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ABSTRACT. Streptide (**1**) is a peptide-derived macrocyclic natural product that has attracted considerable attention since its discovery in 2015. It contains an unprecedented post-translational modification that intramolecularly links the β-carbon (C3) of a residue 2 lysine with the C7 of a residue 6 tryptophan, thereby forming a 20-membered cyclic peptide. Herein, we report the first total synthesis of streptide that confirms the regiochemistry of the lysine-tryptophan crosslink and provides an unambiguous assignment of the stereochemistry (3*R* vs 3*S*) of the lysine-2 C3 center. Both the 3*R* and the originally assigned 3*S* lysine diastereomers were independently prepared by total synthesis and it is the former, not the latter, that was found to correlate with the natural product. The approach enlists a powerful Pd(0)-mediated indole annulation for the key macrocyclization of the complex core peptide, utilizes an underdeveloped class of hypervalent iodine(III) aryl substrates in a palladium-catalyzed C–H activation/β-arylation reaction conducted on a lysine derivative, and provides access to material with which the role of streptide and related natural products may be examined.

INTRODUCTION

Streptide (**1**) is a peptide-derived macrocyclic natural product that was first isolated from *Streptococcus thermophilus* (Figure 1). Its production is controlled by a quorum sensing system, consisting of a short hydrophobic peptide signal and a cognate transcriptional regulator, that is common to many streptococci, including pathogenic organisms.[1,2] Streptide biosynthesis commences with the ribosomal production of a 30 amino acid peptide that is post-translationally modified by macrocyclization and further processed by proteolysis to deliver the mature nine amino acid cyclic

peptide.[1-3] The structure of streptide and the biosynthetic origin of the macrocycle were disclosed in 2015 through a combination of spectroscopic and enzymatic studies.[3] NMR spectroscopic analyses located the crosslink to the β-carbon (C3) of the residue 2 lysine and C7 of the residue 6 tryptophan, giving rise to a 20-membered cyclic peptide. The α-carbon configurations were experimentally determined to be L while computational studies in conjunction with NMR NOESY constraints suggested a lysine-2 (*S*)-C3 configuration at the new chiral center.[3] Subsequent biosynthetic investigations showed that the crosslink is installed in a single step by StrB, a radical SAM metalloenzyme, via radical-mediated chemistry. More recently, homologs of StrB have been identified in pathogenic streptococci.[4,5] They have been shown to catalyze the same crosslinking reaction on a common AK^{2}GDGW^6 peptide core that contains the identical leader sequence but altered C-termini, depending on the producing strain, indicating that streptide is the first characterized member of a growing family of such post-translationally modified macrocyclic peptides.[6] At present, the role of streptide and its congeners upon secretion remains undefined.

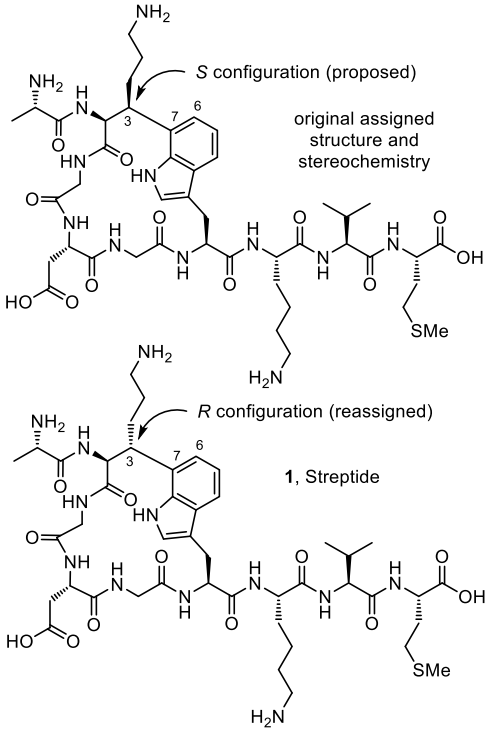


Figure 1. Assigned lysine (3*S*) and revised (3*R*) structure of streptide (**1**).

In efforts that culminated in the total synthesis of the chloropeptins and complestatins,[7] we introduced an intramolecular palladium(0)-mediated indole annulation for macrocyclization of their strained biaryl ring systems that contain crosslinks to either C6 or C7 of the indole of a (*R*)-tryptophan embedded within their peptide cores.[7,8] Not only did this reaction permit synthesis of their strained 16- or 17-membered macrocyclic rings, but it did so in the context of fully functionalized peptides, providing a powerful new macrocyclization reaction that was more effective than conventional alternatives.[9]

Given the unusual structure of streptide, its unprecedented crosslink, its unknown function, and its low natural abundance (1.7 mg/20 L production culture), we embarked on a total synthesis of streptide that would enlist this palladium(0)-mediated macrocyclization reaction as a key step in the assembly of the 20-membered cyclic peptide core. This approach would afford a total synthesis of **1** amenable to the preparation of analogues or congeners, and at the same time provide a formidable test of the generality of the indole annulation macrocyclization. Thus, key to our approach was the use of this intramolecular Larock indole synthesis to close the 20-membered cyclic peptide, enlisting conditions that permit the use of a 2-bromoaniline and its reaction with an alkyne that contains a removable large terminal substituent (–$SiEt_3$) to control the indole cyclization regioselectivity (Figure 2).[10] Not only would this cyclization provide the fully functionalized ring system of **1**, but its use would represent only the second reported application of what has proven to be a powerful macrocyclization reaction for the synthesis of complex natural products. Additionally, a palladium-catalyzed C–H activation reaction[11] on a lysine derivative was planned for diastereoselective installation of a suitably functionalized β-aryl substituent needed for implementation of the indole annulation.

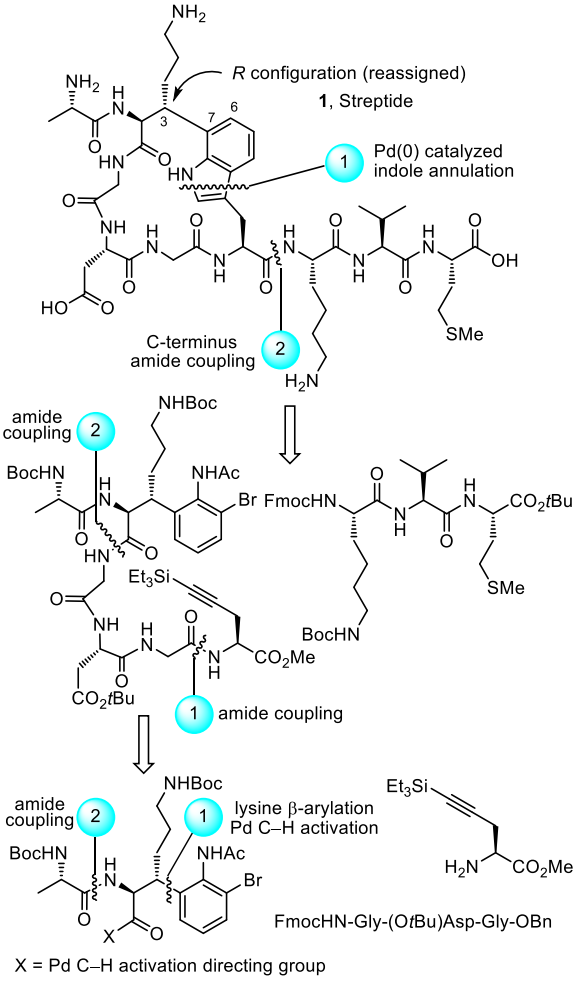


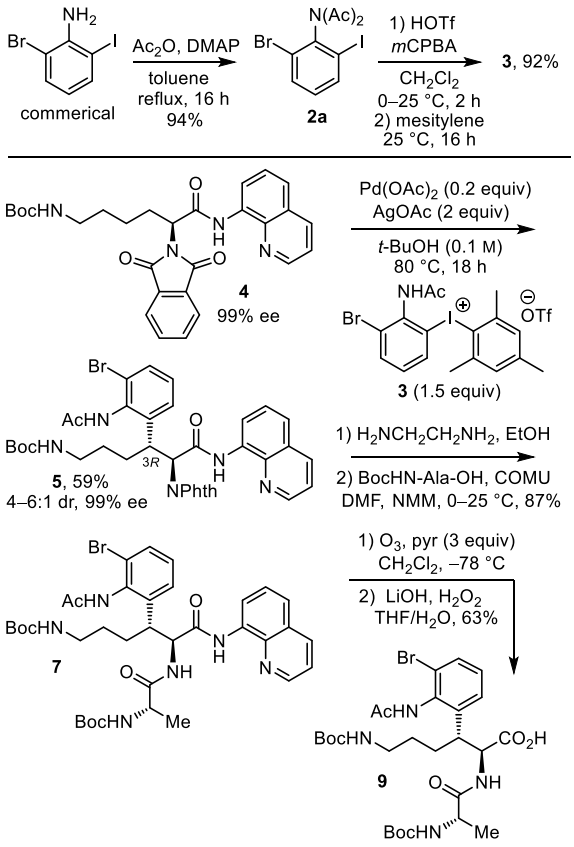
Figure 2. Key retrosynthetic steps for the total synthesis of streptide.

The efforts detailed herein culminated in the first total synthesis of streptide and provided an unambiguous assignment of the regiochemistry and stereochemistry (3*R* vs 3*S*) of the lysine C3 center. Both the 3*R* and the originally proposed 3*S* lysine diastereomers were independently prepared by total synthesis and subsequent NMR and HPLC analysis demonstrated that the 3*R* diastereomer correlates with the natural product. In addition to demonstrating the generality of the indole annulation macrocyclization reaction, the approach led to the use of an underutilized class of hypervalent iodine(III) aryl substrates in the palladium-catalyzed C–H activation/β-arylation functionalization of a lysine derivative. With an approach at hand to synthesize streptide and related analogues, the function of this natural product and its congeners can now be explored.

RESULTS AND DISCUSSION

β-Arylation of lysine derivatives by palladium-catalyzed C–H activation. Since the initial report by Corey[12] utilizing Daugulis' bidentate 8-aminoquinoline directing group,[13] additional advances in Pd-catalyzed β-functionalization of amino acids have been reported,[14] including recent functionalizations reported by Yu without added directing groups.[15] Despite these reports, Pd-catalyzed β-aryl functionalization of amino acids at non-benzylic secondary alkyl centers possessing β-hydrogens is still underdeveloped.[16] This challenge is compounded when intercepting an intermediate Pd(II) alkyl species with a sterically demanding aryl halide coupling partner as required for our work (i.e. ortho-substitution).[17] To effectively install the appropriately functionalized aryl unit for the key indole annulation macrocyclization reaction, we found that the use of a diaryliodonium salt allowed introduction of a sterically hindered 3-bromo-2-acetamidophenyl group without competitive reactions of the requisite aryl bromide in a reaction in which the empirically-derived conditions also proved important, perhaps accelerating the reaction or suppressing competitive β-hydride elimination and C–Pd homolysis.[18,19] This β-arylation reaction was efficient, scalable, diastereoselective and conducted with an unsymmetrical diaryliodonium salt, bearing a non-transferable sterically large mesityl group.[18] Thus, treatment of **4**, prepared from L-lysine (Supporting Information), and the iodonium salt **3**,[20] prepared in two steps (86%) from commercially available 6-bromo-2-iodoaniline, with $Pd(OAc)_2$ (0.2 equiv) and AgOAc (2.0 equiv) in *t*-BuOH (0.1 M) provided **5** in good yield and diastereoselectivity (60%, 4–6:1 dr) under mild reaction conditions (80 °C, 18 h). This was observed without competitive α-epimerization of the product (>99% ee), intramolecular trap of the intermediate Pd(II)-palladacycle by the distal carbamate, or β-hydride elimination (Scheme 1). The outcome of the reaction allowed us to carry both the 3*R* (major) and 3*S* (minor) diastereomers forward to complete the synthesis of both streptide stereoisomers. Fortuitously, though unanticipated, it was the major 3*R* diastereomer **5**, not the minor 3*S* diastereomer **6**, of this reaction that afforded streptide.

Scheme 1



Initial efforts to install a functionalized aryl group at the β-position of lysine were conducted with N-acetyl-6-bromo-2-iodoaniline (**2b**) and N-acetyl-2,6-dibromoaniline and were only modestly successful. The native directing group ability of the carboxylic acid was explored[15] as well as removable carboxylic acid-derived directing groups (e.g., -CONHOMe and -CONHC$_6$F$_5$ carboxamides), albeit unsuccessfully.[11] Notably, when **4** was employed in early studies, the intermediate Pd(II) C–H insertion product was often intercepted in an intramolecular amination reaction by the distal carbamate (NHBoc) to give a pyrrolidine. Thus, a screen was conducted with the bis-phthalimide protected lysine derivative **10**, lacking a distal carbamate NH and incorporating the strongly coordinating 8-aminoquinoline directing group.[13] The screen, which enlisted **2b** as the coupling partner, was conducted with varied solvents, palladium sources, bases, and additives, leading to confirmed product formation. However, even with extensive optimization, product **11** was only observed at near stoichiometric loadings of Pd(OAc)$_2$ (0.4 equiv) with excess aryl iodide (4 equiv), and then only afforded **11** in low yield (27%, 0.4 equiv of Pd(OAc)$_2$, 1.5 equiv of AgOAc, 0.07 M *t*-BuOH, 110 °C, 48 h) (Figure 3). As a result, efforts were refocused on alternatives to the coupling partner **2b**, expecting that a stronger oxidizing reagent may better access the needed Pd(IV) oxidative insertion intermediate than an aryl iodide with our hindered substrate. Unsymmetrical diaryliodonium salts such as **3**, bearing a nontransferable aryl

group, were explored as coupling partners. Although such reagents have been used effectively,[18,19] Sanford's early mechanistic studies indicated that their participation in oxidative addition at Pd(II) is still the rate-determining step in a $Pd^{II/IV}$ cycle.[18] This likely has discouraged their subsequent exploration in couplings that might suffer competing reactions of the intermediate Pd(II) palladacycle. Treatment of **10** even with near stoichiometric (vs excess) amounts of the iodonium salt **3** (1.2 equiv, 0.2 equiv of $Pd(OAc)_2$, 1.2 equiv of K_2CO_3) provided **11** in good yield (60%) with excellent diastereoselectivity (>20:1 dr) under mild reaction conditions (110 °C, 16 h) without the need for other heavy metal additives (e.g., Ag(I)) (Figure 3). In the optimization of the reaction of **10** with **3**, no reaction was observed at 25–60 °C, an increased loading of base did not further improve conversions and use of toluene versus $ClCH_2CH_2Cl$ led to competitive β-hydride elimination. The extension of these observations to **4**, as shown in Scheme 1, now proved possible and provided **5** under mild conditions (*t*-BuOH, 80 °C, 18 h) but required inclusion of AgOAc in the reaction mixture to avoid distal carbamate interception of the intermediate Pd(II) C–H insertion product.

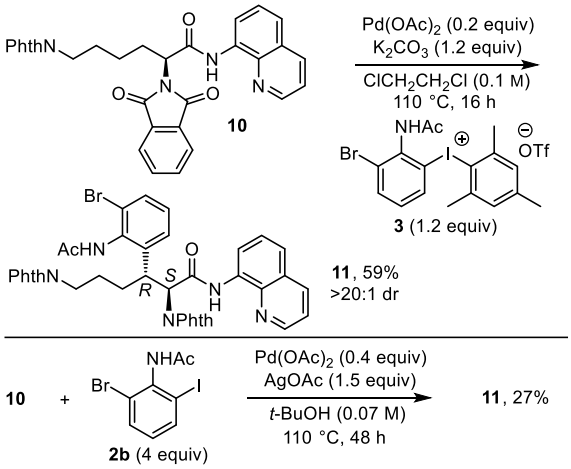


Figure 3. Initial key observations on C–H activation and subsequent arylation of a lysine derivative.

The stereochemistry of **5** and **6** was assigned initially based on the original work of Corey[12] for Pd-catalyzed β-arylation of phthaloyl-protected L-amino acids that contain the 8-aminoquinolyl directing group (>20–4:1 dr favoring 2*S*,3*R*), in which the initial C–H insertion preferentially forms the sterically favored intermediate 5-membered *trans*-palladacycle. In Corey's work, the stereochemical assignments were unambiguously established by X-ray structures of several of the major products,[12] and such work subsequently has been extended to crystallographic characterization of the intermediate *trans*-palladacycles themselves by Yu and Chen.[14f,g] Our initial assignments based on this work were confirmed with the single crystal X-ray structure determinations of **12** and **13**, derived from the major and minor diastereomers **5** and **6**, respectively (Figure 4).[20]

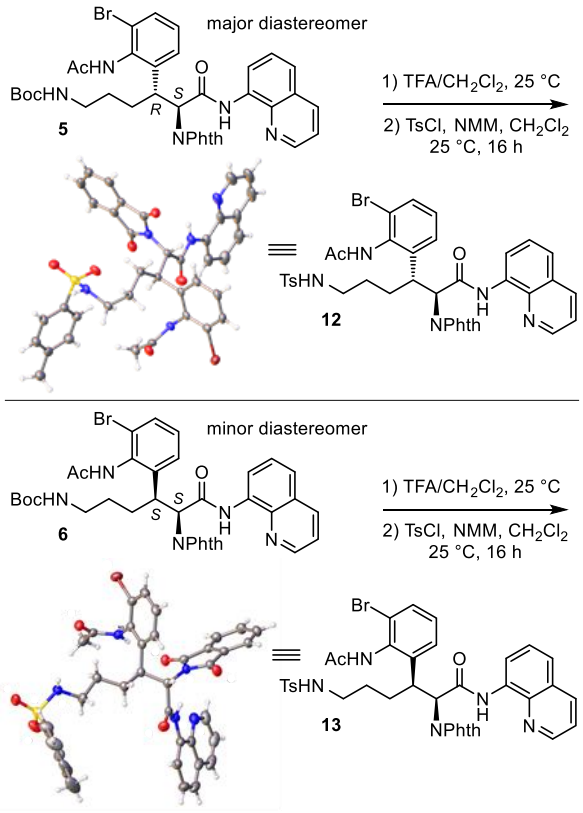
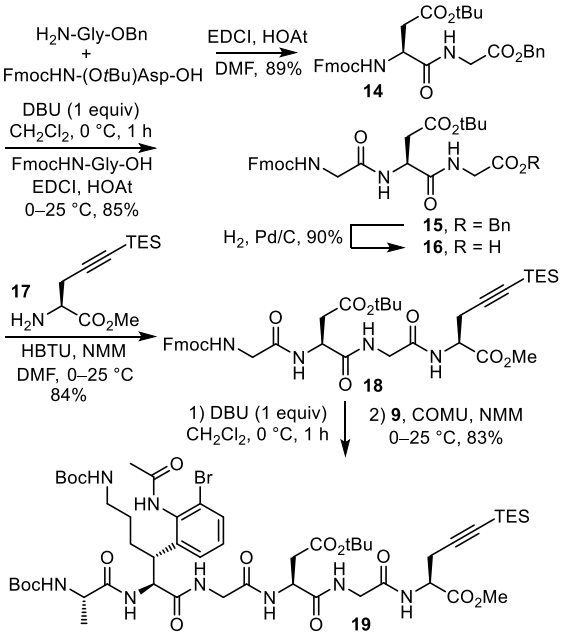


Figure 4. Relative and absolute stereochemistry of **5** and **6** established by X-ray diffraction analysis.[20]

The major diastereomer **5** and its accompanying minor isomer **6** were not easily separated by chromatography at this stage but were found to be readily separated after installation of the protected N-terminal alanine residue. Thus, phthalimide deprotection of the diastereomeric mixture of **5** and **6** (4–6:1) with 1,2-diaminoethane (EtOH, 0–25 °C, 18 h) provided the corresponding free amines (99%), which were immediately coupled with BocHN-Ala-OH (1.2 equiv, 1.2 equiv of 1-cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), 1.0 equiv of N-methylmorpholine (NMM), DMF, 0–25 °C, 18 h, 75–87% combined yield) to provide the alanine-linked lysine-2 2*S*,3*R* (**7**) and 2*S*,3*S* (**8**) diastereomers (Scheme 1). At this point, the lysine C3 diastereomers were easily separated by column chromatography. Removal of the 8-aminoquinoline directing group from the major isomer **7** was accomplished by mild oxidative deprotection (via ozonolysis),[21] which cleaves the quinolyl group to a reactive imide intermediate that we found can be hydrolyzed selectively to provide not only a carboxamide (NH_4OH, THF, 25 °C, 18 h, 67%), but also the carboxylic acid. Thus, ozonolysis of **7** in the presence of pyridine[22] (3 equiv) at –78 °C (CH_2Cl_2, 5–10 min) followed by treatment of the isolated reaction product with H_2O_2–LiOH (THF/H_2O, 0 °C, 1 h) directly provided the carboxylic acid **9** in good yield (63%, Scheme 1). Whereas ozonolysis in absence of pyridine led to substantial amounts of carboxamide product upon hydrolysis, its use as an ozonolysis additive[22] tempered the oxidation reaction and thereby helped suppress the competitive carboxamide formation in the subsequent intermediate imide hydrolysis.

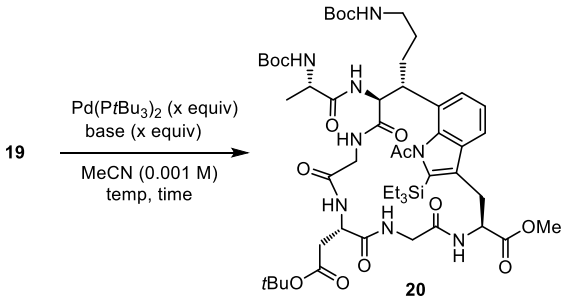
Assembly of the N-terminal core hexapeptide and Larock macrocyclization. The linear peptide **19**, incorporating **9** and the partner triethylsilyl alkyne that serve as the precursors for the crosslinked tryptophan, was prepared by coupling H_2N-Gly-OBn with FmocHN-(O*t*Bu)Asp-OH promoted by N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride and 1-hydroxy-7-azabenzotriazole (EDCI–HOAt, DMF) to afford the dipeptide **14** in 89% yield (Scheme 2). Deprotection of **14** (1 equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), CH_2Cl_2, 0 °C, 1.5 h) under conditions found to suppress cyclization followed by coupling of the released free amine in the same vessel with FmocHN-Gly-OH (EDCI–HOAt, 16 h) provided **15** in 85% yield. Benzyl ester removal by hydrogenolysis (H_2, Pd/C, THF, 23 °C, 16 h) afforded the tripeptide **16** (90%), which was coupled with **17**[7a] (preparation supplied in Supporting Information, 1.07 equiv of HBTU, 1.07 equiv of NMM, DMF, 0–23 °C, 16 h) to provide **18**. (2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was found to be the optimal coupling reagent of those examined, providing **18** in good yield (84%) and under conditions that do not result in α-epimerization adjacent to the methyl ester. After in situ Fmoc deprotection of **18** (DBU, CH_2Cl_2, 0 °C, 1 h), the linear hexapeptide **19** of the streptide macrocyclic core was accessed by coupling the liberated amine with **9** (1.2 equiv of COMU, 1.0 equiv of NMM, CH_2Cl_2, 0–25 °C, 18 h, 83%).

Scheme 2



Macrocyclization of **19** was first examined under conditions we previously introduced (1.1 equiv of $Pd(OAc)_2$, 1.3 equiv of 1,1'-bis(di-*tert*-butylphosphino)ferrocene (dtbpf), 1.3 equiv of Et_3N, toluene/MeCN (1:1), 100 °C, 1 h), permitting use of an aryl bromide.[7] The reaction provided **20** in good albeit variable yield (50%, Figure 5). The addition of Bu_4NBr (1 equiv), which is thought to increase catalyst stability by preventing Pd black formation,[23] maintained but did not improve the yield of **20**

(45%). However, the reaction remained variable, especially on small scale, complicating its optimization and scale up. The use of other ammonium salts or additives (e.g., LiCl) did not improve this further. Whereas other Pd catalysts such as $PdCl_2(MeCN)_2$, $Pd_2(dba)_3$, $PdCl_2$(dtbpf), and (o-tol$_3$P)$_3$Pd failed to provide the macrocyclization product or did so poorly as noted in our previous work,[7,8] (t-Bu$_3$P)$_2$Pd also proved to be effective and provided consistent conversions as reported by Reisman.[24] Further optimization focused on the use of this catalyst (Figure 5). Macrocycle **20** was obtained in 60% yield under even milder reaction conditions (80 °C, 0.001 M MeCN, 1 h), using Et_3N or dicyclohexyl methylamine (Cy_2NMe, 1.3 equiv) and (t-Bu$_3$P)$_2$Pd (1.1 equiv) (entries 4 and 8). Although Et_3N could be used as the added base (entry 4), its lower boiling point led to more variable yields on small scale (entry 4 vs 3), and this is likely the source of the variable conversions we initially observed with its use with $Pd(OAc)_2$. Prolonged reaction times beyond 1 h in addition to increased base loading also provided the desired product in comparable, but not improved, yields (entries 7 and 9). Lastly, a catalyst loading of 0.6 equiv of (t-Bu$_3$P)$_2$Pd matched the conversion of its stoichiometric use (58%, 2 h, entry 6 vs 8 and 9), whereas use of 0.2 equiv of (t-Bu$_3$P)$_2$Pd also provided **20**, albeit in lower yield (34%, 4 h, entry 5). Because such reactions work effectively with catalytic $Pd(OAc)_2$ or (t-Bu$_3$P)$_2$Pd on simpler non-peptide substrates,[8,24] it is likely that the precursor **19** or the macrocycle **20** act to sequester the catalyst, requiring the higher catalyst loading.[7,8] However, further efforts to optimize the reaction for sub-stoichiometric use of the Pd catalyst were not pursued as it was not central for the work detailed herein. Nonetheless, it is remarkable that the Larock cyclization, adapted for use with an aryl bromide, performs effectively for formation of a 20-membered macrocycle on a complex substrate that contains 6 amides, 2 carbamates and 2 esters, one of which is prone to epimerization.



entry	Pd (equiv)	base (equiv)	temp (°C)	time	yield
1	$Pd(OAc)_2$ (1.1)[a]	Et_3N (1.3)	100	1 h	50%
2	$Pd(OAc)_2$ (1.1)[a,b]	Et_3N (1.3)	100	1 h	45%
3	Pd(Pt-Bu$_3$)$_2$ (1.1)	Et_3N (1.3)	100	1 h	28%
4	Pd(Pt-Bu$_3$)$_2$ (1.2)	Et_3N (1.3)	80	1 h	58%
5	Pd(Pt-Bu$_3$)$_2$ (0.2)	Cy_2NMe (2.5)	80	4 h	34%
6	Pd(Pt-Bu$_3$)$_2$ (0.6)	Cy_2NMe (2.5)	80	2 h	58%
7	Pd(Pt-Bu$_3$)$_2$ (1.2)	Cy_2NMe (2.5)	80	4 h	50%
8	**Pd(Pt-Bu$_3$)$_2$ (1.1)**	**Cy_2NMe (1.3)**	**80**	**1 h**	**60%**
9	Pd(Pt-Bu$_3$)$_2$ (1.1)	Cy_2NMe (1.3)	80	2 h	54%

[a]1.3 equiv dtbpf, toluene/MeCN (1:1). [b]1 equiv Bu_4NBr.

Figure 5. Representative results of the macrocyclization optimization.

With **20** in hand, its selective or exhaustive deprotections were examined. This not only set the stage for completion of the total synthesis of streptide, but it also permitted the examination of the conformational properties of **1** and the core macrocycle. TES removal under mild conditions (3 equiv of Bu_4NF, THF, 0 °C, 78%) provided **21** (Figure 6). Whereas hydrolysis of the C-terminal methyl ester of **20** with LiOH or related reagents was challenging due to competing epimerization and partial indole N-acetyl deprotection, the hydrolysis to provide **22** was accomplished cleanly with Me_3SnOH (88–93%).[25] Finally, studies on the single-step global deprotection of **22** provided complex mixtures unless a trialkylsilane was included in the reaction mixture, acting to trap cationic intermediates arising from the deprotections. Thus, both **23** (71%) and **24** (94%) were obtained in excellent yields from **22** following HCl or trifluoroacetic acid (TFA) treatment and are indicative of clean TES removal along with cleavage of the *t*-butyl carbamates and *t*-butyl ester and a slower hydrolysis of the N-acetylindole.

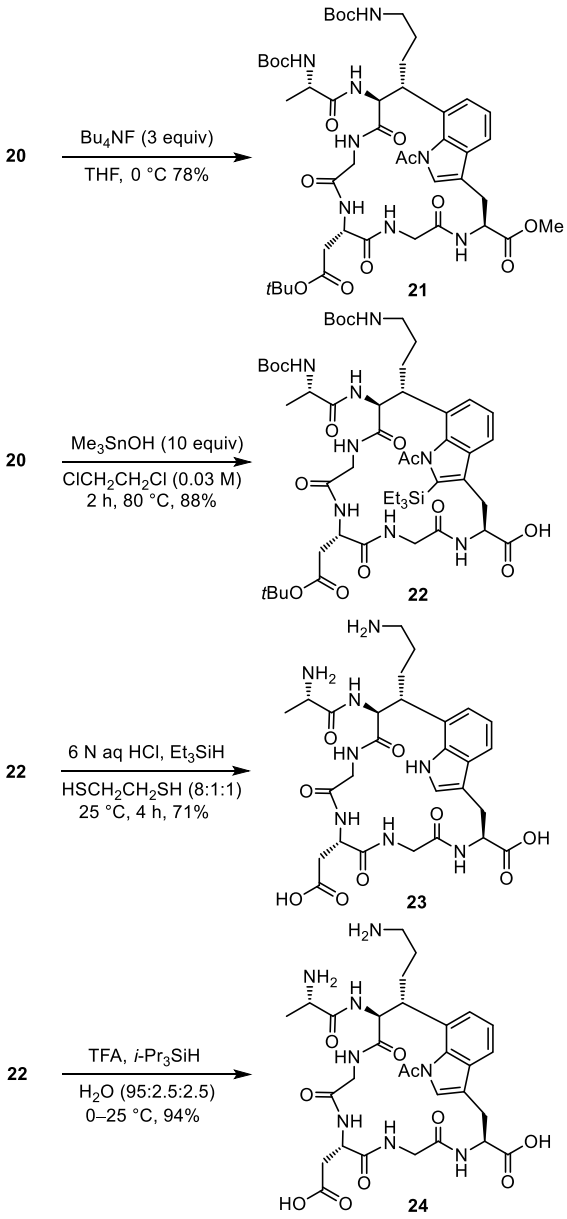
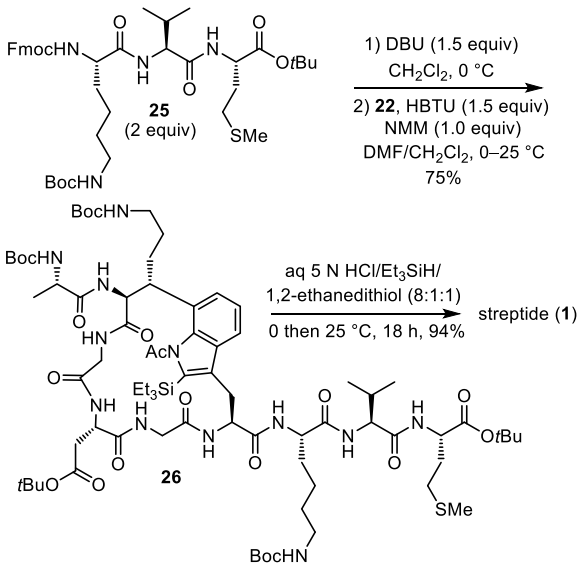


Figure 6. Deprotection studies on the macrocyclic core.

Macrocycle **20** and each of the partial or complete deprotection products **21**–**24** displayed relatively sharp ^{1}H NMR spectra consistent with adoption of rigid or rapidly interconverting conformations. At 1 °C but not at 25 °C, only **20** displayed a limited number of distinct (e.g., indole C4 CH) and sharp ^{1}H NMR signals (CD_3OD) attributable to two (major and minor) conformations (Supporting Information Figures S1–S6). Although not examined in detail, these comparisons of **20** and **21** suggest that **20**, as well as **21**, do not display atropisomeric behavior at room temperature.

Completion of the total synthesis of streptide. As detailed above, selective hydrolysis of the methyl ester **20** to provide **22** was achieved with use of Me_3SnOH (4–10 equiv, $ClCH_2CH_2Cl$, 80 °C, 4.5–2 h, 88–93%), leaving the indole N-acetyl group untouched and avoiding detectable epimerization (Figure 6). Typical hydrolysis reactions mediated by LiOH, with or without added H_2O_2, either led to apparent competitive epimerization or partial deprotection of the indole N-acetyl group. Fmoc deprotection of the tripeptide **25** (2 equiv, synthesis in Supporting Information) with DBU (1.5 equiv, 0 °C, 1 h) followed by coupling of the free amine with **22** (2 equiv of HBTU, 1 equiv of NMM, DMF/CH_2Cl_2, 0–25 °C, 18 h) provided **26** (75%, >99:1 dr), constituting a fully protected streptide (Scheme 3). Although not investigated in detail, alternative amide coupling reagents such as COMU provided only trace product formation, whereas use of EDCI–HOAt (CH_2Cl_2) and HATU (DMF) led to apparent competitive α-epimerization but provided the products in good yield (56% and 67%, respectively). A single-step global deprotection of **26** to provide streptide (**1**) with removal of 7 protecting groups was achieved by treatment with aqueous 5 N HCl/Et_3SiH/1,2-ethanedithiol (8:1:1) at room temperature. This smoothly and cleanly provided streptide (**1**) in high yield (82–94%) after purification by reverse-phase HPLC, completing the first reported total synthesis of streptide.

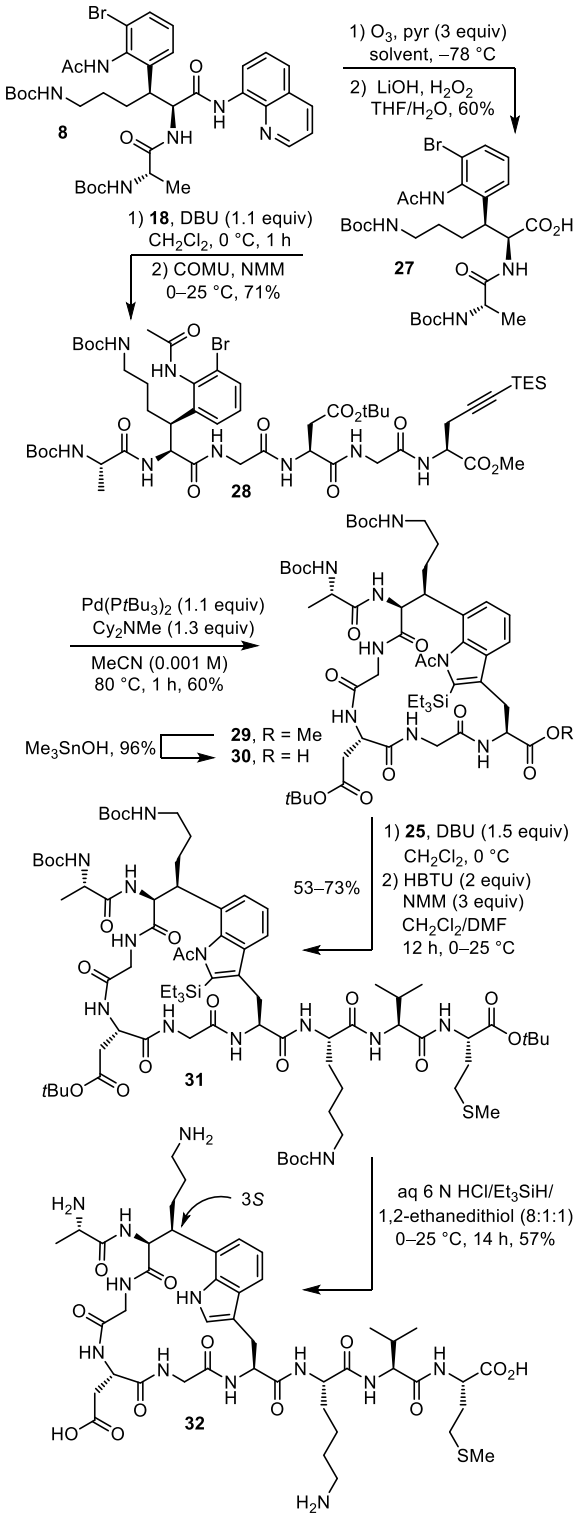
Scheme 3



Comparison of the spectroscopic properties of synthetic **1** with the ^{1}H and ^{13}C NMR spectra reported for authentic streptide provided a near identical match. In the course of the initial characterization of synthetic **1**, we found that trace amounts of acid (TFA) residual from our HPLC purification can impact the chemical shifts observed in the NMR spectra and it is possible concentration dependent distinctions were also observed. This initial and unexpected correlation with natural streptide was particularly strong for the ^{1}H NMR spectrum, whereas there were a few larger chemical shift differences in the ^{13}C NMR. It was not clear whether the unexpected spectroscopic correlation of synthetic **1**, containing the lysine 3*R* (not the proposed 3*S*) stereochemistry, with natural streptide represented a true correlation or one that was fortuitously close. Consequently, and as originally planned, the lysine 3*S*-diastereomer of streptide was also prepared.

Total synthesis of the lysine-2 3*S*-diastereomer of streptide. The lysine-2 C3 configuration in streptide could not be unambiguously established by spectroscopic methods in the original structure determination studies.[3] Instead, a computational approach was employed to assess correspondence between the computed streptide structures and constraints derived from NOESY correlations using the CYANA algorithm. The diastereomer that satisfies more constraints would give a lower target function (*f*). Both *S* and *R* C3 configurations could be acceptably fit to the ^{1}H NMR data (e.g.; *S* vs *R*: *f* = 0.042 vs 0.054, rmsd = 1.62 vs 1.72 Å, and accounting for 29 vs 27 observed NOEs between calculated and observed values)[3] with the *S*-diastereomer providing a slightly better fit. In addition, the original characterization of the natural product and its spectroscopic data were collected on limited amounts of sample. To unambiguously assign the configuration at the lysine C3, the lysine 3S-diastereomer **8**, arising from the minor diastereomer **6** generated in the C–H functionalization reaction, was carried through the identical synthetic sequence (Scheme 4). Notably, the Pd(0)-promoted macrocyclization of **28** provided **29** in conversions (60%) identical to that observed with the diastereomer **19** without alteration of the reaction conditions (80 °C, 0.001 M CH_3CN, 1 h). This synthesis provided **32**, the lysine-2 C3 *S*-diastereomer.

Scheme 4



Reisolation of and comparison with natural streptide. To facilitate the comparison of the synthetic streptide diastereomers with the authentic natural product, streptide was isolated from 20 L of *S. thermophilus* production cultures using previously established procedures,[3] yielding approximately 1.7 mg of pure material. After completion of the synthetic work and with the benefit of having freshly isolated streptide available, detailed NMR and HPLC-MS comparisons were conducted. Upon side-by-side examination, natural streptide and the synthetic lysine-2 3*R* diastereomer clearly correlated. The

^{1}H and ^{13}C NMR data were essentially identical, whereas the 3*S* diastereomer was easily distinguishable with divergent chemical shifts at the lysine-2 α-^{1}H (4.81 ppm for the 3*R* diastereomer and authentic streptide, 5.02 for the 3*S* diastereomer) and lysine-2 β-^{1}H (3.52 ppm for the 3*R* diastereomer and authentic streptide, 4.03 ppm for the 3*S* diastereomer), among others (Supporting Information Figure S20–S23). Moreover, we were able to demonstrate that the ^{1}H NMR and ^{13}C NMR spectra of **1** display subtle condition-dependent shifts and changes largely centered within or adjacent to the acidic residues (Met^{9}, Asp^{4}, Gly^{3} and Gly^{5}), accounting for the uncertainty in our initial correlation with the original published spectra (Supporting Information Figures S17–S19 and accompanying discussion). However, changes in the chemical shifts for Ala^{1}, Lys^{2} and Trp^{6} were not observed (< 0.01 ppm differences), indicating a lack of any impact of either the protonation state or any major conformational change of the macrocycle under the conditions examined. In agreement with the NMR comparisons, high-resolution HPLC-MS analysis unambiguously demonstrated co-elution of authentic streptide with the 3*R* diastereomer **1**, but not with the 3*S* diastereomer **32**, which could be separated from both when injected as mixtures (Figure 7). Together, these results confirm the first chemical synthesis and establish the full structural assignment of streptide.

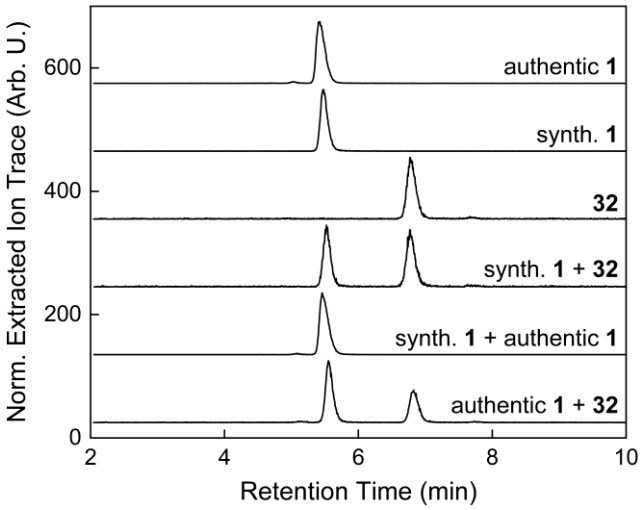


Figure 7. HR-HPLC-MS analysis of authentic and synthetic streptides. The sample loaded is labeled on top of the resulting elution profile. Streptide elution was monitored by absorbance at 280 nm (not shown) and by MS total ion count. Shown is the HR-MS-extracted ion chromatogram for streptide (*m/z* 989.489) in each trace. Authentic streptide co-elutes with lysine-2 C3 diastereomer **1** (3*R*) but not with **32** (3*S*). See Supporting Information for details.

With knowledge of the absolute configuration of the lysine-2 C3, we generated a computational model of streptide in CYANA using constraints derived from NOESY data that were collected on authentic **1**.[3,26] The crosslinked Lys/Trp residues were added manually to the CYANA residue library and the configuration of the lysine-2 C3 was explicitly defined as *R*. The calculation provided possible

solution conformers, with the conformer that had the lowest target function, and therefore exhibited the best agreement with the experimental constraints, depicted in Figure 8. It features an indole-based cyclophane, a (3,7)indolophane, in which the encircling peptide adopts a rigid conformation enforced by the amide bonds within the macrocycle and the bridging indole. The three-dimensional model of **1** facilitates future docking studies to possible target sites.

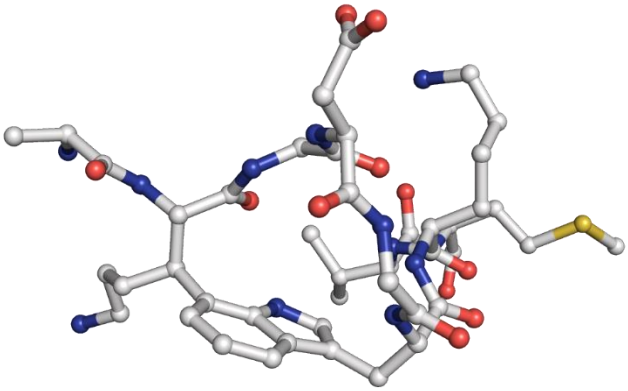


Figure 8. Computational model of the three-dimensional structure of streptide.

CONCLUSION

Herein, we report the first total synthesis of streptide that provided confirmation of the site (regiochemistry) and established the configuration (stereochemistry, 3*R* vs 3*S*) of the residue 2 lysine C3 central to the unusual crosslink in this natural product. Both the 3*R* and the initial computationally assigned 3*S* lysine diastereomers were independently prepared by total synthesis, involving a longest linear synthetic sequence of 11 steps, and it was the 3*R* diastereomer that was found to correlate with the natural product. The approach utilized a powerful Pd(0)-mediated indole annulation for macrocyclization of the core 20-membered cyclic peptide, enlisted an underutilized hypervalent iodine(III) aryl substrate in a key palladium-catalyzed C–H activation and β-arylation reaction conducted on a lysine derivative, and provides access to material with which the role of streptide and related natural products may be examined.

ASSOCIATED CONTENT

Supporting Information

Full experimental details. The Supporting Information is available free of charge on the ACS Publications website at DOI: XX.

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Notes

The authors declare no competing financial interest.

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