Isolation, Total Synthesis and the Structure Determination of the Antifungal Macrocyclic Depsipeptide, Tetraselide

Hiroki Nakahara, Goh Sennari, Haruki Azami, Hayama Tsutsumi, Yoshihiko Noguchi, Yoshihiro Watanabe, Yuki Inahashi, Masato Iwatsuki, Tomoyasu Hirose,* Toshiaki Sunazuka*

Ōmura Satoshi Memorial Institute and Graduate School of Infection Control Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan.

Abstract: Macrocyclic peptides and depsipeptides are the emerging class of a new modality in drug discovery research. Tetraselide, an antifungal cyclic peptide isolated from a marine-derived filamentous fungus, possesses the unique amphiphilic structural feature that represents the five consecutive β-hydroxy-amino acid and fatty acid moieties. Because the structure elucidation of the naturally occurring product left six stereocenters ambiguous, we implemented bioinformatic analyses, chemical degradation study and chiral pool fragment synthesis to identify two of the undetermined stereochemistry. Convergent total synthesis of four remaining plausible isomers of tetraselide was accomplished via liquid-phase peptide synthesis using the soluble hydrophobic tag auxiliaries. The key advance involves fragment coupling by the serine/threonine ligation reaction and head-to-tail macrolactamization of the carrier-supported precursors that enabled systematic elaboration of the amphiphilic cyclic peptides. Ultimately, we determined the absolute structure of this natural product.

Introduction

Over the last decade, cyclic peptides have drawn much attention in drug discovery efforts as an attractive modality.[1] Their unique properties such as high binding affinity and selectivity targeting protein-protein interactions (PPIs) with low toxicity potentially serve as complementarily advantageous therapeutics to antibodies and small drug molecules.[2] Although cell permeability and oral bioavailability are, in general, considered as challenges,[3] there are emerging research that *N*-methyl amides[4] and/or depsipeptides[5] enhance membrane permeation.[6] Despite chemical accessibility of this class of peptide molecules, the vast majority in therapeutical cyclic peptides has been developed based on natural products. Therefore, exploration of new cyclic peptides and depsipeptides from natural sources plays important roles as the foundations of drug leads in medicinal chemistry research.

Our research group has been working on in quest of novel secondary metabolites of the microorganism from underexplored natural sources.[7] During the course of our screening program to explore antifungal natural products,[8] we isolated a macrocyclic depsipeptide, namely tetraselide (**1**, Figure 1A), from a culture broth of *Trichoderma* sp., the marine-derived filamentous fungus. As a result of NMR and LC-MS/MS analyses (see the Supporting Information, SI), we identified that the structure of **1** consists of six polar amino acids such as Orn, Thr, Ser, Ser, Ser, Ser, two hydrophobic Ala and Gly residues, and one β-hydroxy-γ-methyl-fatty acid.[9] The five consecutive β-hydroxy-amino acid residues represent its unique structural feature that has been rarely seen in natural products. The modified Marfey method[10] to analyze the absolute configurations of the amino acids revealed the presence of L-Ala, L-Orn, and D-*allo*-Thr. The four consecutive Ser moiety was found to be a 3:1

mixture of L- and D-isomers, although the position of the D-Ser residue remained ambiguous. Consequently, because six stereocenters of **1** including the C3 and C4 positions of the β-hydroxy-γ-methyl-fatty acid moiety could not be determined, chemical synthesis was required to fully characterize the absolute structure of the natural product **1**.

Since the invention of the Merrifield resin,[11] solid-phase peptide synthesis (SPPS) has become one of the most common methods to elaborate peptide compounds.[12] Although SPPS serves as a robust and reliable method, excess amounts of reagents and coupling partners are often required to achieve sufficient reactivity due to the heterogeneous nature of the reaction. As such, convergent synthetic approach, which would require the preparation of peptide fragments over several steps, is generally considered inefficient, whereas linear synthesis is more commonly employed in SPPS.[13] Additionally, typical head-to-tail macrocyclization in SPPS needs to remove the solid-phase carrier, necessitating manipulation of the corresponding cyclization precursor in liquid phase. The solubility and polarity of peptides without carrier support sometimes result in troublesome handling, particularly with amphiphilic and zwitterionic peptides.[14]

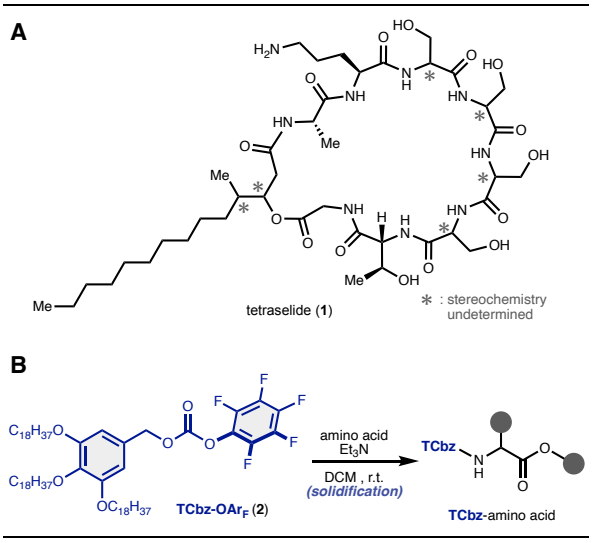


Figure 1. Natural product tetraselide (**1**) and our previous work.

As an alternative approach to SPPS, our group has been interested in liquid-phase peptide synthesis (LPPS)[15] using soluble hydrophobic tags[16] and applied this method to the total syntheses such as argifin,[17] kozupeptine,[18] verticilide,[19] and emodepside derivatives.[20] Recently, we developed a carbonate-type tag reagent TCbz-OAr$_F$ (**2**) that enabled to attach the tag-carrier to the amine group of amino acids (Figure 1B).[21] Orthogonal to the typical approach that relies on installation of the carrier molecule to the C-terminus, we thought to develop de novo synthetic strategy using the tag-reagent **2**. Herein, we report convergent total synthesis and the structure determination of tetraselide (**1**) via LPPS utilizing two tag-carrier molecules. The homogeneous reaction circumstance of LPPS enabled convergent fragment coupling of two carrier-supported peptides with an equimolar amount. Because the TCbz group was installed to the amine group of the Orn side chain, we successfully synthesized head-to-tail macrocyclic peptides being supported on a carrier molecule. This strategy facilitated systematic syntheses of all plausible isomers of the structurally ambiguous four consecutive Ser moiety and allowed us to determine the structure of **1**.

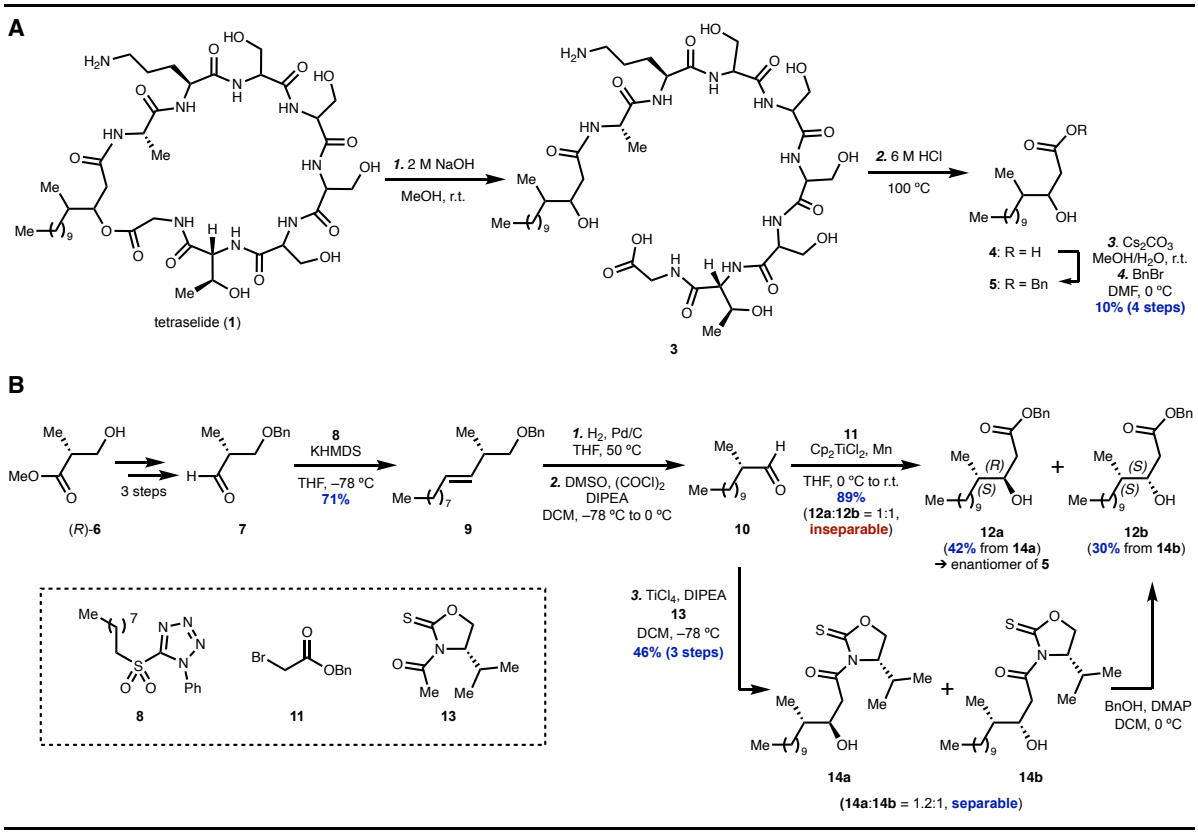
Results and Discussion

Biosynthetic analysis, chemical degradation and chiral pool synthesis to elucidate the stereochemistry.

As described earlier, our preliminary efforts for the structural elucidation of tetraselide (**1**) suggested that there are 16 possible diastereomers—four of the consecutive Ser moiety and four of the fatty acid moiety. Our failures to clarify the structure of **1** using Edman degradation[22] and X-ray crystallographic analysis led us to implement biosynthetic analysis to narrow down the structural candidates of **1**. We thought that the stereocenters of the β-hydroxy-γ-methyl fatty acid moiety in **1** would be predictable because the absolute stereochemistry of compounds produced by PKS relies on the amino acid sequence of each PKS domain. As a result of the bioinformatic analysis, we found that the keto reductase (KR) domain in the PKS was classified as the B-type, which generally reduces the ketone group to a D-hydroxy group.[23] In addition, we identified that the Tyr residue—one of the proton donors in the enoyl reductase (ER) domain—was substituted with Leu, suggesting that the methyl group would likely be the D-configuration.[24] These results indicated that the β-hydroxy-γ-methyl fatty acid moiety in **1** could be 3*S*-hydroxy and 4*R*-methyl configurations. To support this hypothesis, we next attempted chemical degradation study of the naturally occurring **1** and synthesis of the degraded fragment using a chiral pool method.

Our degradation experiments of **1** began with hydrolysis of the ester moiety by treating with 2 M NaOH, yielding linear peptide **3** (Scheme 1A). Subjection of **3** to the acidic conditions using 6 M HCl at 100 °C to hydrolyze amide bonds afforded fatty acid **4** as a major component after acid-extraction. In order to facilitate UV detection for the purification by reverse-phase HPLC, selective benzylation of the acid moiety in **4** provided ester **5** in 10% overall yield over four steps from **1**. With successfully degraded alcohol **5** in hand, we sought to chemically synthesize fatty acid derivatives to confirm the absolute stereochemistry.

In order to implement enantiospecific synthesis for the structural determination of **5**, we thought to employ a chiral pool starting material. Because the (*S*)-enantiomer of Roche ester **6** is much more expensive and less available, we began our investigation with (*R*)-**6** (Scheme 1B). Following the known 3-step sequence to lead to aldehyde **7**,[25] Julia-Kocienski olefination[26] of **7** using PT-sulfone **8** afforded alkene **9** in 71% yield as a single isomer. Hydrogenation of the double bond and simultaneous hydrogenolysis of the benzyl group in **9**, which was followed by Swern oxidation, produced aldehyde **10**. Subsequently, Reformatsky reaction of **10** using bromoacetate **11** gave rise to a 1:1 mixture of the diastereomers (**12a** and **12b**) in 89% yield over three steps, which were inseparable in our hands. Fortunately, during our investigation to achieve the diastereoselective aldol reaction, we found that treatment of **10** with chiral auxiliary **13** under the conditions using $TiCl_4$ in the presence of DIPEA[27] afforded the desired adducts **14a** and **14b**, which were easily separable by column chromatography. After separating the diastereomers, benzyl esterification of each **14a** and **14b** using BnOH and DMAP provided alcohols **12a** (42% yield) and **12b** (30% yield), respectively. The stereochemistry of the hydroxy groups in **12a** and **12b** was determined by the modified Mosher method (see the SI).[28]



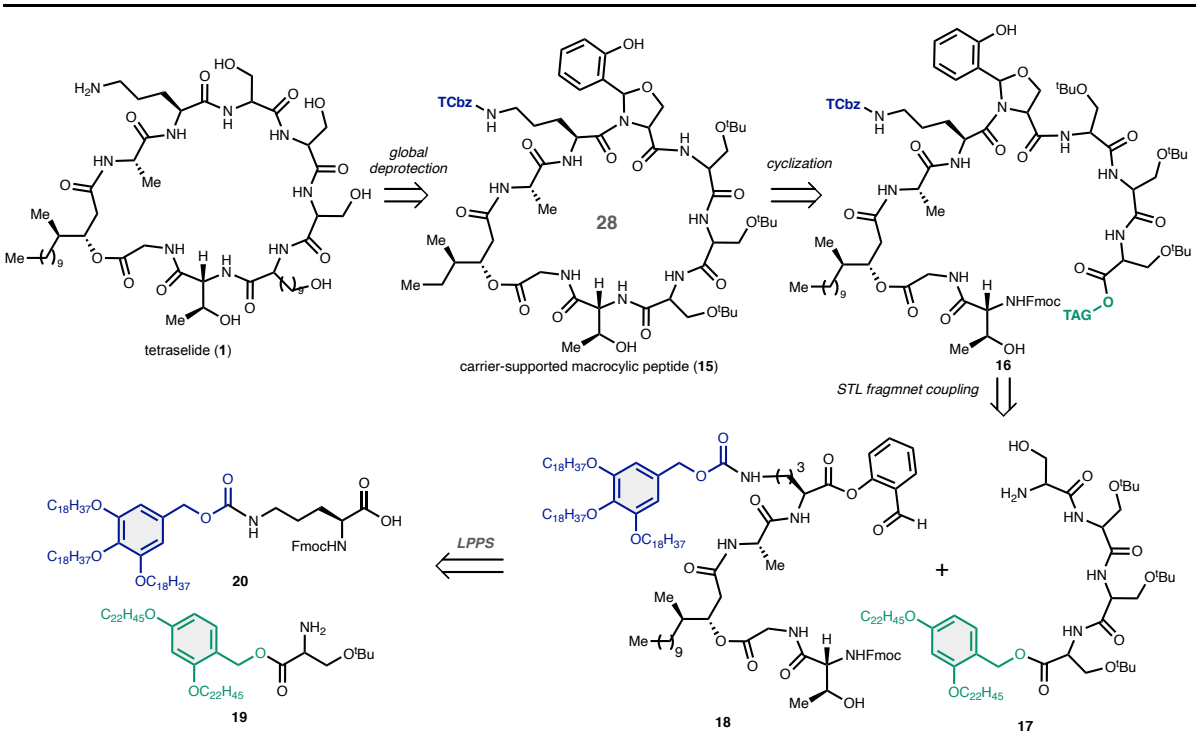
Scheme 1. Chemical degradation and chiral pool synthesis of the fatty acid.

The NMR comparison of the synthesized alcohols **12a** and **12b** to degraded **5** showed that the *anti*-configuration of the methyl and hydroxy groups is the natural form. Because the optical rotation of **12a** was opposite to that of **5**, we determined the absolute configuration of the β-hydroxy-γ-methyl-fatty acid moiety in tetraselide (**1**) is (3*S*, 4*R*). This result is consistent with our bioinformatic analysis of the PKS of **1**. Nevertheless, we could narrow down the plausible structure of natural product **1** to four isomers in the consecutive Ser residue. With this invaluable information, we commenced the total synthesis of all four isomers.

Synthetic strategy.

To determine the structure of tetraselide (**1**), we envisioned a convergent route that could efficiently synthesize all four plausible isomers which differ the position of the D-isomer in the four consecutive Ser moiety. (Scheme 2). In this regard, we thought that natural product **1** could be divided into two carrier-supported peptide fragments (**17** and **18**) by disconnection at the C-terminus of Orn and the N-terminus of Thr. In the forward sense, head-to-tail macrolactamization of **16** at the Thr-Ser moiety after cleavage of the C-terminal tag would forge the 28-membered macrocycle in **15**. To suppress problematic epimerization via the oxazolone formation at the C-terminus for convergent fragment coupling,[29] we thought to synthesize the macrocyclization precursor **16** using the Ser/Thr ligation reaction[30] between the N-terminus of the four consecutive Ser fragment **17** and salicylaldehyde ester **18**. In this way, the Ser fragment **17** would be prepared without protection of the hydroxyl group at the N-terminal side chain through one-pot LPPS[20] using tag-supported Ser **19**. The western fragment **18** could be synthesized by peptide elongation from TCbz-supported Orn **20**, which we have developed previously.[21]

Overall, our strategy using the TCbz group at the side-chain amine group would allow the systematic synthesis of head-to-tail macrocyclic peptides with the carrier molecule supported.



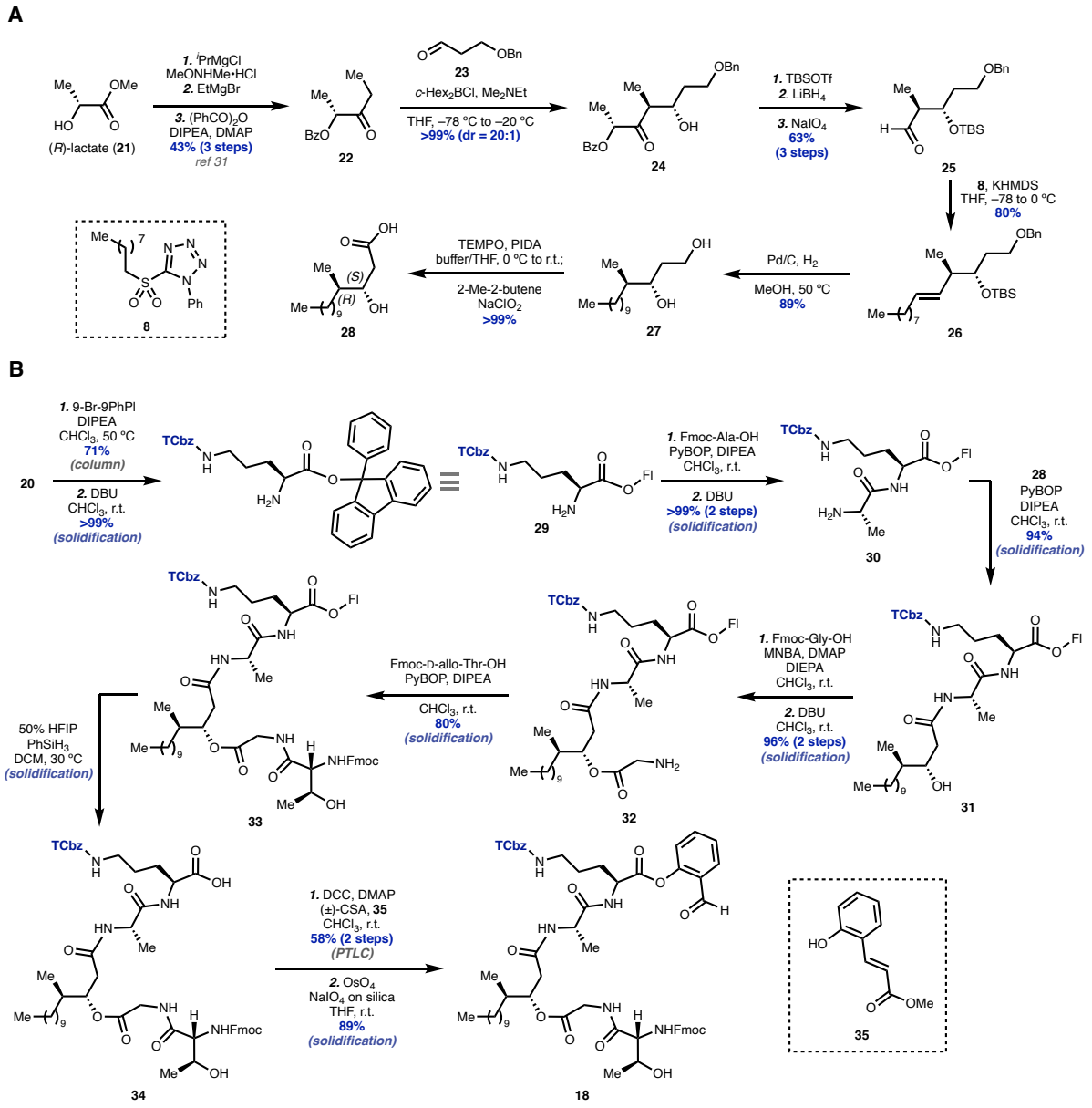
Scheme 2. Retrosynthesis of tetraselide (**1**).

Western fragment synthesis.

The western fragment synthesis commenced with diastereoselective elaboration of the fatty acid moiety in the desired enantioenriched form (Scheme 3A). We found that an aldol reaction of the know ketone **22**, prepared from (*R*)-lactate **21** over three steps, with aldehyde **23** under the conditions reported by Paterson[31] gave rise to the desired *anti*-diastereomer **24** in excellent yield and selectivity (*d.r.* = 20:1). Protecting group manipulations and oxidative cleavage afforded aldehyde **25**, which was then subjected to the Julia–Kocienski olefination conditions using PT-sulfone **8**, providing alkene **26** in 80% yield as a single isomer. Simultaneous hydrogenation of the double bond and hydrogenolysis of the benzyl group in **26** gave diol **27** in 89% yield, followed by selective oxidation of the primary alcohol in **27** to the corresponding carboxylic acid, affording **28** in >99% yield. In this way, we prepared the desired fatty acid **28** in overall 10 steps from commercially available **21**.

With fatty acid **28** in hand, we then undertook peptide elongation by investigation of the protecting group at the C-terminus of Fmoc-Orn(TCbz)-OH (**20**). As a result, the 9-phenylfluorenyl (Fl) group was installed to the acid group of **20** in 71% yield followed by removal of the Fmoc group to provide amine **29** quantitatively (Scheme 3B). Condensation of **29** with Fmoc-Ala-OH and subsequent removal of the Fmoc group produced **30** in quantitative yield over two steps. Dipeptide **30** was condensed with fatty acid **28** (66% yield), followed by esterification of alcohol **31** with Fmoc-Gly-OH using MNBA and deprotection of the amine group, providing **32** in 96% yield over two steps. Subjection of **32** to the condensation conditions with Fmoc-D-allo-Thr-OH to produce **33** (80% yield) and subsequent deprotection of the acid group under the mild conditions provided **34**. Lastly,

salicylaldehyde ester **18** was synthesized by esterification of **34** with phenol **35** (58% yield over 2 steps from **33**) and oxidative cleavage of the corresponding unsaturated ester (89% yield). Overall, we prepared the western fragment **18** in a total of 11 steps from Fmoc-Orn(TCbz)-OH (**20**).



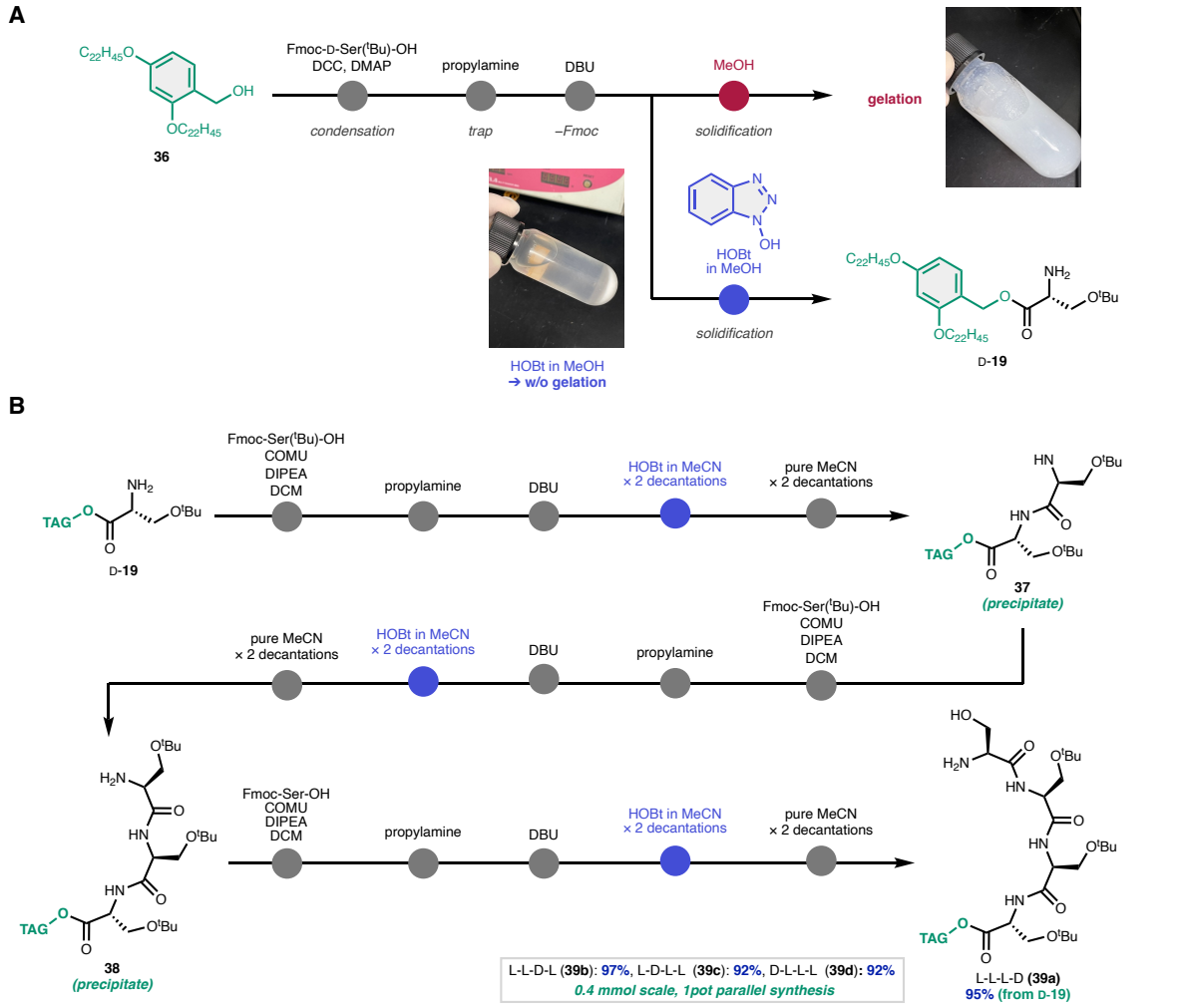
Scheme 3. Western fragment synthesis.

Consecutive Ser fragment synthesis.

We began investigation of the Ser fragment synthesis with a one-pot protocol similar to that we previously developed in the total synthesis of emodepsides.[20] Considering the orthogonality to the TCbz group, we chose tag-carrier **36** reported by Chiba and coworkers[32] which could be selectively removed at the late-stage under mild conditions. Esterification of **36** with Fmoc-D-Ser(ᵗBu)-OH using DCC and DMAP, which was followed by trapping the excess activated ester reagent residue with propylamine and cleavage of the Fmoc group using DBU to furnish the corresponding amine D-**19**. Unexpectedly, the typical purification procedure of LPPS using the tag-

carrier by solidification with MeOH led to gelation of the mixture and diminished the yield (Scheme 4A). To our delight, we found that solidification using MeOH containing HOBt as a moderate acidic additive, which we previously employed to prevent the undesired cross condensation in the total synthesis of argifin,[17] successfully suppressed the problematic gelation. We assumed that the addition of HOBt affects the intermolecular hydrogen bonding network of the carrier-supported Ser derivative in the solution of MeOH.

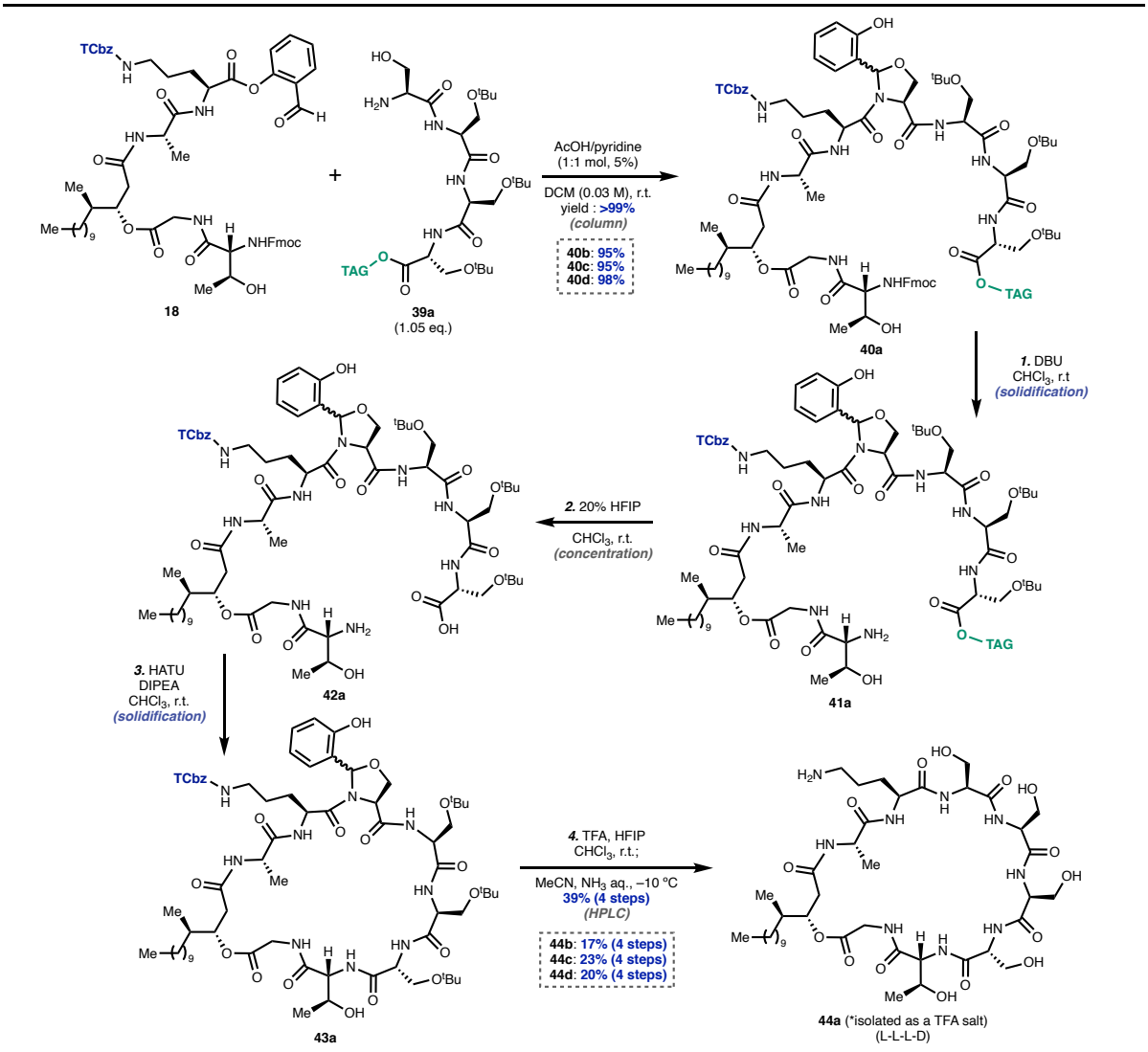
With this simple and practical procedure to handle with the carrier-supported polar peptide, we attempted one-pot synthesis of the consecutive Ser fragment (Scheme 4B). The carrier-supported Ser D-**19** was subjected to the condensation conditions using COMU with Fmoc-Ser(*t*Bu)-OH, and subsequent treatment with propylamine and DBU to remove the Fmoc group. A solution of HOBt in MeCN was used for solidification, which the precipitate was washed once with MeCN containing HOBt, followed by additional pure MeCN twice to provide dipeptide **37**. This procedure was repeated using Fmoc-Ser(*t*Bu)-OH to afford tripeptide **38**, and then analogously with Fmoc-Ser-OH to lead to tetrapeptide **39a** without events. Of note, we synthesized all four plausible isomers of the Ser fragments (**39b-d**) in a range of 92–97% yield. As this method could be performed on a 0.4 mmol scale, we demonstrated the one-pot parallel synthesis to rapidly access the four fragments at scale.



Scheme 4. Pot-economical Ser fragment synthesis.

Total synthesis and the absolute structure determination of the natural product.

With both fragments in hand, we investigated fragment coupling by the STL reaction (Scheme 5). After a survey of the buffered reaction conditions that could promote imine formation with maintaining the solubility of carrier-supported peptide fragments, we found that treatment of **18** and **39a** with 5% AcOH/pyridine (1:1 mol/mol) in DCM (0.03 M) gave rise to the desired coupled product **40a** in quantitative yield as a diastereomixture of the *N,O*-acetal moiety. Remarkably, the STL reaction proceeded efficiently with only a slight excess (1.05 eq.) of **39a** without epimerization. After sequential removal of the Fmoc group and the carrier-molecule at the C-terminus in **40a**, amino acid **42a** was subjected to the macrocyclization conditions using HATU in the presence of DIPEA, affording head-to-tail macrocyclic peptide **43a**. Finally, deprotection of **43a** completed the synthesis of the desired peptide **44a** in 39% yield over four steps, which all spectra data were consistent with that of tetraselide (**1**). Analogously, we prepared other three isomers **44b-d** in four steps (17–23% yield) and determined the order of the four consecutive Ser moiety in natural product **1** is L-L-L-D (shown as compound **44a**).



Scheme 5. Fragment coupling and total synthesis of tetraselide.

In conclusion, we have achieved the first total synthesis of tetraselide to determine its absolute structure. Motivated by our group's long-standing interest in exploring new natural products, this macrocyclic depsipeptide was isolated as a potent antifungal compound from the marine-derived filamentous fungus. We have implemented bioinformatic analysis to predict two ambiguous stereocenters in the fatty acid moiety, as well as chemical degradation and chiral pool synthesis to elucidate the stereochemistry. With this information, a convergent synthetic strategy using two carrier-supported peptide fragments has been developed to synthesize four plausible isomers of the natural product. The western fragment was synthesized via unconventional LPPS based on our previous work using the TCbz group, while we established one-pot parallel synthesis of the four Ser fragments. The challenging peptide fragment coupling was accomplished utilizing the Ser/Thr ligation reaction. Head-to-tail macrocyclization of the carrier-supported precursors using the TCbz auxiliary allowed us to handle the macrocyclic peptides with a systematic way. Overall, our work highlights the power of current LPPS to enable practical convergent synthesis of macrocyclic peptides.

Acknowledgements

The authors are grateful to Distinguished Emeritus Professor Satoshi Ōmura (Kitasato University) for his helpful support and suggestions. We thank Drs. Yuriko Nagano (Japan Agency for Marine-Earth Science and Technology) and Kenichi Nonaka (Teikyo University of Science) for the deposition of the fungal strain. This study was partially supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grants No. 22K06535 (T.H.), 23K06053 (Y.N.), and Research Support Project for Life Sciences Research and Drug Discovery (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)) from AMED (Grant Number JP24ama121035). The authors thank Dr. Kenichiro Nagai, Ms. Noriko Sato, and Ms. Reiko Seki (School of Pharmacy, Kitasato University) for spectroscopic assistance.

References

[1] (a) Zorzi, A.; Deyle, K.; Heinis, C. Cyclic peptide therapeutics: past, present and future. *Curr. Opin. Chem. Biol.* **2017**, *38*, 24–29. (b) Morrison, C. Constrained peptides' time to shine? *Nat. Rev. Drug Discovery* **2018**, *17*, 531–533.

[2] Vinogradov, A. A.; Yin, Y.; Suga, H. Macrocyclic Peptides as Drug Candidates: Recent Progress and Remaining Challenges. *J. Am. Chem. Soc.* **2019**, *141*, 4167–4181.

[3] (a) Dougherty, P. G.; Sahni, A.; Pei, D. Understanding Cell Penetration of Cyclic Peptides. *Chem. Rev.* **2019**, *119*, 10241–10287. (b) Ohta, A.; Tanada, M.; Shinohara, S.; Morita, Y.; Nakano, K.; Yamagishi, Y.; Takano, R.; Kariyuki, S.; Iida, T.; Matsuo, A.; Ozeki, K.; Emura, T.; Sakurai, Y.; Takano, K.; Higashida, A.; Kojima, M.; Muraoka, T.; Takeyama, R.; Kato, T.; Kimura, K.; Ogawa, K.; Ohara, K.; Tanaka, S.; Kikuchi, Y.; Hisada, N.; Hayashi, R.; Nishimura, Y.; Nomura, K.; Tachibana, T.; Irie, M.; Kawada, H.; Torizawa, T.; Murao, N.; Kotake, T.; Tanaka, M.; Ishikawa, S.; Miyake, T.; Tamiya, M.; Arai, M.; Chiyoda, A.; Akai, S.; Sase, H.; Kuramoto, S.; Ito, T.; Shiraishi, T.; Kojima, T.; Iikura, H. Validation of a New Methodology to Create Oral Drugs beyond the Rule of 5 for Intracellular Tough Targets. *J. Am. Chem. Soc.* **2023**, *145*, 24035–24051.

[4] (a) Biron, E.; Chatterjee, J.; Ovadia, O.; Langenegger, D.; Brueggen, J.; Hoyer, D.; Schmid, H. A.; Jelinek, R.; Gilon, C.; Hoffman, A.; Kessler, H. Improving Oral Bioavailability of Peptides by Multiple N-Methylation: Somatostatin Analogues. *Angew. Chem. Int. Ed.* **2008**, *47*, 2595–2599. (b) White, T. R.; Renzelman, C. M.; Rand, A. C.; Rezai, T.; McEwen, C. M.; Gelev, V. M.; Turner, R. A.; Linington, R. G.; Leung, S. S. F.; Kalgutkar, A. S.; Bauman, J. N.; Zhang, Y.; Liras, S.; Price, D. A.; Mathiowetz, A. M.; Jacobson, M. P.; Lokey, R. S. On-Resin N-Methylation of Cyclic Peptides for Discovery of Orally Bioavailable Scaffolds. *Nat. Chem. Biol.* **2011**, *7*, 810–817. (c) Wang, C. K.; Northfield, S. E.; Colless, B.; Chaousis, S.; Hamernig, I.; Lohman, R.-J.; Nielsen, D. S.; Schroeder, C. I.; Liras, S.; Price, D. A.; Fairlie, D. P.; Craik, D. J. Rational Design and Synthesis of an Orally Bioavailable Peptide Guided by NMR Amide Temperature Coefficients. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 17504–17509.

[5] (a) Sivanathan, S.; Scherkenbeck, J. Cyclodepsipeptides: A Rich Source of Biologically Active Compounds for Drug Research. *Molecules* **2014**, *19*, 12368–12420. (b) Liu, S.-X.; Ou-Yang, S.-Y.; Lu, Y.-F.; Guo, C.-L.; Dai, S.-Y.; Li, C.; Yu, T.-Y.; Pei, Y.-H. Recent Advances on Cyclodepsipeptides: Biologically Active Compounds for Drug Research. *Front. Microbiol.* **2023**, *14*, 1276928.

[6] (a) Weinmüller, M.; Rechenmacher, F.; Kiran Marelli, U.; Reichart, F.; Kapp, T. G.; Räder, A. F. B.; Di Leva, F. S.; Marinelli, L.; Novellino, E.; Muñoz-Félix, J. M.; Hodivala-Dilke, K.; Schumacher, A.; Fanous, J.; Gilon, C.; Hoffman, A.; Kessler, H. Overcoming the Lack of Oral Availability of Cyclic Hexapeptides: Design of a Selective and Orally Available Ligand for the Integrin Avβ3. *Angew. Chem. Int. Ed.* **2017**, *56*, 16405–16409. (b) Schumacher-Klinger, A.; Fanous, J.; Merzbach, S.; Weinmüller, M.; Reichart, F.; Räder, A. F. B.; Gitlin-Domagalska, A.; Gilon, C.; Kessler, H.; Hoffman, A. Enhancing Oral Bioavailability of Cyclic RGD Hexa-Peptides by the Lipophilic Prodrug Charge Masking Approach: Redirection of Peptide Intestinal Permeability from a Paracellular to Transcellular Pathway. *Mol. Pharmaceutics* **2018**, *15*, 3468–3477. (c) Hosono, Y.; Uchida, S.; Shinkai, M.; Townsend, C. E.; Kelly, C. N.; Naylor, M. R.; Lee, H.-W.; Kanamitsu, K.; Ishii, M.; Ueki, R.;

Ueda, T.; Takeuchi, K.; Sugita, M.; Akiyama, Y.; Lokey, S. R.; Morimoto, J.; Sando, S. Amide-to-Ester Substitution as a Stable Alternative to N-Methylation for Increasing Membrane Permeability in Cyclic Peptides. *Nat. Commun.* **2023**, *14*, 1416. (d) Cao, L.; Xu, Z.; Shang, T.; Zhang, C.; Wu, X.; Wu, Y.; Zhai, S.; Zhan, Z.; Duan, H. Multi_CycGT: A Deep Learning-Based Multimodal Model for Predicting the Membrane Permeability of Cyclic Peptides. *J. Med. Chem.* **2024**, *67*, 1888–1899.

[7] Ōmura, S. A Splendid Gift from the Earth: The Origins and Impact of the Avermectins (Nobel Lecture). *Angew. Chem. Int. Ed.* **2016**, *55*, 10190–10209.

[8] (a) Watanabe, Y.; Suga, T.; Narusawa, S.; Iwatsuki, M.; Nonaka, K.; Nakashima, T.; Shinohara, Y.; Shiotsuki, T.; Ichimaru, N.; Miyoshi, H.; Asami, Y.; Ōmura, S.; Shiomi, K. Decatamariic Acid, a New Mitochondrial Respiration Inhibitor Discovered by Pesticidal Screening Using Drug-Sensitive Saccharomyces Cerevisiae. *J. Antibiot.* **2017**, *70*, 395–399. (b) Watanabe, Y.; Suga, T.; Narusawa, S.; Iwatsuki, M.; Nonaka, K.; Nakashima, T.; Shinohara, Y.; Shiotsuki, T.; Ichimaru, N.; Miyoshi, H.; Asami, Y.; Ōmura, S.; Shiomi, K. Decatamariic Acid, a New Mitochondrial Respiration Inhibitor Discovered by Pesticidal Screening Using Drug-Sensitive Saccharomyces Cerevisiae. *J. Antibiot.* **2017**, *70*, 395–399. (c) Watanabe, Y.; Asami, Y.; Narusawa, S.; Hashimoto, S.; Iwatsuki, M.; Nonaka, K.; Shinohara, Y.; Shiotsuki, T.; Ichimaru, N.; Miyoshi, H.; Ōmura, S.; Shiomi, K. Ascosteroside D, a New Mitochondrial Respiration Inhibitor Discovered by Pesticidal Screening Using Insect ADP/ATP Carrier Protein-Expressing Saccharomyces Cerevisiae. *J. Antibiot.* **2018**, *71*, 146–148. (d) Watanabe, Y.; Yoshida, Y.; Tokiwa, T.; Higo, M.; Ban, S.; Ikeda, A.; Noguchi, Y.; Hirose, T.; Sunazuka, T.; Nonaka, K.; Yaguchi, T.; Iwatsuki, M. Hakuhybotric Acid, a New Antifungal Polyketide Produced by a Mycoparasitic Fungus *Hypomyces Pseudocorticiicola* FKI-9008. *J. Gen. Appl. Microbiol.* **2022**, *68*, 200–206. (e) Watanabe, Y.; Takahashi, S.; Ito, S.; Tokiwa, T.; Noguchi, Y.; Azami, H.; Kojima, H.; Higo, M.; Ban, S.; Nagai, K.; Hirose, T.; Sunazuka, T.; Yaguchi, T.; Nonaka, K.; Iwatsuki, M. Hakuhybotrol, a Polyketide Produced by *Hypomyces Pseudocorticiicola*, Characterized with the Assistance of 3D ED/MicroED. *Org. Biomol. Chem.* **2023**, *21*, 2320–2330. (f) Azami, H.; Watanabe, Y.; Sakai, K.; Nakahara, H.; Kojima, H.; Tokiwa, T.; Nonaka, K.; Noguchi, Y.; Nagano, Y.; Hirose, T.; Sunazuka, T.; Matsui, H.; Arima, N.; Abe, K.; Hanaki, H.; Iwatsuki, M. Antifungal Profile against Candida Auris Clinical Isolates of Tyroscherin and Its New Analog Produced by the Deep-Sea-Derived Fungal Strain Scedosporium Apiospermum FKJ-0499. *J. Antibiot.* **2024**, *77*, 156–162.

[9] Of note, the same planar structure has been reported in the patent (JP 2004-168685). However, the reported NMR spectra did not match with those of tetraselide (**1**).

[10] Harada, K.; Fujii, K.; Hayashi, K.; Suzuki, M.; Ikai, Y.; Oka, H. Application of d,l-FDLA Derivatization to Determination of Absolute Configuration of Constituent Amino Acids in Peptide by Advanced Marfey's Method. *Tetrahedron Letters* **1996**, *37*, 3001–3004.

[11] Merrifield, R. B. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.

[12] Jaradat, D. M. M. Thirteen decades of peptide synthesis: key developments in solid phase peptide synthesis and amide bond formation utilized in peptide ligation. *Amino Acids*, **2018**, *50*, 39–68.

[13] Martin, V.; Egelund, P. H. G.; Johansson, H.; Thordal Le Quement, S.; Wojcik, F.; Sejer Pedersen, D. Greening

the Synthesis of Peptide Therapeutics: An Industrial Perspective. *RSC Adv.* **2020**, *10*, 42457–42492.

[14] (a) Isidro-Llobet, A.; Kenworthy, M. N.; Mukherjee, S.; Kopach, M. E.; Wegner, K.; Gallou, F.; Smith, A. G.; Roschangar, F. Sustainability Challenges in Peptide Synthesis and Purification: From R&D to Production. *J. Org. Chem.* **2019**, *84*, 4615–4628. (b) Mueller, L. K.; Baumruck, A. C.; Zhdanova, H.; Tietze, A. A. Challenges and Perspectives in Chemical Synthesis of Highly Hydrophobic Peptides. *Front. Bioeng. Biotechnol.* **2020**, *8*, 162.

[15] (a) Bayer, E.; Mutter, M. Liquid Phase Synthesis of Peptides. *Nature* **1972**, *237*, 512–513. (b) Mutter, M.; Bayer, E. Rapid Procedure for Liquid-Phase Peptide Synthesis: The Crystallization Method. *Angew. Chem. Int. Ed.* **1974**, *13*, 88–89.

[16] Tamiaki, H.; Obata, T.; Azefu, Y.; Toma, K. A Novel Protecting Group for Constructing Combinatorial Peptide Libraries. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 733–738.

[17] Hirose, T.; Kasai, T.; Akimoto, T.; Endo, A.; Sugawara, A.; Nagasawa, K.; Shiomi, K.; Ōmura, S.; Sunazuka, T. Solution-Phase Total Synthesis of the Hydrophilic Natural Product Argifin Using 3,4,5-Tris(Octadecyloxy)Benzyl Tag. *Tetrahedron* **2011**, *67*, 6633–6643.

[18] (a) Hayashi, Y.; Fukasawa, W.; Hirose, T.; Iwatsuki, M.; Hokari, R.; Ishiyama, A.; Kanaida, M.; Nonaka, K.; Také, A.; Otoguro, K.; Ōmura, S.; Shiomi, K.; Sunazuka, T. Kozupeptins, Antimalarial Agents Produced by Paracamarosporium Species: Isolation, Structural Elucidation, Total Synthesis, and Bioactivity. *Org. Lett.* **2019**, *21*, 2180–2184. (b) Hayashi, Y.; Hirose, T.; Iwatsuki, M.; Ōmura, S.; Sunazuka, T. Synthesis of the Antimalarial Peptide Aldehyde, a Precursor of Kozupeptin A, Utilizing a Designed Hydrophobic Anchor Molecule. *Org. Lett.* **2019**, *21*, 8229–8233.

[19] Watanabe, A.; Noguchi, Y.; Hirose, T.; Monma, S.; Satake, Y.; Arai, T.; Masuda, K.; Murashima, N.; Shiomi, K.; Ōmura, S.; Sunazuka, T. Efficient Synthesis of a Ryanodine Binding Inhibitor Verticilide Using Two Practical Approaches. *Tetrahedron Lett.* **2020**, *61*, 151699.

[20] Noguchi, Y.; Sekikawa, S.; Nogaki, Y.; Satake, Y.; Murashima, N.; Kirisawa, T.; Schiffer, G.; Köebberling, J.; Hirose, T.; Sunazuka, T. Pot-Economical Synthesis of Cyclic Depsipeptides Using a Hydrophobic Anchor Molecule toward the Construction of an Unnatural Peptide Library. *Tetrahedron* **2022**, *128*, 133100.

[21] Nakahara, H.; Sennari, G.; Noguchi, Y.; Hirose, T.; Sunazuka, T. Development of a Nitrogen-Bound Hydrophobic Auxiliary: Application to Solid/Hydrophobic-Tag Relay Synthesis of Calpinactam. *Chem. Sci.* **2023**, *14*, 6882–6889.

[22] Edman, P. Method for Determination of the Amino Acid Sequence in Peptides. *Acta Chem. Scand.* **1950**, *4*, 283–293.

[23] (a) Keatinge-Clay, A. T. A Tylosin Ketoreductase Reveals How Chirality Is Determined in Polyketides. *Chemistry & Biology* **2007**, *14*, 898–908. (b) Keatinge-Clay, A. T. Stereocontrol within Polyketide Assembly Lines. *Nat. Prod. Rep.* **2016**, *33*, 141–149.

[24] Weissman, K. J. Polyketide Stereocontrol: A Study in Chemical Biology. *Beilstein J. Org. Chem.* **2017**, *13*, 348–371.

[25] Pullin, R. D. C.; Rathi, A. H.; Melikhova, E. Y.; Winter, C.; Thompson, A. L.; Donohoe, T. J. Tethered Aminohydroxylation: Synthesis of the β-Amino Acid of Microsclerodermins A and B. *Org. Lett.* **2013**, *15*, 5492–5495.

[26] Blakemore, P. R. The Modified Julia Olefination: Alkene Synthesis via the Condensation of Metallated Heteroarylalkylsulfones with Carbonyl Compounds. *J. Chem. Soc., Perkin Trans. 1* **2002**, *23*, 2563–2585.

[27] Crimmins, M. T.; She, J. An Improved Procedure for Asymmetric Aldol Additions with N-Acyl Oxazolidinones, Oxazolidinethiones and Thiazolidinethiones. *Synlett* **2004**, *8*, 1371–1374.

[28] Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. High-Field FT NMR Application of Mosher's Method. The Absolute Configurations of Marine Terpenoids. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.

[29] Carpino, L. A.; El-Faham, A. The Diisopropylcarbodiimide/ 1-Hydroxy-7-Azabenzotriazole System: Segment Coupling and Stepwise Peptide Assembly. *Tetrahedron* **1999**, *55*, 6813–6830.

[30] Li, X.; Lam, H. Y.; Zhang, Y.; Chan, C. K. Salicylaldehyde Ester-Induced Chemoselective Peptide Ligations: Enabling Generation of Natural Peptidic Linkages at the Serine/Threonine Sites. *Org. Lett.* **2010**, *12*, 1724–1727.

[31] (a) Paterson, I; Wallace, D. J.; Velázquez, S. M. Studies in polypropionate synthesis: High π-face selectivity in *syn* and *anti* aldol reactions of chiral boron enolates of lactate-derived ketones. *Tetrahedron Lett.* **1994**, *35*, 9083–9086. (b) Paterson, I.; Wallace, D. J. Manipulation of the aldol adducts from lactate-derived ketones. A versatile chiral auxiliary for the asymmetric synthesis of β-hydroxy carbonyl compounds. *Tetrahedron Lett.* **1994**, *35*, 9087–9090. (c) Lam, N. Y. S.; Stockdale, T. P.; Anketell, M. J.; Paterson, I. Conquering Peaks and Illuminating Depths: Developing Stereocontrolled Organic Reactions to Unlock Nature's Macrolide Treasure Trove. *Chem. Commun.* **2021**, *57*, 3171–3189.

[32] Tana, G.; Kitada, S.; Fujita, S.; Okada, Y.; Kim, S.; Chiba, K. A Practical Solution-Phase Synthesis of an Antagonistic Peptide of TNF-α Based on Hydrophobic Tag Strategy. *Chem. Commun.* **2010**, *46*, 8219.