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Total Synthesis and Reassignment of the Structures of the Antimicrobial Lipodepsipeptides Circulocin γ and δ

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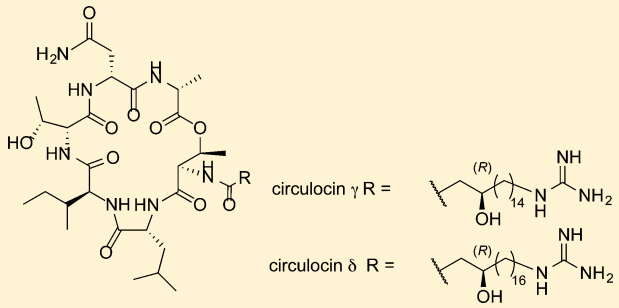
Supporting Information

ABSTRACT: The structures of the naturally occurring antimicrobial lipodepsipeptides circulocin γ and circulocin δ have been reported to comprise a common cyclic depsipeptide core attached to 3-hydroxy,ω-guanidino fatty acid chains differing in length by two methylene units, but analysis of the reported data suggested that the originally reported structures had incorrect side chain lengths. The total synthesis of both side chain epimers of the originally reported structure of circulocin γ bearing a 19-guanidino-3-hydroxynonadecanoyl (GHND) side chain has been accomplished using a modular approach involving synthesis of the cyclic depsipeptide and side chain fragments followed by a late stage coupling reaction. This revealed that the originally reported structure for circulocin γ bearing the GHND side chain is incorrect and that this structure is actually that of circulocin δ. It has also enabled the absolute configuration of the side chain hydroxy group of the natural product to be assigned as (*R*). Subsequent synthesis of the analogue bearing a 17-guanidino-3-(*R*)-hydroxyheptadecanoyl (GHHD) side chain provided confirmation that this revised structure is that of circulocin γ.



INTRODUCTION

The rapid increase in multidrug resistant bacterial and fungal infections in recent years has led to an urgent need for the development of new antimicrobial agents.[1−5] Naturally occurring cyclic lipo(depsi)peptides are promising lead structures for antimicrobial drug discovery, with a number of compounds from this class such as daptomycin already in clinical use. Strains of *Paenibacillus* and *Bacillus* are known producers of bioactive cyclic lipopeptides, and a number of antimicrobial compound classes including the iturins,[6] surfactins[7,8] and fusaricidins (or LI-F class)[9,10] have been isolated from these microorganisms.[11] These compounds are often produced as mixtures of closely related structures, and different strains of microorganism can sometimes produce structures of high similarity. For example, the fusaricidin class of cyclic lipodespipeptides, which contain a cyclic depsihexapeptide core with a 15-guanidino-3-hydroxypentadecanoyl (GHPD) side chain attached to the cycle via the nitrogen atom of an L-threonine residue, are obtained as a mixture of at least 12 compounds from the L-1129 strain of *Paenibacillus polymyxa*[9,10] as well as from other strains of *Paenibacillus*.[12,13] The components of this mixture differ only in the compositions of three of the amino acids (L-Thr, D-*allo*-Thr and D-Ala are conserved) in the cyclic depsipeptide core, while the GHPD side chain remains constant throughout the series. The promising antimicrobial activity displayed by the fusaricidin cyclic peptides against both fungi and bacteria (including some drug resistant strains) has led to the synthesis and evaluation of the antimicrobial activity of a number of analogues of these compounds, leading to the conclusion that the guanidino functionality of the GHPD side chain is crucial for activity.[14] A recent total synthesis of fusaricidin A (LI-F04a) **1** enabled the assignment of the absolute stereochemistry of the stereogenic center at C3 of the GHPD side chain as (*R*)-configured[15] and an analogue lacking the hydroxy group at this position had significantly reduced antifungal activity.[16]

The GHDP side chain of the fusaricidin peptides is unique, with most other classes of antimicrobial cyclic lipodepsipeptides bearing fatty acid side chains terminating in methyl groups. However, the structures of the circulocins (**2**−**5**), a series of four cyclic lipodepsipeptides isolated from the J2154 strain of *Bacillus circulans*, are reported to contain similar guanidino-terminated fatty acid side chains with a C3-hydroxy group, albeit of different lengths to those reported for the fusaricidin series. (Figure 1).[17] The circulocins exhibit antibiotic activity against clinically relevant species of Gram-positive bacteria, including piperacillin-resistant staphylococci and vancomycin-resistant enterococci, as well as antifungal activity against *Candida albicans*. Of the four peptides isolated, circulocins α and β **2** and **3** are reported to have cyclic depsipentapeptide cores, while circulocins γ and δ are reportedly cyclic depsihexapeptides (**4** and **5**) with striking similarities to the fusaricidin class (Figure 1). In particular, circulocins γ and δ have a cyclic hexadepsipeptide core that is almost identical to that of fusaricidin A, with the only differences being substitution of D-Val and L-Val residues by D-Leu and L-Ile, respectively.

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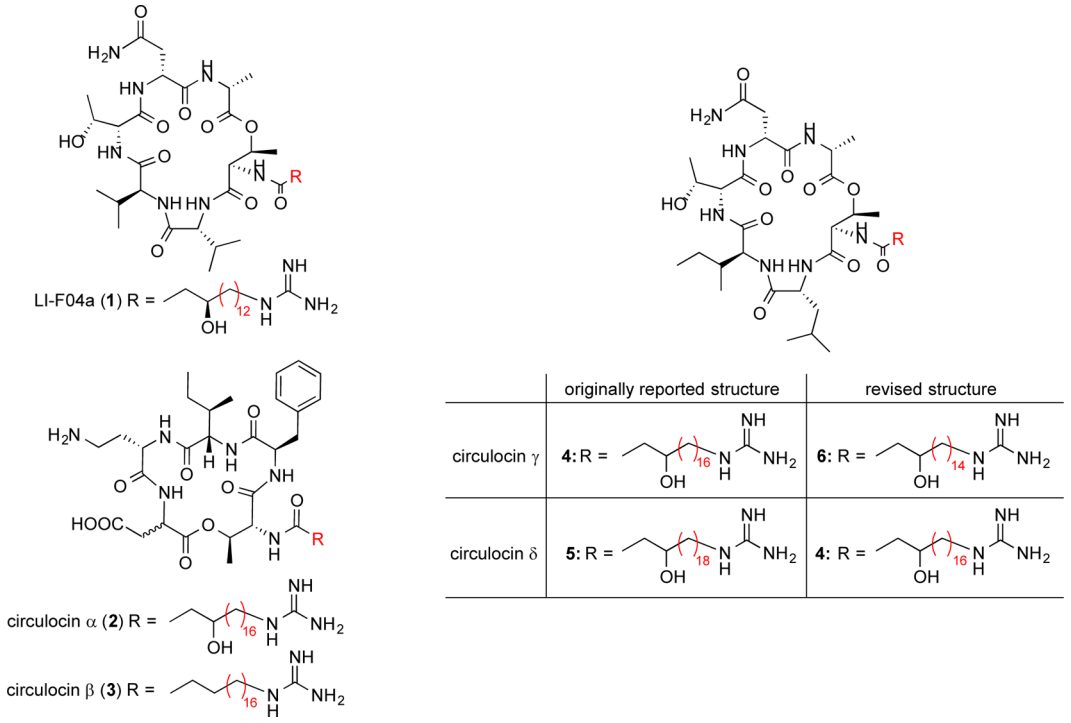
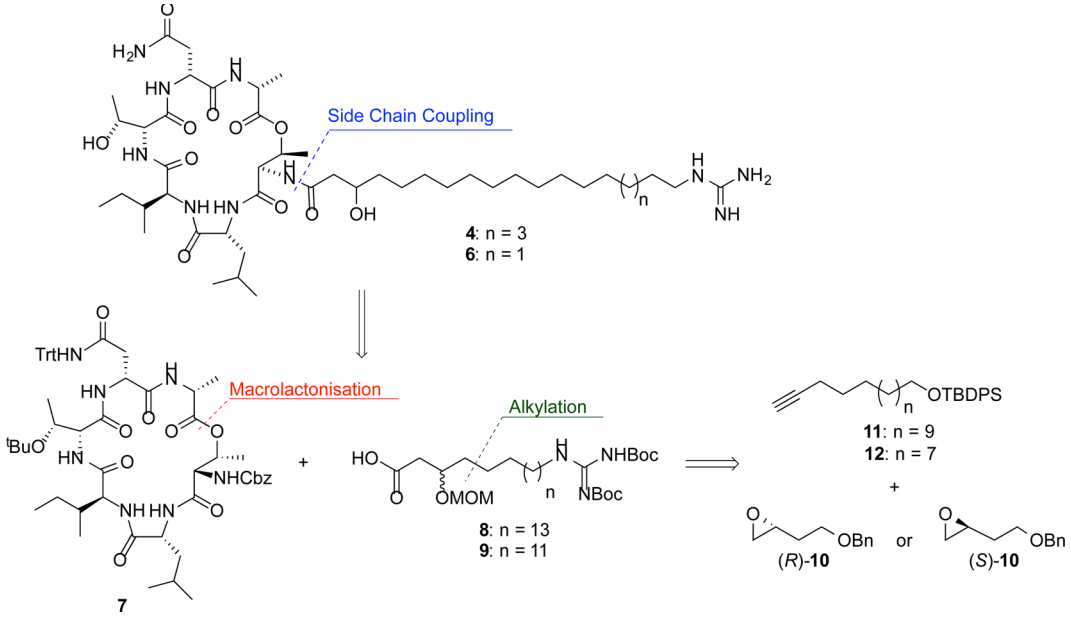


Figure 1. Structure of fusaricidin A and the reported[17] and revised structures of circulocins α–δ.

Scheme 1. Retrosynthesis



The structures proposed by He et al.[17] for circulocins γ **4** and δ **5** differ only in the lengths of the guanidino-terminated side chains, with that in circulocin γ **4** reported to be a 19-guanidino-3-hydroxynonadecanoic acid (GHND) side chain, while that in circulocin δ **5** is reported to be a 21-guanidino-3-hydroxy-heneicosanoic acid (GHHC) side chain (Figure 1). However, close inspection of the data reported by He et al. for circulocins γ and δ suggests that the reported side chain lengths are incorrect. Notably, the molecular formulas for the proposed structures ($C_{47}H_{86}N_{10}O_{11}$ for circulocin γ and $C_{49}H_{90}N_{10}O_{11}$ for circulocin δ) do not match the molecular formulas provided by high resolution mass spectrometry (m/z = 939.6243, corresponding to $MH^+ = C_{45}H_{83}N_{10}O_{11}$ and m/z = 967.6556, corresponding to $MH^+ = C_{47}H_{87}N_{10}O_{11}$ for circulocins γ **4** and δ **5**, respectively). The molecular mass of the proposed structures differ from those obtained by high resolution mass spectrometry by the mass of two methylene units (C_2H_4) in each case. We postulated that the most likely reason for this difference was a mis-assignment of the number of methylene units in the alkyl side chains and that the correct structures of circulocins γ (**6**) and δ (**4**) bear 17-guanidino-3-hydroxy-heptadecanoic acid (GHHD) and 19-guanidino-3-hydroxyno-nadecanoic acid (GHND) side chains, respectively.

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To clarify the structures of circulocins γ and δ we undertook the total synthesis of the revised structures **4** and **6**. Additionally, in order to confirm the absolute stereochemistry of the stereogenic center at C3 of the side chains and determine whether it was identical to that observed for fusaricidin A **1**,[14] we prepared both side chain epimers of the revised structure of circulocin δ **4**.

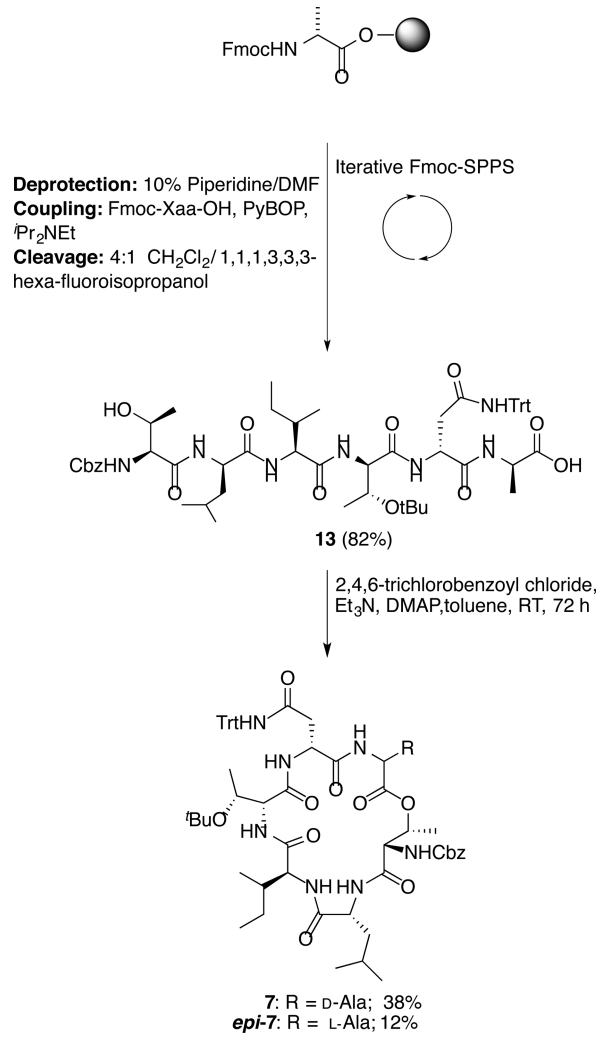
RESULTS AND DISCUSSION

Given that both **4** and **6** contain an identical cyclic depsipeptide core, we chose to employ a convergent synthetic strategy, similar to that used previously in our total synthesis of **1**,[15] to provide ready access to compounds of varying side chain length and to both side chain epimers of **4** in an efficient manner (Scheme 1). This route allows the cyclic peptide core **7** and the required GHND and GHHD side chains (**8** and **9**, respectively) to be synthesized independently and combined in late stage coupling reactions. We envisaged that the protected GHND and GHHD side chains **8** and **9** should be readily accessible via alkylation of the chiral epoxides (*R*)-**10** and (*S*)-**10** with alkynes **11** and **12**, respectively.

In order to prepare the cyclic depsipeptide core of circulocins γ and δ using our previously optimized macrolactonization approach,[15,16] we first prepared the linear hexapeptide **13** by standard solid phase peptide synthesis protocols using 2-chlorotrityl chloride resin as the solid support, PyBOP/Hünig's base as the coupling agent and an Fmoc/*t*-Bu protecting group strategy (Scheme 2). The *N*-terminal threonine residue was incorporated as the Cbz-protected derivative to allow later removal of this protecting group while leaving the side chain protecting groups intact. Cyclization of **13** was then performed using the modified Yamaguchi lactonization conditions[18] described in our previous publication, which were optimized to mimimize epimerization of the C-terminal residue during the macrocyclization reaction to prepare the core of fusaricidin A.[15,16] This gave the desired cyclic peptide **7** in a 38% isolated yield after purification by HPLC, but the formation of a significant quantity (12%) of the C-terminal epimerized cyclic peptide *epi*-**7** was also observed (Scheme 2). In efforts to improve the yield of the desired cyclic product, we also attempted the cyclization of **13** using 2-methyl-6-nitrobenzoic anhydride (MNBA),[19] however this did not lead to improved yields or reduced quantities of the epimer (**7** and *epi*-**7** were obtained in 34 and 14% yields, respectively). Alternative approaches to the cyclic product **7**, involving macrolactamizations at the D-Asn-D-Ala; D-Leu-L-Ile and L-Thr-D-Leu junctions were also explored, however analysis of the crude reaction mixtures indicated only low yields of the required cyclic product and the presence of diasteromeric mixtures, so these were not investigated further. It is interesting to note that the seemingly minor changes to the linear peptide structure resulting from the replacement of the two Val residues present in the fusaricidin A core by the D-Leu and L-Ile residues required for the circulocin core had a significant effect on the isolated yield and amount of epimerization occurring in the cyclization reaction. This highlights the impact of linear peptide sequence in determining the success of the cyclization reaction, with small changes significantly affecting the reaction outcome.

With the cyclic core of circulocins γ and δ in hand, we turned our attention to the synthesis of the GHND and GHHD side chains. We focused initially on the synthesis of both enantiomers of side chain **8**, which would provide access to our revised structure of circulocin δ, since this would allow us

Scheme 2. Synthesis of Cyclic Depsipeptide 7



to confirm both the validity of our structural revisions (given that this is identical to the structure of circulocin γ originally proposed by He et al.[17]) and the absolute stereochemistry of the C3 alcohol in **4**.

To enable the assignment of the stereochemistry of the C3 alcohol in circulocin δ **4**, both enantiomers of the side chain **8** were required. It was envisioned that this could be achieved by $BF_3{\cdot}OEt_2$ promoted alkylation of both enantiomers of the epoxide **10** with the alkyne **11** using similar methods to those employed previously in our synthesis of fusaricidin A.[15] In order to prepare the alkyne chain of the required length, we commenced with propargyl alcohol **14** as outlined in Scheme 3. The dianion of **14** was prepared upon treatment with 2 equiv of *n*-BuLi in a solution of THF and HMPA. This was then treated with lauryl bromide to give the corresponding 1-hydroxy 2-alkyne **15**[20] in 70% yield. Treatment of this acetylenic alcohol with 6.5 equiv of potassium 3-aminopropylamide (KAPA) in 3-aminopropylamine in the acetylenic zipper reaction[21] gave the required terminal alkyne **16** in 80% yield and the resulting primary alcohol was then protected as a TBDPS ether to give **11** in 83% yield. The terminal alkyne **11** was then reacted with both enantiomers of epoxide **10** and the resulting secondary alcohols were used to synthesize the required pair of enantiomeric side chains following similar procedures to those developed previously for the synthesis of the 15-

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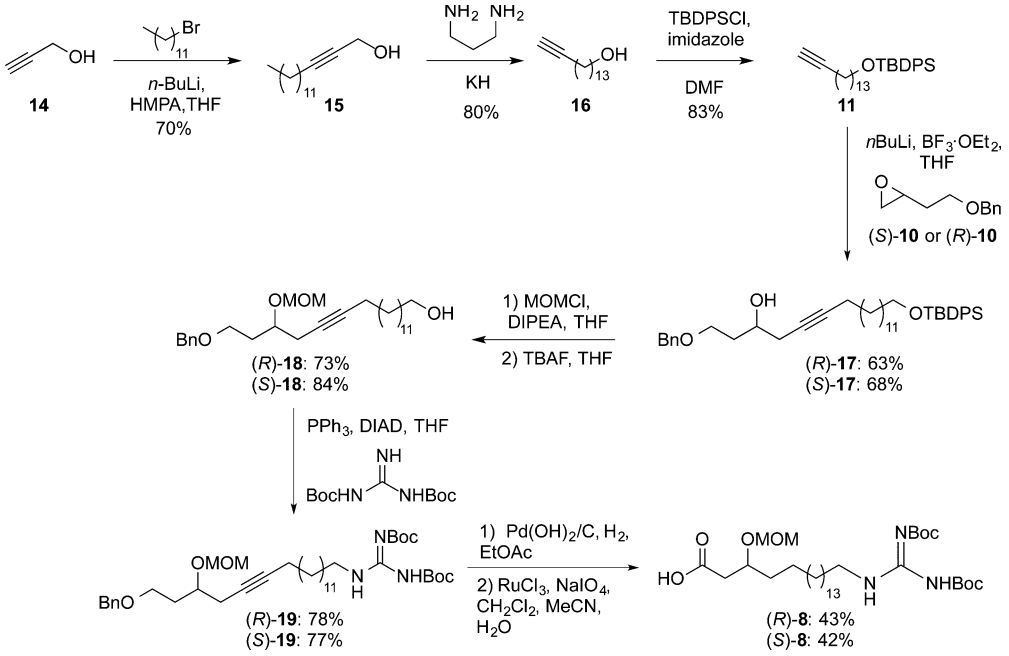
Scheme 3. Synthesis of Both Enantiomers of the GHND Side Chain 8

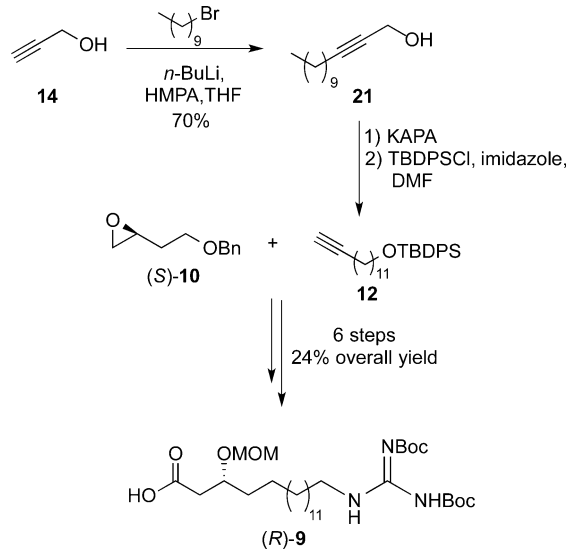


guanidino-3-hydroxypentadecanoyl (GHPD) side chain.[15] Briefly, $BF_3{\cdot}OEt_2$ promoted alkylation of terminal alkyne **11** with the chiral epoxide (*S*)-**10** gave the secondary alcohol (*R*)-**17** in 63% yield. The protection of the secondary alcohol as a MOM ether followed by silyl deprotection proceeded smoothly to give the primary alcohol (*R*)-**18** in 73% yield. The primary alcohol was then converted to the protected guanidine (*R*)-**19** in 78% yield upon treatment with di-*tert*-butoxycarbonylguanidine under Mitsonobu conditions.[22] Concomitant reduction of the internal alkyne and hydrogenolysis of the benzyl ether using a palladium hydroxide on charcoal catalyst were followed by oxidation of the resulting primary alcohol using ruthenium tetroxide[23] to give the carboxylic acid (*R*)-**8** in 43% yield over the two steps. The (*S*)-enantiomer was synthesized in a similar manner, commencing with the opposite enantiomer of the chiral epoxide, (*R*)-**10**, to give (*S*)-**8** in 18% overall yield.

Synthesis of the (*R*)-(GHHD) side chain was performed using the same procedures but starting from the reaction of propargyl alcohol **14** with 1-bromodecane to give alkyne **21**, which was subsequently isomerized and protected to give the terminal alkyne **12**.[20] Reaction of **12** with epoxyalcohol (*S*)-**10** and subsequent manipulation as described above for the longer side chain gave [(*R*)-**9**] in 24% yield over the 6 steps (Scheme 4).

In order to complete the total synthesis of both side chain epimers of the revised structure of circulocin δ **4**, the N-terminal Cbz protecting group was removed from the L-Thr residue of the cyclic peptide core **7** by hydrogenolysis with an excess of Pd/C catalyst in THF. This reaction was sluggish with some over-reduced side products evident, as previously observed for deprotection of the core of fusaricidin A,[15] but under these conditions the free amine **22** was obtained in 59% yield after RP-HPLC purification (Scheme 5). Amine **22** was then condensed with the side chain fragment (*R*)-**8** using solution phase peptide coupling conditions employing an excess of the coupling reagent HATU and Hünig's base in DMF, followed by global removal of the acid labile protecting groups with 90:5:5 TFA:CH_2Cl_2:H_2O to give (*R*)-**4** in 24%

Scheme 4. Synthesis of (*R*)-9



yield over the two steps after RP-HPLC purification (Scheme 5). The side chain (*S*)-epimer [(*S*)-**4**] was prepared in a similar manner from **7**, but without purification of the intermediates. Using this method (*S*)-**4** was obtained in 3.2% yield over the three steps from **7** (Scheme 5). This yield was significantly lower than when the product was isolated after each step (14%), predominantly because purification of the final product was difficult. However, sufficient material to allow the side chain absolute stereochemistry to be established was obtained. Similarly, **22** was also condensed with the side chain fragment (*R*)-**9**, followed by global protecting group removal to give (*R*)-**6** in 22% overall yield.

The experimentally obtained ^{1}H NMR and ^{13}C NMR spectra of both compounds (*R*)-**4** and (*R*)-**6** were very similar to each other and to those reported for both circulocins δ and γ by He et al.,[17] suggesting that the original error in the determination of the structures of circulocins δ and γ was not in the

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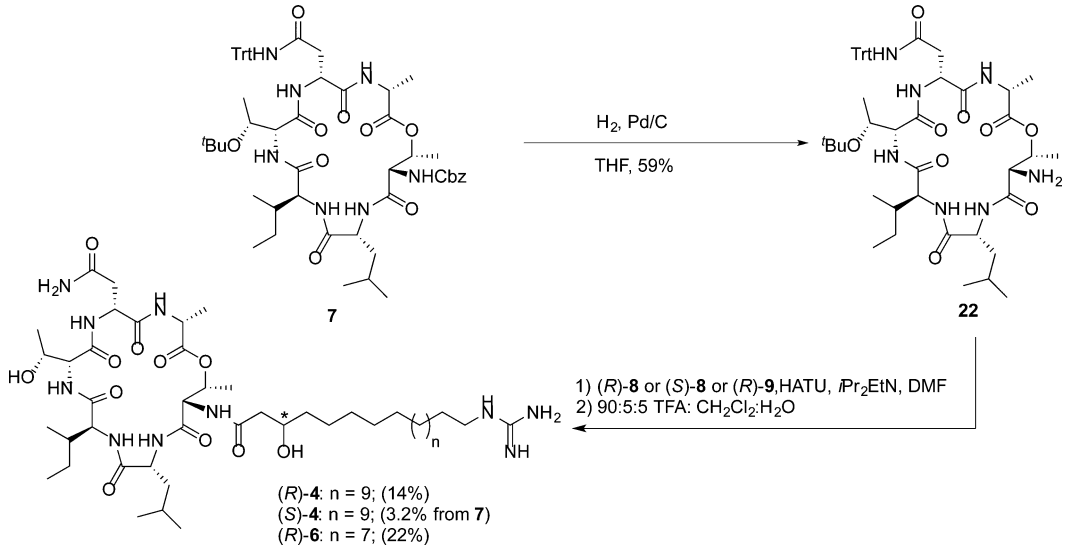
Scheme 5. Synthesis of Final Compounds



assignment of the peptide core, as if this was the case the spectra of the synthesized compounds would be significantly different to that reported for the natural products. Compounds (*R*)-**4** and (*S*)-**4** were synthesized with a 19-guanidino-3-hydroxy-nonadecanoic acid side chain, originally assigned to circulocin γ. The fact that the molecular formulas for these compounds, as determined by HRMS in the original paper (Table 1), were the same as that for the proposed structure of

Table 1. HRMS Data for Originally Reported[17] and Synthetic Material

	originally reported $[M + H]^+$	synthetic material $[M + H]^+$
circulocin γ	939.6243	939.6240
circulocin δ	967.6556	967.6572

circulocin δ together with a comparison of all data for our synthetic **4** with that reported by He et al. (see below) indicate that in the original report the length of the alkyl chains of circulocin δ and circulocin γ were mis-assigned. We therefore propose that circulocin γ actually has a C17 (GHHD) side chain and circulocin δ a C19 (GHND) side chain.

To confirm the revised structure of circulocin δ, the physical and spectroscopic data for (*R*)-**4** and (*S*)-**4** were compared to the data reported for the natural product (see Supporting Information for tables showing comparative data). The ^{13}C NMR spectra of the synthetic compounds were very similar and a close to match to the data reported in the literature for the natural product (Table S2, Supporting Information). However, close inspection indicated that while the ^{13}C NMR spectrum of (*R*)-**4** had a maximum $\Delta\delta$ of 0.3 ppm compared to the data for the natural product, that of (*S*)-**4** had larger discrepancies with the greatest difference being observed for the carbon at the stereogenic center of the side chain ($\Delta\delta$ of 0.9 ppm). As was the case with fusaricidin A,[15] the ^1H NMR spectrum of the (*R*)-isomer was almost identical to the data for the natural product, with a maximum $\Delta\delta$ of 0.02 ppm, while the ^1H NMR spectrum of the (*S*)-isomer was slightly inconsistent with that of the natural product, with a maximum $\Delta\delta$ of 0.08 ppm (Table S1, Supporting Information). The most obvious differences between the two spectra are in the chemical shifts of the diastereotopic protons attributable to the prochiral carbon C2 (Figure 2), together with the coupling constants between the proton at C3 and the diastereotopic protons on C2. In (*R*)-**4** the C2 protons are observed as two doublets of doublets at 2.45 and 2.32 ppm, respectively. This is identical to the reported chemical shifts for these protons in the natural product. For (*S*)-**4** the first of these doublets of doublets was observed at 2.43 ppm, but the second was observed at 2.24 ppm, 0.06 ppm upfield of the reported chemical shift. There was also a difference in the coupling constants between the proton at C3 and the diastereotopic protons on C2. In (*R*)-**4** the coupling constants between these protons are 5.4 and 6.4 Hz. This is very similar to the coupling constants reported for the natural product (4.9 and 6.4 Hz for these protons). In the case of (*S*)-**4** the coupling constants are 9.0 and 3.7 Hz respectively, which is

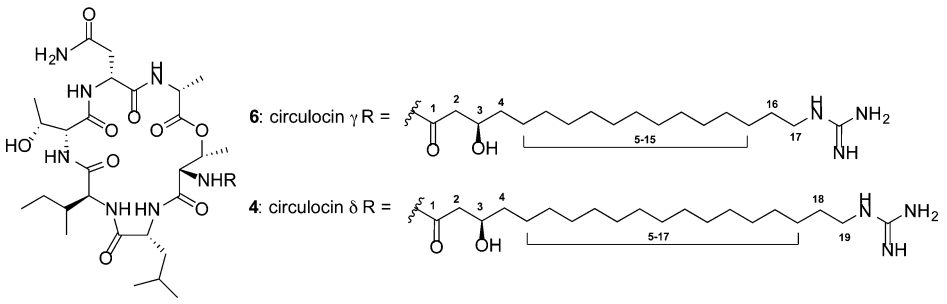


Figure 2. Revised structures of circulocin γ and δ with side chain carbons numbered.

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significantly different to the data reported for the natural product. There was also a difference in the observed chemical shift of the diastereotopic protons on C4 between (*R*)-**4** and (*S*)-**4**. In (*R*)-**4** this multiplet is observed at 1.37 ppm, in the natural product this resonance is reported at 1.36 ppm, while in (*S*)-**4** it was observed 0.08 ppm downfield at 1.44 ppm. Finally, comparison of the optical rotations of the synthetic material with the (*R*)-configured side chain (+10°) and that of the (*S*)-configured side chain (+2°) with those reported for the natural product (+12.9°)[17] indicated that the natural product is most likely to bear a side chain with (*R*)-stereochemistry at C3.

Given the similarities between the structures of circulocin γ and δ, and the trend for lipodepsipeptides bearing a C3 hydroxyl group to have the *R*-configuration at this stereocenter,[11] only a single isomer of our revised structure for circulocin γ was prepared, in which the C3 hydroxy group was *R*-configured. The HRMS data for the synthetic material was found to be a close match to that originally reported for the natural product (Table 1) confirming the molecular formula of this compound as $C_{45}H_{83}N_{10}O_{11}$. The optical rotation of the synthetic (*R*)-**6** (+8.2, *c* 0.08, MeOH) is close to that reported for the isolated natural product (+12.3, *c* 0.31, MeOH). The ^{1}H NMR and ^{13}C NMR data obtained for the synthetic (*R*)-**6** also match well with the data reported for the natural product, with a maximum $\Delta\delta$ of 0.03 ppm in the ^{1}H NMR data. Similarly there is a maximum deviation of only 0.2 ppm in the ^{13}C NMR spectrum. The coupling constants of the critical C2 protons on the alkyl chain of the synthetic material are also a good match to those of the natural product (4.9 compared to 5.0 Hz and 6.2 compared to 6.5 Hz). Analysis of the data indicates that for both compounds **4** and **6**, the C3 *R*-isomer corresponds to the natural product. The fact that in both the circulocin and fusaricidin compounds the C3 alcohol is (*R*)-configured indicates a common biosynthetic pathway and that the other closely related peptides that have been isolated are most likely to also have the (*R*)-configuration.

CONCLUSIONS

In summary, we have completed the first reported total syntheses of circulocin δ **4** and its C3 epimer (*S*)-**4**, together with the (*R*)-isomer of circulocin γ **6**. The total syntheses of **4** and **6** have enabled us to revise the structures of these compounds. We have found that both circulocin γ and circulocin δ have a different length alkyl chain than was originally reported by He et al.[12] We argue that, based on our data together with that reported originally, circulocin δ has a C19 alkyl chain, rather than the C21 chain reported. This was confirmed by comparison of the reported HRMS, ^{1}H and ^{13}C NMR data for circulocin δ with that of the synthesized compounds (*R*)-**4** and (*S*)-**4**. Similarly based upon the available experimental information we propose that circulocin γ **6** has a C17 alkyl chain, rather than the C19 chain reported. In addition, the synthesis of both side chain epimers of the revised structure of circulocin δ has also allowed the complete stereochemical assignment of these natural products, confirming the absolute configuration of the stereogenic center at C3 as (*R*). For both fusaricidin A and circulocin δ, subtle yet significant differences can be observed in the ^{1}H NMR and ^{13}C NMR spectra of the (*R*)- and the (*S*)-isomers. These may be useful in the future for assigning the stereochemistry in related peptides and similar systems, for example heptadepsin, a cyclic depsipeptide that was recently isolated from *Paenibacillus* sp. BML771−113F9 by Umezawa and co-workers.[24]

EXPERIMENTAL SECTION

General Experimental Methods. Materials, solvents, instrumentation, and general methods were essentially as described in previous publications from our laboratory.[15,16] ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded at a frequency of 300 or 400 MHz. ^{13}C Nuclear magnetic resonance spectra were recorded at a frequency of 100 or 150 MHz. Low resolution mass spectra (MS) were recorded on an ion trap mass spectrometer (ESI). High resolution mass spectra (HRMS) were recorded on a 7.0 T Fourier transform ion cyclotron resonance mass spectrometer (FTICR) with an analytical electrospray source. Infrared spectra (IR) were obtained with an FT-IR spectrometer with ATR unit. Flash column chromatography was carried out using Kieselgel 60 silica gel (SiO_2, 0.04−0.065 μm) with the indicated solvents. Analytical, preparative and semipreparative reverse phase HPLC (RP-HPLC) was performed using a multisolvent delivery system and pump with a 2998 photodiode array detector or a programmable wavelength detector operating at 254 and 214 nm. Analytical HPLC employed a Sunfire C18 column (2.1 × 150 mm column, 5 μm particle size, flow rate of 0.8 mL min^{-1}). Preparative RP-HPLC employed a Sunfire Prep C18 OBD column (19 × 150 mm, 5 μm particle size, flow rate 7 mL min^{-1}). Semipreparative RP-HPLC employed a Sunfire C18 column (10 × 250 mm, 5 μm particle size, flow rate 4 mL min^{-1}). The mobile phase consisted of eluents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile). For all HPLC runs a gradient of 30−100% B over 40 min was used, unless otherwise stated. Most reagents were commercially available reagent grade chemicals and were used without further purification. CH_2Cl_2, methanol and triethylamine were distilled from calcium hydride, THF was distilled from sodium and benzophenone before use. DMF was obtained as peptide synthesis grade and stored over 4 Å molecular sieves. All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. Compounds **12**, **15**, **16** and **21** were prepared using similar methods to those reported by Breit et al.[20]

General Procedure 1. *Resin Loading.* 2-Chlorotrityl chloride resin (0.50 g, 1.4 mmol/g) was swollen with CH_2Cl_2 (5 mL) for 30 min. To this was added a solution of Fmoc-amino acid (0.88 mmol) in 4:1 CH_2Cl_2:DMF (5 mL). The mixture was treated with diisopropylethylamine (0.67 mL, 3.5 mmol) and left to stir overnight. The resin was then collected by filtration and washed with CH_2Cl_2 (3 × 20 mL), 17:2:1 CH_2Cl_2:methanol:diisopropylethylamine (3 × 20 mL) followed by CH_2Cl_2 (5 × 20 mL). The resin was then dried under reduced pressure. The loading was determined by deprotection of the resin with 10% piperidine in DMF (2 × 5 mL for 3 min) and then measuring the absorbance of the piperidine-fulvene adduct at λ = 301 nm.

General Procedure 2. *Iterative Peptide Assembly.* The desired peptide was synthesized by coupling amino acids manually in polypropylene syringes with sintered discs (Torviq). The following sequence of steps was used: deprotection, washing, coupling, and washing. *Deprotection*: The resin was treated with 10% piperidine in DMF (2 × 5 mL for 3 min). Resin loading was determined after each coupling step by measuring the absorbance of the piperidine-fulvene adduct at λ = 301 nm. *Washing*: The resin was sequentially washed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL) and DMF (5 × 5 mL). *Coupling:* One of two coupling methods was employed. *Method (A):* A solution of the Fmoc-amino acid (4 equiv), PyBOP (4 equiv) and diisopropylethylamine (8 equiv) was dissolved in DMF (1 mL) and added to the resin. The resin was then shaken on an orbital shaker (175 rpm) for 2 h. For the amino acids Fmoc-D-Leu-OH, Fmoc-L-Ile-OH and Cbz-L-Thr-OH a double coupling method was performed where the solution was discarded and a further preactivated solution was added prior to the washing step. *Method (B):* Fmoc-D-*allo*-Thr(*tert*-butyl)-OH was coupled by adding preactivated acid (1.5 equiv), PyBOP (2 equiv) and diisopropylethylamine (2 equiv) to the resin and then agitating on an orbital shaker (175 rpm) for 16 h.

General Procedure 3. *Epoxide Coupling.* To a cooled (−78 °C) solution of alkyne (1.5 equiv) dissolved in dry THF (10 mL), was added *n*-BuLi (2.0 M in hexanes; 1.5 equiv; 7.0 mmol) under an atmosphere of argon. This solution was stirred for 0.5 h, before boron

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trifluoride diethyl etherate complex (1.5 equiv) was added. The epoxide (1 equiv) dissolved in dry THF (10 mL), was cooled to −78 °C and then added dropwise to the alkyne solution. This mixture was stirred for 2 h at −78 °C before it was warmed to room temperature and quenched with satd. NH_4Cl (50 mL). This solution was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), and evaporated. Chromatography on silica gel (5:1 hexanes: Et_2O), afforded the alcohol as a colorless oil.

General Procedure 4. *MOM Protection.* The alcohol (1 equiv) was dissolved in THF (10 mL) and diisopropylethylamine (20 equiv) was added slowly under an atmosphere of argon. The solution was then cooled to 0 °C and chloromethyl methyl ether (10 equiv) was added dropwise. The solution was stirred for 16 h. The reaction mixture was then quenched with water (100 mL) and extracted with Et_2O (3 × 100 mL). The combined organic extracts were then washed with brine (100 mL), dried (Na_2SO_4) and the solvent evaporated. Chromatography on silica gel (4:1 hexanes:EtOAc), afforded the protected alcohol as a colorless oil.

General Procedure 5. *Silyl Deprotection.* The protected alcohol (1 equiv) was dissolved in dry THF (25 mL). To this solution was added tetrabutylammonium fluoride (1 M solution in THF, 2 equiv). This solution was stirred for 16 h, before being concentrated under reduced pressure. Chromatography on silica gel (2:1 hexanes:Et_2O), afforded the deprotected alcohol, as a colorless oil.

General Procedure 6. *Guanidinylation.* The alcohol (1 equiv) was dissolved in dry THF (10 mL) and added to a solution of 1,3-bis(*tert*-butoxycarbonyl)guanidine (2 equiv) and triphenylphosphine (2 equiv) in dry THF (10 mL) under an atmosphere of argon. This solution was then cooled to 0 °C and diisopropyl azodicarboxylate (2 equiv) was added. The solution was then stirred at room temperature for 16 h. The solvent was removed under reduced pressure and the residue subjected to flash chromatography (4:1 hexanes:EtOAc) to give the product as a colorless oil.

General Procedure 7. *Hydrogenolysis.* The alkyne (1 equiv) was dissolved in EtOAc (10 mL) and 10% $Pd(OH)_2/C$ (10% w/w; 50 mg) catalyst was added. The reaction flask was evacuated and purged with H_2 three times and the solution was stirred under an atmosphere of H_2 for 16 h. The solution was then filtered through a pad of Celite and the solvent removed under reduced pressure. The residue was purified by flash chromatography (4:1 hexanes:EtOAc). This yielded the desired alcohol as a colorless oil.

General Procedure 8. *Oxidation.* Sodium periodate (2.5 equiv) was added to a solution of the alcohol (1 equiv) in dichloromethane (2.0 mL), acetonitrile (2.0 mL) and water (3.2 mL). After 5 min ruthenium trichloride (10 mol %) was added and the mixture stirred for 2 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel (9:1 $CHCl_3$:MeOH) to afford the acid as a colorless oil.

Cbz-L-Thr-D-Leu-L-Ile-Val-D-*allo*-Thr(*tert*-butyl)-D-Asn(Trt)-D-Ala-OH (13). The hexapeptide **13** was synthesized following the general procedures for solid phase peptide synthesis (procedures 1 and 2). The peptide was cleaved from the resin by agitating with (4:1 v/v CH_2Cl_2:1,1,1,3,3,3-hexafluoro-2-propanol) (3 × 5 mL) for 20 min. The solvent was removed under reduced pressure and the residue azeotropically distilled with toluene (3 × 30 mL). The residue was then purified by flash chromatography (4:1 chloroform:methanol). This afforded **13** as an amorphous colorless solid (0.18 g, 82%, based on 0.23 mmol scale); mp 167−170 °C (decomp.); $[\alpha]^{20}_D$ +21.7 (*c* 0.41, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.63 (1H, s), 8.23 (1H, d, *J* = 8.3 Hz), 7.95 (1H, d, *J* = 6.5 Hz), 7.37−7.19 (20H, m), 5.13 (1H, d, *J* = 12.5 Hz), 5.09 (1H, d, *J* = 12.5 Hz), 4.66 (1H, dd, *J* = 8.6, 5.1 Hz), 4.54 (1H, dd, *J* = 8.3, 4.5 Hz), 4.44 (1H, m), 4.33 (1H, q, *J* = 7.3 Hz), 4.24 (1H, dq, *J* = 6.6, 4.5 Hz), 4.18−4.07 (3H, m), 2.92 (1H, dd, *J* = 15.7, 5.1 Hz), 2.85 (1H, dd, *J* = 15.7, 8.3 Hz), 1.88 (1H, m), 1.60 (5H, m), 1.36 (3H, d, *J* = 7.3 Hz), 1.19 (3H, d, *J* = 6.6 Hz), 1.17 (9H, m), 1.03 (3H, d, *J* = 6.6 Hz), 0.95 (3H, d, *J* = 6.9 Hz), 0.92−0.85 (9H, m), (6 NH and OH protons not observed); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5, 175.0, 174.9, 173.8, 172.5, 172.1, 171.7, 158.5, 145.9, 138.1, 130.1, 129.5, 129.1, 128.9, 128.7, 127.8, 75.5, 71.8, 68.6, 67.8, 67.3, 62.6, 60.3, 60.2, 53.1, 51.8, 50.0, 41.4, 39.2, 36.9, 28.5, 26.4, 25.9, 23.6, 21.8, 20.1, 18.8, 18.1, 15.8, 11.2, (2 signals obscured or overlapping); MS (ESI) *m*/*z* 1064 [(M + H)$^+$ 100%], HRMS (ESI, MNa^+) Calcd for $C_{58}H_{77}N_7O_{12}Na$, 1086.5522, found 1086.5532; IR (CH_2Cl_2) 3311, 2972, 1713, 1651, 1519 cm^{-1}.

Cyclic Depsipeptide (7). The peptide **13** (0.18 g, 0.16 mmol) was dissolved in dry toluene (40 mL) and added dropwise over 16 h to a solution of triethylamine (33 μL, 0.25 mmol), 2,4,6 trichlorobenzoyl chloride (39 μL, 0.25 mmol) and DMAP (80 mg, 0.66 mmol) in toluene (170 mL, final concentration of acid 0.001 M) under an atmosphere of argon. After the addition, the solution was stirred for 72 h and then the toluene was removed under reduced pressure. The crude product was purified by flash chromatography (9:1 $CHCl_3$:MeOH) to give **7** as a colorless solid (66 mg, 38%); mp 260 °C (decomp.); $[\alpha]^{20}_D$ +15 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (1H, br s), 8.38 (4H, m), 7.68 (1H, br s), 7.35−7.17 (21H, m), 5.24 (1H, dq, *J* = 6.6, 2.0 Hz), 5.10 (1H, d, *J* = 12.7 Hz), 5.04 (1H, d, *J* = 12.7 Hz), 4.70 (1H, m), 4.40 (1H, dd, *J* = 7.6, 7.6), 4.23 (2H, m), 4.18 (1H, dd, *J* = 9.0, 2.0 Hz), 4.11 (1H, dd, *J* = 6.6, 6.6), 3.94 (1H, m), 3.11 (1H, dd, *J* = 15.0, 6.3 Hz), 2.50 (1H, m), 1.78 (3H, m),1.60−1.55 (2H, m), 1.40 (5H, m), 1.15 (1H, d, J = 6.0 Hz), 1.11 (12H, m, H3), 1.05 (1H, d, *J* = 6.1 Hz), 0.84−0.78 (12H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 171.0, 170.4, 170.3, 170.0, 169.6, 168.1, 156.4, 145.2, 137.0, 128.6, 128.3, 128.2, 127.8, 127.4, 126.3, 73.6, 70.2, 69.5, 65.8, 65.8, 59.7, 59.4, 56.4, 50.4, 50.2, 47.8, 42.4, 37.7, 36.9, 28.1, 24.1, 23.9, 22.5, 21.7, 19.0, 17.4, 16.4, 15.4, 11.0;, (2 signals obscured or overlapping); MS (ESI) *m*/*z* 1046 [(M + H)$^+$ 100%], HRMS (ESI, MNa^+) Calcd for $C_{58}H_{75}N_7O_{11}Na$, 1068.5417, found 1068.5420; IR (CH_2Cl_2) 3288, 2973, 1736, 1665, 1635, 1523 cm^{-1}.

1-[(*tert*-Butyldiphenylsilyl)oxy]pentadec-15-yne (11). Pentadec-14-yn-1-ol (**16**) (1.7 g, 7.6 mmol) was dissolved in DMF (20 mL). To this solution was added *tert*-butyldiphenylsilyl chloride (2.3 mL, 9.1 mmol) and imidazole (1.3 g, 19 mmol). The solution was then stirred for 2 h before being quenched with satd. NH_4Cl (100 mL) and extracted with Et_2O (3 × 100 mL). The combined organic fractions were then washed with brine (100 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue chromatographed on silica gel (9:1 hexanes:Et_2O), to give **11** as a colorless oil (1.3 g, 83%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (4H, dd, *J* = 8.0, 1.7 Hz), 7.44−7.36 (6H, m), 3.66 (2H, t, *J* = 6.6 Hz), 2.19 (2H, dt, *J* = 7.1, 2.7 Hz), 1.94 (1H, t, *J* = 2.7 Hz), 1.60−1.50 (6H, m), 1.41−1.26 (16H, m), 1.06 (9 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6, 134.2, 129.5, 127.6, 84.8, 68.0, 64.0, 32.6, 29.6, 29.5, 29.4, 29.1, 28.8, 28.5, 26.9, 25.8, 19.2, 18.4, (3 signals overlapping or obscured); MS (APCI) *m*/*z* 463 [(M + H)$^+$, 100%], 371 (60), 417 (50) ; IR (CH_2Cl_2) 2927, 2855 cm^{-1}.

(*R*)-1-(Benzyloxy)-19-[(*tert*-butyldiphenylsilyl)oxy]nonadec-5-yn-3-ol [(*R*)-17]. Treatment of alkyne **11** (3.1 g, 6.7 mmol), with epoxide (*S*)-**10** (0.80 g, 4.5 mmol) according to general procedure 3 gave the alcohol (*R*)-**17** (1.8 g, 63%) as a colorless oil; $[\alpha]^{20}_D$ −1.7 (*c* 0.39, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (4H, dd, *J* = 8.0, 1.7 Hz), 7.43−7.27 (11H, m), 4.52, (2H, s), 3.92 (1H, m), 3.73−3.62 (2H, m), 3.65 (2H, t, *J* = 6.4), 2.36 (2H, dt, *J* = 6.4, 2.5 Hz), 2.14 (2H, tt, *J* = 7.4, 2.5 Hz), 1.94−1.78 (2H, m), 1.56 (4H, m), 1.52−1.44 (2H, m), 1.34 (2H, m), 1.25 (14H, m), 1.04 (9H, s), (OH not observed); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 135.6, 134.2, 129.5, 128.4, 127.7, 127.7, 127.5, 82.9, 76.1, 73.3, 69.7, 68.6, 64.0, 35.5, 32.6, 29.6(4) (4C), 29.6(0), 29.4, 29.2, 29.0, 28.9, 27.6, 26.9 (3C), 25.8, 19.2, 18.8; MS (ESI) *m*/*z* 664 [(M + Na)$^+$, 100%]; HRMS (ESI, MNa^+) Calcd for $C_{42}H_{60}O_3SiNa$, 663.4204, found 663.4204; IR (CH_2Cl_2) 3410, 2925, 2853 cm^{-1}.

(*S*)-1-(Benzyloxy)-19-[(*tert*-butyldiphenylsilyl)oxy]nonadec-5-yn-3-ol [(*S*)-17]. Treatment of alkyne **11** (2.6 g, 5.7 mmol) with epoxide (*S*)-**10** (0.63 g, 3.5 mmol) according to general procedure 3 afforded the alcohol (*S*)-**17** (1.4 g, 68%) as a colorless oil; $[\alpha]^{20}_D$ +1.18 (*c* 1.0, $CHCl_3$). Other characterization data were identical to those reported for compound (*S*)-**17**.

(*R*)-1-(Benzyloxy)-19-[(*tert*-butyldiphenylsilyl)oxy]-3-(methoxymethoxy)-pentadec-5-yne [(*R*)-23]. Treatment of alcohol

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(*R*)-**17** (1.2 g, 2.1 mmol) according to general procedure 4 gave alcohol (*R*)-**23** (1.1 g, 86%); $[\alpha]^{20}_{D}$ −15 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (4H, dd, *J* = 8.0, 1.7), 7.43−7.27 (11H, m), 4.71 (1H, d, *J* = 6.9 Hz), 4.64 (1H, d, *J* = 6.9 Hz), 4.50 (2H, s), 3.84 (1H, m), 3.66 (2H, t, *J* = 6.6 Hz), 3.67−3.54 (2H, m), 3.37 (3H, s), 2.44 (2H, m), 2.14 (2H, tt, *J* = 7.0, 2.5 Hz), 2.04−1.85 (2H, m), 1.59−1.44 (4H, m), 1.34 (4H, m), 1.25 (14H, s), 1.05 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 135.6, 134.2, 129.5, 128.3, 127.7, 127.7, 127.5, 96.0, 82.3, 76.1, 73.6, 72.9, 66.8, 64.0, 55.5, 34.4, 32.6, 29.7, 29.6(4) (4 C), 29.6(0), 29.4, 29.2, 29.0. 28.9, 26.9 (3 C), 25.8, 19.2, 18.8; MS (ESI) *m*/*z* 708 [(M + Na)$^+$ 100%]; HRMS (ESI, MNa^+) Calcd for $C_{44}H_{64}O_4SiNa$, 707.4466, found 707.4455; IR (CH_2Cl_2) 2927, 2854 cm^{-1}. Anal. Calcd for $C_{44}H_{64}O_4Si$: C, 77.1; H, 9.4. Found: C, 77.4; H, 9.6.

(*R*)-19-(Benzyloxy)-13-(methoxymethoxy)-pentadec-14-yn-1-ol [(*R*)-18]. Treatment of (*R*)-**23** (1.1 g, 1.8 mmol) according to general procedure 5 gave the alcohol (*R*)-**18** (0.68 g, 85%); $[\alpha]^{20}_{D}$ −18.4 (*c* 1.16, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.26 (5H, m), 4.71 (1H, d, *J* = 7.0 Hz), 4.64 (1H, d, *J* = 7.0 Hz), 4.50 (2H, s), 3.83 (1H, m), 3.65−3.57 (4H, m), 3.37 (3H, s), 2.43 (2H, m), 2.13 (2H, tt, *J* = 7.1, 2.5 Hz), 2.04−1.85 (2H, m), 1.60−1.40 (6H, m), 1.40−1.26 (16H, m), (OH not observed), ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 128.3, 127.6, 127.5, 95.9, 82.3, 76.1, 73.6, 72.9, 66.7, 63.1, 55.5, 34.4, 32.8, 29.5(9), 29.5 (7) (3 C), 29.5(2), 29.4, 29.1, 29.0, 28.9, 25.7, 25.1, 18.8; MS (ESI) *m*/*z* 469 [(M + Na)$^+$ 100%], HRMS (ESI, MNa^+) Calcd for $C_{28}H_{46}O_4Na$, 469.3288, found 469.3284; IR (CH_2Cl_2) 3410, 2925, 2853 cm^{-1}.

(*S*)-1-(Benzyloxy)-19-[(*tert*-butyldiphenylsilyl)oxy]-3-(methoxymethoxy)-pentadec-5-yne [(*S*)-23]. Treatment of alcohol (*S*)-**17** (1.2 g, 2.1 mmol) according to general procedure 4 gave alcohol (*S*)-**23** (1.1 g, 86%); $[\alpha]^{20}_{D}$ +11.5 (*c* 1.0, $CHCl_3$). Other characterization data were identical to those reported for compound (*R*)-**23**.

(*S*)-19-(Benzyloxy)-13-(methoxymethoxy)-nonadec-14-yn-1-ol [(*S*)-18]. Treatment of (*S*)-**23** (0.88 g, 1.3 mmol) according to general procedure 5 gave (*S*)-**18** (0.60 g, 98%); $[\alpha]^{20}_{D}$ +15.0 (*c* 1.4, $CHCl_3$). Other characterization data were identical to those reported for compound (*R*)-**18**.

(*R*)-1-(Benzyloxy)-19-{[*N*,*N*′-bis(*tert*-butoxycarbonyl)]-guanidino}-3-(methoxymethoxy)-nonadec-5-yne [(*R*)-19]. Treatment of alcohol (*R*)-**18** (0.82 g, 1.84 mmol) according to general procedure 6 gave (*R*)-**19** (0.98 g, 78%); $[\alpha]^{20}_{D}$ −13.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (2H, s br), 7.34−7.26 (5H), 4.71 (1H, d, *J* = 6.6 Hz), 4.64 (1H, d, *J* = 6.6 Hz), 4.50 (2H, s br), 3.90−3.80 (3H, m), 3.64−3.55 (2H, m), 3.37 (3H, s), 2.43 (2H, dt, *J* = 6.0, 2.5 Hz), 2.13 (2H, tt, *J* = 7.0, 2.2 Hz), 2.03−1.84 (2H, m), 1.58−1.42 (22H, m), 1.36−1.25 (18H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 160.8, 155.2, 138.4, 128.3, 127.7, 127.5, 95.9, 83.4, 82.3, 78.6, 76.1, 73.5, 72.9, 66.7, 55.5, 44.7, 34.3, 29.7 (4 C), 29.6, 29.3, 29.2, 29.0, 28.9, 28.8, 28.3 (3 C), 28.0 (3 C), 26.7, 25.1, 18.8; MS (ESI) *m*/*z* 711 [(M + Na)$^+$ 100%], 688 (50); HRMS (ESI, MH$^+$) Calcd for $C_{39}H_{66}N_3O_7$, 688.4895, found 688.4886; IR ($CHCl_3$) 3393, 2923, 2858, 1713, 1645, 1611, 1511 cm^{-1}. Anal. Calcd for $C_{39}H_{65}N_3O_7$: C, 68.1; H, 9.5, N, 6.1. Found: C, 68.1; H, 9.7, N, 6.1.

(*S*)-1-(Benzyloxy)-19-{[*N*,*N*′-bis(*tert*-butoxycarbonyl)]-guanidino}-3-(methoxymethoxy)-nonadec-5-yne [(*S*)-19]. Treatment of alcohol (*S*)-**18** (0.60 g, 1.3 mmol) according to general procedure 6 gave (*S*)-**19** (0.69 g, 77%); $[\alpha]^{20}_{D}$ +11.0 (*c* 0.42, $CHCl_3$). Other characterization data were identical to those reported for compound (*R*)-**19**.

(*R*)-19-{[*N*,*N*′-Bis(*tert*-butoxycarbonyl)]guanidino}3-(methoxymethoxy)-nonadecan-1-ol [(*R*)-24]. Treatment of alkyne (*R*)-**19** (0.93 g, 1.2 mmol) according to general procedure 7 gave the desired alcohol (*R*)-**24** (0.52 g, 71%); $[\alpha]^{20}_{D}$ −24.0 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.30 (2H, s br), 4.67 (1H, d, *J* = 6.9 Hz), 4.63 (1H, d, *J* = 6.9 Hz), 3.86 (2H, m, CH_2), 3.81−3.66 (3H, m), 3.38 (3H, s), 2.52 (1H, s br), 1.83−1.62 (2H, m), 1.56−1.51 (4H, m), 1.50 (9H, s), 1.47 (9H, s), 1.27−1.23 (26H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 160.7, 155.1, 95.8, 83.3, 78.6, 76.4, 59.8, 55.7, 44.6, 36.6, 34.6, 29.8, 29.7 (4 C), 29.6 (4 C), 29.5, 29.2, 28.7, 28.3 (3 C), 28.0 (3 C), 26.6, 25.2; MS (ESI) *m*/*z* 602 [(M + H)$^+$ 100%], 502 (50); HRMS (ESI, MH$^+$) Calcd for $C_{32}H_{64}N_3O_7$, 602.4739, found 602.4731; IR (CH_2Cl_2) 3386, 2926, 2855, 1713, 1641, 1610, 1510 cm^{-1}.

(*S*)-19-{[*N*,*N*′-Bis(*tert*-butoxycarbonyl)]guanidino}3-(methoxymethoxy)-nonadecan-1-ol [(*S*)-24]. Treatment of alkyne (*S*)-**19** (0.69 g, 1.0 mmol) according to general procedure 7 gave the desired alcohol (*S*)-**24** (0.51 g, 85%); $[\alpha]^{20}_{D}$ +15.0 (*c* 0.40, $CHCl_3$). Other characterization data were identical to those reported for compound (*R*)-**24**.

(*R*)-19-{[*N*,*N*′-Bis(*tert*-butoxycarbonyl)]guanidino}3-(methoxymethylenoxy)-nonadecanoic acid [(*R*)-8]. Treatment of the alcohol (*R*)-**24** (0.24 g, 0.4 mmol) according to general procedure 8 gave the acid (*R*)-**8** (0.15 g, 60%); $[\alpha]^{20}_{D}$ −2.6 (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (3H, s br), 4.66 (2H, dd, *J* = 13.7, 7.0 Hz), 3.97 (1H, m), 3.86 (2H, t, *J* = 7.3 Hz), 3.35 (3H, s), 2.58 (1H, dd, *J* = 15.7, 7.1 Hz), 2.50 (1H, dd, *J* = 15.7, 5.4 Hz), 1.66−1.52 (4H, m), 1.50 (9H, s), 1.47 (9H, s), 1.28−1.24 (26H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5, 163.6, 160.5, 155.2, 95.9, 83.4, 78.7, 74.5, 55.5, 44.7, 40.0, 34.7, 29.6(1), 29.6(0), 29.5(3) (7 C), 29.5(1), 29.2, 28.7, 28.3 (3 C), 28.0 (3 C), 26.6, 25.1; MS (ESI) *m*/*z* 616 [(M + H)$^+$ 100%]; HRMS (ESI, MH$^+$) Calcd for $C_{32}H_{62}N_3O_8$, 616.4531, found 616.4534; IR (CH_2Cl_2) 3379, 2978, 2925, 2854, 1712, 1639, 1610, 1510 cm^{-1}.

(*S*)-19-{[*N*,*N*′-Bis(*tert*-butoxycarbonyl)]guanidino}3-(methoxymethylenoxy)-nonadecanoic acid [(*S*)-8]. Treatment of the alcohol (*S*)-**24** (0.50 g, 0.90 mmol) according to general procedure 8 gave the acid (*S*)-**8** (0.30 g, 54%); $[\alpha]^{20}_{D}$ +1.5 (*c* 0.40, $CHCl_3$). Other characterization data were identical to those reported for compound (*R*)-**8**.

Circulocin *δ* [(*R*)-4]. Cyclic peptide **7** (30 mg, 29 μmol) was dissolved in 10 mL of dry THF and Pd/C catalyst (10% w/w, 0.1 g) was added. The reaction flask was evacuated and purged with H_2 three times and the solution was left stirring under an atmosphere of H_2 for 24 h. More Pd/C catalyst (10% w/w, 50 mg) was then added and the solution was left stirring under an atmosphere of H_2 for another 24 h. The solution was then filtered through a pad of Celite and the solvent removed under reduced pressure. The residue was purified by preparative RP-HPLC using the standard conditions. The amine **22** was isolated after lyophilization as an amorphous white solid (15 mg, 59%). MS (ESI) *m*/*z* 912 [(M + H)$^+$ 100%], HRMS (ESI, MNa^+) Calcd For $C_{50}H_{69}N_7O_9Na$, 934.5049, found 934.5064.

A solution of the acid (*R*)-**8** (18.2 mg, 30 μmol), HATU (13.5 mg, 36 μmol) and diisopropylethylamine (10 μL, 59 μmol) in DMF (0.5 mL) was added to a solution of amine **22** (9 mg, 10 μmol) in DMF (0.5 mL). The mixture was shaken on an orbital shaker (175 rpm) for 16 h. The crude reaction mixture was then purified by RP-HPLC using the standard conditions. The protected compound **30** was then treated with a solution of TFA:H_2O:CH_2Cl_2 (90:5:5) for 3 h. The solvent was removed under reduced pressure and the residue azeotropically distilled with toluene (3 × 30 mL). The residue was then purified by semipreparative RP-HPLC (0−50% B over 40 min). This gave [(*R*)-**4**] after lyophilization as a colorless solid (2.3 mg, 24%); mp 217−220 °C (decomp.); $[\alpha]^{20}_{D}$ +10 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38 (1H, br m), 8.37 (1H, br m), 8.09 (1H, d, *J* = 7.0 Hz), 8.06 (1H, d, *J* = 8.5 Hz), 7.46 (1H, br m), 7.45 (1H, d, *J* = 8.0 Hz), 7.42 (1H, br s), 7.35 (1H, d, *J* = 7.3 Hz), 7.30−6.80 (3H, br m), 7.00 (1H, br s), 5.30 (1H, dq, *J* = 6.5, 2.5 Hz), 5.02 (1H, d, *J* = 5.0 Hz), 4.93 (1H, d, *J* = 4.0 Hz), 4.66 (1H, dd, *J* = 7.7, 7.8 Hz), 4.39 (1H, dd, *J* = 8.5, 2.2 Hz), 4.28 (1H, m), 4.20 (1H, m), 3.97 (1H, m), 3.92 (2H, m), 3.81 (1H, m), 3.07 (1H, dd, *J* = 7.0, 7.0 Hz), 3.05 (1H, dd, *J* = 7.0, 7.0 Hz), 2.78 (1H, dd, *J* = 15, 7.3 Hz), 2.59 (1H, dd, *J* = 15, 6.2), 2.45 (1H, dd, *J* = 13.2, 5.4), 2.32 (1H, dd, *J* = 13.2, 6.4 Hz), 1.77 (1H, m), 1.43 (1H, m), 1.42 (2H, m), 1.40 (1H, m), 1. 39 (2H, m), 1.37 (2H, m), 1.25 (26H, m), 1.18 (4H, m), 1.12 (3H, m), 1.10 (3H, m), 0.84 (6H, d, *J* = 6.5 Hz), 0.81 (3H, d, *J* = 7.0 Hz), 0.79 (3H, t, *J* = 7.3 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.7, 172.4, 171.5, 171.4, 170.6, 170.2, 169.7, 167.9, 156.5, 70.3, 67.5, 65.4, 60.1, 56.6, 56.6, 50.3, 49.9, 47.6, 42.7, 42.3, 40.5, 36.4, 36.4, 35.8, 28.9, 28.5, 28.3, 25.9, 25.4, 24.0, 23.9, 22.6, 22.2, 19.3, 17.0, 16.0, 15.3, 10.6; MS (ESI) *m*/*z* 968 [(M +

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H)$^+$ 100%], HRMS (ESI, MH$^+$) Calcd for $C_{47}H_{87}N_{10}O_{11}$, 967.6550, found 967.6572.

***epi*-Circulocin δ [(*S*)-4].** Cyclic peptide **7** (44 mg, 42 μmol) was dissolved in 20 mL of dry THF and Pd/C catalyst (10% w/w, 0.10 g) was added. The reaction flask was evacuated and purged with H_2 three times and the solution was left stirring under an atmosphere of H_2 for 24 h. More Pd/C catalyst (10% w/w, 50 mg) was then added and the solution was left stirring under an atmosphere of H_2 for another 24 h. The solution was then filtered through a pad of Celite and the solvent removed under reduced pressure. The amine was then used without further purification

A solution of the acid (*S*)-**8** (34 mg, 55 μmol), HATU (25 mg, 66 μmol) and diisopropylethylamine (20 μL, 0.11 mmol) in DMF (0.5 mL) was added to a solution of amine **22** (16.8 mg, 18.4 μmol) in DMF (0.5 mL). The mixture was shaken on an orbital shaker (175 rpm) for 16 h. The crude reaction mixture was then treated with a solution of TFA:H_2O:CH_2Cl_2 (90:5:5) for 3 h. The solvent was removed under reduced pressure and the residue azeotropically distilled with toluene (3 × 30 mL). The residue was then purified by semipreparative RP-HPLC (0−50% B over 40 min). This gave the (*S*)-isomer of circulocin δ (*S*)-**4** after lyophilization as a colorless solid (1.3 mg, 3.2%); mp 216−219 °C (decomp.); $[\alpha]^{20}_D$ +2.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 (1H, d, *J* = 7.4 Hz), 8.39 (1H, d, *J* = 4.2 Hz), 8.07 (1H, d, *J* = 8.3 Hz), 8.02 (1H, d, *J* = 7.0 Hz), 7.47 (2H, br m), 7.45 (1H, br s), 7.30−6.80 (3H, br s), 7.28 (1H, d, *J* = 8.2), 7.00 (1H, br s), 5.29 (1H, dq, *J* = 2.0, 6.7 Hz), 4.93 (1H, br s), 4.81 (1H, d, *J* = 4.7 Hz), 4.70 (1H, m), 4.36 (1H, dd, *J* = 8.2, 2.0 Hz), 4.30 (1H, m), 4.13 (1H, t, *J* = 7.0 Hz), 3.95 (2H, m), 3.91 (1H, m), 3.79 (1H, m), 3.10 (1H, d, *J* = 6.6 Hz), 3.05 (1H, d, *J* = 6.6 Hz), 2.85 (1H, dd, *J* = 15.0, 6.6 Hz), 2.57 (1H, dd, *J* = 15.0, 7.2 Hz), 2.43 (1H, dd, *J* = 13.1, 9.0 Hz), 2.24 (1H, dd, *J* = 13.1, 3.7 Hz), 1.75 (1H, m), 1.44 (4H, m), 1.24 (30H, m), 1.19 (4H, d, *J* = 7.0 Hz), 1.17 (3H, d, *J* = 6.6 Hz), 1.09 (3H, d, *J* = 5.5 Hz), 0.87−0.78 (12H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 172.3, 172.2, 171.1, 170.4, 170.6, 169.7, 168.1, 156.6, 70.1, 68.4, 65.4, 59.7, 56.9, 56.8, 50.3, 49.7, 47.7, 43.0, 42.2, 40.5, 36.5, 36.4, 35.4, 28.5−29.0 (11 C), 28.2, 25.8, 25.6, 23.7, 23.6, 22.6, 21.9, 18.9, 16.8, 15.8, 15.0, 10.3; MS (ESI) *m*/*z* 968 [(M + H)$^+$ 100%], HRMS (ESI, MNH$^+$) Calcd for $C_{47}H_{87}N_{10}O_{11}$, 967.6550, found 967.6563.

1-[(*tert*-Butyldiphenylsilyl)oxy]tridec-12-yne (12). Tridec-12-yn-1-ol (**21**) (1.6 g, 8.2 mmol) was dissolved in DMF (20 mL). To this solution was added *tert*-butyldiphenylsilyl chloride (2.5 mL, 9.6 mmol) and imidazole (1.4 g, 20 mmol). The solution was then stirred for 2 h before being quenched with satd. NH_4Cl (100 mL) and extracted with Et_2O (3 × 100 mL). The combined organic fractions were then washed with brine (100 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue chromatographed on silica gel (9:1 hexanes:Et_2O), to give **12** as a colorless oil (3.6 g, quant). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, 4H, *J* = 7.5 Hz), 7.46−7.37 (m, 6H), 3.68 (t, 2H, *J* = 6.5 Hz), 2.20 (dt, 2H, *J* = 6.9, 2.5 Hz), 1.95 (t, 1H, *J* = 2.5 Hz), 1.63−1.50 (m, 4H), 1.49−1.33 (m, 4H), 1.29 (s,10H), 1.08 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.7, 134.3, 129.6, 127.7, 84.9, 68.2, 64.2, 32.8, 29.7, 29.6, 29.5, 29.3, 28.9, 28.7, 25.9, 27.0, 19.4, 18.5.

(*R*)-1-(Benzyloxy)-17-[(*tert*-butyldiphenylsilyl)oxy]heptadec-5-yn-3-ol [(*R*)-25]. Treatment of alkyne **12** (0.73 g, 0.17 mmol), with epoxide (*S*)-**10** (0.24 g, 0.11 mmol) according to general procedure 3 gave the alcohol (*R*)-**25** (0.55 g, 80%); $[\alpha]^{20}_D$ −1.0 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (4H, dd, *J* = 6.5, 1.5 Hz), 7.43−7.28 (11H, m), 4.54 (2H, s), 3.94 (1H, m), 3.76−3.73 (1H, m), 3.67 (3H, m, 3H), 2.95 (1H, br s), 2.38 (2H, d, *J* = 4.5 Hz), 2.16 (2H, *J* = 7.0 Hz), 1.92−1.89 (1H, m), 1.87−1.81 (1H, m), 1.57 (2H, m), 1.49 (2H, m), 1.36 (4H, m), 1.26 (10H, m), 1.06 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.1, 135.7, 134.3, 129.6, 128.6, 127.9, 127.8, 127.7, 83.0, 76.3, 73.4, 69.9, 68.8, 64.1, 35.6, 32.7, 29.7(4), 29.7(0), 29.6(7), 29.5, 29.3, 29.2, 29.1, 27.7, 27.0, 25.9, 19.3, 18.9; MS (ESI) *m*/*z* 635 [(M + Na)$^+$ 100%]; HRMS (ESI, MNa$^+$) Calcd for $C_{40}H_{56}O_3NaSi$ 635.3891, found 635.3884; IR (thin film) 3450, 3075, 3050, 2925, 2855, 1470, 1425 cm^{-1}.

(*R*)-1-Benzyloxy-17-(*tert*-butyldiphenylsiloxy)-3-(methoxymethoxy)-heptadec-5-yne [(*R*)-26]. Treatment of alcohol (*R*)-**25** (0.54 g, 0.88 mmol) according to general procedure 4 gave protected alcohol (*R*)-**26** (0.52, 90%): $[\alpha]^{20}_D$ −13 (*c* 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (4H, d, *J* = 6.5 Hz), 7.43−7.28 (11H, m), 4.72 (1H, d, *J* = 6.5 Hz), 4.66 (1H, d, *J* = 6.5 Hz), 4.52 (2H, s), 3.85 (1H, m), 3.67 (2H, t, *J* = 6.5 Hz), 3.64−3.57 (2H, m), 3.38 (3H, s), 2.45 (2H, m), 2.15 (2H, m), 2.07−1.98 (1H, m), 1.95−1.88 (1H, m), 1.58 (1H, m), 1.49 (2H, m), 1.36−1.32 (5H, m), 1.26 (10H, s), 1.07 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6, 135.7, 134.3, 129.6, 128.5, 127.8, 127.8, 127.7, 96.1, 82.4, 76.2, 73.7, 73.1, 66.9, 64.1, 55.6, 34.5, 32.7, 29.74, 29.72, 29.68, 29.5, 29.3, 29.2, 29.1, 27.0, 25.9, 25.3, 19.4, 19.0; MS (APCI) *m*/*z* 674 [(M + NH$_4$)$^+$ 100%]; HRMS (APCI, MNH$_4^+$) calc. for $C_{42}H_{64}NO_4Si$ 674.4599, found 674.4595; IR (thin film) 3060, 2930, 2855, 1465, 1455, 1425 cm^{-1}.

(*R*)-17-(Benzyloxy)-15-(methoxymethoxy)-heptadec-12-yn-1-ol [(*R*)-27]. Treatment of protected alcohol (*R*)-**26** (0.35 g, 0.53 mmol) according to general procedure 5 gave alcohol (*R*)-**27** (0.18 g, 83%); $[\alpha]^{20}_D$ −15 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (4H, m), 7.27 (1H, m), 4.71 (1H, d, *J* = 6.9 Hz), 4.64 (1H, d, *J* = 6.9 Hz), 4.51 (1H, d, *J* = 11.9 Hz), 3.83 (1H, m), 3.62 (2H, t, *J* = 6.6 Hz), 3.61−3.56 (2H, m), 3.36 (3H, s), 2.45−2.41 (2H, m), 2.15−2.11 (2H, m), 2.03−1.95 (1H, m), 1.92−1.85 (1H, m), 1.55 (2H, m), 1.49−1.42 (3H, m), 1.34−1.26 (15H, m).^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6, 128.5, 127.8, 127.7, 96.1, 82.4, 76.2, 73.7, 73.1, 66.9, 63.2, 55.7, 34.5, 32.9, 29.7, 29.6(5), 29.6(3), 29.5, 29.3, 29.1, 29.0, 25.9, 25.3, 18.9; MS (ESI) *m*/*z* 441 [(M + Na)$^+$ 100%]; HRMS (ESI, MNa$^+$) Calcd for $C_{26}H_{42}NaO_4$ 441.2975, found 441.2975; IR (thin film) 3300, 3060, 2925, 2855, 1455 cm^{-1}.

(*R*)-1-(Benzyloxy)-17-{[*N*,*N*′-bis(*tert*-butoxycarbonyl)]-guanidino}-3-(methoxymethoxy)-heptadec-5-yne [(*R*)-28]. Treatment of alcohol (*R*)-**27** (0.17 g, 0.42 mmol) according to general procedure gave (*R*)-**28** (0.22 g, 81%); $[\alpha]^{20}_D$ −13 (*c* 0.15, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (1H, br s), 9.28 (1H, br s), 7.35 (4H, m), 7.28 (1H, m), 4.72 (1H, d, *J* = 6.9 Hz), 4.65 (1H, d, *J* = 6.9 Hz), 4.51 (2H, s), 3.90 (2H, dd, *J* = 7.6, 7.4 Hz), 3.85 (1H, m), 3.65−3.56 (2H, m), 3.38 (3H, s), 2.45 (2H, m), 2.15 (2H, m), 2.05−1.97 (1H, m), 1.94−1.86 (1H, m), 1.59−1.43 (4H, m), 1.53 (9H, s) 1.51 (9H, s), 1.40−1.32 (2H, m), 1.28 (12H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 160.9, 155.3, 138.6, 128.4, 127.7, 127.6, 96.0, 83.5, 82.4, 78.7, 76.2, 73.6, 73.0, 66.8, 55.6, 44.8, 34.5, 29.7, 29.4, 29.3, 29.1, 29.0, 28.9, 28.8, 28.4, 28.1, 26.8, 25.3, 18.9 (1 signal obscured or overlapping); MS (ESI) *m*/*z* 660 [(M + H)$^+$ 100%]; HRMS (ESI, MH$^+$) Calcd for $C_{37}H_{62}N_3O_7$ 660.4582, found 660.4580; IR (thin film) 3380, 3060, 2975, 2925, 2855, 1710, 1640, 1610, 1505, 1455 cm^{-1}.

(*R*)-17-{[*N*,*N*′-Bis(*tert*-butoxycarbonyl)]guanidino}3-(methoxymethoxy)-heptadecan-1-ol [(*R*)-29]. Treatment of alkyne (*R*)-**28** (0.11 g, 0.17 mmol) according to general procedure 7 gave the desired alcohol (*R*)-**29** (0.079 g, 81%); $[\alpha]^{20}_D$ −19 (*c* 3.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (2H, br s), 4.67 (1H, d, *J* = 6.6 Hz), 4.63 (1H, d, *J* = 6.5 Hz), 3.86 (2H, m), 3.77−3.67 (3H, m), 3.38 (3H, s), 2.41 (1H, br s), 1.78 (1H, m), 1.66 (1H, m), 1.59−1.46 (4H, m), 1.49 (9H, s), 1.47 (9H, s), 1.23 (22H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.1, 160.9, 155.3, 96.0, 83.5, 78.8, 76.6, 60.0, 55.8, 44.8, 36.8, 34.7, 29.9 (4 C), 29.8 (3 C), 29.7, 29.4, 28.9, 28.4, 28.1, 26.8, 25.4; MS (ESI) *m*/*z* 596 [(M + Na)$^+$ 100%]; HRMS (ESI, MNa$^+$) Calcd for $C_{30}H_{59}N_3NaO_7$ 596.4245, found 596.4242; IR (thin film) 3380, 2975, 2925, 2855, 1710, 1610, 1510, 1455 cm^{-1}.

(*R*)-17-{[*N*,*N*′-Bis(*tert*-butoxycarbonyl)]guanidino}-3-(methoxymethoxy)-heptadecanoic acid ([(*R*)-9]. Treatment of the alcohol (*R*)-**29** (0.065 g, 0.11 mmol) according to general procedure 8 gave the acid (*R*)-**9** (0.040 g, 60%); $[\alpha]^{20}_D$ −2.4 (*c* 0.43, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (2H, br s), 4.70 (2H, d, *J* = 7.0 Hz), 3.98 (1H, m), 3.86 (2H, dd, *J* = 7.5, 7.5 Hz), 3.37 (3H, s), 3.00 (1H, br s), 2.61−2.51 (2H, m), 1.64−1.10 (4H, m), 1.52 (9H, s), 1.49 (9H, s), 1.25 (22H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.5, 164.1, 160.9, 155.4, 96.1, 83.6, 78.9, 74.8, 55.8, 44.9, 40.1, 34.9, 29.8 (2 C), 29.7 (2 C), 29.7(5), 29.7(4), 29.7(2), 29.6(7), 29.5, 28.9, 28.5, 28.2, 26.9, 25.3; MS (ESI) *m*/*z* 588 [(M + H)$^+$ 100%]; HRMS (ESI, MH$^+$) Calcd

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for $C_{30}H_{58}N_3O_8$ 588.4218, found 588.4219; IR (thin film) 3380, 2975, 2925, 1790, 1710, 1610, 1510, 1455 cm^{-1}.

Circulocin γ [(*R*)-6]. A solution of the acid (*R*)-**9** (9.5 mg, 16 μmol), HATU (7.5 mg, 20 μmol) and diisopropylethylamine (10 μL, 59 μmol) in DMF (0.5 mL) was added to a solution of amine **22** (10 mg, 11 μmol) in DMF (0.5 mL). The mixture was shaken on an orbital shaker (175 rpm) for 16 h. The crude reaction mixture was then purified by column chromatography (CH_2Cl_2/MeOH 99:1) to give the coupled product **31** (12.0 mg, 72%). The protected compound **31** was then treated with a solution of TFA:H_2O:CH_2Cl_2 (90:5:5) for 3 h. The solvent was removed under reduced pressure and the residue azeotropically distilled with toluene (3 × 30 mL). The residue was then purified by semipreparative RP-HPLC (0−50% B over 40 min). This gave [(*R*)-**6**] after lyophilization as a colorless solid (3.5 mg, 31% from **9**). $[\alpha]^{20}_D$ +8.2 (*c* 0.08, MeOH); ^{1}H NMR (500 MHz, DMSO) δ 8.50 (1H, br s), 8.39 (1H, m), 8.13 (1H, m), 8.13−8.08 (1H, br s), 7.49 (1H, d, *J* = 9.2 Hz), 7.47−7.39 (5H, m), 7.02 (1H, s), 5.30 (1H, m), 4.64 (1H, m), 4.41 (1H, d, *J* = 8.3 Hz), 4.31 (1H, m), 4.20 (1H, m), 3.95 (3H, m), 3.81 (1H, m), 3.06 (2H, m), 2.76 (1H, dd, *J* = 14.5, 5.6 Hz), 2.61 (1H, dd, *J* = 14.5, 6.6 Hz), 2.47 (1H, dd, *J* = 13.3, 4.7 Hz), 2.31 (1H, dd, *J* = 13.6, 6.5 Hz), 1.77 (1H, m), 1.49−1.43 (1H, m), 1.47−1.40 (2H, m), 1.40 (1H, m), 1.38 (4H, m), 1.32 (2H, m), 1.23 (22H, s), 1.18−1.12 (1H, m), 1.19 (3H, d, *J* = 7.0 Hz), 1.13 (3H, d, *J* = 6.2 Hz) 1.10 (3H, d, *J* = 5.0 Hz), 0.83−0.81 (12H, m); ^{13}C NMR (HMQC-/HMBC-NMR) (500 MHz, DMSO) δ 172.9, 171.6, 171.5, 170.4, 170.1, 169.5, 167.8, 70.3, 67.5, 65.4, 60.1, 56.7, 56.5, 50.3, 50.1, 47.6, 42.6, 42.1, 40.4, 36.5, 35.6, 28.6, 25.8, 28.3, 24.1, 23.9, 22.3, 19.2, 16.7, 15.8, 15.2, 10.6; the guanidinyl carbon signal could not be observed using the applied parameters; MS (ESI) *m*/*z* 940 [(M + H)$^+$ 100%]; HRMS (ESI, MH$^+$) Calcd for $C_{45}H_{83}N_{10}O_{11}$ 939.6237, found 939.6240 [M + H$^+$]; IR (thin film) 3310, 2930, 2855, 1670, 1635, 1545, 1450 cm^{-1}.

■ ASSOCIATED CONTENT

Supporting Information

Comparison tables listing NMR data for synthesized compounds versus data reported for the isolated natural products; ^{1}H and ^{13}C NMR spectra for all new compounds; HPLC traces for final products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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