

Cite this: *Chem. Sci.*, 2025, 16, 6060 All publication charges for this article have been paid for by the Royal Society of Chemistry

Isolation, total synthesis and structure determination of antifungal macrocyclic depsipeptide, tetraselide†

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

Macrocyclic peptides, including depsipeptides, are an emerging new modality in drug discovery research. Tetraselide, an antifungal cyclic peptide isolated from a marine-derived filamentous fungus, possesses a unique amphiphilic structural feature consisting of five consecutive β -hydroxy-amino acid residues and fatty acid moieties. Because the structure elucidation of the naturally occurring product left six stereocenters ambiguous, we implemented bioinformatic analyses, chemical degradation studies and chiral pool fragment synthesis to identify two of the undetermined stereocenters. Convergent total synthesis of the four remaining plausible isomers of tetraselide was accomplished via liquid-phase peptide synthesis (LPPS) using soluble hydrophobic tag auxiliaries. The key advances involve fragment coupling by the serine/threonine ligation (STL) 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.

Received 22nd January 2025
Accepted 3rd March 2025

DOI: 10.1039/d5sc00566c

rsc.li/chemical-science

Introduction

Over the last decade, cyclic peptides have drawn much attention in drug discovery efforts as an attractive modality due to their unique properties such as high binding affinity and selectivity targeting protein–protein interactions (PPIs) with low toxicity.¹ Thus, cyclic peptides can potentially serve as advantageous therapeutics complementary to antibodies and small drug molecules.² Although cell permeability and oral bioavailability are generally considered to be challenging,³ there is emerging research that *N*-methyl amides⁴ and/or depsipeptides⁵ enhance membrane permeation.⁶ Despite chemical accessibility of this class of peptide molecules, the vast majority of therapeutic cyclic peptides have been developed based on the structures of natural products. Therefore, exploration of new cyclic peptides and depsipeptides from natural sources plays an important role as a foundation of drug leads in medicinal chemistry.

Our research group has been on a quest for novel secondary metabolites of microorganisms from underexplored natural sources.⁷ During the course of our screening program to explore antifungal natural products,⁸ we isolated a macrocyclic depsipeptide, tetraselide (**1**, Fig. 1A), from a culture broth of

Trichoderma sp. FKJ-0225, a marine-derived filamentous fungus. As a result of NMR spectroscopic and LC-MS/MS analyses (see the ESI†), we identified six polar amino acids, Orn, Thr, Ser, Ser, Ser, Ser, two hydrophobic Ala and Gly residues, and a β -hydroxy- γ -methyl fatty acid in the structure of **1**.⁹ The five consecutive β -hydroxy-amino acid residues represent a unique structural feature that has rarely been seen in natural products.¹⁰ The advanced Marfey method¹¹ to analyze the absolute configuration of amino acids revealed the presence of L-Ala, L-Orn, and D-allo-Thr. The four consecutive Ser moieties were found to be a 3 : 1 mixture of L- and D-isomers, although the position of the D-Ser residue in the sequence 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,¹² solid-phase peptide synthesis (SPPS) has become one of the most common methods to elaborate peptide compounds (Fig. 1B).¹³ 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, the convergent synthetic approach, which requires the preparation of peptide fragments through their isolation/purification over several steps, is generally considered to be less efficient than the more commonly employed SPPS strategies.¹⁴ Despite this, several scalable approaches for peptide fragment coupling in solution have

Omura Satoshi Memorial Institute, Graduate School of Infection Control Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan. E-mail: thiore@lisci.kitasato-u.ac.jp; sunazuka@lisci.kitasato-u.ac.jp

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d5sc00566c>

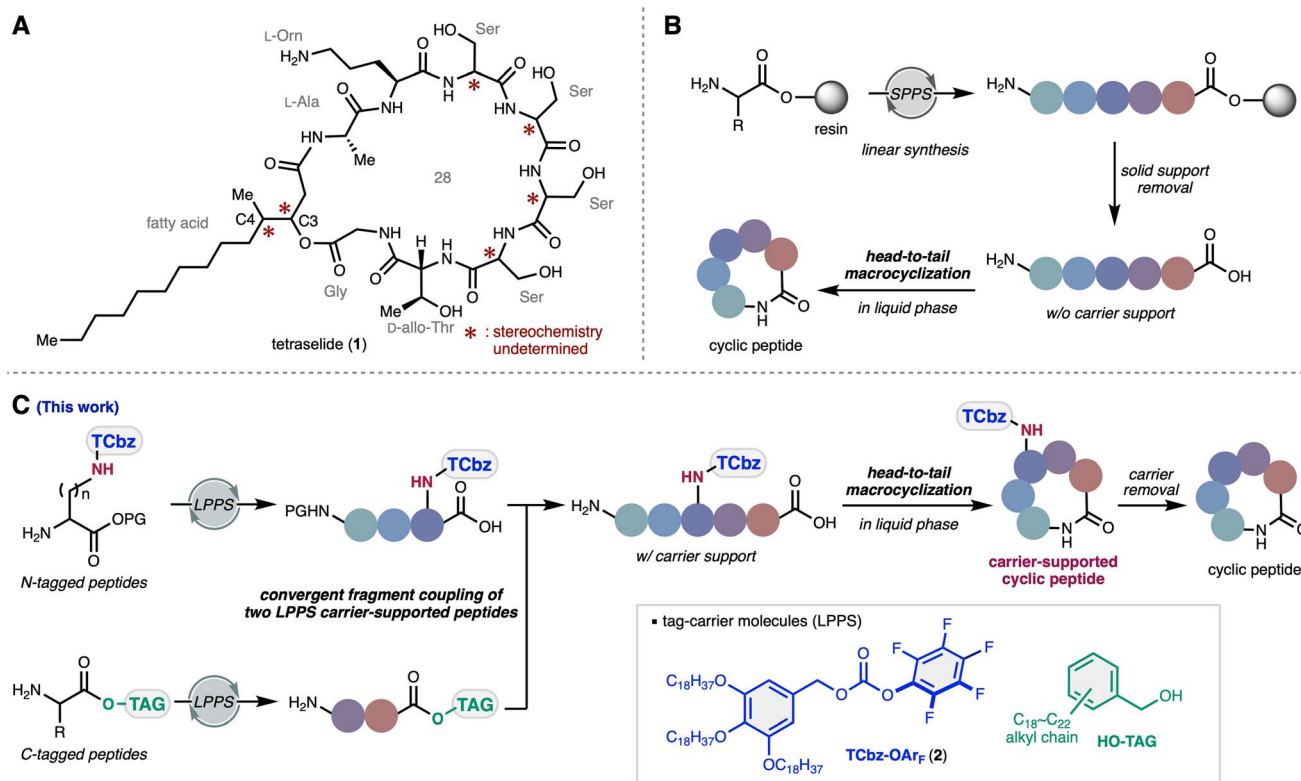


Fig. 1 Natural product tetraselide and peptide synthesis strategy. (A) The structure of tetraselide. (B) Typical SPPS strategy for cyclic peptides. (C) Our convergent strategy to synthesize macrocyclic peptides. SPPS: solid-phase peptide synthesis. LPPS: liquid-phase peptide synthesis. PG: protecting group.

been developed.¹⁵ Additionally, head-to-tail macrocyclization on the solid-phase requires side chain anchoring strategies or removal from the solid-phase carrier necessitates a solution phase cyclization.¹⁶ The solubility and polarity of peptides without the carrier support sometimes result in troublesome handling, particularly with amphiphilic and zwitterionic peptides.¹⁷

As an alternative approach to SPPS, our group has been interested in liquid-phase peptide synthesis (LPPS)¹⁸ using soluble hydrophobic tags.¹⁹ We applied this method to the total syntheses of argifin,²⁰ kozupeptin,²¹ verticillide,²² and emodepside derivatives.²³ Recently, we also developed a carbonate-type tag reagent TCbz-OAr_F (2) that enabled attachment of the tag-carrier to the amine group of amino acids (Fig. 1C).²⁴ Orthogonal to the typical approach that relies on installation of the carrier molecule to the C-terminus, we thought to develop a *de novo* synthetic strategy using the tag-reagent 2. Herein, we report the convergent total synthesis and structure determination of tetraselide (1) *via* LPPS utilizing two tag-carrier molecules. The homogeneous reaction conditions of LPPS enabled convergent fragment coupling of two carrier-supported peptides in equimolar amounts. Because the TCbz group was installed on the amine group of the Orn side chain, we successfully synthesized head-to-tail macrocyclic peptides supported on a carrier molecule. This strategy facilitated systematic convergent syntheses of all plausible isomers of the four structurally

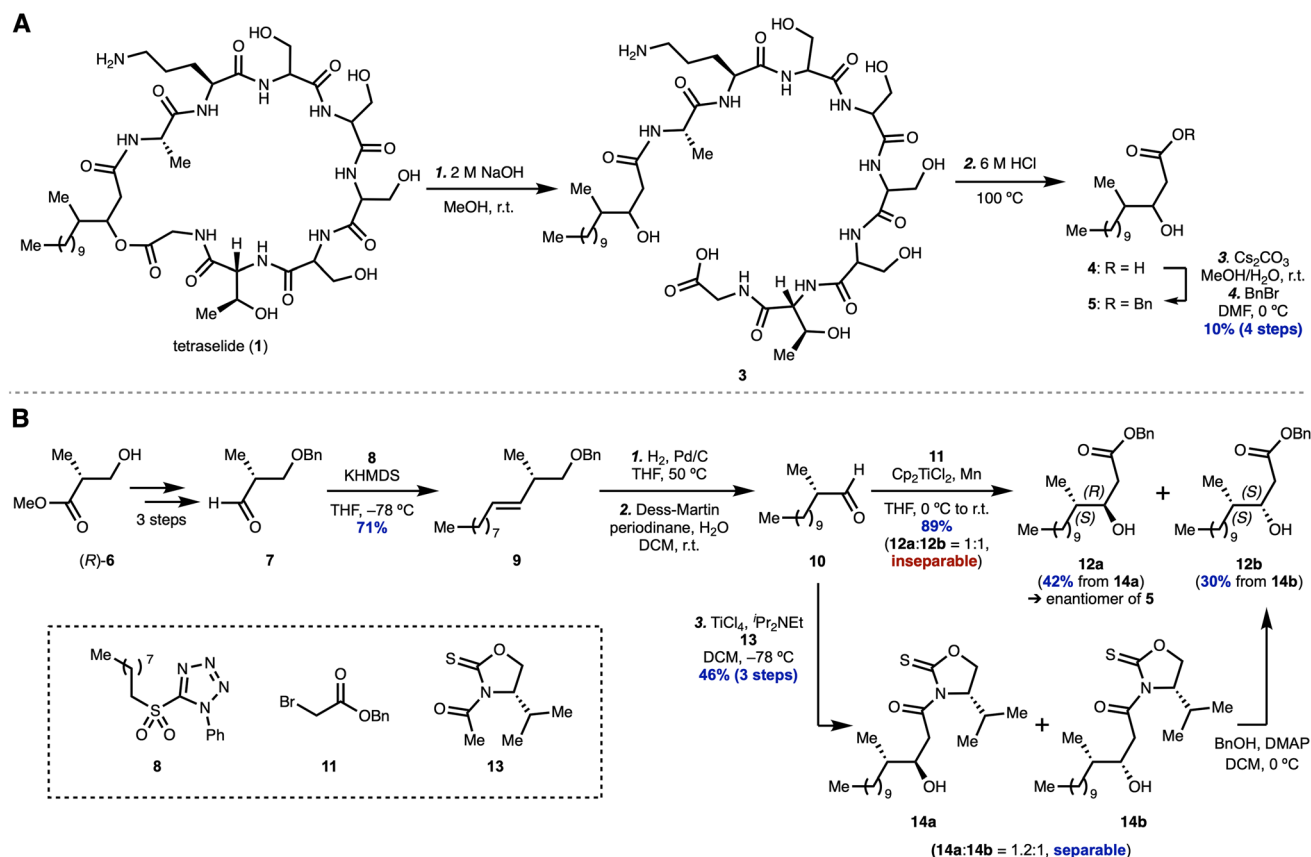
ambiguous consecutive Ser moieties and allowed us to determine the structure of 1.

Results and discussion

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

As described earlier, our preliminary efforts for the structural elucidation of tetraselide (1) suggested that there were 16 possible diastereomers—four of the consecutive Ser moieties and four of the fatty acid moiety. Our failure to clarify the structure of 1 using Edman degradation²⁵ and X-ray crystallographic analysis led us to implement biosynthetic analysis to narrow down the structural candidates of 1. First, from the draft genome sequence of the FKJ-0225 strain, we found five genes (*ttsA* to *ttsE*, ESI Fig. S12A,† accession number: LC847109) associated with the biosynthesis of 1 by mining a candidate biosynthetic gene cluster using antiSMASH 7.0 (ref. 26) and 2ndfind.²⁷ This indicated that the β-hydroxy-γ-methyl fatty acid moiety produced by the polyketide synthase (PKS; *ttsA*) is transferred to the non-ribosomal peptide synthase (NRPS; *ttsE*). Subsequent peptide elongation from L-Ala to Gly, followed by macrolactonization at the C-terminus of Gly with the hydroxy group at C3, furnishes tetraselide (ESI Fig. S12B†). To predict the position of the D-Ser residue, Clabofold²⁸ and AutoDock²⁹ were used to conduct docking simulations of the adenylation (A) domains in the modules that construct the consecutive Ser





Scheme 1 Initial experimentations to support our hypothesis through bioinformatic analysis. (A) Chemical degradation of the naturally occurring product. (B) Chiral pool synthesis of fatty acids.

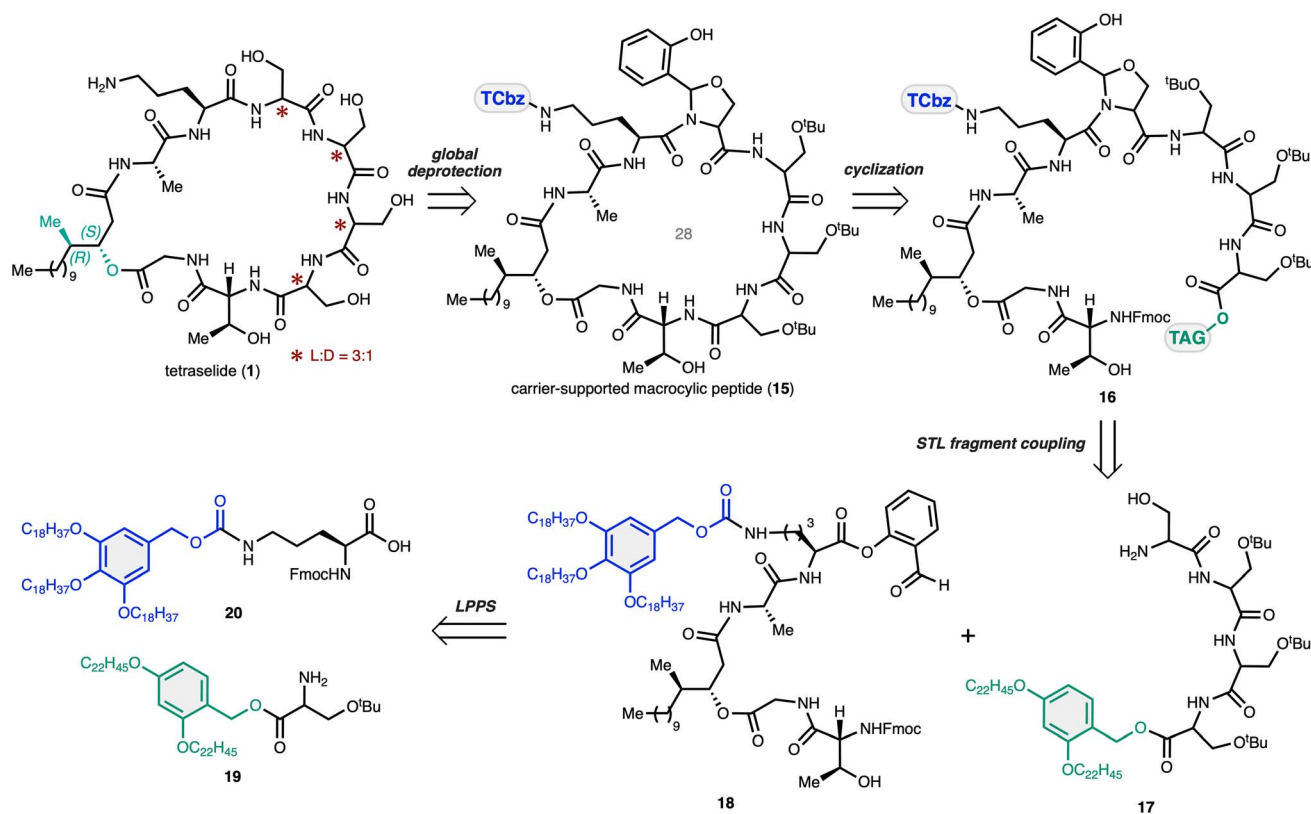
moiety. Consequently, all A domains are likely responsible for the recognition of only L-Ser (ESI Fig. S13[†]) and the corresponding epimerization (E) domains are not involved, suggesting that L-Ser might be epimerized at a condensation (C) domain in the modules.³⁰ As a result of phylogenetic analysis of the C domains (ESI Fig. S14[†]), we speculated that the Ser residue next to Thr might be the D-form, although the mechanism of epimerization is unclear at this stage (see the ESI[†] for details).

We expected 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 PKSs relies on the amino acid sequence of each PKS domain. As a result of the bioinformatic analysis (ESI Fig. S15[†]), 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.³¹ In addition, we identified that the Tyr residue—one of the proton donors in the enoyl reductase (ER) domain—is substituted with Leu, suggesting that the methyl group would likely be in the D-configuration.³² These results indicated that the β -hydroxy- γ -methyl fatty acid moiety in **1** could be in the (3*S*)-hydroxy and (4*R*)-methyl configurations. To support this hypothesis, we next attempted a chemical degradation study of the naturally occurring **1** and synthesis of the degraded fragment using a chiral pool approach.

Our degradation experiments of **1** began with hydrolysis of the ester moiety by treatment with 2 M NaOH, yielding linear peptide **3** (Scheme 1A). Peptide **3** was subjected to 6 M HCl at 100 °C to hydrolyze the amide bonds, affording fatty acid **4** as the major component after acid extraction. In order to facilitate UV detection for 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 next chemically synthesized fatty acid derivatives to confirm the absolute stereochemistry.

In order to implement enantiospecific synthesis for the structural determination of **5**, we employed a chiral pool starting material. We began our investigation with Roche ester (*R*)-**6** because the (*S*)-enantiomer is more expensive and less available (Scheme 1B). Following the known three-step sequence to produce aldehyde **7**,³³ Julia-Kocienski olefination³⁴ of **7** using PT-sulfone **8** afforded alkene **9** in 71% yield as a single isomer. Hydrogenation of the double bond and simultaneous hydrolysis of the benzyl group in **9**, which was followed by Dess-Martin oxidation, produced aldehyde **10**. Reformatsky reaction of **10** using bromoacetate **11** gave rise to an inseparable 1 : 1 mixture of diastereomers (**12a** and **12b**) in 89% yield over three steps. Fortunately, during our investigation to achieve a diastereoselective aldol reaction, we found that treatment of **10** with chiral auxiliary **13** using TiCl_4 in the presence of $^i\text{Pr}_2\text{NEt}$ ³⁵





Scheme 2 Retrosynthesis of tetraselide using two carrier supported peptides for convergent assembly in LPPS.

afforded the desired adducts **14a** and **14b**, which were easily separable by column chromatography. After separating the diastereomers, benzyl esterification of **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 (ESI Fig. S18†).³⁶

A comparison of the ¹H NMR spectra for the synthesized alcohols **12a** and **12b** with degraded **5** revealed that the methyl and hydroxy groups in the natural form are in the anti-configuration. Because the optical rotation of **12a** was opposite to that of **5**, we assigned the absolute configuration of the β-hydroxy-γ-methyl fatty acid moiety in tetraselide (**1**) as (3*S*, 4*R*). This result is consistent with our bioinformatic analysis of the PKS of **1**. Nevertheless, we narrowed down the plausible structure of natural product **1** to four isomers in the consecutive Ser residues. 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 that differed in the position of the *D*-isomer in the four consecutive Ser moieties (Scheme 2). In this regard, 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

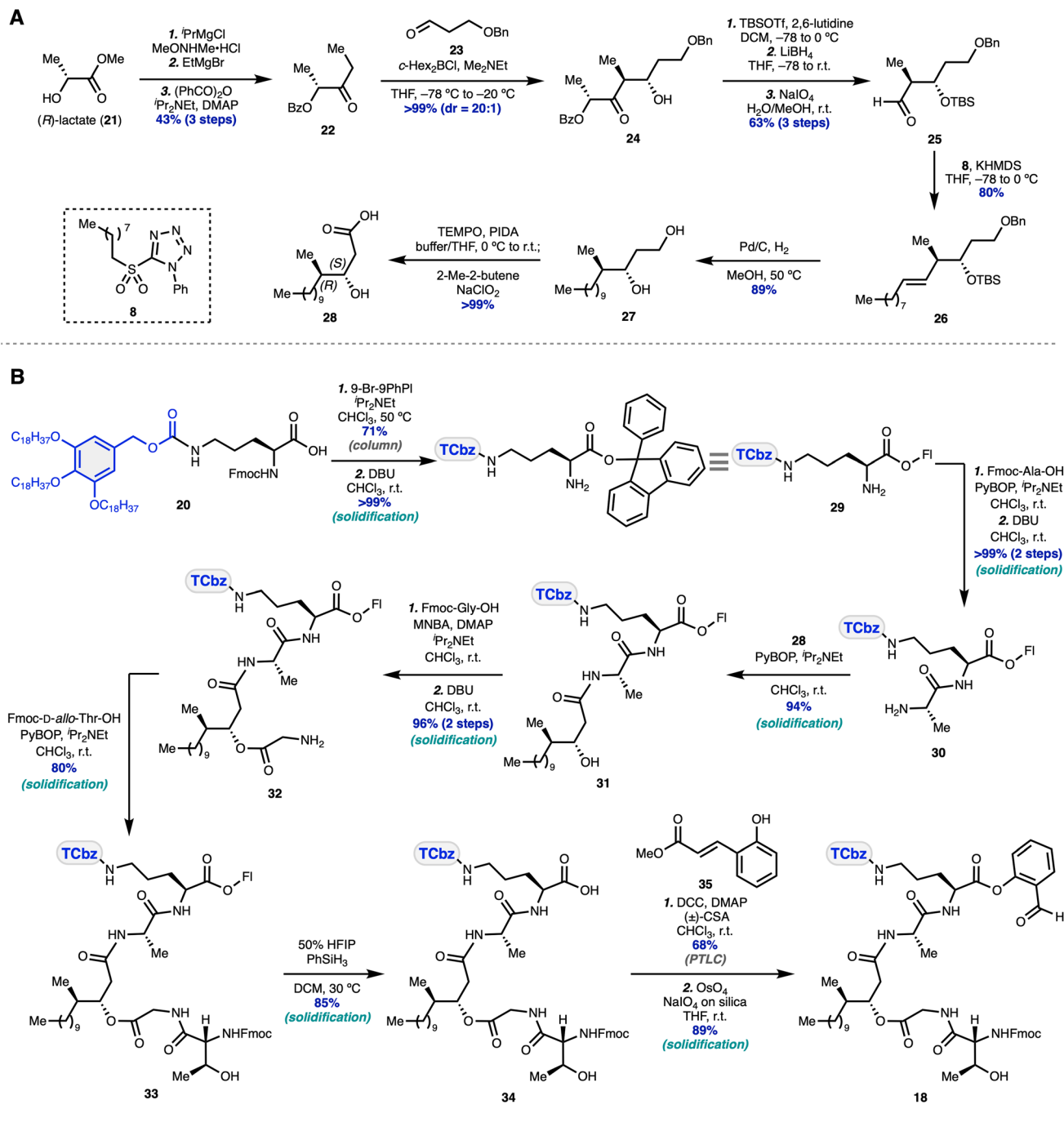
sense, head-to-tail macrolactamization of **16** at the Thr-Ser moiety after cleavage of the C-terminal tag would forge the 28-membered macrocycle in **15**. We expected that the side chain bound Tcbz group would allow us to assemble the macrocyclic peptides in a concise fashion by solidification, followed by global deprotection to furnish tetraselide and its constitutional isomers.

To suppress problematic epimerization *via* oxazolone formation at the C-terminus for convergent fragment coupling³⁷ and to promote facile macrocyclization, we thought to synthesize the macrocyclization precursor **16** using the serine/threonine ligation (STL) reaction³⁸ between the N-terminus of the four consecutive Ser fragment **17** and salicylaldehyde ester **18**. In this way, Ser fragment **17** could be prepared without protection of the hydroxy group at the N-terminal side chain through one-pot LPPS²³ using tag-supported Ser **19**. The western fragment **18** could be synthesized by peptide elongation from Tcbz-supported Orn **20**, which we developed previously.²⁴

Western fragment synthesis

The western fragment synthesis commenced with diastereoselective elaboration of the fatty acid moiety in the desired enantioenriched form (Scheme 3A). The aldol reaction of the known ketone **22**,³⁹ prepared from (*R*)-lactate **21** over three steps, with aldehyde **23** under the conditions reported by Paterson⁴⁰ gave rise to the desired *anti*-diastereomer **24** in excellent yield and selectivity (d.r. = 20:1). Protecting group



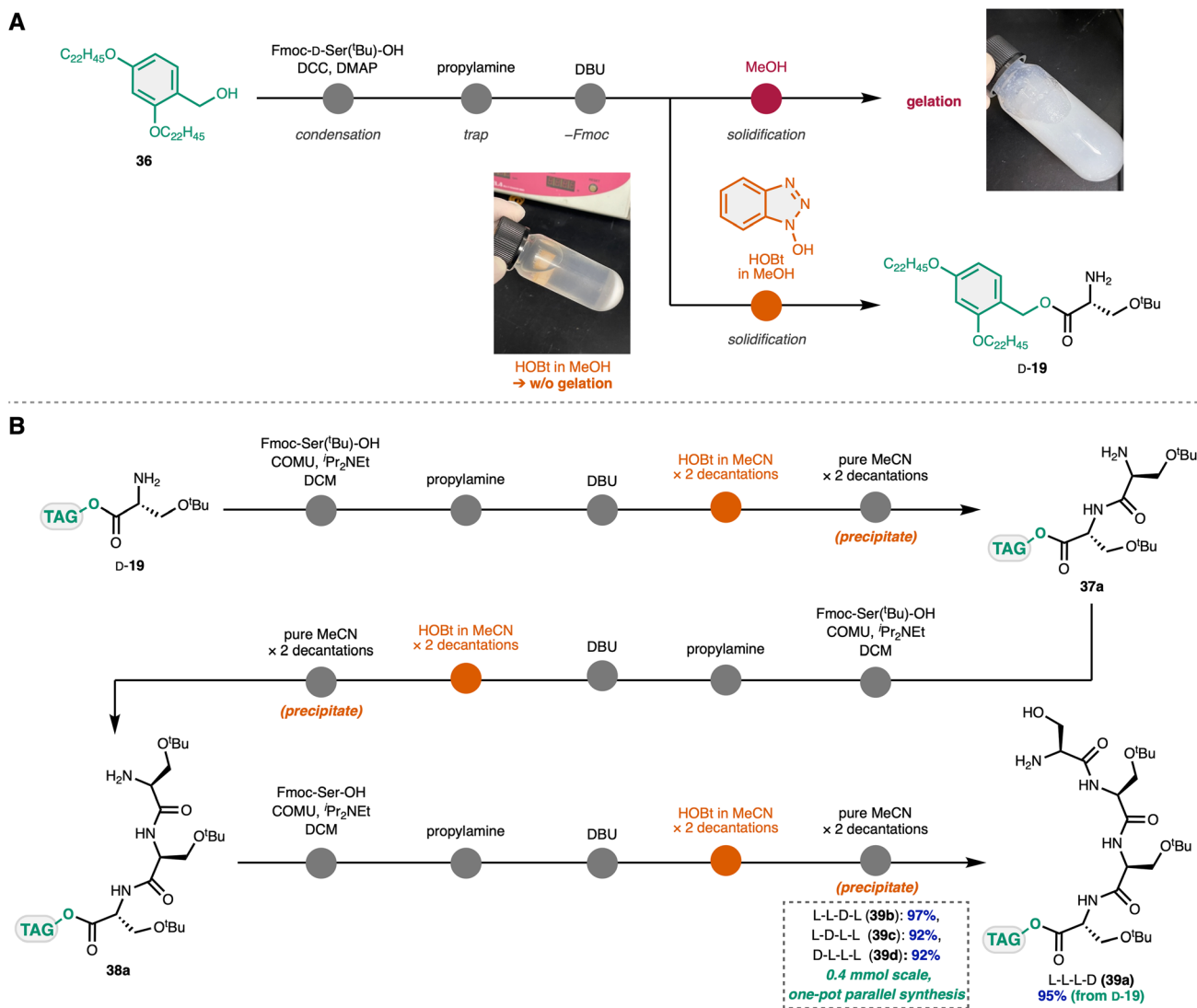


Scheme 3 Western fragment synthesis. (A) Diastereoselective synthesis of the fatty acid fragment in an enantiospecific fashion. (B) Tcbz group-enabled LPPS to elaborate the desired western fragment. The typical procedure for our LPPS is shown in the gray box.

manipulations and oxidative cleavage afforded aldehyde **25**, which was subjected to the Julia–Kocienski olefination condition 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





Scheme 4 One-pot Ser fragment synthesis. (A) Synthesis of tag-supported Ser 19. (B) Rapid peptide elongation using a one-pot protocol.

alcohol in **27** to the corresponding carboxylic acid, affording **28** in >99% yield. In this way, the desired fatty acid **28** was prepared in 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**) (see the ESI† for details). Consequently, the 9-phenylfluorenyl (Fl) group was installed on the acid moiety 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** (94% yield), followed by esterification of alcohol **31** with Fmoc-Gly-OH using MNBA (2-methyl-6-nitrobenzoic anhydride) 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 mild conditions provided **34** in 85% yield. Finally,

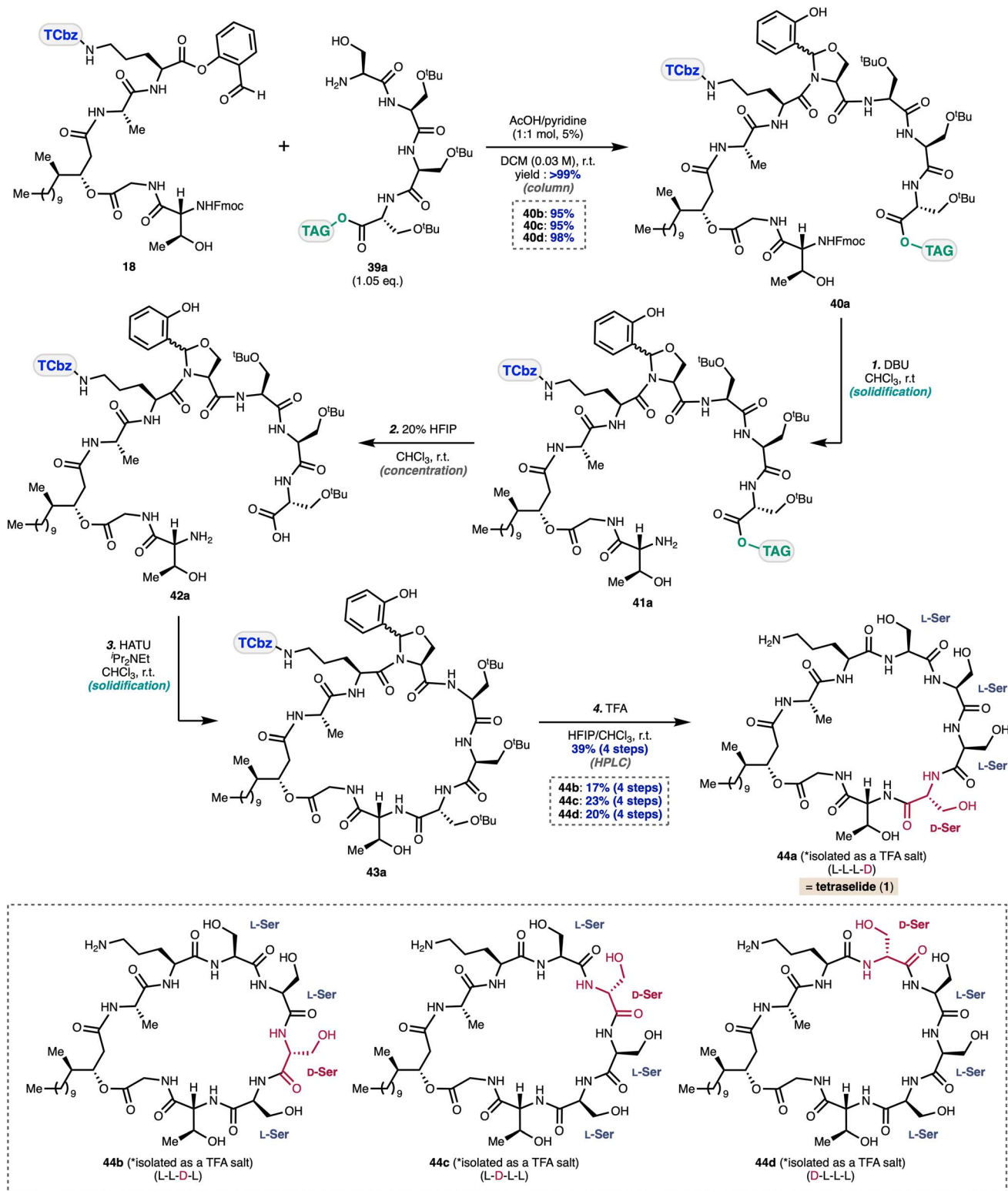
salicylaldehyde ester **18** was synthesized by esterification of **34** with phenol **35** (68% yield) and oxidative cleavage of the corresponding unsaturated ester (89% yield). Thus, the western fragment **18** was prepared in a total of 11 steps from Fmoc-Orn(TCbz)-OH (**20**).

Consecutive Ser fragment synthesis

We began our investigation of the Ser fragment synthesis with a one-pot protocol similar to that we previously developed in the total synthesis of emodepsides.²³ Considering the orthogonality to the Tcbz group in the downstream deprotection step, we chose tag-carrier **36** reported by Chiba and coworkers⁴¹ 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, followed by trapping of the excess activated ester reagent residue with propylamine and cleavage of the Fmoc group using DBU, furnished the corresponding amine **D-19**.

Unexpectedly, the typical purification procedure of LPPS using the tag-carrier by solidification with MeOH led to gelation

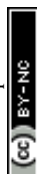




Scheme 5 Fragment coupling using the STL reaction, and complete synthesis of tetraselide and its constitutional isomers through systematic elaboration of the carrier-supported cyclic peptides.

of the mixture and diminished the yield (Scheme 4A). To our delight, we found that solidification using MeOH containing HOBt as a moderately acidic additive, which we previously employed to prevent the undesired cross condensation in the

total synthesis of argifin,¹⁹ successfully suppressed the problematic gelation. The addition of HOBt may affect the intermolecular hydrogen bonding network of the carrier-supported Ser derivative in the MeOH solution.



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

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 buffered reaction conditions that could promote imine formation while maintaining the solubility of the carrier-supported peptide fragments,⁴² we found that treatment of **18** and **39a** with 5% AcOH/pyridine (1 : 1 mol mol^{−1}) in DCM (0.03 M) gave rise to the desired coupled product **40a** in quantitative yield as a diastereomeric mixture of the *N,O*-acetal moiety.⁴³ Remarkably, the STL reaction proceeded efficiently with only a slight excess (1.05 eq.) of **39a** without epimerization of the peptide backbone. This highlights the power of convergent LPPS strategy under the homogeneous reaction conditions.

After cleavage of the Fmoc group in **40a** under basic conditions, the carrier molecule at the C-terminus in **41a** was selectively removed under mild conditions using HFIP to furnish amino acid **42a**. Macrolactamization of **42a** using HATU in the presence of ⁱPr₂NEt afforded head-to-tail macrocyclic peptide **43a**. Finally, global deprotection of the amino acids in **43a** including the TCbz group was accomplished by treatment with TFA in HFIP/CHCl₃. At this stage, the solid residue derived from the carrier molecules was removed by filtration and washed with MeCN. The crude filtrate was purified by reverse-phase HPLC, affording the desired peptide **44a** in 39% yield over four steps. Analogously, we prepared the other three isomers **44b–d** in four steps (17–23% yield). All spectral data of synthesized **44a** were consistent with those of naturally occurring tetraselide (**1**), thus determining the order of the four consecutive Ser moieties in the natural product **1** as *L-L-L-D*. Consequently, our rapid systematic syntheses of all plausible isomers of tetraselide through the assembly of the carrier-supported macrocyclic peptides allowed us to reveal the absolute structure of the natural product.

Conclusions

We 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 a marine-derived filamentous fungus. We 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 was developed to synthesize the four plausible isomers of the natural product.

The western fragment was synthesized *via* unconventional LPPS based on our previous work using the TCbz group. We also established the one-pot parallel synthesis of the four Ser fragments. The challenging peptide fragment coupling was accomplished utilizing the serine/threonine ligation reaction. Head-to-tail macrocyclization of the carrier-supported precursors using the TCbz auxiliary allowed us to handle the macrocyclic peptides in a systematic way.

Overall, our work highlights the power of LPPS to enable practical convergent syntheses of macrocyclic peptides. The synthesized isomers were evaluated for *in vitro* antifungal activities that were used for our screening method to explore the natural product (see the ESI† for details). Further investigations for antifungal properties as well as structure–activity relationship studies are currently underway at our institute.

Data availability

All data associated with this publication are provided in the ESI.†

Author contributions

T. H. and T. S. supervised the project. The design of this work was conceptualized by G. S. and T. H. with input from H. N. and H. T. H. N., H. A., and Y. W. carried out the experimental work. H. T. performed bioinformatic analyses and docking simulations. The experimental data were recorded by H. N., H. A., and Y. W. The ESI† was written by H. N., G. S., H. A., H. T. and Y. W. and reviewed by I. M. and T. H. The manuscript was written by H. N., G. S. and H. T. and reviewed by all authors.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

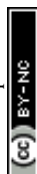
The authors are grateful to Distinguished Emeritus Professor Satoshi Ōmura (Kitasato University) for his helpful support and suggestions. We thank Drs Yuriko Nagano (Japan Agency for Marine-Earth Science and Technology) and Kenichi Nonaka (Teikyo University of Science) for the deposition of the fungal strain. This work was supported by a Grant-in-Aid for Transformative Research Areas (A) “Latent Chemical Space” [JP24H01789] for G. S. from the Ministry of Education, Culture, Sports, Science and Technology, Japan. This study was partially supported by Japan Society for the Promotion of Science (JSPS)



KAKENHI Grant No. 24K18256 (G. S.), 23K06053 (Y. N.), 21K05293 (Y. W.), 22K06535 (T. H.), 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 No. JP24ama121035). The authors thank Dr Kenichiro Nagai, Ms Noriko Sato, and Ms Reiko Seki (School of Pharmacy, Kitasato University) for spectroscopic assistance.

Notes and references

- (a) A. Zorzi, K. Deyle and C. Heinis, *Curr. Opin. Chem. Biol.*, 2017, **38**, 24–29; (b) C. Morrison, *Nat. Rev. Drug Discovery*, 2018, **17**, 531–533.
- A. A. Vinogradov, Y. Yin and H. Suga, *J. Am. Chem. Soc.*, 2019, **141**, 4167–4181.
- (a) P. G. Dougherty, A. Sahni and D. Pei, *Chem. Rev.*, 2019, **119**, 10241–10287; (b) A. Ohta, M. Tanada, S. Shinohara, Y. Morita, K. Nakano, Y. Yamagishi, R. Takano, S. Kariyuki, T. Iida, A. Matsuo, K. Ozeki, T. Emura, Y. Sakurai, K. Takano, A. Higashida, M. Kojima, T. Muraoka, R. Takeyama, T. Kato, K. Kimura, K. Ogawa, K. Ohara, S. Tanaka, Y. Kikuchi, N. Hisada, R. Hayashi, Y. Nishimura, K. Nomura, T. Tachibana, M. Irie, H. Kawada, T. Torizawa, N. Murao, T. Kotake, M. Tanaka, S. Ishikawa, T. Miyake, M. Tamiya, M. Arai, A. Chiyoda, S. Akai, H. Sase, S. Kuramoto, T. Ito, T. Shiraishi, T. Kojima and H. Iikura, *J. Am. Chem. Soc.*, 2023, **145**, 24035–24051.
- (a) E. Biron, J. Chatterjee, O. Ovadia, D. Langenegger, J. Brueggem, D. Hoyer, H. A. Schmid, R. Jelinek, C. Gilon, A. Hoffman and H. Kessler, *Angew. Chem., Int. Ed.*, 2008, **47**, 2595–2599; (b) T. R. White, C. M. Renzelman, A. C. Rand, T. Rezai, C. M. McEwen, V. M. Gelev, R. A. Turner, R. G. Linington, S. S. F. Leung, A. S. Kalgutkar, J. N. Bauman, Y. Zhang, S. Liras, D. A. Price, A. M. Mathiowetz, M. P. Jacobson and R. S. Lokey, *Nat. Chem. Biol.*, 2011, **7**, 810–817; (c) C. K. Wang, C. K. S. E. Northfield, B. Colless, S. Chaousis, I. Hamernig, R.-J. Lohman, D. S. Nielsen, C. I. Schroeder, S. Liras, D. A. Price, D. P. Fairlie and D. J. Craik, *Proc. Natl. Acad. Sci. U.S.A.*, 2014, **111**, 17504–17509.
- (a) S. Sivanathan and J. Scherkenbeck, *Molecules*, 2014, **19**, 12368–12420; (b) S.-X. Liu, S.-Y. Ou-Yang, Y.-F. Lu, C.-L. Guo, S.-Y. Dai, C. Li, T.-Y. Yu and Y.-H. Pei, *Front. Microbiol.*, 2023, **14**, 1276928.
- (a) M. Weinmüller, F. Rechenmacher, U. Kiran Marelli, F. Reichart, T. G. Kapp, A. F. B. Räder, F. S. Di Leva, L. Marinelli, E. Novellino, J. M. Muñoz-Félix, K. Hodivala-Dilke, A. Schumacher, J. Fanous, C. Gilon, A. Hoffman and H. Kessler, *Angew. Chem., Int. Ed.*, 2017, **56**, 16405–16409; (b) A. Schumacher-Klinger, J. Fanous, S. Merzbach, M. Weinmüller, F. Reichart, A. F. B. Räder, A. Gitlin-Domagalska, C. Gilon, H. Kessler and A. Hoffman, *Mol. Pharmaceutics*, 2018, **15**, 3468–3477; (c) Y. Hosono, S. Uchida, M. Shinkai, C. E. Townsend, C. N. Kelly, M. R. Naylor, H.-W. Lee, K. Kanamitsu, M. Ishii, R. Ueki, T. Ueda, K. Takeuchi, M. Sugita, Y. Akiyama, S. R. Lokey, J. Morimoto and S. Sando, *Nat. Commun.*, 2023, **14**, 1416; (d) L. Cao, Z. Xu, T. Shang, C. Zhang, X. Wu, Y. Wu, S. Zhai, Z. Zhan and H. Duan, *J. Med. Chem.*, 2024, **67**, 1888–1899.
- S. Ōmura, *Angew. Chem., Int. Ed.*, 2016, **55**, 10190–10209.
- (a) Y. Watanabe, T. Suga, S. Narusawa, M. Iwatsuki, K. Nonaka, T. Nakashima, Y. Shinohara, T. Shiotsuki, N. Ichimaru, H. Miyoshi, Y. Asami, S. Ōmura and K. Shiomi, *J. Antibiot.*, 2017, **70**, 395–399; (b) Y. Watanabe, Y. Asami, S. Narusawa, S. Hashimoto, M. Iwatsuki, K. Nonaka, Y. Shinohara, T. Shiotsuki, N. Ichimaru, H. Miyoshi, S. Ōmura and K. Shiomi, *J. Antibiot.*, 2018, **71**, 146–148; (c) Y. Watanabe, Y. Yoshida, T. Tokiwa, M. Higo, S. Ban, A. Ikeda, Y. Noguchi, T. Hirose, T. Sunazuka, K. Nonaka, T. Yaguchi and M. Iwatsuki, *J. Gen. Appl. Microbiol.*, 2022, **68**, 200–206; (d) Y. Watanabe, S. Takahashi, S. Ito, T. Tokiwa, Y. Noguchi, H. Azami, H. K. M. Higo, S. Ban, K. Nagai, T. Hirose, T. Sunazuka, T. Yaguchi, K. Nonaka and M. Iwatsuki, *Org. Biomol. Chem.*, 2023, **21**, 2320–2330; (e) H. Azami, Y. Watanabe, K. Sakai, H. Nakahara, H. Kojima, T. Tokiwa, K. Nonaka, Y. Noguchi, Y. Nagano, T. Hirose, T. Sunazuka, H. Matsui, N. Arima, K. Abe, H. Hanaki and M. Iwatsuki, *J. Antibiot.*, 2024, **77**, 156–162.
- 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).
- F. Rosconi, D. Davyt, V. Martínez, M. Martínez, J. A. Abin-Carriquiry, H. Zane, A. Butler, E. M. De Souza and E. Fabiano, *Environ. Microbiol.*, 2013, **15**, 916–927.
- K. Harada, K. Fujii, K. Hayashi, M. Suzuki, Y. Ikai and H. Oka, *Tetrahedron Lett.*, 1996, **37**, 3001–3004.
- R. B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149–2154.
- (a) D. M. M. Jaradat, *Amino Acids*, 2018, **50**, 39–68; (b) N. Wang, P. Saidharedy and X. Jiang, *Nat. Prod. Rep.*, 2020, **37**, 246–275; (c) H. Liu, L. Bai and X. Jiang, *Tetrahedron Lett.*, 2024, **151**, 155314.
- V. Martin, P. H. G. Egelund, H. Johansson, S. Thordal Le Quement, F. Wojcik and D. Sejer Pedersen, *RSC Adv.*, 2020, **10**, 42457–42492.
- B. Bray, *Nat. Rev. Drug Discovery*, 2003, **2**, 587–593.
- (a) A. Thakkar, T. B. Trinh and D. Pei, *ACS Comb. Sci.*, 2013, **15**, 120–129; (b) L. Digal, S. C. Samson, M. A. Stevens, A. Ghorai, H. Kim, M. C. Mifflin, K. R. Carney, D. L. Williamson, S. Um, G. Nagy, D.-C. Oh, M. C. Mendoza and A. G. Roberts, *ACS Chem. Biol.*, 2024, **19**, 81–88.
- (a) A. Isidro-Llobet, M. N. Kenworthy, S. Mukherjee, M. E. Kopach, K. Wegner, F. Gallou, A. G. Smith and F. Roschangar, *J. Org. Chem.*, 2019, **84**, 4615–4628; (b) L. K. Mueller, A. C. Baumruck, H. Zhdanova and A. A. Tietze, *Front. Bioeng. Biotechnol.*, 2020, **8**, 162.
- (a) E. Bayer and M. Mutter, *Nature*, 1972, **237**, 512–513; (b) M. Mutter and E. Bayer, *Angew. Chem., Int. Ed.*, 1974, **13**, 88–89.



- 19 H. Tamiaki, T. Obata, Y. Azefu and K. Toma, *Bull. Chem. Soc. Jpn.*, 2001, **74**, 733–738.
- 20 T. Hirose, T. Kasai, T. Akimoto, A. Endo, A. Sugawara, K. Nagasawa, K. Shiomi, S. Ōmura and T. Sunazuka, *Tetrahedron*, 2011, **67**, 6633–6643.
- 21 (a) Y. Hayashi, W. Fukasawa, T. Hirose, M. Iwatsuki, R. Hokari, A. Ishiyama, M. Kanaida, K. Nonaka, A. Také, K. Otoguro, S. Ōmura, K. Shiomi and T. Sunazuka, *Org. Lett.*, 2019, **21**, 2180–2184; (b) Y. Hayashi, T. Hirose, M. Iwatsuki, S. Ōmura and T. Sunazuka, *Org. Lett.*, 2019, **21**, 8229–8233.
- 22 A. Watanabe, Y. Noguchi, T. Hirose, S. Monma, Y. Satake, T. Arai, K. Masuda, N. Murashima, K. Shiomi, S. Ōmura and T. Sunazuka, *Tetrahedron Lett.*, 2020, **61**, 151699.
- 23 Y. Noguchi, S. Sekikawa, Y. Nogaki, Y. Satake, N. Murashima, T. Kirisawa, G. Schiffer, J. Köebberling, T. Hirose and T. Sunazuka, *Tetrahedron*, 2022, **128**, 133100.
- 24 H. Nakahara, G. Sennari, Y. Noguchi, T. Hirose and T. Sunazuka, *Chem. Sci.*, 2023, **14**, 6882–6889.
- 25 P. Edman, *Acta Chem. Scand.*, 1950, **4**, 283–293.
- 26 K. Blin, S. Shaw, H. E. Augustijn, Z. L. Reitz, F. Biermann, M. Alanjary, A. Fetter, B. R. Terlouw, W. W. Metcalf, E. J. N. Helfrich, G. P. van Wezel, M. H. Medema and T. Weber, *Nucleic Acids Res.*, 2023, **51**, W46–W50.
- 27 A Web-Based Support Tool to Find Secondary Metabolite Biosynthetic Gene Cluster, <https://biosyn.nih.gov/jp/2ndfind/>.
- 28 M. Mirdita, K. Schütze, Y. Moriwaki, L. Heo, S. Ovchinnikov and M. Steinegger, *Nat. Methods*, 2022, **19**, 679–682.
- 29 (a) O. Trott and A. J. Olson, *J. Comput. Chem.*, 2010, **31**, 455–461; (b) J. Eberhardt, D. Santos-Martins, A. F. Tillack and S. Forli, *J. Chem. Inf. Model.*, 2021, **61**, 3891–3898.
- 30 C. Rausch, I. Hoof, T. Weber, W. Wohlleben and D. H. Huson, *BMC Evol. Biol.*, 2007, **7**, 78.
- 31 (a) A. T. Keatinge-Clay, *Chem. Biol.*, 2007, **14**, 898–908; (b) A. T. Keatinge-Clay, *Nat. Prod. Rep.*, 2016, **33**, 141–149.
- 32 K. J. Weissman, *J. Org. Chem.*, 2017, **13**, 348–371.
- 33 R. D. C. Pullin, A. H. Rathi, E. Y. Melikhova, C. Winter, A. L. Thompson and T. J. Donohoe, *Org. Lett.*, 2013, **15**, 5492–5495.
- 34 P. R. Blakemore, *J. Chem. Soc., Perkin Trans. 1*, 2002, **23**, 2563–2585.
- 35 M. T. Crimmins and J. She, *Synlett*, 2004, **8**, 1371–1374.
- 36 I. Ohtani, T. Kusumi, Y. Kashman and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092–4096.
- 37 L. A. Carpino and A. El-Faham, *Tetrahedron*, 1999, **55**, 6813–6830.
- 38 X. Li, H. Y. Lam, Y. Zhang and C. K. Chan, *Org. Lett.*, 2010, **12**, 1724–1727.
- 39 L. C. Dias and E. C. Polo, *J. Org. Chem.*, 2017, **82**, 4072–4112.
- 40 (a) I. Paterson, D. J. Wallace and S. M. Velázquez, *Tetrahedron Lett.*, 1994, **35**, 9083–9086; (b) I. Paterson and D. J. Wallace, *Tetrahedron Lett.*, 1994, **35**, 9087–9090; (c) N. Y. S. Lam, T. P. Stockdale, M. J. Anketell and I. Paterson, *Chem. Commun.*, 2021, **57**, 3171–3189.
- 41 G. Tana, S. Kitada, S. Fujita, Y. Okada, S. Kim and K. Chiba, *Chem. Commun.*, 2010, **46**, 8219.
- 42 H. Wu, Z. Sun and X. Li, *Angew. Chem., Int. Ed.*, 2023, **62**, e202310624.
- 43 (a) H. Y. Lam, Y. Zhang, H. Liu, J. Xu, C. T. T. Wong, C. Xu and X. Li, *J. Am. Chem. Soc.*, 2013, **135**, 6272–6279; (b) Z. Sun, Z. Shang, N. Forelli, K. H. L. Po, S. Chen, S. F. Brady and X. Li, *Angew. Chem., Int. Ed.*, 2020, **59**, 19868–19872.

