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Synthetic Studies Toward the Skyllamycins: Total Synthesis and Generation of Simplified Analogues

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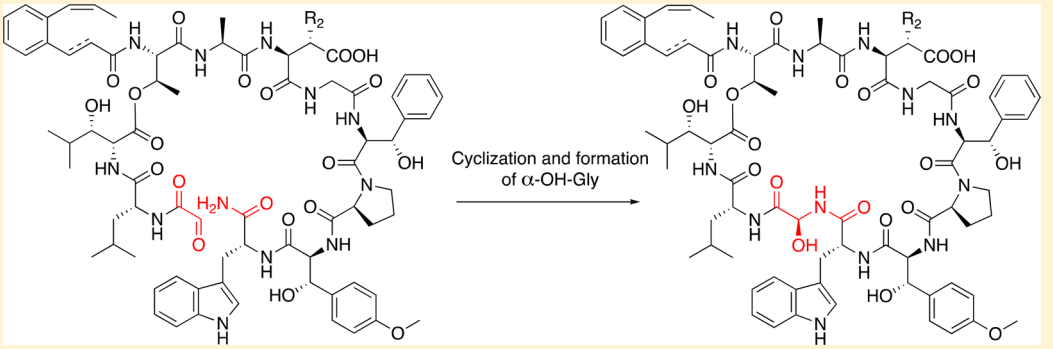
Supporting Information



ABSTRACT: Herein, we report our synthetic studies toward the skyllamycins, a highly modified class of nonribosomal peptide natural products which contain a number of interesting structural features, including the extremely rare α-OH-glycine residue. Before embarking on the synthesis of the natural products, we prepared four structurally simpler analogues. Access to both the analogues and the natural products first required the synthesis of a number of nonproteinogenic amino acids, including three β-OH amino acids that were accessed from the convenient chiral precursor Garner's aldehyde. Following the preparation of the suitably protected nonproteinogenic amino acids, the skyllamycin analogues were assembled using a solid-phase synthetic route followed by a final stage solution-phase cyclization reaction. To access the natural products (skyllamycins A−C) the synthetic route used for the analogues was modified. Specifically, linear peptide precursors containing a C-terminal amide were synthesized via solid-phase peptide synthesis. After cleavage from the resin the N-terminal serine residue was oxidatively cleaved to a glyoxyamide moiety. The target natural products, skyllamycins A−C, were successfully prepared via a final step cyclization with concomitant formation of the unusual α-OH-glycine residue. Purification and spectroscopic comparison to the authentic isolated material confirmed the identity of the synthetic natural products.

INTRODUCTION

The skyllamycins are a family of nonribosomal cyclic depsipeptide natural products produced by *Streptomyces* sp. Skyllamycin A **1** was first isolated in 2001 by Matsuda and coworkers who showed that it possessed inhibitory activity against the platelet-derived growth factor (PDGF) signaling pathway.[1] This signaling plays a role in cell migration and proliferation and, importantly, aberrant signaling has been implicated in a number of human disease states, including cancer.[2] Skyllamycin A was found to specifically inhibit the interaction of the PDGF B-type dimer to the PDGF β-receptor. At the time of isolation, the stereochemistry of the amino acids was not assigned. Subsequently, Süssmuth and coworkers reisolated skyllamycin A (**1**), along with the unmethylated skyllamcyin B **2**, and carried out a thorough investigation of the biosynthetic pathways that assemble these interesting natural products.[3] Skyllamycins A (**1**), B (**2**), and C (**3**), the latter a reduced congener, were independently isolated in 2014 during our search for *Pseudomonas aeruginosa* biofilm inhibitors.[4] Skyllamycins B and C were both shown to inhibit the formation of biofilms, while skyllamycin B was also capable of clearing preattached biofilms. Biofilms play a significant role in the ability of bacteria to evade antibiotics.[5] Given the established threat of antimicrobial resistance,[6] mechanisms such as biofilm formation that bacteria use to evade antibiotics have become important targets, and it is now recognized that novel methods by which to clear biofilm forming bacterial infections are urgently needed.

The skyllamycins are highly modified and possess a number of unusual amino acids (Figure 1). Specifically, they contain two D-amino acids, D-Leu and D-Trp, as well as β-Me-Asp residue (blue) and a pseudo N-terminal cinnamic acid residue (green), unique to this family of natural products. Related structural motifs are present in other natural products such as the pepticinnamins[7] and the recently isolated coprisamides.[8]

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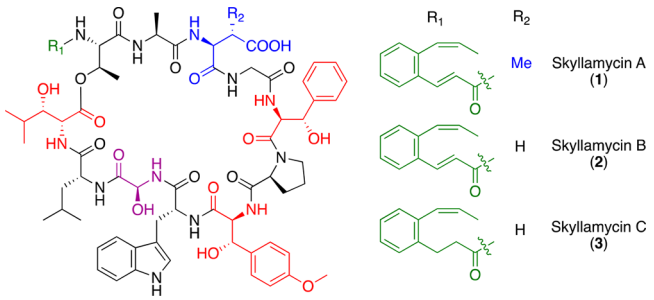
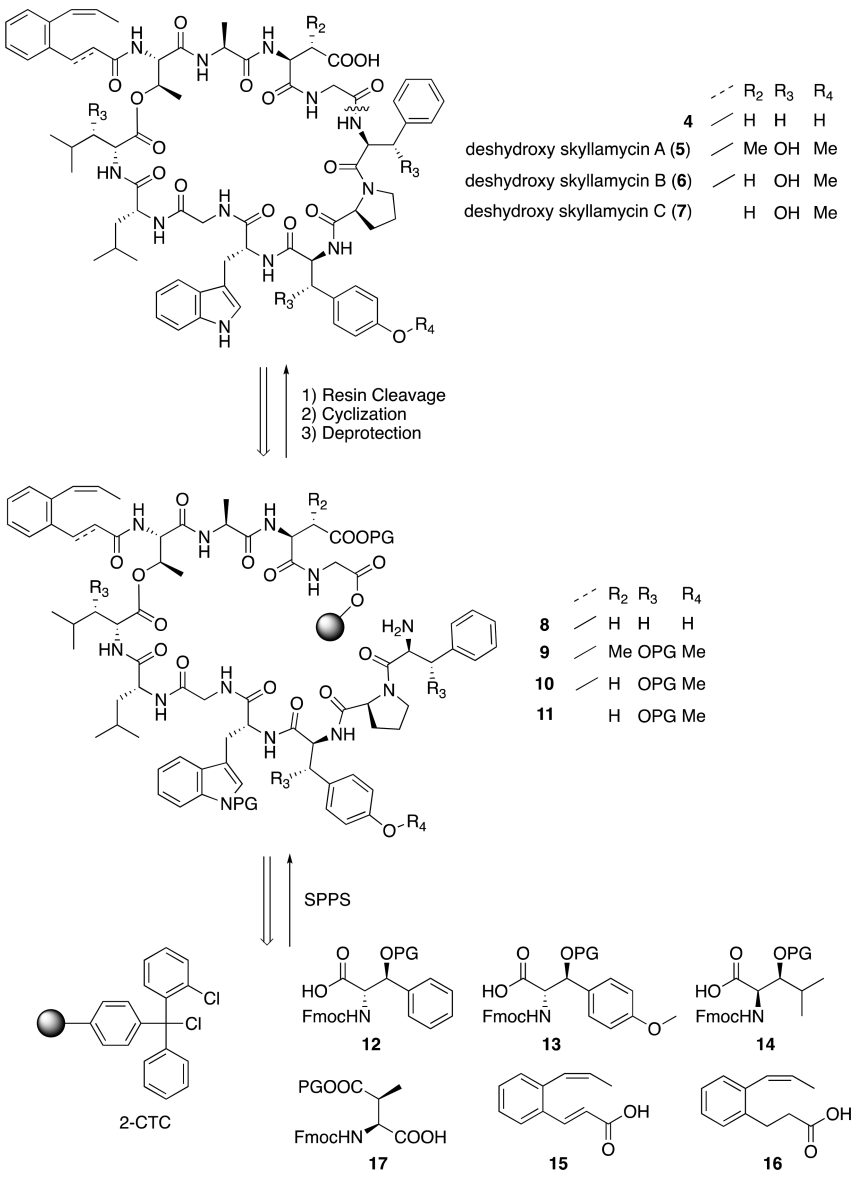


Figure 1. Structure of skyllamycins A–C **1**–**3**.

They are highly hydroxylated, containing three β-OH amino acids namely Phe, D-Leu, and an *O*-Me Tyr residue (red). Most unusually, they contain an α-OH-Gly residue (magenta) which to date has only been reported in the linear natural product spergualin,[9,10] but has not been reported in any other cyclic peptide natural products. While rare in natural products, α-OH-Gly residues are common in biological systems as precursors to C-terminal amides, particularly among peptide hormones which often require a C-terminal amide for full activity.[11]

In the second isolation report, Süssmuth and coworkers determined the biosynthetic origins of the unusual modifications present in the skyllamycins.[3] Further studies carried out by Cryle and coworkers revealed that a single P450 monooxygenase was responsible for the installation of the hydroxyl functionality in the three β-OH amino acids, in each case generating the 3*S* stereochemistry.[12] A crystal structure of this P450 enzyme bound to the peptidyl-carrier protein domain of the nonribosomal peptide synthetase was subsequently determined by Cryle and coworkers.[13] This important work shed light on the structural and biosynthetic origins of the stereoselective installation of the hydroxyl moiety. While the gene responsible for the installation of the α-OH-Gly is known, the exact timing of the formation of this unusual modification remains elusive. Following their initial biosynthetic studies,

Scheme 1. Retrosynthetic Analysis of Skyllamycin Analogues 4–7[a]



[a]PG = protecting group.

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Süssmuth and coworkers confirmed the configuration of the amino acids in the natural products by a combination of Marfey's analysis and chiral HPLC/GC.[14] The unusual α-OH-Gly residue was not stable to the hydrolytic cleavage conditions employed to convert the natural products back to their amino acid building blocks. As such, the authors were unable to determine the absolute stereochemistry of this residue. However, using a combination of intramolecular distances estimated from NMR-NOESY measurements and molecular dynamics simulations, the α-OH-Gly residue was proposed to have the (*S*)-configuration. Importantly, it was determined that when in this conformation, the natural products possess a strong internal hydrogen bonding network involving five strong intramolecular H-bonds, with the α-OH-Gly as well as the hydroxyl groups of three β-OH residues all participating. When in the (*R*)-configuration, this hydrogen bonding network is less extensive, and the α-OH-Gly residue is not involved. The authors therefore concluded that the hydrogen bonding network stabilizes the α-OH-Gly residue in the (*S*)-configuration.

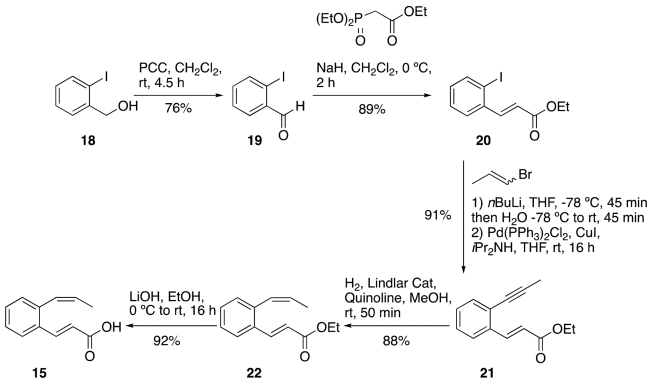
Due to their highly unusual and synthetically challenging structures and interesting biological activities we began a program directed toward the total synthesis of the skyllamycin natural products as well as the generation of simplified analogues to assess the importance of particular modifications for activity. We recently reported the first total synthesis of skyllamycins A–C **1**–**3**,[15] and herein we report full details of the total synthesis of these natural products (including the synthesis of all modified amino acids) as well as the generation of four simplified analogues.

RESULTS AND DISCUSSION

Before we embarked on the synthesis of the natural products we first wanted to investigate the synthesis of simplified skyllamycin analogues **4**–**7** (Scheme 1). We envisioned that these analogues would be easier to access synthetically compared to the natural products, and would serve to provide insight into the importance of the unusual α-OH-Gly residue for antibiofilm activity. We first targeted a simplified skyllamycin analogue **4** to validate our synthetic strategy toward these analogues. Notably, analogue **4** contains only the cinnamic acid residue and does not possess any of the structurally complex β-OH amino acids or the challenging α-OH-Gly residue. Following the successful preparation of this simplified analogue, we envisaged the synthesis of the so-called deshydroxy skyllamycins **5**–**7**. These analogues contain all the modified amino acids except for the α-OH-Gly residue. We reasoned that these molecules would allow us to develop robust synthetic routes to the noncommercially available amino acids as well as optimize the proposed solid-phase chemistry to access the natural product skeleton. Furthermore, these analogues would provide initial structure activity data on biofilm inhibition, particularly the importance of the α-OH-Gly residue. Retrosynthetically, we envisioned that the simplified analogue **4** and deshydroxy skyllamycins **5**–**7** could be generated after resin cleavage, cyclization, and deprotection from the corresponding resin-bound linear peptides **8**–**11**. We decided to utilize the Phe-Gly junction as the cyclization point as the C-terminal Gly residue cannot epimerize. Resin-bound linear peptides **8**–**11** could be synthesized beginning from 2-Cl-trityl chloride (2-CTC) resin using a Fmoc solid-phase peptide synthesis protocol (SPPS). To perform the proposed synthesis, a number of suitably protected noncommercially available amino acids, specifically the three β-OH amino acids **12**–**14**, two cinnamic acids (**15** and **16**), and finally a β-Me-Asp **17** required synthesis.

We started with the preparation of cinnamoyl building block **15**, which was inspired by the synthesis of the related pentenyl phenyl acrylic acid carried out by Jiang and coworkers[16] (Scheme 2). Synthesis began with the pyridinium chlorochromate mediated oxidation of *o*-iodobenzyl alcohol **18** to afford aldehyde **19**. This was reacted with triethyl phosphonacetate in a Horner–Wadsworth–Emmons reaction to afford the (*E*)-alkene **20** exclusively in excellent yield. The key step was a the Sonogashira[17] cross-coupling reaction between iodide **20** and propyne generated in situ by reaction of 1-bromopropene and *n*-BuLi.[18] Gratifyingly, this proceeded in excellent yield, providing alkyne **21**. The alkyne was subsequently reduced under Lindlar reduction conditions to afford diene **22**,[19] followed by base-mediated hydrolysis of the ethyl ester to afford cinnamic acid **15** in excellent overall yield.

Scheme 2. Synthesis of Cinnamic Acid 15



With building block **15** in hand, we began the synthesis of simplified analogue **4** (Scheme 3). Toward this end, Fmoc-Gly-OH was loaded to 2-CTC resin; the resin was capped, and Fmoc deprotection was performed using a solution of 10 vol % piperidine in DMF. Following this, the peptide was extended utilizing standard Fmoc-SPPS conditions, namely iterative coupling with (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as the coupling reagent, *N*-methylmorpholine (NMM) as the base in DMF, followed by Fmoc deprotection steps to yield resin-bound tetrapeptide **23**. Cinnamic acid **15** was then coupled using the more active coupling reagent 1-*bis*(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), and Hünig's base in DMF to yield resin-bound **24**. The key on-resin esterification reaction with Fmoc-D-Leu-OH was next carried out, mediated by *N*,*N*′-diisopropylcarbodiimide (DIC) and *N*,*N*-dimethylamino pyridine (DMAP) to yield branched resin-bound peptide **25**. Further extension of the peptide followed by cleavage from resin under mildly acidic conditions using 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) in CH_2Cl_2 yielded linear side chain protected peptide **26**. The crude linear peptide was then treated under cyclization conditions, namely 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM·BF_4) in DMF at high dilution (0.01 M) to yield protected cyclic peptide **27**. After removal of the solvent, the crude cyclic peptide was subjected to acidic deprotection using a cocktail of

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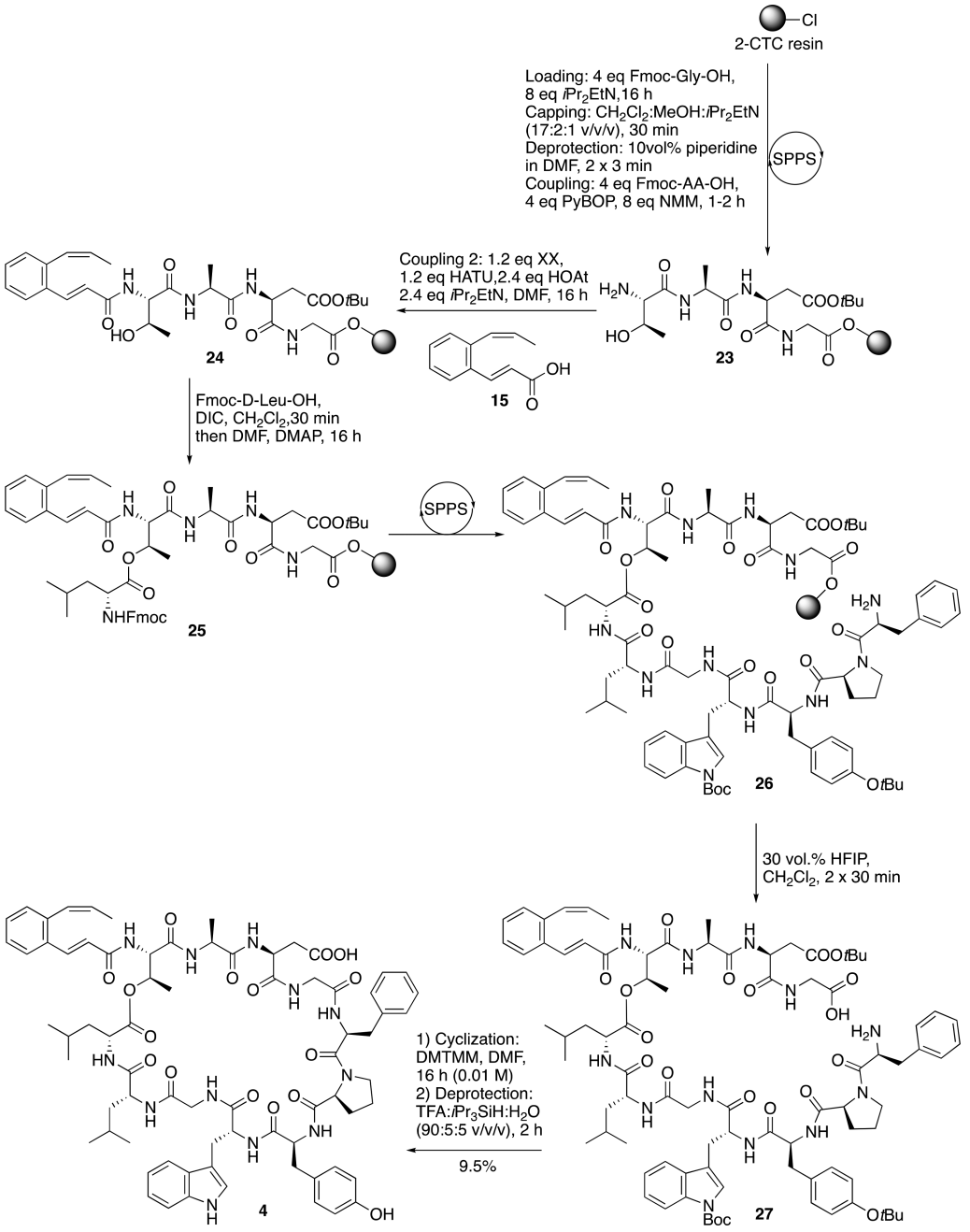
Scheme 3. Synthesis of Simplified Skyllamycin Analogue 4



trifluoroacetic acid (TFA), triisopropylsilane, and water to remove the side chain protecting groups. This yielded simplified skyllamycin analogue **4** in 9.5% yield (25 steps, 91% per step) after purification by reverse-phase HPLC.

With this synthetic route to the proposed deshydroxy skyllamycins verified through the synthesis of **4**, we next sought to target deshydroxy skyllamycins A−C **5**−**7**, which differ from the natural products only by the absence of the unusual α-OH-Gly residue. As such, all the nonproteinogenic amino acids first required synthesis in suitably protected form Fmoc-SPPS. We began the synthesis of reduced cinnamic acid **16** present in skyllamycin C **7** from alkene **20** prepared earlier (Scheme 4). Due to the presence of the alkyl iodide, the double bond could not be reduced using standard Pd-catalyzed hydrogenation. As such, we carried out a three step procedure to afford reduced aryl iodide **28**. Initially, we hydrolyzed the ethyl ester with LiOH, followed by the key reduction step, mediated by hydrazine hydrate and catalytic guanidine nitrate[20] to yield the reduced carboxylic acid. It was necessary to hydrolyze the ester to prevent the formation of the corresponding acyl hydrazide during the ensuing reduction reaction. Finally, the acid was re-esterified, affording iodide **28** in 94% yield over the three step sequence. This was subsequently carried through the same synthetic sequence as described above for cinnamic acid **15**, namely Sonogashira coupling with propyne to afford **29**, Lindlar reduction to diene **30**, and ester hydrolysis to yield reduced cinnamic acid **16** in excellent yield over the seven step synthetic route.

Having synthesized both cinnamic acids, we next turned our attention to the construction of the three β-OH amino acids

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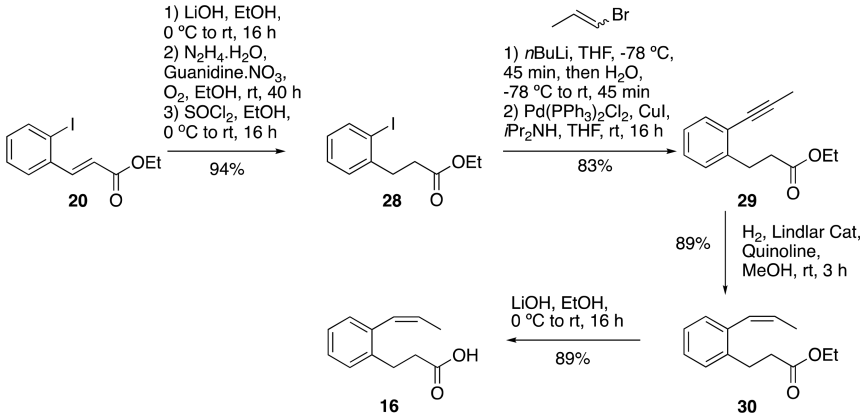
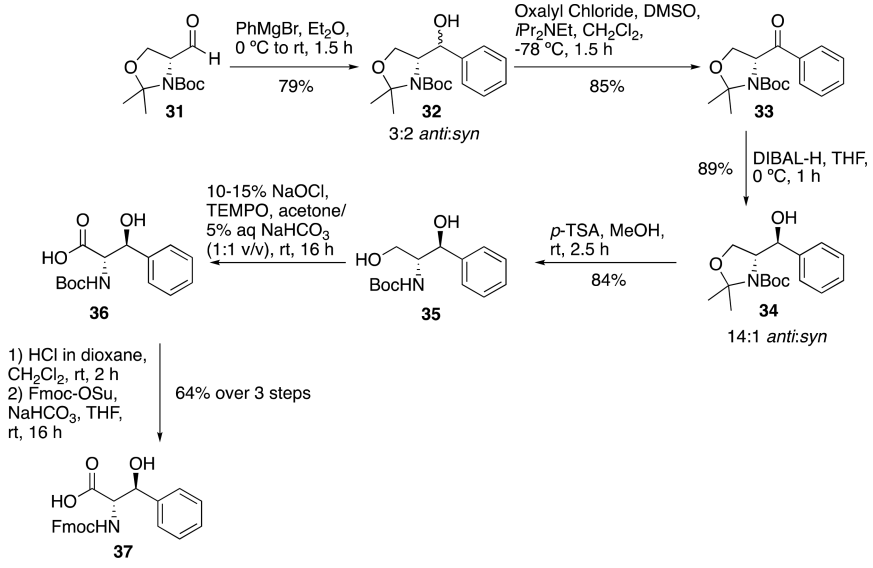
Scheme 4. Synthesis of Reduced Cinnamic Acid 16



Scheme 5. Synthesis of Fmoc-β-OH-Phe 37



present in the natural product family. As discussed above, all three contain the 3*S* stereochemistry at the β-position. Furthermore, both β-OH-Phe and β-OH-*O*-Me-Tyr present in the skyllamycins are L-configured, i.e. they possess the 2*S* stereochemistry. On the basis of previous work carried out in our laboratory on the synthesis of β-seleno/thio-Phe for peptide ligation,[21,22] we decided to use Garner's aldehyde[23] for the synthesis of (2*S*,3*S*)-β-OH-Phe. Garner's aldehyde is a convenient chiral starting material for the synthesis of β-OH amino acids and has been widely used in the synthetic community.[24] Our synthesis began with Grignard addition of MgPhBr to (*R*)-Garner's aldehyde **31**, which led to an inseparable mixture of diastereomers **32** (Scheme 5). Subsequent oxidation of the alcohol to the ketone **33** under Swern conditions followed by diastereoselective reduction with DIBAL-H yielded **34** with the desired *anti*-stereochemistry.[25] Removal of the oxazolidine moiety with *p*-TSA afforded diol **35** in good yield. This was then carried through a reaction sequence requiring only a single purification. Specifically, the primary alcohol of **35** was first oxidized selectively to the acid using a TEMPO-mediated oxidation with NaOCl as the stoichiometric oxidant, affording acid **36**. Following this, Boc-deprotection mediated by HCl in dioxane and a final Fmoc-protection using Fmoc-OSu in mixed THF and saturated aqueous $NaHCO_3$ yielded suitably protected β-OH-Phe **37** in excellent yield over the three steps.

We next turned our attention to the (2*R*,3*S*)-β-OH-Leu residue, which forms the key ester bond with the side chain alcohol of the threonine residue in the natural products. As a result, the β-OH functionality required protection to prevent oligomerization during the esterification reaction. We envisioned this could be accomplished through the use of a novel pseudoproline protecting group strategy. While pseudoprolines are widely used as turn-inducers to introduce kinks into the growing peptide chain during Fmoc-SPPS,[26] they are not often employed as a protecting group for the side-chain hydroxyl groups. To begin the synthesis, inspired by Joullié and coworkers,[27] (*S*)-Garner's aldehyde **38** was reacted with *i*PrMgBr to yield alcohol **39** with exclusive (3*S*)-stereochemistry in moderate yield (Scheme 6). The desired protected amino acid could then be accessed in a five-step sequence involving only one purification step. Specifically, removal of the oxazolidine moiety, in this case using dilute HCl in THF, afforded diol **40**. Selective oxidation of the primary alcohol then yielded acid **41**. Removal of the Boc group and installation of an Fmoc group gave **42**, which was then subject to final protection to yield the desired oxazolidine **43** in 53% over the five-step sequence. Importantly, the ease and scalability of the

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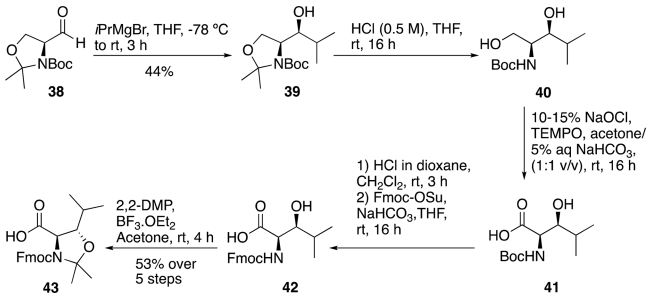
Scheme 6. Synthesis of Suitably Protected (2*R*,3*S*)-*β*-OH-Leu 43



final steps allowed for the generation of multigram quantities of this amino acid cassette suitable for direct incorporation into Fmoc-SPPS.

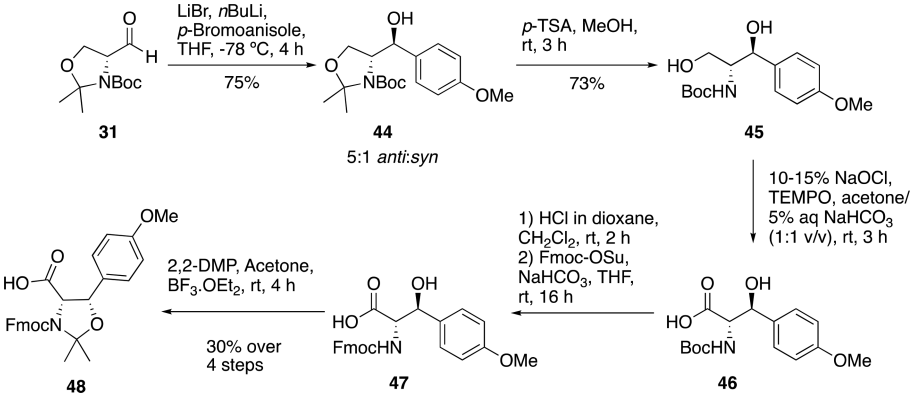
The final *β*-OH amino acid that required synthesis was (2*S*,3*S*)-*β*-OH-*O*-Me-Tyr (Scheme 7). This was again prepared from (*R*)-Garner's aldehyde **31** and was inspired by the work of Hamada and coworkers in their synthesis of the *β*-OH-Tyr diastereomers for the synthesis of papuamides.[28] The first step involved the addition of the organo-lithium species generated from *p*-Br-anisole to (*R*)-Garner's aldehyde to yield alcohol **44** as a mixture of diastereomers (5:1 *anti*:*syn*). Removal of the oxazolidine with *p*-TSA yielded diol **45** which could be recrystallized to slightly improve the diastereomeric ratio (9:1 *anti*:*syn*, 62% recovery). Subsequent oxidation to acid **46**, Boc removal and Fmoc protection to **47**, and final oxazolidine protection yielded the desired final building block **48**. Importantly, at this final stage, the diastereomers could be separated by silica column chromatography to yield purified amino acid **48** as a single diastereomer in good yield over the four steps.

Having prepared *β*-OH amino acids **37**, **43**, and **48**, we investigated the synthesis of deshydroxy skyllamycin B **6** (Scheme 8). Beginning from resin-bound peptide **24** synthesized above, we carried out on-resin esterification with *β*-OH-Leu residue **43** to afford resin-bound branched peptide **49**. Pleasingly, this proceeded efficiently, and we could optimize this coupling to reduce the number of equivalents of this precious building block used. Initially, the next amino acid required, Fmoc-D-Leu-OH, was coupled using the PyBOP coupling conditions outlined earlier. Unfortunately, upon cleavage of a small portion of resin and HPLC-MS analysis, no coupling was observed. We turned to other more active coupling reagents such as HATU and COMU; however, unfortunately, complete conversion was never observed. We presumed this was due to the steric bulk of the oxazolidine protecting group and so investigated more forcing conditions. Pleasingly, treating resin-bound **49** with Fmoc-D-Leu-OH, HATU, HOAt, and Hünig's base at 50 °C under microwave irradiation for 1 h afforded the desired resin-bound **50**. Extension of the peptide utilizing standard PyBOP conditions for Fmoc-Gly-OH and Fmoc-D-Trp(Boc)-OH yielded **51** and was followed by coupling with the more active HATU for precious *β*-OH-Tyr to afford resin-bound **52**. We then coupled Fmoc-Pro-OH using the microwave conditions described above and analyzed a small portion of cleaved resin by HPLC-MS. Unfortunately, at this point, the chromatogram showed a number of peaks with the desired peptide. We reasoned that under the strongly acidic cleavage conditions (TFA:iPr$_3$SiH:H$_2$O, 90:5:5 v/v/v) used to remove the *t*Bu- and Boc-protecting groups the *β*-OH-Tyr group was undergoing acid-catalyzed dehydration. Indeed, this issue was reported by Lipton and coworkers in their synthesis of callipeltin A which also contains a *β*-OH-Tyr residue.[29]

This required us to revise our synthetic strategy, specifically the choice of alternate protecting groups which would be cleaved under more mildly acidic conditions. With this in mind, we left the indole ring of the Trp residue unprotected (Scheme 9). We also employed the hyper acid-labile phenylisopropyl (Ph*i*Pr) protecting group for the side chain of the Asp residue, which can be cleaved with 1 vol % TFA in CH_2Cl_2.[30] Interestingly, during these initial studies, the oxazolidine protecting groups were never observed when analyzing peptides cleaved from the resin by HPLC-MS. This was true even when the peptide was cleaved from resin using the very mildly acidic HFIP conditions. While the hyper-acid lability of these protecting groups is not fully understood, it is possible that the bulky amino acid side chains enhances the acid lability. Importantly, this unexpected acid lability meant that these protecting groups would still be suitable to be used in the revised synthetic strategy. As such, we targeted resin-bound protected linear peptide **53**.

The final amino acid required for the synthesis of the deshydroxy skyllamycin analogues was the (2*S*,3*S*)-*β*-Me-Asp. Having altered our synthetic strategy to the analogues, the more acid-labile Ph*i*Pr protecting group was required on the side-chain carboxylic acid of the building block (Scheme 10). Toward this end, H$_2$N-*β*-Me-Asp(*t*Bu)-OH **54** was first synthesized following a report by Goodman and coworkers.[31]

Scheme 7. Synthesis of Suitably Protected (2*S*,3*S*)-*β*-OH-*O*-Me-Tyr 48



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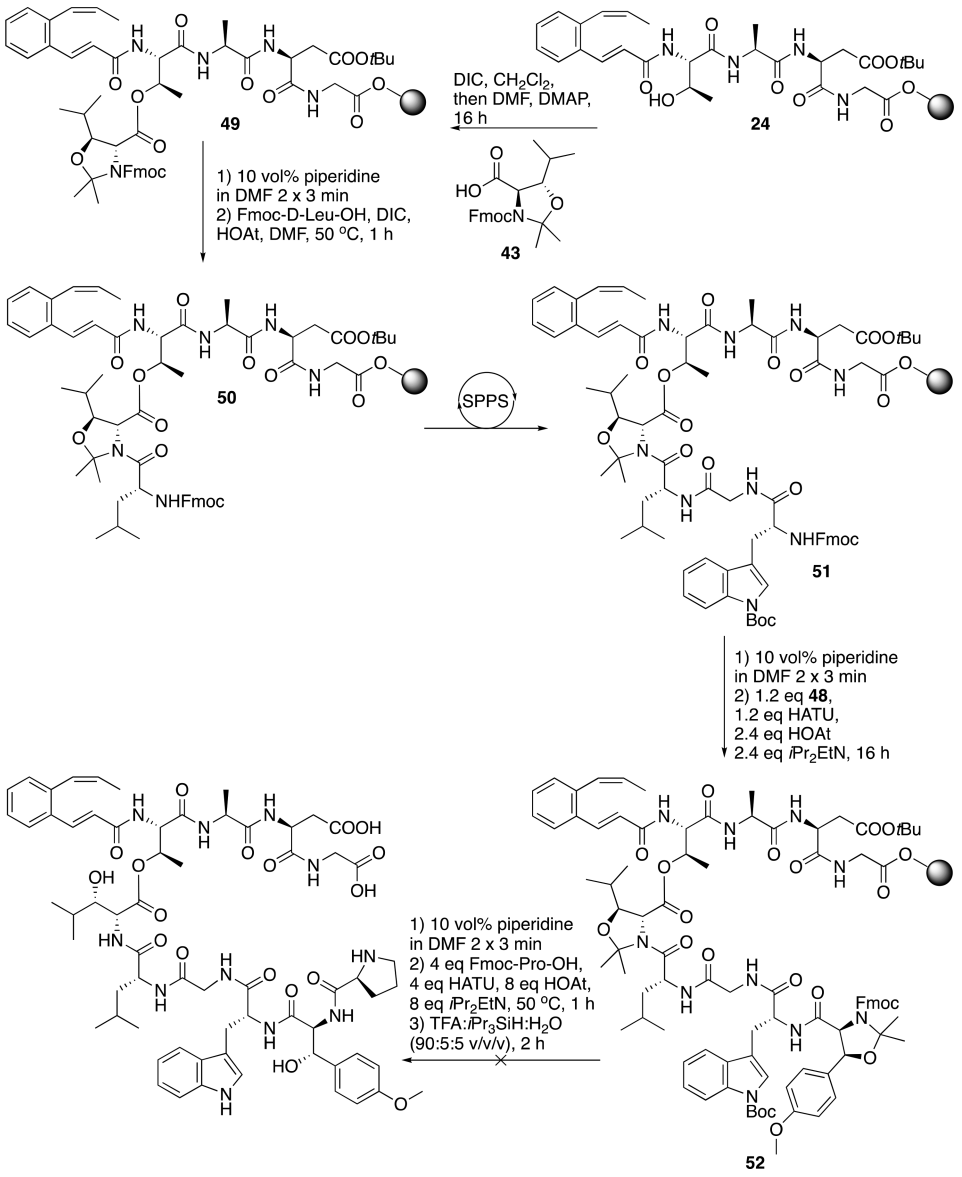
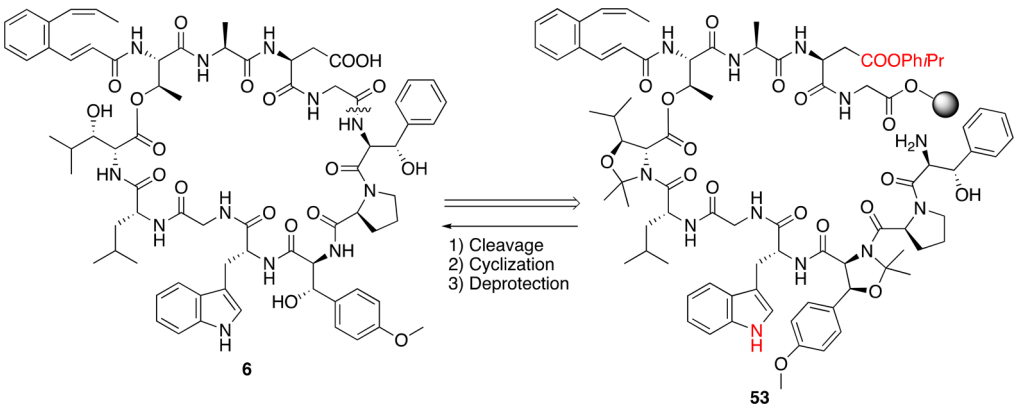
Scheme 8. Initial Attempted Synthesis of Deshydroxy Skyllamycin B 6



Scheme 9. Revised Synthetic Approach to Deshydroxy Skyllamycin B 6



An Fmoc and an allyl protecting group were installed on the amine and carboxylic acid, respectively, to afford **55**, followed by the removal of the side-chain *t*-butyl and installation of the desired Phi*Pr* protecting group, yielding **56**. Final removal of the allyl ester yielded the suitably protected amino acid **57** ready for incorporation into Fmoc-SPPS.

With all the required suitably protected nonproteinogenic amino acids in hand, we next embarked on the synthesis of

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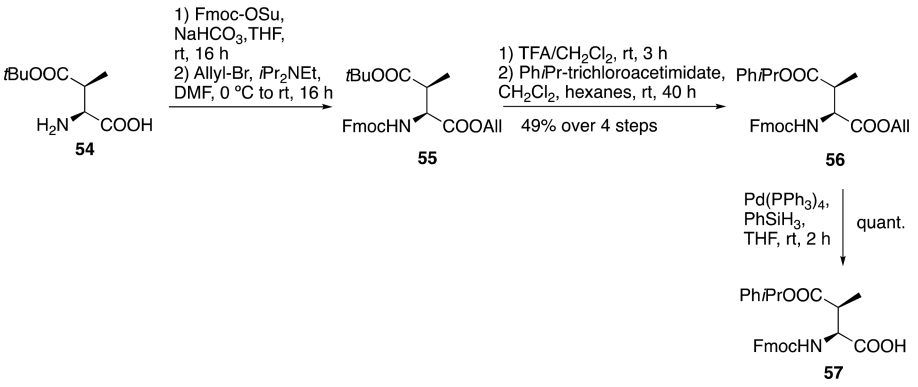
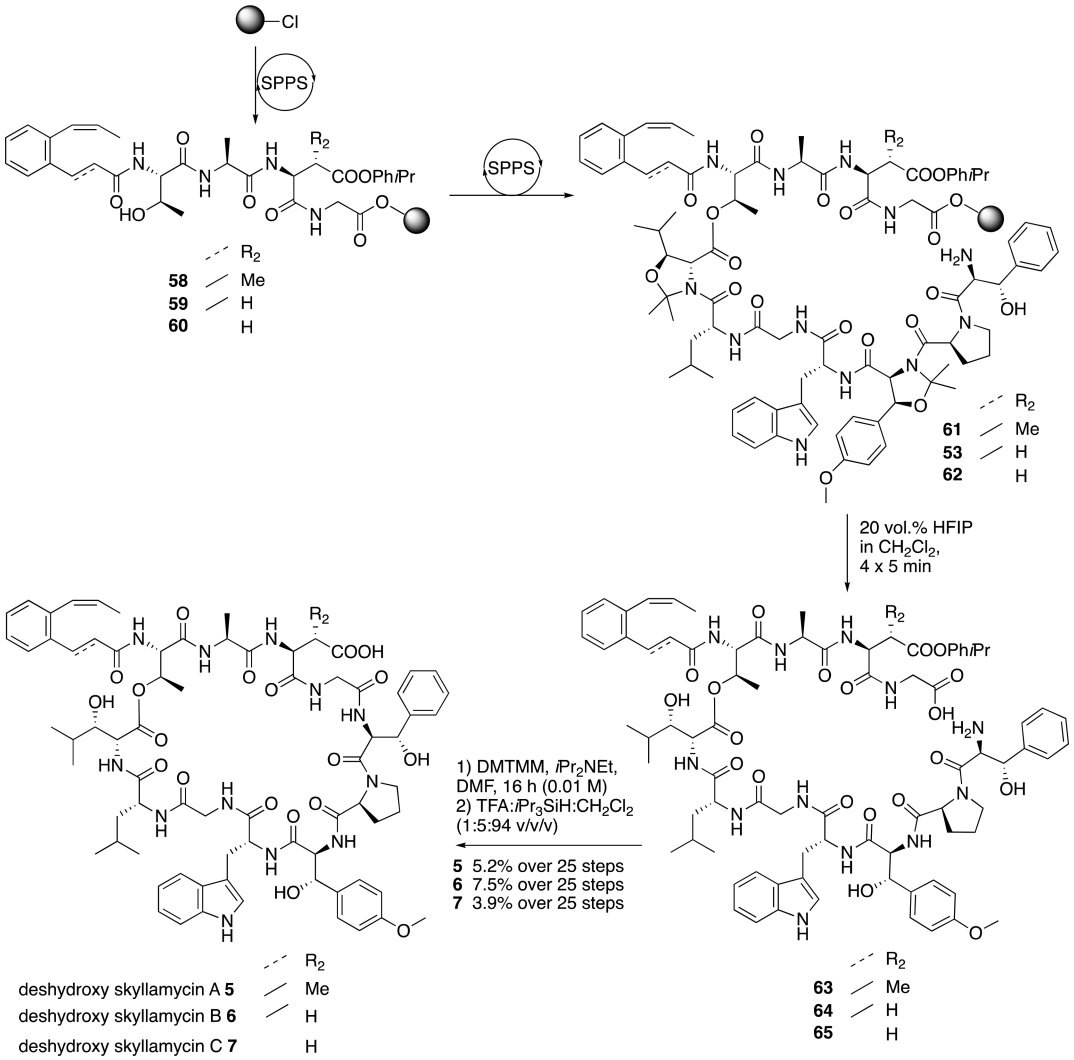
Scheme 10. Synthesis of Suitably Protected β-Me-Asp 57



Scheme 11. Synthesis of Deshydroxy Skyllamycins A–C 5–7



deshydroxy skyllamycins A–C **5**–**7** via our revised synthetic strategy. We began by loading Fmoc-Gly-OH to 2-CTC resin (Scheme 11). To this was coupled either Asp or β-Me-Asp, both suitably protected with the PhiPr protecting group. The peptide chains were then extended under PyBOP coupling conditions followed by coupling of the appropriate cinnamic acid to yield resin-bound **58**–**60**. The key on-resin esterification was then carried out using β-OH-Leu building block **43**, followed by the optimized microwave coupling of Fmoc-D-Leu-OH to the resin. Extension of the peptide using standard PyBOP conditions for Fmoc-Gly-OH and Fmoc-D-Trp-OH and the more active HATU for precious β-OH-Tyr. Microwave coupling of Fmoc-Pro-OH under the above-described conditions was then carried out followed by final coupling of the Fmoc-β-OH-Phe building block **37** to yield resin-bound linear peptides **61**, **53**, and **62**. Pleasingly, when a small amount of peptide was cleaved from resin with side chain deprotection using 1 vol % TFA in CH_2Cl_2 and analyzed by

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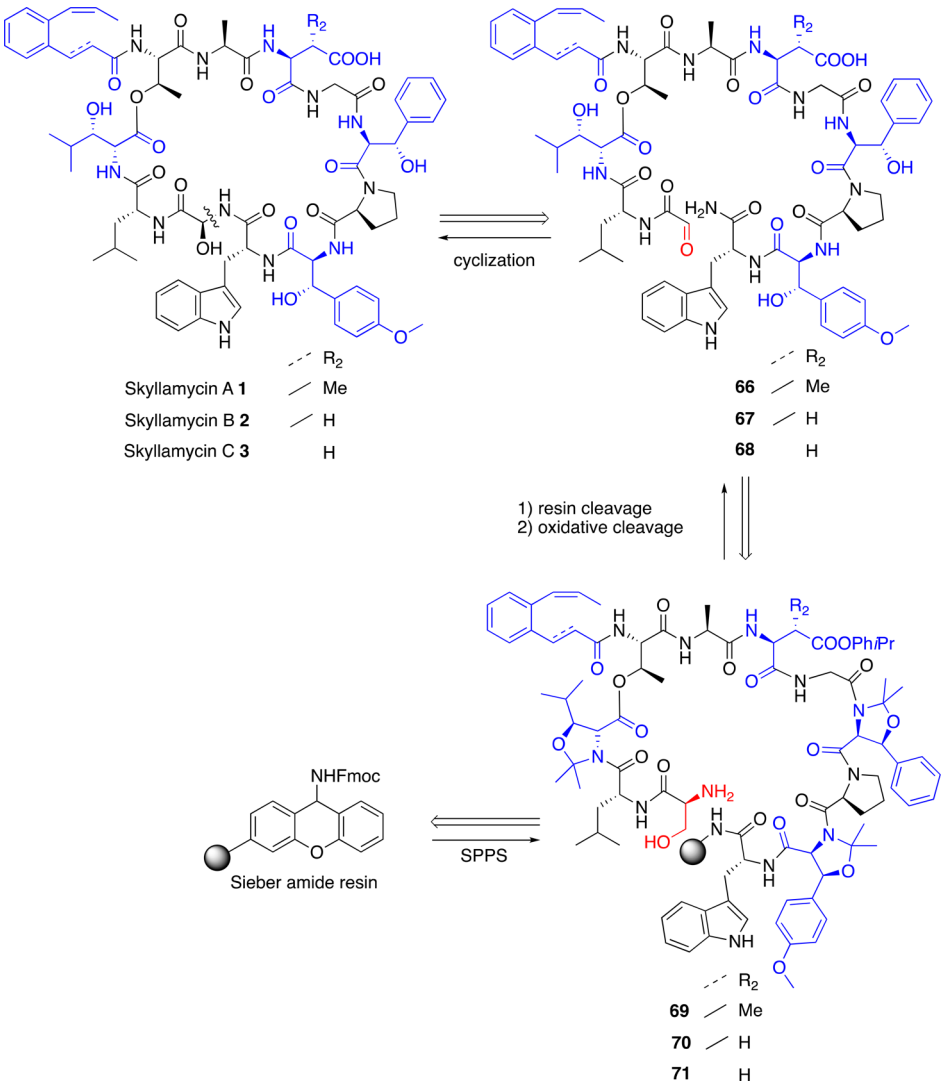
Scheme 12. Retrosynthetic Analysis of Skyllamycins A−C 1−3



HPLC-MS, we observed the desired deprotected product without any decomposition.

Initial experiments carried out to cleave the peptides from resin under standard conditions (30% HFIP in CH_2Cl_2, 30 min) led to partial removal of the hyper acid labile Ph*i*Pr group. The cleavage conditions were thus optimized to 20% HFIP in CH_2Cl_2 for 4 × 5 min treatments, which resulted in no unwanted Ph*i*Pr cleavage and generation of protected linear peptides **63**−**65**. With the linear peptides in hand, we then carried out the cyclization under the optimized reaction conditions described for simplified skyllamycin analogue **4**. Pleasingly, these conditions (DMTMM·BF_4, DMF, 0.01 M) resulted in complete consumption of starting material and conversion to the desired product. Removal of the DMF, followed by mild cleavage of the Ph*i*Pr with 1% TFA in CH_2Cl_2, with *i*Pr_3SiH as a cation scavenger and final purification by reverse-phase HPLC yielded the desired deshydroxy skyllamycins A−C **5**−**7** in 4−8% over 25 steps (88−90% per step).

Having successfully completed the synthesis of these skyllamycin analogues, we next sought to apply the optimized synthetic route to the native natural products. The most interesting feature of these natural products is the unusual α-OH-Gly residue, which is known to be stable in the mature cyclic peptide.[3] Indeed, the intramolecular hydrogen bonding network present in the natural products is thought to stabilize this unusual residue. However, we reasoned that this residue would not be sufficiently stable to repeated SPPS conditions and a final acidic cleavage,[32] which was verified during attempts to synthesize the amino acid (not shown). As such, to assemble the natural products, we envisaged we could carry out a final cyclization and concomitant installation of the α-OH-Gly residue in one step (Scheme 12). This could be carried out by reaction between the C-terminal amide and an N-terminal aldehyde **66**−**68**, which in turn could be accessed via an oxidative cleavage reaction from the N-terminal serine residue. Resin-bound linear peptide precursors **69**−**71** in turn could be synthesized on Sieber amide resin, beginning from the Trp residue, which would reveal a C-terminal amide upon cleavage.[33] In contrast to the synthesis of deshydroxy skyllamycins A−C **5**−**7**, the key on-resin esterification step in this synthetic sequence would now be carried out after installation of both the β-OH-Tyr and β-OH-Phe residues. As such, to prevent unwanted esterification during the synthesis, we protected the β-OH-Phe as the oxazolidine to yield **72** (Scheme 13).

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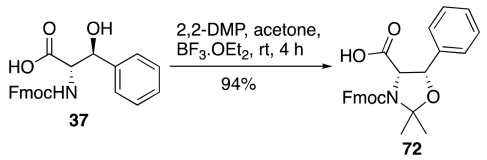
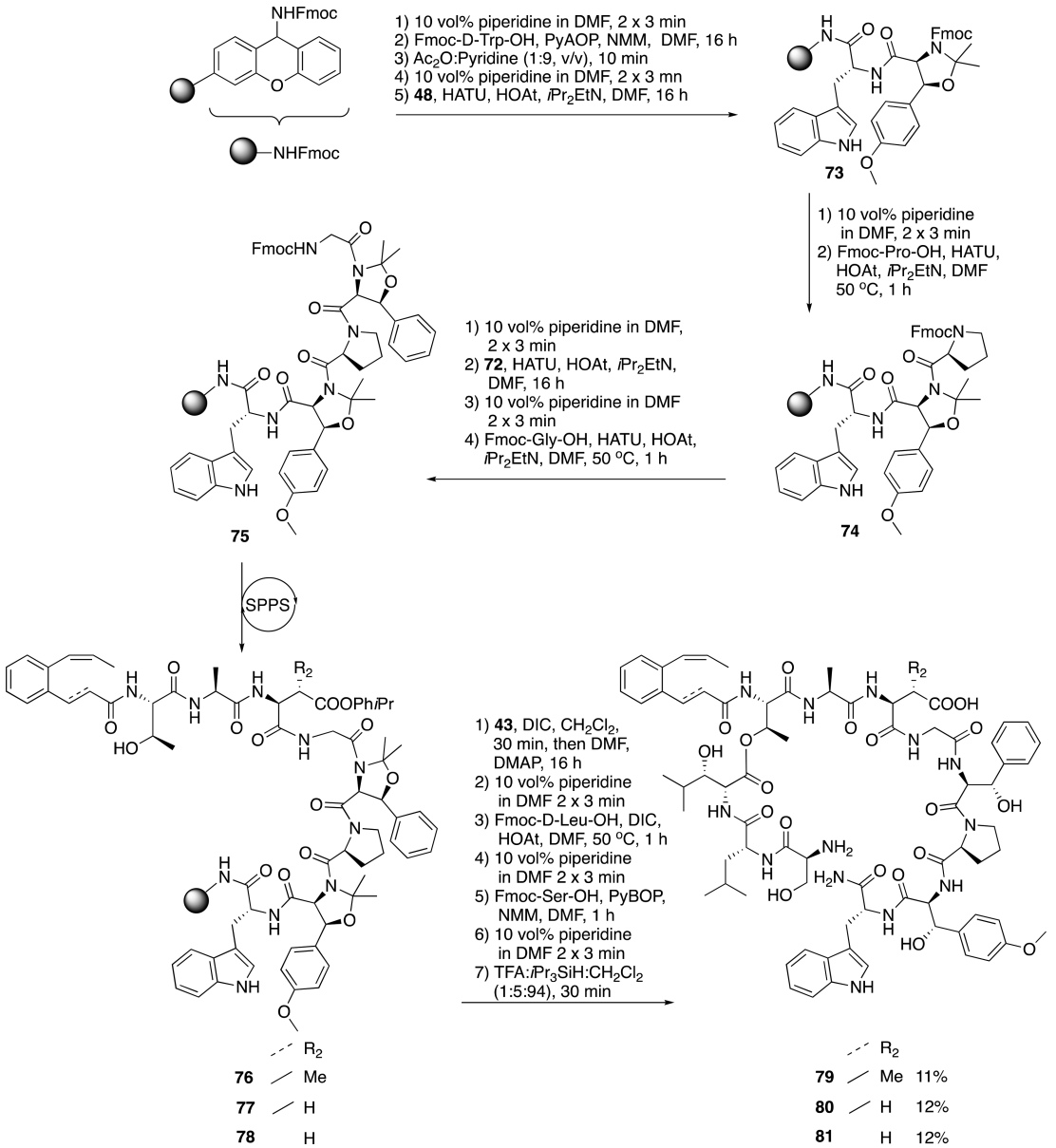
Scheme 13. Synthesis of Oxazolidine Protected β-OH-Phe 72



Construction of the linear peptide began with loading of acid-labile Sieber amide resin with Fmoc-D-Trp-OH followed by coupling of β-OH-Tyr **48** to yield resin-bound dipeptide **73** (Scheme 14). Subsequent Fmoc-deprotection and microwave-assisted coupling of Fmoc-Pro-OH yielded resin-bound tripeptide **74**. This was followed by coupling of protected β-OH-Phe **72** and microwave-assisted coupling of Fmoc-Gly-OH. This yielded the key resin-bound pentapeptide **75**, which was used as the key intermediate for the synthesis of the three natural products.

Resin-bound **75** was then coupled with either Fmoc-Asp(Ph*i*Pr)-OH or Fmoc-β-Me-Asp(Ph*i*Pr)-OH **57** and the peptide further elongated before coupling of the appropriate cinnamic acid to yield resin-bound **76**–**78**. The key on-resin esterification was then performed to yield the desired branched peptides. At this stage, when analyzing a sample of the cleaved peptide, we noticed the presence of a small number of peptides containing two or three β-OH-Leu moieties. Considering the lability of the oxazolidine protecting groups to acid, we hypothesize that these were removed at some point during the SPPS. While surprising, this is potentially due to conformational effects exerted by the peptide backbone. However, by reducing the number of equivalents (to 3 equiv) and then retreating the peptide with a further 0.5 equiv, we were able to achieve near quantitative esterification with minimal over-esterification.

Scheme 14. Synthesis of Linear Peptides 79–81



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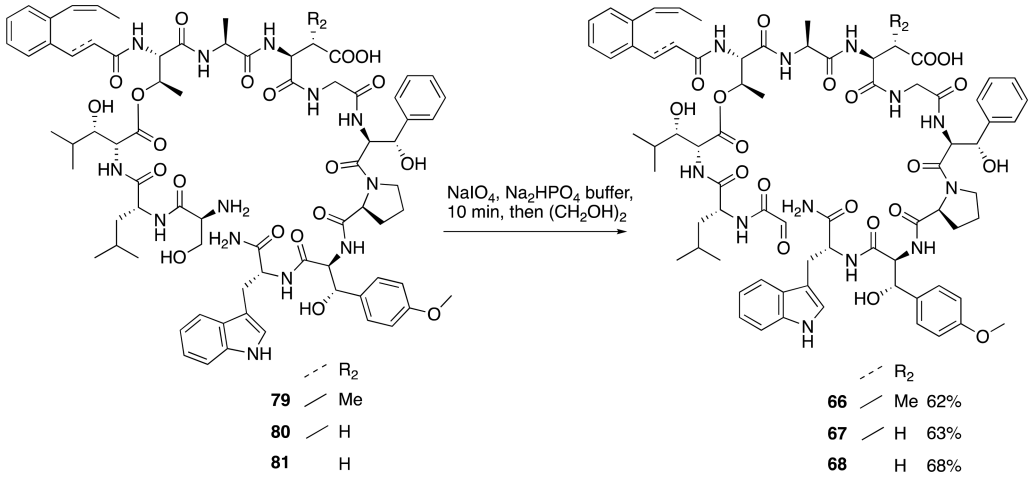
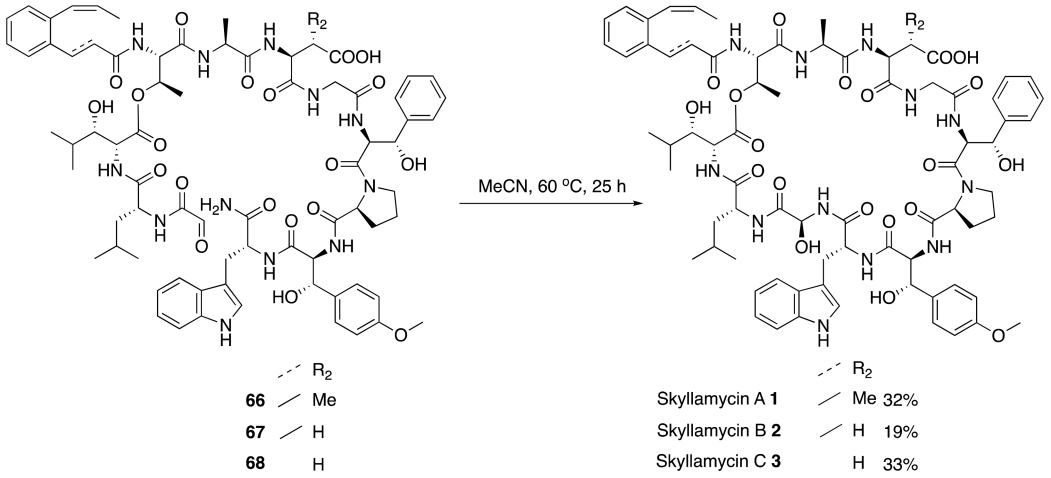
Scheme 15. Synthesis of Aldehydes 66−68



Scheme 16. Synthesis of Skyllamycins A−C 1−3



Next, we carried out microwave-assisted coupling of Fmoc-D-Leu-OH under the previously optimized conditions, namely HATU, HOAt, and Hünig's base. Pleasingly, this reaction proceeded efficiently for the synthesis of skyllamycins B and C. Unfortunately, when the same conditions were used for skyllamycin A and a small portion of crude peptide was analyzed, we noticed the presence of a large amount of an unwanted byproduct with a mass corresponding to that of the target peptide with the loss of water. This was presumably due to the formation of an aspartimide, a common byproduct during Fmoc-SPPS.[34] We hypothesize that this was only the case during the synthesis of skyllamycin A due a conformational effect induced by the β-Me group on the Asp residue. Interestingly, this was never observed during the synthesis of deshydroxy skyllamycin A **5**, and this may be attributed to the proximity of the β-Me-Asp residue to the resin with the steric bulk of the 2-Cl-Trt resin linker preventing formation of the aspartimide. Aspartimides are generally formed due to excess base present in the Fmoc deprotection reaction; however, in this case, it was proposed that the large excess of Hünig's base and extended microwave heating had led to the formation of this byproduct. As such, we ran trials of base-free conditions to affect the coupling of Fmoc-D-Leu-OH to the oxazolidine protected β-OH-Leu residue. Specifically, the transformation was carried out using DIC and HOAt with microwave heating at 50 °C with no external base added. Pleasingly, this successfully forged the desired bond with no aspartimide formation. In subsequent syntheses of skyllamycins B **2** and C **3**, these optimized base-free microwave conditions were used when carrying out this coupling. A final coupling of Fmoc-Ser-OH followed by cleavage from the resin and PhiPr cleavage under mildly acidic conditions furnished the desired unprotected linear peptides **79−81**.

Having prepared the linear peptides **79−81** we embarked on the end-game of the synthesis of the skyllamycins. Specifically, we treated peptides **79−81** with $NaIO_4$ in a mixture of mildly basic Na_2HPO_4 buffer (pH 9) and MeCN[35] (Scheme 15). Pleasingly, this led to rapid oxidative cleavage to the aldehyde in 10 min, at which point a solution of excess ethylene glycol was added to quench the reaction. It was important to keep the pH of the solution controlled, as initial attempts using aqueous $NaHCO_3$ led to hydrolysis of the ester bond. The linear peptide aldehydes were then immediately purified by RP-HPLC and lyophilized to furnish aldehydes **66−68** in yields of 62−68%.

Having successfully obtained linear peptide aldehydes **66−68**, the proposed cyclization reaction was first investigated on skyllamycin B. We reasoned that the C-terminal amide and N-terminal aldehyde would react under mildly acidic conditions and, as such, incubated peptide **67** in a solution of 1% TFA in MeCN for 20 h. Pleasingly, monitoring by HPLC-MS revealed

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the consumption of starting material and the presence of two new peaks, both with the desired mass of the natural product. The two compounds were then isolated and analyzed by ^{1}H NMR; however, unfortunately, both compounds had very different spectra to the isolated natural product. We hypothesized that these products might be cyclic hemiacetals, formed by attack of one of the β-OH groups onto the aldehyde, which would afford an identical mass to the target natural products.

Gratifyingly, the next cyclization conditions we attempted proved successful. Specifically, we incubated linear aldehyde **67** in MeCN at 60 °C and analyzed the reaction by HPLC-MS (Scheme 16). After 25 h, all starting material **67** was consumed, and a new peak with the desired mass and different retention time to those observed previously appeared. This peak was isolated in 42% yield after HPLC, analyzed by ^{1}H NMR and the compared to the authentic natural material. Unfortunately, the NMR analysis revealed that the isolated peak was in fact a 1:1 mixture of two compounds; however, one set of signals overlapped with the isolated material. This mixture was then subject to careful UPLC-MS coinjection studies with the isolated natural product which revealed that the synthetic mixture did indeed contain the natural product. Using a highly optimized HPLC protocol, we separated the mixture and isolated skyllamycin B **2** in 19% yield. This purified material was further subject to comprehensive NMR studies and possessed an almost identical spectrum to that of the isolated material. While the identity of the compound which coeluted with skyllamycin B was not definitively confirmed, we suspect that it was the epimer at the newly formed α-OH-Gly position.

Having successfully synthesized skyllamycin B, we next applied the optimized conditions to the synthesis of skyllamycins A **1** and C **3**. Pleasingly, this resulted in the formation of new peaks with the desired mass by HPLC-MS. Interestingly, in both of these cases, the HPLC-MS profiles were very different to those observed for skyllamycin B, implying that slight changes in structure made a large change in the elution profile of the natural products. Importantly, the peaks resolved much more efficiently, and skyllamycins A and C could be isolated in 32 and 33% yields, respectively. The synthesized natural products were then compared to the isolated material by UPLC-MS coinjection and shown to have identical elution times. Synthetic skyllamycins A **1** and C **3** were also comprehensively analyzed by NMR and produced spectra which were extremely consistent with those of the isolated natural products, suggesting the synthetic material was identical to the isolated natural products. All three natural products exhibited highly analogous circular dichroism spectra, which strongly suggests that the synthetic and isolated material are the same enantiomer.

Given our ongoing interest in the development of antibiofilm lead compounds against *P. aeruginosa*, the simplified analogue **4**, deshydroxy skyllamycins A−C **5**−**7**, and both isolated and synthetic skyllamycins A−C **1**−**3** were tested in our image-based biofilm inhibition assay.[4] Evaluation of biofilm coverage from these images revealed moderate activities for all compounds at the top tested concentrations. Surprisingly, neither removal of the hydroxyl group from the hydroxyglycine motif (compounds **5**−**7**) nor removal of the β-OH moieties from the three β-OH amino acids and elimination of the methyl group from the -OMe tyrosine (compound **4**) dramatically impacted activity. This result suggests that variation at these positions is well-tolerated, paving the way for the development of simplified skyllamycin analogue libraries using standard SPPS reagents and methods.

CONCLUSION

In summary, the total synthesis of skyllamycins A−C as well as the generation of four simplified analogues is described. We efficiently synthesized the nonproteinogenic amino acids present in the natural products that were suitable for direct incorporation into Fmoc-SPPS. Simplified skyllamycin analogue **4** and deshydroxy skyllamycins A−C **5**−**7** were first rapidly assembled using an efficient SPPS protocol and solution phase macrolactamization strategy. An alternate SPPS strategy needed to be employed for the synthesis of the linear precursors **79**−**81** of the skyllamycin natural products. The linear peptide precursors were successfully converted to skyllamycins A−C **1**−**3** using a novel cyclization strategy that proceeded with concomitant formation of the unusual α-OH-Gly residue in the final step. The synthetic natural products were consistent with the structures of the isolated natural products as confirmed by spectroscopic studies. The reported study provides insight into these highly modified natural products, and the synthetic route is amenable to the generation of further analogues for detailed structure−activity relationships against bacterial biofilms as well as other phenotypic screens. Studies toward this end will serve as future research efforts in our laboratories.

EXPERIMENTAL SECTION

All reactions were carried out in dried glassware under an argon atmosphere and at room temperature (22 °C) unless aqueous conditions were used or unless otherwise specified. Reactions undertaken at −78 °C utilized a bath of dry ice and acetone. Reactions carried out at 0 °C employed a bath of water and ice. Anhydrous THF, CH_2Cl_2, MeCN, DMF, toluene, and MeOH were obtained using a PureSolv solvent purification system with water detectable only in low ppm levels. Reactions were monitored by thin layer chromatography (TLC) on aluminum backed silica plates (Merck Silica Gel 60 F254). Visualization of TLC plates was undertaken with an ultraviolet (UV) light at λ = 254 nm and staining with solutions of vanillin, ninhydrin, phosphomolybdic acid (PMA), potassium permanganate, or sulfuric acid, followed by exposure of the stained plates to heat. Silica flash column chromatography (Merck Silica Gel 60 40−63 μm) was undertaken to purify crude reaction mixtures using solvents as specified.

All commercially available reagents were used as obtained from Sigma-Aldrich, Merck, or Acros Organics. Amino acids, coupling reagents, and resins were obtained from NovaBiochem or GL Biochem, and peptide synthesis grade DMF was obtained from Merck or Labscan. All noncommercially available reagents were synthesized according to literature procedures as referenced. Microwave-assisted peptide couplings were carried out using a Biotage Initiator$^+$ Alstra microwave peptide synthesizer equipped with an inert gas manifold. Fmoc-strategy solid-phase peptide synthesis (Fmoc-SPPS) procedures were employed using HMPB functionalized polyethylene glycol resin (HMPB-NovaPEG), 2-CTC functionalized polystyrene resin, or Sieber amide functionalized resin within fritted syringes (purchased from Torviq). All reagent equivalents are in regard to the amount of amino acid loaded to resin.

^{1}H NMR spectra were obtained using a Bruker DRX 400 or DRX 500 at frequencies of 400 or 500 MHz, respectively, in $CDCl_3$, acetone-d_6, CD_3OD, or DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and coupling constants in Hertz (Hz). The residual solvent peaks were used as internal standards without the use of tetramethylsilane (TMS). ^{1}H NMR data is reported as follows: chemical shift values (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. = broad, ap. = apparent),

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coupling constant(s), and relative integral. ^{13}C NMR spectra were obtained using a Bruker DRX 400 or DRX 500 at 100 or 125 MHz in $CDCl_3$, MeOD, acetone-d_6, or DMSO-d_6 unless otherwise specified. ^{13}C NMR data are reported as chemical shift values (ppm). Any rotamers were confirmed by saturation transfer experiments or the presence of in-phase cross-peaks in a NOESY spectrum. Low-resolution mass spectra for novel compounds were recorded on a Bruker amaZon SL mass spectrometer (ESI) operating in positive mode or on a Shimadzu 2020 (ESI) mass spectrometer operating in positive mode. High resolution mass spectra were recorded on a Bruker-Daltronics Apex Ultra 7.0T Fourier transform (FTICR) mass spectrometer. Circular dichroism spectra were recorded on a Chirascan qCD from 600 to 200 nm at a 1 nm resolution at a scan rate of 0.5 scans/sec.

LC-MS was performed either on a Shimadzu 2020 LC-MS instrument with an LC-M20A pump, SPD-20A UV/vis detector and a Shimadzu 2020 (ESI) mass spectrometer operating in positive mode, or on a Shimadzu UHPLC-MS equipped with the same modules as above but with an SPD-M30A diode array detector. Separations on the LC-MS system were performed on a Waters Sunfire 5 μm, 2.1 × 150 mm (C18) column. On the UHPLC-MS system, separations were performed on a Waters Acquity 1.7 μm, 2.1 × 50 mm (C18) column. These separations were performed using a mobile phase of 0.1 vol % formic acid in water (Solvent A) and 0.1 vol % formic acid in MeCN (Solvent B) using linear gradients. Preparative reverse-phase HPLC was performed using a Waters 500 pump with a 2996 photodiode array detector and a Waters 600 Multisolvent Delivery System.

Fmoc-SPPS General Protocols. *Fmoc Deprotection.* A given resin-bound peptide was washed with CH_2Cl_2 (5×) and DMF (5×) before being treated with a solution of 10 vol % piperidine in DMF (2 × 3 min). The resin was again washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×).

PyBOP Coupling Conditions. A given resin-bound peptide was washed with CH_2Cl_2 (5×) and DMF (5×) before being treated with a solution of 10 vol % piperidine in DMF (2 × 3 min). The resin was again washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×). The resin was shaken for 1 h at room temperature with a solution of the desired Fmoc-protected amino acid (4 equiv), PyBOP (4 equiv), and 4-methylmorpholine (NMM) (8 equiv) in DMF (0.1 M in regard to loaded peptide). The coupling solution was discharged and the resin washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×).

HATU Coupling Conditions. A given resin-bound peptide was washed with CH_2Cl_2 (5×) and DMF (5×) before being treated with a solution of 10 vol % piperidine in DMF (2 × 3 min). The resin was again washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×). The resin was shaken for 16 h at room temperature with a solution of the desired Fmoc-protected amino acid (1.1−1.5 equiv), HATU (1.1−1.5 equiv), HOAt (2.2−3 equiv), and iPr$_2$NEt (2.2−3 equiv) in DMF (0.1 M in regard to loaded peptide). The coupling solution was discharged and the resin washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×).

On-Resin Esterification Conditions. To a solution of oxazolidine protected Fmoc-D-Leu-OH or Fmoc-β-OH-D-Leu-OH **43** (4 equiv compared to the resin-bound peptide) in CH_2Cl_2 (0.06 M) at 0 °C was added DIC (1 equiv compared to amino acid). The solution was warmed to room temperature and stirred for 30 min before being concentrated under a stream of N_2. The resultant slurry was dissolved in DMF (0.1 M in regard to loaded peptide) and sucked into the fritted syringe containing resin-bound peptide. Subsequently, a solution of DMAP (catalytic, ∼6 small crystals) in DMF (0.15 mL) was sucked up, and the resin was shaken at room temperature for 16 h. The coupling solution was discharged and the resin washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×).

Microwave Coupling Conditions to Oxazolidine Protected Amino Acid. The resin was transferred to a Biotage microwave peptide synthesis reaction vessel and treated with a solution of the desired Fmoc-protected amino acid (4 equiv), HATU (4 equiv), HOAt (8 equiv), and iPr$_2$NEt (8 equiv) in DMF (0.1 M in regard to loaded peptide) under microwave irradiation at 50 °C for 1 h (sealed reaction vessel, temperature monitored via internal probe). The resin was then transferred to a fritted syringe; the coupling solution was discharged, and the resin washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×).

2-CTC Resin Loading. 2-CTC resin (maximum loading 0.9 mmol/g) in a fritted syringe was swollen in CH_2Cl_2 (5 mL) for 30 min before being washed with a solution of 20 vol % iPr$_2$NEt in CH_2Cl_2 (2 × 5 mL). The resin was then shaken with a solution of Fmoc-Gly-OH (0.6 equiv relative to resin functionalization) and iPr$_2$NEt (1.2 equiv relative to resin functionalization) in CH_2Cl_2 at room temperature for 16 h. The resin was then washed CH_2Cl_2 (5×), DMF (5×), and CH_2Cl_2 (5×), and then treated with a solution of CH_2Cl_2:MeOH:iPr$_2$NEt (v/v/v, 17:2:1, 5 mL) for 30 min. The resin was then washed CH_2Cl_2 (5×), DMF (5×), and CH_2Cl_2 (10×) before being dried under high vacuum and accurately split by weighing before the resin loading was determined. Loaded resin was treated with a solution of 10 vol % piperidine in DMF (2 × 3 min) which was then diluted (100 μL in 10 mL) and the absorbance analyzed at λ = 301 nm to determine number of μmol of amino acid loaded to resin.

2-CTC Resin Cleavage Conditions. Resin-bound peptide was washed with CH_2Cl_2 (5×) and DMF (5×) before being treated with a solution of 10 vol % piperidine in DMF (2 × 3 min). The resin was again washed with DMF (5×), CH_2Cl_2 (5×), DMF (5×), and CH_2Cl_2 (20×). The resin was treated with a 20 vol % solution of HFIP in CH_2Cl_2 (∼5 mL, 4 × 4 min) and the solution transferred to a round-bottom flask and diluted with CH_2Cl_2 (30 mL) before being concentrated under a stream of N_2 and dried in vacuo.

Sieber Amide Loading. Sieber amide resin (550 mg, maximum loading 0.73 mmol/g) was placed in a fritted syringe and swollen in CH_2Cl_2 (10 mL) for 30 min before being washed with DMF (5×). The resin was then treated with a solution of 10 vol % piperidine in DMF (2 × 3 min) and washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×) before being shaken with a solution of Fmoc-D-Trp-OH (255 mg, 600 μmol), PyAOP (312 mg, 600 μmol), and *N*-methylmorpholine (131 μL, 1.2 mmol) in DMF (4 mL) at room temperature for 16 h. The resin was then washed DMF (5×) and CH_2Cl_2 (5×) and then treated with a solution of pyridine:Ac_2O (v/v, 9:1, 5 mL) for 10 min. The resin was then washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×) before the resin loading was determined. Loaded resin was treated with a solution of 10 vol % piperidine in DMF (2 × 3 min) which was then diluted and the absorbance analyzed at λ = 301 nm to determine number of μmol of amino acid loaded to resin.

Sieber Amide Resin Cleavage Conditions. Resin-bound peptide was washed with CH_2Cl_2 (5×) and DMF (5×) before being treated with a solution of 10 vol % piperidine in DMF (2 × 3 min). The resin was again washed with DMF (5×), CH_2Cl_2 (5×), DMF (5×) and CH_2Cl_2 (20×). The resin was treated with a solution of TFA:iPr$_3$SiH:CH_2Cl_2 (1:5:94, v/v/v) in CH_2Cl_2 (0.001 M wrt. resin loading) and the solution transferred to a round-bottom flask and diluted with CH_2Cl_2 (30 mL) before being concentrated under a stream of N_2 and dried in vacuo. Linear peptides were purified via HPLC using a Waters Sunfire C18 OBD 19 × 150 mm column, using a 0−50 vol % MeCN in H_2O (0.1% TFA) focused gradient (0−40 vol % MeCN over 5 min, 40−50 vol % over 15 min) at a flow rate flow rate of 16 mL min^{-1} and lyophilized.

General Procedures for Modified Amino Acid Synthesis. *General Procedure A: Selective Oxidation of the Primary Alcohol.* To a solution of amino-diol (1 equiv) in acetone (0.2 M) was added TEMPO (0.2 equiv) followed by aqueous $NaHCO_3$ (5% g/100 mL) to bring the solution to 0.1 M. The mixture was cooled to 0 °C, and sodium hypochlorite (3.5 equiv, 10−15% by weight) was added portionwise over 25 min. The mixture was warmed to room temperature and stirred for 3−16 h (until judged complete by TLC analysis). The reaction mixture was diluted with water and the aqueous phase was washed with Et_2O (2×) then acidified to pH 2 with aqueous HCl (1 M). The aqueous phase was extracted with EtOAc (3×) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo.

General Procedure B: Boc Deprotection Followed by Fmoc Protection. To a solution of amino acid (1 equiv) in CH_2Cl_2 (0.16 M) was added HCl in dioxane (10 equiv, 4 M). The solution was stirred at

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room temperature for 1.5−3 h (until judged complete by TLC analysis), and the solvent was removed in vacuo. To a solution of the crude residue in a mixture of THF:saturated aqueous $NaHCO_3$ (2:1 v/v, 0.1 M) was added Fmoc-OSu (1.05 equiv). The reaction was stirred for 16 h at room temperature before being poured onto water and washed with Et_2O (2×). The aqueous phase was then acidified to pH 1 with aqueous HCl (1 M) and extracted with EtOAc (3×) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo.

General Procedure C: Oxazolidine Protection of β-OH Amino Acid. To a solution of alcohol (1 equiv) in acetone (0.14 M) was added 2,2-dimethoxypropane (10 equiv) followed by $BF_3.OEt_2$ (0.1 equiv). The reaction mixture was stirred at room temperature for 5−16 h (until judged complete by TLC analysis). The reaction mixture was poured onto saturated aqueous NH_4Cl and extracted with EtOAc (3×), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo.

General Procedures for Peptide Cyclization. *General Procedure D: Peptide Cyclization Conditions.* Crude cleaved peptide was suspended in DMF (0.01 M) before a solution of DMTMM·BF_4 (1.5 equiv) in DMF (0.01 M) with respect to peptide) was added, to give an overall concentration of 0.005 M of crude peptide, followed by iPr_2NEt (2 equiv) and stirred at room temperature for 16 h. The reaction mixture was analyzed by HPLC-MS and concentrated under a stream of N_2. The crude solid was diluted with CH_2Cl_2 (∼30 mL) and further concentration by a stream of N_2 and dried in vacuo.

General Procedure E: Cyclic Peptide Deprotection Conditions. To a solution of crude cyclized peptide in CH_2Cl_2 (0.001 M wrt. crude linear peptide) was added iPr_3SiH (5% v/v of CH_2Cl_2) followed by TFA (1% v/v of CH_2Cl_2). The reaction mixture was stirred for 30 min and then diluted with CH_2Cl_2 (equal to initial volume) and concentrated under a stream of N_2 and dried in vacuo. Cyclic peptides were purified via HPLC using a Waters Sunfire C18 OBD 19 × 150 mm column, using a 0−60 vol % MeCN in H_2O (0.1% TFA) focused gradient (0−50 vol % MeCN over 5 min, 50−60 vol % over 15 min) at a flow rate of 16 mL min^{-1}.

General Procedures for the Synthesis of Skyllamycins A−C (1−3). *General Procedure F: Oxidative Cleavage Reaction.* To a solution of linear peptide bearing an *N*-terminal serine residue (**79**−**81**) (6.5−7 μmol) in a mixture of aqueous Na_2HPO_4 (1 M, pH 8.4) (400 μL) and MeCN (400 μL) was added an aqueous solution of $NaIO_4$ (53−58 μL, 2 equiv, 10 mg in 200 μL of H_2O). The mixture was mixed using a vortex mixer before being allowed to react for 10 min. To the mixture was added an aqueous solution of ethylene glycol (35−38 μL, 10 equiv, 20 μL in 200 μL of H_2O). The mixture was then diluted up to 3.5 mL with a mixture of MeCN and H_2O (with 0.1% TFA) and purified via HPLC using a Waters Sunfire C18 OBD 19 × 150 mm column, using a 0−60 vol % MeCN in H_2O (0.1% TFA) focused gradient (0−50 vol % MeCN over 5 min, 50−60 vol % over 15 min) at a flow rate flow rate of 16 mL min^{-1} and lyophilized.

General Procedure G: Cyclization Reaction. Linear peptide aldehyde (**66**−**68**) (5.8−6.8 mg) was dissolved in MeCN (4 mL) in 2 separate microcentrifuge tubes. The solutions were incubated at 60 °C, and the reaction was monitored by HPLC-MS. After 25 h, each microcentrifuge tube was diluted up to 3.5 mL total volume with H_2O and then purified over two runs via HPLC using a Waters Sunfire C18 OBD 19 × 150 mm column, using a 0−60 vol % MeCN in H_2O (0.1% TFA) focused gradient (0−50 vol % MeCN over 5 min, 50−60 vol % over 15 min) at a flow rate flow rate of 16 mL min^{-1}. All peaks were collected and lyophilized.

Synthesis of Cinnamic Acid 15. *2-Iodobenzaldehyde (**19**).* To a solution of *m*-iodo benzyl alcohol **18** (3.00 g, 12.8 mmol) in CH_2Cl_2 (30 mL) was added pyridinium chlorochromate (3.04 g, 15.14 mmol). The mixture was left to stir at room temperature for 3.5 h at which point TLC showed the presence of starting material. A further portion of pyridinium chlorochromate (0.5 g, 2.5 mmol) was added, and the mixture was stirred for a further 1 h. The mixture was concentrated in vacuo and crude residue was purified by flash chromatography (eluent: 3:7 v/v EtOAc/petroleum benzines) to yield aldehyde **19** as a pale yellow solid (2.42 g, 76%).

^1H NMR ($CDCl_3$, 500 MHz) δ (ppm) 10.07 (d, *J* = 0.7 Hz, 1H), 7.95 (dd, *J* = 7.90, 0.75 Hz 1H), 7.88 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.28 (td, *J* = 7.6, 1.8 Hz, 1H); LRMS (+ESI) *m*/*z* 233 $[M + Na]^+$; mp 38.2−39.9 °C. These data are in agreement with those previously reported by Tummatorn and Dudley.[38]

*(E)-Ethyl 3-(2-Iodophenyl)acrylate (**20**).* To a solution of NaH (265 mg, 6.65 mmol, 60% dispersion in oil) in CH_2Cl_2 (24 mL) at 0 °C was added triethyl phosphonoacetate (0.96 mL, 4.87 mmol) dropwise. The mixture was stirred at this temperature for 5 min. A solution of aldehyde **19** (1.1 g, 4.43 mmol) in CH_2Cl_2 (19 mL) was added dropwise to the above mixture, and the resultant mixture was stirred at 0 °C for 2 h. The reaction mixture was quenched with cold H_2O (10 mL) and poured onto H_2O (20 mL). The aqueous layer was extracted with EtOAc (4 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 3:97 → 5:95 v/v EtOAc/petroleum benzines) to yield iodide **20** as a yellow oil which solidified on freezing (1.2 g, 89%).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.90 (d, *J* = 15.8 Hz, 1H), 7.90 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.56 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.38−7.34 (m, 1H), 7.05 (td, *J* = 7.6, 1.6 Hz, 1H), 6.31 (d, *J* = 15.8 Hz, 1H), 4.29 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H); LRMS (+ESI) *m*/*z* 325 $[M + Na]^+$. These data are in agreement with those previously reported by Sun et al.[16]

*(E)-Ethyl 3-(2-(Prop-1-yn-1-yl)phenyl)acrylate (**21**).* To a solution of 1-bromo-1-propene (0.46 mL, 5.35 mmol) in THF (7.1 mL) at −78 °C was added *n*-BuLi (2.8 mL, 7.08 mmol, 2.5 M in hexanes) dropwise. The solution was stirred at this temperature for 45 min. H_2O (128 μL, 7.08 mmol) was added, and the resultant solution was warmed to room temperature. After 45 min, a solution of iodide **20** (535 mg, 1.77 mmol) in THF (2.2 mL) was added dropwise, followed by CuI (34 mg, 0.18 mmol), iPr_2NH (5.2 mL), and $Pd(PPh_3)_2Cl_2$ (62 mg, 0.089 mmol). The resultant mixture was stirred at room temperature for 16 h. The reaction mixture was then poured onto saturated aqueous NH_4Cl (30 mL) and extracted with Et_2O (3 × 30 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 2:98 → 5:95 v/v EtOAc/petroleum benzines) to yield alkyne **21** as a yellow solid (344 mg, 91%).

^1H NMR ($CDCl_3$, 500 MHz) δ (ppm) 8.21 (d, *J* = 16.1 Hz, 1H), 7.62−7.61 (m, 1H), 7.47−7.45 (m, 1H), 7.31−7.29 (m, 2H), 6.51 (d, *J* = 16.1 Hz, 1H), 4.29 (q, *J* = 7.1 Hz, 2H), 2.15 (s, 3H), 1.37 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 167.1, 143.0, 135.8, 133.1, 129.7, 127.9, 126.1, 125.1, 119.5, 92.5, 77.2, 60.6, 14.5, 4.7; LRMS (+ESI) *m*/*z* 215 $[M + H]^+$; HRMS (+ESI) *m*/*z*: $[M + Na]^+$ Calcd for $C_{14}H_{14}O_2Na$ 237.0886; Found: 237.0886; IR ν_{max} (ATR) 2980, 2916, 2247, 2212, 1710, 1633, 1478, 1315, 1268, 1176 cm^{-1}; mp 37.6−39.4 °C.

*(E)-Ethyl 3-(2-((Z)-Prop-1-en-1-yl)phenyl)acrylate (**22**).* To a solution of alkyne **21** (257 mg, 1.2 mmol) in MeOH (11.8 mL) was added quinoline (154 μL, 1.24 mmol) and Lindlar's catalyst (258 mg, 0.12 mmol, 5% Pd by weight). The solution was stirred under an atmosphere of H_2 for 20 min. A further portion of Lindlar's catalyst (200 mg) was added, and the mixture was stirred under an atmosphere of H_2 for 50 min. The mixture was then filtered over Celite and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 1:99 → 5:95 v/v EtOAc/petroleum benzines) to yield diene **22** as a colorless oil (230 mg, 88%).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.89 (d, *J* = 16.0 Hz, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.36−7.21 (m, 3H), 6.58 (dd, *J* = 11.4, 1.5 Hz, 1H), 6.39 (d, *J* = 16.0 Hz, 1H), 5.97−5.92 (m, 1H), 4.26 (q, *J* = 7.1 Hz, 2H), 1.66 (dd, *J* = 7.0, 1.8 Hz, 3H), 1.33 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 167.2, 143.1, 138.1, 133.0, 130.2, 129.6, 129.4, 127.9, 127.2, 126.6, 119.2, 60.5, 14.5 14.4; LRMS (+ESI) *m*/*z* 217 $[M + H]^+$; HRMS (+ESI) *m*/*z*: $[M + Na]^+$ Calcd for $C_{14}H_{16}O_2Na$ 239.1043; Found 239.1043; IR ν_{max} (ATR) 2979, 1708, 1632, 1477, 1445, 1311, 1268, 1165 cm^{-1}.

*(E)-3-(2-((Z)-Prop-1-en-1-yl)phenyl)acrylic acid (**15**).* To a solution of diene **22** (189 mg, 0.87 mmol) in ethanol (2.8 mL, 95%) at 0 °C was added LiOH (84 mg, 3.5 mmol). The solution was warmed to

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room temperature and stirred for 16 h. The mixture was cooled to 0 °C and acidified to pH 1 with aqueous HCl (1 M). The mixture was extracted with EtOAc (4 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to yield acid **15** as an off-white solid (152 mg, 92%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.01 (d, J = 16.0 Hz, 1H), 7.66 (dd, J = 7.6, 0.7 Hz, 1H), 7.38−7.24 (m, 4H), 6.59 (dd, J = 11.4, 1.5 Hz, 1H), 6.42 (d, J = 16.0, 1H), 5.98 (dq, J = 11.4, 7.0 Hz, 1H), 1.66 (dd, J = 7.0, 1.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 172.4, 145.7, 138.5, 132.6, 130.3, 130.2, 129.8, 127.8, 127.3, 126.8, 118.1, 14.6; LRMS (+ESI) m/z 189 $[M + H]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{12}H_{12}O_2Na$ 211.0730; Found 211.0730; IR ν_{max} (ATR) 3014 (broad), 1678, 1623, 1479, 1422, 1332, 1300, 1288, 1224 cm^{-1}; mp 128.7−132.2 °C.

Synthesis of Reduced Cinnamic Acid 16. *Ethyl 3-(2-Iodophenyl)propanoate (**28**).* To a solution of ester **20** (500 mg, 1.65 mmol) in ethanol (5.3 mL, 95%) at 0 °C was added LiOH (159 mg, 6.62 mmol). The solution was warmed to room temperature and stirred for 16 h. The mixture was cooled to 0 °C and acidified to pH 1 with aqueous HCl (0.1 M). The mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield acid as a white solid (447 mg) which was used without further purification.

To a solution of the above crude acid (447 mg, 1.63 mmol) in a solution of ethanol (5 mL) and EtOAc (5 mL) was added guanidine nitrate (36.5 mg, 0.163 mmol) followed by hydrazine hydrate (217 μL, 4.89 mmol). The reaction mixture was stirred under an atmosphere of O_2 for 16 h, at which point an aliquot was analyzed by ^{1}H NMR. Starting material was detected, and so the reaction mixture was stirred under O_2 for a further 16 h. The mixture was then concentrated under a stream of N_2. Water (30 mL) was added and then acidified to pH 1 with aqueous HCl (1 M). The mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield reduced acid as a white solid (446 mg), which was used without further purification.

To a solution of the above crude reduced acid (398 mg, 1.44 mmol) in ethanol (6 mL) at 0 °C was added $SOCl_2$ (526 μL, 7.20 mmol) dropwise. The solution was warmed to room temperature and stirred for 16 h. The solution was concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 5:95 v/v EtOAc/petroleum benzines) to yield reduced ethyl ester **28** as a colorless oil (413 mg, 94%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm); 7.82 (d, J = 8.4 Hz, 1H), 7.30−7.22 (m, 2H), 6.90 (ddd, J = 7.9, 6.6, 2.5, 1H), 4.14 (q, J = 7.2 Hz, 2H), 3.05 (t, J = 7.6 Hz, 2H), 2.62 (t, J = 8.2 Hz, 2H), 1.25 (d, J = 7.1 Hz, 3H); LRMS (+ESI) m/z 327 $[M + Na]^+$. These data are in agreement with those previously reported by Tummatorn and Dudley.[36]

*Ethyl 3-(2-(Prop-1-yn-1-yl)phenyl)propanoate (**29**).* To a solution of 1-bromo-1-propene (0.57 mL, 6.71 mmol) in THF (5 mL) at −78 °C was added *n*-BuLi (3.58 mL, 8.96 mmol, 2.5 M in hexanes) dropwise. The solution was stirred at this temperature for 45 min. H_2O (161 μL, 8.96 mmol) was added, and the resultant solution was warmed to room temperature. After 45 min, a solution of iodide **28** (340 mg, 1.12 mmol) in THF (1.5 mL) was added dropwise, followed by CuI (43 mg, 0.22 mmol), iPr$_2$NH (3.12 mL), and $Pd(PPh_3)_2Cl_2$ (79 mg, 0.112 mmol). The resultant mixture was stirred at room temperature for 16 h. The reaction mixture was then poured onto saturated aqueous NH_4Cl (30 mL), and extracted with Et_2O (3 × 30 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 5:95 v/v EtOAc/petroleum benzines) to yield alkyne **29** as a colorless oil (202 mg, 83%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.37 (d, J = 7.6 Hz, 1H), 7.21−7.11 (m, 3H), 4.13 (q, J = 7.2 Hz, 2H), 3.08 (t, J = 7.6, 2H), 2.65 (t, J = 8.1 Hz, 2H), 2.09 (s, 3H), 1.24 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 173.3, 142.4, 132.4, 128.8, 127.9, 126.3, 123.6, 90.1, 78.1, 60.5, 35.0, 30.1, 14.4, 4.6; LRMS (+ESI) m/z 239 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{16}O_2Na$ 239.1043; Found 239.1043; IR ν_{max} (ATR) 2980, 2917, 2252, 1730, 1485, 1447, 1371, 1293, 1181, 1155 cm^{-1}.

*(Z)-Ethyl 3-(2-(Prop-1-en-1-yl)phenyl)propanoate (**30**).* To a solution of alkyne **29** (102 mg, 0.47 mmol) in MeOH (4.7 mL) was added quinoline (58 μL, 0.47 mmol) and Lindlar's catalyst (100 mg, 0.047 mmol, 5% Pd by weight). The solution was stirred under an atmosphere of H_2 for 1 h. A further portion of Lindlar's catalyst (100 mg) was added, and the mixture was stirred under an atmosphere of H_2 for 45 min. The reaction was monitored by TLC every 20 min, and if the reaction was not complete, a further portion of Lindlar's catalyst (200−300 mg) was added. The reaction was dosed a total of 6 times to push it to completion. The mixture was then filtered over Celite and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 5:95 v/v EtOAc/petroleum benzines) to yield alkene **30** as a colorless oil (85 mg, 83%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.22−7.17 (m, 4H), 6.53 (ap. dd, J = 11.5, 1.7 Hz, 1H), 5.87 (dq, J = 11.5, 7.0 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 2.93 (t, J = 7.8 Hz, 2H), 2.54 (t, J = 8.3 Hz, 2H), 1.73 (dd, J = 7.0, 1.8 Hz, 3H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 173.2, 138.8, 136.4, 129.8, 129.0, 128.4, 127.8, 127.1, 126.1, 60.5, 35.1, 28.9, 14.4, 14.3; LRMS (+ESI) m/z 241 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{18}O_2Na$ 241.1199; Found 241.1199; IR ν_{max} (ATR) 2980, 1730, 1447, 1371, 1288, 1252, 1178, 1157, 1113 cm^{-1}.

*(Z)-3-(2-(Prop-1-en-1-yl)phenyl)propanoic acid (**16**).* To a solution of alkene **30** (79 mg, 0.36 mmol) in ethanol (4 mL, 95%) at 0 °C was added LiOH (35 mg, 1.45 mmol). The solution was warmed to room temperature and stirred for 16 h. The mixture was cooled to 0 °C and acidified to pH 1 with aqueous HCl (1 M). The mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to yield acid **16** as an off-white solid (62 mg, 89%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 7.24−7.14 (m, 4H), 6.53 (dq, J = 11.4, 1.8 Hz, 1H), 5.88 (dq, J = 11.4, 7.0 Hz, 1H), 2.94 (t, J = 7.7 Hz, 2H), 2.61 (t, J = 8.3 Hz, 2H), 1.73 (dd, J = 7.0, 1.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) 179.6, 138.4, 136.4, 129.9, 129.0, 128.3, 128.0, 127.2, 126.3, 34.8, 28.5, 14.4; LRMS (+ESI) m/z 213 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{12}H_{14}O_2Na$ 213.0886; Found 213.0886; IR ν_{max} (ATR) 3014 (broad), 1701, 1435, 1407, 1319, 1275, 1215 cm^{-1}; mp 62.1−65.0 °C.

Synthesis of Oxazolidine Protected Fmoc-β-OH-Leu-OH 43. *(S)-tert-Butyl 4-((S)-1-Hydroxy-2-methylpropyl)-2,2-dimethyloxazolidine-3-carboxylate (**39**).* Magnesium granules (3.9 g, 159.5 mmol) were heated under vacuum with a heat gun for 2 min. THF (10 mL) and iodine (10 crystals) were then added, and the mixture was stirred vigorously under argon for 15 min. A solution of isopropyl bromide (9 mL, 05.9 mmol) in THF (35 mL) was added dropwise over 20 min, by which point the mixture turned a dull gray color and began to self-reflux. It was heated at reflux for 1 h and then cooled to room temperature. The solution was then added dropwise via cannula over 15 min to a solution of (*S*)-Garner's aldehyde (**38**) (7.3 g, 31.9 mmol) at −78 °C. The solution was stirred for 2 h at this temperature and then warmed to room temperature and stirred for 1 h. The mixture was then diluted with Et_2O (100 mL) and quenched with saturated aqueous NH_4Cl (200 mL). The aqueous phase was extracted with Et_2O (2 × 100 mL), and the combined organic layers were washed with saturated aqueous NH_4Cl (2 × 150 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 8:92 → 10:90 v/v EtOAc/petroleum benzines) to yield a single diastereomer of alcohol **39** as a white crystalline solid (3.57 g, 41%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 4.09−3.96 (br. s, 1H), 3.93 (dd, J = 9.3, 5.8 Hz, 1H), 3.76 (d, J = 9.1 Hz, 1H), 3.50 (br. d, J = 7.4 Hz, 1H), 1.66 (septd, J = 6.8, 2.4 Hz, 1H), 1.60 (s, 3H), 1.51 (s, 3H), 1.49 (s, 9H), 1.03 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H); LRMS (+ESI) m/z 296 $[M + Na]^+$; $[\alpha]_D$ = −51.1° (c 0.45, CH_2Cl_2); mp 84.6−87.3 °C. These data are in agreement with those previously reported by Williams et al.[27]

*(4R,5S)-3-(((9H-Fluoren-9-yl)methoxy)carbonyl)-5-isopropyl-2,2-dimethyloxazolidine-4-carboxylic acid (**43**).* To a solution of alcohol

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39 (3.46 g, 12.65 mmol) in THF (400 mL) was added aqueous HCl (17.0 mL, 0.5 M). The solution was stirred at room temperature for 16 h at which point it was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield diol **40** as a colorless oil, which was used without further purification. Crude diol **40** (3.49 g, 12.65 mmol) was oxidized by general procedure A to produce carboxylic acid **41**, which was used without further purification. Crude acid **41** (2.48 g, 10 mmol) was subject to protecting group manipulation via general procedure B to produce Fmoc amino acid **42**, which was used without further purification. Crude Fmoc amino acid **42** (3.20 g, 8.65 mmol) was oxazolidine protected using general procedure C. Crude oxazolidine protected amino acid **43** was purified by flash chromatography (eluent: 10:90 → 60:40 v/v EtOAc/petroleum benzines) to yield oxazolidine protected amino acid **43** as an off-white crystalline solid (2.72 g, 53% from alcohol **39**).

^{1}H NMR ($CDCl_3$, 500 MHz, rotamers) δ (ppm) 8.52 (br. s, 1H), 7.78−7.67 (m, 2H), 7.58−7.49 (m, 2H), 7.40−7.25 (m, 4H), 4.66 (d, J = 4.2 Hz, 1H), 4.42 (m, 1H), 4.22−4.05 (m, 2H), 3.87 (br. t, J = 6.1 Hz, 0.5H) and 3.79 (br. t, J = 6.4 Hz, 0.5H), 1.91−1.71 (m, 1H), 1.60 (m, 3H), 1.09−0.87 (m, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz, rotamers) δ (ppm) 176.6 and 176.1, 153.1 and 152.0, 144.2 and 144.0, 143.8 and 143.7, 141.7 and 141.6, 141.5 and 141.4, 127.8, 127.3 and 127.2, 127.2 and 127.2, 125.0, 124.5 and 124.5, 120.0 and 120.0, 96.0 and 95.0, 83.9 and 83.9, 67.2 and 66.9, 62.6 and 61.8, 47.4, 32.1 and 31.7, 26.9, 24.8 and 24.7, 18.8 and 18.5, 17.7, and 17.6 LRMS (+ESI) m/z 432 [M + Na]$^+$; HRMS (+ESI) m/z: [M + Na]$^+$ Calcd for $C_{24}H_{27}NO_5Na$ 432.1781; Found 432.1782; IR ν_{max} (ATR) 2961, 2924, 1714, 1697, 1411, 1348, 1259 cm^{-1}; $[\alpha]_D$ = +29.6° (c 0.48, CH_2Cl_2); mp 59.5−60.5 °C.

Synthesis of Fmoc-β-OH-Phe-OH (37). *(4R)-tert-Butyl 4-(hydroxy(phenyl)methyl)-2,2-dimethyloxazolidine-3-carboxylate (**32**).* Magnesium granules (545 mg, 22.4 mmol) were heated under vacuum with a heat gun for 2 min. Ether (4 mL) and iodine (2 crystals) were then added, and the mixture was stirred vigorously under argon for 20 min. A solution of bromobenzene (1.24 mL, 11.8 mmol) in THF (12 mL) was added dropwise over 10 min, by which point the mixture turned a dull gray color and began to self-reflux. The reaction was refluxed for 1 h and then cooled to 0 °C. To the reaction mixture was then added dropwise a solution of (*R*)-Garner's aldehyde (**31**) (2.00 g, 8.72 mmol). The reaction mixture was stirred for 20 min at this temperature and then warmed to room temperature and stirred for 1 h. The mixture was then quenched with saturated aqueous NH_4Cl (100 mL). The aqueous phase was extracted with EtOAc (3 × 70 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 20:80 v/v EtOAc/petroleum benzines) to yield alcohol **32** as a white solid (2.12 g, 79%, *syn*/*anti* 2:2.7).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.43−7.18 (m, 10H), 5.05 (m, 1H), 4.74 (d, J = 8.8 Hz, 1H), 4.40−3.50 (m, 3H), 1.60−1.33 (m, 15H); LRMS (+ESI) m/z 330 [M + Na]$^+$; $[\alpha]_D$ = +8.0° (c 0.5, CH_2Cl_2); mp 79.9−84.0 °C. These data are in agreement with those previously reported by Malins et al.[21]

*(R)-tert-Butyl 4-Benzoyl-2,2-dimethyloxazolidine-3-carboxylate (**33**).* To a solution of oxalyl chloride (0.90 mL, 10.6 mmol) in CH_2Cl_2 (16 mL) at −78 °C was added a solution of dimethyl sulfoxide (1.20 mL, 18.1 mmol) in CH_2Cl_2 (4.3 mL) dropwise. The reaction mixture was stirred at this temperature for 10 min. A solution of alcohol **32** (1.63 g, 5.3 mmol) in CH_2Cl_2 (4.3 mL) was added dropwise, and the mixture was stirred at −78 °C for 1 h. iPr$_2$NEt (3.25 mL, 18.6 mmol) was added, and the mixture was stirred at this temperature for 10 min and then warmed to room temperature. The reaction mixture was poured onto saturated aqueous NH_4Cl (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 70 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 20:80 v/v EtOAc/petroleum benzines) to yield ketone **33** as a white solid (1.38 g, 85%).

^{1}H NMR ($CDCl_3$, 400 MHz, rotamers) δ (ppm) 7.93−7.89 (m, 2H), 7.62−7.44 (m, 3H), 5.47 (dd, J = 7.4, 2.9 Hz, 0.4H) and 5.36 (dd, J = 7.7, 3.7 Hz, 0.6H), 4.31 (ddd, J = 9.0, 7.6, 4.8, 1H) and 3.98−3.90 (m, 1H), 1.76 (s, 2H) and 1.73 (s, 1H), 1.60 (s, 2H) and 1.56 (s, 1H), 1.50 (s, 4H) and 1.28 (s, 5H); LRMS (+ESI) m/z 328 [M + Na]$^+$; $[\alpha]_D$ = +59.6° (c 0.26, CH_2Cl_2); mp 114.6−117.5 °C These data are in agreement with those previously reported by Malins et al.[21]

*(R)-tert-Butyl 4-((S)-Hydroxy(phenyl)methyl)-2,2-dimethyloxazolidine-3-carboxylate (**34**).* To a solution of ketone **33** (1.38 g, 4.51 mmol) in THF (190 mL) at 0 °C was added diisobutyl aluminum hydride (13.5 mL, 1 M in hexanes, 13.5 mmol) dropwise over 30 min. The solution was stirred at this temperature for 30 min. MeOH (10 mL) was added, and the reaction mixture was poured onto ice cold HCl (150 mL, 1M). The aqueous phase was extracted with EtOAc (3 × 70 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 20:80 v/v EtOAc/petroleum benzines) to yield a 14:1 mixture of diastereomers of alcohol **34** as a white solid (1.23 g, 89%).

^{1}H NMR ($CDCl_3$, 500 MHz, 328 K) δ (ppm) 7.41−7.26 (m, 5H), 5.11 (s, 1H), 4.24−4.21 (m, 1H), 4.07 (dd, J = 9.1, 2.0 Hz, 1H), 3.86−3.84 (m, 1H), 1.55−1.45 (m, 15H); LRMS (+ESI) m/z 330 [M + H]$^+$; $[\alpha]_D$ = +22.9° (c 0.48, CH_2Cl_2); mp 96.2−102.0 °C. These data are in agreement with those previously reported by Malins et al.[21]

*tert-Butyl ((1S,2R)-1,3-Dihydroxy-1-phenylpropan-2-yl)-carbamate (**35**).* To a solution of oxazolidine **34** (800 mg, 2.6 mmol) in MeOH (27 mL) was added *p*-toluene sulfonic acid (198 mg, 0.46 mmol). The mixture was stirred at room temperature for 2.5 h. The reaction mixture was poured onto saturated aqueous $NaHCO_3$ (50 mL). The aqueous phase was extracted with EtOAc (3 × 30 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 50:50 v/v EtOAc/petroleum benzines) to yield a 14:1 mixture of diastereomers of diol **35** as a white solid (510 mg, 74%).

^{1}H NMR ($CDCl_3$, 400 MHz, 328 K) δ (ppm) 7.41−7.24 (m, 5H), 5.30 (d, J = 7.6 Hz, 1H), 5.02−4.96 (m, 1H), 3.83−3.73 (m, 1H), 3.65−3.57 (m, 1H), 3.34 (d, J = 3.7 Hz, 1H), 2.67 (brs, 1H), 1.43 (s, 9H); LRMS (+ESI) m/z 290 [M + Na]$^+$; $[\alpha]_D$ = +2.1° (c 0.27, CH_2Cl_2); mp 80.8−84.2 °C. These data are in agreement with those previously reported by Williams et al.[27]

*(2S,3S)-2-(((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-hydroxy-3-phenylpropanoic Acid (**37**).* Diol **35** (479 mg, 1.79 mmol) was oxidized by general procedure A to produce carboxylic acid **36**, which was used without further purification. Crude acid **36** (371 mg, 1.32 mmol) was subject to protecting group manipulation via general procedure B to afford Fmoc amino acid **37**, which was purified by flash chromatography (eluent: 70:30 → 100:0 + 0.1% AcOH v/v EtOAc/petroleum benzines) to yield Fmoc protected amino acid **37** as an off-white crystalline solid (463 mg, 64% from diol **35**).

^{1}H NMR (MeOD, 400 MHz) δ (ppm) 7.76 (d, J = 7.6 Hz, 2H), 7.57−7.50 (m, 2H), 7.46−7.19 (m, 9H), 4.99 (d, J = 7.3 Hz, 1H), 4.52 (d, J = 7.3 Hz, 1H), 4.30−4.04 (m, 3H); ^{13}C NMR (MeOD, 100 MHz) δ (ppm) 174.0, 158.1, 145.2, 145.1, 142.5, 142.4, 142.0, 129.2, 128.9, 128.7, 128.1, 128.0, 126.3, 126.2, 120.9, 75.1, 68.2, 61.3 48.2; LRMS (+ESI) m/z 426 [M + Na]$^+$; HRMS (+ESI) m/z: [M + Na]$^+$ Calcd for $C_{24}H_{21}NO_5Na$ 426.1312; Found 436.1313; IR ν_{max} (ATR) 3408, 3314, 3064, 3083, 1711, 1522, 1450, 1414, 1332, 1260, 1233 cm^{-1}; $[\alpha]_D$ = +24.1° (c 0.19, CH_2Cl_2:MeOH, 0.91:0.9); mp 164.1−173.0 °C

Synthesis of Oxazolidine Protected Fmoc-β-OH-Phe-OH (72). *(4S,5S)-3-(((9H-Fluoren-9-yl)methoxy)carbonyl)-2,2-dimethyl-5-phenyloxazolidine-4-carboxylic Acid (**72**).* Fmoc-β-OH-Phe-OH (**37**) (450 mg, 1.15 mmol) was protected via general procedure C. Crude oxazolidine protected amino acid **72** was purified by flash chromatography (eluent: 30:70 → 45:55 v/v EtOAc/petroleum benzines) to yield oxazolidine protected amino acid **72** as an off-white crystalline solid (465 mg, 94%).

^{1}H NMR ($CDCl_3$, 500 MHz, rotamers) δ (ppm) 7.78 (ap. d, J = 7.88 Hz, 0.7H) and 7.68 (ap. dd, J = 5.7, 7.5 Hz, 1.3H), 7.57 (dd, J = 3.5, 7.5 Hz, 0.7H) and 7.47 (ap. t, J = 7.0 Hz, 1.3H), 7.44−7.16 (m, 9H), 5.34 (d, J = 6.8 Hz, 0.65H) and 5.20 (d, J = 6.7 Hz, 0.35H), 4.76 (dd, J = 4.2, 10.8 Hz, 0.35H) and 4.39 (dd, J = 6.3, 10.8 Hz, 0.65H),

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4.69 (dd, J = 4.4, 11.0 Hz, 0.35H) and 4.30 (dd, J = 6.8, 10.8 Hz), 4.52−4.45 (m, 1H), 4.23 (ap. t, J = 4.2 Hz, 0.35H) and 4.02 (ap. t, J = 6.6 Hz, 0.65H), 1.87 (s, 2H) and 1.21 (s, 1H), 1.63 (s, 2H) and 1.02 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz, rotamers) δ (ppm) 173.6 and 173.4, 152.9 and 151.8, 144.0, 143.9 and 143.6, 141.8 and 141.4, 141.6 and 141.3, 134.2, 129.0 and 128.8, 128.5 and 128.4, 127.9 and 127.9, 127.8 and 127.8, 127.4 and 127.2, 127.3 and 127.2, 126.5 and 126.4, 125.1 and 125.0, 124.6 and 124.5, 120.1 and 120.1, 120.0 and 120.0, 95.5 and 94.7, 78.2 and 77.4, 67.3 and 66.9, 64.6 and 64.0, 47.4 and 47.3, 25.5 and 25.4, 25.0, and 24.3 (extra signals due to further rotational conformations in the Fmoc-region); LRMS (+ESI) m/z 466 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{27}H_{25}NO_5Na$ 466.1624; Found 466.1624; IR ν_{max} (ATR) 2923, 1744, 1706, 1451, 1410, 1348, 1248, 1216, 1190, 1159 cm^{-1}; $[\alpha]_D$ = +21.4° (c 0.5, CH_2Cl_2); mp 60.6−71.2 °C

Synthesis of Oxazolidine Protected Fmoc-*β*-OH-*O*-Me-Tyr-OH (48). *(R)-tert-Butyl 4-((S)-Hydroxy(4-methoxyphenyl)methyl)-2,2-dimethyloxazolidine-3-carboxylate (**44**).* To a solution of dry LiBr (6.66 g, 88.8 mmol) in THF (140 mL) was added *p*-bromoanisole (3.97 mL, 31.53 mmol). The reaction mixture was cooled to −78 °C, and *n*-BuLi (13.2 mL, 33.02 mmol, 2.5 M in hexanes) was added dropwise. The reaction mixture was stirred at this temperature for 45 min. To the reaction mixture was added a solution of (*R*)-Garner's aldehyde (**31**) (3.44 g, 15.01 mmol) in THF (16 mL) dropwise. The reaction mixture was stirred at −78 °C for 4 h before being quenched with saturated aqueous NH_4Cl (30 mL). The mixture was warmed to room temperature and poured onto saturated aqueous NH_4Cl (100 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 15:85 → 25:75 v/v EtOAc/petroleum benzines) to yield a 5.1:1 mixture of diastereomers of alcohol **44** as a colorless oil (3.80 g, 75%, *anti*/*syn* 5.1:1).

^{1}H NMR ($CDCl_3$, 400 MHz, 328 K) major diastereomer (*anti*) δ (ppm) 7.27 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.04−4.98 (m, 1H), 4.23−4.07 (m, 1H), 4.02 (dd, J = 9.4, 2.2 Hz, 1H), 3.88−3.80 (m, 1H), 3.78 (s, 3H), 1.56−1.42 (m, 15H); minor diastereomer (*syn*) δ (ppm) 7.27 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 4.70 (dd, J = 8.6, 3.5 Hz, 1H), 4.23−4.07 (m, 1H), 3.78 (s, 3H), 3.73−3.61 (m, 2H), 1.56−1.42 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz, 328 K) major diastereomer (*anti*) δ (ppm) 159.7, 159.3, 133.6, 127.4, 114.0, 94.8, 80.8, 74.0, 64.0, 63.5, 55.4, 28.6 24.0; minor diastereomer (*syn*) δ (ppm) 159.7, 159.3, 133.6, 128.5, 114.1, 94.8, 80.8, 77.2, 65.0, 63.5, 55.4, 28.6, 26.7 LRMS (+ESI) m/z 360 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{27}NO_5Na$ 360.1781; Found 360.1782; IR ν_{max} (ATR) 3476, 2977, 2933, 1695, 1512, 1457, 1391, 1248, 1173 cm^{-1}; $[\alpha]_D$ = +28.7° (c 1.0, CH_2Cl_2)

*tert-Butyl ((1S,2R)-1,3-Dihydroxy-1-(4-methoxyphenyl)propan-2-yl)carbamate (**45**).* To a solution of oxazolidine **44** (3.64 mg, 10.78 mmol) in methanol (107 mL) was added *p*-toluene sulfonic acid (820 mg, 4.31 mmol). The mixture was stirred at room temperature for 3 h. The reaction mixture was poured onto saturated aqueous $NaHCO_3$ (150 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 50:50 → 60:40 v/v EtOAc/petroleum benzines) to yield a 5.1:1 mixture of diastereomers of diol **45** as a white solid (2.31 g, 73%, *anti*/*syn* 5.1:1).

^{1}H NMR ($CDCl_3$, 400 MHz, 328 K) major diastereomer (*anti*) δ (ppm) 7.31−7.23 (m, 2H), 6.90−6.83 (m, 2H), 5.24 (d, J = 7.8 Hz, 1H), 4.93−4.89 (m, 1H) 3.79 (s, 3H), 3.80−3.70 (m, 2H), 3.64−3.56 (m, 1H), 3.37 (d, J = 3.8 Hz, 1H), 2.79 (s, 1H), 1.42 (s, 9H); minor diastereomer (*syn*) δ (ppm) 5.16 (d, J = 7.9 Hz, 1H), 4.89−4.86 (m, 1H), 3.78 (s, 3H), 3.26 (s, 1H), 2.73 (s, 1H), 1.36 (s, 9H) (some signals for the minor diastereomer are overlapped with the major diastereomer); ^{13}C NMR ($CDCl_3$, 100 MHz, 328 K) major diastereomer (*anti*) δ (ppm) 159.5, 156.3 133.5, 127.4, 114.2, 80.0, 75.7, 62.2, 57.1, 55.5, 28.5; minor diastereomer (*syn*) δ (ppm) 159.6, 156.7, 133.7, 127.5, 114.2, 80.0, 74.1, 64.0, 57.7, 55.5, 28.5; LRMS (+ESI) m/z 320 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{23}NO_5Na$ 320.1468; Found 320.1469; IR ν_{max} (ATR) 3398, 3370, 2975, 2933, 1686, 1612, 1512, 1457, 1392, 1366, 1247, 1171 cm^{-1}; $[\alpha]_D$ = −1.5° (c 0.32, CH_2Cl_2); mp 100.6−102.9 °C.

*(4S,5S)-3-(((9H-Fluoren-9-yl)methoxy)carbonyl)-5-(4-methoxyphenyl)-2,2-dimethyloxazolidine-4-carboxylic Acid (**48**).* Diol **45** (892 mg, 3.00 mmol) was oxidized by general procedure A to produce carboxylic acid **46**, which was used without further purification. Crude acid **46** (720 mg, 2.31 mmol) was subject to protecting group manipulation via general procedure B to afford Fmoc amino acid **47**, which was used without further purification. Crude Fmoc amino acid **47** was oxazolidine protected using general procedure C. The product was purified by flash chromatography (eluent: 35:65 → 60:40 v/v EtOAc/petroleum benzines) to yield oxazolidine protected amino acid **48** as an off-white crystalline solid (438 mg, 30% from diol **45**, single diastereomer).

^{1}H NMR (DMSO-d_6, 500 MHz, rotamers) δ (ppm) 12.47 (brs, 0.5H) and 12.13 (brs, 0.5H), 7.93−7.85 (m, 2H), 7.73−7.62 (m, 2H), 7.45−7.25 (m, 5H), 7.17 (d, J = 8.9 Hz, 1H), 6.64 (d, J = 8.7 Hz, 1H) and 6.83 (d, J = 8.7 Hz, 1H); 5.50 (d, J = 6.8 Hz, 0.5H) and 5.22 (d, J = 6.8 Hz, 0.5H), 4.79 (dd, J = 10.9 and 3.8 Hz, 0.5H) and 4.70 (dd, J = 10.7 and 3.5 Hz, 0.5H), 4.66 (d, J = 6.70 Hz, 0.5H) and 4.30 (d, J = 6.8 Hz, 0.5H), 4.35−4.32 (m, 0.5H) and 4.18−4.13 (m, 0.5H), 4.27−4.18 (m, 1H), 3.76 (s, 1.5H) and 3.70 (s, 1.5H), 1.79 (s, 1.5H) and 1.59 (s, 1.5H), 0.96 (s, 1.5H) and 0.76 (s, 1.5H) ^{13}C NMR (DMSO-d_6, 125 MHz, rotamers) δ (ppm) 170.5 and 170.1, 159.2 and 159.1, 151.8 and 151.2, 144.2 and 144.1, 143.6 and 143.6, 141.2 and 141.1, 140.6 and 140.6, 128.0 and 127.8, 127.8 and 127.7, 127.5, 127.2 and 127.2, 127.1, 127.0 and 127.0, 125.4 and 125.3, 124.4 and 124.3, 120.1 and 120.1, 120.0, 113.4 and 113.2, 93.8 and 93.1, 77.1 and 76.2, 67.0 and 66.0, 64.1 and 63.6, 55.0 and 55.0, 46.7 and 46.6, 25.2 and 23.9, 25.1, and 23.9 (extra signals due to further rotational conformations in the Fmoc-region); LRMS (+ESI) m/z 496 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{28}H_{27}NO_6Na$ 496.1730; Found 496.1730; IR ν_{max} (ATR) 2922, 2853, 1707, 1614, 1515, 1411, 1348, 1249, 1177 cm^{-1}; $[\alpha]_D$ = +30.1° (c 0.29, CH_2Cl_2); mp 213.0−214.9 °C.

Synthesis of Fmoc-*β*-Me-Asp(Phi*Pr*)-OH (57). *(2S,3S)-1-Allyl 4-(2-phenylpropan-2-yl) 2-((((9H-fluoren-9-yl)methoxy)carbonyl)-amino)-3-methylsuccinate (**56**).* To a solution of amino acid **54** (306 mg, 1.51 mmol) in THF (10 mL) was added saturated aqueous $NaHCO_3$ (5 mL) and Fmoc-OSu (538 mg, 1.59 mmol), and the reaction was stirred overnight at room temperature. The reaction mixture was poured onto water (20 mL) and extracted with Et_2O (2 × 30 mL). The aqueous phase was then acidified with 1 M HCl to pH 4 and extracted with EtOAc (3 × 40 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield the crude acid (562 mg).

To a solution of crude acid (526 mg, 1.32 mmol) in DMF (2.6 mL) at 0 °C was added *i*Pr$_2$NEt (0.46 mL, 2.64 mmol) followed by allyl bromide (0.23 mL, 2.64 mmol). The reaction mixture was warmed to room temperature and stirred for 16 h before being diluted with EtOAc (50 mL) and washed with HCl (3 × 30 mL, 0.1 M), saturated aqueous NaCl (30 mL), and the organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to yield allyl ester **55**.

To a solution of crude allyl ester (532 mg, 1.14 mmol) in CH_2Cl_2 (5.5 mL) was added TFA (5.5 mL) at room temperature for 3 h before CH_2Cl_2 (20 mL) was added, and the mixture was concentrated in vacuo. The crude solid was dissolved in CH_2Cl_2 (4 mL), to which a solution of Phi*Pr* trichloroacetimidate (603 mg, 2.28 mmol) in hexanes (8 mL) was added. The reaction mixture was stirred at room temperature for 16 h, at which point it was judged incomplete by TLC analysis. A further portion of Phi*Pr* trichloroacetimidate (300 mg, 1.14 mmol) in CH_2Cl_2 (4 mL) was added and stirred for a further 16 h. The reaction mixture was concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 20:80 v/v EtOAc/petroleum benzines) to yield Phi*Pr* ester **56** as a colorless oil (390 mg, 49% over 4 steps).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.78 (dd, J = 7.5, 0.6 Hz, 2H), 7.60 (dd, J = 7.4, 3.7 Hz, 2H), 7.45−7.29 (m, 8H), 7.27 (m, 1H), 5.96−5.82 (m, 1H), 5.57 (d, J = 9.1 Hz, 1H), 5.34 (dd, J = 17.1, 1.0 Hz, 1H), 5.26 (dd, J = 10.4, 1.0 Hz, 1H), 4.77 (dd, J = 9.2, 4.6 Hz,

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1H), 4.65 (d, J = 5.8 Hz, 2H), 4.50−4.34 (m, 2H), 4.25 (t, J = 7.2 Hz, 1H), 3.06 (qd, J = 7.3, 4.6 Hz, 1H), 1.80 (s, 3H), 1.78 (s, 3H), 1.27 (d, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 171.4, 170.5, 156.1, 145.4, 143.9, 143.8, 141.4, 131.5, 128.3, 127.8, 127.2, 127.2, 125.2, 125.2, 124.4, 120.1, 119.2, 82.8, 67.4, 66.4, 55.9, 47.2, 42.9, 28.7, 28.1, 13.1 LRMS (+ESI) m/z 550 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{32}H_{33}NO_6Na$ 550.2200; Found 550.2209; IR ν_{max} (ATR) 3347, 2923, 2853, 1727, 1509, 1449, 1261, 1201, 1136, 1100 cm^{-1}; $[\alpha]_D$ = +7.5° (c 0.28, CH_2Cl_2).

*(2S,3S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methyl-4-oxo-4-((2-phenylpropan-2-yl)oxy)butanoic Acid (**57**).* To a solution of allyl ester **56** (108 mg, 0.205 mmol) in THF (2 mL) was added $Pd(PPh_3)_4$ (12 mg, 0.01 mmol) followed by $PhSiH_3$ (0.05 mL, 0.41 mmol). The reaction mixture was stirred at room temperature for 2 h before being concentrated in vacuo. The crude product was purified by flash chromatography (eluent: 30:70 → 100:0 v/v EtOAc/petroleum benzines) to yield acid **57** as a white solid (105 mg, quant).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.76 (d, J = 7.4 Hz, 2H), 7.57 (dd, J = 4.5, 6.9 Hz, 2H), 7.44−7.17 (m, 9H), 5.57 (d, J = 8.9 Hz, 1H), 4.71 (dd, J = 4.6, 8.8 Hz, 1H), 4.47−4.34 (m, 2H), 4.22 (ap. t, J = 7.1 Hz, 1H), 3.11−2.99 (m, 1H), 1.76 (s, 6H), 1.28 (d, J = 7.24 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 174.9, 171.8, 156.3, 145.4, 143.9, 143.8, 141.5, 128.4, 127.9, 127.3, 127.3, 125.2, 124.4, 120.1, 83.1, 67.5, 55.8, 47.3, 42.8, 28.6, 28.3, 13.2; LRMS (+ESI) m/z 510 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{29}H_{29}NO_6Na$ 510.1887; Found 510.1895; IR ν_{max} (ATR) 2923, 2853, 1719, 1517, 1449, 1248, 1219, 1137, 1101 cm^{-1}; $[\alpha]_D$ = +4.2° (c 0.38, CH_2Cl_2); mp 52.5- 65.0 °C.

SPPS of Simplified Skyllamycin Analogue 4. *Simplified Skyllamycin **4**.* Fmoc-Gly-OH (50 μmol) was loaded to 2-CTC resin as per the general procedures. Iterative Fmoc-SPPS was carried out to couple Fmoc-Asp(*t*Bu)-OH, Fmoc-Ala-OH, and Fmoc-Thr-OH utilizing standard PyBOP coupling conditions. Cinnamoyl moiety **15** was coupled using HATU coupling conditions. Fmoc-D-Leu-OH (141 mg, 8 equiv) was dissolved in anhydrous CH_2Cl_2 (5 mL) and cooled to 0 °C. *N,N'*-diisopropylcarbodiimide (31 μL, 4 equiv) was added to this solution which was then warmed to room temperature and stirred for 30 min. The reaction mixture was concentrated under a stream of nitrogen and subsequently redissolved in DMF (0.5 mL). This solution, along with a solution of DMAP (catalytic, 6 crystals) in DMF (0.1 mL), was shaken with the resin for 16 h at room temperature before the solution was expelled and the resin washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×).

Iterative Fmoc SPPS was continued with the subsequent couplings of Fmoc-D-Leu-OH, Fmoc-Gly-OH, Fmoc-D-Trp(Boc)-OH, Fmoc-Tyr(O*t*Bu)-OH, Fmoc-Pro-OH, and Fmoc-Phe-OH all performed using PyBOP coupling conditions. The terminal Fmoc group was removed, and the resin-bound peptide **26** was washed with DMF (5×), CH_2Cl_2 (5×), DMF (5×), CH_2Cl_2 (20×). The resin was treated with a 30 vol % solution of HFIP in CH_2Cl_2 (∼5 mL, 2 × 30 min), and the solution was transferred to a round-bottom flask and diluted with CH_2Cl_2 (30 mL) before being concentrated under a stream of N_2 and dried in vacuo to afford **27**. A portion of the crude linear peptide (30% by weight) was then subject to cyclization (general procedure D) and global deprotection (general procedure E) followed by RP-HPLC purification and lyophilization to afford the desired cyclic peptide **4** in (1.98 mg, 9.5%).

LRMS (+ESI) m/z calculated mass 1413.7 $[M + Na]^+$: m/z observed 1412.9 $[M + Na]^+$; HRMS (+ESI) m/z: $[M+2Na]^{2+}$ Calcd for $C_{73}H_{90}N_{12}O_{16}Na_2$ 718.3191; Found 718.3196; Analytical HPLC R_t 12.6 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm). ^{1}H NMR and ^{13}C NMR data is listed in Table S1.

Synthesis of Deshydroxy Skyllamycins A−C (5−7). *Deshydroxy Skyllamycin A (**5**).* Fmoc-Gly-OH was loaded to 2-CTC as per the general procedures to afford 75 μmol of resin-bound amino acid. Fmoc-β-Me-Asp(Ph*i*Pr)-OH (**57**) was coupled under HATU conditions followed by PyBOP coupling of Fmoc-Ala-OH and Fmoc-Thr-OH before coupling of cinnamoyl moiety **15** using HATU to yield resin-bound peptide **58**. From here, on-resin esterification with protected Fmoc-β-OH-Leu-OH **43**, followed by microwave-assisted coupling of Fmoc-D-Leu-OH as detailed in the general procedures, afforded resin-bound depsipeptide. Next, coupling of Fmoc-Gly-OH and Fmoc-D-Trp-OH was carried out using PyBOP coupling conditions followed by coupling of protected Fmoc-β-OH-*O*-Me-Tyr-OH **48** using HATU conditions. Microwave-assisted coupling of Fmoc-Pro-OH, followed by coupling of Fmoc-β-OH-Phe-OH (**37**) using HATU, afforded the complete resin-bound linear peptide **61**. Resin-bound linear peptide **61** was cleaved from resin according to the general SPPS protocols to yield crude linear peptide **63** (42.7 mg, 27 μmol, assumed to be 100% pure). This was subject to cyclization (general procedure D) and global deprotection (general procedure E). Deshydroxy skyllamycin A (**5**) was afforded as a fluffy white solid after RP-HPLC and lyophilization (5.8 mg, 5.2%).

LRMS (+ESI) m/z calculated mass 1467.7 $[M + H]^+$, 1489.7 $[M + Na]^+$: m/z observed 1467.0 $[M + H]^+$, 1489.0 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + Na]^+$ Calcd for $C_{75}H_{94}N_{12}O_{19}Na$ 1489.6650; Found 1489.6658; Analytical HPLC R_t 12.8 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm). ^{1}H NMR and ^{13}C NMR data are listed in Table S2.

*Deshydroxy Skyllamycin B (**6**).* Fmoc-Gly-OH was loaded to 2-CTC as per the general procedures to afford 80 μmol of resin-bound amino acid. Fmoc-Asp(Ph*i*Pr)-OH was coupled under HATU conditions followed by PyBOP coupling of Fmoc-Ala-OH and Fmoc-Thr-OH before coupling of cinnamoyl moiety **15** using HATU to yield resin-bound peptide **59**. From here, on-resin esterification with protected Fmoc-β-OH-Leu-OH **43**, followed by microwave-assisted coupling of Fmoc-D-Leu-OH as detailed in the general procedures afforded resin-bound depsipeptide. Next, coupling of Fmoc-Gly-OH and Fmoc-D-Trp-OH was carried out using PyBOP coupling conditions followed by coupling of protected Fmoc-β-OH-*O*-Me-Tyr-OH **48** using HATU conditions. Microwave-assisted coupling of Fmoc-Pro-OH, followed by coupling of Fmoc-β-OH-Phe-OH (**37**) using HATU, afforded the complete resin-bound linear peptide **53**. Resin-bound linear peptide **53** was cleaved from resin according to the general SPPS protocols to yield crude linear peptide **64** (57.8 mg, 36 μmol, assumed to be 100% pure). This was subject to cyclization (general procedure D) and global deprotection (general procedure E). Deshydroxy skyllamycin B (**6**) was afforded as a fluffy white solid after RP-HPLC and lyophilization (8.6 mg, 7.4%).

LRMS (+ESI) m/z calculated mass 1453.7 $[M + H]^+$, 1475.7 $[M + Na]^+$: m/z observed 1453.0 $[M + H]^+$, 1475.3 $[M + Na]^+$; HRMS (+ESI) m/z: $[M+2Na]^{2+}$ Calcd for $C_{74}H_{92}N_{12}O_{19}Na_2$ 749.3193; Found 749.3196; Analytical HPLC R_t 12.4 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm). ^{1}H NMR and ^{13}C NMR data are listed in Table S3.

*Deshydroxy Skyllamycin C (**7**).* Fmoc-Gly-OH was loaded to 2-CTC as per the general procedures to afford 80 μmol of resin-bound amino acid. Fmoc-Asp(Ph*i*Pr)-OH was coupled under HATU conditions followed by PyBOP coupling of Fmoc-Ala-OH and Fmoc-Thr-OH before coupling of reduced cinnamoyl moiety **16** using HATU to yield resin-bound peptide **60**. From here, on-resin esterification with protected Fmoc-β-OH-Leu-OH **43**, followed by microwave-assisted coupling of Fmoc-D-Leu-OH as detailed in the general procedures. Next, coupling of Fmoc-Gly-OH and Fmoc-D-Trp-OH was carried out using PyBOP coupling conditions followed by coupling of protected Fmoc-β-OH-*O*-Me-Tyr-OH **48** using HATU conditions. Microwave-assisted coupling of Fmoc-Pro-OH, followed by coupling of Fmoc-β-OH-Phe-OH (**37**) using HATU, afforded the complete resin-bound linear peptide **62**. Resin-bound linear peptide **62** was cleaved from resin according to the general SPPS protocols to yield crude linear peptide **65** (43.4 mg, 27 μmol, assumed to be 100% pure). This was subject to cyclization (general procedure D) and global deprotection (general procedure E). Deshydroxy skyllamycin C (**7**) was afforded as a fluffy white solid after RP-HPLC and lyophilization (4.5 mg, 3.9%).

LRMS (+ESI) m/z calculated mass 1455.7 $[M + H]^+$, 1477.7 $[M + Na]^+$: m/z observed 1455.0 $[M + H]^+$, 1477.3 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + 2Na]^{2+}$ Calcd for $C_{74}H_{94}N_{12}O_{19}Na_2$ 750.3271;

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Found 750.3284; Analytical HPLC R_t 12.5 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm). ^1H NMR and ^{13}C NMR data is listed in Table S4.

Synthesis of Skyllamycin A (1). *Skyllamycin A: Linear Peptide **79**.* Fmoc-D-Trp-OH was loaded to Sieber amide resin as per the general procedures, and after Fmoc-loading it was determined that 120 μmol of amino acid was loaded to resin. Oxazolidine protected Fmoc-β-OH-*O*-Me-Tyr-OH **48** was coupled to the resin under HATU conditions, followed by microwave-assisted coupling of Fmoc-Pro-OH. Oxazolidine protected Fmoc-β-OH-Phe-OH **72** was then coupled using HATU conditions, followed by microwave-assisted coupling of Fmoc-Gly-OH to yield key resin-bound intermediate **75**. Fmoc-β-Me-Asp(Phi*Pr*)-OH (**57**) was next coupled using HATU conditions followed by the iterative coupling of Fmoc-Ala-OH and Fmoc-Thr-OH using PyBOP coupling conditions. Cinnamoyl moiety **15** was coupled using HATU conditions followed by on-resin esterification of oxazolidine protected Fmoc-β-OH-Leu-OH **43**.

The peptide was washed with CH_2Cl_2 (5×) and DMF (5×) before being treated with a solution of 10 vol % piperidine in DMF (2 × 3 min). The resin was again washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×). The resin was transferred to a Biotage microwave peptide synthesis vessel and treated with a solution of the desired Fmoc-D-Leu-OH (4 equiv), DIC (4 equiv) and HOAt (4 equiv) in DMF (0.1 M in regard to loaded peptide) under microwave irradiation at 50 °C for 1 h. The resin was then transferred to a fritted syringe, the coupling solution was discharged and the resin washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×). The resin was thoroughly dried, and split and to 60 μmol of resin-bound peptide Fmoc-Ser-OH was coupled using PyBOP coupling conditions. The resin was cleaved using the conditions described in the general procedures to afford the linear peptide **79** as a white fluffy solid after lyophilization (10.1 mg, 11%).

LRMS (+ESI) *m*/*z* calculated mass 1514.7 $[M + H]^+$: *m*/*z* observed 1513.8 $[M + H]^+$; HRMS (+ESI) *m*/*z*: $[M+2H]^{2+}$ Calcd for $C_{76}H_{101}N_{13}O_{20}$ 757.8637; Found 757.8645; Analytical HPLC R_t 9.8 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm).

*Skyllamycin A: Aldehyde **66**.* Linear peptide **79** (10.1 mg, 6.2 μmol) was subject to oxidative cleavage conditions (general procedure F). Aldehyde **66** was obtained as a white fluffy solid after RP-HPLC and lyophilization (5.8 mg, 62%).

LRMS (+ESI) *m*/*z* calculated mass 1501.7 $[M+H_2O+H]^+$, 1523.7 $[M + H_2O + Na]^+$: *m*/*z* observed 1501.0 $[M + H_2O + H]^+$, 1524.1 $[M + H_2O + Na]^+$; HRMS (+ESI) *m*/*z*: $[M + Na]^+$ Calcd for $C_{75}H_{94}N_{12}O_{20}Na$ 1505.6600; Found 1505.6624; Analytical HPLC R_t 12.2 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm).

*Skyllamycin A (**1**).* Aldehyde **66** (5.8 mg, 3.8 μmol) was subject to cyclization conditions (general procedure G). Skyllamycin A (**1**) was isolated as a white fluffy solid after RP-HPLC and lypohilization (1.98 mg, 32%).

LRMS (+ESI) *m*/*z* calculated mass 1483.7 $[M + H]^+$, 1505.7 $[M + Na]^+$: *m*/*z* observed 1483.0 $[M + H]^+$, 1505.1 $[M + Na]^+$; HRMS (+ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{75}H_{95}N_{12}O_{20}$ 1483.6780; Found 1483.6768; Analytical HPLC R_t 12.9 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm). ^1H NMR and ^{13}C NMR data are listed in Table S5.

Synthesis of Skyllamycin B (2). *Skyllamycin B: Linear Peptide **80**.* Fmoc-D-Trp-OH was loaded to Sieber amide resin as per the general procedures and after Fmoc-loading it was determined that 120 μmol of amino acid was loaded to resin. Oxazolidine protected Fmoc-β-OH-*O*-Me-Tyr-OH **48** was coupled to the resin under HATU conditions, followed by microwave-assisted coupling of Fmoc-Pro-OH. Oxazolidine protected Fmoc-β-OH-Phe-OH **72** was then coupled using HATU conditions, followed by microwave-assisted coupling of Fmoc-Gly-OH to yield key resin-bound intermediate **75**. Fmoc-Asp(Phi*Pr*)-OH was next coupled using HATU conditions followed by the iterative coupling of Fmoc-Ala-OH and Fmoc-Thr-OH using PyBOP coupling conditions. Cinnamoyl moiety **15** was coupled using HATU conditions followed by on-resin esterification of oxazolidine protected Fmoc-β-OH-Leu-OH **43**. Fmoc-D-Leu-OH was coupled using microwave-assisted coupling conditions. The resin was thoroughly dried and split, and to 60 μmol of resin-bound peptide, Fmoc-Ser-OH was coupled using PyBOP coupling conditions. The resin was cleaved using the conditions described in the general procedures to afford the linear peptide **80** as a white fluffy solid after lyophilization (10.8 mg, 12%).

LRMS (+ESI) *m*/*z* calculated mass 1500.7 $[M + H]^+$: *m*/*z* observed 1499.8 $[M + H]^+$; HRMS (+ESI) *m*/*z*: $[M+2H]^{2+}$ Calcd for $C_{75}H_{99}N_{13}O_{20}$ 750.8559; Found 750.8567; Analytical HPLC R_t 9.8 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm).

Skyllamycin B: Aldehyde 67. *2*. Linear peptide **80** (10.8 mg, 6.7 μmol) was subject to oxidative cleavage conditions (general procedure F). Aldehyde **67** was obtained as a white fluffy solid after RP-HPLC and lyophilization (6.2 mg, 63%).

LRMS (+ESI) *m*/*z* calculated mass 1469.7 $[M + H]^+$, 1509.7 $[M + H_2O+Na]^+$: *m*/*z* observed 1468.9 $[M + H]^+$, 1509.1 $[M+H_2O+Na]^+$; HRMS (+ESI) *m*/*z*: $[M + Na]^+$ Calcd for $C_{74}H_{92}N_{12}O_{20}Na$ 1491.6443; Found: 1491.6471; Analytical HPLC R_t 12.1 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm).

*Skyllamycin B (**2**).* Aldehyde **67** (6.2 mg, 4.2 μmol) was subject to cyclization conditions (general procedure G). A mixture of skyllamycin B (**2**) and its epimer were isolated as a white fluffy solid after RP-HPLC and lypohilization (2.60 mg, 42%). This mixture was subject to further RP-HPLC purification. Specifically, the mixture of epimers was subject to HPLC using a Phenomenex Kinetix XB-C18 4.6 × 250 mm column, using a gradient of 45% MeOH, 21% MeCN, 34% H_2O ramped to 48.7% MeOH, 21% MeCN, 30.3% H_2O over 20 min, then ramped to 69% MeOH, 21% MeCN, 10% H_2O over 2 min, then held for 3 min with a flow rate of 1.5 mL min^{-1} to yield skyllamycin B (**2**) as a white fluffy solid (1.20 mg, 46% from mixture of epimers, 19% overall).

LRMS (+ESI) *m*/*z* calculated mass 1469.7 $[M + H]^+$, 1491.6 $[M + Na]^+$: *m*/*z* observed 1468.8 $[M + H]^+$, 1490.7 $[M + Na]^+$; HRMS (+ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{74}H_{93}N_{12}O_{20}$ 1469.6624; Found 1469.6600; Analytical HPLC R_t 12.4 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm). ^1H NMR and ^{13}C NMR data is listed in Table S6.

Synthesis of Skyllamycin C (3). *Skyllamycin C: Linear Peptide **81**.* Fmoc-D-Trp-OH was loaded to Sieber amide resin as per the general procedures and after Fmoc-loading it was determined that 120 μmol of amino acid was loaded to resin. Oxazolidine protected Fmoc-β-OH-*O*-Me-Tyr-OH **48** was coupled to the resin under HATU conditions, followed by microwave-assisted coupling of Fmoc-Pro-OH. Oxazolidine protected Fmoc-β-OH-Phe-OH **72** was then coupled using HATU conditions, followed by microwave-assisted coupling of Fmoc-Gly-OH to yield key resin-bound intermediate **75**. Fmoc-Asp(Phi*Pr*)-OH was next coupled using HATU conditions followed by the iterative coupling of Fmoc-Ala-OH and Fmoc-Thr-OH using PyBOP coupling conditions. Reduced cinnamoyl moiety **16** was coupled using HATU conditions followed by on-resin esterification of oxazolidine protected Fmoc-β-OH-Leu-OH **43**. Fmoc-D-Leu-OH was coupled using microwave-assisted coupling conditions. The resin was thoroughly dried and split, and to 60 μmol of resin-bound peptide Fmoc-Ser-OH was then coupled using PyBOP coupling conditions. The resin was cleaved using the conditions described in the general procedures to afford the linear peptide **81** as a white fluffy solid after lyophilization (11.0 mg, 12%).

LRMS (+ESI) *m*/*z* calculated mass 1502.7 $[M + H]^+$: *m*/*z* observed 1501.7 $[M + H]^+$; HRMS (+ESI) *m*/*z*: $[M + 2H]^{2+}$ Calcd for $C_{75}H_{101}N_{13}O_{20}$ 751.8637; Found 751.8644; Analytical HPLC R_t 9.9 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm).

*Skyllamycin C: Aldehyde **68**.* Linear peptide **81** (11.0 mg, 6.8 μmol) was subject to oxidative cleavage conditions (general procedure F). Aldehyde **68** was obtained as a white fluffy solid after RP-HPLC and lyophilization (6.8 mg, 68%).

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LRMS (+ESI) m/z calculated mass 1489.7 $[M + H_2O + H]^+$, 1511.7 $[M + H_2O + Na]^+$: m/z observed 1489.0 $[M + H_2O + H]^+$, 1510.9 $[M + H_2O + Na]^+$; HRMS (+ESI) m/z: $[M + 2Na]^{2+}$ Calcd for $C_{74}H_{94}N_{12}O_{20}Na_2$ 758.3246; Found 758.3250; Analytical HPLC R_t 12.1 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm).

Skyllamycin C (3). Aldehyde **68** (6.8 mg, 4.6 μmol) was subject to cyclization conditions (general procedure G). Skyllamycin C (**3**) was isolated as a white solid after RP-HPLC and lypohilization (2.16 mg, 33%).

LRMS (+ESI) m/z calculated mass 1471.7 $[M + H]^+$, 1593.7 $[M + Na]^+$: m/z observed 1471.0 $[M + H]^+$, 1592.7 $[M + Na]^+$; HRMS (+ESI) m/z: $[M + H]^+$ Calcd for $C_{74}H_{94}N_{12}O_{20}$ 1471.6780; Found 1471.6770; Analytical HPLC R_t 12.6 min (0−100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 15 min, λ = 230 nm). ^1H NMR and ^{13}C NMR data are listed in Table S7.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b00898.

Synthesis of compound **54**, NMR shifts and assignments for compounds **1**−**7**, NMR spectra, and raw biofilm inhibition data (PDF)

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Notes

The authors declare no competing financial interest.

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