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Solid-Phase Synthesis of Macrocyclic Peptides via Side-Chain Anchoring of the Ornithine δ-Amine

Evert Peterse,[a] Nico Meeuwenoord,[a] Hans van den Elst,[a] Gijsbert A. van der Marel,[a] Hermen S. Overkleeft,[a] and Dmitri V. Filippov*[a]

Cyclic peptides represent a popular class of macrocyclic drug candidates and therefore their solid phase synthesis has attracted much attention. In this contribution we present an efficient method of side-chain anchoring for ornithine and lysine residues to be used in the standard Fmoc-based synthesis of cyclic peptides via on-resin cyclization. We demonstrate that the side chain of ornithine and lysine protected with *N*-Boc-group can efficiently be converted to the isocyanate which is then immobilized on Wang-type resin in almost quantitative yield. We further show the synthesis of four biologically active cyclic peptides employing the side chain ornithine anchoring. Our method is at least on a par with the previously reported methodologies in terms of yield and the purity of the final products and is arguably operationally more straightforward.

Introduction

Cyclic peptides encompass a major class of macrocyclic compounds, varying widely in structure, ring size, functional group patterns and biological activities. Cyclic peptides, and also macrocycles in general, have found wide application as antibiotics, ever since the discovery, in the 1940's, of gramicidin S and tyrothricin. Tyrothricin is a mixture of peptides first isolated from the species *Aneurinibacillus migulanus* (formerly known as *Bacillus brevis*) and contains the cyclic decapeptide tyrocidine. In an effort to isolate tyrothricin from *B. brevis*, Gause *et al.* discovered gramicidin S, which already for decades is prescribed as antibiotic for topical infections.[1,2] Both tyrocidine and gramicidin S belong to the so-called 'head-to-tail' cyclic peptide compounds, in which the macrocycle is exclusively made up from the peptide backbone. Cyclic peptides exist as well in which amino acid side chain functionalities are part of the macrocyclic structure.

Compared to the synthesis of linear peptides, the synthesis of head-to-tail cyclic peptides is inherently more complicated, predominantly due to the need to condense the N-terminal amine with the C-terminal carboxylate to form an amide at a certain stage in the synthesis procedure. This cyclization may occur either on-resin (whence a solid phase peptide synthesis procedure is followed, which is often the case) or off-resin, and both procedures require additional functional (protective) group manipulations compared to the solid phase synthesis of linear peptides (Figure 1). Off-resin cyclisation, for instance, requires the use of side chain amine/carboxylic acid protective groups orthogonal to the N-terminal one and that can withstand conditions to cleave the linear precursor from the resin (Figure 1A). In contrast, on-resin cyclisation requires immobilization of the first amino acid building block through a side chain functionality, rather than the C-terminal α-carboxyl group, as is standard practice in the solid phase synthesis of linear peptides (Figure 1B). A major intrinsic advantage of on-resin cyclization is that due to the inherent pseudo-dilution effect, the occurrence of intermolecular condensations is diminished compared to that in solution procedures.[3] Of note as well are simultaneous cyclization/cleavage procedures that have seen some usage but that appear limited in application to relatively simple macrocyclic peptides.[4,5]

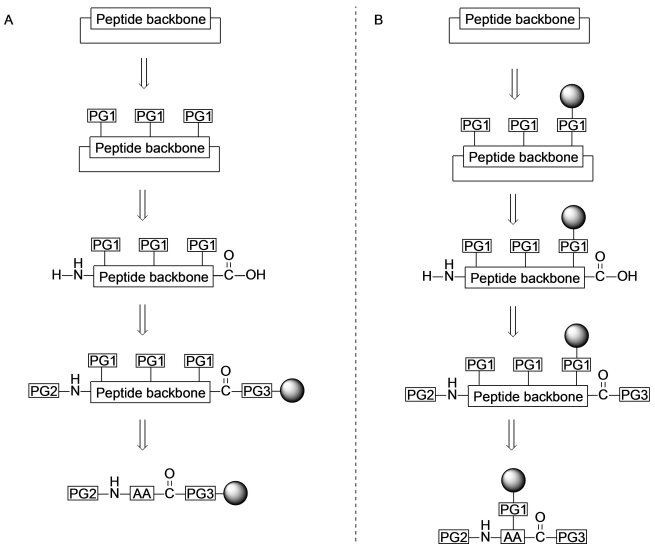

Figure 1. A. Off-resin cyclization strategy for head-to-tail cyclic peptides (PG = protecting group). B. The major alternative strategy for head-to-tail cyclic peptides involving on-resin cyclization for which a trifunctional amino acid is required.

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We here describe the development of optimized protocols for the synthesis of head-to-tail cyclic peptides through on-resin cyclization, with particular focus on the nature of the amino acid selected for attachment to the resin through its side chain, the nature of the connecting functionality and the chemistry used to bring the linkage about. Such a chain anchoring methodology requires amino acids with three functional groups that can be addressed individually, and for which new chemistries may need to be developed. Anchoring through carboxamide or carboxylic acid side-chain functionalities can be achieved using established peptide coupling methodologies, and leads to aspartic, glutamic, asparagine or glutamine residues in the final products.[6,7] Other amino acids that have been attached to a resin *via* their side-chain and for which new chemistries have been developed include cysteine,[8] histidine,[9,10] serine,[11,12] threonine,[11,12] tyrosine,[13] lysine,[14,15] tryptophan,[16] arginine[17] and phenylalanine.[18]

With the objective to utilize the ornithine δ-amine as well as the lysine ε-amine for anchoring to a solid support through an acid-labile functionality, we selected the para-hydroxymethylphenyloxy (Wang) linker in combination with a carbamate as connecting functionality (Figure 2A). This side chain anchoring system is featured in several studies, in which it was created through nucleophilic attack of the free lysine ε-amine or ornithine δ-amine onto an activated carbonate as depicted in Figure 2A (route a).[14,15,19] Carbamates can however also be generated by reacting an alcohol with an isocyanate (Figure 2A, route b) and, since this strategy has not been used for lysine ε-amine **1** and ornithine δ-amine **2**, we decided to explore the isocyanate route for the immobilization of the side chain amines (Figure 2). The main advantage of this approach is the ability to drive the anchoring process to completion by adding additional isocyanate without the need to re-activate the resin itself. Both **1** and **2** were equipped with an N-terminal Fmoc group and an orthogonal C-terminal allyl protective group, allowing Fmoc-based solid phase peptide synthesis (SPPS) procedure with on-resin cyclisation in the final stage. Thus, results here entail the preparation of **1** and **2** following *route b* in efficiencies at least equal to those reported in the literature based on *route a* as well as implementation of anchored ornithine δ-amine **2** in the synthesis of four representative head-to-tail cyclic peptide antibiotics, including gramicidin S and tyrocidine (Figure 2B). These well-known peptides were selected to allow a comparison of the synthetic efficiency of our methodology to that of published syntheses.

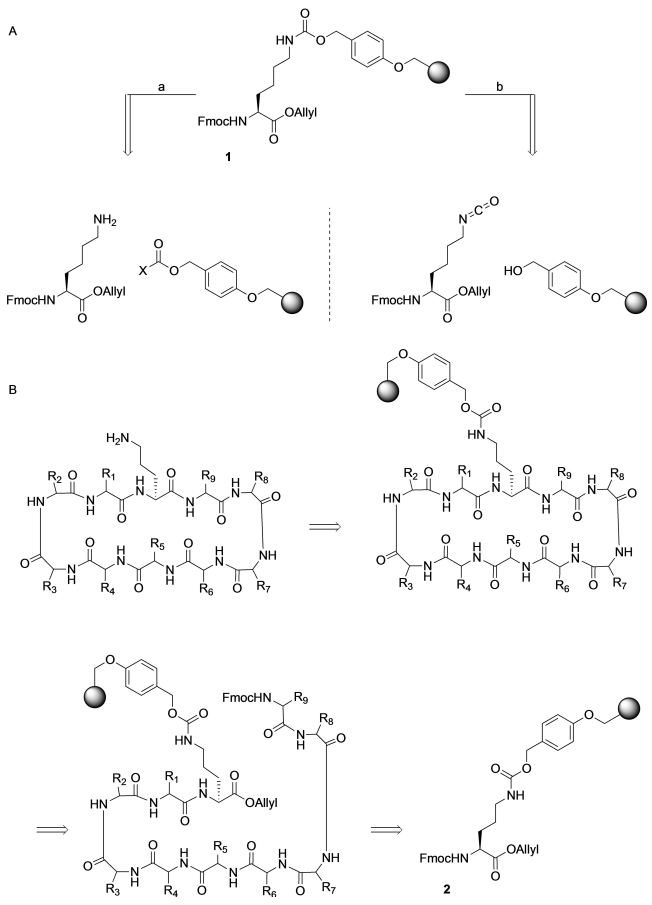
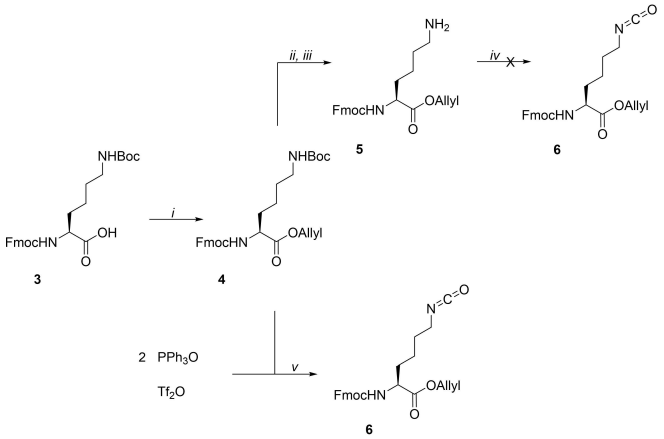


Figure 2. A. Different strategies to anchor the lysine side-chain to a resin through a conventional approach that involves an electrophilic resin (route a)[14,15,19] or a strategy with a nucleophilic resin resulting in the same linkage (route b). B. Retrosynthesis of head-to-tail cyclic peptides using an on-resin cyclization strategy.

Results and Discussion

First, the synthesis of a suitably and orthogonally protected lysine for ensuing side chain anchoring to resins was undertaken. We opted to test the anchoring methodology first for lysine since the required commercially available Fmoc amino acid Fmoc–Lys(Boc)–OH is approximately ten times less expensive than Fmoc–Orn(Boc)–OH. Starting from *N*-α-Fmoc-*N*-ε-Boc lysine **3** alkylation of the carboxylate with allyl bromide and silver carbonate as the base afforded orthogonally protected lysine **4** (Scheme 1).



Scheme 1. Reagents and conditions: (i) Ag_2CO_3, allyl bromide, DMF, 0 °C to rt, 2.5 hr, 95% (ii) $SnCl_4$, DCM, EA, rt, 1 hr, 88% (iii) DMAP, MeCN, rt, 5 min. (iv) Boc_2O, DMAP, MeCN, rt, 10 min., 68% (**4**) (v) PPh_3O, Tf_2O, DCM, 0 °C, 30 min, then **4**, DCM, 0 °C to rt, 3 hr, 19%.

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Initially, the conversion of the amine in the side chain of lysine to the corresponding isocyanate was explored using the method of Knölker *et al.*[20] Thus, removal of the Boc protective group in **4** using tin(IV) chloride to furnish **5** as hydrochloric acid salt, was followed by treatment with DMAP in MeCN to give a solution of the free amine **5**, that was subsequently added to a mixture of DMAP and di-*tert*-butyldicarbonate (1 : 1.4, DMAP:Boc_2O). However, instead of isocyanate **6** urethane **4** was found to be formed as the major product (68 % isolated yield). Knölker and co-workers also noticed an increase in formation of the Boc-protected amine over the preferred isocyanate when utilizing less sterically hindered amines.

Therefore attention was changed to methods that transform carbamates directly into isocyanates.[21–25] As lysine **4** contains two carbamates (*N*-α-Fmoc and *N*-ε-Boc), any successful method to turn **4** into **6** should transform the latter carbamate into the isocyanate while leaving the former untouched. Cho and co-workers described a method to convert an *N*-Boc carbamate to the corresponding isocyanate (Scheme 1).[26] Their procedure involves the use of Hendrickson's dehydrating reagent[27,28] and the results for the investigated carbamates, varying in the nature of the alkoxy substituent indicate that the efficiency of the isocyanate formation correlates with the acid lability of the carbamate. The authors suggest that triflic acid that is formed during the reaction aids the formation of isocyanate by removing the acid-labile *tert*-butyl group. We therefore hypothesized that the methodology should be applicable to convert lysine **4** into isocyanate **6**, entailing the selective transformation of the acid-labile *O*-^{t}Bu carbamate (Boc) in the presence of the acid-stable *O*-fluorenylmethyl carbamate (Fmoc).

Hendrickson reagent was prepared *in situ* by mixing triphenylphosphine oxide and triflic anhydride after which a solution of *N*-Boc/*N*-Fmoc carbamate **4** in dichloromethane was added. After three hours all starting material had been converted to a more apolar compound as monitored by TLC analysis. IR spectroscopic analysis of the crude reaction mixture revealed a strong peak at 2262 cm^{-1}, indicating the presence of an isocyanate. Purification by silica gel column chromatography afforded an orange oil (19 % yield in case compound **6** was the isolated product), that was characterized by NMR spectroscopy. ^{1}H NMR showed the presence of the aromatic protons of fluorenylmethyl group as well as the absence of methyl protons characteristic for a *tert*-butyl group, and ^{13}C NMR displayed at 122 ppm a signal characteristic for an isocyanate carbon. Heteronuclear multiple-bond correlation spectroscopy (HMBC) showed correlation between the isocyanate carbon and the ε-protons of the lysine, while no correlation was observed with the α-proton indicating that the Fmoc-carbamate was intact (Figure 3). All these observations made us conclude that isocyanate **6** was indeed the compound formed.

The low yield of isocyanate **6** is presumably due to instability on the silica gel column as it has been shown that silica promotes the hydrolysis of isocyanates.[29] Therefore, silica gel purification was skipped in the synthesis of a next batch and the resulting crude isocyanate **6** was added to Wang-type resin **8** to furnish side-chain anchored resin **1**. Conditions for

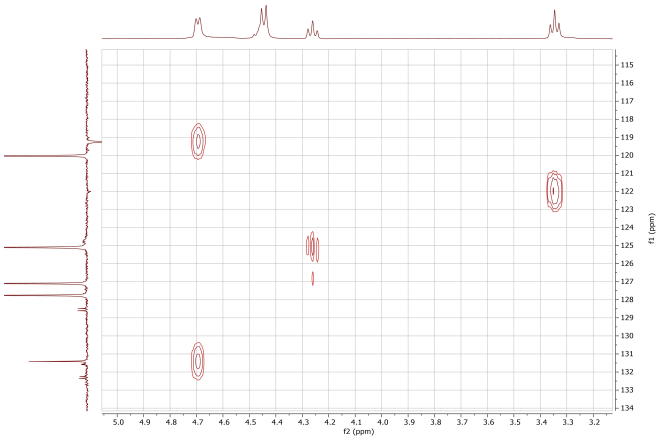
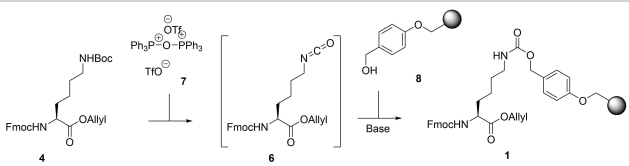


Figure 3. HMBC spectrum shows correlation between the isocyanate carbon and the ε-protons of the lysine on the right.

the formation of carbamate **1** had to be optimized (Table 1) because triflic acid present in the crude mixture should be neutralized and a catalyst such as a tertiary amines is needed to induce carbamate formation.[30] In addition, while the catalytic activity of tertiary amines generally increases as basicity increases, the base-labile Fmoc group limits the choice of catalysts. In the first attempt, 8.5 equivalents *N*-methylmorpholine[31] was added (7.2 eq. to neutralize the triflic acid and the remaining 1.3 eq. to act as base) to the solution of crude isocyanate **6** and this solution was transferred to a reaction vessel containing anhydrous resin **8**, after which the

Table 1. Screening of catalysts in the condensation of isocyanate **8** and resin-bound alcohol **8**.[a]



Entry	Catalyst/Base	Equivalent catalyst	Excess base[b]	Yield[c]
1	*N*-methylmorpholine		1.3	< 5 %
2	1-methylimidazole		1.3	< 5 %
3	DMAP		1.3	Fmoc cleavage
4	Ti(O^{t}Bu)$_4$, *N*-methylmorpholine	1.0	0.3	55 %
5	Ti(O^{t}Bu)$_4$, *N*-methylmorpholine	3.0	0.3	57 %
6	Zr(IV)acac, *N*-methylmorpholine	1.0	0.3	94 %
7	Dibutyltindilaurate, *N*-methylmorpholine	1.0	0.3	> 99 %
8	Dibutyltindilaurate, 1-methylimidazole	1.0	0.3	44 %

[a] Reactions were performed at a 0.1 mmol scale with 3.0 equivalents of **4** compared to resin **8** over 17 hrs. [b] Excess in equivalents compared to resin **8**. [c] Yield was determined by multiplying the dry weight of the resin with the loading determined by UV-Vis absorption following the procedure of Eissler *et al.*[32]

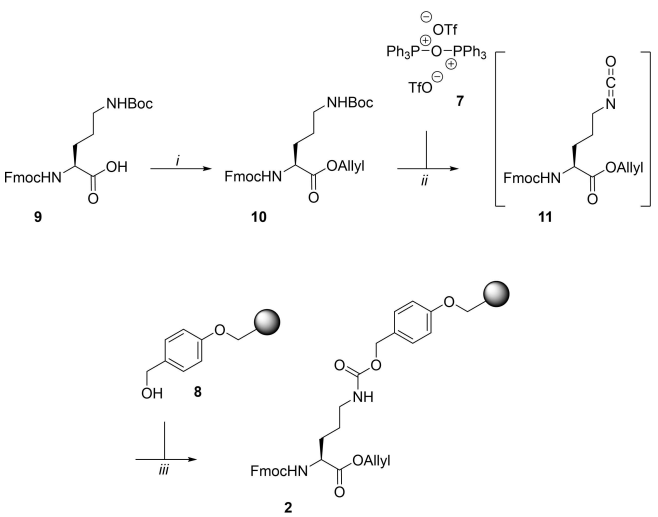
suspension was shaken for 17 hours. The resin was isolated and dried under vacuum and the loading was determined by measuring absorption at 301.0 nm after Fmoc removal with piperidine.[32] Less than 5% loading yield was obtained (Table 1, entry 1).

Guided by the procedure of Yoganathan *et al.*[33] for the addition of alcohols to isocyanates, 1-methylimidazole and 4-dimethylaminopyridine (DMAP)[34] were assessed on their efficacy to condense **6** with resin **8** to provide **1**. Usage of 1-methylimidazole furnished anchored lysine **1** in less than a 5% yield (Entry 2) whereas DMAP led to partial Fmoc cleavage (Entry 3). Titanium(IV) *tert*-butoxide to catalyze the condensation of alcohols with isocyanates, as reported by the group of Arbour[35] was combined with *N*-methylmorpholine to neutralize triflic acid. With 1.0 eq. $Ti(O^tBu)_4$ resin **1** was obtained in 55% yield (Entry 4), an outcome that could not be improved by increasing the amount of catalyst to 3.0 eq. (Entry 5). Finally, zirconium(IV) acetylacetonate and dibutyltin dilaurate,[36,37] in combination with *N*-methylmorpholine as the base proved highly effective in effecting the desired transformation, giving anchored lysine **1** in 94% yield for zirconium(IV) acetylacetonate and >99% yield for dibutyltin dilaurate (Entry 6 & 7*)*. Comparing the two catalysts, dibutyltin dilaurate is more efficient in the synthesis of the urethane while zirconium(IV) acetylacetonate is considerably less toxic.[38,39] *N*-methylmorpholine proved to be the superior base as 1-methylimidazole with dibutyltin dilaurate as the catalyst gave urethane **1** in 44% yield (*Entry 8*). Beta-turn modified gramicidin S analogues containing arylated sugar amino acids display antimicrobial and hemolytic activity comparable to the natural product.

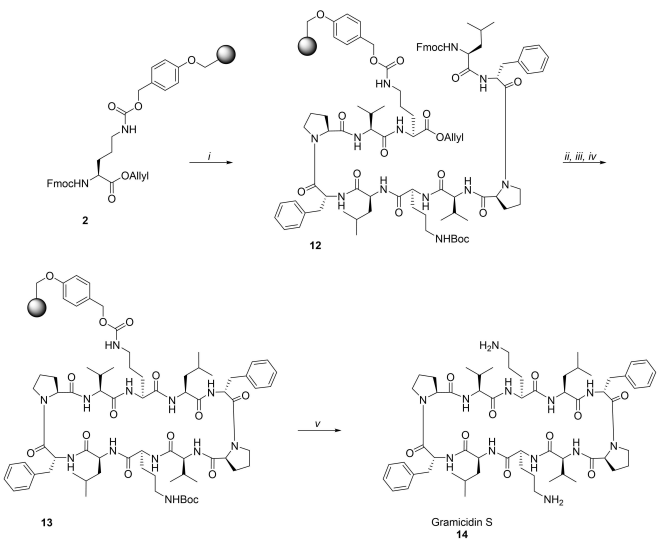
Having suitable conditions available we anchored the δ-amine of ornithine residue to the Wang-type resin to obtain immobilized Fmoc-ornithine **2** using the same sequence of reactions as described for resin **1**. As shown in Scheme 2 *N*-α-Fmoc-*N*-δ-Boc ornithine **9** was allylated to furnish fully protected ornithine **10** that was subsequently converted into isocyanate **11** by Hendrickson's reagent **7**. To the obtained solution of **11** was added *N*-methylmorpholine and dibutyltin dilaurate and the mixture was transferred to a vessel containing TentaGel S PHB resin **8**, resulting in the formation of ornithine-functionalized resin **2** in 94% yield.

Now the stage was set for the synthesis of the head-to-tail cyclic peptides. First the construction of gramicidin S **14** was undertaking, using resin **2**, on an automated peptide synthesizer with standard Fmoc based solid phase peptide synthesis protocols (SPPS, Scheme 3). Each of the nine peptide coupling cycles started with two treatments of 20% (v/v) piperidine in DMF for three minutes to deprotect the Fmoc group. The subsequent condensation was achieved using commercially available amino acids in combination with the activator HCTU and *N*-methylmorpholine for one hour at room temperature. Unreacted amines were capped by two treatments of 10% (v/v) Ac_2O in DMF. After nine cycles, immobilized fully protected linear peptide **12** was ready to be cyclized.

First the allyl ester in immobilized peptide **12** was deprotected in a reaction vessel outside of the peptide synthesizer as free amines may poison the palladium catalyst.[40] Treatment of resin **12** with palladium tetrakis(triphenylphosphine) in the presence of phenylsilane as a scavenger for 90 minutes resulted in complete removal of the allyl group as established by LC-MS analysis of the products after the cleavage from the resin. The Fmoc was removed by two treatments with 20% (v/v) piperidine in DMF for ten minutes to furnish free N-terminus. For the key on-resin cyclization step, the phosphonium activator benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) was chosen as the coupling reagent over uronium- and



Scheme 2. Reagents and conditions: (i) Ag_2CO_3, allyl bromide, DMF, 0 °C to rt, 2.5 hr, 91% (ii) DCM, rt, 15 min. (iii) dibutyltin dilaurate, N-methylmorpholine, DCM, rt, 24 hr, 94%.



Scheme 3. Reagents and conditions: (i) SPPS: (a) piperidine, DMF, rt, 2×3 min. (b) Fmoc–AA–OH, HCTU, *N*-methylmorpholine, DMF, rt, 1 hr (c) Ac_2O, DMF, rt, 2×3 min. (ii) $Pd(PPh_3)_4$, $PhSiH_3$, DCM, DMF, rt, 1.5 hrs (iii) piperidine, DMF, rt, 2×10 min. (iv) benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, 1-hydroxybenzotriazole hydrate, N-methylmorpholine, DMF, rt, 2.5 hrs (v) TFA:TIPS:H2O (190:5:5), rt, 3 hrs, 43%.

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iminium-based salts as phosphonium-based coupling reagents do not suffer from the formation of guanidine derivatives as has been reported for the uronium- and iminium-based reagents.[41,42] 1-Hydroxybenzotriazole (HOBt) was also added to provide more efficient coupling and to lower racemization of the activated amino acid.[43] It is noteworthy, that the PyBOP/HOBt combination has been extensively used in the past for the solution phase cyclization of Gramicidine S an its analogues.[44–46] These cyclisation conditions with *N*-methylmorpholine as the base were applied to the resin and the suspension was shaken for 2.5 hours giving cyclized peptide **13**. Liberation of gramicidin S **14** from the resin was achieved by treating resin **13** with a cleavage cocktail (190:5:5, TFA-TIPS-H_2O) for three hours. The crude peptide was obtained by evaporation and subsequently purified by size exclusion chromatography to afford gramicidin S in 43% yield. Analysis by analytical reversed-phase HPLC revealed that gramicidin S was obtained with a purity of 96% (Table 2, entry 1). In comparison, an on-resin cyclization approach by Andreu and co-workers furnished gramicidin S in a 24% yield with a purity of *ca.* 90%.[19] The major difference in their approach is the method of resin anchoring of the ornithine residue which involved transforming the resin into an activated carbonate species before treatment with ornithine δ-amine (Figure 2A). Extensive research investigating an off-resin cyclization strategy in the synthesis of gramicidin S was conducted by Wadhwani *et al.*[47] The highest yield and purity was obtained when the peptide synthesis was started from the D-phenylalanine residue affording gramicidin S in a 69% overall yield with a 95% purity. When the solid phase synthesis started with Fmoc–Orn(Dde)–OH the yield and purity dropped to 17% and 70% respectively.

To investigate the scope of the procedure three additional natural head-to-tail cyclic peptides that feature ornithine residues, namely tyrocidin A, loloatin A and streptocidin A, were synthesized in the same manner.[2,48–50] Tyrocidin A was obtained in a 66% yield with a purity of 73% (Entry 2). In the synthesis of tyrocidin A, a concurrent cyclization and cleavage approach was employed by ösapay and co-workers using the Kaiser oxime resin furnishing tyrocidin A in a yield of 55% with a >95% purity.[51] This strategy has also been employed by the group of Guo in the syntheses of gramicidin S (25% yield, 87% purity), tyrocidin A (25% yield, >95% purity), loloatin A (28% yield, 90% purity) and streptocidin A (16% yield, 94% purity).[52–55] We further synthesized loloatin A and streptocidin A in a 59% and 40% yield with a purity of 93% and 53% respectively (*Entry 3* and *4*). A reported synthesis of loloatin A by Scherkenbeck *et al.* also employed an on-resin cyclization strategy but here the side-chain of the asparagine residue was used to anchor the growing peptide to the resin.[56] This strategy gave loloatin A in an overall yield of 31% with a 97% purity. Tyrocidin and streptocidin were obtained in a lower purity, which based on the observed masses of the formed product mixtures, may be because in both cases incomplete detritylation of the -Asn-Gln- motif had occurred. Perhaps longer exposure to trifluoroacetic acid could alleviate this problem. The synthesis of streptocidin A in turn appeared accompanied by formation of other minor side products as well.

Table 2. Synthesis of cyclic peptides using side-chain anchoring.

Entry	Peptide	Yield[a]	Purity[b]
1	*cyclo*(-Leu-*D*Phe-Pro-Val-Orn-)$_2$ Gramicidin S	43%	96%
2	*cyclo*(-Leu-*D*Phe-Pro-Phe-*D*Phe-Asn-Gln-Tyr-Val-Orn-) Tyrocidin A	66%	73%
3	*cyclo*(-Leu-*D*Tyr-Pro-Phe-*D*Phe-Asn-Asp-Tyr-Val-Orn-) Loloatin A	59%	93%
4	*cyclo*(-Leu-*D*Phe-Pro-Leu-*D*Trp-Asn-Gln-Tyr-Val-Orn-) Streptocidin A	40%	53%

[a] Yield after size exclusion chromatography. [b] Chromatographic purity based on area percentage.

Conclusion

We successfully anchored the δ- and ε-amine of ornithine and lysine respectively as TFA-sensitive carbamates on a Wang-type resin with a novel method. The method involves the formation of an isocyanate which was readily achieved from the *N*-Boc carbamate using Hendrickson's reagent following a procedure by Cho *et al.*[26] In the coupling of the isocyanate to a TentaGel Wang-type resin, dibutyltin dilaurate and zirconium(IV) acetylacetonate were found to be efficient in catalyzing this transformation with *N*-methylmorpholine acting as the base. The choice of base proved to be influential as 1-methylimidazole hampered the catalysis of dibutyltin dilaurate. The side-chain anchoring procedure, which is fully compatible with ensuing Fmoc-SPPS, was then applied to the synthesis of several natural head-to-tail cyclic peptides including gramicidin S and tyrocidin A. The peptides were synthesized in 40–66% yields with a purity ranging from 53 to 96%, a result that compares well to those reported previously for alternative synthesis procedures.

Experimental Section

All solvents and reagents were obtained commercially and used as received. Reactions were carried out under a nitrogen atmosphere, unless indicated otherwise. Reaction progress and chromatography fractions were monitored by thin layer chromatography (TLC) on silica-gel-coated aluminium sheets with a F254 fluorescent indicator purchased from Merck (Silica gel 60 F_{254}). Visualization was achieved by UV absorption by fluorescence quenching or permanganate stain (4 g $KMnO_4$ and 2 g K_2CO_3 in 200 mL of H_2O). Silica gel column chromatography was performed using Screening Devices silica gel 60 (particle size of 40–63 μm, pore diameter of 60 Å) with the indicated eluent. Analytical reversed-phase high-performance liquid chromatography (RP-HPLC) was performed on a Thermo Finnigan Surveyor HPLC system with a Phenomenex Gemini C_{18} column (4.6 mm x 50 mm, 3 μm particle size) with a flow rate of 1 mL/min and a solvent gradient of 10–90% solvent B over 8 min coupled to a LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI^+). Nuclear magnetic resonance (^1H and ^{13}C APT NMR) spectra were recorded on a Brüker AV-400 or a Brüker AV-500 in the given solvent. Chemical shifts are reported in parts per million (ppm) with

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the residual solvent or tetramethylsilane (0 ppm) as reference. High-resolution mass spectrometry (HRMS) analysis was performed with a Thermo Finnigan LTQ Orbitrap mass spectrometer equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 ml/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150–2000) and dioctyl phthalate (m/z = 391.28428) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a Thermo Finnigan calibration mixture. Nominal and exact m/z values are reported in daltons.

Fmoc-Lys(Boc)-OAllyl 4

Standard Fmoc-protected lysine building block **3** (4.7 g, 10 mmol, 1.0 equiv.) was dissolved in DMF (40 mL, 0.25 M) and the solution was cooled to 0 °C. Silver carbonate (3.6 g, 13 mmol, 1.3 equiv.) was added and the reaction was stirred for 15 min. The cooling bath was removed, allyl bromide (4.0 mL, 46 mmol, 4.6 equiv.) was added and the mixture was stirred for an additional 2.5 hrs. The suspension was then filtered, diluted with EA and subsequently washed with 10% (w/v) aq. $KHSO_4$ and H_2O. The organic layer was dried ($MgSO_4$), filtered and the volatiles were removed under reduced pressure. Purification by silica gel column chromatography (1:4, EA – Pentane to 3:2, EA – Pentane) furnished allyl ester **4** (4.8 g, 9.5 mmol, 95%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H, C*H*-arom), 7.65–7.52 (m, 2H, C*H*-arom), 7.40 (t, J = 7.5 Hz, 2H, C*H*-arom), 7.32 (tt, J = 7.5, 1.3 Hz, 2H, C*H*-arom), 5.91 (ddt, J = 16.4, 10.3, 5.8 Hz, 1H, $OCH_2CH{=}CH_2$), 5.45–5.23 (m, 3H, N*H*Fmoc, $OCH_2CH{=}CH_2$), 4.68–4.62 (m, 2H, $OCH_2CH{=}CH_2$), 4.57 (s, 1H, N*H*Boc), 4.47–4.33 (m, 3H, CH_2-Fmoc, α-Lys), 4.23 (t, J = 7.0 Hz, 1H, C*H*-Fmoc), 3.16–3.07 (m, 2H, ε-Lys), 1.95–1.82 (m, 1H, β-Lys), 1.79–1.67 (m, 1H, β-Lys), 1.43 (s, 13H, δ-Lys, CH_3-Boc, γ-Lys).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.3 (*C*OOAllyl), 156.2 (*C*=O-Boc), 156.1 (*C*=O-Fmoc), 144.0 (*C*q-arom), 143.9 (*C*q-arom), 141.4 (*C*q-arom), 131.6 ($OCH_2CH{=}CH_2$), 127.8 (*C*H-arom), 127.2 (*C*H-arom), 125.2 (*C*H-arom), 120.1 (*C*H-arom), 120.1 (*C*H-arom), 119.2 ($OCH_2CH{=}CH_2$), 83.0 ($C(CH_3)_3$), 67.2 (CH_2-Fmoc), 66.2 ($OCH_2CH{=}CH_2$), 53.9 (α-Lys), 47.3 (*C*H-Fmoc), 40.2 (ε-Lys), 32.3 (β-Lys), 29.7 (δ-Lys), 28.5 (CH_3-Boc), 22.5 (γ-Lys).

HRMS (ESI-Orbitrap) calcd. for $C_{29}H_{36}N_2O_6Na$ $[M+Na]^+$ 531.24656, found 531.24641.

Fmoc-Lys-OAllyl 5

Boc-protected amine **4** (50 mg, 98 μmol, 1.0 equiv.) was dissolved in EA (0.98 mL, 0.10 M) and $SnCl_4$ (1.0 M in DCM, 0.39 mL, 0.39 mmol, 4.0 equiv.) was added. The reaction was stirred at room temperature for 1 hr. Afterwards, the reaction mixture was evaporated and dissolved in a small amount of MeOH. Product crashed out upon addition of Et_2O and was collected by filtration affording amine **5** (38 mg, 86 μmol, 88%) as the hydrochloric acid salt.

^{1}H NMR (400 MHz, MeOD) δ 7.79 (d, J = 7.5 Hz, 2H, C*H*-arom), 7.66 (t, J = 7.5 Hz, 2H, C*H*-arom), 7.39 (t, J = 7.4 Hz, 2H, C*H*-arom), 7.30 (t, J = 7.4 Hz, 2H, C*H*-arom), 5.93 (ddt, J = 16.1, 10.8, 5.6 Hz, 1H, $OCH_2CH{=}CH_2$), 5.32 (dq, J = 17.2, 1.7 Hz, 1H, $OCH_2CH{=}CH_2$), 5.21 (dq, J = 10.5, 1.4 Hz, 1H, $OCH_2CH{=}CH_2$), 4.62 (dt, J = 5.6, 1.5 Hz, 2H, $OCH_2CH{=}CH_2$), 4.43–4.28 (m, 2H, CH_2-Fmoc), 4.25–4.16 (m, 2H, C*H*-Fmoc, α-Lys), 2.97–2.85 (m, 2H, ε-Lys), 1.95–1.82 (m, 1H, β-Lys), 1.80–1.62 (m, 3H, β-Lys, δ-Lys), 1.56–1.38 (m, 2H, γ-Lys).

^{13}C NMR (101 MHz, MeOD) δ 173.5 (*C*OOAllyl), 158.7 (*C*=O-Fmoc), 145.2 (*C*q-arom), 142.5 (*C*q-arom), 133.3 ($OCH_2CH{=}CH_2$), 128.8 (*C*H-arom), 128.1 (*C*H-arom), 126.2 (*C*H-arom), 126.1 (*C*H-arom), 120.9 (*C*H-arom), 118.7 ($OCH_2CH{=}CH_2$), 67.9 (CH_2-Fmoc), 66.7 $OCH_2CH{=}CH_2$), 55.2 (α-Lys), 48.3 (*C*H-Fmoc), 40.5 (ε-Lys), 31.9 (β-Lys), 27.9 (δ-Lys), 23.8 (γ-Lys).

Fmoc-Lys(CO)-OAllyl 6

Triphenylphosphine oxide (0.16 g, 0.58 mmol, 2.4 equiv.) was dissolved in DCM (8.0 mL, 30 mM) and the solution was cooled to 0 °C. Triflic anhydride (48 μL, 0.29 mmol, 1.2 equiv.) was added and the reaction was stirred at 0 °C for 30 min. during which time a white precipitate was formed. Lysine building block **4** (0.12 g, 0.24 mmol, 1.0 equiv.) was added to the suspension and the reaction was stirred for 3 hrs allowing the mixture to gradually warm to room temperature. The solution was loaded onto a silica gel column and purified by silica gel column chromatography (1:4, EA – Pentane) to give isocyanate **6** (20 mg, 46 μmol, 19%) as an orange oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79–7.73 (m, 2H, C*H*-arom), 7.63–7.54 (m, 2H, C*H*-arom), 7.40 (tt, J = 7.5, 1.5 Hz, 2H, C*H*-arom), 7.31 (tt, J = 7.5, 1.1 Hz, 2H, C*H*-arom), 5.91 (ddt, J = 16.6, 11.0, 5.9 Hz, 1H, $OCH_2CH{=}CH_2$), 5.39–5.22 (m, 3H, N*H*Fmoc, $OCH_2CH{=}CH_2$), 4.68–4.61 (m, 2H, $OCH_2CH{=}CH_2$), 4.45–4.34 (m, 3H, CH_2-Fmoc, α-Lys), 4.22 (t, J = 7.0 Hz, 1H, C*H*-Fmoc), 3.31 (t, J = 6.6 Hz, 2H, ε-Lys), 1.93–1.85 (m, 1H, β-Lys), 1.76–1.57 (m, 3H, β-Lys, δ-Lys), 1.55–1.36 (m, 2H, γ-Lys).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1 (*C*OOAllyl), 156.0 (*C*=O-Fmoc), 144.0 (*C*q-arom), 143.8 (*C*q-arom), 141.4 (*C*q-arom), 131.5 ($OCH_2CH{=}CH_2$), 127.9 (*C*H-arom), 127.2 (*C*H-arom), 125.2 (*C*H-arom), 122.1 (NCO), 120.1 (*C*H-arom), 120.1 (*C*H-arom), 119.3 ($OCH_2CH{=}CH_2$), 67.1 (CH_2-Fmoc), 66.3 ($OCH_2CH{=}CH_2$), 53.7 (α-Lys), 47.3 (*C*H-Fmoc), 42.8 (ε-Lys), 32.2 (β-Lys), 30.7 (δ-Lys), 22.3 (γ-Lys).

IR (thin film) ν (cm^{-1}) 2262, 1719, 1700, 1451, 1183, 1085, 759, 741.

Fmoc-Lys(TentaGel S PHB)-OAllyl 1

A solution containing triphenylphosphine oxide (0.20 g, 0.72 mmol, 7.2 equiv.) in DCM (3.0 mL, 0.12 M) was cooled to 0 °C and triflic anhydride (1.0 M in DCM, 0.36 mL, 0.36 mmol, 3.6 equiv.). The reaction was stirred at 0 °C for 30 min. during which a white precipitate was formed. A solution of *N*-Boc protected lysine **4** (0.15 g, 0.30 mmol, 3.0 equiv.) in DCM (0.34 mL, 0.88 M) was then added to the suspension and the cooling bath was removed. The reaction was stirred for 5 min. followed by the addition of *N*-methylmorpholine (83 μL, 0.75 mmol, 7.5 equiv.) and dibutyltin dilaurate (59 μL, 0.10 mmol, 1.0 equiv.). The solution was transferred to TentaGel S PHB resin (0.27 mmol/g, 0.37 g, 0.10 mmol, 1.0 equiv.) which was co-evaporated previously with 1,4-dioxane (3x) and the suspension was shaken for 18 hrs. The suspension was filtered and the resin was washed with DCM (4x) and Et_2O (4x). Drying the resin over N_2 afforded functionalized resin **1** (0.43 g, 0.10 mmol, >99%) with a loading of 0.24 mmol/g. A fraction of the resin (5.0 mg) was subjected to a cleavage cocktail (190:5:5, TFA – H_2O – TIPS) for 2 hrs and analyzed by LC–MS.

LC–MS (ESI^+) calcd. for $C_{24}H_{29}N_2O_4$ $[M+H]^+$ 409.21, observed 409.25 with a retention time of 5.34 min.

Fmoc-Orn(Boc)-OAllyl 10

Fmoc–Orn(Boc)–OH **9** (2.0 g, 4.4 mmol, 1.0 equiv.) was dissolved in DMF (18 mL, 0.25 M) and cooled to 0 °C. Silver carbonate (1.6 g, 5.7 mmol, 1.3 equiv.) was added and the reaction was stirred for 15 min. Allyl bromide (1.8 mL, 20 mmol, 4.6 equiv.) was added,

cooling bath was removed and the mixture was stirred at room temperature for 3 hrs. The suspension was filtered and the filtrate was diluted with DCM and subsequently washed with 10% (w/v) aq. $KHSO_4$. The organic phase was dried ($MgSO_4$), filtered and the volatiles were removed under reduced pressure. Purification by silica gel column chromatography (1:4, EA – Pentane to 2:3, EA – Pentane) afforded allyl ester **10** (2.0 g, 4.1 mmol, 91%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dq, J = 7.7, 1.0 Hz, 2H, C*H*-arom), 7.64–7.57 (m, 2H, C*H*-arom), 7.41 (tq, J = 7.5, 1.0 Hz, 2H, C*H*-arom), 7.32 (tt, J = 7.4, 1.2 Hz, 2H, C*H*-arom), 5.91 (ddt, J = 16.4, 10.9, 5.8 Hz, 1H, $OCH_2CH{=}CH_2$), 5.45 (d, J = 8.3 Hz, 1H, N*H*Fmoc), 5.37–5.24 (m, 2H, $OCH_2CH{=}CH_2$), 4.65 (d, J = 5.8 Hz, 2H, $OCH_2CH{=}CH_2$), 4.57 (s, 1H, N*H*Boc), 4.45–4.36 (m, 3H, CH_2-Fmoc, α-Orn), 4.22 (t, J = 6.9 Hz, 1H, C*H*-Fmoc), 3.22–3.10 (m, 2H, δ-Orn), 1.96–1.84 (m, 1H, β-Orn), 1.77–1.49 (m, 3H, β-Orn, γ-Orn), 1.44 (s, 9H, CH_3-Boc).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1 (*C*OOAllyl), 156.1 (*C*=O-Boc), 156.1 (*C*=O-Fmoc), 143.9 (*C*q-arom), 141.5 (*C*q-arom), 131.6 ($OCH_2CH{=}CH_2$), 127.9 (*C*H-arom), 127.2 (*C*H-arom), 125.2 (*C*H-arom), 120.1 (*C*H-arom), 120.1 (*C*H-arom), 119.3 ($OCH_2CH{=}CH_2$), 67.1 (CH_2-Fmoc), 66.2 ($OCH_2CH{=}CH_2$), 53.8 (α-Orn), 47.3 (*C*H-Fmoc), 40.1 (δ-Orn), 30.0 (β-Orn), 28.5 (CH_3-Boc), 26.3 (γ-Orn).

HRMS (ESI-Orbitrap) calcd. for $C_{28}H_{34}N_2O_6Na$ $[M+Na]^+$ 517.23091, found 517.23091.

Fmoc-Orn(TentaGel S PHB)-OAllyl 2

A solution containing triphenyl phosphine oxide (1.0 g, 3.6 mmol, 7.2 equiv.) in DCM (14 mL, 0.26 M) was cooled to 0 °C and triflic anhydride (1.0 M in DCM, 1.8 mL, 1.8 mmol, 3.6 equiv.) was added. The reaction was stirred at 0 °C for 30 min. forming a white precipitate. A solution of allyl ester **10** (0.74 g, 1.5 mmol, 3.0 equiv.) in DCM (1.7 mL, 0.88 M) was added and the cooling bath was removed. The reaction was stirred at room temperature for 15 min. followed by the addition of *N*-methylmorpholine (0.41 mL, 3.8 mmol, 7.5 equiv.) and dibutyltin dilaurate (0.30 mL, 0.5 mmol, 1.0 equiv.). The solution was transferred to TentaGel S PHB resin (0.27 mmol/g, 1.9 g, 0.50 mmol, 1.0 equiv.) which was previously co-evaporated with 1,4-dioxane (3x) and the suspension was shaken for 24 hrs. The reaction mixture was filtered and the resin was washed with DCM (4x) and Et_2O (4x). Drying the resin over N_2 furnished functionalized resin **2** (2.1 g, 0.47 mmol, 94%) with a loading of 0.23 mmol/g.

Gramicidin S 14
cyclo(-Leu-*D*Phe-Pro-Val-Orn-)$_2$

Functionalized resin **2** (0.44 g, 0.10 mmol, 1.0 equiv.) was elongated using the Tribute peptide synthesizer. Amino acids were presented as solids and 0.20 M HCTU in DMF was used as activator, 0.50 M *N*-methylmorpholine in DMF as the activator base, 20% (v/v) piperidine in DMF as the deprotection agent and a 90:10, DMF-Ac_2O mixture as the capping agent. Coupling of each amino acid occurred at room temperature for 1 hr followed by a capping step (2x 3 min.) betwixt two washing steps. Subsequently, Fmoc was deprotected using the deprotection agent (2x 3 min.) followed by two more washing steps. After furnishing the linear peptide, the resin was washed with DMF (4x) and DCM (4x). Resin was suspended in a mixture of DCM and DMF (1:1, DCM – DMF, 4.0 mL, 25 mM) and phenylsilane (31 μL, 0.25 mmol, 2.5 equiv.) and Pd-$(PPh_3)_4$ (29 mg, 25 μmol, 25 mol%) were added. The resin was shaken for 90 min. while being protected from light. The suspension was filtered and the resin was washed with DCM (3x), 0.50% (w/v) sodium diethyldithiocarbamate in DMF (2x) and DMF (3x). To the resin was added 20% (v/v) piperidine in DMF (5.0 mL, 20 mM) and the resin was shaken for 10 min. Suspension was filtered and 20% (v/v) piperidine in DMF (5.0 mL, 20 mM) was added to the residue. Resin was shaken for 10 min. followed by filtration and washing with DMF (6x). To the resin was added DMF (4.0 mL, 25 mM). Subsequently, 1-hydroxybenzotriazole hydrate (68 mg, 0.50 mmol, 5.0 equiv.), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (0.26 g, 0.50 mmol, 5.0 equiv.) and *N*-methylmorpholine (0.11 mL, 1.0 mmol, 10 equiv.) were added to the suspension and the reaction was stirred for 2.5 hrs. The suspension was filtered and the residue was washed with DMF (3x) and DCM (6×). A cleavage mixture (190:5:5, TFA – H_2O – TIPS, 10 mL, 10 mM) was then added to the resin and the resulting suspension was shaken for 3 hrs. The suspension was filtered and the volatiles of the filtrate were removed under a stream of N_2. Residue was dissolved in a mixture of DCM and MeOH (1:1, MeOH – DCM) and purified by size exclusion chromatography (Sephadex LH-20, 1:1, MeOH – DCM) to give gramicidin S **14** (49 mg, 43 μmol, 43%) as a white solid.

^{1}H NMR (400 MHz, CD_3OH, 4.94 ppm suppressed) δ 8.94 (br s, 2H, N*H*-*D*Phe), 8.72 (dd, J = 12.0, 9.4 Hz, 4H, N*H*-Leu, N*H*-Orn), 7.71 (d, J = 9.0 Hz, 2H, N*H*-Val), 7.37–7.20 (m, 10H, C*H*-arom-*D*Phe), 4.66 (q, J = 7.6 Hz, 2H, α-Leu), 4.54–4.45 (m, 2H, α-*D*Phe), 4.37–4.32 (m, 2H, α-Pro), 4.15 (t, J = 8.8 Hz, 2H, α-Val), 3.73 (t, J = 9.5 Hz, 2H, δ-Pro), 3.15–2.82 (m, 8H, β-*D*Phe, δ-Orn, β-*D*Phe, δ-Orn), 2.50–2.42 (m, 2H, δ-Pro), 2.32–2.22 (m, 2H, β-Val), 2.09–1.96 (m, 4H, β-Pro, β-Orn), 1.81–1.34 (m, 18H, β-Orn, γ-Pro, β-Pro, γ-Pro, γ-Orn, β-Leu, γ-Leu, β-Leu), 0.98–0.84 (m, 24H, γ-Val, δ-Leu).

Note: α-Orn is in the suppressed region of 4.94 ppm.

^{13}C NMR (101 MHz, CD_3OH) δ 173.5 (*C*ONH), 173.5 (*C*ONH), 173.4 (*C*ONH), 172.8 (*C*ONH), 172.4 (*C*ONH), 136.8 (*C*q-arom-*D*Phe), 130.3 (*C*H-arom-*D*Phe), 129.6 (*C*H-arom-*D*Phe), 128.5 (*C*H-arom-*D*Phe), 61.9 (α-Pro), 60.3 (α-Val), 55.9 (α-*D*Phe), 52.4 (α-Orn), 51.4 (α-Leu), 47.9 (δ-Pro), 42.0 (β-Leu), 40.5 (δ-Orn), 31.9 (β-Val), 30.2 (β-Orn), 28.0 (β-Pro), 26.8 (γ-Orn), 25.6 (γ-Val), 24.4 (γ-Pro), 23.1 (δ-Leu), 23.0 (δ-Leu), 19.6 (γ-Val), 19.4 (γ-Val).

HRMS (ESI-Orbitrap) calcd. for $C_{60}H_{93}N_{12}O_{10}$ $[M+H]^+$ 1141.71321, found 1141.68680.

Loloatin A 15
cyclo(-Leu-*D*Tyr-Pro-Phe-*D*Phe-Asn-Asp-Tyr-Val-Orn-)

Following the same procedure as for gramicidin S afforded loloatin A **15** (76 mg, 59 μmol, 59%) as a white solid.

^{1}H NMR (400 MHz, CD_3OH, 4.94 ppm suppressed) δ 9.52–9.33 (m, 2H, N*H*-Asn, N*H*-*D*Tyr), 9.23 (s, 1H, N*H*-Orn), 9.00 (br s, 1H, N*H*-*D*Phe), 8.86 (d, J = 9.4 Hz, 1H, N*H*-Tyr), 8.63 (d, J = 8.7 Hz, 1H, N*H*-Leu), 8.43 (d, J = 4.3 Hz, 1H, N*H*-Asp), 8.14 (s, 1H, δ-Asn), 7.93 (d, J = 8.9 Hz, 1H, N*H*-Val), 7.64–7.49 (m, 2H, δ-Asn, N*H*-Phe), 7.26–7.11 (m, 10H, C*H*-arom-Phe, C*H*-arom-*D*Phe), 7.05 (d, J = 8.1 Hz, 2H, C*H*-arom-*D*Tyr), 6.81 (d, J = 8.1 Hz, 2H, C*H*-arom-Tyr), 6.69 (d, J = 8.1 Hz, 2H, C*H*-arom-*D*Tyr), 6.46 (d, J = 8.0 Hz, 2H, C*H*-arom-Tyr), 5.94–5.83 (m, 1H, α-*D*Phe), 5.53–5.45 (m, 1H, α-Orn), 4.68 (s, 1H, α-Asn), 4.60–4.51 (m, 2H, α-Tyr, α-Phe), 4.47–4.38 (m, 2H, α-*D*Tyr, α-Asp), 4.15 (d, J = 8.1 Hz, 1H, α-Pro), 3.42–3.33 (m, 3H, β-Asn, β-*D*Phe, δ-Pro), 3.27–2.75 (m, 8H, β-Asn, β-*D*Tyr, β-Tyr, δ-Orn, β-*D*Phe, δ-Orn), 2.58–2.49 (m, 1H, β-Asp), 2.46–2.14 (m, 7H, β-Asp, β-Phe, δ-Pro, β-Orn, β-Phe, β-Val), 1.89–1.43 (m, 6H, γ-Orn, β-Leu, γ-Leu, β-Leu, β-Pro), 1.38–1.32 (m, 1H, β-Pro), 1.19–1.04 (m, 13H, γ-Val, γ-Pro, δ-Leu), 0.46 (s, 1H, γ-Pro).

Note: α-Leu and α-Val are in the suppressed region of 4.94 ppm.

^{13}C NMR (101 MHz, CD_3OH) δ 175.2 (*C*OOH), 173.8 (*C*ONH$_2$), 173.7 (*C*ONH), 173.7 (*C*ONH), 173.5 (*C*ONH), 173.4 (*C*ONH), 173.3 (*C*ONH), 172.9 (*C*ONH), 172.8 (*C*ONH), 172.1 (*C*ONH), 172.1 (*C*ONH), 172.0 (*C*ONH), 157.9 (*C*qOH-Tyr), 157.2 (*C*qOH-Tyr), 138.9 (*C*q-Phe), 138.8 (*C*q-Phe), 131.6 (*C*H-arom-Tyr), 130.8 (*C*H-arom-Phe), 130.6 (*C*H-arom-Phe), 130.0 (*C*H-arom-Phe), 129.2 (*C*H-arom-Phe), 129.0 (*C*q-arom-Tyr), 127.6 (*C*H-arom-Phe), 127.2 (*C*q-arom-Tyr), 116.2 (*C*H-arom-Tyr), 116.1 (*C*H-arom-Tyr), 61.4 (α-Pro), 59.5 (α-Tyr), 58.5 (α-Val), 56.3 (α-*D*Tyr), 55.3 (α-Phe), 54.7 (α-*D*Phe), 54.1 (α-Asp), 52.6 (α-Orn), 52.3 (α-Leu), 50.9 (α-Asn), 47.6 (δ-Pro), 43.1 (β-Leu), 41.3 (β-*D*Phe), 40.6 (δ-Orn), 38.7 (β-Phe), 38.2 (β-Tyr), 37.3 (β-*D*Tyr), 36.6 (β-Asn), 36.5 (β-Asp), 33.2 (β-Val), 32.9 (β-Orn), 29.7 (β-Pro), 26.2 (γ-Leu), 24.4 (γ-Orn), 23.8 (δ-Leu), 23.3 (γ-Pro), 22.9 (δ-Leu), 19.6 (γ-Val), 19.1 (γ-Val).

HRMS (ESI-Orbitrap) calcd. for $C_{65}H_{85}N_{12}O_{15}$ $[M+H]^+$ 1273.62519, found 1273.62505.

Tyrocidin A 16
***cyclo*(-Leu-*D*Phe-Pro-Phe-*D*Phe-Asn-Gln-Tyr-Val-Orn-)**

Following the same procedure as for gramicidin S afforded tyrocidin A- **16** (75 mg, 59 μmol, 66%) as a white solid.

^{1}H NMR (400 MHz, CD_3OH, 4.94 ppm suppressed) δ 9.40–9.27 (m, 2H, N*H*-Asn, N*H*-*D*Phe), 9.15–9.02 (m, 2H, N*H*-Orn, N*H*-Gln), 8.92 (d, *J* = 9.7 Hz, 1H, N*H*-*D*Phe), 8.74 (d, *J* = 9.8 Hz, 1H, N*H*-Tyr), 8.50 (br s, 1H, N*H*-Leu), 8.10 (s, 1H, δ-Asn), 7.88 (d, *J* = 9.1 Hz, 1H, N*H*-Val), 7.57 (s, 1H, δ-Asn), 7.52 (d, *J* = 9.0 Hz, 1H, N*H*-Phe), 7.37 (d, *J* = 2.4 Hz, 1H, ε-Gln), 7.34–7.10 (m, 15H, C*H*-arom-Phe, C*H*-arom-*D*Phe), 6.91 (d, *J* = 2.3 Hz, 1H, ε-Gln), 6.86 (d, *J* = 8.0 Hz, 2H, C*H*-arom-Tyr), 6.50 (d, *J* = 7.8 Hz, 2H, C*H*-arom-Tyr), 5.87–5.77 (m, 1H, α-*D*Phe), 5.47 (q, *J* = 8.2 Hz, 1H, α-Orn), 4.70–4.51 (m, 3H, α-Asn, α-Tyr, α-Phe), 4.49–4.43 (m, 1H, α-*D*Phe), 4.13 (d, *J* = 8.0 Hz, 1H, α-Pro), 4.04 (q, *J* = 5.8, 5.1 Hz, 1H, α-Gln), 3.39–3.32 (m, 2H, δ-Pro, β-Asn), 3.30–3.04 (m, 6H, β-*D*Phe, β-Asn, β-*D*Phe, β-Tyr), 3.01–2.79 (m, 3H, δ-Orn, β-*D*Phe, δ-Orn), 2.41 (t, *J* = 13.2 Hz, 1H, β-Phe), 2.33–1.87 (m, 7H, β-Phe, δ-Pro, β-Val, β-Orn, γ-Gln, β-Orn), 1.85–1.55 (m, 6H, γ-Orn, β-Leu, β-Gln, γ-Leu), 1.52–1.41 (m, 2H, β-Pro, β-Leu), 1.37–1.33 (m, 1H, β-Pro), 1.15–1.00 (m, 13H, γ-Val, γ-Pro, δ-Leu), 0.48–0.34 (m, 1H, γ-Pro).

Note: α-Leu and α-Val are in the suppressed region of 4.94 ppm.

^{13}C NMR (101 MHz, CD_3OH) δ 178.4 (*C*ONH$_2$-Gln), 175.1 (*C*ONH$_2$-Asn), 173.9 (*C*ONH), 173.8 (*C*ONH), 173.4 (*C*ONH), 173.4 (*C*ONH), 173.3 (*C*ONH), 173.1 (*C*ONH), 173.0 (*C*ONH), 172.9 (*C*ONH), 172.2 (*C*ONH), 172.0 (*C*ONH), 157.2 (*C*qOH-Tyr), 138.9 (*C*q-*D*Phe), 138.7 (*C*q-*D*Phe), 136.8 (*C*q-Phe), 130.9 (*C*H-arom-Tyr), 130.8 (*C*H-arom-Phe), 130.5 (*C*H-arom-Phe), 130.0 (*C*H-arom-Phe), 129.5 (*C*H-arom-Phe), 129.3 (*C*H-arom-Phe), 129.2 (*C*H-arom-Phe), 129.1 (*C*q-arom-Tyr), 128.4 (*C*H-arom-Phe), 127.7 (*C*H-arom-Phe), 127.6 (*C*H-arom-Phe), 116.3 (*C*H-arom-Tyr), 61.4 (α-Pro), 59.6 (α-Val), 58.3 (α-Tyr), 56.7 (α-Gln), 56.0 (α-*D*Phe), 55.4 (α-Phe), 54.6 (α-*D*Phe), 52.4 (α-Orn), 52.3 (α-Leu), 50.9 (α-Asn), 47.6 (δ-Pro), 42.9 (β-Leu), 41.4 (β-*D*Phe), 40.6 (δ-Orn), 38.7 (β-Phe), 38.3 (β-Tyr), 37.3 (β-*D*Phe), 36.5 (β-Asn), 33.2 (β-Val), 32.6 (β-Orn), 31.6 (γ-Gln), 29.9 (β-Pro), 26.8 (β-Gln), 26.3 (γ-Leu), 24.4 (γ-Orn), 23.8 (δ-Leu), 23.3 (γ-Pro), 22.7 (δ-Leu), 19.5 (γ-Val), 19.1 (γ-Val).

HRMS (ESI-Orbitrap) calcd. for $C_{66}H_{88}N_{13}O_{13}$ $[M+H]^+$ 1270.66191, found 1270.66186.

Streptocidin A 17
***cyclo*(-Leu-*D*Phe-Pro-Leu-*D*Trp-Asn-Gln-Tyr-Val-Orn-)**

Following the same procedure as for gramicidin S afforded streptocidin A-**17** (45 mg, 35 μmol, 40%) as a white solid.

^{1}H NMR (400 MHz, CD_3OH, 4.94 ppm suppressed) δ 10.40 (s, 1H, N*H*-arom-Trp), 9.45 (d, *J* = 7.5 Hz, 1H, N*H*-Asn), 9.22 (br s, 1H, N*H*-*D*Phe), 9.07 (s, 1H, N*H*-Gln), 8.98 (br s, 1H, N*H*-Orn), 8.80 (d, *J* = 9.7 Hz, 1H, N*H*-Tyr), 8.38–8.27 (m, 1H, N*H*-Leu), 8.22 (d, *J* = 9.5 Hz, 1H, N*H*-*D*Trp), 8.08 (s, 1H, δ-Asn), 7.89 (d, *J* = 9.2 Hz, 1H, N*H*-Val), 7.60 (d, *J* = 8.0 Hz, 1H, C*H*-arom-*D*Trp), 7.54 (s, 1H, δ-Asn), 7.43–7.39 (m, N*H*-Leu), 7.39 (s, 1H, ε-Gln)), 7.34–7.20 (m, 6H, C*H*-arom-*D*Phe, C*H*-arom-*D*Trp), 7.09–6.96 (m, 2H, C*H*-arom-*D*Trp), 6.93 (s, 1H, ε-Gln), 6.90–6.82 (m, 3H, C*H*-arom-*D*Trp, C*H*-arom-Tyr), 6.55–6.44 (m, 2H, C*H*-arom-Tyr), 5.87–5.74 (m, 1H, α-*D*Trp), 5.55–5.45 (m, 1H, α-Orn), 4.75–4.64 (m, 1H, α-Asn), 4.63–4.55 (m, 1H, α-Tyr), 4.47–4.39 (m, 1H, α-*D*Phe), 4.26–4.20 (m, 1H, α-Pro), 4.17–4.08 (m, 1H, α-Leu), 4.05 (q, *J* = 6.1, 5.2 Hz, 1H, α-Gln), 3.62–3.56 (m, 1H, δ-Pro), 3.29–3.21 (m, 2H, β-Asn, β-*D*Trp), 3.19–2.96 (m, 7H, β-Asn, β-*D*Trp, β-Tyr, β-*D*Phe, δ-Orn), 2.91–2.78 (m, 1H, δ-Orn), 2.35 (q, *J* = 8.6 Hz, 1H, δ-Pro), 2.27–2.21 (m, 1H, β-Val), 2.07–1.96 (m, 4H, β-Orn, γ-Gln, β-Orn), 1.92–1.86 (m, 1H, β-Pro), 1.84–1.70 (m, 4H, γ-Orn, β-Gln), 1.65–1.35 (m, 5H, β-Pro, γ-Pro, γ-Leu, β-Leu), 1.28–1.17 (m, 2H, β-Leu, γ-Leu), 1.14 (d, *J* = 6.7 Hz, 3H, γ-Val), 1.11 (d, *J* = 6.8 Hz, 3H, γ-Val), 1.02–0.92 (m, 7H, δ-Leu, β-Leu), 0.63 (d, *J* = 6.6 Hz, 6H, δ-Leu), −0.15 (s, 1H, β-Leu).

Note: α-Leu and α-Val are in the suppressed region of 4.94 ppm.

^{13}C NMR (101 MHz, CD_3OH) δ 179.4 (*C*ONH$_2$-Gln), 178.4 (*C*ONH$_2$-Asn), 175.1 (*C*ONH), 174.7 (*C*ONH), 174.5 (*C*ONH), 174.0 (*C*ONH), 173.9 (*C*ONH), 173.3 (*C*ONH), 173.1 (*C*ONH), 173.0 (*C*ONH), 172.1 (*C*ONH), 172.0 (*C*ONH), 157.2 (*C*q-OH-Tyr), 138.2 (*C*q-arom-*D*Phe), 136.8 (*C*q-arom-*D*Trp), 130.9 (*C*H-arom-Tyr), 130.7 (*C*H-arom-*D*Phe), 130.5 (*C*H-arom-*D*Phe), 129.5 (*C*H-arom-*D*Phe), 129.1 (*C*q-arom-Tyr), 128.4 (*C*q-arom-*D*Trp), 125.3 (*C*H-arom-*D*Trp), 122.5 (*C*H-arom-*D*Trp), 120.1 (*C*H-arom-*D*Trp), 120.0 (*C*H-arom-*D*Trp), 116.2 (*C*H-arom-Tyr), 112.2 (*C*H-arom-*D*Trp), 111.1 (*C*q-arom-*D*Trp), 61.7 (α-Pro), 59.6 (α-Val), 58.3 (α-Tyr), 56.6 (α-Gln), 56.0 (α-*D*Phe), 53.2 (α-*D*Trp), 52.6 (α-Orn), 52.4 (α-Leu), 52.4 (α-Leu), 50.9 (α-Asn), 47.7 (δ-Pro), 42.3 (β-Leu), 40.6 (δ-Orn), 40.4 (β-Leu), 38.2 (β-Tyr), 37.8 (β-*D*Phe), 36.5 (β-Asn), 33.0 (β-Val), 32.9 (β-Orn), 31.6 (γ-Gln), 31.6 (β-Trp), 30.1 (β-Pro), 26.7 (β-Gln), 26.2 (γ-Leu), 25.5 (γ-Leu), 24.3 (γ-Orn), 24.0 (δ-Leu), 23.6 (γ-Pro), 23.4 (δ-Leu)), 22.2 (δ-Leu), 20.7 (δ-Leu), 19.5 (γ-Val), 19.3 (γ-Val).

HRMS (ESI-Orbitrap) calcd. for $C_{65}H_{91}N_{14}O_{13}$ $[M+H]^+$ 1275.68846, found 1275.68889.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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