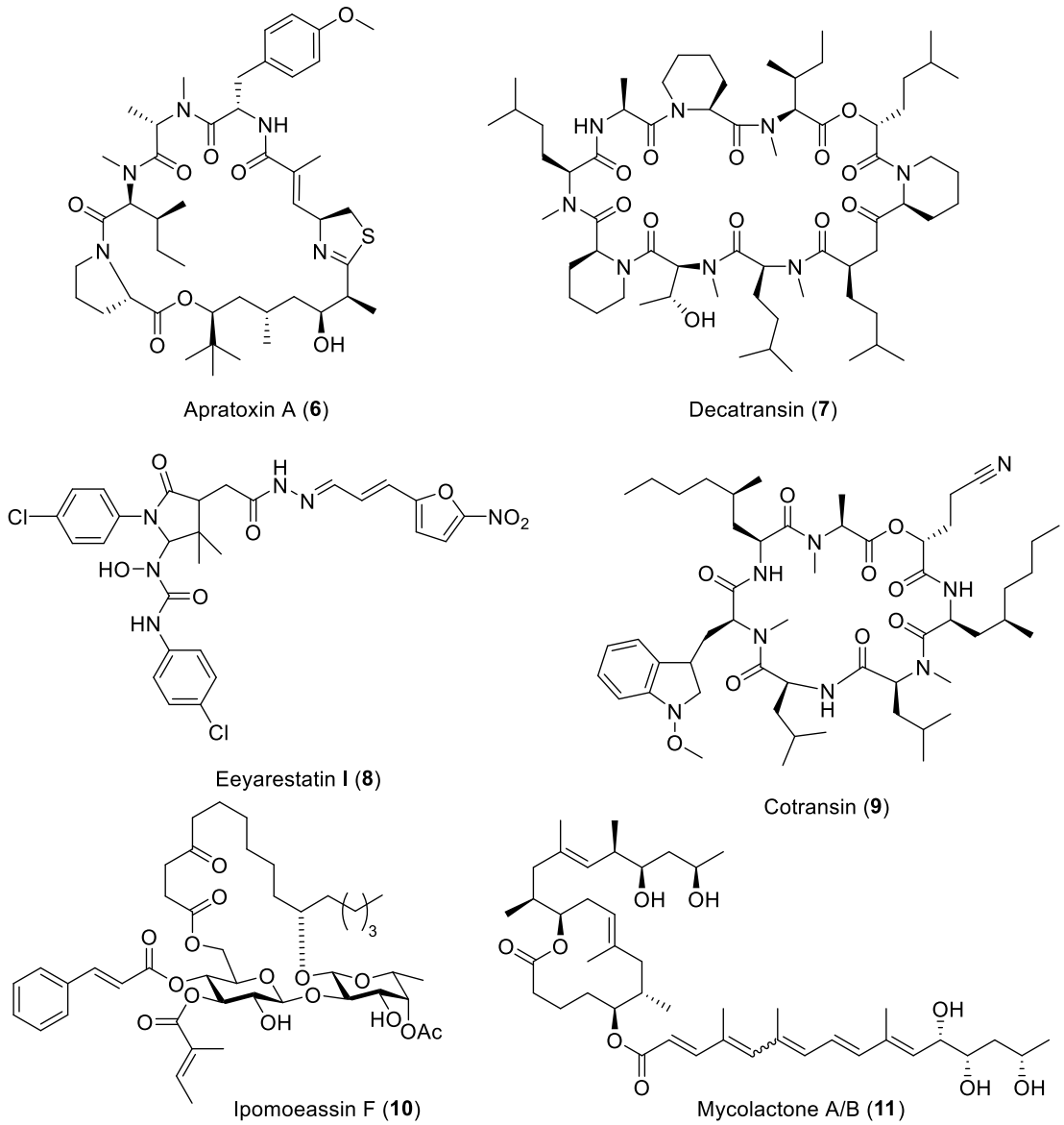


Figure 3. Structures of Sec61α inhibitors

ている coibamide A に種々の修飾を施すことにより、細胞選択性に違いを出し、正常な細胞への毒性効果を低減した新しい抗がん剤のシーズを創製する可能性がある。

こうした背景のもと、著者は、coibamide A の魅力的な生物活性及び標的分子との相互作用に必要な構造要素を明らかにすべく、coibamide A の構造活性相関研究に取り組んだ。

第二節　Coibamide A の大環状構造の構造活性相関研究

所属研究室では、coibamide A (**1**) の化学合成による構造決定に向けた取り組みや抗がん剤としての応用を目指した構造活性相関研究を実施してきた。このうち、一般的に化学的および生物学的安定性が低いとされるマクロラクトン構造を改変した構造活性相関研究の過程において、環状構造の母骨格を維持したままマクロラクトン部位をアルキル鎖に変換した誘導体 **12a** が中程度の活性を示すことを見出している（Figure 4）[20]。このように、マクロラクトン部分周辺の構造は、微細な変換であっても coibamide A の生物活性に大きな影響を与えることが明らかとなっていた。こうした背景のもと、著者は、ペプチド **12a** の 2 箇所の MeSer(Me)を MeAla に置換した誘導体 **13a** を設計し、ペプチド **13a** をリードとした構造活性相関研究を行った。

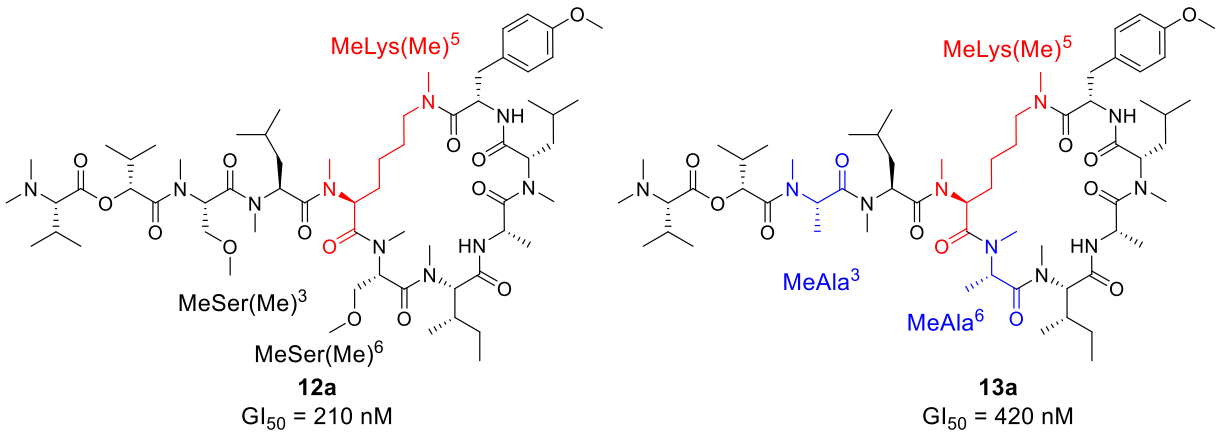
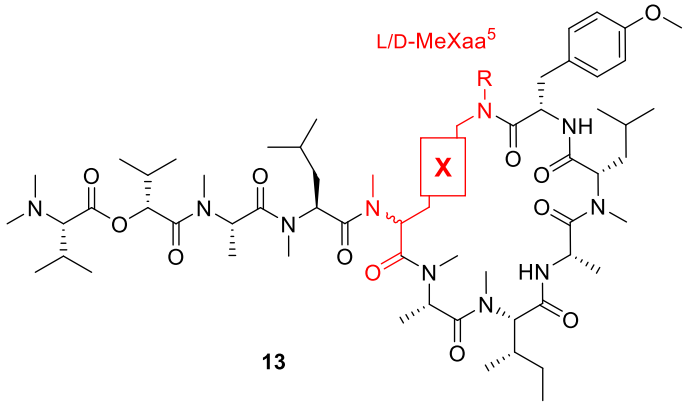


Figure 4. Structures of Coibamide A MeLys(Me) Analogues. GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

ペプチド **13a** はペプチド **12a** より活性は低下したものの、リード化合物として十分な活性を示した。引き続き、coibamide A の D-MeAla[11]–MeThr[5] のエステル部位の構造最適化を行うことを目的として、MeLys(Me)[5] の立体化学、側鎖アルキル鎖長、側鎖アミノ基上の修飾様式（N^{ε}-メチル基）の異なる各種誘導体 **13b–h** を設計・合成した。得られたペプチドの細胞増殖抑制活性を評価したところ、リシン側鎖に N^{ε}-メチル基を有さない誘導体 **13e** ではペプチド **13a** と比較して活性の低下は 2 分の 1 程度であった。このことから、5 残基目のリシンの側鎖メチル基が coibamide A の環構造に与える影響は小さいことが示唆された。一方、他の誘導体では活性が消失もしくは大幅に低下した。この結果から、5 残基目のアミノ酸は L-MeLys(Me)が最適であり、立体化学や側鎖アルキル鎖長の変化により環構造が大幅に変化することが示唆された（Table 1）。

Coibamide A の主鎖のペプチド結合上の N-メチル基および大環状構造を構成するアミノ酸の立体化学がペプチドのコンフォメーションや生物活性に与える影響を調べることを目的

Table 1. Structure−Activity Relationships of Analogues with Lys and Orn in Place of the Ester Linkage of Coibamide A



peptide	MeXaa[5]	GI_{50} (μM)[a]
13a	L-MeLys(Me)	0.42 ± 0.03
13b	D-MeLys(Me)	8.3 ± 2.2
13c	L-MeOrn(Me)	9.6 ± 3.2
13d	D-MeOrn(Me)	>10
13e	L-MeLys	0.85 ± 0.02
13f	D-MeLys	>10
13g	L-MeOrn	>10
13h	D-MeOrn	>10

[a] GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

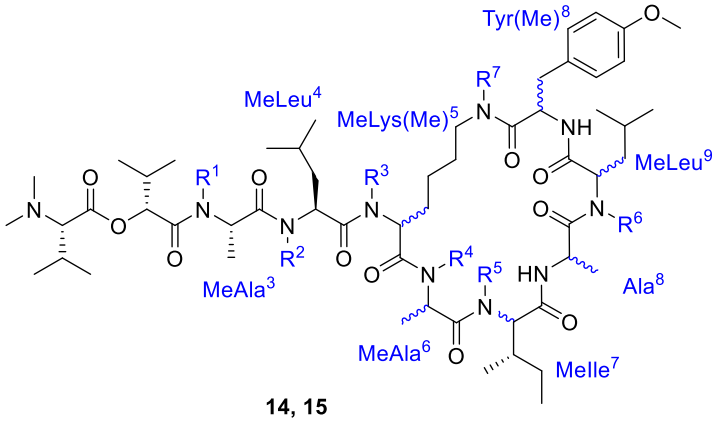
として、ペプチド **13a** の *N*-メチルアミノ酸を *N*-メチル基修飾のないアミノ酸に置換した誘導体 **14a–f**、および、大環状構造を構成するアミノ酸の立体配置が異なる誘導体 **15a–e** を合成し、生物活性を評価した（Table 2）。

環外部分の配列に含まれる *N*-メチルアミノ酸の *N*-メチル基を除去した誘導体 **14a** 及び **14b** では、ペプチド **13a** と比較して活性が低下したが、低下の度合いは 10 分の 1 未満で、他の誘導体よりも相対的に小さかった。一方、大環状骨格上の *N*-メチル基を除去した誘導体 **14c–f** は、活性が消失もしくは大幅に低下した。大環状構造を構成するアミノ酸の *N*-メチル基は MeLys(Me)[5] の N^{ε}-メチル基を除いて生物活性に重要であり、その寄与は環外部分の配列中の主鎖構造の *N*-メチル基と比較して高いことが示唆された。

また、大環状構造を構成するアミノ酸のうち、MeLeu[9] を D-MeLeu に置換した誘導体 **15d** では 10 分の 1 程度の低下にとどまった。一方で、この他のアミノ酸の立体配置を変更した誘導体 **13b**、**15a–c** および **15e** では、生物活性が 15 分の 1 以下にまで低下した。これらの結果から、MeLeu[9] の立体配置が環構造や生物活性に与える影響は相対的に小さいことが示唆された。大環状構造を構成するアミノ酸の立体配置を変更した誘導体 **13b** および **15a–e** は、

いずれもペプチド **13a** と比較して活性が低下したことから、ペプチド **13a** のアミノ酸立体配置の組み合わせが最適であることが示唆された。

Table 2. Modification of the Macrocyclic Structure by Substitution with *N*-Demethylated and D-Amino Acids



14, 15

peptide	modification	GI_{50} (μM)[a]
N-Demethyl amino acid		
13a	-	0.42 ± 0.03
14a	Ala^3	2.2 ± 0.6
14b	Leu^4	3.4 ± 0.6
14c	$Lys(Me)^5$	6.4 ± 1.7
14d	Ala^6	7.5 ± 3.2
14e	Ile^7	>10
14f	Leu^9	3.9 ± 0.4
13e	$MeLys^5$	0.85 ± 0.02
D-Amino acid		
13b	D-$MeLys(Me)^5$	8.3 ± 2.2
15a	D-$MeAla^6$	>10
15b	D-*allo*-Ile^7	>10
15c	D-Ala^8	>10
15d	D-$MeLeu^9$	2.6 ± 0.9
15e	D-$Tyr(Me)^{10}$	>10

[a] GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

第三節　Coibamide A の Tyr(Me) 部位の構造最適化による高活性誘導体の創製

所属研究室では、光親和性修飾基を有するプローブを用いた coibamide A (**1**) の標的分子の同定を試みる研究に取り組んできた。これまでに、coibamide A の Tyr(Me)[10] 部位に 2-amino-3-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)phenyl]propanoic acid (Tdf) を導入したプローブを設計・合成し、このプローブが Sec61α と共有結合を形成することを明らかにした[9]。このことは、coibamide A の Tyr(Me)[10] 側鎖が Sec61α 上の相互作用部位の近傍に位置し、Sec61α による分子認識に重要な役割を果たしていることを示唆している。

一方、恩田らは、Sec61α に作用して細胞増殖抑制活性を示す環状デプシペプチドとして知られている apratoxin A (**6**) の構造活性相関研究において、α,β-不飽和アミドおよびチアゾリン環部分を変更した誘導体 **16** を報告した（Figure 4）[21]。ペプチド **16** は apratoxin A と比較して細胞増殖抑制活性が 40 分の 1 以下にまで低下したものの、ペプチド **16** の Tyr(Me) を β-(4-biphenylyl)alanine（Bph）に置換した誘導体 **17** は **16** の約 100 倍強力な細胞増殖抑制活性を示した。

これらの知見をもとに、著者は、apratoxin A と同じ標的分子に作用する coibamide A においても Tyr(Me) 部位の修飾により細胞増殖抑制活性が向上することを期待して、構造活性相関研究を行った。

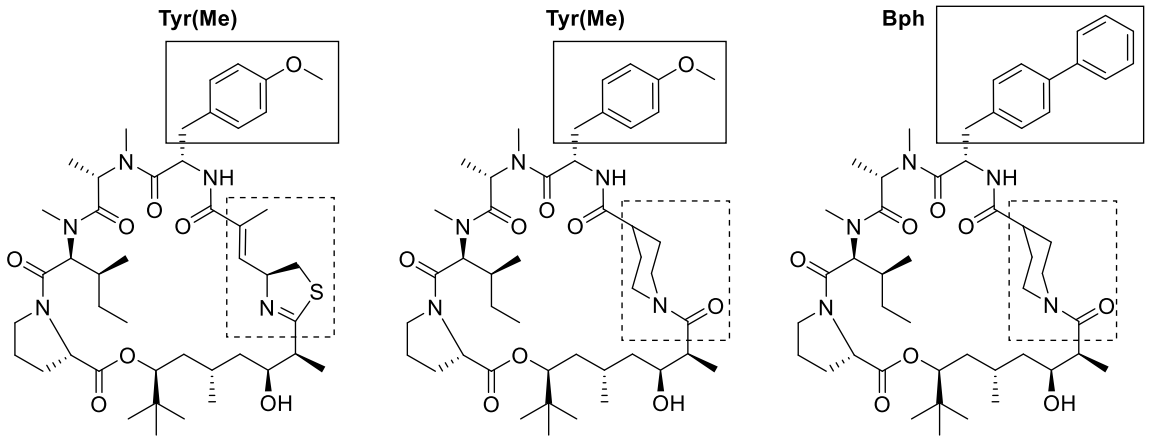
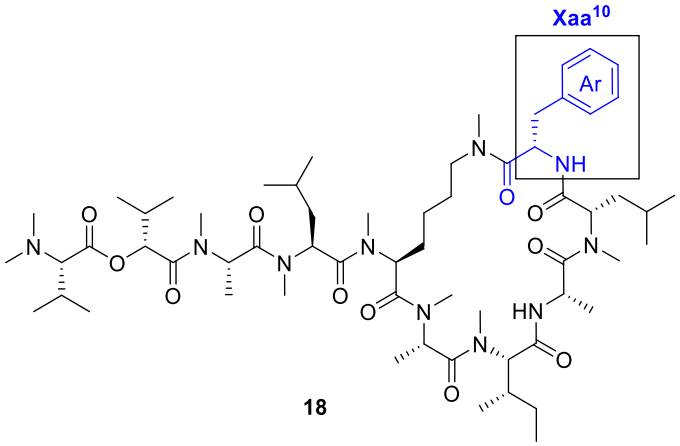


Figure 4. Structure–Activity Relationship of Apratoxin A Derivatives. GI_{50} values are the concentrations for 50% growth inhibition of HCT116 cells (n = 3).

まず、ベンゼン環上のパラ位の置換基を変更した誘導体 **18a–i** の合成および生物活性の評価を行った（Table 3）。Phe を導入した誘導体 **18a** の生物活性は 10 分の 1 程度であり、ニトロ基、シアノ基、*t*-ブトキシ基をそれぞれ導入した誘導体 **18b**、**18d** および **18h** はペプチド **13a** と比較して数分の 1 程度にまで活性が低下したものの、**18i** をはじめとする多くの誘導体が **13a** と同程度の生物活性を示した。一方、Bph を導入した誘導体 **18j** はペプチド **13a** の

Table 3. Structure−Activity Relationships of Coibamide A Analogues with Modification at the Tyr(Me)^{10} Moiety of Coibamide A



peptide	Xaa^{10} [a]	GI$_{50}$ (μM)[b]
13a	Tyr(Me) [Phe(4-OMe)]	0.42 ± 0.03
18a	Phe	4.0 ± 0.8
18b	Phe(4-NO_2)	1.1 ± 0.2
18c	Phe(4-CF_3)	0.37 ± 0.07
18d	Phe(4-CN)	1.5 ± 0.5
18e	Phe(4-N_3)	0.38 ± 0.05
18f	Phe(4-Cl)	0.71 ± 0.19
18g	Phe(4-*t*-Bu)	0.61 ± 0.21
18h	Phe(4-O*t*-Bu) [Tyr(*t*-Bu)]	1.0 ± 0.1
18i	Phe(4-OCF_3) [Tyr(CF_3)]	0.32 ± 0.03
18j	Bph [Phe(4-Ph)]	0.060 ± 0.016
18k	2-Pal	>10
18l	3-Pal	>10
18m	4-Pal	>10
18n	MePhe	>10
18o	Tic	>10
18p	1-Nal	4.8 ± 0.5
18q	2-Nal	0.28 ± 0.03

[a]2-Pal, β-(2-pyridyl)alanine; 3-Pal, β-(3-pyridyl)alanine; 4-Pal, β-(4-pyridyl)alanine; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; 1-Nal, β-(1-naphthyl)alanine; 2-Nal, β-(2-naphthyl)-alanine; Bph, β-(4-biphenylyl)alanine. [b]GI$_{50}$ values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

7 倍ほど強力な細胞増殖抑制活性を示した［GI$_{50}$ (**18j**) = 60 nM］。

Bph の導入により活性が向上したことから、さらに他の芳香環の導入により活性が向上す

ることを期待して、誘導体 **18k–q** の合成および生物活性の評価を行った。ピリジン環を導入した誘導体 **18k–m** では活性が消失した。*N*-メチル基を導入した誘導体 **18n** や、芳香環のコンフォメーションを固定することが可能な 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid（Tic）を導入した誘導体 **18o** でも活性が消失したことから、Tyr(Me)^{10} 部位の *N*-アルキル化などの大環状構造のコンフォメーションに変化を及ぼす修飾は、生物活性に不利であることが示唆された。興味深いことに、β-(1-naphthyl)alanine (1-Nal) を導入した誘導体 **18p** ではペプチド **13a** と比較して生物活性は 10 分の 1 程度にまで低下した一方で、β-(2-naphthyl)alanine (2-Nal) を導入した誘導体 **18q** では生物活性がわずかに向上した［GI_{50} (**18p**) = 4.8 μM; GI_{50} (**18q**) = 0.28 μM］。これらの結果から、芳香環のパラ位の置換基の存在とその種類が生物活性に重要であることが示唆された。

上述の構造活性相関研究から得られた知見をもとに coibamide A（天然型）の生物活性の向上を目指した検討を行った。細胞増殖抑制活性の向上が期待できる Bph を coibamide A に導入した誘導体 **19a**、および、その MeAla^3/MeAla^6 置換体 **19b** を設計した。ペプチド **19a** および **19b** の細胞増殖抑制活性を評価したところ、**19a** は天然物の 10 倍以上強力な生物活性を示した［GI_{50} (**19a**) = 0.11 nM］。また、MeAla 置換体 **19b** も、天然物の 5 倍以上高い活性を示した［GI_{50} (**19b**) = 0.25 nM］（Figure 5）。

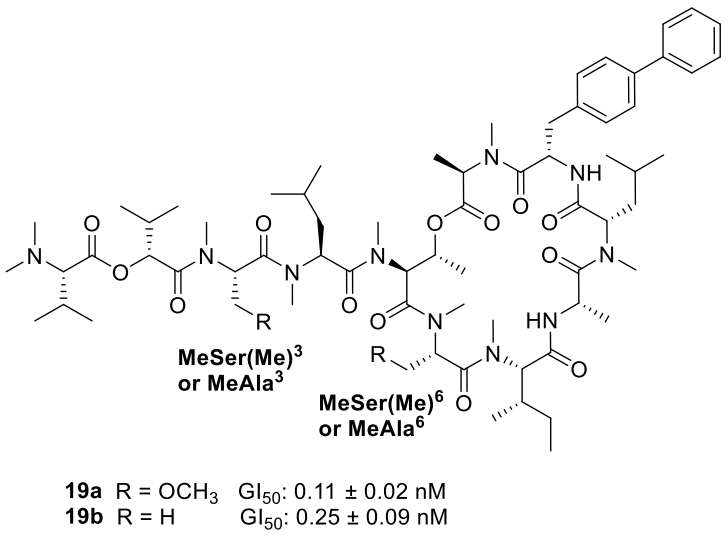


Figure 5. Structures of Biphenylylalanine (Bph)-containing Coibamide A Analogues and Their Cytotoxicities. GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

第四節　Coibamide A のマクロラクトン部位の構造活性相関研究

著者の研究開始時点までに、所属研究室では、coibamide A (**1**) のマクロラクトン部位を改変した誘導体の合成を行っていた。この過程において、エステル結合をメチレン鎖とした誘導体 **12a** が中程度の活性を示し、炭素―炭素二重結合とした誘導体 **12b** の活性が消失することを明らかにしている[20]。これらの誘導体では、天然物の MeThr の β-メチル基と D-MeAla の α-メチル基に相当する 2 つのメチル基を欠いており、天然物におけるこれらのメチル基が標的部位と直接相互作用していた可能性や、これらのメチル基が生物活性に必要なコンフォメーションをとることに寄与している可能性が示唆された。こうした背景のもと、著者は、coibamide A の MeThr の β-メチル基と D-MeAla の α-メチル基に相当するメチル基を導入することで、生物活性の向上が期待できるのではないかと考え、誘導体 **20a** および **20b** を設計・合成した（Figure 6）。

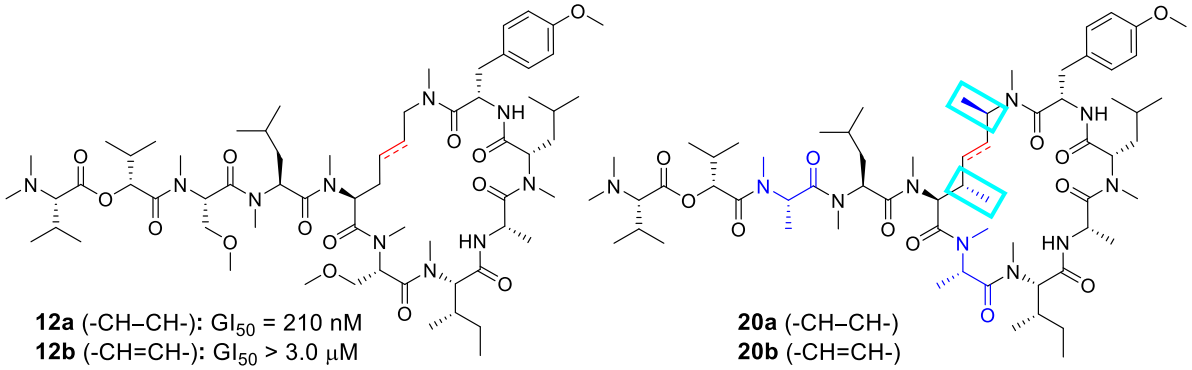


Figure 6. Design of Coibamide A Derivatives with 3,6-Dimethyl Lysines. GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

設計したペプチドを合成するためには、リシンアナログを合成する必要があった。Fmoc 法を用いた固相合成によりペプチドを合成するべく、これに適用可能な保護リシンアナログとして、**21a** および **21b** を設計した（Figure 7）。まず、これらを合成するためには、α 位のアミノ基、リシンの側鎖上の β-メチル基、ε-メチル基の計 3 つの不斉点の立体配置を制御する必要がある。また、アルケンを有する **21b** については、2 つの不斉炭素に挟まれたアルケン部位を trans 型とする必要がある。さらに、これらのリシンアナログでは、ペプチド合成

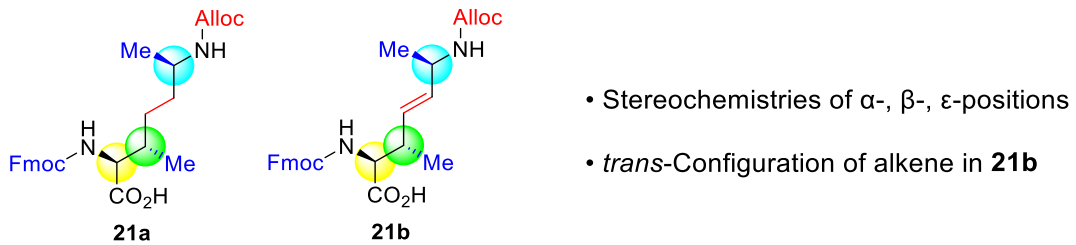
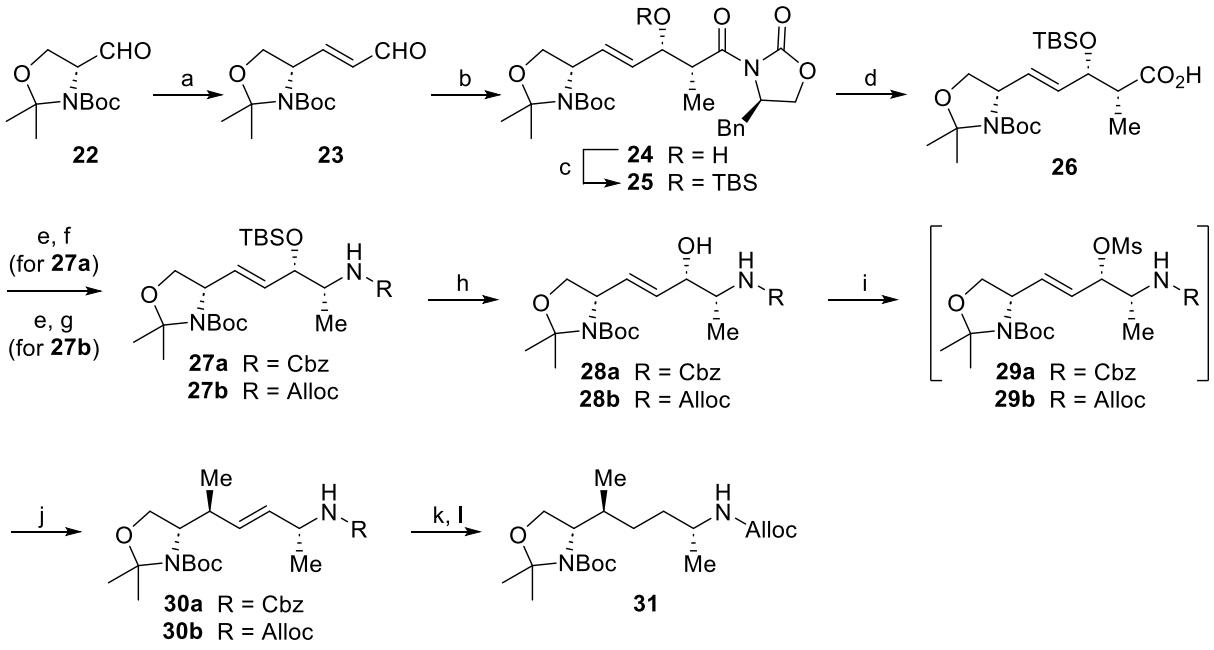


Figure 7. Design of Protected 3,6-Dimethyl Lysine Derivatives

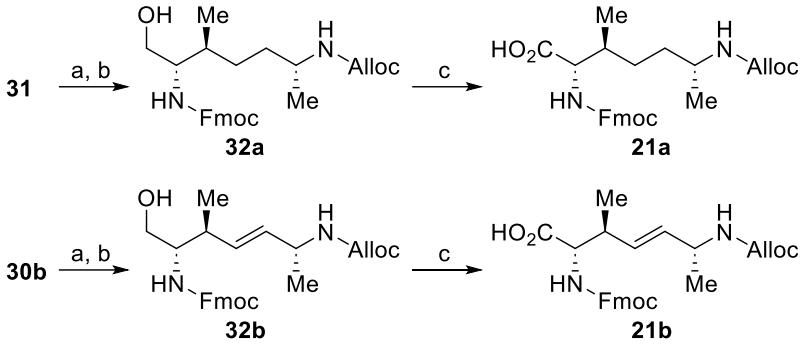
の際に主鎖と側鎖の 2 つのアミノ基を区別する必要があることから、α-アミノ基の保護基として Fmoc 基を、ε-アミノ基の保護基として Alloc 基を利用することとした。

2 つのリシンアナログは、D-Ser 由来の Garner アルデヒド [22] を出発原料として合成した。まず、Garner アルデヒド **22** への Wittig 反応 [23] により 2 炭素ユニットを増炭した後、得られたアルデヒド **23** に対して Evans アルドール反応を行うことで、D-MeAla の α-メチル基に相当するメチル基を有するアルコール **24** を立体選択的に得た。その後、ヒドロキシ基を TBS 保護し、不斉補助基を加水分解により除去して、カルボン酸 **26** を合成した。カルボン酸 **26** の立体配置は、X 線結晶構造解析により確認した。続いて、カルボン酸 **26** の DPPA 処理により得られる酸アジドの Curtius 転位反応によりイソシアナートを得た後、リシンの側鎖保護基に対応したアルコールとの反応により保護アミン **27** を得た。この際、飽和炭素鎖からなるリシンアナログの前駆体 **27a** は、後の工程でアルケンの還元が必要となることを踏まえて、側鎖保護基を一旦 Cbz 基で保護した。引き続き、TBS 基を脱保護した後、水酸基をメシル化した基質 **29a,b** に対して、有機銅試薬を用いた *anti*-S_N2'型アルキル化反応 [24,25] によりメチル基を立体選択的に導入し、**30a** および **30b** を得た。Cbz 体 **30a** は、接触還元によるオレフィンの還元と Cbz 基の脱保護の後、Alloc 保護することで化合物 **31** とした（Scheme 1）。



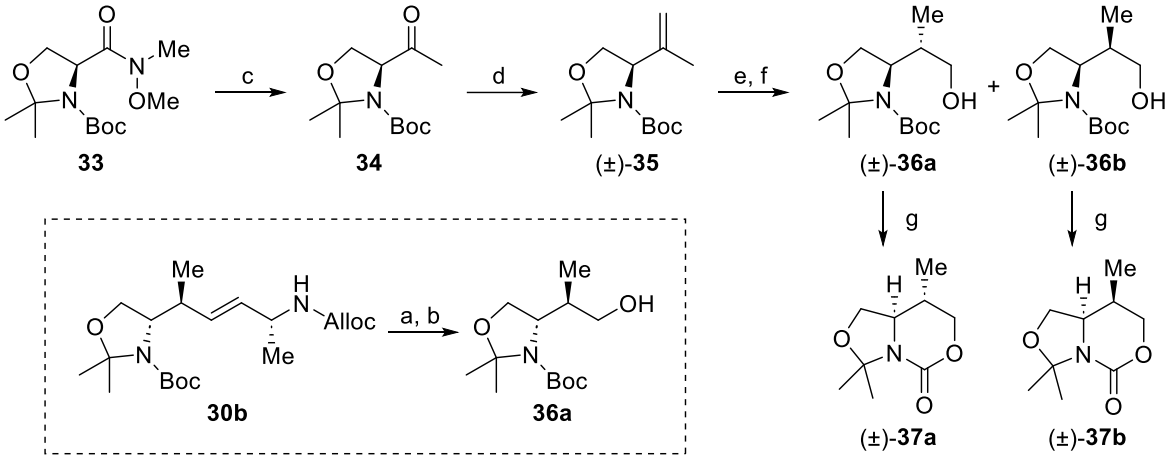
Scheme 1. Stereoselective Synthesis of the Lys Frameworks. *Reagents and conditions*: (a) Ph_3P=CHCHO, CH_2Cl_2, rt, 75%; (b) (*R*)-4-benzyl-3-propanoyl-2-oxazolinone, *n*-Bu_2BOTf, DIEA, CH_2Cl_2, 75%; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 98%; (d) $LiOH·H_2O$, H_2O_2, THF, H_2O, 0 °C, 75%; (e) DPPA, Et_3N, toluene, 50 °C; (f) BnOH, toluene, 50 °C, 84% (2 steps); (g) allyl alcohol, toluene, 50 °C, 90% (2 steps); (h) TBAF, THF, rt, 80% (**28a**) and 83% (**28b**); (i) MsCl, pyridine, $CHCl_3$, 0 °C; (j) MeMgBr, CuCN, LiCl, THF, –78 °C, 79% (**30a**) and 75% (**30b**) (2 steps); (k) 10% Pd/C, MeOH, rt; (l) Alloc-Cl, DIEA, THF, 0 °C to rt, 85% (2 steps from **30a**).

最後に、アセトナイドの除去と Boc 基の除去、Fmoc 保護を行った後、生成したアルコールの酸化反応を行うことにより、目的のリシンアナログ **21a** および **21b** を合成した（Scheme 2）。



Scheme 2. Synthesis of Protected Lys Derivatives with Two Methyl Groups. *Reagents and conditions*: (a) TFA, $CHCl_3$, 0 °C to rt; (b) Fmoc-OSu, DIEA, MeCN, H_2O, rt, 71% (**32a**) and 87% (**32b**) (2 steps); (c) AZADOL, NaClO, $NaClO_2$, MeCN, phosphate buffer (pH 7.0), 0 °C, 75% (**21a**) and 71% (**21b**).

リシンアナログの β 位に導入されたメチル基の立体配置は、別途化学合成した標品の NMR 解析により確認した（Scheme 3）。まず、アルケン **30b** をオゾン酸化に付した後、$NaBH_4$, で還元処理することでアルコール **36a** を得た。これとは別に、アルコールの 2 つのジアステレオマー**36a** および **36b** を、Weinreb アミド **33** から合成した。すなわち、アミド **33** を MeLi で処理することでメチルケトンを得た後、Wittig 反応によりアルケン(±)-**35** を得た。続いて、ヒドロホウ素化―酸化反応によりアルケン部位を変換した後、カラムクロマトグラフィーに



Scheme 3. Synthesis of an Authentic Samples for the Determination of the Stereochemistry of Compound **30b**. *Reagents and conditions*: (a) O_3, MeOH, –78 °C; (b) $NaBH_4$, –78 °C to rt, 43%; (c) MeLi, THF–Et_2O, –78 °C; (d) Ph_3P-$CH_3^+Br^-$, *t*-BuOK, rt, 56% (2 steps); (e) $BH_3 \cdot SMe_2$, THF, 0 °C; (f) H_2O_2, rt, 12% ((±)-**36a**) and 45% ((±)-**36b**); (g) NaH, THF, 0 °C, then reflux, 67% ((±)-**37a**) and 71% ((±)-**37b**).

よりジアステレオマーを分離することで、アルコール(±)-**36a,b** を得た。このプロセスにより得られた(±)-**36a** の NMR スペクトルは、アルケン **30b** のオゾン処理により得られたアルコール **36a** と一致した。

引き続き、アルコール(±)-**36a,b** をそれぞれ塩基性条件での環化反応に付すことで、1,3-オキサジナン-2-オン(±)-**37a,b** に変換した。化合物(±)-**37a,b** の 8a 位の水素原子に関する ^{1}H-NMR 解析において、(±)-**37a** では 8-メチル基との間に、(±)-**37b** では 8 位の水素原子との間にそれぞれ NOE 相関が認められた（Figure 8）。これらのことから、化合物(±)-**37a** の 8a 位の水素原子と 8-メチル基はシス配置であり、リシンアナログ前駆体 **30b** に導入されたメチル基は所望の立体配置で導入されていることが示唆された。

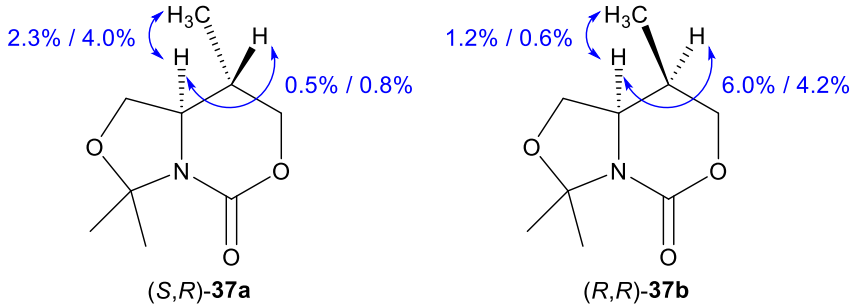
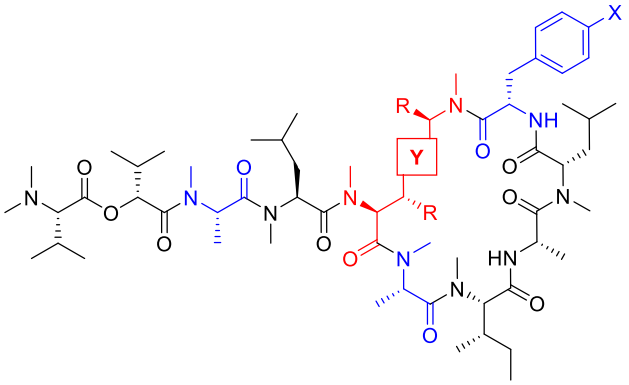


Figure 8. NOE Correlations for the Determination of the Stereochemistries in Lys Derivatives.

合成したリシンアナログを導入した coibamide A 誘導体の合成を行った。得られた誘導体の活性を評価したところ、β 位および ε 位にメチル基を導入したアルキル誘導体 **20a** の細胞増殖抑制活性は、無置換体 **13a** よりも大幅に向上した［GI_{50} (**20a**) = 41 nM］（Table 4）。また、エステル結合部分を炭素―炭素二重結合とした誘導体 **20b** の細胞増殖抑制活性は、単結合のペプチド **20a** よりもさらに強力な生物活性を示し、その活性はエステル体 **4** とほぼ同等の値を示した［GI_{50} (**20b**) = 2.6 nM］。これらの結果より、エステル周辺の 2 つのメチル基と sp^2 炭素の存在が生物活性に寄与している可能性が示唆された。メチル基を導入することで活性発現に重要なコンフォメーションを取りやすくなるとともに、sp^2 炭素の存在により構造が好ましい形に固定化されたことが生物活性の向上の一因であると考えられる。

Table 4. Structure−Activity Relationships of Analogues with a Modified Lysine.



Peptide	R	X	Y	GI_{50} (nM)[a]
4	Me	OMe [Tyr(Me)]	-CO-O-	3.1 ± 0.8
13a	H	OMe [Tyr(Me)]	$-CH_2CH_2-$	420 ± 30
18j	H	Ph (Bph)	$-CH_2CH_2-$	60 ± 16
20a	Me	OMe [Tyr(Me)]	$-CH_2CH_2-$	41 ± 4
20b	Me	OMe [Tyr(Me)]	-(*E*)-CH=CH-	2.6 ± 0.4

[a] GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

先の検討において、coibamide A のエステル結合を構成する sp^2 炭素の存在もしくはペプチド **20b** に新たに導入された炭素—炭素二重結合が活性コンフォメーションに有利であることが示唆されたことから、引き続き、この部位をアミド結合とした誘導体の活性を調査した。基底状態のアミド結合 **38** は電子の非局在化に伴う共鳴構造 **A** をとり、カルボニル炭素と窒素原子間の結合が二重結合性を帯びた平面構造をとることから、これらの修飾を施した誘導体においても生物活性が維持されることを期待した（Figure 9）[26]。

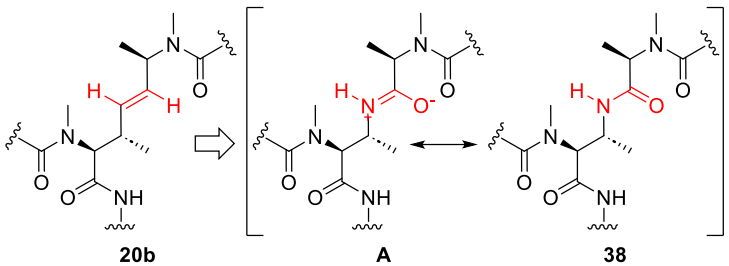
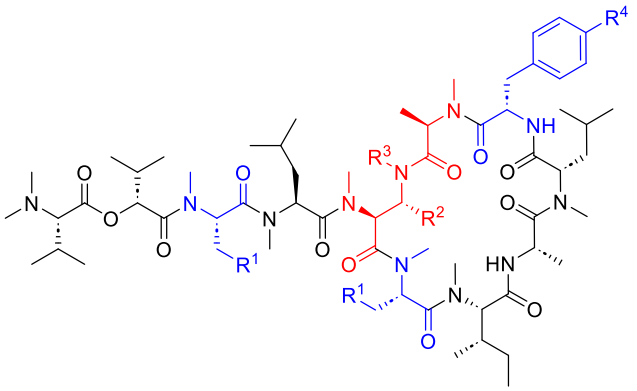


Figure 9. Design of Coibamide A Derivatives with an Amide Linkage at the D-MeAla[11]–MeThr[5] Position.

エステル結合からアミド結合への変換の効果を評価するために、ペプチド **4** の MeThr を N^{α}-メチル-2,3-ジアミノ酪酸（MeDab）に置換した誘導体 **38a** を設計し、これらの細胞増殖抑制活性を評価した。このペプチド **38a** は、エステル結合からなるペプチド **4** とほぼ同等の活性を示した［GI_{50} (**38a**) = 3.6 nM］。この結果をもとに、coibamide A の D-MeAla[11]–MeThr[5] のエステル結合をアミド結合に変換した誘導体 **38b** と、さらに Tyr(Me)[10] を Bph に置換した誘導体 **38c** を設計した。ペプチド **38b** および **38c** の生物活性を評価したところ、**38b** は天然物と同等の活性を示し［GI_{50} (**38b**) = 1.3 nM］、Bph 置換体 **38c** は天然物や **38b** よりも強力な生物活性を示した［GI_{50} (**38c**) = 0.27 nM］（Table 5）。これらの結果から、coibamide A のエステル結合からアミド結合への変換が可能であることが示唆された。一方、MeThr の β-メチル基に相当するメチル基を持たない N^{α}-メチル-2,3-ジアミノプロピオン酸（MeDap）に置換し

Table 5. Structure−Activity Relationships of Analogues with an Amide Linkage.



Peptide	R^1	R^2	R^3	R^4	GI_{50} (nM)[a]
Coibamide A (**1**)					1.5 ± 0.4
4					3.1 ± 0.8
38a	H [MeAla]	Me	H	OMe [Tyr(Me)]	3.6 ± 1.4
38b	OMe [MeSer(Me)]	Me	H	OMe [Tyr(Me)]	1.3 ± 0.3
38c	OMe [MeSer(Me)]	Me	H	Ph (Bph)	0.27 ± 0.10
39a	H [MeAla]	H	H	OMe [Tyr(Me)]	48 ± 10
39b	H [MeAla]	H	Me	OMe [Tyr(Me)]	6500

[a] GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

た誘導体 **39a,b** では、いずれも細胞増殖抑制活性が低下した。すなわち、β-アミノ基にメチル基修飾を持たない NH アミド体 **39a** は、ペプチド **4** の約 16 分の 1 の生物活性であった［GI_{50} (**39a**) = 48 nM］。また、ペプチド **39a** の β-アミノ基に *N*-メチル基修飾を付した NMe アミド体 **39b** では、大幅な生物活性の低下が認められた［GI_{50} (**39b**) = 6500 nM］。

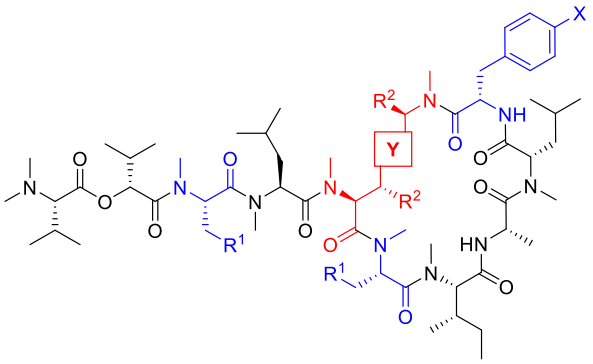
これらのことから、coibamide A のマクロラクトン部位を構成するエステル結合から NH アミド結合への変換では coibamide A と同様のコンフォメーションを維持することが可能であり、新たに導入されたアミド NH は水素結合供与体として生物活性に悪影響を及ばさないことが示唆された。また、アミド誘導体においても、coibamide A の $MeThr^5$ の β-メチル基に相当するメチル基が生物活性に重要であることが示唆された。

第五節　Coibamide A 誘導体の生物活性評価

第四節までの構造活性相関研究では、A549 細胞に対する細胞増殖抑制活性を指標として、coibamide A 誘導体の評価を進めてきた。本研究で得られた coibamide A 誘導体のうち、A549 細胞に対する強力な生物活性を示したペプチドについて、複数の細胞株に対する細胞毒性、並びに、タンパク質分泌阻害活性を評価した。

まず、A549 細胞（ヒト肺胞基底上皮腺癌細胞）に加えて U87 細胞（ヒト神経膠芽腫細胞）と HCT116 細胞（ヒト結腸癌細胞）に対する細胞毒性を評価した（Table 6）。Coibamide A (**1**) は、HCT116 細胞に対して A549 細胞に対する活性と同程度の生物活性を示したが、U87 細胞には 30 分の 1 程度まで活性が低下した。一連の coibamide A 誘導体のうち、Bph を導入したペプチド **19a** がすべての細胞株に対して最も強力な生物活性を示した。Coibamide A の D-MeAla^{11}–MeThr^{5} 部分の 2 つの側鎖メチル基に相当するメチル基を持たないペプチド **13a**、**18j**、および **39a** は、すべての細胞株に対してメチル基を有する他のペプチドよりも弱い活性を示しており、これらのメチル基の適切な配置が細胞毒性に重要な役割を果たしているこ

Table 6. Cytotoxicities of Coibamide A Derivatives against U87, HCT116, and A549 Cells



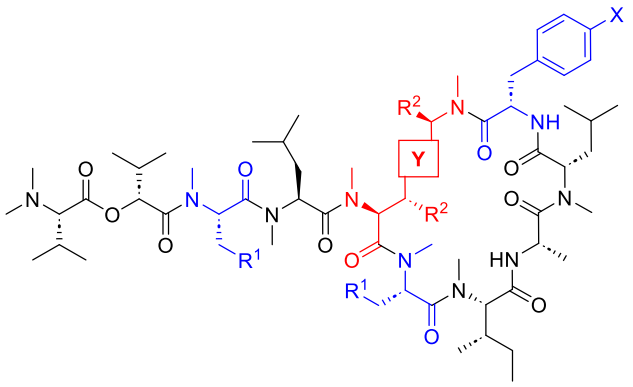
Peptide	R_1	R_2	X	Y	U87 CC_{50} (nM)[a]	HCT116 CC_{50} (nM)[a]	A549 GI_{50} (nM)[b]
1	OMe	Me	OMe	-CO-O-	50 ± 6	2.7 ± 0.5	1.5 ± 0.4
4	H	Me	OMe	-CO-O-	2.6 ± 0.1	3.9 ± 0.5	3.1 ± 0.8
13a	H	H	OMe	-CH_2CH_2-	830 ± 110	2,300 ± 100	420 ± 30
18j	H	H	Ph	-CH_2CH_2-	55 ± 2	130 ± 30	60 ± 16
19a	OMe	Me	Ph	-CO-O-	1.4 ± 0.1	1.6 ± 0.1	0.11 ± 0.22
20a	H	Me	OMe	-CH_2CH_2-	57 ± 2	130 ± 30	41 ± 4
20b	H	Me	OMe	-(*E*)-CH=CH-	6.2 ± 0.1	11 ± 1	2.6 ± 0.4
38a	H	Me	OMe	-CO-NH-	3.9 ± 0.5	4.5 ± 0.5	3.6 ± 1.4
38b	OMe	Me	OMe	-CO-NH-	2.2 ± 0.4	3.4 ± 0.6	1.3 ± 0.25
38c	OMe	Me	Ph	-CO-NH-	0.6 ± 0.2	0.80 ± 0.23	0.27 ± 0.10
39a	H	H	OMe	-CO-NH-	48 ± 3	60 ± 4	48 ± 10

[a] CC_{50} values are the concentrations for 50% viability after 72-hr incubation of cells (n = 3). [b] GI_{50} values are the concentrations for 50% growth inhibition of A549 cells (n = 3).

とが示唆された。

Coibamide A 誘導体によるタンパク質分泌抑制活性を、*Gaussia* ルシフェラーゼ（GLuc）を細胞外に分泌するレポーターを組み込んだ U87 細胞株を用いて評価した（Table 7）。Coibamide A とその MeAla^3/MeAla^6 置換体 **4** はいずれも強力なタンパク質分泌抑制活性を示し、また、Bph^{10} 置換体 **18j** および **19a** はさらに低濃度で効果的なタンパク質分泌抑制活性を示した。一方、D-MeAla^{11}–MeThr^5 部位をリシン誘導体に変換した **13a** および **20a,b** や、アミド置換体のうち MeThr の β-メチル基に相当するメチル基を欠く誘導体 **39a** ではタンパク質分泌抑制活性が減弱した。興味深いことに、エステル結合をアミド結合に置換した **38a–c** はいずれも強力なタンパク質分泌抑制活性を示し、特に天然物と同じ MeSer(Me)^3/MeSer(Me)^6 を有する **38b** および **38c** では、coibamide A より 10 倍以上強力な阻害活性を示した。

Table 7. Protein Secretion Inhibition of Coibamide A Derivatives



Peptide	R^1	R^2	X	Y	IC_{50} (nM)[a]
1	OMe	Me	OMe	-CO-O-	1.1 ± 0.3
4	H	Me	OMe	-CO-O-	2.0 ± 0.3
13a	H	H	OMe	-CH_2CH_2-	19 ± 4
18j	H	H	Ph	-CH_2CH_2-	0.86 ± 0.11
19a	OMe	Me	Ph	-CO-O-	0.79 ± 0.14
20a	H	Me	OMe	-CH_2CH_2-	18 ± 1
20b	H	Me	OMe	-(*E*)-CH=CH-	13 ± 2
38a	H	Me	OMe	-CO-NH-	0.71 ± 0.09
38b	OMe	Me	OMe	-CO-NH-	0.04 ± 0.02
38c	OMe	Me	Ph	-CO-NH-	0.08 ± 0.03
39a	H	H	OMe	-CO-NH-	30 ± 3.9

[a] IC_{50} values are the concentrations for 50% of secretory function in U87-GLuc cells after 18-hr incubation in the presence of the compound (n = 3). During the protein secretion assay (18-hr incubation for U87-Gluc cells), no effect on cell viability was observed (at 300 nM for peptide **1** and 3.0 μM for other peptides).

以上の比較により、coibamide A 誘導体の 3 種類の腫瘍細胞株への細胞毒性の構造活性相関の傾向と、タンパク質分泌阻害活性の構造活性相関の傾向には違いがあることが判明した。すべての coibamide A 誘導体が特定のタンパク質分泌機能のみを同じ作用様式で阻害することで細胞への作用を示すのであれば、このような差異は認められないはずである。このため、このような差異が生じることとなった 2 つの可能性について考察した。

1 つ目の可能性として、coibamide A およびその誘導体が Sec61α 以外の標的分子にも作用して細胞増殖抑制活性を示すケースが考えられる。このケースでは、coibamide A の D-MeAla^{11}–MeThr^{5} に相当する部位にアミド結合を有する誘導体 **38b** および **38c** は Sec61α またはその他のタンパク質分泌機能を調節するタンパク質に対する阻害活性を介して強力な細胞毒性を示したのに対し、アルキル鎖を有する **20a** および **20b** の細胞増殖抑制活性は Sec61α 以外の標的に作用することによるものであったと解釈することができる。天然物に見られる D-MeAla^{11}–MeThr^{5} のエステル結合は Sec61α に対する阻害活性に有効であることに加えて、その他の標的分子に対してもある程度の作用を有していたと考えられる。また、Tyr(Me)^{10} の Bph への置換は、エステル体 **19a** およびアミド体 **38c** ではその他の標的分子に対する活性の向上に寄与し、アルキル体 **18j** では Sec61α の阻害活性の向上に寄与したと考えられる。

2 つ目の可能性として、Sec61α を介したタンパク質分泌阻害活性の基質特異性が coibamide A 誘導体の化学構造により異なるケースが考えられる。このケースでは、例えば、coibamide A とペプチド **38b** を比較すると coibamide A の D-MeAla^{11}–MeThr^{5} に相当する部位のアミド結合への変換は、Gluc（タンパク質分泌活性の指標として用いたレポータータンパク質）の分泌に対する阻害活性が著しく向上した一方で、細胞増殖に影響するタンパク質の分泌阻害活性は coibamide A と同等であった可能性があると解釈することができる。また、アルキル鎖からなる誘導体 **13a** と **20a** を比較すると、ペプチド **13a** への MeThr の β-メチル基と D-MeAla の α-メチル基に相当する 2 つのメチル基の導入は、Gluc の分泌阻害活性には影響を与えなかったのに対し、細胞増殖に関わるタンパク質の分泌阻害活性には効果を示したと考えることができる。

第六節　小括

著者は、coibamide A (**1**) の D-MeAla^{11}–MeThr^{5} 部位を MeLys(Me) に置換した誘導体をリード化合物とした構造活性相関研究ならびに高活性誘導体の創製に向けた検討を行った。大環状構造の環の大きさ、アミノ酸の立体配置、*N*-メチル基の有無の違いによる比較を行い、coibamide A 由来の大環状骨格を構成する主鎖骨格が生物活性に最適であることを明らかにした。最適な大環状構造からなる coibamide A 誘導体の Tyr(Me)^{10} 部位の構造最適化を試みたところ、Bph に変換することにより細胞増殖抑制活性が向上することを明らかにした。Coibamide A のエステル結合を炭素―炭素二重結合やアミド結合に置換して細胞増殖抑制活性が維持されたことから、この観点では生物学的等価体として有効であることが示唆された。一方、タンパク質分泌抑制活性の観点では、エステル結合は炭素―炭素二重結合やアミド結合とは異なる活性を示し、必ずしも生物学的に等価な機能を示さないことが明らかとなった。また、Coibamide A およびその誘導体のタンパク質分泌阻害活性と細胞増殖抑制活性の構造活性相関を比較したところ、一部の誘導体については相関が認められないものがあった。この結果は、coibamide A が Sec61α 以外の標的分子にも作用して細胞増殖抑制活性を示しているケースと Sec61α を介する基質特異的なタンパク質分泌阻害を示しているケースが考えられる。Coibamide A に微細な修飾を加えることで正常細胞への毒性を軽減しつつ、腫瘍細胞や細菌、ウイルスの生存に関わるタンパク質分泌抑制活性のみを示す Sec61 阻害剤の創製の可能性を示している。

Experimental section

Synthetic General Method

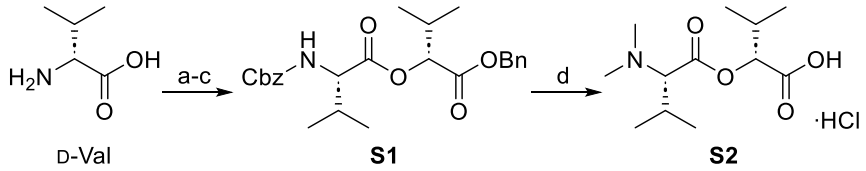
^{1}H and ^{13}C NMR spectra were recorded using a JEOL ECA-500 or JEOL ECZ600R spectrometer. Chemical shifts are reported in δ (ppm), relative to Me_4Si (in $CDCl_3$) as an internal standard for ^{1}H, and referenced to the residual solvent signal for ^{13}C. Exact mass (HRMS) data were recorded on a JEOL SX-102A (FAB) or Shimadzu LC-ESI-IT-TOF-MS (ESI-TOF) equipment. Optical rotations were measured using a JASCO P-1020 polarimeter. For flash chromatography, Wakogel C-300E (Wako) was employed. For analytical HPLC, a Cosmosil 5C18-ARII column (4.6 × 250 mm, Nacalai Tesque, Inc.) was employed with a linear gradient of CH_3CN (with 0.05% (v/v) TFA) or (with 0.1% (v/v) TFA) in H_2O, and eluting products were detected by UV at 220 nm. Preparative HPLC was performed using a Cosmosil 5C18-ARII preparative column (20 × 250 mm, Nacalai Tesque, Inc.). The compound purity for the bioassays was determined to be >90% by HPLC analysis.

Fmoc-MeSer(Me)-OH. Fmoc-MeSer(Me)-OH was synthesized by the identical procedure reported previously.[27,28] To a suspension of Fmoc-Ser(Me)-OH (5.74 g, 16.8 mmol) in toluene (300 mL), paraformaldehyde (3.33 g, 111 mmol) and $TsOH·H_2O$ (320 mg, 1.68 mmol) were added, and the mixture was refluxed for 2 h. The solution was washed with aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. After concentration, the crystalline product was dissolved in $CHCl_3$/TFA (1:1, 170 mL), and Et_3SiH (8.04 mL, 50.4 mmol) was added. The solution was stirred at room temperature for 22 h followed by concentration to give an oily residue. Purification by flash chromatography on silica gel ($CHCl_3$:MeOH = 1:0 to 20:1) provided Fmoc-MeSer(Me)-OH as a colorless oil (5.87 g, 98% for 2 steps). The spectral data were in good agreement with those previously reported.[28]

Alloc-Bph-OH. To a suspension of H-Bph-OH (419 mg, 1.74 mmol) in THF (0.91 mL) and H_2O (1.93 mL), allyl chloroformate (185 μL, 1.74 mmol) and 2.0 M aqueous NaOH (1.74 mL) were added at room temperature. After being stirred for 3.5 h, the reaction mixture was concentrated. The residue was acidified with 2.0 M HCl, and extracted with EtOAc. The extract was washed with brine, and dried over $MgSO_4$. After concentration, the residue was recrystallized from *n*-hexane-EtOAc to provide Alloc-Bph-OH as a white solid (489 mg, 86%): $[\alpha]^{25}_{D}$ +76.2 (*c* 0.52, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.12-3.28 (m, 2H), 4.57 (d, *J* = 5.5 Hz, 2H), 4.69-4.76 (m, 1H), 5.14-5.24 (m, 2H), 5.28 (d, *J* = 17.0 Hz, 1H) 5.87-5.93 (m, 1H), 7.25 (d, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.48-7.62 (m, 4H); $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$) δ: 37.3, 54.5, 66.0, 118.0, 127.0, 127.3, 127.4, 128.8, 129.7, 132.4, 134.5, 140.1, 140.6, 155.8, 176.1; HRMS (ESI-TOF) calcd for $C_{19}H_{20}NO_4$ $[M+H]^+$: 326.1387; found: 326.1388.

Preparation of N-Terminal Me_2Val^1-D-Hva^2 Moiety.

Cbz-Val-D-Hva-OBn (S1). To a solution of D-valine (27.9 g, 238 mmol) in 0.5 M H_2SO_4 (950 mL), a solution of $NaNO_2$ (98.2 g, 1.43 mol) in H_2O (325 mL) was added dropwise at 0 °C. The mixture



D-Val **S1** **S2**

Scheme S1. Synthesis of N-Terminal Me_2Val^1-D-Hva^2 Moiety (**S2**). *Reagents and conditions*: (a) $NaNO_2$, H_2SO_4, H_2O; (b) BnBr, Et_3N, DMF, (c) Cbz-Val-OH, EDCI·HCl, DMAP, CH_2Cl_2, 44% (3 steps); (d) H_2, Pd/C, H_2, HCHO, *i*-PrOH-H_2O, 68%.

was stirred for 10 h at room temperature. The whole was extracted with Et_2O and the extract was washed with brine, and dried over $MgSO_4$. The filtrate was concentrated under reduced pressure to give crude H-D-Hva-OH (25.4 g), which was used for the next step without further purification. To a stirred solution of crude H-D-Hva-OH (20.7 g, ca. 175 mmol) and Et_3N (48.8 mL, 350 mmol) in dry DMF (100 mL), BnBr (19.8 mL, 166 mmol) was added dropwise at 0 °C. The mixture was stirred for 18 h at room temperature. The resulting mixture was diluted with EtOAc and the whole was washed with citric acid aq., $NaHCO_3$ aq., and brine, and dried over $MgSO_4$. The filtrate was concentrated under reduced pressure to give crude H-D-Hva-OBn (18.9 g), which was used for the next step without further purification. To a mixture of H-D-Hva-OBn (18.9 g, ca. 90.6 mmol), Cbz-Val-OH (27.3 g, 109 mmol) and DMAP (1.11 g, 9.06 mmol) in dry CH_2Cl_2 (180 mL), EDCI·HCl (34.6 g, 181 mmol) was added at 0 °C. The mixture was stirred for 24 h at room temperature. After concentration, the residue was diluted with EtOAc. The whole was washed with citric acid aq., $NaHCO_3$ aq., and brine, and dried over $MgSO_4$. Purification by flash column chromatography on silica gel (*n*-hexane:EtOAc = 5:1) provided compound **S1** as a colorless oil (38.0 g, 44% for 3 steps): $[\alpha]^{26}_D$ +15.3 (*c* 0.79, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 0.89-1.00 (m, 12H), 2.19-2.43 (m, 2H), 4.44 (dd, *J* = 9.5, 4.0 Hz, 1H), 4.90 (d, *J* = 4.0 Hz, 1H), 5.11-5.21 (m, 4H), 5.27 (d, *J* = 9.5 Hz, 1H), 7.24-7.40 (m, 10H); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ: 17.0, 17.2, 18.7, 19.0, 30.0, 31.1, 59.1, 66.9, 67.0, 77.4, 128.10, 128.13, 128.3, 128.4, 128.46, 128.49, 135.1, 136.1, 156.1, 169.0, 171.4; HRMS (ESI-TOF) calcd for $C_{25}H_{31}NNaO_6$ $[M+Na]^+$: 464.2044; found: 464.2044.

Me_2Val-D-Hva-OH·HCl (S2). To a solution of Cbz-Val-D-Hva-OBn **S1** (304 mg, 0.688 mmol) in *i*-PrOH (2.4 mL) were added 10% Pd/C (42.4 mg) and formaldehyde (37%, 513 μL, 6.88 mmol). The mixture was stirred under H_2 at room temperature for 24 h. The resulting mixture was filtered using a membrane filter (Advantec T050A025A, 0.50 μm) and concentrated under reduced pressure. The residue was purified by a CombiFlash® Rf 150 apparatus using a C18 reversed phase column to provide TFA salt. To replace TFA counterions with chloride ions, the product was lyophilized in the presence of HCl, which gave compound **S2** as a white solid (133 mg, 68%): $[\alpha]^{25}_D$ +8.59 (*c* 0.55, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.00 (d, *J* = 7.0 Hz, 3H), 1.02-1.09 (m, 6H), 1.31 (d, *J* = 6.5 Hz, 3H), 2.34-2.44 (m, 2H), 3.00 (s, 6H), 3.88 (d, *J* = 7.0 Hz, 1H), 5.02 (d, *J* = 3.5 Hz, 1H); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ: 16.7, 17.8, 18.9, 20.0, 27.5, 29.2, 41.6, 71.9, 78.8, 167.0, 169.8; HRMS (ESI-TOF)

calcd for $C_{12}H_{24}NO_4$ $[M+H]^+$: 246.1700; found: 246.1699.

General Procedure for Solid-Phase Peptide Synthesis.

Loading of an amino acid on the solid support. A solution of Fmoc amino acid (0.198 mmol) and DIEA (138 μL, 0.792 mmol) in dry CH_2Cl_2 (2.0 mL), was reacted with (2-Cl)Trt chloride resin (204 mg, 0.326 mmol). The reaction was continued for 2 h at room temperature.

Deprotection of Fmoc group. The Fmoc-protected peptidyl resin was treated with 20% piperidine/DMF for 20 min.

Coupling reaction using HATU/DIEA. DIEA (104 μL, 0.600 mmol) was added to a solution of Fmoc amino acid (0.30 mmol) and HATU (110 mg, 0.290 mmol) in DMF. The whole was poured into the peptidyl resin (0.10 mmol), and the reaction was continued for 1.5 h at 40 °C. For the coupling of N-terminal ester moiety (**S2**), an excess amount of DIEA (157 μL, 0.900 mmol) was used.

Coupling reaction using DIC/HOBt. DIC (46 μL, 0.30 mmol) was added to a solution of Fmoc amino acid (0.30 mmol) and HOBt·H_2O (46 mg, 0.30 mmol) in DMF. The whole was poured into the peptidyl resin (0.100 mmol), and the reaction was continued for 1.5 h at 40 °C.

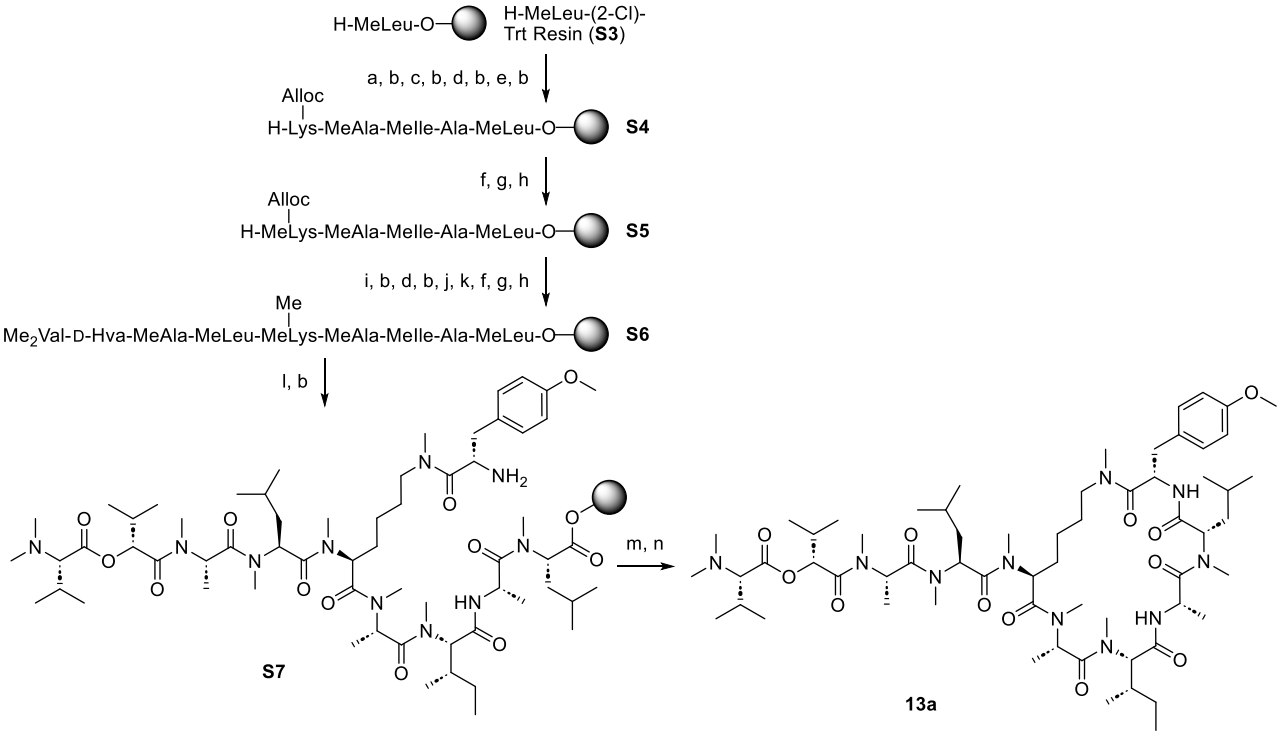
N-Methylation on solid support.[29] 2,4,6-Collidine (132 μL, 1.00 mmol) was added to a solution of NsCl (111 mg, 0.500 mmol) in NMP. The whole was poured into the peptidyl resin (0.100 mmol), and the reaction was continued for 15 min at room temperature. After removal of the reagent solution, a solution of MeOH (20 μL, 0.50 mmol) and Ph_3P (131 mg, 0.500 mmol) in dry THF was added into the peptidyl resin. DEAD (228 μL, 0.500 mmol) was added dropwise, and the reaction was continued for 30 min at room temperature. This reaction was repeated twice. To a suspension of the peptidyl resin in NMP, DBU (75 μL, 0.50 mmol) and 2-mercaptoethanol (70 μL, 1.0 mmol) were added, and the reaction was continued for 5 min. This deprotection process was repeated twice.

Deprotection of Alloc group. To the peptidyl resin (0.100 mmol) were added $PhSiH_3$ (247 μL, 2.00 mmol) and $Pd(PPh_3)_4$ (23 mg, 0.020 mmol) in dry CH_2Cl_2, and the reaction was continued for 10 min.

Cleavage from the resin. The peptidyl resin was treated with 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP)/CH_2Cl_2 (3:7) for 2 h at room temperature. After filtration of the residual resin, the filtrate was concentrated under reduced pressure to give a crude peptide, which was used for the next step without further purification.

Synthesis of [MeAla^3, MeLys(Me)^5, MeAla^6]-Coibamide A

[MeAla^3, MeLys(Me)^5, MeAla^6]-Coibamide A (13a). The linear peptide was constructed by solid-phase peptide synthesis on peptidyl resin **S3** (0.490 mmol/g, 204 mg, 0.100 mmol). After the cleavage from the resin **S7** as described above, EDCI·HCl (192 mg, 1.00 mmol) was added to a solution of linear peptide, HOAt (136 mg, 1.00 mmol), and DIEA (697 μL, 4.00 mmol) in dry DMF (100 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and the stirring was continued for 18 h. The reaction mixture was concentrated and the residue was purified by RP-HPLC to give compound **13a** (5.9 mg, 4.4% from resin) as a white powder: ^{1}H NMR (500 MHz, $CDCl_3$, mixture of



Scheme S2. Synthesis of [MeAla^3, MeLys(Me)^5, MeAla^6]-Coibamide A (**13a**). *Reagents and conditions*: (a) Fmoc-Ala-OH·H_2O, HATU, DIEA, DMF, 40 °C; (b) 20% piperidine/DMF, rt; (c) Fmoc-MeIle-OH, HOBt·H_2O, DIC, DMF, 40 °C; (d) Fmoc-MeAla-OH, HATU, DIEA, DMF, 40 °C; (e) Fmoc-Lys(Alloc)-OH, HATU, DIEA, DMF, 40 °C; (f) NsCl, 2,4,6-collidine, NMP, rt; (g) Ph_3P, DEAD, MeOH, THF, rt; (h) 2-mercaptoethanol, DBU, NMP, rt; (i) Fmoc-MeLeu-OH, HATU, DIEA, DMF, 40 °C; (j) Me_2Val-D-Hva-OH, HATU, DIEA, NMP, 40 °C; (k) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt; (l) Fmoc-Tyr(Me)-OH, HATU, DIEA, NMP, 40 °C; (m) 30% HFIP/CH_2Cl_2, rt; (n) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt.

rotamers) δ: 0.69-1.04 (m, 27H), 1.08-1.15 (m, 4H), 1.15-1.22 (m, 4H), 1.22-1.29 (m, 4H), 1.29-1.37 (m, 4H), 1.37-1.76 (m, 9H), 1.76-1.97 (m, 2H), 2.00-2.29 (m, 3H), 2.42-2.53 (m, 1H), 2.57-2.74 (m, 3H), 2.74-3.15 (m, 27H), 3.78 (s, 2H), 3.79 (s, 1H), 3.83-3.90 (m, 1H), 4.23 (d, J = 11.0 Hz, 1H), 4.29-4.41 (m, 0.5H), 4.85-5.17 (m, 3.5H), 5.35-5.57 (m, 4H), 6.47 (d, J = 8.5 Hz, 0.5H), 6.74-6.87 (m, 2.5H), 6.99 (d, J = 8.5 Hz, 0.5H), 7.07 (d, J = 8.5 Hz, 1H), 7.08-7.18 (m, 0.5H), 8.02 (br s, 0.5H), 8.28 (d, J = 10.0 Hz, 0.5H); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 9.9, 11.3, 14.1, 14.37, 14.44, 15.3, 15.5, 15.6, 16.3, 16.4, 18.0, 18.3, 18.69, 18.74, 18.8, 19.0, 20.0, 20.8, 21.2, 21.3, 21.57, 21.62, 22.2, 22.6, 23.0, 23.2, 24.4, 24.65, 24.74, 24.9, 25.3, 26.1, 26.4, 27.78, 27.81, 28.6, 28.7, 28.8, 28.9, 29.0, 29.3, 29.55, 29.60, 29.7, 29.9, 30.1, 32.2, 32.5, 33.0, 33.4, 35.2, 37.3, 37.4, 37.5, 37.7, 38.9, 39.4, 42.1, 43.9, 46.3, 48.3, 48.6, 49.7, 49.8, 50.2, 50.5, 50.8, 51.1, 51.3, 52.3, 52.9, 54.9, 58.4, 65.2, 70.6, 70.6, 113.4, 113.8, 127.7, 127.8, 129.9, 130.2, 158.3, 158.4, 167.3, 167.4, 167.6, 168.2, 168.6, 169.4, 170.2, 170.3, 170.4, 170.5, 170.9, 171.0, 171.1, 172.1, 172.4, 172.4; HRMS (ESI-TOF) calcd for $C_{62}H_{107}N_{10}O_{12}$ $[M+H]^+$: 1183.8064; found: 1183.8070.

[MeAla^3, D-MeLys(Me)^5, MeAla^6]-Coibamide A (13b). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **13b** (10.3 mg, 8% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{106}N_{10}NaO_{12}$ $[M+Na]^+$: 1205.7884; found: 1205.7864.

[MeAla^3, MeOrn(Me)^5, MeAla^6]-Coibamide A (13c). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **13c** (27.6 mg, 22% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{105}N_{10}O_{12}$ $[M+H]^+$: 1169.7908; found: 1169.7909.

[MeAla^3, D-MeOrn(Me)^5, MeAla^6]-Coibamide A (13d). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **13d** (9.5 mg, 7% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{104}N_{10}NaO_{12}$ $[M+Na]^+$: 1191.7727; found: 1191.7740.

[MeAla^3, MeLys^5, MeAla^6]-Coibamide A (13e). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.490 mmol/g, 204 mg, 0.100 mmol) was converted into **13e** (25.6 mg, 20% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{105}N_{10}O_{12}$ $[M+H]^+$: 1169.7908; found: 1169.7909.

[MeAla^3, D-MeLys^5, MeAla^6]-Coibamide A (13f). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **13f** (17.2 mg, 13% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{105}N_{10}O_{12}$ $[M+H]^+$: 1169.7908; found: 1169.7906.

[MeAla^3, MeOrn^5, MeAla^6]-Coibamide A (13g). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **13g** (19.4 mg, 15% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{60}H_{103}N_{10}O_{12}$ $[M+H]^+$: 1155.7751; found: 1155.7756.

[MeAla^3, D-MeOrn^5, MeAla^6]-Coibamide A (13h). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **13h** (19.8 mg, 16% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{60}H_{103}N_{10}O_{12}$ $[M+H]^+$: 1155.7751; found: 1155.7753.

[Ala^3, MeLys(Me)^5, MeAla^6]-Coibamide A (14a). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **14a**

(6.9 mg, 5% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{104}N_{10}NaO_{12}$ $[M+Na]^+$: 1191.7727; found: 1191.7742.

[MeAla^3, Leu^4, MeLys(Me)^5, MeAla^6]-Coibamide A (14b). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **14b** (5.4 mg, 4% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{106}N_{10}O_{12}$ $[M+2H]^{2+}$: 585.3991; found: 585.3975.

[MeAla^3, Lys(Me)^5, MeAla^6]-Coibamide A (14c). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **14c** (8.5 mg, 7% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{105}N_{10}O_{12}$ $[M+H]^+$: 1169.7908; found: 1169.7922.

[MeAla^3, MeLys(Me)^5, Ala^6]-Coibamide A (14d). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **14d** (8.8 mg, 7% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{106}N_{10}O_{12}$ $[M+2H]^{2+}$: 585.3991; found: 585.3976.

[MeAla^3, MeLys(Me)^5, MeAla^6, Ile^7]-Coibamide A (14e). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **14e** (11.6 mg, 9% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{106}N_{10}O_{12}$ $[M+2H]^{2+}$: 585.3991; found: 585.3990.

[MeAla^3, MeLys(Me)^5, MeAla^6, Leu^9]-Coibamide A (14f). According to the procedure described for the preparation of **13a**, H-Leu-(2-Cl)-Trt resin (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **14f** (11.6 mg, 9% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{104}N_{10}NaO_{12}$ $[M+Na]^+$: 1191.7727; found: 1191.7734.

[MeAla^3, MeLys(Me)^5, D-MeAla^6]-Coibamide A (15a). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **15a** (3.5 mg, 3% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{107}N_{10}O_{12}$ $[M+H]^+$: 1183.8064; found: 1183.8070.

[MeAla^3, MeLys(Me)^5, MeAla^6, D-*allo*-MeIle^7]-Coibamide A (15b). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **15b** (2.3 mg, 2% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{107}N_{10}O_{12}$ $[M+H]^+$: 1183.8064; found: 1183.8066.

[MeAla^3, MeLys(Me)^5, MeAla^6, D-Ala^8]-Coibamide A (15c). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.690 mmol/g, 145 mg, 0.100 mmol) was converted into **15c** (3.2 mg, 3% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{106}N_{10}NaO_{12}$ $[M+Na]^+$: 1205.7884; found: 1205.7830.

[MeAla^3, MeLys(Me)^5, MeAla^6, D-MeLeu^9]-Coibamide A (15d). According to the procedure described for the preparation of **13a**, H-D-MeLeu-(2-Cl)-Trt resin (0.570 mmol/g, 175 mg, 0.100 mmol) was converted into **15d** (13.4 mg, 10% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{107}N_{10}O_{12}$ $[M+H]^+$: 1183.8064; found: 1183.8063.

[MeAla^3, MeLys(Me)^5, MeAla^6, D-Tyr(Me)^{10}]-Coibamide A (15e). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.490 mmol/g, 204 mg, 0.100 mmol) was converted into **15e** (4.4 mg, 3% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{107}N_{10}O_{12}$ $[M+H]^+$: 1183.8064; found: 1183.8059.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe^{10}]-Coibamide A (18a). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18a** (4.2 mg, 3% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{105}N_{10}O_{11}$ $[M+H]^+$: 1153.7959; found: 1153.7958.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe(4-NO$_2$)^{10}]-Coibamide A (18b). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18b** (17.4 mg, 13% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{104}N_{11}O_{13}$ $[M+H]^+$: 1198.7810; found: 1198.7809.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe(4-CF$_3$)^{10}]-Coibamide A (18c). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18c** (32.8 mg, 25% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{104}F_3N_{10}O_{11}$ $[M+H]^+$: 1221.7833; found: 1221.7832.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe(4-CN)^{10}]-Coibamide A (18d). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18d** (23.9 mg, 18% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{104}N_{11}O_{11}$ $[M+H]^+$: 1178.7911; found: 1178.7911.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe(4-N$_3$)^{10}]-Coibamide A (18e). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18e** (5.8 mg, 4% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{104}N_{13}O_{11}$ $[M+H]^+$: 1194.7973;

found: 1194.7974.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe(4-Cl)^{10}]-Coibamide A (18f). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18f** (25.9 mg, 20% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{61}H_{104}ClN_{10}O_{11}$ $[M+H]^+$: 1187.7569; found: 1187.7564.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe(4-*t*-Bu)^{10}]-Coibamide A (18g). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18g** (36.1 mg, 27% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{65}H_{113}N_{10}O_{11}$ $[M+H]^+$: 1209.8585; found: 1209.8582.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe(4-O*t*-Bu)^{10}]-Coibamide A (18h). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18h** (31.7 mg, 24% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{65}H_{113}N_{10}O_{12}$ $[M+H]^+$: 1225.8534; found: 1225.8534.

[MeAla^3, MeLys(Me)^5, MeAla^6, Phe(4-OCF$_3$)^{10}]-Coibamide A (18i). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18i** (21.8 mg, 16% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{104}F_3N_{10}O_{12}$ $[M+H]^+$: 1237.7782; found: 1237.7784.

[MeAla^3, MeLys(Me)^5, MeAla^6, Bph^{10}]-Coibamide A (18j). According to the procedure described for the preparation of **13a**, peptidyl resin **S3** (0.490 mmol/g, 204 mg, 0.100 mmol) was converted into **18j** (16.2 mg, 12% from resin) as a white powder: ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.66-1.79 (m, 51H), 1.79-2.00 (m, 2H), 2.00-2.32 (m, 3H), 2.41-2.85 (m, 9H), 2.85-3.31 (m, 22H), 3.78-3.95 (m, 1H), 4.21 (m, 0.5H), 4.28-4.46 (m, 0.5H), 4.74-5.21 (m, 4H), 5.21-5.69 (m, 4H), 6.52 (d, *J* = 8.5 Hz, 0.5H), 6.83 (br s, 0.5H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.31-7.39 (m, 1H), 7.39-7.48 (m, 2H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.55-7.69 (m, 3H), 7.97 (br s, 0.5H), 8.52 (d, *J* = 9.5 Hz, 0.5H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 10.2, 11.7, 14.5, 14.7, 14.8, 15.5, 15.8, 16.0, 16.7, 16.9, 18.3, 18.6, 19.1, 19.18, 19.22, 19.3, 20.4, 21.2, 21.6, 21.9, 22.0, 22.7, 23.0, 23.3, 23.6, 24.8, 25.0, 25.1, 25.2, 25.4, 26.6, 26.9, 28.16, 28.20, 29.00, 29.04, 29.1, 29.3, 29.7, 29.91, 29.94, 30.1, 30.2, 30.3, 30.4, 32.5, 32.8, 33.5, 33.8, 35.6, 37.7, 37.8, 37.9, 38.5, 39.5, 39.9, 42.5, 44.4, 46.6, 48.8, 49.0, 50.0, 50.1, 50.4, 50.6, 51.5, 51.6, 52.6, 53.4, 58.8, 65.5, 70.8, 70.9, 126.8, 126.9, 127.0, 127.3, 127.4, 127.5, 128.8, 128.9, 129.7, 130.3, 135.1, 135.4, 139.7, 139.9, 140.1, 140.5, 167.7, 167.8, 167.9, 168.5, 169.1, 169.9, 170.6, 170.8, 170.9, 171.25, 171.29, 171.34, 172.5, 172.6, 172.7; HRMS (ESI-TOF) calcd for $C_{67}H_{109}N_{10}O_{11}$ $[M+H]^+$: 1229.8272; found: 1229.8254.

[MeAla^3, MeLys(Me)^5, MeAla^6, 2-Pal^{10}]-Coibamide A (18k). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18k** (7.1 mg, 5% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{60}H_{105}N_{11}O_{11}$ $[M+2H]^{2+}$: 577.8992; found: 577.8993.

[MeAla^3, MeLys(Me)^5, MeAla^6, 3-Pal^{10}]-Coibamide A (18l). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18l** (31.3 mg, 23% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{60}H_{105}N_{11}O_{11}$ $[M+2H]^{2+}$: 577.8992; found: 577.8992.

[MeAla^3, MeLys(Me)^5, MeAla^6, 4-Pal^{10}]-Coibamide A (18m). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18m** (25.0 mg, 18% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{60}H_{105}N_{11}O_{11}$ $[M+2H]^{2+}$: 577.8992; found: 577.8991.

[MeAla^3, MeLys(Me)^5, MeAla^6, MePhe^{10}]-Coibamide A (18n). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18n** (2.0 mg, 2% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{107}N_{10}O_{11}$ $[M+H]^{+}$: 1167.8115; found: 1167.8116.

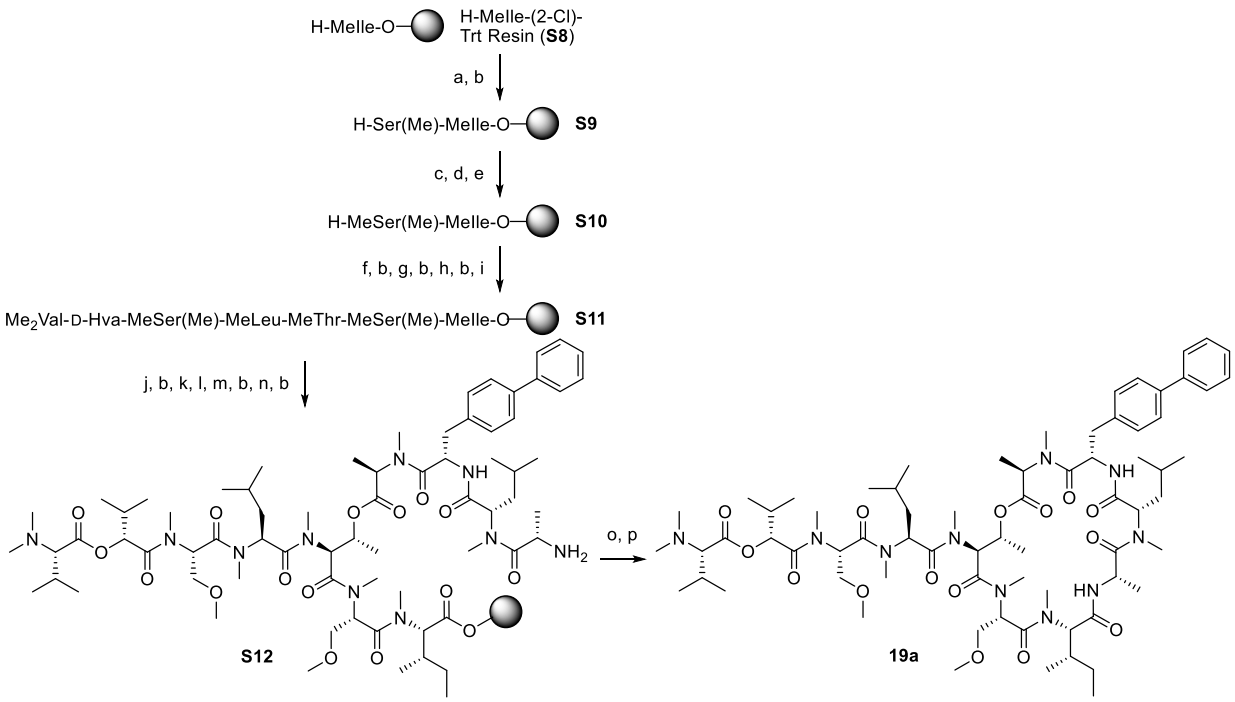
[MeAla^3, MeLys(Me)^5, MeAla^6, Tic^{10}]-Coibamide A (18o). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18o** (8.9 mg, 7% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{62}H_{105}N_{10}O_{11}$ $[M+H]^{+}$: 1165.7959; found: 1165.7959.

[MeAla^3, MeLys(Me)^5, MeAla^6, 1-Nal^{10}]-Coibamide A (18p). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18p** (5.8 mg, 4% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{65}H_{107}N_{10}O_{11}$ $[M+H]^{+}$: 1203.8115; found: 1203.8114.

[MeAla^3, MeLys(Me)^5, MeAla^6, 2-Nal^{10}]-Coibamide A (18q). According to the procedure described for the preparation of **13a**, peptidyl resin **S6** (0.100 mmol) was converted into **18q** (5.5 mg, 4% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{65}H_{107}N_{10}O_{11}$ $[M+H]^{+}$: 1203.8115; found: 1203.8112.

Synthesis of [Bph^{10}]-Coibamide A

The peptide was assembled on H-MeIle-(2-Cl)Trt resin by Fmoc-SPPS. Coupling of Ser(Me)^6 followed by on-resin N-methylation[29] provided peptidyl resin **S10**. MeThr^5, MeLeu^4, and MeSer(Me)^3



Scheme S3. Synthesis of [Bph[10]]-coibamide A (**19a**). *Reagents and conditions*: (a) Fmoc-Ser(Me)-OH, DIC, HOAt, DMF, 40 °C; (b) 20% piperidine/DMF, rt; (c) NsCl, 2,4,6-collidine, NMP, rt; (d) Ph_3P, DEAD, MeOH, THF, rt; (e) 2-mercaptoethanol, DBU, NMP, rt; (f) Fmoc-MeThr-OH, DIC, HOAt, DMF, 40 °C; (g) Fmoc-MeLeu-OH, DIC, HOAt, DMF, 40 °C (h) Fmoc-MeSer(Me)-OH, DIC, HOAt, DMF, 40 °C; (i) Me_2Val-D-Hva-OH, HATU, DIEA, DMF, 40 °C; (j) Fmoc-D-MeAla-OH, DIC, DMAP, 1,2-DCE, rt; (k) Alloc-Bph-OH, DIC, HOAt, DMF, 40 °C; (l) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt; (m) Fmoc-MeLeu-OH, DIC, HOBt·H_2O, DMF, 40 °C; (n) Fmoc-Ala-OH·H_2O, DIC, HOAt, DMF, 40 °C; (o) 30% HFIP/CH_2Cl_2, rt; (p) HATU, DIEA, CH_2Cl_2.

were efficiently coupled onto *N*-methylamino acids using DIC/HOAt. Coupling of the N-terminal Me_2Val[1]-D-Hva[2] moiety using HATU/DIEA gave the peptidyl resin **S11** Subsequently, Fmoc-D-MeAla-OH was coupled onto the MeThr[5] hydroxy group using DIC/DMAP. To avoid the diketopiperazine formation under basic condition for deprotection of the Fmoc group, Alloc protection was employed for Bph[10], which can be removed under neutral condition. After coupling of Alloc-Bph-OH using DIC/HOAt followed by deprotection of the Alloc group, MeLeu[9] and Ala[8] were coupled using DIC/HOBt and DIC/HOAt, respectively. Cleavage of the open-chain peptide **S12** from the resin and macrocyclization with HATU/DIEA in CH_2Cl_2[30] gave the desired product **19a**.

[Bph[10]]-Coibamide A (19a). The linear peptides were constructed by solid-phase peptide synthesis on peptidyl resin **S8** (0.712 mmol/g, 422 mg, 0.300 mmol). Fmoc- or Alloc- protected amino acids (0.900 mmol) were coupled at 40 °C by using DIC (139 µL, 0.900 mmol) and HOAt (122 mg, 0.900 mmol) in DMF. Fmoc-D-MeAla-OH (488 mg, 1.50 mmol) was coupled onto the hydroxy group of MeThr[5] at room temperature using DIC (232 µL, 1.50 mmol) and DMAP (55 mg, 0.45 mmol) in dry DCE. After

cleavage from the resin **S12** as described above, HATU (570 mg, 1.50 mmol) and DIEA (1.05 mL, 6.00 mmol) were added to a solution of crude linear peptide in dry CH_2Cl_2 (300 mL). The reaction mixture was stirred for 12 h. After the mixture was concentrated, the residue was purified by RP-HPLC to give compound **19a** (26.5 mg, 6.1% from resin) as a white powder: ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.61-1.17 (m, 33H), 1.17-1.48 (m, 11H), 1.48-1.61 (m, 2H), 1.61-1.73 (m, 1H), 2.01-2.15 (m, 1H), 2.15-2.33 (m, 2H), 2.35-2.87 (m, 9H), 2.87-3.06 (m, 15H), 3.06-3.43 (m, 12H), 3.44-3.57 (m, 1H), 3.57-3.73 (m, 2H), 3.73-3.82 (m, 1H), 3.82-3.95 (m, 2H), 4.71-4.87 (m, 1H), 5.06 (d, J = 4.5 Hz, 1H), 5.13-5.23 (m, 1H), 5.23-5.30 (m, 1H), 5.30-5.46 (m, 1H), 5.46-5.59 (m, 1H), 5.68-5.84 (m, 1H), 5.84-6.05 (m, 1H), 6.20-6.44 (m, 1H), 6.54-6.70 (m, 1H), 6.82-7.11 (m, 1H), 7.25 (d, J = 7.0 Hz, 1H), 7.29-7.39 (m, 2H), 7.39-7.60 (m, 6H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 11.6, 12.9, 13.0, 15.8, 16.6, 17.5, 18.6, 18.7, 19.0, 19.1, 21.0, 21.11, 21.14, 21.5, 22.1, 23.1, 23.2, 23.3, 23.4, 24.3, 25.3, 28.2, 28.9, 29.1, 29.7, 29.9, 30.1, 30.2, 30.4, 31.0, 32.0, 36.6, 37.6, 38.8, 39.7, 47.0, 50.0, 51.3, 51.5, 51.7, 52.9, 53.1, 53.3, 58.8, 63.6, 64.7, 68.2, 68.6, 68.9, 69.0, 71.0, 77.6, 126.7, 126.88, 126.93, 127.1, 127.2, 127.4, 128.8, 129.7, 130.0, 130.1, 135.3, 140.0, 140.5, 167.2, 168.0, 168.5, 168.6, 169.15, 169.22, 169.6, 170.0, 170.3, 170.6, 170.7, 171.2, 171.6, 172.0; HRMS (ESI-TOF) calcd for $C_{70}H_{114}N_{10}O_{15}$ $[M+2H]^{2+}$: 667.4227; found: 667.4227.

[MeAla^3, MeAla^6, Bph^{10}]-Coibamide A (19b). According to the procedure described for the preparation of **19a**, peptidyl resin **S8** (0.712 mmol/g, 422 mg, 0.300 mmol) was converted into **19b** (30.3 mg, 7.3% from resin) as a white powder: ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.62-0.84 (d, J = 6.0 Hz, 3H), 0.86-0.99 (m, 15H), 0.99-1.06 (m, 8H), 1.08 (d, J = 7.0 Hz, 3H), 1.11 (d, J = 7.0 Hz, 3H), 1.13-1.24 (m, 8H), 1.24-1.34 (m, 9H), 1.34-1.45 (m, 2H), 1.45-1.59 (m, 2H), 1.64-1.74 (m, 1H), 2.01-2.10 (m, 1H), 2.15-2.30 (m, 2H), 2.33-2.72 (m, 4H), 2.76 (s, 3H), 2.87 (s, 3H), 2.89-3.23 (m, 20H), 3.63-3.83 (m, 2H), 3.87 (d, J = 9.0 Hz, 1H), 4.74-4.90 (m, 1H), 5.01 (d, J = 4.5 Hz, 1H), 5.06-5.71 (m, 6H), 6.32-6.61 (m, 1H), 7.23-7.31 (m, 2H), 7.35 (t, J = 7.5 Hz, 1H), 7.44 (t, J = 7.5 Hz, 2H), 7.46-7.60 (m, 4H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 11.5, 11.7, 12.7, 12.8, 14.0, 14.4, 14.6, 15.6, 15.8, 16.0, 16.7, 18.5, 18.9, 19.0, 21.0, 21.2, 21.9, 23.0, 23.2, 23.4, 24.1, 25.1, 25.2, 28.0, 28.9, 29.0, 29.5, 29.6, 29.9, 30.1, 30.7, 32.1, 36.6, 37.5, 38.0, 38.7, 39.5, 46.4, 46.7, 50.0, 50.2, 50.3, 50.6, 51.1, 51.2, 51.4, 51.6, 57.3, 64.6, 68.8, 70.8, 70.9, 77.1, 126.6, 126.7, 126.8, 127.0, 127.1, 127.3, 128.7, 129.6, 130.0, 134.9, 137.1, 139.1, 140.0, 140.3, 140.6, 167.4, 167.6, 167.7, 168.5, 169.2, 169.9, 170.4, 170.6, 171.4, 171.5, 171.8, 171.9, 172.8, 173.7; HRMS (ESI-TOF) calcd for $C_{68}H_{108}N_{10}NaO_{13}$ $[M+Na]^+$: 1295.7990; found: 1295.7976.

***tert*-Butyl (*S*,*E*)-2,2-dimethyl-4-(3-oxoprop-1-en-1-yl)oxazolidine-3-carboxylate (23).** To a stirred solution of D-Garner aldehyde **22** (1.00 g, 4.34 mmol) in dry CH_2Cl_2 (7 mL) was added triphenylphosphoranylidene-acetaldehyde (1.34 g, 4.34 mmol) at room temperature. The reaction mixture was stirred for 46 h. The solution was concentrated and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 7/1) to give compound **23** (0.836 g, 75%) as a yellow

oil. The spectral data were in good agreement with those previous reported.[23]

***tert*-Butyl (*S*)-4-{(3*S*,4*R*,*E*)-5-[(*R*)-4-benzyl-2-oxooxazolidin-3-yl]-3-hydroxy-4-methyl-5-oxopent-1-en-1-yl}-2,2-dimethyloxazolidine-3-carboxylate (24).** To a stirred solution of (*R*)-4-benzyl-3-propionyl-2-oxazolidinone (754 mg, 3.23 mmol) in dry CH_2Cl_2 (24 mL) under argon were added *n*-Bu_2BOTf (1.0 M in CH_2Cl_2; 3.88 mL, 3.88 mmol) and DIEA (676 μL, 3.88 mmol) at –78 °C. After being stirred for 1 h, the reaction mixture was warmed to 0 °C and stirred for 30 min. To this mixture was added a solution of **23** (826 mg, 3.23 mmol) in CH_2Cl_2 (8 mL) at –78 °C. After being stirred for 1 h, the mixture was warmed to –10 °C and stirred for 1 h. The mixture was diluted with pH 7.0 phosphate buffer solution (4 mL) and 30% H_2O_2 in MeOH (1:2, 12 mL) and stirred overnight. The whole was concentrated under reduced pressure and extracted with Et_2O. The extract was washed with aqueous saturated $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 4/1 to 2/1) to give compound **24** (1.18 g, 75%) as a colorless oil: $[\alpha]^{26}_D$ –28.1 (*c* 1.02, $CHCl_3$); IR (neat): 3496 (OH), 1778 (C=O), 1690 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: δ: 1.24 (d, *J* = 7.0 Hz, 3H), 1.45 (s, 9H), 1.45 (s, 1.5H), 1.51 (s, 1.5H), 1.57 (s, 1.5H), 1.62 (s, 1.5H), 2.79 (dd, *J* = 13.4, 9.5 Hz, 1H), 2.82–2.94 (m, 1H), 3.25 (dd, *J* = 13.4, 3.2 Hz, 1H), 3.75 (dd, *J* = 8.8, 1.6 Hz, 1H), 3.80–3.92 (m, 1H), 4.00–4.06 (m, 1H), 4.15–4.27 (m, 2H), 4.28–4.32 (m, 0.5H), 4.38–4.45 (m, 0.5H), 4.47–4.56 (m, 1H), 4.65–4.74 (m, 1H), 5.56–5.69 (m, 1H), 5.72–5.81 (m, 1H), 7.20 (d, *J* = 7.1 Hz, 2H), 7.28 (m, 1H), 7.33 (m, 2H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 10.9, 11.2, 23.5, 24.8, 26.7, 27.4, 28.4, 37.8, 55.1, 58.5, 58.6, 66.2, 67.9, 68.2, 71.3, 71.9, 79.7, 94.0, 127.4, 128.9, 129.0, 129.4, 130.4, 131.1, 131.3, 134.9, 151.9, 153.0, 176.7; HRMS (ESI-TOF) calcd for $C_{26}H_{36}N_2$ Na O_7 $[M+Na]^+$: 511.2415; found: 511.2415.

***tert*-Butyl (*S*)-4-{(3*S*,4*R*,*E*)-5[(*R*)-4-benzyl-2-oxooxazolidin-3-yl]-3-[(*tert*-butyldimethylsilyl)-oxy]-4-methyl-5-oxopent-1-en-1-yl}-2,2-dimethyloxazolidine-3-carboxylate (25).** To a stirred solution of **24** (2.03 g, 4.16 mmol) in dry CH_2Cl_2 (16 mL) under argon were added TBSOTf (1.15 mL, 5.00 mmol) and 2,6-lutidine (1.74 mL, 17.0 mmol) at 0 °C. The reaction mixture was warmed to room temperature and was stirred for 2 h. The reaction was quenched with aqueous saturated $NaHCO_3$. The whole was extract with EtOAc and the extract was washed with saturated citric acid and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 3/1) to give compound **25** (2.45 g, 98%) as a colorless oil: $[\alpha]^{24}_D$ –7.5 (*c* 1.02, $CHCl_3$); IR (neat): 1782 (C=O), 1696 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.01 (s, 3H), 0.01 (s, 3H), 0.88 (s, 9H), 1.21 (d, *J* = 6.5 Hz, 3H), 1.45 (s, 9H), 1.47 (s, 1.5H), 1.51 (s, 1.5H), 1.55 (s, 1.5H), 1.59 (s, 1.5H), 2.73–2.82 (m, 1H), 3.23–3.31 (m, 1H), 3.65–3.72 (m, 1H), 3.87–3.97 (m, 1H), 4.00 (m, 1H), 4.11–4.18 (m, 1.5H), 4.28 (m, 0.5H), 4.31–4.42 (m, 2H), 4.55–4.63 (m, 1H), 5.60–5.74 (m, 2H), 7.21 (d, *J* = 7.1 Hz, 2H), 7.28 (m, 1H), 7.33 (dd, *J* = 7.3, 7.3 Hz, 2H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: −5.2, −4.3, −0.03, 11.9,

12.7, 18.1, 23.4, 24.7, 25.7, 26.7, 26.9, 27.3, 28.4, 37.7, 41.9, 44.4, 55.7, 58.0, 66.0, 67.8, 68.6, 73.2, 74.5, 79.6, 79.9, 93.5, 93.8, 127.2, 128.9, 129.1, 129.4, 130.2, 131.2, 132.1, 135.3, 135.5, 151.7, 153.2, 174.3, 174.8; HRMS (ESI-TOF) calcd for $C_{32}H_{50}N_2NaO_7Si$ $[M+Na]^+$: 625.3279; found: 625.3273.

(2*R*,3*S*,*E*)-5-[(*S*)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyloxazolidin-4-yl]-3-[(*tert*-butyldimethylsilyl)oxy]-2-methylpent-4-enoic acid (26). To a stirred solution of **25** (84 mg, 0.14 mmol) in THF (2 mL) and H_2O (0.5 mL) were added $LiOH·H_2O$ (11.8 mg, 0.281 mmol) and 30% H_2O_2 in H_2O (100 μL) at 0 °C. The reaction mixture was stirred at the same temperature for 4 h. The reaction was quenched with aqueous saturated Na_2SO_3. The mixture was acidified with saturated citric acid to pH 3 and extracted with EtOAc. The extract was washed with water and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 3/1) to give compound **26** (46.6 mg, 75%) as colorless block crystals: mp 105-109 °C; $[\alpha]^{25}_D$ +40.4 (*c* 1.04, $CHCl_3$); IR (neat): 1703 (C=O); ^1H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.07 (s, 4.5H), 0.09 (s, 1.5H), 0.90 (br s, 9H), 1.10 (br s, 3H), 1.41–1.62 (m, 15H), 2.58–2.66 (m, 1H), 3.69 (m, 1H), 3.99–4.04 (m, 1H), 4.28–4.34 (m, 0.5H), 4.36–4.42 (m, 1.5H), 5.51–5.75 (m, 2H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: –5.3, –5.2, –4.2, 0.0, 11.3, 11.6, 18.5, 23.3, 24.7, 25.7, 26.8, 27.5, 28.4, 45.4, 45.5, 58.2, 67.8, 68.4, 74.2, 74.6, 79.8, 80.2, 93.4, 94.3, 129.9, 130.1, 131.8, 132.7, 151.8, 176.4; HRMS (ESI-TOF) calcd for $C_{22}H_{41}NNaO_6Si$ $[M+Na]^+$: 466.2595; found: 466.2594.

X-ray Crystallographic Analyses of 26

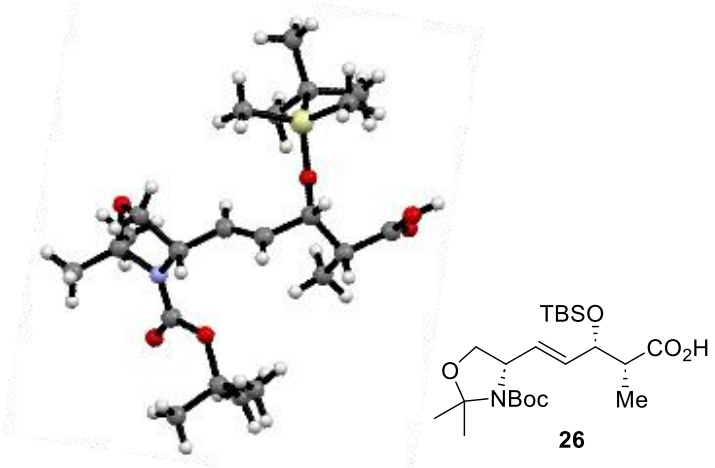


Figure S1. Crystal Structure of Carboxylic Acid **26**.

The data of the compound **26** ($C_{22}H_{41}NO_6Si$) was collected with a Rigaku XtaLAB AFC11 (RCD3): quarter-chi single diffractometer using mirror monochromated Cu*Kα* radiation at 93 K. The substance was crystallized from *n*-hexane as colorless block crystals and solved in monoclinic space group $P2_1/_n$ with $Z = 2$. The structure was solved by direct methods (SHELXT-2018/2) and refined by the full-matrix least-squares on F2 (SHELXL-2018/3). All non-hydrogen atoms were refined anisotropically

and all hydrogen atoms were placed using AFIX instructions. The crystal data are as follows: $C_{22}H_{41}NO_6Si$, FW: 443.65. a = 6.7065(1), b = 22.0348(2), c = 9.0980(1), V =1293.05(3) Å^3, Z = 2, D_{calc} = 1.139 g/cm^3, μ = 1.078 mm^{-1}, R_1 = 0.0313 ($I > 2\sigma(I)$), wR_2 = 0.0895 (all data), GOF = 1.057. The CCDC deposition number: CCDC 2239668.

***tert*-Butyl (*S*)-4-[(3*S*,4*R*,*E*)-4-{[(benzyloxy)carbonyl]amino}-3-[(*tert*-butyldimethylsilyl)oxy]-pent-1-en-1-yl]-2,2-dimethyloxazolidine-3-carboxylate (27a).** To a stirred solution of **26** (518 mg, 1.17 mmol) in dry toluene (5 mL) were added diphenylphosphoryl azide (377 μL, 1.75 mmol) and triethylamine (488 μL, 3.5 mmol) at room temperature. The reaction mixture was warmed to 50 °C and stirred for 24 h. To this mixture was added benzyl alcohol (602.4 μL, 5.84 mmol) at 50 °C. The reaction mixture was stirred at the same temperature for 24 h. Then, water was added to the mixture at room temperature. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 5/1) to give compound **27a** (540 mg, 84 % in 2 steps) as a colorless oil: $[\alpha]^{25}_D$ +56.8 (*c* 1.02, $CHCl_3$); IR (neat): 1699 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.00 (s, 6H), 0.89 (s, 9H), 1.06 (d, *J* = 6.3 Hz, 3H), 1.43 (s, 9H), 1.48–1.60 (m, 6H), 3.69 (d, *J* = 8.6 Hz, 1H), 3.75–4.02 (m, 2H), 4.27–4.39 (m, 2H), 4.78–4.87 (m, 1H), 5.08 (s, 2H), 5.53–5.71 (m, 2H), 7.33 (m, 5H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: –5.1, –4.5, 13.9, 14.5, 18.2, 23.4, 25.0, 25.8, 26.7, 28.4, 28.4, 51.3, 51.5, 58.3, 58.5, 66.4, 66.5, 67.8, 68.4, 73.8, 74.0, 79.6, 80.0, 93.3, 94.0, 127.9, 128.1, 130.1, 131.9, 136.6, 151.8, 155.7; HRMS (ESI-TOF) calcd for $C_{29}H_{48}N_2NaO_6Si$ $[M+Na]^+$: 571.3174; found: 571.3170.

***tert*-Butyl (*S*)-4-[(3*S*,4*R*,*E*)-4-{[(allyloxy)carbonyl]amino}-3-[(*tert*-butyldimethylsilyl)oxy]pent-1-en-1-yl]-2,2-dimethyloxazolidine-3-carboxylate (27b).** To a stirred solution of **26** (1.07 g, 2.41 mmol) in dry toluene (10 mL) were added diphenylphosphoryl azide (622 μL, 2.89 mmol) and triethylamine (1.01 mL, 7.24 mmol) at room temperature. The reaction mixture was warmed to 50 °C and stirred for 24 h. To this mixture was added allyl alcohol (828 μL, 12.1 mmol) at 50 °C. The reaction mixture was stirred at the same temperature for 24 h. Then, water was added to the mixture at room temperature. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 5/1) to give compound **27b** (1.08 g, 90 % in 2steps) as a colorless oil: $[\alpha]^{25}_D$ +62.5 (*c* 1.01, $CHCl_3$); IR (neat): 1698 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.02 (s, 3H), 0.04 (s, 3H), 0.91 (s, 9H), 1.06 (d, *J* = 6.3 Hz, 3H), 1.39–1.64 (m, 15H), 3.68–3.78 (m, 2H), 4.02 (m, 1H), 4.26–4.42 (m, 2H), 4.55 (m, 2H), 4.76–4.90 (m, 1H), 5.16–5.23 (m, 1H), 5.29 (d, *J* = 17.2 Hz, 1H), 5.50–5.59 (m, 0.5H), 5.60–5.78 (m, 1.5H), 5.85–5.97 (m, 1H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: –5.2, –4.5, 13.9, 14.5, 18.1, 23.4, 24.9, 25.8, 25.8, 26.6, 27.4, 28.4, 51.2, 51.4, 58.3, 58.5, 65.2, 65.3, 67.7, 68.4, 73.8, 74.0, 79.6, 80.0, 93.3, 93.9, 117.3, 117.5, 130.0, 131.0, 132.9, 133.1, 151.8, 155.5; HRMS (FAB) calcd for

$C_{25}H_{47}N_2O_6Si$ $[M+H]^+$: 499.3198; found: 499.3202.

***tert*-Butyl (*S*)-4-[(3*S*,4*R*,*E*)-4-{[(benzyloxy)carbonyl]amino}-3-hydroxypent-1-en-1-yl]-2,2-dimethyloxazolidine-3-carboxylate (28a).** To a stirred solution of **27a** (582 mg, 1.06 mmol) in THF (5 mL) under argon was added TBAF (1.0 M solution in THF; 5.31 mL, 5.31mmol) at room temperature. After being stirred for 1 h, the reaction mixture was diluted with aqueous saturated NH_4Cl. The whole was extracted with Et_2O and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 2/1) to give compound **28a** (369 mg, 80%) as a colorless oil: $[\alpha]^{24}_D$ +36.8 (*c* 1.02, $CHCl_3$); IR (neat): 3353 (OH), 1697 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 1.12 (d, *J* = 6.9 Hz, 3H), 1.42 (s, 9H), 1.46–1.63 (m, 6H), 2.27–2.56 (m, 1H), 3.68-3.75 (m, 1H), 3.80–3.99 (m, 1H), 4.03 (dd, *J* = 9.2, 6.3 Hz, 1H), 4.22–4.39 (m, 2H), 4.89–5.19 (m, 3H), 5.53–5.79 (m, 2H), 7.28–7.39 (m, 5H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 14.9, 15.3, 15.9, 23.5, 25.1, 26.7, 27.1, 28.3, 28.5, 51.2, 51.4, 58.6, 59.1, 65.9, 66.7, 67.8, 68.3, 73.8, 74.3, 79.8, 80.3, 93.6, 94.1, 128.1, 128.2, 128.5, 130.2, 130.4, 131.0, 131.8, 136.5, 151.8, 152.1, 156.9; HRMS (FAB) calcd for $C_{23}H_{35}N_2O_6$ $[M+H]^+$: 435.2490; found: 435.2505.

***tert*-Butyl (*S*)-4-[(3*S*,4*R*,*E*)-4-{[(allyloxy)carbonyl]amino}-3-hydroxypent-1-en-1-yl]-2,2-dimethyloxazolidine-3-carboxylate (28b).** To a stirred solution of **27b** (1.40 g, 2.81 mmol) in THF (14 mL) under argon was added TBAF (1.0 M solution in THF; 14.0 mL, 14.0 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was diluted with aqueous saturated NH_4Cl. The whole was extracted with Et_2O and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 2/1) to give compound **28b** (895 mg, 83%) as a colorless oil: $[\alpha]^{25}_D$ +43.5 (*c* 1.00, $CHCl_3$); IR (neat): 3361 (OH), 1694 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 1.11 (d, *J* = 6.9 Hz, 3H), 1.38–1.64 (m, 15H), 2.56– 2.80 (m, 1H), 3.69–3.74 (m, 1H), 3.77-3.95 (m, 1H), 4.03 (dd, *J* = 8.9, 6.2 Hz, 1H), 4.21–4.26 (m, 1H), 4.26–4.39 (m, 1H), 4.48–4.59 (m, 2H), 4.98–5.21 (m, 3H), 5.53–5.79 (m, 2H), 5.83–5.94 (m, 1H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 14.9, 15.8, 23.5, 25.1, 26.7, 27.1, 28.4, 51.1, 51.4, 58.6, 59.1, 65.5, 67.8, 68.2, 73.8, 74.2, 79.7, 80.3, 93.6, 94.0, 117.6, 117.8, 130.2, 130.5, 131.1, 132.7, 132.9, 151.8, 152.1, 156.7; HRMS (ESI-TOF) calcd for $C_{19}H_{32}N_2NaO_6$ $[M+Na]^+$: 407.2153; found: 407.2152.

***tert*-Butyl (*S*)-4-[(2*S*,5*R*,*E*)-5-{[(benzyloxy)carbonyl]amino}hex-3-en-2-yl]-2,2-dimethyloxazolidine-3-carboxylate (30a).** To a stirred solution of **28a** (257 mg, 0.591 mmol) in $CHCl_3$ (1 mL) under argon were added pyridine (952 μL, 11.8 mmol) and MsCl (458 μL, 5.91 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was diluted with water. The whole was extracted with EtOAc and extract was washed with saturated citric acid and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give compound **29a**, which was used further purification. To a

stirred solution of LiCl (250 mg, 5.91 mmol) and CuCN (265 mg, 2.95 mmol) in dry THF (3 mL) under argon was added MeMgBr (1.02 M solution in THF; 2.90 mL, 2.95 mmol) at –78 °C. The reaction mixture was warmed to 0 °C and stirred for 15 min. To this mixture was added a solution of **29a** in dry THF (2 mL) at –78 °C. After being stirred for 30 min, the reaction mixture was diluted with aqueous saturated NH_4Cl and 28% ammonia solution. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 2/1) to give compound **30a** (202 mg, 79% in 2 steps) as a colorless oil: $[\alpha]^{25}_D$ +7.5 (*c* 1.00, $CHCl_3$); IR (neat): 1698 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.97 (d, *J* = 6.7 Hz, 3H), 1.21 (d, *J* = 6.1 Hz, 3H), 1.45 (br s, 12H), 1.54 (s, 2H), 1.60 (s, 1H), 2.62–2.71 (m, 1H), 3.68–3.90 (m, 3H), 4.19–4.29 (m, 1H), 4.58–4.69 (m, 1H), 5.08 (s, 2H), 5.36–5.59 (m, 2H), 7.28–7.38 (m, 5H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 14.3, 15.0, 21.0, 21.3, 22.8, 24.3, 26.0, 27.0, 28.4, 28.5, 38.7, 48.0, 48.4, 60.8, 61.2, 64.4, 64.5, 66.5, 79.7, 80.1, 93.6, 94.2, 128.1, 128.1, 128.5, 131.6, 132.1, 132.8, 136.6, 152.3, 155.4; HRMS (FAB) calcd for $C_{24}H_{37}N_2O_5$ $[M+H]^+$: 433.2697; found: 433.2715.

***tert*-Butyl (*S*)-4-[(2*S*,5*R*,*E*)-5-{[(allyloxy)carbonyl]amino}hex-3-en-2-yl]-2,2-dimethyloxazolidine-3-carboxylate (30b).** To a stirred solution of **28b** (438 mg, 1.14 mmol) in $CHCl_3$ (2 mL) under argon were added pyridine (1.83 mL, 22.8 mmol) and MsCl (881.2 μL, 11.4 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was diluted with water. The whole was extracted with EtOAc and extract was washed with saturated citric acid and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give compound **29b**, which was used further purification. To a stirred solution of LiCl (482 mg, 11.4 mmol) and CuCN (510 mg, 5.69 mmol) in distilled THF (6 mL) under argon was added MeMgBr (1.02 M solution in THF; 5.57 mL, 5.69 mmol) at –78 °C. The reaction mixture was warmed to 0 °C and stirred 15 min. To this mixture was added a solution of **29b** in dry THF (4 mL) at –78 °C. After being stirred for 30 min, the reaction mixture was diluted with aqueous saturated NH_4Cl and 28% ammonia solution. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 2/1) to give compound **30b** (327 mg, 75% in 2 steps) as a colorless oil: $[\alpha]^{25}_D$ +16.9 (*c* 1.00, $CHCl_3$); IR (neat): 1698 (C=O); ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ: 0.98 (d, *J* = 6.9 Hz, 3H), 1.21 (m, 3H), 1.47 (s, 12H), 1.58 (s, 2H), 1.60 (s, 1H), 2.62–2.72 (m, 1H), 3.72–3.91 (m, 3H), 4.23 (m, 1H), 4.50– 4.64 (m, 3H), 5.21 (d, *J* = 10.3 Hz, 1H), 5.30 (d, *J* = 17.2 Hz, 1H), 5.38–5.58 (m, 2H), 5.86–5.96 (m, 1H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 14.3, 15.0, 21.0, 21.3, 22.8, 24.3, 26.0, 27.0, 28.4, 28.5, 38.7, 48.0, 48.4, 60.8, 61.2, 64.5, 64.5, 65.4, 79.7, 80.1, 93.6, 94.3, 117.5, 131.6, 132.1, 132.7, 133.0, 152.3, 152.8, 155.3; HRMS (ESI-TOF) calcd for $C_{20}H_{34}N_2NaO_5$ $[M+Na]^+$: 405.2360; found: 405.2360.

***tert*-Butyl (*S*)-4-[(2*S*,5*R*)-5-{[(allyloxy)carbonyl]amino}hexan-2-yl]-2,2-dimethyloxazolidine-3-carboxylate (31).** To a stirred solution of **30a** (170 mg, 0.393 mmmol) in MeOH (17 mL) was added 10% Pd/C (34 mg) at room temperature and the mixture was flushed with H_2 gas (1 atm). After being stirred for 1 h, the reaction mixture was filtered through membrane filter. The filtrate was concentrated under reduced pressure to give the corresponding amine, which was used without further purification. To a stirred solution of the above amine in THF were added allyl chloroformate (49.9 μL, 0.472 mmol) and DIEA (82.2 μL, 0.472 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture warmed to room temperature and stirred for 2 h. Water was added to this mixture at room temperature. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 2/1) to give compound **31** (129 mg, 85%) as a colorless oil: $[\alpha]^{24}_D$ +2.6 (*c* 1.02, $CHCl_3$); IR (neat): 1697 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.86 (d, *J* = 6.8 Hz, 3H), 1.09–1.31 (m, 5H), 1.35–1.64 (m, 17H), 1.85–2.08 (m, 1H), 3.56–3.71 (m, 1H), 3.72–3.91 (m, 3H), 4.43–4.66 (m, 3H), 5.20 (d, *J* = 10.3 Hz, 1H), 5.29 (d, *J* = 17.2 Hz, 1H), 5.84−5.97 (m, 1H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 14.4, 14.5, 21.5, 22.8, 24.3, 26.1, 26.9, 28.5, 30.1, 30.3, 34.3, 34.6, 35.3, 35.4, 47.3, 47.6, 60.8, 61.2, 63.7, 63.9, 65.3, 79.6, 80.1, 93.5, 94.0, 117.5, 117.6, 133.0, 133.1, 152.4, 153.0, 155.7; HRMS (FAB) calcd for $C_{20}H_{37}N_2O_5$ $[M+H]^+$: 385.2697; found: 385.2702.

(9*H*-Fluoren-9-yl)methyl allyl [(2*S*,3*S*,6*R*)-1-hydroxy-3-methylheptane-2,6-diyl]dicarbamate (32a). To a stirred solution of **31** (108 mg, 0.281 mmol) in CH_2Cl_2 (1 mL) was added TFA in CH_2Cl_2 (1:3, 4 mL) at 0 °C. After being stirred for 30 min, the mixture was warmed to room temperature and stirred for 1 h. The mixture was concentrated under reduced pressure to give the corresponding amine, which was used without further purification. To a stirred solution of the above amine in MeCN (2 mL) and H_2O (2 mL) were added Fmoc-OSu (123.2, 0.365 mmol) in MeCN (1 mL) and DIEA (196 μL, 1.12 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 h. Then, water was added to the mixture at room temperature. The whole was extracted with EtOAc and the extract was washed with saturated citric acid and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 1/1) to give compound **32a** (92.7 mg, 71%) as a white amorphous solid: $[\alpha]^{25}_D$ – 14.6 (*c* 0.80, DMF); IR (neat): 3321 (OH), 1698 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.79–0.86 (m, 0.5H), 0.93 (d, *J* = 6.4 Hz, 2.5H), 1.14 (d, *J* = 6.2 Hz, 3H), 1.39–1.50 (m, 2H), 1.61–1.88 (m, 2H), 2.16–2.30 (m, 1H), 3.40–3.77 (m, 4H), 4.19–4.26 (m, 1H), 4.33–4.72 (m, 5H), 5.06–5.24 (m, 3H), 5.74–5.96 (m, 1H), 7.34 (dd, *J* = 7.4, 7.4 Hz, 2H), 7.39 (dd, *J* = 7.4, 7.4 Hz, 2H), 7.60 (d, *J* = 7.3 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 2H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 16.2, 22.1, 28.2, 32.6, 33.7, 46.3, 47.3, 56.9, 63.3, 65.5, 66.7, 117.6, 119.9, 125.0, 125.1, 127.0, 127.0, 127.7, 132.8, 141.3, 143.9, 156.2, 157.1; HRMS (FAB) calcd for $C_{27}H_{35}N_2O_5$ $[M+H]^+$: 467.2541; found: 467.2543.

(9*H*-Fluoren-9-yl)methyl allyl [(2*R*,5*S*,6*S*,*E*)-7-hydroxy-5-methylhept-3-ene-2,6-diyl]dicarbamate (32b). To a stirred solution of **30b** (403 mg, 1.05 mmol) in CH_2Cl_2 (2 mL) was added TFA in CH_2Cl_2 (1:3, 8 mL) at 0 °C. After being stirred for 30 min, the mixture was warmed to room temperature and stirred for 1 h. The mixture was concentrated under reduced pressure to give the corresponding amine, which was used without further purification. To a stirred solution of the above amine in MeCN (4 mL) and H_2O (4 mL) were added Fmoc-OSu (462 mg, 1.37 mmol) in MeCN (2 mL) and DIEA (733 μL, 4.21 mmol) at room temperature. The reaction mixture was stirred room temperature for 2 h. Then, water was added to the mixture at room temperature. The whole was extracted with EtOAc and the extract was washed with saturated citric acid and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 1/1) to give compound **32b** (425 mg, 87%) as a white amorphous solid: $[\alpha]^{26}_D$ +17.8 (*c* 1.04, $CHCl_3$); IR (neat): 3314 (OH), 1688 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 1.03 (d, *J* = 6.3 Hz, 3H), 1.20 (d, *J* = 5.7 Hz, 3H), 2.23–2.66 (m, 2H), 3.27–3.72 (m, 3H), 4.10–4.25 (m, 2H), 4.31–4.59 (m, 4H), 4.79 (d, *J* = 6.9 Hz, 1H), 5.10–5.30 (m, 3H), 5.38–5.47 (m, 1H), 5.50–5.62 (m, 1H), 5.76–5.95 (m, 1H), 7.30 (dd, *J* = 7.4, 7.4 Hz, 2H), 7.38 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.75 (d, *J* = 7.4 Hz, 2H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 16.7, 21.0, 28.4, 37.3, 47.3, 48.6, 57.0, 63.8, 65.5, 66.7, 117.7, 120.0, 125.1, 127.0, 127.1, 127.7, 131.4, 132.7, 132.8, 132.9, 141.3, 143.9, 143.9, 155.5, 157.0; HRMS (ESI-TOF) calcd for $C_{27}H_{32}N_2NaO_5$ [M+Na]+: 487.2203; found: 487.2203.

(2*S*,3*S*,6*R*)-2-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-6-{[(allyloxy)carbonyl]amino}-3-methylheptanoic acid (21a). To a stirred solution of **32a** (92 mg, 0.197 mmol) in MeCN (4.5 mL) and pH 7.0 phosphate buffer solution (1.5 mL) were added AZADOL (6.04 mg, 0.0394 mmol), $NaClO_2$ (44.5 mg, 0.394 mmol) and NaClO solution (119.5 μL, 0.197 mmol) at 0 °C. After being stirred for 1.5 h, the reaction mixture was diluted with 1 M HCl. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by preparative thin-layer chromatography over silica gel ($CHCl_3$/MeOH = 9/1) to give compound **21a** (71 mg, 75%) as a colorless amorphous solid: $[\alpha]^{25}_D$ +12.7 (*c* 0.613, $CHCl_3$); IR (neat): 1701 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.83–1.01 (m, 3H), 1.10–1.30 (m, 6H), 1.44–1.51 (m, 1H), 1.98–2.12 (m, 1H), 3.66–3.75 (m, 1H), 4.19–4.24 (m, 1H), 4.31–4.64 (m, 5H), 5.13–5.41 (m, 2H), 5.56 (d, *J* = 8.1 Hz, 1H), 5.81–6.05 (m, 1H), 7.25 (dd, *J* = 7.3, 7.3 Hz, 2H), 7.51 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.57–7.61 (m, 2H), 7.75 (d, *J* = 7.5 Hz, 2H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ: 14.1, 15.8, 16.3, 21.5, 21.7, 22.7, 28.2, 29.7, 31.6, 34.2, 34.6, 35.4, 46.2, 46.8, 47.1, 59.3, 65.5, 66.0, 66.2, 66.9, 67.1, 117.6, 119.9, 125.2, 127.1, 127.7, 129.0, 132.6, 132.8, 141.2, 143.8, 144.0, 156.2, 156.7, 176.6; HRMS (FAB) calcd for $C_{27}H_{33}N_2O_6$ $[M+H]^+$: 481.2334; found: 481.2331.

(2*S*,3*S*,6*R*,*E*)-2-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-6-{[(allyloxy)carbonyl]amino}-3-methylhept-4-enoic acid (21b). To a stirred solution of **32b** (139 mg, 0.299 mmol) in MeCN (6 mL) and pH 7.0 phosphate buffer solution (2 mL) were added AZADOL (9.14 mg, 0.0597 mmol), $NaClO_2$ (67.5 mg, 0.597 mmol) and NaClO solution (181 μL, 0.299 mmol) at 0 °C. After being stirred for 1.5 h, the reaction mixture was diluted with 1 M HCl. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by preparative thin-layer chromatography over silica gel ($CHCl_3$/MeOH = 9/1) to give compound **21b** (102 mg, 71%) as a colorless amorphous solid: $[\alpha]^{24}_D$ +18.2 (*c* 0.991, $CHCl_3$); IR (neat): 1708 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.04 (d, *J* = 6.9 Hz, 3H), 1.15 (d, *J* = 5.2 Hz, 3H), 2.73–2.90 (m, 1H), 4.06–4.22 (m, 3H), 4.29–4.48 (m, 4H), 5.01–5.51 (m, 4H), 5.71–5.83 (m, 2H), 7.26 (t, *J* = 7.4 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.53–7.59 (m, 2H), 7.72 (d, *J* = 7.4 Hz, 2H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ: 16.2, 20.9, 29.7, 38.6, 47.1, 48.7, 59.1, 65.6, 66.9, 67.3, 117.8, 119.9, 124.9, 125.2, 127.0, 127.7, 130.0, 132.7, 133.1, 141.2, 143.8, 144.0, 155.9, 156.7, 176.7; HRMS (FAB) calcd for $C_{27}H_{30}N_2NaO_6$ $[M+Na]^+$: 501.1996; found: 501.2003.

***tert*-Butyl (*S*)-4-[(*R*)-1-hydroxypropan-2-yl]-2,2-dimethyloxazolidine-3-carboxylate (36a).** O_3 gas was bubbled into a stirred solution of **30b** (49.5 mg, 0.129 mmol) in MeOH (5 mL) at −78 °C for 20 min. O_2 was then bubbled through the solution for 10 min to remove excess O_3. The reaction mixture was added $NaBH_4$ (49.0 mg, 1.29 mmol). The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched with saturated citric acid at 0 °C. The whole was extracted with EtOAc and the extract was washed with aqueous saturated $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silicagel (hexane/EtOAc = 2/1) to give compound **36a** (14.5 mg, 43%) as a white amorphous solid: $[\alpha]^{23}_D$ –5.58 (*c* 0.63, $CHCl_3$); IR (neat): 3253 (OH), 1688 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 1.03 (d, *J* = 6.8 Hz, 3H), 1.47–1.52 (m, 15H), 1.61–1.70 (m, 1H), 3.34–3.41 (m, 1H), 3.58–3.63 (m, 1H), 3.74–3.85 (m, 2H), 3.91 (dd, *J* = 8.9, 5.3 Hz, 2H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 14.8, 24.4, 27.5, 28.3, 38.6, 59.1, 63.3, 66.2, 81.2, 93.5, 154.3; HRMS (ESI-TOF) calcd for $C_{13}H_{25}NNaO_4$ $[M+Na]^+$: 282.1676; found:282.1677.

***tert*-Butyl 2,2-dimethyl-4-(prop-1-en-2-yl)oxazolidine-3-carboxylate ((±)-35).** (±)-**35** was synthesized by the identical procedure reported previously.[31,32] To a stirred solution of amide **33** (1.00 g, 3.47 mmol) in dry THF (10 mL) under argon was added MeLi (1.09 M solution in Et_2O; 6.37 mL, 6.93mmol) at –78 °C. After being stirred for 2 h, the reaction mixture was diluted with aqueous saturated NH_4Cl. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give corresponding ketone, which was used without further purification. To a stirred solution of methyl triphenylphosphonium bromide (3.72 g, 10.4 mmol) in dry THF (10 mL) under argon was added *t*-BuOK (1.17 g, 10.4 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 10 min. To this mixture

was added a solution of the above ketone in dry THF (5 mL) at room temperature. After being stirred for 10 min, the reaction mixture was diluted with brine. The whole was extracted with EtOAc and the extract was dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 7/1) to give compound (±)-**35** (472 mg, 56%) as a colorless oil. The spectral data were in good agreement with those previous reported.[32]

***tert*-Butyl (*S*/*R*)-4-[(*R*/*S*)-1-hydroxypropan-2-yl]-2,2-dimethyloxazolidine-3-carboxylate ((±)-36)**. To a stirred solution of (±)-**35** (300 mg, 1.24 mmol) in dry THF (9 mL) under argon was added $BH_3 \cdot SMe_2$ (2.0 M in THF solution; 2.47 mL, 4.97 mmol) at 0 °C. After stirred for 4 h, aqueous solutions of 30% H_2O_2 and saturated NH_4Cl were added to the mixture, which was warmed to room temperature and stirred overnight. The whole was extracted with EtOAc and the extract was washed with brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 4/1) to give compound (±)-**36a** (37.0 mg, 12%) as a white amorphous solid and compound (±)-**36b** (144 mg, 45%) as a colorless oil.

(±)-**36a**: IR (neat): 3286 (OH), 1690 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 1.04 (d, J = 6.8 Hz, 3H), 1.48–1.52 (m, 15H), 1.62–1.69 (m, 1H), 3.35–3.43 (m, 1H), 3.59–3.65 (m, 1H), 3.79–3.86 (m, 2H), 3.92 (dd, J = 8.9, 5.3 Hz, 1H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 14.8, 24.4, 27.6, 28.4, 38.6, 59.2, 63.4, 66.3, 81.2, 93.5, 154.3; HRMS (ESI-TOF) calcd for $C_{13}H_{25}NNaO_4$ $[M+Na]^+$: 282.1676; found: 282.1676.

(±)-**36b**: IR (neat): 3486 (OH), 1695 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ: 0.85 (d, J = 6.8 Hz, 3H), 1.48 (s, 12H), 1.58 (s, 3H), 1.85–1.94 (m, 1H), 3.23–3.31 (m, 1H), 3.42–3.50 (m, 1H), 3.79–3.84 (m, 1H), 4.00–4.07 (m, 1H), 4.12–4.19 (m, 1H), 4.24–4.30 (m, 1H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 11.3, 23.9, 26.1, 28.2, 40.1, 56.7, 64.6, 66.4, 80.8, 94.3, 154.5; HRMS (ESI-TOF) calcd for $C_{13}H_{25}NNaO_4$ $[M+Na]^+$: 282.1676; found: 282.1676.

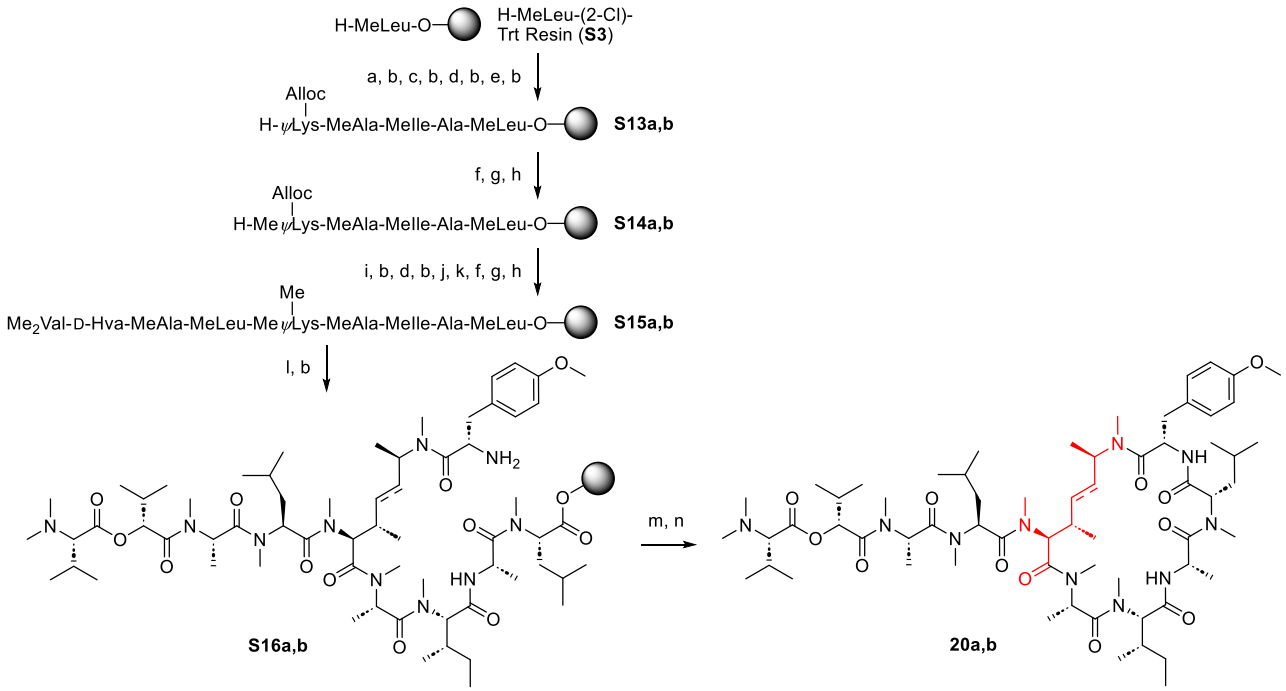
(8*R/S*,8a*S/R*)-3,3,8-Trimethyltetrahydro-3*H*,5*H*-oxazolo[3,4-*c*][1,3]oxazin-5-one ((±)-**37a**). To a stirred solution of NaH (34 mg, 0.848 mmol) in dry THF (0.5 mL) under argon was added a solution of (±)-**36a** (44.0 mg, 0.170 mmol) in dry THF (1 mL) at 0 °C. The reaction mixture was refluxed for 15 min. Then, aqueous saturated NH_4Cl was added to the mixture at room temperature. The whole was extracted with EtOAc and the extract was washed with aqueous saturated $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silicagel (hexane/EtOAc = 1/1) to give compound (±)-**37a** (21.1 mg, 67%) as colorless block crystals: mp 103-105 °C; IR (neat): 1687 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.97 (d, J = 6.6 Hz, 3H), 1.57 (s, 3H), 1.61 (s, 3H), 1.81–1.92 (m, 1H), 3.39 (ddd, J = 10.0, 10.0, 5.6 Hz, 1H), 3.54 (dd, J = 9.9, 8.5 Hz, 1H), 3.83 (dd, J = 11.3, 11.3 Hz, 1H), 4.16–4.24 (m, 2H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ: 12.6, 23.7, 25.9, 31.2, 60.5, 68.7, 72.2, 95.5, 149.5; HRMS (ESI-TOF)

calcd for $C_9H_{15}NNaO_3$ $[M+Na]^+$: 208.0944; found: 208.0944.

(8*R/S*,8a*R/S*)-3,3,8-Trimethyltetrahydro-3*H*,5*H*-oxazolo[3,4-*c*][1,3]oxazin-5-one ((±)-**37b**). To a stirred solution of NaH (110 mg, 2.76 mmol) in dry THF (2 mL) under argon was added a solution of (±)-**36b** (143 mg, 0.552 mmol) in dry THF (4 mL) at 0 °C. The reaction mixture was refluxed for 15 min. Then, aqueous saturated NH_4Cl was added to the mixture at room temperature. The whole was extracted with EtOAc and the extract was washed with aqueous saturated $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silicagel (hexane/EtOAc = 1/1) to give compound (±)-**37b** (72.7 mg, 71%) as a colorless oil: IR (neat): 1688 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.06 (d, *J* = 7.1 Hz, 3H), 1.56 (s, 3H), 1.62 (s, 3H), 2.15–2.23 (m, 1H), 3.64 (dd, *J* = 10.1, 8.7 Hz, 1H), 3.89–3.95 (m, 1H), 4.04 (dd, *J* = 8.6, 5.8 Hz, 1H), 4.11 (dd, *J* = 11.0, 1.7 Hz, 1H), 4.28 (dd, *J* = 11.0, 2.6 Hz, 1H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ: 10.4, 23.4, 25.9, 26.1, 57.7, 65.7, 72.9, 94.6, 149.2; HRMS (ESI-TOF) calcd for $C_9H_{15}NNaO_3$ $[M+Na]^+$: 208.0944; found: 208.0944.

Synthesis of Coibamide A Derivatives with a Modified Lysine (20a,b).

Synthesis of compound 20a. The linear peptides were constructed by solid-phase peptide synthesis on peptidyl resin **S3** (0.490 mmol/g, 102 mg, 0.0500 mmol). After the cleavage from the resin **S16a** as described above, EDCI·HCl (96 mg, 0.500 mmol) was added to a solution of crude linear peptide, HOAt (68 mg, 0.500 mmol), and DIEA (348 μL, 2.00 mmol) in dry DMF (50 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and the stirring was continued for 18 h. The reaction mixture was concentrated in vacuo and the residue was purified by RP-HPLC to give compound **20a** (3.7 mg, 3% from resin) as a white powder: ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ: 0.43-1.59 (m, 50H), 1.59-2.29 (m, 1.5H), 2.47-2.67 (m, 3H), 2.67-2.78 (m, 2.5H), 2.78-3.10 (m, 19H), 3.19-3.30 (m, 2H), 3.69-3.82 (m, 2.5H), 3.82-3.92 (m, 1H), 3.92-4.06 (m, 0.5H), 4.41-4.72 (m, 0.5H), 4.72-4.87 (m, 1H), 4.87-4.96 (m, 0.5H), 4.96-5.06 (m, 1.5H), 5.06-5.18 (m, 1H), 5.18-5.34 (m, 2H), 5.34-5.66 (m, 1.5H), 6.58-6.83 (m, 1.5H), 6.83-6.94 (m, 0.5H), 6.94-7.18 (m, 1.5H), 8.67-8.98 (m, 0.5H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 10.1, 12.0, 14.0, 14.1, 14.4, 14.7 15.4, 15.5, 16.1, 16.8, 16.9, 18.0, 18.7, 18.8, 19.1, 19.2, 20.7, 21.3, 21.5, 22.6, 23.3, 23.47, 23.52, 24.5, 24.6, 25.0, 25.2, 25.5, 26.8, 26.9, 28.2, 28.25, 28.28, 29.1, 29.6, 29.89, 29.93, 30.1, 30.4, 31.4, 31.6, 32.7, 35.6, 37.2, 37.4, 45.2, 46.3, 46.9, 49.5, 49.7, 50.2, 50.4, 51.2, 51.3, 55.1, 55.2, 56.5, 56.7, 65.7, 70.8, 113.6, 115.5, 117.5, 127.5, 130.9, 158.7, 162.2, 162.5, 167.4, 167.7, 168.1, 169.5, 170.6, 170.7, 171.5, 172.6, 173.6, 173.7; HRMS (FAB) calcd for $C_{64}H_{111}N_{10}O_{12}$ $[M+H]^+$: 1211.8378; found: 1211.8407.

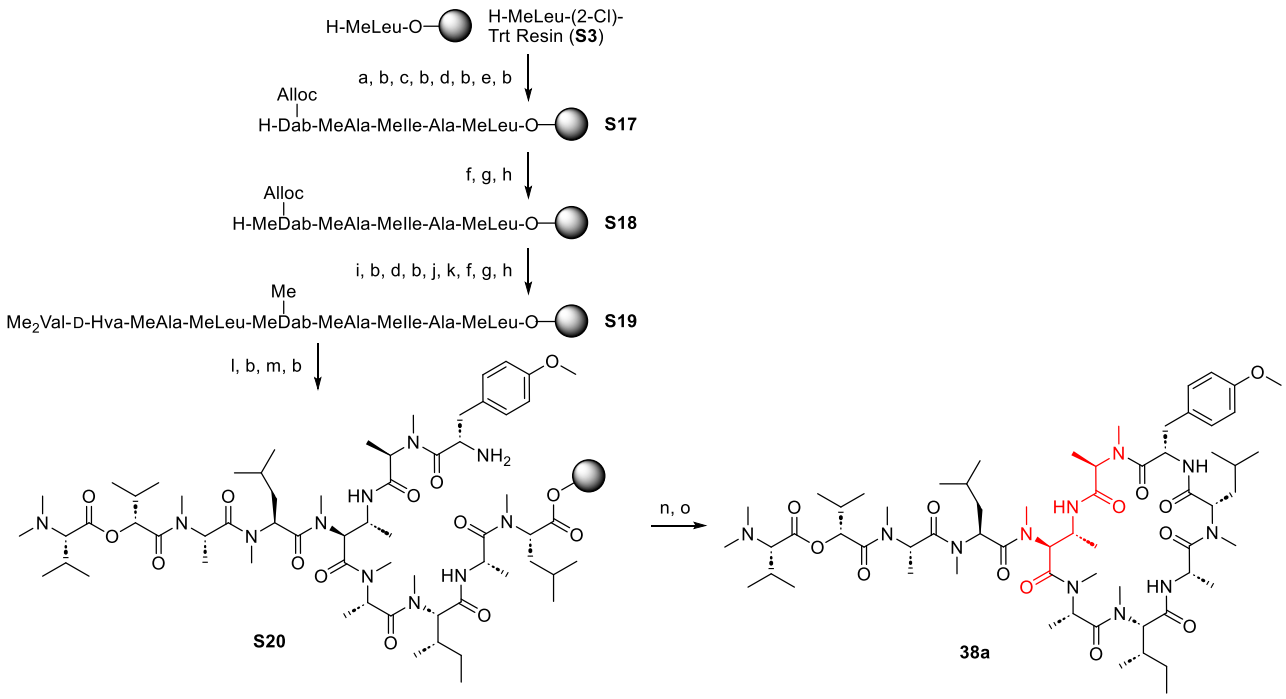


Scheme S4. Synthesis of Coibamide A Derivatives with a Modified Lysine (**20a,b**). *Reagents and conditions*: (a) Fmoc-Ala-OH·H_2O, HATU, DIEA, DMF, 40 °C; (b) 20% piperidine/DMF, rt; (c) Fmoc-MeIle-OH, HOBt·H_2O, DIC, DMF, 40 °C; (d) Fmoc-MeAla-OH, HATU, DIEA, DMF, 40 °C; (e) Fmoc- ψLys(Alloc)-OH, HATU, DIEA, DMF, 40 °C; (f) NsCl, 2,4,6-collidine, NMP, rt; (g) Ph_3P, DEAD, MeOH, THF, rt; (h) 2-mercaptoethanol, DBU, NMP, rt; (i) Fmoc-MeLeu-OH, HATU, DIEA, DMF, 40 °C; (j) Me_2Val-D-Hva-OH·HCl, HATU, DIEA, NMP, 40 °C; (k) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt; (l) Fmoc-Tyr(Me)-OH, HATU, DIEA, NMP, 40 °C; (m) 30% HFIP/CH_2Cl_2, rt; (n) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt.

Synthesis of compound 20b. According to the procedure described for the preparation of **20a**, peptidyl resin **S3** (0.650 mmol/g, 77 mg, 0.0500 mmol) was converted into **20b** (1.2 mg, 1% from resin) as a white powder: ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ: 0.75-2.37 (m, 63H), 2.37-2.63 (m, 2H), 2.63-3.30 (m, 25H), 3.65-3.99 (m, 3.5H), 4.80-4.91 (m, 0.5H), 4.91-5.02 (m, 1H), 5.02-5.28 (m, 2H), 5.28-5.43 (m, 1H), 5.43-5.52 (m, 1H), 5.52-5.67 (m, 0.5H), 6.59-6.95 (m, 2H), 6.95-7.17 (m, 2H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 11.7, 14.1, 14.4, 14.7, 16.0, 17.3, 17.6, 18.6, 18.8, 18.9, 19.1, 19.3, 21.0, 22.1, 22.7, 23.2, 24.6, 24.8, 25.0, 25.3, 28.2, 29.0, 29.3, 29.7, 30.0, 30.1, 30.6, 31.9, 32.6, 37.5, 38.1, 46.8, 50.0, 51.1, 51.5, 55.3, 65.1, 71.0, 113.8, 113.9, 127.6, 130.2, 130.4, 158.5, 167.6, 168.0, 168.7, 169.6, 170.6, 172.2; HRMS (FAB) calcd for $C_{64}H_{109}N_{10}O_{12}$ $[M+H]^+$: 1209.8221; found: 1209.8239.

Synthesis of Coibamide A Derivative with a D-MeAla-MeDab Linkage (38a)

Synthesis of compound 38a. The linear peptides were constructed by solid-phase peptide synthesis



Scheme S5. Synthesis of Coibamide A Derivative with a D-MeAla-MeDab Linkage (**38a**). *Reagents and conditions*: (a) Fmoc-Ala-OH·H_2O, HATU, DIEA, DMF, 40 °C; (b) 20% piperidine/DMF, rt; (c) Fmoc-MeIle-OH, HOBt·H_2O, DIC, DMF, 40 °C; (d) Fmoc-MeAla-OH, HATU, DIEA, DMF, 40 °C; (e) Fmoc-Dab(Alloc)-OH, HATU, DIEA, DMF, 40 °C; (f) NsCl, 2,4,6-collidine, NMP, rt; (g) Ph_3P, DEAD, MeOH, THF, rt; (h) 2-mercaptoethanol, DBU, NMP, rt; (i) Fmoc-MeLeu-OH, HATU, DIEA, DMF, 40 °C; (j) Me_2Val-D-Hva-OH, HATU, DIEA, NMP, 40 °C; (k) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt; (l) Fmoc-D-MeAla-OH, HOBt·H_2O, DIC, DMF, 40 °C; (m) Fmoc-Tyr(Me)-OH, HATU, DIEA, NMP, 40 °C; (n) 30% HFIP/CH_2Cl_2, rt; (o) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt.

on peptidyl resin **S3** (0.490 mmol/g, 206 mg, 0.100 mmol). After the cleavage from the resin **S20** as described above, EDCI·HCl (192 mg, 1.00 mmol) was added to a solution of crude linear peptide, HOAt (136 mg, 1.00 mmol), and DIEA (697 µL, 4.00 mmol) in dry DMF (100 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and the stirring was continued for 18 h. The reaction mixture was concentrated in vacuo and the residue was purified by RP-HPLC to give compound **38a** (10.4 mg, 8% from resin) as a white powder: ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ: 0.53-1.72 (m, 48H), 1.90-2.15 (m, 2H), 2.15-2.36 (m, 2.5H), 2.36-2.59 (m, 4H), 2.59-2.82 (m, 6H), 2.82-3.30 (m, 23H), 3.52-3.89 (m, 5H), 4.15-4.24 (m, 0.5H), 4.40-4.68 (m, 1.5H), 4.68-4.91 (m, 1.5H), 4.91-5.15 (m, 2.5H), 5.34-5.68 (m, 2.5H), 5.68-6.07 (m, 1H), 6.07-6.20 (m, 0.5H), 6.62-6.88 (m, 2.5H), 6.88-7.01 (m, 0.5H), 7.01-7.18 (m, 2H), 7.30-7.43 (m, 0.5H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 11.7, 11.8, 12.6, 12.8, 16.0, 17.3, 17.4, 17.5, 18.0, 18.5, 18.59, 18.64, 18.9, 19.0, 20.8, 22.2, 22.4, 22.7, 23.1, 23.4, 24.3, 24.9, 25.0, 25.2, 25.5, 28.2, 28.8, 29.3, 29.4, 29.5, 29.7, 29.8, 30.0, 30.1, 30.17, 30.21, 30.3, 30.9, 32.1, 34.0, 35.9, 36.9, 37.4, 37.9, 38.1, 39.0, 44.3,

44.4, 46.5, 47.1, 51.0, 51.4, 51.7, 51.8, 51.9, 52.3, 52.7, 52.9, 53.0, 55.30, 55.33, 56.0, 58.5, 58.75, 58.80, 58.86, 58.91, 64.6, 64.7, 64.8, 68.4, 68.6, 68.88, 68.93, 71.3, 71.4, 113.9, 114.3, 126.9, 127.8, 130.1, 130.2, 158.8, 159.1, 167.1, 167.7, 167.8, 169.0, 169.1, 169.6, 169.8, 170.0, 170.1, 170.3, 170.4, 170.8, 171.7, 171.9, 172.6, 173.0; HRMS (FAB) calcd for $C_{67}H_{107}N_{11}NaO_{13}$ $[M+Na]^+$: 1248.7942; found: 1248.7963.

Synthesis of compound 38b. According to the procedure described for the preparation of **38a**, peptidyl resin **S3** (0.490 mmol/g, 206 mg, 0.100 mmol) was converted into **38b** (15.0 mg, 11% from resin) as a white powder: ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ: 0.71-1.70 (m, 48H), 1.87-2.14 (m, 1H), 2.14-2.32 (m, 2H), 2.41-2.55 (m, 3H), 2.67-2.76 (m, 3H), 2.76-3.26 (m, 25H), 3.26-3.39 (m, 6H), 3.47-3.92 (m, 9H), 4.16-4.23 (m, 0.5H), 4.40-4.60 (m, 1H), 4.70-4.86 (m, 1.5H), 4.94-5.10 (m, 2H), 5.31-5.40 (m, 0.5H), 5.40-5.47 (m, 0.5H), 5.67-5.89 (m, 2H), 6.00-6.10 (m, 0.5H), 6.10-6.22 (m, 0.5H), 6.61-6.73 (m, 0.5H), 6.73-6.89 (m, 2.5H), 6.89-6.97 (m, 0.5H), 6.97-7.12 (m, 2H), 7.12-7.23 (m, 0.5H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 11.7, 11.8, 12.6, 12.8, 16.0, 17.3, 17.4, 17.5, 18.0, 18.5, 18.59, 18.64, 18.9, 19.0, 19.4, 20.8, 22.2, 22.4, 22.7, 23.1, 23.4, 24.3, 24.9, 25.0, 25.2, 25.5, 28.2, 28.8, 29.3, 29.4, 29.5, 29.7, 29.8, 30.0, 30.1, 30.17, 30.21, 30.3, 30.9, 32.1, 34.0, 35.9, 36.9, 37.4, 37.87, 37.95, 38.1, 39.0, 44.3, 44.4, 46.5, 47.1, 51.0, 51.4, 51.7, 51.7, 51.8, 51.9, 52.3, 52.7, 52.9, 53.0, 55.29, 55.33, 56.0, 58.5, 58.9, 64.6, 64.7, 64.8, 68.4, 68.6, 68.88, 68.93, 71.3, 71.4, 113.9, 114.3, 126.9, 127.8, 130.1, 130.2, 158.8, 159.1, 167.1, 167.7, 168.8, 169.0, 169.1, 169.6, 169.8, 170.0, 170.1, 170.3, 170.4, 170.8, 171.7, 171.9, 172.6, 173.0; HRMS (FAB) calcd for $C_{65}H_{112}N_{11}O_{15}$ $[M+H]^+$: 1286.8334; found: 1229.8353.

Synthesis of compound 38c. According to the procedure described for the preparation of **38a**, peptidyl resin **S3** (0.490 mmol/g, 206 mg, 0.100 mmol) was converted into **38c** (10.5 mg, 7% from resin) as a white powder: ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ: 0.74-1.78 (m, 48H), 1.88-2.15 (m, 1H), 2.15-2.39 (m, 2H), 2.39-2.61 (m, 3H), 2.61-2.81 (m, 3H), 2.81-3.27 (m, 25H), 3.27-3.43 (m, 6H), 3.43-3.57 (m, 1H), 3.57-3.93 (m, 6H), 4.05-4.26 (m, 0.5H), 4.33-4.64 (m, 1H), 4.69-4.79 (m, 0.5H), 4.79-4.94 (m, 1H), 4.94-5.12 (m, 2H), 5.30-5.40 (m, 0.5H), 5.40-5.46 (m, 0.5H), 5.59-5.95 (m, 2.5H), 6.00-6.11 (m, 0.5H), 6.11-6.23 (m, 0.5H), 6.68-6.74 (m, 0.5H), 6.88-6.97 (m, 0.5H), 7.19-7.26 (m, 1.5H), 7.32-7.40 (m, 1.5H), 7.40-7.48 (m, 2H), 7.48-7.53 (m, 1.5H), 7.53-7.62 (m, 3H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 11.6, 11.8, 12.5, 12.8, 14.1, 16.0, 17.1, 17.2, 17.5, 17.9, 18.5, 18.6, 18.8, 19.0, 19.1, 19.3, 20.7, 22.1, 22.2, 22.3, 22.4, 22.6, 23.1, 23.3, 24.2, 24.9, 25.0, 25.2, 25.5, 28.1, 28.7, 29.26, 29.35, 29.7, 29.8, 29.9, 30.05, 30.1, 30.17, 30.24, 30.4, 30.8, 31.9, 32.1, 34.0, 36.2, 37.2, 37.3, 37.86, 37.34, 38.92, 44.2, 44.3, 46.4, 47.1, 51.0, 51.8, 52.0, 52.6, 53.0, 53.1, 56.0, 57.6, 58.5, 58.7, 58.79, 58.83, 58.9, 64.5, 64.7, 64.8, 68.3, 68.5, 68.80, 68.84, 71.0, 71.1, 126.90, 126.93, 127.2, 127.4, 127.5, 128.8, 128.9, 129.49, 129.55, 134.1, 134.8, 140.2, 140.3, 140.4, 140.6, 162.1, 162.3, 167.1, 167.7, 168.1, 168.2, 168.4, 168.7, 168.8, 168.9, 169.4, 169.6, 169.97, 170.05, 170.3, 170.5, 171.0, 171.7, 171.8, 172.5, 172.9; HRMS (FAB) calcd for $C_{70}H_{114}N_{11}O_{14}$ $[M+H]^+$:

1332.8541; found: 1332.8560.

Synthesis of compound 39a. According to the procedure described for the preparation of **38a**, peptidyl resin **S3** (0.590 mmol/g, 171 mg, 0.100 mmol) was converted into **39a** (7.0 mg, 5% from resin) as a white powder: ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ: 0.62-2.30 (m, 52H), 2.43-2.54 (m, 3H), 2.54-3.16 (m, 27H), 3.58-3.92 (m, 6H), 3.92-4.13 (m, 0.5H), 4.13-4.26 (m, 1H), 4.50-4.64 (m, 1H), 4.82-5.15 (m, 4H), 5.15-5.29 (m, 0.5H), 5.29-5.44 (m, 1.5H), 5.44-5.58 (m, 1H), 5.58-5.74 (m, 0.5H), 6.35-6.51 (m, 0.5H), 6.57-6.75 (m, 1H), 6.75-6.91 (m, 2H), 7.03-7.15 (m, 2H), 7.53-7.77 (m, 0.5H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 11.6, 11.8, 12.6, 12.9, 14.5, 14.8, 15.6, 16.0, 16.9, 17.1, 17.2, 18.7, 18.8, 18.9, 19.1, 19.22, 19.25, 19.3, 21.3, 22.1, 22.5, 22.7, 22.8, 24.8, 25.0, 25.1, 25.2, 28.2, 29.0, 29.1, 29.2, 29.4, 29.5, 30.0, 30.1, 30.2, 30.3, 30.5, 32.3, 33.2, 33.6, 35.8, 36.8, 37.1, 37.5, 37.7, 39.0, 46.1, 46.4, 48.3, 49.0, 50.1, 50.3, 50.8, 51.2, 51.4, 51.9, 52.4, 55.3, 55.4, 58.2, 58.9, 64.6, 65.1, 70.9, 113.9, 114.3, 126.7, 127.9, 130.1, 130.3, 158.8, 159.2, 167.80, 167.85, 168.0, 168.1, 168.5, 170.0, 170.7, 171.0, 171.4, 172.0, 172.1, 172.7, 173.5, 173.9; HRMS (ESI-TOF) calcd for $C_{62}H_{105}N_{11}NaO_{13}$ $[M+Na]^+$: 1234.7786; found: 1234.7778.

Synthesis of compound 39b. According to the procedure described for the preparation of **38a**, peptidyl resin **S3** (0.590 mmol/g, 171 mg, 0.100 mmol) was converted into **39b** (3.1 mg, 2% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{63}H_{107}N_{11}NaO_{13}$ $[M+Na]^+$: 1248.7942; found: 1248.7942.

Growth Inhibition Assay Using A549 Cells[33]

A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented with 10% (v/v) fetal bovine serum at 37 °C in a 5% CO_2-incubator. Growth inhibition assays using A549 cells were performed in 96-well plates (BD Falcon). A549 cells were seeded at 1000 cells/well in 90 μL of culture media, respectively, and were cultured for 24 h. Chemical compounds in DMSO were diluted 50-fold with the culture medium in advance. 30 μL of the chemical diluents were added. The final volume of DMSO in the medium was equal to 0.5% (v/v). The cells under chemical treatment were incubated for a further 72 h. The wells in the plates were washed twice with the cultured medium without phenol-red. After 1-hour incubation with 100 μL of the medium, the cell culture in each well was supplemented with 20 μL of the MTS reagent (Promega), followed by incubation for additional 40 min. Absorbance at 490 nm of each well was measured using a Wallac 1420 ARVO SX multilabel counter (Perkin Elmer). Three experiments were performed per condition and the averages of inhibition rates in each condition were evaluated to determine IC_{50} values using the GraphPad Prism software.

Viability Assays Using U87 MG Cells and HCT116 Cells

Human U87 MG glioblastoma and HCT116 colon cancer cells were from American Type Culture

Collection (ATCC, Manasses, VA). U87 MG cells were cultured in Minimum Essential Medium (MEM) with Earl's salts and L-glutamine (Corning Life Sciences), supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and 100 U/mL penicillin and 100 mg/mL streptomycin (1% penicillin/streptomycin). HCT116 cells were cultured in McCoy's 5A medium supplemented with 10% FBS and 1% penicillin/streptomycin. All cells were grown under standard conditions and maintained at 37 ºC in an atmosphere of 5% CO_2. For analysis of biological activity, cells were seeded at 3,000 cells/well into 96-well plates and allowed to attach overnight. On the day of the experiment, all cells were treated at the same time with the test compound or vehicle (0.1% DMSO) and plates returned to the cell culture incubator for 72 h. The viability of cells was measured at 72 h using a CellTiter-Glo® Luminescent Cell Viability Assay (Promega Corp) and luminescent signals measured using a multi-mode microplate reader (Biotek Synergy HT).

Protein Secretion Assay

The generation of U87 MG cells expressing the secreted reporter *Gaussia* luciferase (GLuc) to yield the U87-GLuc cells for reporter assays has been described previously.[34,35] U87-GLuc cells were cultured in Minimum Essential Medium (MEM) with Earl's salts and L-glutamine (Corning Life Sciences), supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and 100 U/mL penicillin and 100 mg/mL streptomycin (1% penicillin/streptomycin). All cells were grown under standard conditions and maintained at 37 ºC in an atmosphere of 5% CO_2. For analysis of biological activity, cells were seeded at 3,000 cells/well into 96-well plates and allowed to attach overnight. On the day of the experiment, all cells were treated at the same time with the test compound or vehicle (0.1% DMSO) and plates returned to the cell culture incubator for 18 h. For analysis of secretory function in U87-GLuc cells at 18 h, 20 µL of conditioned cell culture medium was removed from each well and transferred to new 96-well white-walled plates. Subsequently, 50 µL of 1.68 µM coelenterazine was injected into each well (final concentration = 1.2 µM), and luminescent signals were measured using a multi-mode microplate reader (Biotek Synergy HT) with Gen5® software and compared across conditions (3 sec wait, 0.5 sec integration time following coelenterazine injection). The viability of all U87-GLuc cells was measured at 18 h using a CellTiter-Glo® Luminescent Cell Viability Assay (Promega Corp) and luminescent signals measured using the same microplate reader.

参考文献

1. Medina, R. A.; Goeger, D. E.; Hills, P.; Mooberry, S. L.; Huang, N.; Romero, L. I.; Ortega-Barría, E.; Gerwick, W. H.; McPhail, K. L. *J. Am. Chem. Soc.* **2008**, *130*, 6324.
2. He, W.; Qiu, H.-B. Chen, Y.-J.; Xi, J.; Yao, Z.-J. *Tetrahedron Lett.* **2014**, *55*, 6109.
3. Nabika, R.; Suyama, T. L.; Hau, A. M.; Misu, R.; Ohno, H.; Ishmael, J. E.; McPhail, K. L.; Oishi, S.; Fujii, N. *Bioorg. Med. Chem. Lett.* **2015**, 25, 302-306.
4. Yao, G.; Pan, Z.; Wu, C.; Wang, W.; Fang, L.; Su, W. *J. Am. Chem. Soc.* **2015**, *137*, 13488.
5. Yao, G.; Wang, W.; Ao, L.; Cheng, Z.; Wu, C.; Pan, Z.; Liu, K.; Li, H.; Su, W.; Fang, L. *J. Med. Chem.* **2018**, *61*, 8908.
6. Hau, A. M.; Greenwood, J. A.; Löhr, C. V.; Serrill, J. D.; Proteau, P. J.; Ganley, I. G.; McPhail, K. L.; Ishmael, J. E. *PLoS One.* **2013**, *8*, e65250.
7. Wan, X.; Serrill, J. D.; Humphreys, I. R.; Tan, M.; McPhail, K. L.; Ganley, I. G.; Ishmael, J. E. *Mar. Drugs* **2018**, *16*, 77.
8. Serrill, J. D.; Wan, X.; Hau, A. M.; Jang, H. S.; Coleman, D. J.; Indra, A. K.; Alani, A. W. G.; McPhail, K. L.; Ishmael, J. E. *Invest. New Drugs* **2016**, *34*, 24.
9. Tranter, D.; Paatero, A. O.; Kawaguchi, S.; Kazemi, S.; Serrill, J. D.; Kellosalo, J.; Vogel, W. K.; Richter, U.; Mattos, D. R.; Wan, X.; Thornburg, C. C.; Oishi, S.; McPhail, K. L.; Ishmael, J. E.; Paavilainen, V. O. *ACS Chem. Biol.* **2020**, *15*, 2125.
10. Huang, K.-C.; Chen, Z.; Jiang, Y.; Akare, S.; Kolber-Simonds, D.; Condon, K.; Agoulnik, S.; Tendyke, K.; Shen, Y.; Wu, K.-M.; Mathieu, S.; Choi, H.-w.; Zhu, X.; Shimizu, H.; Kotake, Y.; Gerwick, W. H.; Uenaka, T.; Woodall-Jappe, M.; Nomoto, K. *Mol. Cancer Ther.* **2016**, *15*, 1208.
11. Paatero, A. O.; Kellosalo, J.; Dunyak, B. M.; Almaliti, J.; Gestwicki, J. E.; Gerwick, W. H.; Taunton, J.; Paavilainen, V. O. *Cell Chem. Biol*. **2016**, *23*, 561.
12. Junne, T.; Wong, J.; Studer, C.; Aust, T.; Bauer, B. W.; Beibel, M.; Bhullar, B.; Bruccoleri, R.; Eichenberger, J.; Estoppey, D.; Hartmann, N.; Knapp, B.; Krastel, P.; Melin, N.; Oakeley, E. J.; Oberer, L.; Riedl, R.; Roma, G.; Schuierer, S.; Petersen, F.; Tallarico, J. A.; Rapoport, T. A.; Spiess, M.; Hoepfner, D. *J. Cell Sci.* **2015**, *128*, 1217.
13. Cross, B. C. S.; McKibbin, C.; Callan, A. C.; Roboti, P.; Piacenti, M.; Rabu, C.; Wilson, C. M.; Whitehead, R.; Flitsch, S. L.; Pool, M. R.; High, S.; Swanton, E. *J. Cell Sci*. **2009**, *122*, 4393.
14. Gamayun, I.; O'Keefe, S.; Pick, T.; Klein, M.-C.; Nguyen, D.; McKibbin, C.; Piacenti, M.; Williams, H. M.; Flitsch, S. L.; Whitehead, R. C.; Swanton, E.; Helms, V.; High, S.; Zimmermann, R.; Cavalié, A. *Cell Chem. Biol.* **2019**, *26*, 571.
15. Besemer, J.; Harant, H.; Wang, S.; Oberhauser, B.; Marquardt, K.; Foster, C. A.; Schreiner, E. P.; de Vries, J. E.; Dascher-Nadel, C.; Lindley, I. J. D. *Nature* **2005**, *436*, 290.
16. Garrison, J. L.; Kunkel, E. J.; Hegde, R. S.; Taunton, J. *Nature* **2005**, *436*, 285.
17. MacKinnon, A. L.; Garrison, J. L.; Hegde, R. S.; Taunton, J. *J. Am. Chem. Soc*. **2007**, *129*, 14560.
18. Zong, G.; Hu, Z.; O'Keefe, S.; Tranter, D.; Iannotti, M. J.; Baron, L.; Hall, B.; Corfield, K.; Paatero, A. O.; Henderson, M. J.; Roboti, P.; Zhou, J.; Sun, X.; Govindarajan, M.; Rohde, J. M.; Blanchard,

N.; Simmonds, R.; Inglese, J.; Du, Y.; Demangel, C.; High, S.; Paavilainen, V. O.; Shi, W. Q. *J. Am. Chem. Soc.* **2019**, *141*, 8450.

19. Baron, L.; Paatero, A. O.; Morel, J.-D.; Impens, F.; Guenin-Macé, L.; Saint-Auret, S.; Blanchard, N.; Dillmann, R.; Niang, F.; Pellegrini, S.; Taunton, J.; Paavilainen, V. O.; Demangel, C. *J. Exp. Med.* **2016**, *213*, 2885.
20. 川口晋作 京都大学大学院薬学研究科 修士論文 **2018**.
21. Onda, Y.; Masuda, Y.; Yoshida, Y.; Doi, T. *J. Med. Chem.* **2017**, *60*, 6751.
22. Garner, P.; Park, J. M. *Org. Synth.* **1991**, *70*, 18.
23. Zhang, X.; Ni, W.; van der Donk, W. A. *J. Org. Chem.* **2005**, *70*, 6685.
24. Corey, E. J.; Boaz, N. W. *Tetrahedron Lett.* **1984**, *25*, 3063.
25. Ibuka, T.; Nakao, T.; Nishii, S.; Yamamoto, Y. *J. Am. Chem. Soc.* **1986**, *108*, 7420.
26. Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc., Perkin Trans.* **1982**, *1*, 307.
27. Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77.
28. Nabika, R.; Suyama, T. L.; Hau, A. M.; Misu, R.; Ohno, H.; Ishmael, J. E.; McPhail, K. L.; Oishi, S.; Fujii, N. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 302.
29. Biron, E.; Chatterjee, J.; Kessler, H. *J. Pept. Sci.* **2006**, *12*, 213.
30. Pan, Z.; Wu, C.; Wang, W.; Cheng, Z.; Yao, G.; Liu, K.; Li, H.; Fang, L.; Su, W. *Org. Lett.* **2017**, *19*, 4420.
31. Li, P. X.; Evans, C. D.; Wu, Y. Z.; Cao, B.; Hamel, E.; Joullie, M. M. *J. Am. Chem. Soc.* **2008**, *130*, 2351.
32. Hu, X. E.; Kim, N. K.; Ledoussal, B. *Org. Lett.* **2002**, *4*, 4499.
33. Hou, Z.; Nakanishi, I.; Kinoshita, T.; Takei, Y.; Yasue, M.; Misu, R.; Suzuki, Y.; Nakamura, S.; Kure, T.; Ohno, H.; Murata, K.; Kitaura, K.; Hirasawa, A.; Tsujimoto, G.; Oishi, S.; Fujii, N. *J. Med. Chem.* **2012**, *55*, 2899.
34. Badr, C. E.; Hewett, J. W.; Breakefield, X. O.; Tannous, B. A. *PLoS One* **2007**, *2*, e571.
35. Kazemi, S.; Kawaguchi, S.; Badr, C. E.; Mattos, D. R.; Ruiz-Saenz, A.; Serrill, J. D.; Moasser, M. M.; Dolan, B. P.; Paavilainen, V. O.; Oishi, S.; McPhail, K. L.; Ishmael, J. E. *Biochem. Pharmacol.* **2021**, *183*, 114317.

第二章　Vitilevuamide の合成研究

第一節　研究の背景

Vitilevuamide (**1**) は、尾索動物類より単離された 14 残基の二環性環状ペプチドであり、強力な細胞増殖抑制活性を有している（Figure 1）[1,2]。Vitilevuamide は、NCI-60 スクリーニングパネルにおいて、12 種類の腫瘍細胞株に対して nM レベルで細胞増殖抑制活性を示すなど、魅力的な生物活性を有している [3]。また、チューブリンの重合阻害作用を示す化合物を用いた競合実験により、vitilevuamide はビンカアルカロイドやコルヒチンとは違うサイトに結合してチューブリンに作用すると考えられている [4]。しかしながら、天然からの供給量に制限があることや化学合成法が未確立であることなどから、vitilevuamide の詳細な作用機序解析研究は行われていない。

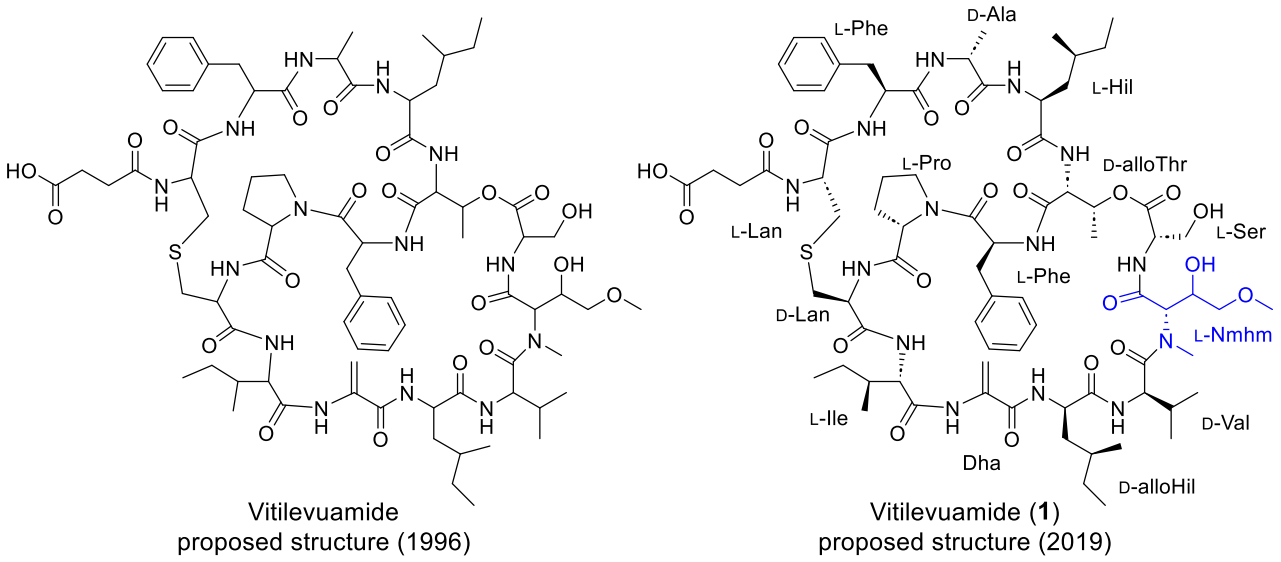


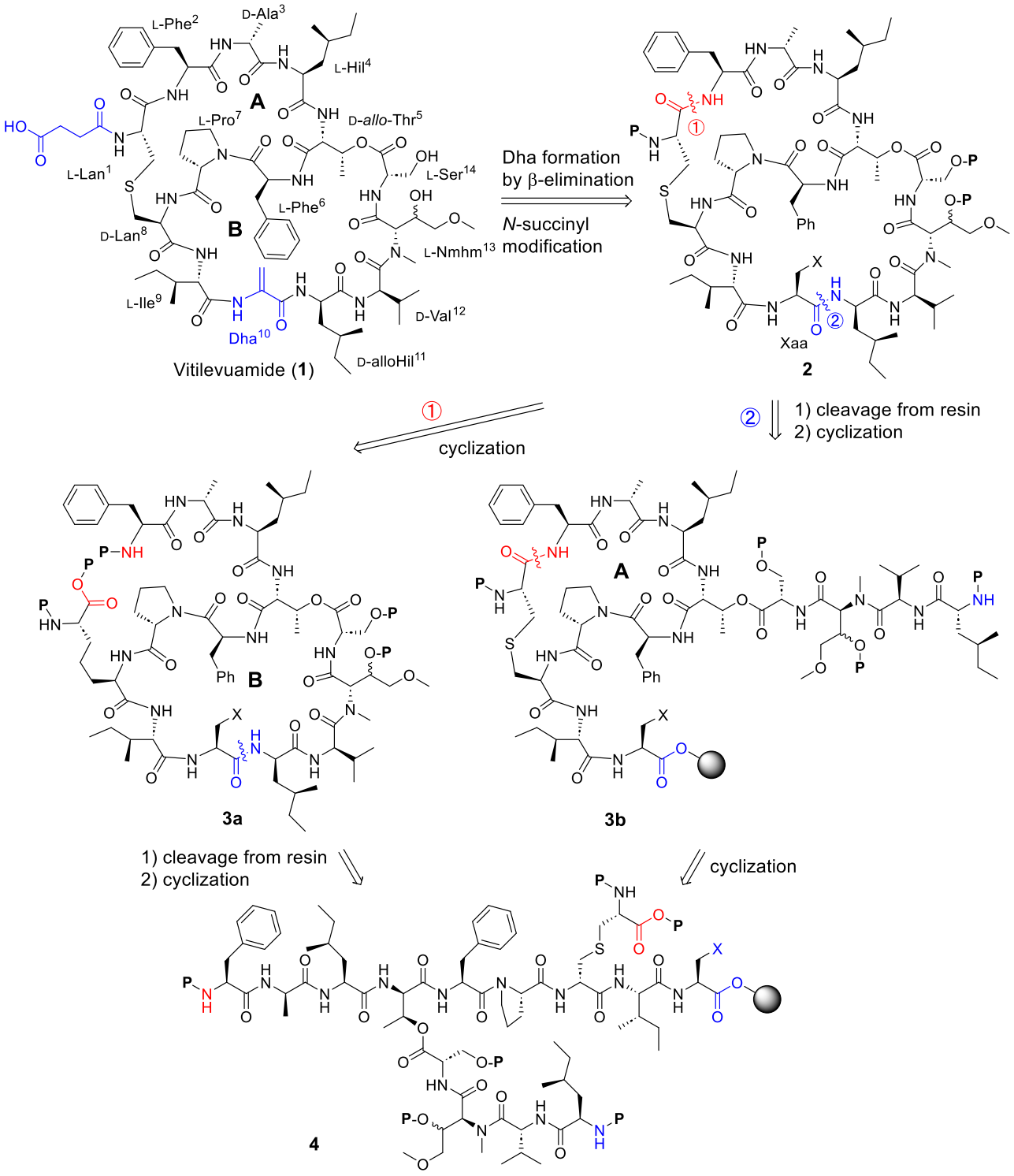
Figure 1. Proposed Structures of Vitilevuamide

Vitilevuamide を構成している 14 残基のアミノ酸には、ランチオニン（Lan）やホモイソロイシン（Hil）など非天然型側鎖を有するアミノ酸が含まれている。1996 年に単離された当初、vitilevuamide の化学構造はアミノ酸配列が確定しているのみで、構成アミノ酸の立体配置は不明であった。その後、vitilevuamide は 2019 年に再単離され、詳細な NMR 解析、キラルアミノ酸分析、並びに、生合成遺伝子クラスターの解析により、ほぼすべてのアミノ酸の立体配置が明らかとなった [3]。しかしながら、β-ヒドロキシメトキシニン（Nmhm）残基がアミノ酸分析の前処理のための酸性条件に不安定であったことから、立体配置の決定が困難であり、Nmhm の β 位のヒドロキシ基の立体配置は明らかとなっていない。

本研究では、立体配置を含む化学構造の決定、並びに、構造最適化研究や標的分子探索のためのプローブの創製に資する vitilevuamide の化学合成プロセスの確立に向けた検討を行った。

第二節　Vitilevuamide の合成計画

Vitilevuamide (**1**) の合成において重要となるのは、デヒドロアラニン（Dha）の構築法、二環性ペプチドの 2 つの環構造の構築順序、および、これらに適用可能な直交性のある保護基の選択である。本研究の重要課題であるこれらの観点をふまえて、著者は、vitilevuamide の

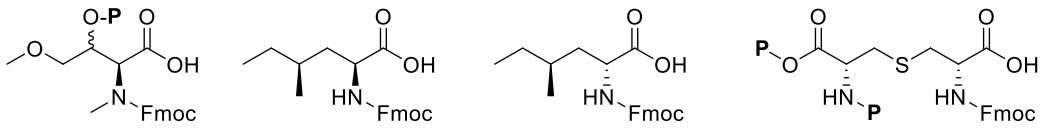


Scheme 1. Retrosynthetic Analysis of Vitilevuamide

合成計画を以下のように立案した（Scheme 1）。

環化反応の際の副反応が懸念されるコハク酸は合成過程の終盤に導入することとし、化学的に不安定であると予想された Dha は、脱離反応により Dha へと変換可能なアミノ酸（Xaa）を用いて前駆体ペプチド **2** を構築することとした。ペプチド **2** の 2 つの大環状構造（A 環および B 環）を構築するための環化位置は、Lan^1–Phe^2 間のペプチド結合（A 環の構築）と Xaa^{10}–D-*allo*-Hil^{11} 間のペプチド結合（B 環の構築）を選択した。Lan^1–Phe^2 間のペプチド結合での環化では、Lan^1 のアミノ基へのカルバメート型保護基の利用により環化反応におけるエピメリ化を抑制できる利点がある。また、Xaa^{10}–D-*allo*-Hil^{11} 間のペプチド結合での環化においては、Xaa^{10} を最後の過程で Dha に変換するため、環化反応における Xaa^{10} のエピメリ化を考慮する必要がない。ペプチド **2** を構築するプロセスは、環化前駆体となる線形ペプチド **4** から B 環を構築してペプチド **3a** を得た後に A 環を構築する経路①と、ペプチド **4** から A 環を構築してペプチド **3b** を得た後に B 環を構築する経路②が想定される。保護ペプチド **4** は、Dha 前駆体（Xaa）を担持したクロロトリチル樹脂上で Fmoc 固相合成法を用いて合成することとした。

また、環化前駆体 **4** を合成するためには、市販のアミノ酸誘導体以外に、非天然型側鎖を有する 3 種類のアミノ酸 **5**–**7**、適切な保護基の組み合わせからなる Lan 誘導体 **8** を用いる必要があるため、これらの合成についても検討を行った（Figure 2）。



Fmoc-L-Nmhm-OH (**5**)　Fmoc-L-Hil-OH (**6**)　Fmoc-D-*allo*-Hil-OH (**7**)　Fmoc-Lan-OH (**8**)

Figure 2. Structures of Protected Nonproteinogenic Amino Acids (**5-8**) for Synthesis of Vitilevuamide. *Abbreviations*: Nmhm: *N*-methyl-β-hydroxymethoxinine; Hil: homoisoleucine; Lan: lanthionine

第三節　モデルペプチドを用いた vitilevuamide の二環性骨格の構築プロセスの確立

Vitilevuamide (**1**) の二環性骨格の合成プロセスの確立に向けて、適切な保護基の選択、デヒドロアラニン（Dha）の構築法の決定を目的とした検討を行った。この際、効率的な検討を行うべく、非天然型側鎖を有するアミノ酸を市販試薬として入手可能なアミノ酸に置き換えたモデル化合物 **9** を設計した（Figure 3）。具体的には、L-Hil[4] および D-*allo*-Hil[11] をそれぞれ L-Leu および D-Leu に、L-Nmhm[13] を L-MeThr に置き換えた誘導体をモデルペプチドとして設定した。これらの変換によるペプチドの主鎖構造への影響は軽微であり、二環性骨格の構築を検討することに支障はないと判断した。

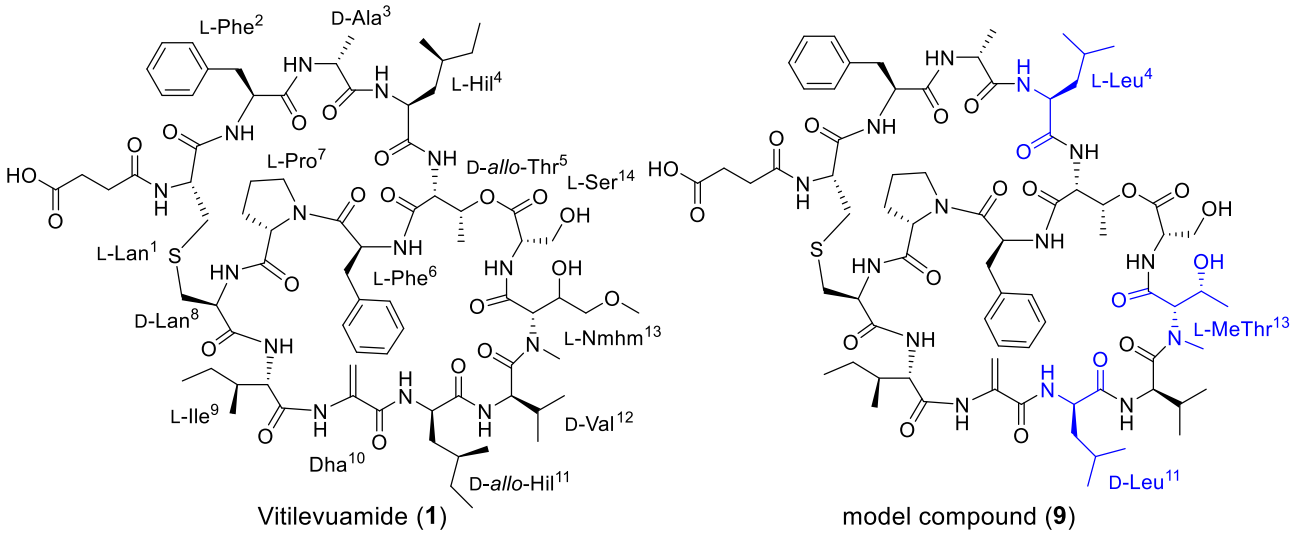
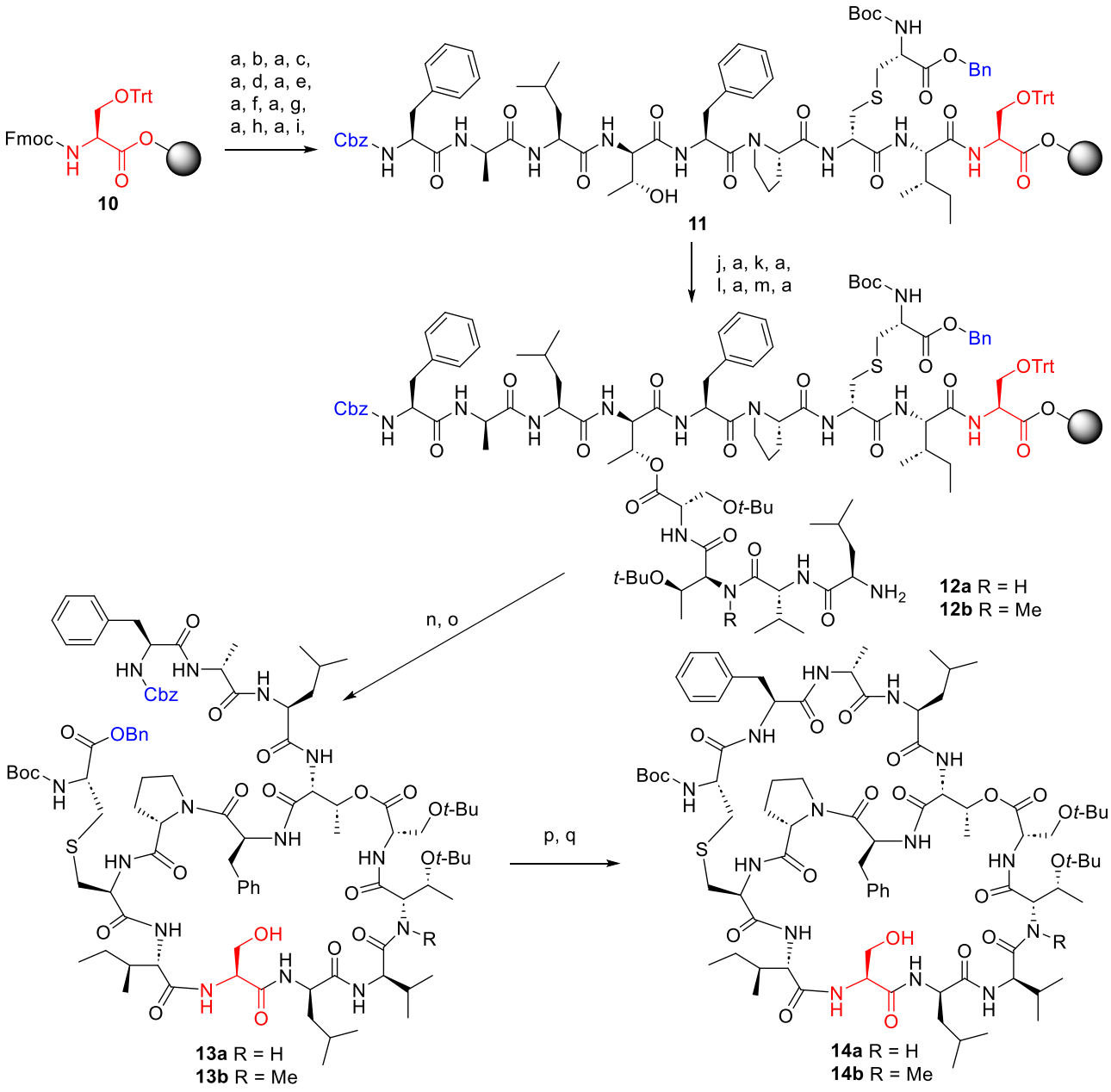


Figure 3. Design of a Model Peptide for the Synthetic Study on Vitilevuamide.

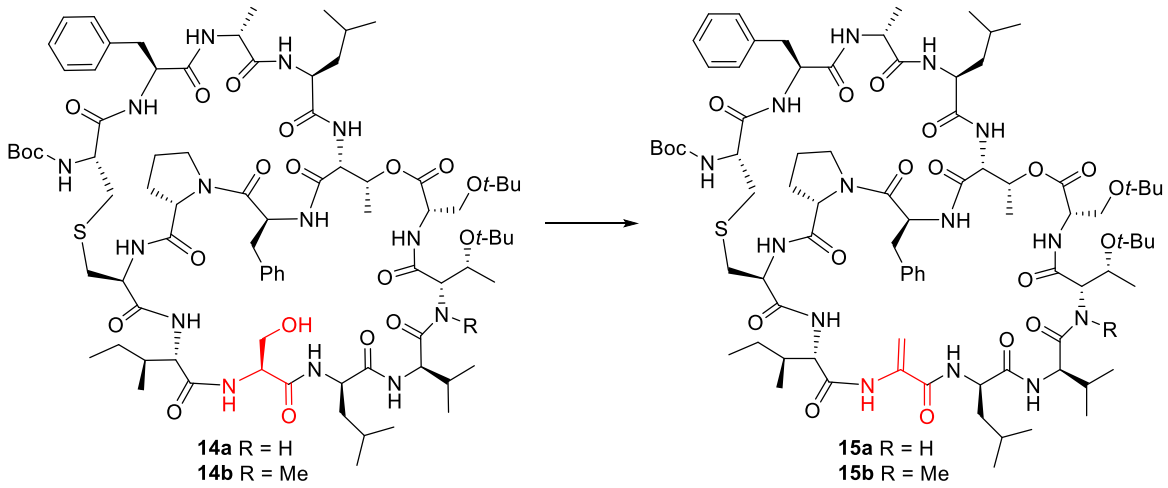
Vitilevuamide の二環性骨格の B 環を最初に構築する経路①の合成プロセスの検討を行った（Scheme 2）。Dha 前駆体として Ser 誘導体を用いることとし、これに相当する Fmoc-Ser(Trt)-OH を担持したクロロトリチル樹脂 **10** を出発原料として利用した。まず、DIC/HOBt を縮合剤として利用した Fmoc 固相合成法により Phe[2] から Ser(Trt)[10] の配列 **11** を構築した。この際、Lan 誘導体は別途合成した Boc/ベンジルエステル体を用いた（実験項参照）。その後、D-*allo*-Thr[5] 側鎖のヒドロキシ基上に、DIC/DMAP を用いて Fmoc-Ser(*t*-Bu)-OH を縮合した。この際、20%程度の Ser(*t*-Bu)[10] のエピメリ化が観察された。引き続き、アミノ酸の縮合を行い、D-Leu[11] までの配列 **12** を構築した。この際、MeThr[13] の *N*-メチルアミノ基上へのアシル化（D-Val[12] の導入）には、縮合剤として DIC/HOAt を用いた。引き続き、1,1,1,3,3,3-hexafluoroisopropanol を利用した樹脂からの切り出し、EDCI/HOAt を用いた希釈条件下での環化反応を行い、B 環を構築した環状ペプチド **13** を得た。最後に、ペプチド **13** の Cbz 基とベンジルエステルを接触還元により脱保護し、この基質に対して希釈条件下での環化反応に付すことで、二環性環状ペプチド **14** を得た。



Scheme 2. Synthetic Study on the Bicyclic Structure of a Vitilevuamide Model Peptide Having a Ser at the Dha[10] Position. *Reagents and conditions*: (a) 20% piperidine/DMF, rt; (b) Fmoc-Ile-OH, DIC, HOBt·H_2O, DMF, rt; (c) Fmoc-D-Lan(Boc-Lan-OBn)-OH, DIC, HOBt·H_2O, DMF, rt; (d) Fmoc-Pro-OH, DIC, HOBt·H_2O, DMF, rt; (e) Fmoc-Phe-OH, DIC, HOAt, DMF, 40 °C; (f) Fmoc-D-*allo*-Thr-OH, DIC, HOBt·H_2O, DMF, rt; (g) Fmoc-Leu-OH, DIC, HOBt·H_2O, DMF, rt; (h) Fmoc-D-Ala-OH, DIC, HOBt·H_2O, DMF, rt; (i) Cbz-Phe-OH, DIC, HOBt·H_2O, DMF, rt; (j) Fmoc-Ser(*t*-Bu)-OH, DIC, DMAP, CH_2Cl_2, DMF, rt; (k) Fmoc-Thr(*t*-Bu)-OH (for **12a**) or Fmoc-MeThr(*t*-Bu)-OH (for **12b**), DIC, HOBt·H_2O, DMF, rt; (l) Fmoc-D-Val-OH, DIC, HOAt, DMF, 40 °C; (m) Fmoc-D-Leu-OH, DIC, HOBt·H_2O, DMF, rt; (n) 30% HFIP/CH_2Cl_2, rt; (o) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt, 17% from resin (**13a**) and 24% from resin (**13b**); (p) 10% Pd/C, H_2, *i*-PrOH, rt; (q) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt, 26% (**14a**) and 22% (**14b**) (2 steps).

続いて、二環性ペプチド **14** の Ser 残基を Dha に変換するための反応条件の検討を行った（Table 1）。まず、MsCl により **14a** のヒドロキシ基を活性化し、引き続き塩基性条件下での脱離反応を試みたところ、Dha 体 **15a** は得られず、オキサゾール環形成を経由して得られたと思われる原料と同一分子量の生成物（原料の異性体）と塩化物イオンの求核攻撃により生成したクロロ体が得られた（entry 1）。活性化剤として TsCl や Ms_2O を利用した際にも同様に、原料の異性体と思われる生成物が得られた（entries 2 および 3）。また、基質 **14b** を Ms_2O で活性化し、脱離反応に用いる塩基として DBU を添加したところ、微量の Dha 体 **15b** が検出されたが、原料の異性体が主生成物として得られた（entry 4）。また、Cu(I) 塩存在下 EDCI で処理する条件[5]を検討したところ、Dha 体 **15b** が検出されたものの、イソウレアの脱離の進行が遅く、また、原料の異性体の生成も確認された（entry 5）。

Table 1. Investigation on Dehydroalanine Formation by β-Elimination of the Ser Side Chain.



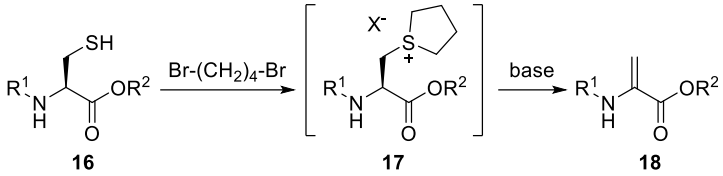
entry	substrate	R	conditions	yield of 15	byproducts
1	**14a**	H	MsCl, Et_3N	N.D.[a]	isomer[b], chloride[c]
2	**14a**	H	TsCl, Et_3N, DMAP	N.D.[a]	isomer[b]
3	**14a**	H	Ms_2O, Et_3N	N.D.[a]	isomer[b]
4	**14b**	Me	Ms_2O, Et_3N, DBU	trace	isomer[b]
5	**14b**	Me	EDCI·HCl, CuCl, Et_3N	trace	isomer[b]

[a] Not detected. [b] Unidentified product with the identical molecular weight mass of the substrate. [c] Unidentified product.

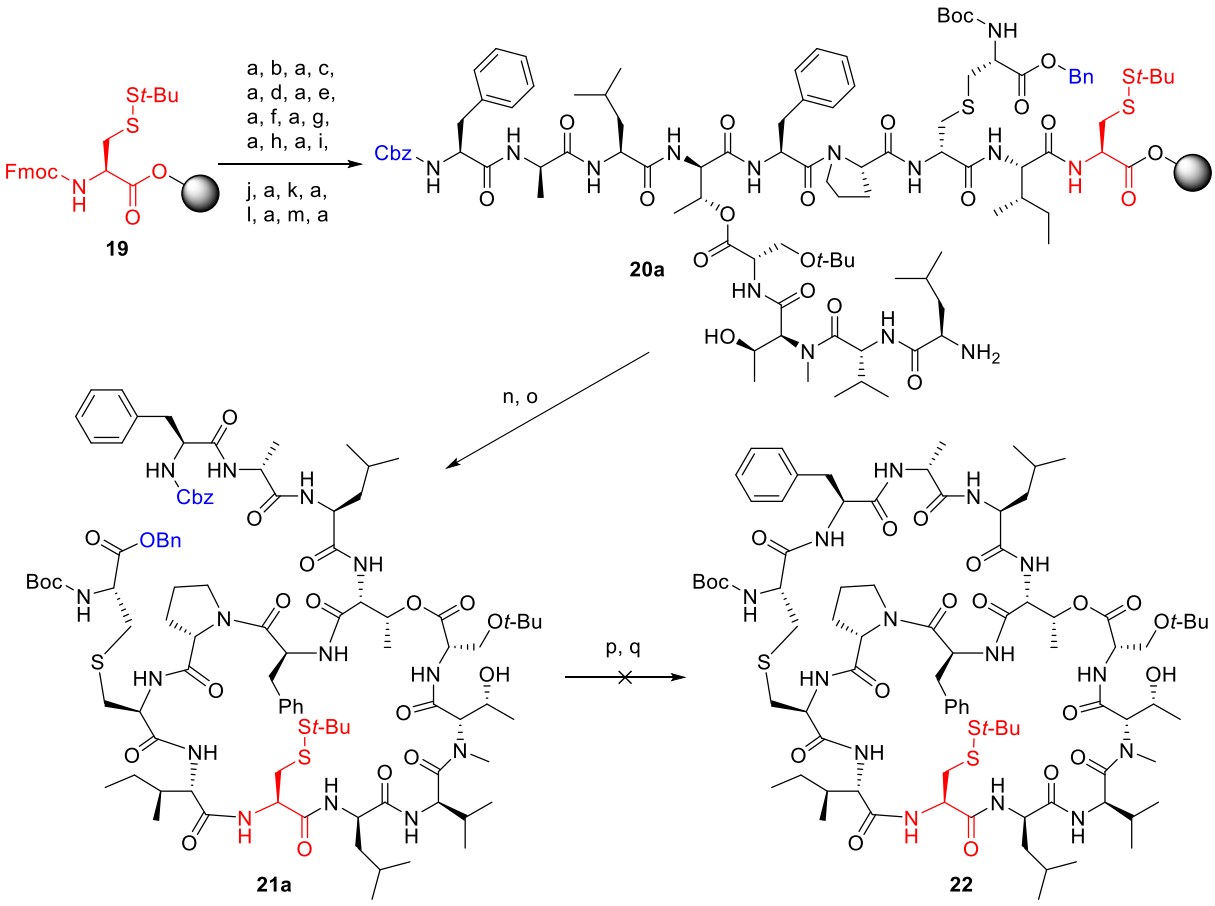
上述のように、Ser 残基を Dha へ変換するプロセスが進行しなかったことから、Dha 前駆体を変更した条件の検討を行った。Chalker らは、Cys 誘導体 **16** の側鎖メルカプト基を塩基性条件下で 1,4-ジブロモブタンによるビスアルキル化の条件に付すと、スルホニウム塩型の脱離基 **17** の形成を経て、β 脱離により Dha 誘導体 **18** へと変換される反応を報告している（Scheme 3）[6]。本反応を vitilevuamide の Dha 部分構造の構築に適用すべく、Cys 誘導体を

含む二環性骨格の構築を検討した。

Fmoc-Cys(S*t*-Bu)-OH を担持したクロロトリチル樹脂 **19** を出発原料として、Scheme 2 と同様のプロセスによる合成を試みた（Scheme 4）。ペプチド鎖の伸長により保護ペプチド樹脂



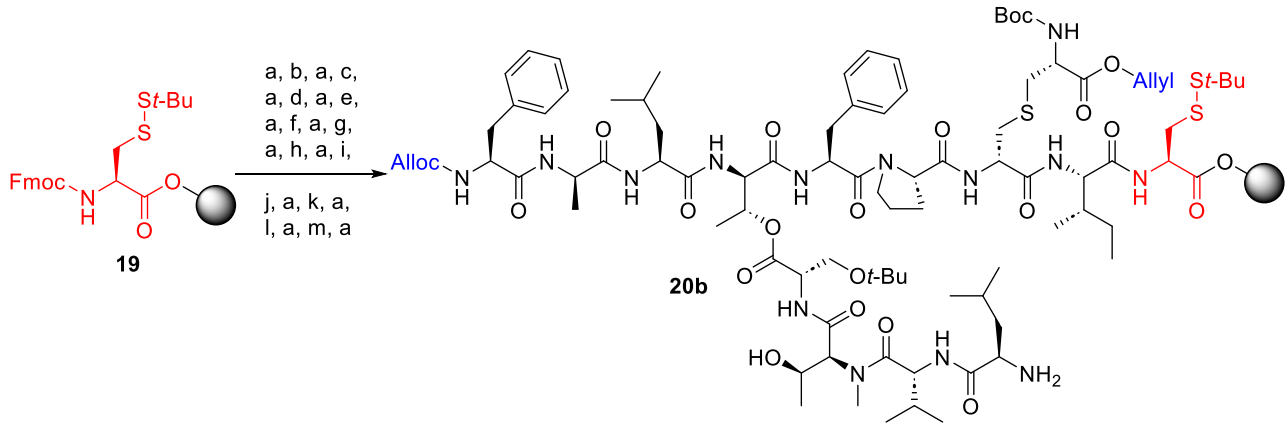
Scheme 3. Dehydroalanine Formation from Cys-type Precursor.



Scheme 4. Synthetic Study of a Vitilevuamide Model Peptide Having a Cys(S*t*-Bu) at the Dha[10] Position Using Cbz/Bn Ester Protective Groups. *Reagents and conditions*: (a) 20% piperidine/DMF, rt; (b) Fmoc-Ile-OH, DIC, HOBt·H_2O, DMF, rt; (c) Fmoc-D-Lan(Boc-Lan-OBn)-OH, DIC, HOBt·H_2O, DMF, rt; (d) Fmoc-Pro-OH, DIC, HOBt·H_2O, DMF, rt; (e) Fmoc-Phe-OH, DIC, HOAt, DMF, 40 °C; (f) Fmoc-D-*allo*-Thr-OH, DIC, HOBt·H_2O, DMF, rt; (g) Fmoc-Leu-OH, DIC, HOBt·H_2O, DMF, rt; (h) Fmoc-D-Ala-OH, DIC, HOBt·H_2O, DMF, rt; (i) Cbz-Phe-OH, DIC, HOBt·H_2O, DMF, rt; (j) Fmoc-Ser(*t*-Bu)-OH, DIC, DMAP, CH_2Cl_2, DMF, rt; (k) Fmoc-MeThr-OH, DIC, HOBt·H_2O, DMF, rt; (l) Fmoc-D-Val-OH, DIC, HOAt, DMF, 40 °C; (m) Fmoc-D-Leu-OH, DIC, HOBt·H_2O, DMF, rt; (n) 30% HFIP/CH_2Cl_2, rt; (o) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt, 7% from resin; (p) 10% Pd/C, H_2, *i*-PrOH, rt; (q) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt.

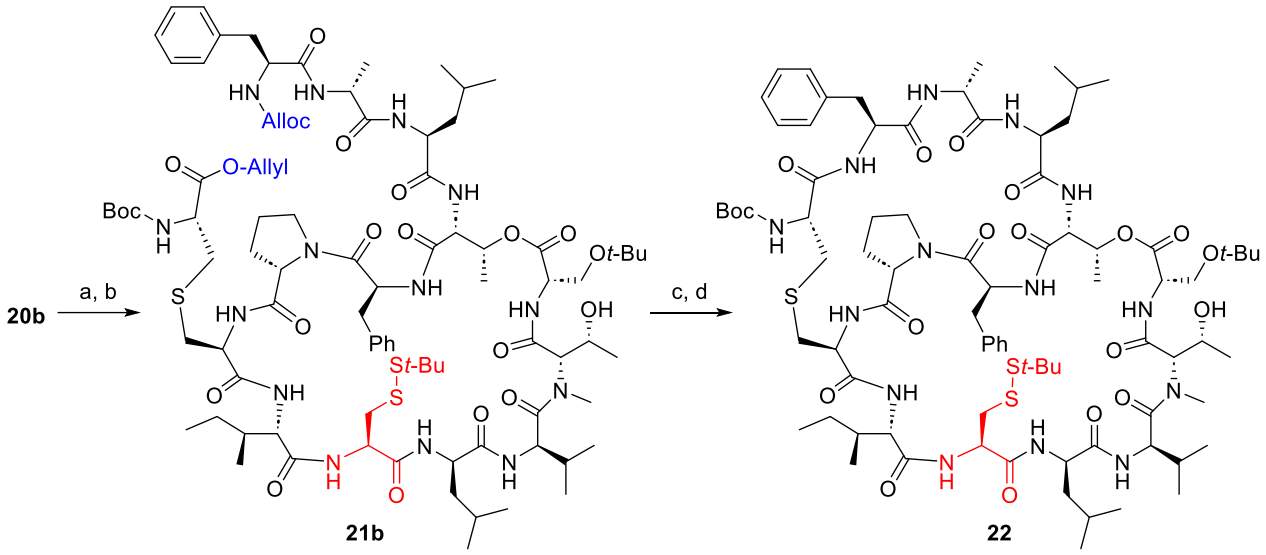
20a を得た後、樹脂からの切り出しと B 環の環化反応により、保護ペプチド **21a** を得ることができた。しかしながら、ペプチド **21a** の接触還元を用いた脱保護を行ったところ、系中が複雑化し、その後 A 環を構築するための環化反応に付しても目的の二環性ペプチド **22** を得ることはできなかった。システインの保護基が接触還元の条件には適合しなかったことが考えられる。

接触還元による脱保護が適切に機能しなかったことから、A 環部分のベンジル系保護基をアリル系保護基に変えて検討を行った。まず、Scheme 4 と同様の経路により、Fmoc-D-Lan-(Boc-Lan-O-Allyl)-OH 及びおよび Alloc-Phe-OH を用いてペプチド鎖の伸長を行い、保護ペプチド樹脂 **20b** を合成した（Scheme 5）。ここで、用いた Lan 誘導体の合成は第四節で説明する。



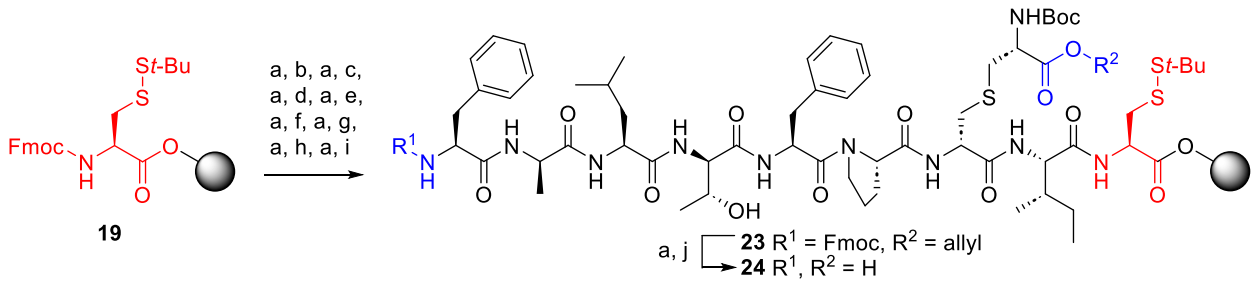
Scheme 5. Synthesis of a Vitilevuamide Model Peptide Precursor Having a Cys(S*t*-Bu) at the Dha[10] Position Using Alloc/Allyl Ester Protective Groups. *Reagents and conditions*: (a) 20% piperidine/DMF, rt; (b) Fmoc-Ile-OH, HOBt·DIC, H_2O, DMF, rt; (c) Fmoc-D-Lan(Boc-Lan-O-Allyl)-OH, DIC, HOBt·H_2O, DMF, rt; (d) Fmoc-Pro-OH, HOBt·DIC, H_2O, DMF, rt; (e) Fmoc-Phe-OH, DIC, HOAt, DMF, 40 °C; (f) Fmoc-D-*allo*-Thr-OH, DIC, HOBt·H_2O, DMF, rt; (g) Fmoc-Leu-OH, DIC, HOBt·H_2O, DMF, rt; (h) Fmoc-D-Ala-OH, DIC, HOBt·H_2O, DMF, rt; (i) Alloc-Phe-OH, DIC, HOBt·H_2O, DMF, rt; (j) Fmoc-Ser(*t*-Bu)-OH, DIC, DMAP, CH_2Cl_2, DMF, rt; (k) Fmoc-MeThr-OH, DIC, HOBt·H_2O, DMF, rt; (l) Fmoc-D-Val-OH, DIC, HOAt, DMF, 40 °C; (m) Fmoc-D-Leu-OH, DIC, HOBt·H_2O, DMF, rt.

引き続き、樹脂からの切り出しの後、B 環の環化反応を経て保護ペプチド **21b** を得た。得られたペプチド **21b** に $Pd(PPh_3)_4/PhSiH_3$ を作用させたところ、Alloc 基およびアリルエステルの脱保護がスムーズに進行したことを確認した（Scheme 6）。続いて、この脱保護体を希釈条件下での環化反応に付すことで、二環性ペプチド **22** を得ることができた。この合成経路では、脱保護した工程で精製操作を含むことや、切り出しから環化までの収率が不十分であることなど、複数の課題があった。



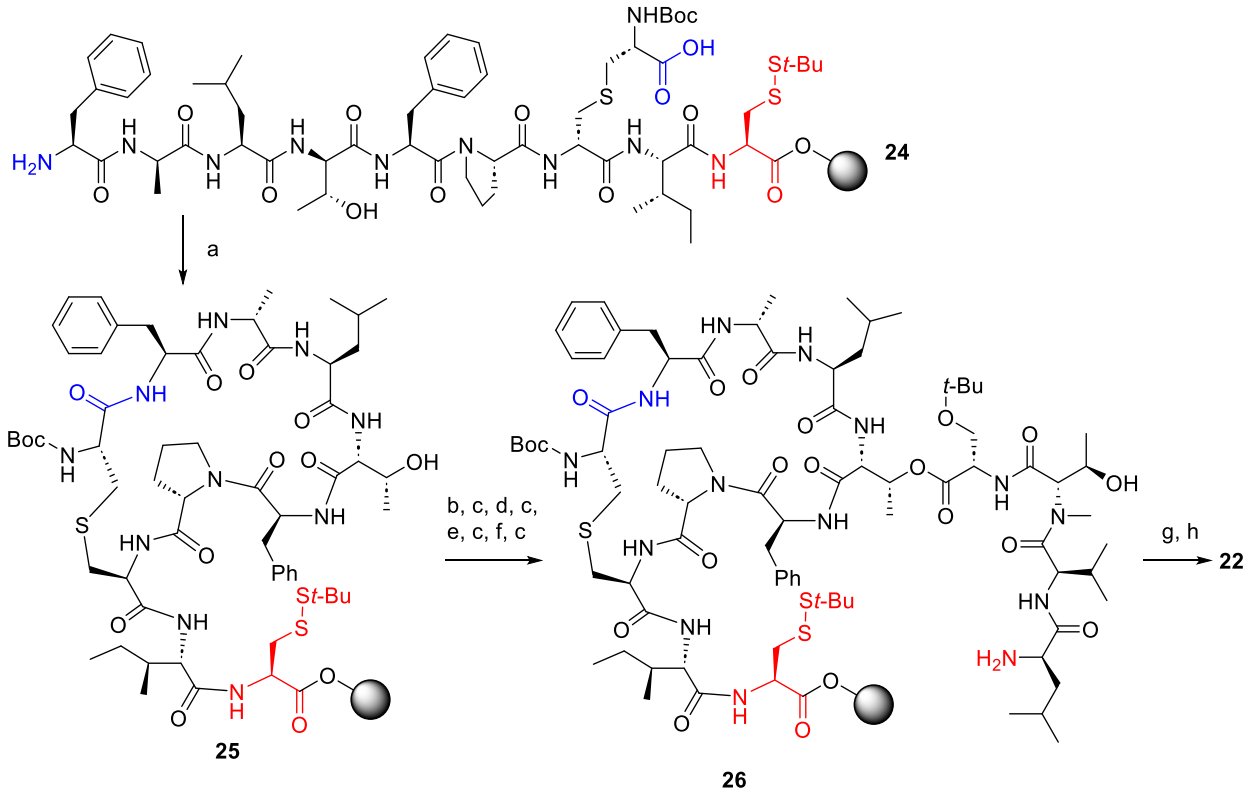
Scheme 6. Synthesis of a Vitilevuamide Model Peptide Having a Cys(S*t*-Bu) at the Dha[10] Position Using Alloc/Allyl Ester Protective Groups. *Reagents and conditions*: (a) 30% HFIP/CH_2Cl_2, rt; (b) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt, 13% from resin **19**; (c) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt; (d) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt, 25% (2 steps).

そこで、二環性ペプチド **22** について、固相樹脂上で A 環を構築する環化反応を先に行った後、B 環を構築する経路②（Scheme 1）の合成プロセスを検討した。まず、Fmoc-Cys(S*t*-Bu)-OH を担持したクロロトリチル樹脂 **19** を出発原料として、Phe[2] から Cys(S*t*-Bu)[10] までのペプチド配列 **23** を構築し、Fmoc 基およびアリルエステルを脱保護することで保護ペプチド **24** を得た（Scheme 7）。この環化前駆体 **24** に対して固相樹脂上で DIC/HOAt を作用させたところ、効率よく環化反応が進行することが観察された（Scheme 8）。引き続き、A 環か



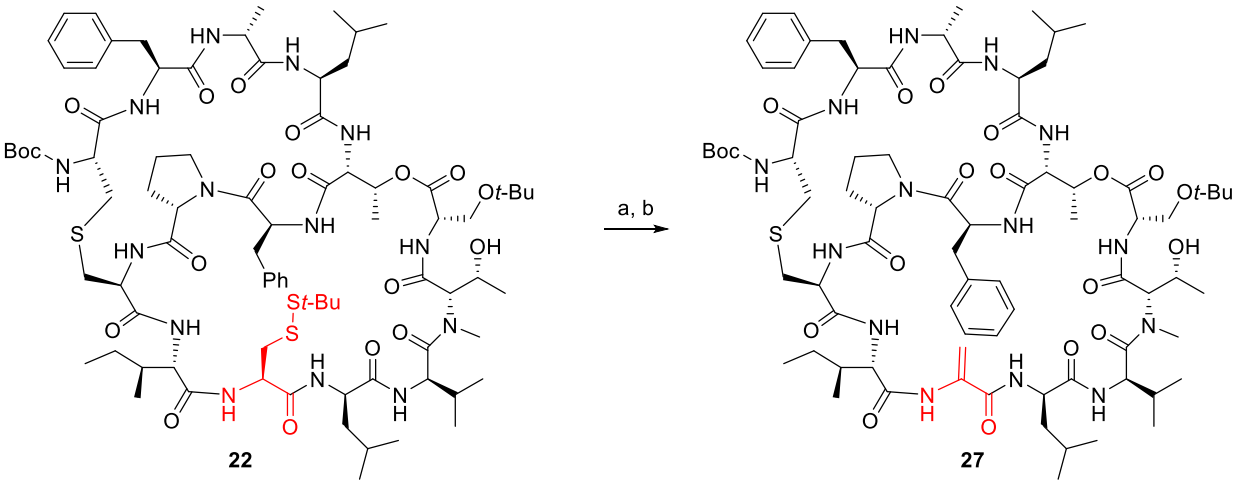
Scheme 7. Synthesis of a Vitilevuamide Model Peptide Precursor for an Alternative Synthetic Route. *Reagents and conditions*: (a) 20% piperidine/DMF, rt; (b) Fmoc-Ile-OH, DIC, HOBt·H_2O, DMF, rt; (c) Fmoc-D-Lan(Boc-Lan-O-Allyl)-OH, DIC, HOBt·H_2O, DMF, rt; (d) Fmoc-Pro-OH, DIC, HOBt·H_2O, DMF, rt; (e) Fmoc-Phe-OH, DIC, HOAt, DMF, 40 °C; (f) Fmoc-D-*allo*-Thr-OH, DIC, HOBt·H_2O, DMF, rt; (g) Fmoc-Leu-OH, DIC, HOBt·H_2O, DMF, rt; (h) Fmoc-D-Ala-OH, DIC, HOBt·H_2O, DMF, rt; (i) Fmoc-Phe-OH, DIC, HOBt·H_2O, DMF, rt; (j) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt.

らなる環状ペプチド **25** の D-*allo*-Thr[5] 側鎖のヒドロキシ基上にペプチド鎖を伸長し、D-Leu[11] までの配列 **26** を構築した。最後に、固相樹脂からの切り出し、および、希釈条件下での環化反応に付すことで B 環を構築し、二環性ペプチド **22** を合成した。本合成経路では、Lan[1] のアリルエステルの脱保護と環化反応を固相樹脂上で行うことで、中間体の精製操作を省略できるとともに、ペプチド **22** の収率が向上した。



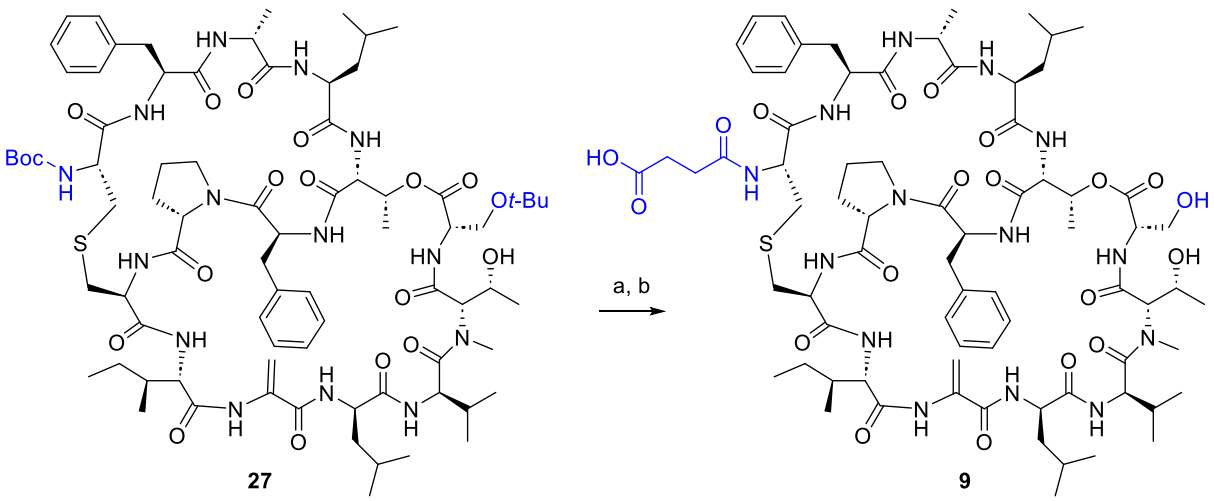
Scheme 8. Alternative Synthetic Route of a Vitilevuamide Model Peptide Having a Cys(S*t*-Bu) at the Dha[10] Position. *Reagents and conditions*: (a) DIC, HOAt, DMF, rt; (b) Fmoc-Ser(*t*-Bu)-OH, DMAP, DIC, CH_2Cl_2, DMF, rt; (c) 20% piperidine/DMF, rt; (d) Fmoc-MeThr-OH, HOBt·H_2O, DIC, DMF, rt; (e) Fmoc-D-Val-OH, HOAt, DIC, DMF, 40 °C; (f) Fmoc-D-Leu-OH, HOBt·H_2O, DIC, DMF, rt; (g) 30% HFIP/CH_2Cl_2, rt; (h) EDCI·HCl, HOAt, DIEA, DMF, 0 °C to rt, 6% from resin **19**.

続いて、Chalker らが報告したビスアルキル化脱離反応[6]を参考にして、二環性ペプチド **22** の Cys 残基から Dha への変換について検討した（Scheme 9）。まず、Cys 上の *tert*-ブチルチオ基を DTT により除去した後、塩基性条件下で 1,4-ジブロモブタンを作用させたところ、望みの脱離反応が進行し、Dha 体 **27** が得られた。この Cys を介して Dha を構築するプロセスでは、Ser を前駆体とした際に得られた異性体の生成は全く観察されなかった。詳細な理由は不明ではあるが、スルホニウム塩の適度な求電子性により置換反応などの副反応が起こりにくく、期待した脱離反応が優先的に進んだと考えられる。



Scheme 9. Dehydroalanine Formation via β-Elimination of the Cys Side Chain. *Reagents and conditions*: (a) DTT, DIEA, DMF, rt; (b) 1,4-dibromobutane, Et_3N, DMF, rt, 62% (2 steps).

最後に、二環性ペプチド **27** からモデルペプチド **9** への変換を検討した（Scheme 10）。まず、ペプチド **27** の Lan[1] の Boc 基と Ser[14] の *t*-Bu 基を TFA 処理により脱保護した。続いて、$NaHCO_3$ 存在下無水コハク酸を作用させることでコハク酸を導入し、vitilevuamide のモデル化合物 **9** を得ることができた。以上のモデルペプチドを用いた検討により、vitilevuamide の二環性骨格とこれに含まれる Dha の構築プロセスを確立することができた。



Scheme 10. Synthesis of Vitilevuamide Model Peptide. *Reagents and conditions*: (a) 95% TFA aq.; (b) succinic anhydride, $NaHCO_3$, MeCN, H_2O rt, 62% (2 steps).

第四節　非天然型側鎖を有する保護アミノ酸の合成

モデル化合物の合成検討により、vitilevuamide (**1**) の合成に必要となる非天然型側鎖を有するアミノ酸 **5**–**8** の構造が確定した（Figure 4）。Nmhm (**5**) は、3 位の立体配置が確定した 2 種類の異性体を調製する必要がある。モデルペプチドの合成に用いた MeThr のヒドロキシ基には保護基を必要としなかったことから、3*R* 体 **5a** と 3*S* 体 **5b** を設計した。2 つの Hil 誘導体は、4 位の立体配置が共通であるものの、α 位の立体配置が異なる 2 つの異性体（L-Hil **6** および D-*allo*-Hil **7**）として合成する必要がある。Lan 誘導体は、Boc/アリルエステル保護体 **8** として合成した。

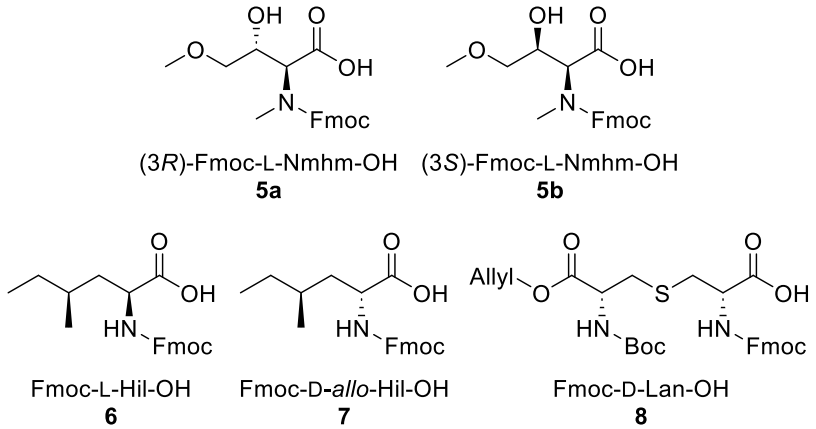


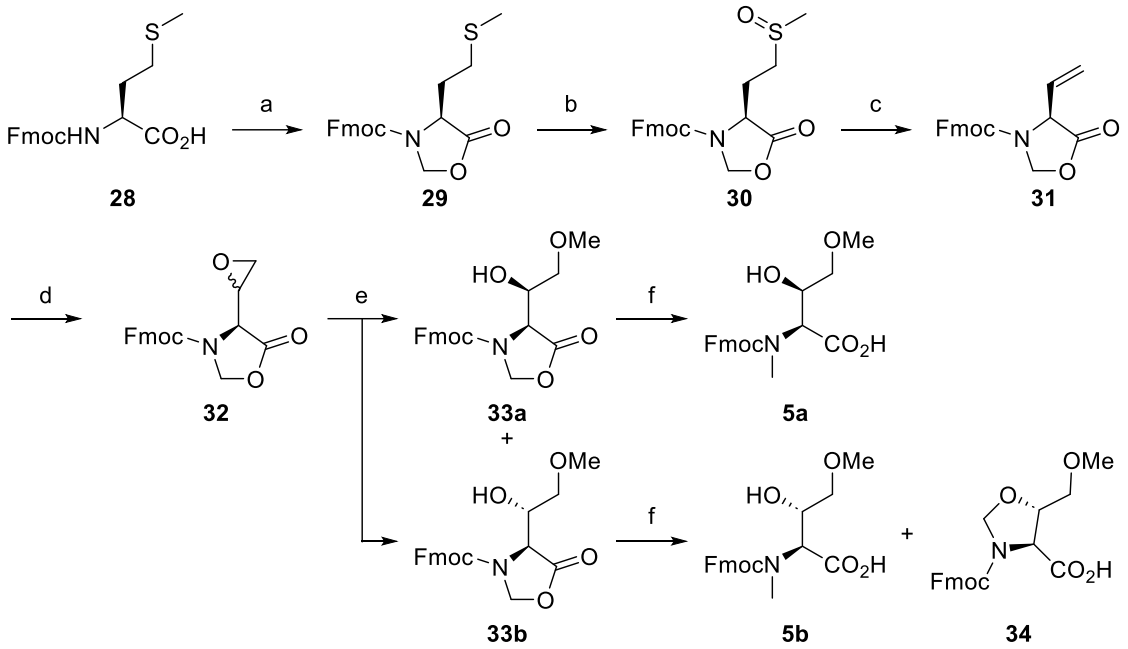
Figure 4. Structures of Protected Nonproteinogenic Amino Acids for the Synthesis of Vitilevuamide.

Fmoc-L-Nmhm-OH の合成

Fmoc-Met-OH を出発原料として合成を行った（Scheme 11）。酸性条件下パラホルムアルデヒドを用いたアセタール形成反応 [7] によりオキサゾリジン **29** とした後、チオエーテルを酸化してスルホキシド **30** とした基質を高温条件下で脱離させることで、末端アルケン **31** を得た [8]。引き続き、mCPBA を用いたエポキシ化反応によりエポキシ体 **32** をジアステレオマー混合物として得た後、過塩素酸存在下メタノール処理によりメトキシ基を導入した [9]。アルコール **33a,b** のジアステレオマー混合物をクロマトグラフィーにより分離し、**33a** の X 線結晶構造解析により、**33a**（3*R* 体）および **33b**（3*S* 体）の立体化学を決定した。最後に、酸性条件下でシラン還元を行い、(3*R*)-Fmoc-L-Nmhm-OH (**5a**) および(3*S*)-Fmoc-L-Nmhm-OH (**5b**) を得た。

Fmoc-L-Hil-OH および Fmoc-D-*allo*-Hil-OH の合成

Hil 誘導体は Schöllkopf 試薬を用いて合成した（Scheme 12）[10]。*R* 体もしくは *S* 体の Schöllkopf 試薬 **35** を強塩基性条件下で (*S*)-1-iode-2-methylbutane と反応させてアルキル化し、**36a** もし

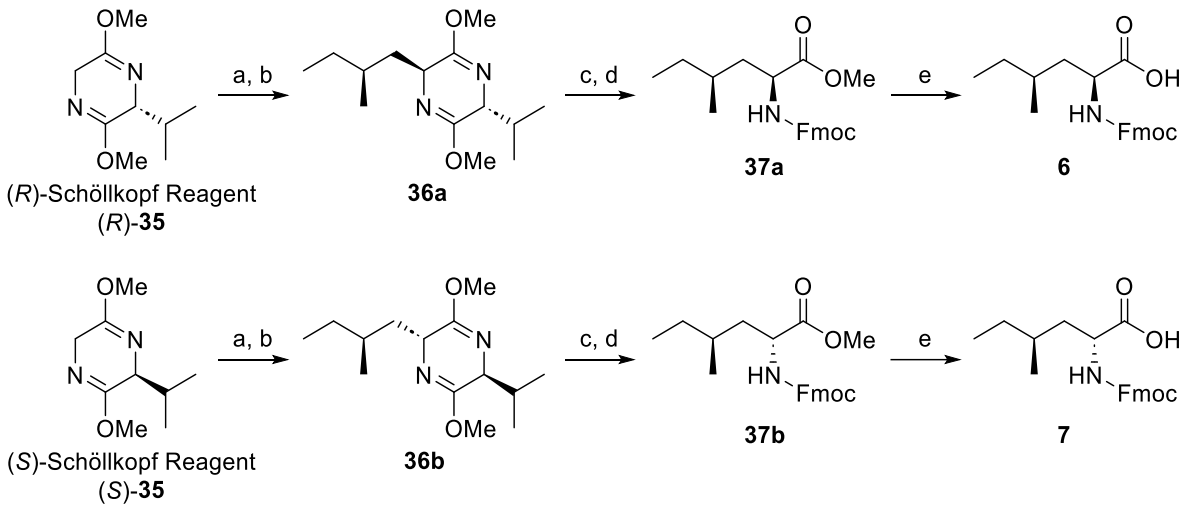


Scheme 11. Synthesis of Fmoc-L-Nmhm-OH. *Reagents and conditions*: (a) $(HCHO)_n$, $TsOH·H_2O$, toluene reflux; (b) $NaIO_4$, THF, MeOH, H_2O, 0 °C to rt; (c) xylene, reflux, 48% (3 steps); (d) *m*-CPBA, CH_2Cl_2, 0 °C to rt, 68%; (e) $HClO_4$, MeOH, rt; then, separation, 16% (**33a**) and 20% (**33b**); (f) TFA, Et_3SiH, CH_2Cl_2, rt, 59% (**5a**), 37% (**5b**) and 29% (**34**).

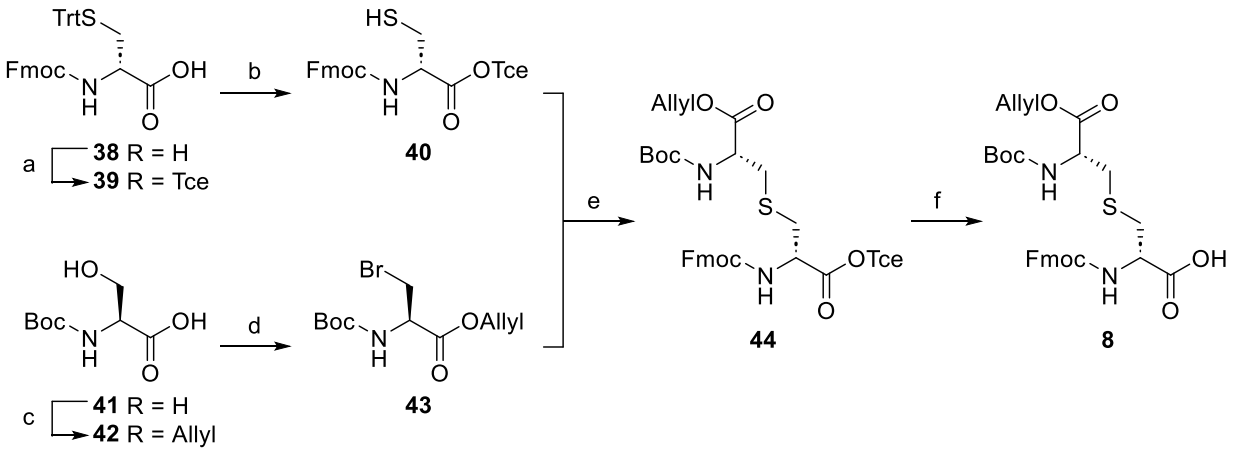
くは **36b** を立体選択的に得た。酸性条件での加水分解および Fmoc 保護を経て、メチルエステル **37a,b** を Schöllkopf 試薬由来のバリン誘導体との混合物として得た。続いて、メチルエステルを加水分解し、HPLC 精製を経て Fmoc-L-Hil-OH (**6**) および Fmoc-D-*allo*-Hil-OH (**7**) をそれぞれ合成した。

Fmoc-D-Lan(Boc-L-Lan-O-Allyl)-OH の合成

Zhu らによる相関移動触媒を用いた保護ランチオニン合成を参考に合成した（Scheme 13）[11]。まず、Fmoc-D-Cys(Trt)-OH (**38**) から、保護基の変換を経て Fmoc-D-Cys-OTce (**40**) を合成した。一方、Boc-Ser-OH (**41**) のアリルエステル保護とブロモ化により、β-ブロモアラニン誘導体 **43** を得た。続いて、TBAB 存在下で酢酸エチルおよび pH 8.5 の炭酸水素ナトリウム水溶液の二層系でシステイン誘導体 **40** とブロモアラニン誘導体 **43** を反応させて、ランチオニン **44** を得た。この際、**43** 由来のデヒドロアラニンに対する **40** の付加反応により得られたと考えられるジアステレオマーが 10%程度生成していることが認められた。最後に、**44** のトリクロロエチルエステルを還元処理により脱保護して、目的の Fmoc-D-Lan(Boc-L-Lan-O-Allyl)-OH (**8**) を合成した。



Scheme 12. Synthesis of Fmoc-L-Hil-OH and Fmoc-D-*allo*-Hil-OH. *Reagents and conditions*: (a) *n*-BuLi, THF, –78 °C; (b) (*S*)-1-iodo-2-methylbutane, –78 °C, 54% (**36a**) and 59% (**36b**); (c) 1 M HCl aq., THF, rt; (d) Fmoc-OSu, CH_2Cl_2, rt; (e) HCl, 1,4-dioxane, H_2O, reflux, 34% (**6**) and 45% (**7**) (3 steps).



Scheme 13. Synthesis of Fmoc-D-Lan(Boc-L-Lan-O-Allyl)-OH. *Reagents and conditions*: (a) 2,2,2-trichloroethanol, EDCI·HCl, DMAP, CH_2Cl_2, –15 °C, 95%; (b) TFA, TIS, CH_2Cl_2, H_2O, rt; (c) Allyl-Br, K_2CO_3, DMF, rt, 88%; (d) CBr_4, Ph_3P, CH_2Cl_2, 0 °C to rt, 79%; (e) TBAB, $NaHCO_3$, EtOAc, H_2O, rt, 51% from **39** (2 steps); (f) zinc dust, NH_4OAc, THF-H_2O, rt, 72%.

第五節　小括

著者は、構造最適化研究や標的分子探索のためのプローブの創製に向けた vitilevuamide (**1**) の化学合成法の確立を目指して、vitilevuamide の化学構造中で立体配置が明らかとされていない Nmhm のヒドロキシ基に関する 2 つの立体異性体の合成研究を行った。モデル化合物を用いた検討により、vitilevuamide の 2 つの大環状構造を構築するプロセスとこれに用いる保護基、並びに、Dha 前駆体となるアミノ酸とその構築プロセスを確定した。また、vitilevuamide の合成に必要となる非天然型側鎖を有する保護アミノ酸の合成法を確立した。本研究で確立したモデル化合物の合成経路と非天然型側鎖を有する保護アミノ酸合成法を用いることで、vitilevuamide の全合成による構造決定が実現するとともに、優れた生物活性プロファイルを有する誘導体の創製、並びに、標的分子の探索や相互作用解析が進展することが期待される。

Experimental section

Synthetic general method

^{1}H and ^{13}C NMR spectra were recorded using a Bruker AV300, BrukerAV500 or JEOL ECZ600R spectrometer. Chemical shifts are reported in δ (ppm), relative to Me_4Si (in $CDCl_3$ and DMSO) or the residual solvent peak in CD_3CN as an internal standard for ^{1}H, and referenced to the residual solvent signal for ^{13}C. Exact mass (HRMS) data were recorded on a Shimadzu JEOL SX-102A (FAB), JEOL GC mate II (EI) LC-ESI-IT-TOF-MS equipment. Optical rotations were measured using a JASCO P-1020 polarimeter. For flash chromatography, Wakogel C-300E (Wako) or Wakogel C-200E (Wako) was employed. For analytical HPLC, a Cosmosil 5C18-ARII column (4.6 × 250 mm, Nacalai Tesque, Inc.) was employed with a linear gradient of CH_3CN (with 0.05% (v/v) TFA) in H_2O, and eluting products were detected by UV at 220 nm. Preparative HPLC was performed using a Cosmosil 5C18-ARII preparative column (20 × 250 mm, Nacalai Tesque, Inc.).

General procedure for solid-phase peptide synthesis.

Loading of an amino acid on the solid support. A solution of Fmoc amino acid (0.82 mmol) and DIEA (572 μL, 3.28 mmol) in dry CH_2Cl_2 (10.0 mL), were reacted with (2-Cl)Trt chloride resin (1.0 g, 1.37 mmol). The reaction was continued for 2 h at room temperature.

Deprotection of Fmoc group. The Fmoc-protected peptidyl resin was treated with 20% piperidine/DMF for 20 min.

Coupling reaction using DIC/HOBt. DIC (46 μL, 0.30 mmol) was added to a solution of Fmoc amino acid (0.30 mmol) and HOBt·H_2O (46 mg, 0.30 mmol) in DMF. The whole was poured into the peptidyl resin (0.100 mmol), and the reaction was continued for 1.5 h at room temperature.

Coupling reaction using DIC/HOAt. DIC (46 μL, 0.30 mmol) was added to a solution of Fmoc amino acid (0.30 mmol) and HOAt (40 mg, 0.30 mmol) in DMF. The whole was poured into the peptidyl resin (0.100 mmol), and the reaction was continued for 1.5 h at 40 °C.

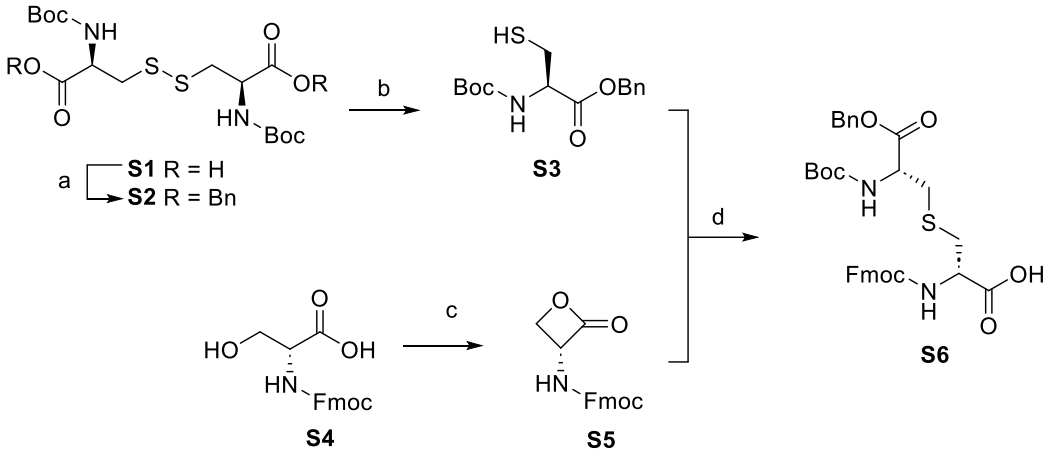
Coupling reaction of esterification. DMAP (18 mg, 0.15 mmol) was added to a solution of Fmoc amino acid (0.50 mmol) in $CHCl_3$/DMF (9/1). The whole was poured into the peptidyl resin (0.100 mmol). To a suspension of the peptidyl resin was added DIC (78 μL, 0.50 mmol), and the reaction was continued for 18 h at room temperature.

Deprotection of Allyl group. To the peptidyl resin (0.100 mmol) were added $PhSiH_3$ (247 μL, 2.00 mmol) and $Pd(PPh_3)_4$ (23 mg, 0.020 mmol) in dry CH_2Cl_2, and the reaction was continued for 10 min.

Cyclisation on solid support. DIC (46 μL, 0.30 mmol) was added to a solution of HOAt (40 mg, 0.30 mmol) in DMF. The whole was poured into the peptidyl resin (0.100 mmol), and the reaction was continued for 1.5 h at room temperature.

Cleavage from the resin. The peptidyl resin was treated with 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP)/CH_2Cl_2 (3:7) for 2 h at room temperature. After filtration of the residual resin, the filtrate was concentrated under reduced pressure to give a crude peptide, which was used for the next step without further purification.

Preparation of Fmoc-D-Lan(Boc-Lan-OBn)-OH.



Scheme S1. Synthesis of Fmoc-D-Lan(Boc-L-Lan-O-Bn)-OH. *Reagents and conditions*: (a) BnBr, K_2CO_3, DMF, rt, 78%; (b) *n*-Bu_3P, H_2O, THF, rt; (c) Ph_3P, DMEAD, THF, –60 °C to rt, 44%; (d) $CsHCO_3$, DMF, rt, 28% from **S5**.

(Boc-Cys-OBn)$_2$ (S2). To a stirred solution of (Boc-Cys)$_2$ **S1** (2.1 g, 4.7 mmol) in DMF (24 mL) were added K_2CO_3 (1.6 g, 11.8 mmol) and BnBr (1.3 mL, 10.3 mmol) at room temperature. After being stirred for 12 h, to this solution was added water. The whole was extract with EtOAc and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 4/1 to 1/1) to give compound **S2** (2.3 g, 78%) as a white solid. The spectral data were in good agreement with those previously reported[12].

(9*H*-Fluoren-9-yl)methyl (*R*)-(2-oxooxetan-3-yl)carbamate (S5). To a stirred solution of Ph_3P (2.4 g, 9.16 mmol) in dry THF (30 mL) was added DMEAD (2.2 g, 9.16 mmol) in dry THF (30 mL) at –60 °C. After being stirred for 30 min, to this solution was added Fmoc-D-Ser-OH (**S4**) in dry THF (30 mL) at –60 °C. The reaction mixture was warmed to room temperature and stirred for 12 h. After water was added to this solution, the whole was extract with EtOAc and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 2/1) to give compound **S5** (1.2 g, 44%) as a white solid. The spectral data were in good agreement with those previously reported[13].

Fmoc-D-Lan(Boc-Lan-OBn)-OH (S6). To a stirred solution of **S2** (502 mg, 0.81 mmol) in dry THF (10 mL) was added Bu_3P (275 μL, 1.2 mmol) at room temperature. After being stirred for 30 min, H_2O (100 μL) was added to this solution at room temperature. After being stirred for 1 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash

chromatography over silica gel (hexane/EtOAc = 5/1) to give thiol **S3** as a colorless oil. Thiol **S3** was dissolved in dry DMF. To this solution was added $CsHCO_3$ (570 mg, 1.47 mmol) at rt. After being stirred for 30 min, **S5** (445 mg, 1.5 mmol) was added to this mixture at room temperature. After being stirred for 12 h, water was added to this solution. The whole was extract with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel ($CHCl_3$/MeOH = 50/1 to 30/1) to give compound **S6** (254 mg, 28%) as a colorless oil: $[\alpha]^{21}_D$ –22.8 (*c* 1.09, $CHCl_3$); IR (neat): 1716 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotamers) δ: 1.36 (s, 5H), 1.43 (s, 4H), 2.65 (t, *J* = 15.4 Hz, 1H), 2.73-3.13(m, 2.5H), 3.26 (d, *J* = 14.5 Hz, 0.5H), 4.09 (br, 0.5H), 4.20 (br, 1H), 4.26-4.44 (m, 1.5H), 4.44-4.77 (m, 2H), 5.06-5.29 (m, 2H), 5.49 (d, *J* = 7.2 Hz, 0.5H), 6.03 (d, *J* = 7.1 Hz, 0.5H), 7.23-7.42 (m, 8H), 7.46 (d, *J* = 6.9 Hz, 0.5H), 7.53 (t, *J* = 8.2 Hz, 1.5H), 7.61 (t, *J* = 9.0 Hz, 1H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.75 (d, *J* = 6.9 Hz, 1H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, mixture of rotamers) δ: 28.1, 28.3, 34.7, 35.1, 35.6, 47.1, 47.3, 53.8, 54.2, 66.1, 67.3, 67.6, 76.6, 80.8, 82.4, 120.0, 124.8, 125.2, 127.1, 127.6, 127.7, 128.6, 135.0, 141.3, 143.7, 155.5, 155.8, 156.0, 157.4, 169.9, 170.7, 173.1, 173.9; HRMS (ESI-TOF) calcd for $C_{33}H_{36}N_2NaO_8S$ $[M+Na]^+$:643.2085; found:643.2090.

Compound 13a. The linear peptide was constructed by solid-phase peptide synthesis on peptidyl resin **10** (0.340 mmol/g, 147 mg, 0.050 mmol). After the cleavage from the resin **12a** as described above, EDCI·HCl (96 mg, 0.50 mmol) was added to a solution of linear peptide, HOAt (68 mg, 0.50 mmol), and DIEA (348 μL, 2.00 mmol) in dry DMF (50 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and the stirring was continued for 18 h. The reaction mixture was concentrated and the residue was purified by RP-HPLC to give compound **13a** (16.4 mg, 17% from resin) as a white powder: LRMS (ESI) *m/z* 1905 $[M+H]^+$.

Compound 13b. According to the procedure described for the preparation of **13a**, peptidyl resin **10** (0.340 mmol/g, 147 mg, 0.05 mmol) was converted into **13b** (23.0 mg, 24% from resin) as a white powder: LRMS (ESI) *m/z* 1918 $[M+H]^+$.

Compound 14a. To a stirred solution of **13a** (11 mg, 5.78 μmmol) in *i*-PrOH (1 mL) were added 10% Pd/C (20 mg) and AcOH (10 μL) at room temperature and the mixture was flushed with H_2 gas (1 atm). After being stirred for 12 h, the reaction mixture was filtered through membrane filter. The filtrate was concentrated under reduced pressure to give the corresponding peptide, which was used without further purification. To a stirred solution of above peptide, HOAt (17 mg, 0.13 mmol), and DIEA (87 μL, 0.5 mmol) in dry DMF (13 mL) was added EDCI·HCl (24 mg, 0.13 mmol) at 0 °C. The reaction mixture was allowed to warm up to room temperature and the stirring was continued for 18 h. The reaction mixture was concentrated, and the residue was purified by RP-HPLC to give compound **14a** (2.5 mg, 26%) as a white powder: LRMS (ESI) *m/z* 1662 $[M+H]^+$.

Compound 14b. According to the procedure described for the preparation of **14a**, **13b** (11 mg, 5.78 μmmol) was converted into **14b** (4.4 mg, 22%) as a white powder: LRMS (ESI) *m/z* 1676 $[M+H]^+$.

Compound 21a. The linear peptide was constructed by solid-phase peptide synthesis on peptidyl resin **19** (0.441 mmol/g, 113 mg, 0.050 mmol). After the cleavage from the resin **20a** as described above, EDCI·HCl (96 mg, 0.50 mmol) was added to a solution of linear peptide, HOAt (68 mg, 0.50 mmol), and DIEA (348 μL, 2.00 mmol) in dry DMF (50 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and the stirring was continued for 18 h. The reaction mixture was concentrated, and the residue was purified by RP-HPLC to give compound **21a** (6.5 mg, 6.6% from resin) as a white powder: LRMS (ESI) *m/z* 1966 $[M+H]^+$.

Compound 21b. The linear peptide was constructed by solid-phase peptide synthesis on peptidyl resin **19** (0.441 mmol/g, 113 mg, 0.050 mmol). After the cleavage from the resin **20b** as described above, EDCI·HCl (96 mg, 0.50 mmol) was added to a solution of linear peptide, HOAt (68 mg, 0.50 mmol), and DIEA (348 μL, 2.00 mmol) in dry DMF (50 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and the stirring was continued for 18 h. The reaction mixture was concentrated, and the residue was purified by RP-HPLC to give compound **21b** (12 mg, 13% from resin) as a white powder: HRMS (ESI-TOF) calcd for $C_{90}H_{140}N_{14}Na_2O_{22}S_3$ $[M+2Na]^{2+}$: 955.4607; found: 955.4615.

Compound 22 (Scheme 6). To a stirred solution of **21b** (6 mg, 3.2 μmol) in dry CH_2Cl_2 (1 mL) were added $Pd(Ph_3P)_4$ (2 mg, 0.02 mmol) and $PhSiH_3$ (24 μL, 0.20 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was concentrated, and the residue was purified by RP-HPLC to give corresponding peptide. To a stirred solution of above peptide, HOAt (6.8 mg, 0.05 mmol), and DIEA (34.8 μL, 0.20 mmol) in dry DMF (5 mL) was added EDCI·HCl (9.6 mg, 0.05 mmol) at 0 °C. The reaction mixture was allowed to warm up to room temperature, and the stirring was continued for 18 h. The reaction mixture was concentrated, and the residue was purified by RP-HPLC to give compound **22** (1.4 mg, 25%) as a white powder.

Compound 22 (Scheme 8). The linear peptide was constructed by solid-phase peptide synthesis on peptidyl resin **19** (0.506 mmol/g, 395 mg, 0.20 mmol). After the cleavage from the resin **26** as described above, EDCI·HCl (192 mg, 1.00 mmol) was added to a solution of linear peptide, HOAt (136 mg, 1.0 mmol), and DIEA (697 μL, 4.00 mmol) in dry DMF (100 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature, and the stirring was continued for 18 h. The reaction mixture was concentrated and the residue was purified by RP-HPLC to give compound **22** (19 mg, 5.5% from resin) as a white powder: ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ: 0.61-2.39 (m, 72H), 2.39-3.98 (m, 18H), 3.98-5.94 (m, 16H), 5.94-6.94 (m, 3H), 6.94-7.58 (m, 14H), 7.58-8.22 (m, 2.5H), 8.22-8.63 (m, 1.5H), 8.63-9.41 (br, 1H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$, mixture of

rotamers) δ: 9.6, 12.0, 15.5, 16.1, 18.3, 20.3, 20.6, 21.1, 21.9, 22.8, 23.2, 23.4, 23.5, 24.6, 24.9, 26.0, 27.4, 27.6, 28.3, 29.3, 29.7, 29.8, 30.0, 30.4, 33.3, 34.9, 36.1, 38.1, 39.7, 41.0, 42.7, 48.6, 48.7, 49.0, 51.6, 52.5, 53.7, 54.2, 55.4, 61.0, 62.8, 66.4, 73.2, 73.5, 79.9, 126.8, 127.0, 127.3, 128.5, 128.6, 128.9, 129.2, 129.5, 136.8, 154.4, 168.5, 169.8, 171.4, 171.6, 172.0, 172.7, 174.0, 175.4; HRMS (ESI-TOF) calcd for $C_{83}H_{130}N_{14}Na_2O_{19}S_3$ $[M+2Na]^{2+}$: 884.4292; found: 884.4293.

Compound 27. To a stirred solution of **22** (19 mg, 0.01 mmmol) in dry DMF (11 mL) were added DIEA (764 μL, 4.4 mmol) and DTT (169.1 mg, 1.1 mmol) at room temperature. After being stirred for 12 h, the reaction mixture was concentrated, and the residue was purified by RP-HPLC to give corresponding peptide. To a stirred solution of above peptide in dry DMF (11 mL) were added Et_3N (611 μL, 4.4 mmol) and 1,4-dibromobutane (130 μL, 1.1 mmol) at room temperature. After being stirred for 12 h, the reaction mixture was concentrated, and the residue was purified by RP-HPLC to give compound **27** (11 mg, 62%) as a white powder: ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotamers) δ: 0.63-1.46 (m, 41H), 1.46-1.91 (m, 5H), 1.93-2.36 (m, 15H), 2.38-3.98 (m, 11H), 4.23-5.17 (m, 10H), 5.98 (br, 0.5H), 6.20-6.65 (m, 1.5H), 6.94-7.25 (m, 5H), 7.27-7.41 (m, 4H), 7.96-8.29 (m, 1H), 8.78 (br, 0.5H), 9.33-9.69 (m, 1H); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ: 10.0, 13.2, 14.1, 14.7, 18.7, 18.9, 19.6, 21.5, 22.7, 23.2, 23.9, 24.4, 24.8, 25.0, 25.4, 27.4, 28.2, 29.3, 29.7, 31.3, 34.6, 35.6, 36.7, 39.6, 43.4, 44.0, 47.9, 50.6, 52.2, 52.6, 52.9, 54.0, 55.0, 59.3, 60.1, 61.5, 62.5, 67.8, 72.9, 73.8, 76.8, 77.0, 77.2, 80.3, 109.1, 127.1, 127.4, 128.5, 128.8, 129.0, 129.3, 135.5, 136.1, 138.4, 154.8, 167.1, 168.0, 168.8, 169.5, 170.2, 170.8, 171.6, 172.7, 173.6; HRMS (ESI-TOF) calcd for $C_{79}H_{120}N_{14}Na_2O_{19}S$ $[M+2Na]^{2+}$: 823.4180; found: 823.4185.

Model compound (9). Peptide **27** (10.8 mg, 6.7 μmmol) was stirred in 95% TFA aq. (17 mL) at room temperarture. After being stirred for 2 h, the mixture was concentrated under reduced pressure to give the corresponding peptide, which was used without further purification. To a stirred solution of above peptide in MeCN (4 mL) were added aqueous saturated $NaHCO_3$ (4 mL) and succinic anhydride (135 mg, 1.4 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was added 1 M HCl. The whole was extract with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by RP-HPLC to give compound **9** (6.5 mg, 62%) as a white powder: ^{1}H NMR (500 MHz, CD_3CN, mixture of rotamers) δ: 0.68-1.40 (m, 38H), 1.41-2.27 (m, 16H), 2.30-5.60 (m, 40H), 6.72-7.75 (m, 18H), 7.95 (br, 1.5H), 8.36 (br, 1.5H), 8.62-9.03 (m, 1H); ^{13}C{^{1}H} NMR (75 MHz, CD_3CN, 50 °C) δ: 10.3, 15.4, 18.7, 19.2, 19.3, 20.2, 21.3, 22.0, 22.9, 23.1, 23.3, 25.3, 25.7, 25.8, 29.5, 30.9, 31.5, 33.3, 37.5, 38.8, 42.9, 48.5, 49.1, 51.8, 53.3, 54.0, 54.4, 54.8, 55.7, 56.7, 60.9, 61.7, 63.0, 63.1, 66.0, 73.5, 109.2, 127.48, 127.53, 129.1, 129.2, 130.0, 137.4, 137.7, 166.2, 168.0, 169.7, 170.0, 170.9, 171.3, 172.0, 172.7, 173.0, 173.8, 173.9, 174.1, 174.4; HRMS (ESI-TOF) calcd for $C_{74}H_{108}N_{14}Na_2O_{20}S$ $[M+2Na]^{2+}$: 795.3685; found: 795.3683.

(9*H*-Fluoren-9-yl)methyl (*S*)-5-oxo-4-vinyloxazolidine-3-carboxylate (31). To a suspension of

Fmoc-L-Met-OH (5.0 g, 13.5 mmol) in toluene (250 mL), paraformaldehyde (3.0 g, 100 mmol) and TsOH·H_2O (537 mg, 2.82 mmol) were added, and the mixture was refluxed for 2 h. The solution was washed with aqueous saturated $NaHCO_3$ and brine, and dried over $NaSO_4$. The filtrate was concentrated under reduced pressure to give compound **29**. To a stirred solution of **29** in MeOH (65 mL) and THF (13 mL) was slowly added $NaIO_4$ (2.74 g, 12.8 mmol) in H_2O (17 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature, and the stirring was continued for 72 h. The reaction mixture was concentrated under reduced pressure, and CH_2Cl_2 was added to the residue. The whole was extract with CH_2Cl_2 and the extract was washed 1 M HCl and brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give compound **30**. The solution of **30** in dry xylene (70 mL) was refluxed for 24 h. The solution was concentrated and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 5/1) to give compound **31** (2.16 g, 48%) as a light yellow oil: $[\alpha]^{23}_D$ +64.7 (*c* 1.00, $CHCl_3$); IR (neat): 1804 (C=O), 1714 (C=O); 1H NMR (300 MHz, $CDCl_3$, 50 °C) δ: 4.19 (t, *J* = 5.7 Hz, 1H), 4.52-4.63 (br, 1H), 4.59 (dd, *J* = 5.6, 1.6 Hz, 2H), 5.10 (d, *J* = 4.52 Hz, 1H), 5.19 (br, 0.5H), 5.25 (br, 0.9H), 5.28 (br, 0.6H), 5.38 (br, 1H), 5.63-5.77 (m, 1H), 7.29 (td, *J* = 7.4, 1.2 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.52 (dd, *J* = 7.4, 0.8 Hz, 2H), 7.74 (d, *J* = 7.5 Hz, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$, 50 °C) δ: 47.2, 56.8, 67.6, 77.5, 119.1, 119.5, 120.1, 120.1, 124.6, 124.7, 127.2, 127.9, 127.9, 129.2, 141.5, 143.3, 143.4, 152.8, 169.7; HRMS (EI) calcd for $C_{20}H_{17}NO_4$ $[M]^+$: 335.1158; found: 335.1154.

(9*H*-Fluoren-9-yl)methyl (4*S*)-4-(oxiran-2-yl)-5-oxooxazolidine-3-carboxylate (32). To a stirred solution of **31** (5.2 g, 15.4 mmol) in CH_2Cl_2 (260 mL) was added mCPBA (13.3 g, 54 mmol, 70%) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 days. The reaction was quenched with aqueous saturated Na_2SO_3. The whole was extract with CH_2Cl_2 and the extract was washed with aqueous saturated $NaHCO_3$ and brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 5/1) to give compound **32** (3.7 g, 68%) as a mixture of both diastereoisomers.

(9*H*-Fluoren-9-yl)methyl (*S*)-4-[(*R*)-1-hydroxy-2-methoxyethyl]-5-oxooxazolidine-3-carboxylate (33a) and (9*H*-fluoren-9-yl)methyl (*S*)-4-[(*S*)-1-hydroxy-2-methoxyethyl]-5-oxooxazolidine-3-carboxylate (33b). To a stirred solution of **32** (3.51 g, 9.98 mmol) in MeOH (30 mL) was added perchloric acid (1.31 mL) at room temperature. The reaction mixture was stirred for 4.5 h. The reaction was quenched with aqueous saturated $NaHCO_3$. The whole was extracted with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by a CombiFlash® Rf 150 apparatus over silica gel (hexane/EtOAc) to give compound **33a** (630 mg, 16%) as needle crystals and compound **33b** (776 mg, 20%) as a colorless oil. **33a**: mp 146-150 °C $[\alpha]^{21}_D$ +83.9 (*c* 1.01, $CHCl_3$); IR (neat): 1799 (C=O), 1711 (C=O); ^1H NMR (300 MHz, DMSO, 50 °C) δ: 3.29 (s, 3H), 3.36 (d, *J* = 6.6 Hz, 0.5H), 3.46-3.56 (m, 1H), 3.98-4.10 (br, 1H), 4.25 (s, 1H), 4.29 (t, *J* = 6.6 Hz, 1H), 4.37-4.45 (m, 2H), 5.17 (d, *J* = 3.6 Hz, 1H), 5.39 (d, *J* = 3.8 Hz,

1H), 5.64 (d, J = 4.9 Hz, 1H), 7.34 (t, J = 7.4 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.68 (d, J = 7.4 Hz, 2H), 7.88 (d, J = 7.5 Hz, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO, 50 °C) δ: 47.1, 58.1, 58.9, 67.7, 69.5, 72.4, 78.7, 120.6, 125.6, 127.6, 128.2, 141.2, 141.3, 144.0, 144.1, 152.7, 170.5; HRMS (EI) calcd for $C_{21}H_{21}NO_6$ $[M]^+$: 383.1369; found: 383.1368.

33b: $[\alpha]^{21}_D$ +96.9 (*c* 1.00, $CHCl_3$); IR (neat): 1801 (C=O), 1715 (C=O); ^1H NMR (300 MHz, DMSO, 50 °C) δ: 3.10-3.22 (m, 2H), 3.19 (s, 3H), 3.97 (q, J = 5.7 Hz, 1H), 4.16 (s, 1H), 4.31 (d, J = 6.0 Hz, 1H), 4.45-4.57 (m, 2H), 5.08 (d, J = 4.2 Hz, 1H), 5.45 (d, J = 4.7 Hz, 1H), 5.46 (d, J = 2.9 Hz, 1H), 7.34 (td, J = 7.4, 1.2 Hz, 2H), 7.42 (t, J = 7.3 Hz, 2H), 7.67 (dd, J = 7.5, 2.9 Hz, 2H), 7.88 (d, J = 7.4 Hz, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO, 50 °C) δ: 47.2, 58.7, 67.7, 70.7, 73.9, 79.5, 120.6, 125.0, 125.5, 127.6, 128.2, 141.3, 144.0, 144.2, 154.1, 172.5; HRMS (EI) calcd for $C_{21}H_{21}NO_6$ $[M]^+$: 383.1369; found: 383.1371.

X-ray Crystallographic Analyses of 33a

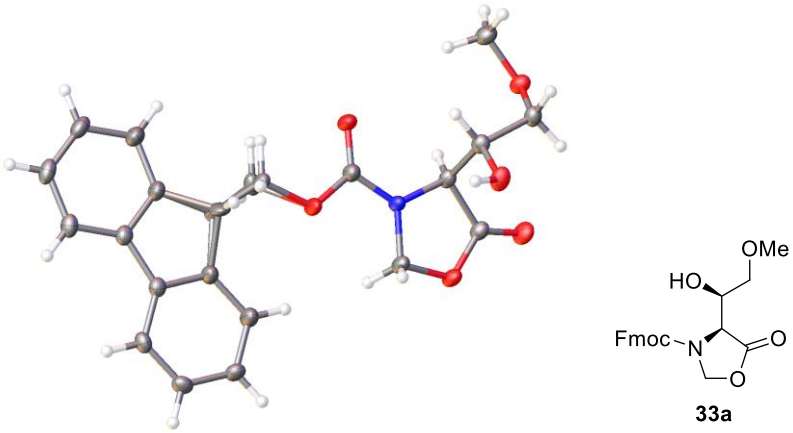


Figure S1. Crystal Structure of Alcohol **33a**.

The data of the compound **33a** ($C_{21}H_{21}NO_6$) was collected with a Rigaku XtaLAB Synergy-I: quarter-chi single diffractometer using mirror monochromated Cu-Kα radiation at 100 K. The substance was crystallized from EtOAc/hexane as colorless needle crystals and solved in monoclinic space group $P2_1/_n$ with Z = 2. The structure was solved by direct methods (SHELXT-2018/2) and refined by the full-matrix least-squares on F2 (SHELXL-2018/3). All non-hydrogen atoms were refined anisotropically and all hydrogen atoms were placed using AFIX instructions. The crystal data are as follows: $C_{21}H_{21}NO_6$, FW: 383.39. a = 12.7080(5), b = 5.8976(1), c = 13.5551(5), V = 902.40(6) Å^3, Z = 2, D_{calc} = 1.411 g/cm^3, μ = 0.864 mm^{-1}, R_1 = 0.0552 ($I > 2\sigma(I)$), $_wR_2$ = 0.1427 (all data), GOF = 1.039.

(2S,3*R*)-Fmoc-Nmhm-OH (5a). To a stirred solution of **33a** (174 mg, 0.453 mmol) in dry CH_2Cl_2 (2.2 mL) were added Et_3SiH (289 µL, 1.81 mmol) and TFA (2.2 mL) at room temperature. The reaction mixture was stirred for 24 h. The solution was concentrated, and the residue was dissolved in EtOAc.

The whole was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by a CombiFlash® Rf 150 apparatus over silica gel ($CHCl_3$/MeOH) to give compound **5a** (105 mg, 59%) as a colorless oil. $[\alpha]^{21}_D$ –83.9 (*c* 0.94, $CHCl_3$); IR (neat): 1715 (C=O); ^{1}H NMR (300 MHz, DMSO, 50 °C) δ: 2.89 (s, 3H), 3.25 (s, 3H), 3.27-3.47 (m, 2H), 3.96-4.04 (m, 1H), 4.18-4.62 (m, 4H), 4.80-5.60 (br, 1H), 7.32 (t, *J* = 7.2 Hz, 2H), 7.42 (t, *J* = 7.8 Hz, 2H), 7.65 (d, *J* = 7.3 Hz, 2H), 7.88 (d, *J* = 7.4 Hz, 2H), 12.2-13.1 (br, 1H); ^{13}C{^{1}H} NMR (75 MHz, DMSO, 50 °C) δ: 14.5, 21.2, 32.2, 47.2, 58.8, 60.2, 60.4, 61.0, 67.4, 69.3, 74.7, 120.4, 120.5, 125.5, 127.5, 128.1, 141.2, 144.3, 156.4, 171.4; HRMS (ESI-TOF) calcd for $C_{21}H_{24}NO_6$ $[M+H]^+$: 386.1598; found: 386.1596.

(2S,3*S*)-Fmoc-Nmhm-OH (5b). To a stirred solution of **33a** (200 mg, 0.521 mmol) in dry CH_2Cl_2 (2.5 mL) were added Et_3SiH (333 μL, 2.08 mmol) and TFA (2.5 mL) at room temperature. The reaction mixture was stirred for 24 h. The solution was concentrated, and the residue was dissolved in EtOAc washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by a CombiFlash® Rf 150 apparatus over silica gel ($CHCl_3$/MeOH) to give compound **5b** (76 mg, 37%) as a colorless oil and byproduct **34** (58 mg, 29%) as a colorless oil.
5b: $[\alpha]^{22}_D$ –72.9 (*c* 0.98, $CHCl_3$); IR (neat): 1712 (C=O); ^{1}H NMR (300 MHz, DMSO, 50 °C) δ: 2.94 (s, 3H), 3.20-3.34 (m, 5H), 4.17-4.41 (m, 4H), 4.61-4.70 (m, 1H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.41 (d, *J* = 7.3 Hz, 2H), 7.65 (dd, *J* = 7.3, 2.7 Hz, 2H), 7.88 (d, *J* = 7.4 Hz, 2H); ^{13}C{^{1}H} NMR (75 MHz, DMSO, 50 °C) δ: 33.1, 47.2, 58.9, 60.4, 67.4, 69.1, 74.6, 120.5, 125.5, 127.5, 128.1, 141.3, 144.30, 144.35, 145.7, 157.0, 171.7; HRMS (ESI-TOF) calcd for $C_{21}H_{24}NO_6$ $[M+H]^+$: 386.1598; found: 386.1598.
34: $[\alpha]^{22}_D$ –89.2 (*c* 1.06, $CHCl_3$); IR (neat): 1719 (C=O); ^{1}H NMR (300 MHz, DMSO, 50 °C) δ: 3.32 (s, 3H), 3.48-3.57 (m, 2H) 4.14-4.41 (m, 5H), 4.76 (d, *J* = 3.8 Hz, 1H), 5.03 (br, 1H), 7.33 (td, *J* = 7.4, 1.1 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.64 (d, *J* = 7.2 Hz, 2H), 7.88 (d, *J* = 7.5 Hz, 2H), 13.1 (br, 1H); ^{13}C NMR (75 MHz, DMSO, 50 °C) δ: 47.1, 58.8, 59.2, 67.6, 72.2, 79.0, 120.3, 120.6, 125.5, 127.2, 127.6, 128.2, 141.2, 144.0, 144.1, 145.7, 152.9, 171.5; HRMS (ESI-TOF) calcd for $C_{21}H_{22}NO_6$ $[M+H]^+$: 384.1442; found: 384.1443.

(2*R*,5*S*)-2-Isopropyl-3,6-dimethoxy-5-[(*S*)-2-methylbutyl]-2,5-dihydropyrazine (36a). To a stirred solution of (*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (200 μL, 1.12 mmol) in dry THF (5 mL) was added *n*-BuLi (1.58 M solution in hexane, 710 μL, 1.12) at –78 °C. After being stirred for 15 min, to this solution was added (*S*)-iodo-2 methylbutane (194 μL, 54%) and stirring was continued for 4 h at –78 °C. The reaction mixture was allowed to warm up to room temperature overnight. The reaction was quenched with saturated aqueous NH_4Cl. The whole was extract with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 19/1) to give compound **36a** (153 mg, 54%) as a colorless oil: $[\alpha]^{23}_D$ +7.1 (*c* 1.01, $CHCl_3$); IR (neat): 2961 (CH_3), 1695 (C=O), 1672 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.69 (d, *J* = 6.8 Hz, 3H), 0.85-0.91

(m, 5H), 1.06 (d, J = 6.9 Hz, 3H), 1.10-1.35 (m, 2H), 1.38-1.49 (m, 1H), 1.61-1.69 (m, 1H), 1.75-1.83 (m, 1H), 2.24-2.33 (m, 1H), 3.49 (d, J = 5.1 Hz, 1H), 3.68 (s, 3H), 3.69 (s, 3H), 3.91-3.95 (m, 1H), 4.00-4.06 (m, 1H); $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$) δ: 11.1, 16.5, 19.1, 19.6, 29.1, 30.1, 31.5, 41.5, 52.2, 52.3, 54.1, 60.4, 163.0, 164.8; HRMS (ESI-TOF) calcd for $C_{14}H_{27}N_2O_2$ $[M+H]^+$: 255.2067; found: 255.2065.

(2*S*,5*R*)-2-Isopropyl-3,6-dimethoxy-5-[(*S*)-2-methylbutyl]-2,5-dihydropyrazine (36b). To a stirred solution of (*S*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (500 μL, 2.8 mmol) in dry THF (12 mL) was added *n*-BuLi (1.58 M solution in hexane, 1.8 mL, 2.8 mmol) at –78 °C. After being stirred for 15 min, to this solution was added (*S*)-iodo-2 methylbutane (466 μL, 3.6 mmol) and stirring was continued for 4 h at –78 °C. The reaction mixture was allowed to warm up to room temperature overnight. The reaction was quenched with saturated aqueous NH_4Cl. The whole was extract with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 19/1) to give compound **36b** (418 mg, 59%) as a colorless oil: $[\alpha]^{22}_D$ –15.3 (*c* 0.31, $CHCl_3$); IR (neat): 2963 (CH_3), 1696 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ: 0.69 (d, J = 6.8 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H), 0.93 (d, J = 6.4 Hz, 3H), 1.06 (d, J = 6.9 Hz, 3H), 1.13-1.25 (m, 1H), 1.25-1.37 (m, 1H), 1.45-1.54 (m, 1H), 1.58-1.65 (m, 1H), 1.67-1.74 (m, 1H), 2.22-2.33 (m, 1H), 3.67 (s, 3H), 3.69 (s, 3H), 3.91-3.95 (m, 1H), 3.99-4.04 (m,1H); $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$) δ: 11.3, 16.6, 19.0, 19.1, 30.2, 30.6, 31.5, 41.5, 52.3, 54.0, 60.5, 162.8, 164.8; HRMS (ESI-TOF) calcd for $C_{14}H_{27}N_2O_2$ $[M+H]^+$: 255.2067; found: 255.2070.

Fmoc-Hil-OH (6). To a stirred solution of **36a** (165 g, 0.65 mmol) in THF (3 mL) was added 1 M HCl (3 mL) at room temperature. After being stirred for 2 h, the reaction mixture quenched with 2 M NaOH. The whole was extract with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give the mixture of corresponding amine, which was used without further purification. To a stirred solution of above amine in dry CH_2Cl_2 (5 mL) was added Fmoc-OSu (263 mg, 0.78 mmol) at room temperature. After being stirred for 12 h, water was added to this solution. The whole was extract with CH_2Cl_2 and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 4/1) to give mixture of compound **37a** and Fmoc-D-Val-OMe. To this mixture in 1,4-dioxane (3 mL) and water, HCl (420 μL, 4.92 mmol, 36%) was added, and the mixture was refluxed for 12 h. The whole was extract with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by RP-HPLC to give compound **6** (80.1 mg, 34%) as a colorless oil: $[\alpha]^{24}_D$ +5.2 (*c* 0.76, $CHCl_3$); IR (neat): 1714 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, J = 6.8 Hz, 3H), 0.95 (d, J = 6.3 Hz, 3H), 1.13-1.25 (m, 1H), 1.39-1.60 (m, 3H), 1.80-1.89 (m, 1H), 4.23 (t, J = 6.9 Hz, 1H), 4.42 (t, J = 6.3 Hz, 3H), 5.16 (d, J = 8.4 Hz, 1H), 7.31 (t, J = 7.4 Hz, 2H), 7.40 (t, J = 7.4 Hz,

2H), 7.59 (t, J = 6.5 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H); $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$) δ: 10.8, 14.2, 19.2, 21.1, 28.4, 30.9, 39.4, 47.2, 52.3, 60.6, 67.1, 120.0, 125.1, 127.1, 127.8, 141.3, 143.7, 143.8, 156.2, 177.9; HRMS (ESI-TOF) calcd for $C_{22}H_{25}NNaO_4$ $[M+Na]^+$: 390.1676; found:390.1674.

Fmoc-D-*allo*-Hil-OH (7). To a stirred solution of **36b** (191 mg, 0.75 mmol) in THF (4 mL) was added 1 M HCl (4 mL) at room temperature. After being stirred for 2 h, the reaction mixture quenched with 2 M NaOH. The whole was extract with EtOAc and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give the mixture of corresponding amine, which was used without further purification. To a stirred solution of above amine in dry CH_2Cl_2 (6 mL) was added Fmoc-OSu (304 mg, 0.90 mmol) at room temperature. After being stirred for 12 h, water was added to this solution. The whole was extract with CH_2Cl_2, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 4/1) to give mixture of compound **37b** and Fmoc-Val-OMe. To a mixture of compound **37b** and Fmoc-Val-OMe in H_2O/1,4-dioxane (1/1, 3 mL), HCl (500 μL, 5.76 mmol, 36%) was added, and the mixture was refluxed for 12 h. The whole was extract with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by RP-HPLC to give compound **7** (124 mg, 45%) as a colorless oil; $[\alpha]^{25}_D$ +5.5 (*c* 1.13, $CHCl_3$); IR (neat): 1714 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ: 0.89 (t, J = 7.3 Hz, 3H), 0.95 (d, J = 6.4 Hz, 3H), 1.20-1.30 (m, 1H), 1.30-1.42 (m, 1H), 1.44-1.55 (m, 1H), 1.65 (t, J = 7.1 Hz, 3H), 4.23 (t, J = 6.8 Hz, 1H), 4.43 (t, J = 6.9 Hz, 3H), 5.09 (d, J = 8.7 Hz, 1H), 7.31 (t, J = 7.4 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.59 (t, J = 5.9 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H); $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$) δ: 11.2, 14.2, 18.4, 29.8, 31.0, 39.2, 47.2, 52.2, 60.5, 67.1, 120.0, 125.1, 127.1, 127.7, 141.3, 143.7, 143.9, 156.3, 177.9; HRMS (ESI-TOF) calcd for $C_{22}H_{25}NNaO_4$ $[M+Na]^+$: 390.1676; found:390.1675.

Fmoc-D-Cys(Trt)-OTce (39). To a stirred solution of **38** (5.0 g, 8.54 mmol) in dry CH_2Cl_2 (34 mL) were added 2,2,2-trichloroethanol (900 μL, 9.39 mmol), DMAP (104 mg, 0.85 mmol) and EDCI·HCl (1.8 g, 9.39 mmol) at –15 °C. After being stirred for 12 h, the reaction was quenched with 10% citric acid. The whole was extract with EtOAc and the extract was washed with aqueous saturated $NaHCO_3$ and brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 5/1) to give compound **39** (5.8 g, 95 %) as a colorless oil: $[\alpha]^{23}_D$ –1.33 (*c* 1.04, $CHCl_3$); IR (neat): 1766 (C=O), 1730 (C=O); ^1H NMR (300 MHz, $CDCl_3$, 50 °C) δ: 2.71 (br, 2H), 4.20 (t, J = 6.9 Hz, 1H), 4.31-4.45 (m, 3H), 4.63 (d, J = 11.8 Hz, 1H), 4.76 (d, J = 11.9 Hz, 1H), 5.14 (br, 1H), 7.16-7.32 (m, 11H), 7.33-7.43 (m, 8H), 7.57 (d, J = 7.2 Hz, 2H), 7.74 (d, J = 7.5 Hz, 2H); $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$, 50 °C) δ: 33.8, 47.2, 53.2, 67.3, 67.4, 74.6, 94.4, 120.0, 125.0, 127.0, 127.1, 127.7, 128.1, 129.5, 141.4, 143.7, 143.8, 144.2, 155.5, 169.1; HRMS (ESI-TOF) calcd for $C_{39}H_{32}Cl_3NNaO_4S$ $[M+Na]^+$: 738.1010; found: 738.1001.

Boc-Ser-OAllyl (42). To a stirred solution of **41** (5.0 g, 24.4 mmol) in DMF (50 mL) were added allyl bromide (2.3 mL, 26.8 mmol) and K_2CO_3 (4.4 g, 31.8 mmol) at room temperature. After being stirred for 12 h, the reaction mixture quenched with water. The whole was extract with EtOAc and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 2/1) to give compound **42** (5.3 g, 88%) as a colorless oil. The spectral data were in good agreement with those previously reported.[14]

Boc-β-bromo-Ala-OAllyl (43). Boc-β-bromo-Ala-OAllyl was synthesized by the identical procedure reported previously.[15] To a stirred solution of **42** (5.3 g, 21.5 mmol) and CBr_4 (8.6 g, 25.8 mmol) in dry CH_2Cl_2 (150 mL) under argon was added Ph_3P (6.8 g, 25.8 mmol) in dry CH_2Cl_2 (50 mL) at 0 ℃. The reaction mixture was warmed to room temperature and stirred for 4 h. The reaction mixture quenched with water. The whole was extract with CH_2Cl_2 and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 7/1) to give compound **43** (5.2 g, 79%) as a colorless oil.

Fmoc-D-Lan(Boc-Lan-OAllyl)-OTce (44). To a stirred solution of **39** (550 mg, 0.77 mmol) in dry CH_2Cl_2 (8 mL) under argon were added triisopropylsilane (236 μL, 1.15 mmol) and TFA (880 μL, 11.5 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was diluted CH_2Cl_2 and water. To this solution was slowly added $NaHCO_3$ (920 mg, 11.5 mmol). The whole was extract with CH_2Cl_2, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give compound **40**. To a stirred solution of **40** in EtOAc (5 mL) under argon was added TBAB (989 g, 3.07 mmol) in aqueous saturated $NaHCO_3$ (adjusted pH 8.5 with 1 M HCl) and **43** (189 mg, 0.61 mmol) in EtOAc (10 mL) at room temperature. After being stirred for 12 h, the whole was extract with EtOAc, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography over silica gel (hexane/EtOAc = 5/1) to give compound **44** (219 mg, 51%) as a colorless oil; $[\alpha]^{24}_D$ +10.0 (*c* 1.03, $CHCl_3$); IR (neat): 1717 (C=O); ^{1}H NMR (300 MHz, DMSO, 50 °C) δ: 2.73 (m, 4H), 4.15-4.45 (m, 5H), 4.58 (dt, *J* = 5.3, 1.5 Hz, 2H), 4.90 (q, *J* = 11.1 Hz, 2H), 5.19 (dd, *J* = 10.5, 1.5 Hz, 1H), 5.32 (dq, *J* = 17.3, 1.7 Hz, 1H), 5.79-5.99 (m, 1H), 7.19 (br, 1H), 7.32 (td, *J* = 7.4, 1.2 Hz, 2H), 7.41 (t, *J* = 7.1 Hz, 2H), 7.70 (d, *J* = 7.1 Hz, 2H), 7.87 (d, *J* = 7.3 Hz, 2H), 7.94 (d, *J* = 7.5 Hz, 1H); ^{13}C{^{1}H} NMR (75 MHz, DMSO, 50 °C) δ: 28.6 33.3, 33.7, 47.2, 54.3, 54.6, 65.5, 66.5, 74.2, 75.9, 95.4, 118.2, 120.5, 125.6, 127.5, 128.1, 132.7, 141.2, 144.2, 155.8, 156.4, 169.8, 170.7, 171.0; HRMS (ESI-TOF) calcd for $C_{31}H_{35}Cl_3N_2NaO_8S$ $[M+Na]^+$: 723.1072; found: 723.1073.

Fmoc-D-Lan(Boc-Lan-OAllyl)-OH (8). To a stirred solution of **44** (81 mg, 0.12 mmol) in THF (6 mL) under argon were added zinc dust (290 mg, 0.45 mmol) and 0.5 M NH_4OAc (1.5 mL) at room

temperature. After being stirred for 12 h, the reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure. The residue was added $CHCl_3$ and H_2O. The whole was extract with $CHCl_3$, and the extract was washed with brine, dried over Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel ($CHCl_3$/MeOH = 50/1 to 10/1) to give compound **8** (47.5 mg, 72%) as a colorless oil; $[\alpha]^{24}_D$ –14.6 (*c* 1.03, $CHCl_3$); IR (neat): 1719 (C=O); ^{1}H NMR (300 MHz, DMSO, 50 °C) δ: 1.38 (s, 9H), 2.74-3.03 (m, 4H), 4.09-4.35 (m, 5H), 4.57 (d, *J* = 5.3 Hz, 2H), 5.19 (dd, *J* = 10.5, 1.3 Hz, 1H), 5.31 (dq, *J* = 17.3, 1.6 Hz, 1H), 5.80-5.97 (m, 1H), 7.19 (br, 1H), 7.32 (td, *J* = 7.4, 1.1 Hz, 2H), 7.41 (t, *J* = 7.1 Hz, 2H), 7.58 (d, *J* = 7.1 Hz, 1H), 7.71 (t, *J* = 7.3 Hz, 2H), 7.87 (d, *J* = 7.4 Hz, 2H); ^{13}C{^{1}H} NMR (75 MHz, DMSO, 50 °C) δ: 28.6, 33.6, 34.0, 47.2, 54.6, 65.4, 66.3, 70.3, 79.0, 118.2, 120.5, 125.7, 127.5, 128.1, 129.3, 132.6, 132.8, 139.7, 141.2, 144.3, 155.8, 171.1, 172.5; HRMS (ESI-TOF) calcd for $C_{29}H_{34}N_2NaO_8S$ $[M+Na]^+$: 593.1928; found: 593.1928.

参考文献

1. Fernandez, A. M. Isolation and characterization of vitilevuamide from the ascidians *Didemnum cuculliferum and Polysyncraton lithostrotum*, University of Utah, doctoral thesis, 1996.
2. Fernandez, A. M.; He, H.; Mcdonald, L. A.; Lassota, P.; Sorensen, E. F.; Edler, M. C.; Barrows, L. R.; Clardy, J. C.; Ireland, C. M. *IUPAC* 1999.
3. Gallegos, D. A. Natural Products Discovery from Unique Microbial Habitats, Oregon State University, doctoral thesis, 2019.
4. Edler, M. C.; Fernandez, A. M.; Lassota, P.; Ireland, C. M.; Barrows, L. R. *Biochem. Pharmacol.* **2002**, *63*, 707.
5. Jimenez, J. C.; Bayo, N.; Chavarria, B.; Lopez-Macia, A.; Royo, M.; Nicolas, E.; Giralt, E.; Albericio, F. *Lett. Pept. Sci*. **2002**, *9*, 135.
6. Chalker, J. M.; Gunnoo, S. B.; Boutureira, O.; Gerstberger, S. C.; Fernández-González, M.; Bernardes, G. J. L.; Griffin, L.; Hailu, H.; Schofield, C. J.; Davis, B. G. *Chem. Sci.* **2011**, *2*, 1666.
7. Annette, E. Buba, A. E.; Koch, S.; Kunz, H.; Löwe, H. *Eur. J. Org. Chem.* **2013**, 4509.
8. Wu, H.; Zhang, Y.; Li, Y.; Xu, J.; Wang, Y.; Li, X. *J. Am. Chem. Soc.* **2021**, *143*, 7808.
9. Jones, R. J.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 1144.
10. Schöllkopf, U. *Tetrahedron* **1983**, *39*, 2085.
11. Zhu, X. M. Schmidt, R. R. *Eur. J. Org. Chem*. **2003**, *20*, 4069.
12. Du, X.; Qian, J.; Wang, Y.; Zhang, M.; Chu, Y.; Li, Y. *Bioorg. Med. Chem.* **2019**, *27*, 2784.
13. Spetzler, J. C.; Høeg-Jensen, T. *J. Peptide Sci*. **1999**, 5, 582.
14. Spicer, C. D.; Pujari-Palmer, M.; Autefage, H.; Insley, G.; Procter, P.; Engqvist, H.; Stevens, M. M. *ACS Cent. Sci.* **2020**, *6*, 226.
15. Bundle, D. R.; Rich, J. R.; Jacques, S.; Yu, H. N.; Nitz, M.; Ling, C. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 7725.

結論

著者は、天然から単離された分岐骨格を有する環状ペプチドおよびその誘導体の合成研究を行った。

第一章では、投げ縄型構造を有する環状ペプチド coibamide A の構造活性相関研究を行った。Coibamide A のマクロラクトン構造周辺の構造の重要性に着目し、各種誘導体を設計・合成するとともに、細胞増殖抑制活性およびタンパク質分泌阻害活性を評価した。その結果、coibamide A の Tyr(Me) 部位を Bph に変換することにより、細胞増殖抑制活性が向上することを明らかにした。また、coibamide A のマクロラクトン部位を形成するエステル結合を炭素―炭素二重結合やアミド結合に置換しても、天然物と同等の細胞増殖抑制活性を示すことを明らかにした。さらに、細胞増殖抑制活性とタンパク質分泌阻害活性を比較することで、coibamide A およびその誘導体が Sec61α 以外の標的分子にも作用することにより、細胞増殖抑制活性を示している可能性を示した。

第二章では、二環性骨格からなる環状ペプチド vitilevuamide の合成研究を行った。非天然型アミノ酸を変換したモデル化合物を用いた検討により、vitilevuamide の 2 つの大環状構造を固相樹脂上での環化反応を活用することで効率的に構築するプロセスを確立した。また、このプロセスに適用可能な合成素子となるアミノ酸の適切な保護基を確定させた。このうち、Dha 前駆体となるアミノ酸について、vitilevuamide の二環性骨格を構築後に Dha へと導くプロセスを確立した。

近年、マクロサイクロ類の医薬への応用が注目を集めており、環状ペプチド類はその代表的な一翼を担うものとして期待されている。魅力的な生物活性を有するユニークな骨格からなる環状ペプチド類も多数報告されているものの、これらの創薬展開や基礎研究への応用のためには、化学合成上の制約をはじめとして未だに解決するべき課題が残されている。本研究において取り扱ったペプチド性天然物ならびにその誘導体の合成研究により得られた知見は、今後新たに見出される環状ペプチド類の構造最適化研究やこれらを活用した創薬研究においても有益なものとなることが期待される。

謝辞

本研究の実施にあたり有益な御指導、御助言をいただきました創薬有機化学分野大野浩章教授、京都薬科大学薬品化学分野大石真也教授に深く感謝申し上げます。

副査として御助言をいただくとともに本論文の細部にわたり御指導をいただきました生体機能化学分野二木史朗教授、システムケモセラピー・制御分子学分野掛谷秀昭教授に深く感謝申し上げます。

研究を進めるにあたって、多くの御助言をいただきました創薬有機化学分野井貫晋輔准教授、創薬有機化学分野有地法人助教、バイオ医薬品化学分野秋葉宏樹助教に深く感謝致します。

本研究の遂行にあたって、タンパク質分泌阻害試験に御協力いただきました Kerry L. McPhail 教授（Oregon State University）、Jane E. Ishmael 教授（Oregon State University）、Daphne R. Mattos 博士（Oregon State University）、高分解能質量測定に御協力いただきました京都薬科大学共同利用機器センター服部恭尚講師に深く感謝申し上げます。

Coibamide A の構造活性相関研究に関して多大な御協力をいただきました喜多村隆志修士、辻岡里菜氏、および vitilevuamide の合成研究に関して多大な御協力をいただきました京都薬科大学薬品化学分野小林数也准教授、横畠綾音氏、三反崎秀真氏、新井拓海氏、新谷越佑氏に感謝致します。

大学院での研究生活を送る上で、ともに研究に励み協力をいただきました創薬有機化学分野並びに京都薬科大学薬品化学分野に在籍した諸氏に感謝致します。

最後に、長きにわたる学生生活をサポートしていただいた家族に心より感謝申し上げます。

2023 年 3 月

鈴木力斗