Solid-Phase Syntheses of Loloatins A−C

Jürgen Scherkenbeck,*[a][‡] Heru Chen,[b] and Richard K. Haynes*[a]

Keywords: Natural products / Peptides / Protecting groups / Resins / Synthesis

The cyclic decapeptides Loloatin A (cyclic L-valyl-L-ornithyl-L-leucyl-D-tyrosyl-L-prolyl-L-phenylalanyl-D-phenylalanyl-L-asparaginyl-L-aspartyl-L-tyrosyl), loloatin B (cyclic L-valyl-L-ornithyl-L-leucyl-D-tyrosyl-L-prolyl-L-phenylalanyl-D-phenylalanyl-L-asparaginyl-L-aspartyl-L-trytophanyl) and loloatin C (cyclic L-valyl-L-ornithyl-L-leucyl-D-tyrosyl-L-prolyl-L-tryptophanyl-D-phenylalanyl-L-asparaginyl-L-aspartyl-L-tryptophanyl) have been synthesized by Fmoc-based solid-phase peptide synthesis, commencing with Asp linked to polystyrene RAM resin through its side chain, and by on-resin cyclization of the linear decapeptide through Asp and Asn, followed by cleavage of Asp from the resin. Through the use of a unique combination of DMF/dichloroethane solvent mixture in the coupling steps, and careful monitoring of both coupling and Fmoc deprotection steps, the final cyclic peptides were obtained in overall yields of 31–37%.

Introduction

Loloatins are a relatively new family of cyclic decapeptides, originally isolated from laboratory cultures of a tropical marine bacterium collected from the Great Barrier Reef off the southern coast of Papua New Guinea.[1]

By a combination of spectroscopic analysis − mainly involving NMR spectroscopy − and chemical degradation, it was demonstrated that the loloatins shared two D-amino acids − D-Phe and D-Tyr (Figure 1) − as characteristic structural features, and that they differed in the amino acids in positions 5, 6, and 10. The loloatins have structural features in common with the tyrocidines, cyclic decapeptides that have been isolated from *Bacillus brevis* species,[2] namely four aromatic amino acid residues, two of which have the D configuration. The presence of two aromatic D-amino acids is also a feature of gramicidin S. However, the loloatins, unlike the tyrocidines, have zwitterionic character due to the presence of both ornithine and aspartic acid residues.

Loloatins A−D display potent antibiotic activity against methicillin-resistant strains of *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* sp. (VRE), and penicillin-resistant *Streptococcus pneumoniae*. Interestingly, only loloatin C also shows antibacterial activity against the gram-negative bacterium *Escherichia coli*, while loloatin D was found to be four times less active than loloatins A−C against *E. coli*. These structure-activity relationships demonstrate that rather subtle changes in the decapeptide structure can have a significant impact on the antimicrobial activity. In all cases, loloatin C appears to be

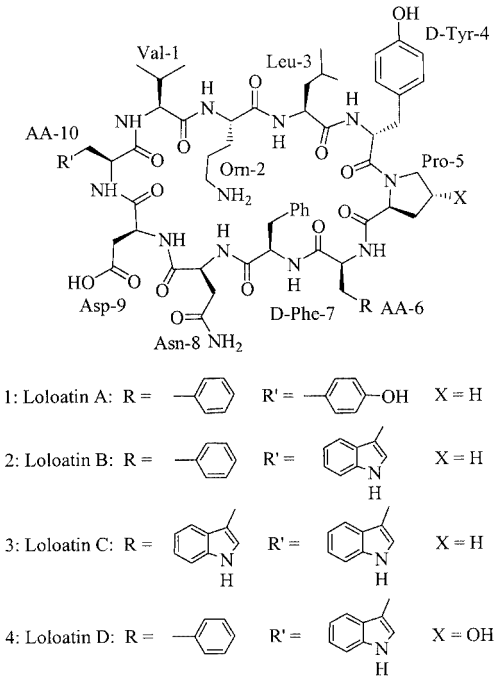


Figure 1. Structures of the four loloatins

[a] Department of Chemistry, The Hong Kong University of Science and Technology
Clear Water Bay, Kowloon, Hong Kong, P. R. China
Fax: (internat.) + 852/2358-1594
E-mail: haynes@ust.hk
[b] Department of Chemistry, The Hong Kong University of Science and Technology
Clear Water Bay, Kowloon, Hong Kong, P. R. China
[‡] Present address: Bayer AG, Central Research
51368 Leverkusen, Germany
Fax: (internat.) + 49-(0)214/3071-157
E-mail: juergen.scherkenbeck.js@bayer-ag.de

the compound with the highest activity. Of significance is the observation that the MICs of loloatins A−C indicate antibacterial activity against gram-positive bacteria at least similar to that of tyrocidine C, the most potent antibiotic in the tyrocidine family. However, in contrast to loloatin C, the tyrocidines do not show activity against gram-negative bacteria.

The biological mechanism of the loloatins has not yet been established. However, the mechanisms of action of the tyrocidines and gramicidins have been extensively examined. Tyrocidine A may play a regulatory role during sporulation of *B. brevis*,[3] probably due to interaction with superhelical chromosomal DNA, resulting in a packaging of the DNA when the bacteria enter the sporulation phase.[4] The tyrocidines have also been shown to interact with phospholipid membranes, creating an ion channel through the membrane.[5] This interruption of membrane function may explain the antimicrobial action of the tyrocidines. In solution, tyrocidine A appears to adopt a single conformation with type II′ and III β-turns and twisted antiparallel strands with intra-annular hydrogen bonds between the pairs of opposing peptide groups.[6] The backbone conformation of residues 2−6 (Figure 2) closely resembles that found in the crystal structure of gramicidin S.[7] Gramicidin S displays potent activity against gram-negative and gram-positive bacteria, and is also fungicidal. Unfortunately, however, gramicidin S is nonspecific, and displays high hemolytic activity, limiting its use as an antibiotic. Like that of tyrocidine A, the primary mode of the antimicrobial action of gramicidin S appears to be due to its ability to disrupt the integrity of the lipid bilayer of the inner membrane of bacterial cells. The two tripeptide Val-Orn-Leu sequences in gramicidin S form an antiparallel β-sheet terminated on each side by type 2′ β-turns formed by the D-Phe-Pro sequences. The key feature of the molecule is its amphiphilicity, due to the lipophilic and positively charged ornithine side chains projecting on one side, and the hydrophobic Leu and Val side chains projecting from the other. This "sidedness" appears to play a crucial role in the maintenance of high levels of antibiotic activity in analogues of gramicidin S.[8]

The isolation of yet another class of cyclic decapeptide structurally akin to the tyrocidines and gramicidin S is clearly important. Those structural features that differentiate the loloatins from the latter require careful analysis in terms of a potentially specific mode of action, a lack of which limits the applicability of tyrocidine A and gramicidin S. This applies in particular to the presence *both* of the Orn and the Asp residues, which should markedly influence the potential amphiphilicity of the lolatins relative to gramicidin S. Evaluation of the biological activity of the loloatins and detailed structural analysis should then provide useful information regarding the design features required to make these families of peptides more selective in their action, and consequently may enable access to functionally useful antibiotics. Therefore, as part of a program aimed at mapping structure-activity relationships in cyclic peptides and developing new drugs based on these entities, it was necessary to prepare quantities of loloatins A−C in order to establish their three-dimensional structures.

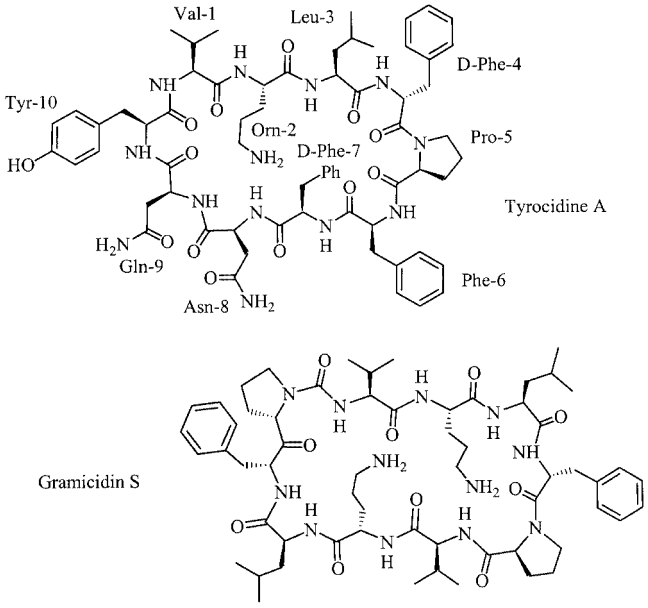


Figure 2. Structures of tyrocidine A and gramicidin S

Results and Discussion

Synthetic Strategy

The fluorenylmethoxycarbonyl (Fmoc) strategy for solid-phase synthesis (Fmoc-SPPS)[9] has found broad application in peptide synthesis, and its effectiveness has been underscored in the construction of notably difficult cyclic peptides.[10] Because of these advantages, we selected this method for our synthesis of the loloatins. The synthesis consists of two parts, chain elongation and the final cyclization. Whilst chain elongation is relatively straightforward, formation of the macrocycle is a difficult challenge, and choice of the most effective strategy is crucial. In solid-phase peptide synthesis, several on-resin cyclization strat-

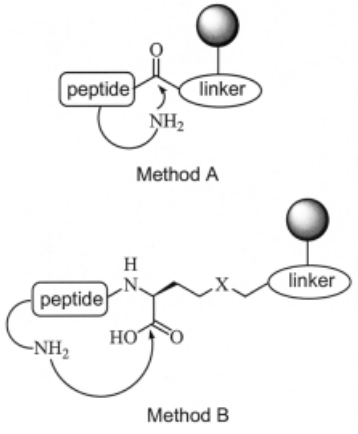
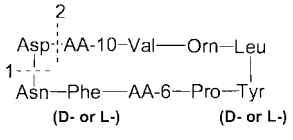


Figure 3. Two methods for peptide cyclization on solid phase

egies for synthesis of cyclic peptides have been developed, and of these, the two illustrated in Figure 3 appeared to be most suitable. Method A involves the use of electrophilic linkers which are labile to free amines,[11] wherein cyclization also effects concomitant cleavage from the solid support. Method B involves attachment of an amino acid side chain to the solid support[12] followed by on-resin cyclization and an ensuing cleavage/deprotection.

From previous experience involving exploratory syntheses of unnatural all-L-loloatin C and smaller cyclic hexapeptides based on the loloatin skeleton, in which cyclization at the carbonyl group attached directly to the linker had proven to be a difficult step, we selected method B for the cyclization step. The method requires the use of an amino acid − Asp or Asn − that can be attached to the resin through a functionalized side chain. Since the direction of peptide bond formation in solid-phase chemistry is from C to N, the use of Asp as the amino acid linked to the resin would require disconnection at site 2 (Figure 4), but use of this site would elicit problems with the sterically demanding amino acids Tyr or Trp, through which ring-closure would have to take place. Another difficulty with this cyclization would be the potential acylation of the indolyl nitrogen atom in tryptophan. Disconnection at site 1 would require the use of Asp as the first amino acid, to be linked to the resin through its side chain. Two advantages are apparent from this choice. The first is that the steric hindrance between Asp and Asn in the cyclization reaction should be significantly less than that between Asp and Trp. Secondly, Asn may be derived from Asp simply by use of a RAM-type resin, which releases an amide after cleavage. For these reasons, the approach based on an on-resin macrocyclization between Asp and Asn was followed.

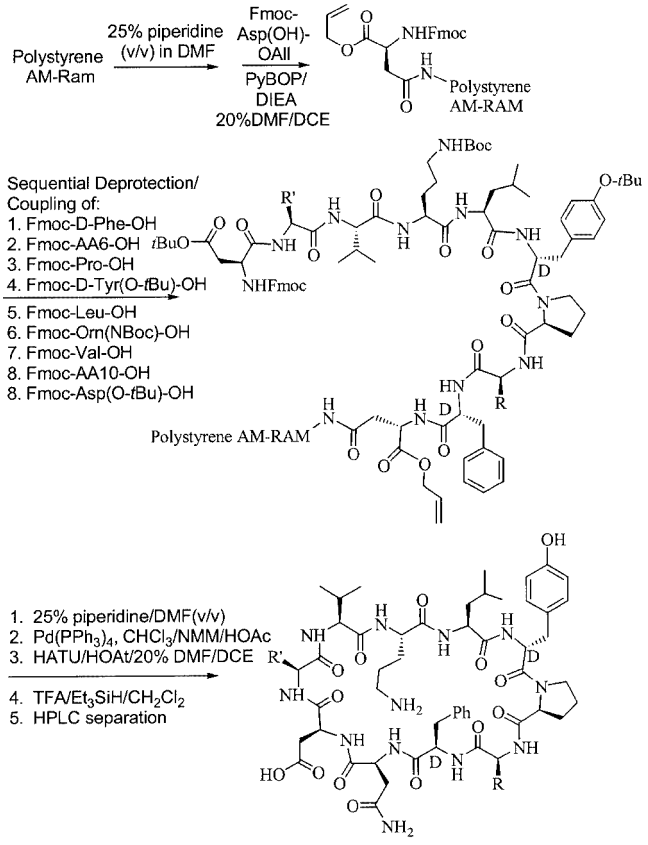


	AA-6	AA-10
Loloatin A	Phe	Tyr
Loloatin B	Phe	Trp
Loloatin C	Trp	Trp

Figure 4. Retrosynthetic analysis of loloatins A−C

Synthesis

For the synthesis of the natural loloatins, PyBOP [benzotriazol-1-yloxy(tripyrrolidino)phosphonium hexafluorophosphate][13] is used as the coupling reagent in each acylation step, while for the cyclization step, HATU [*O*-(7-azabenzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate] together with 1-hydroxy-7-azabenzotriazole (HOAt) is used. After considerable experimentation with standard solvent combinations, we eventually employed a DMF (20%)/1,2-dichloroethane (DCE) mixture, the use of which in solid-phase peptide synthesis is reported here for the first time. DCE is more hydrophobic than dichloromethane, yet possesses very similar solvating properties; the hydrophobicity is believed to enhance the solvation of the peptide-linked resin. For the Fmoc-deprotection steps, two successive treatments with 25% piperidine in DMF were used. Polystyrene AM RAM was used as solid support. The details are shown in Scheme 1.



Scheme 1. Solid-phase total synthesis of the natural loloatins

The reaction scale was usually 0.1−0.2 mmol. For the coupling of the first amino acid (Fmoc-Asp-OAll) to the resin, 0.4 mmol of the derivative and coupling reagent − that is a fourfold excess of each − were used in order to ensure complete acylation. In general, the peptide/resin was agitated with 10 mL of 20% DMF in DCE by passage of a stream of nitrogen through the mixture. In a separate flask, the amino acid derivative and PyBOP were dissolved in the minimum possible amount of DMF, usually about 1.5 mL. Then an equivalent amount of diisopropylethylamine (DIEA) was added to initiate the reaction. After about 10−15 min, the solution was transferred by syringe to the peptide/resin mixture for the coupling reaction. Bromophenol blue (1−2 drops) was added to monitor the coupling process. When the color of the peptide-linked resin had changed from dark blue to the original blue of the dye, the coupling was terminated. The solution was separated from the resin by filtration, and the resin was washed thoroughly with fresh DMF, in order to remove starting materials and by-products.

Deprotection of Fmoc-protected amino groups was carried out carefully, with use of quantitative monitoring.[14]

In this process, ordinarily two treatments of the peptide-linked resin with 25% piperidine in DMF were employed. Reaction times were 25 and 5 min, respectively. Both solutions were separated by filtration, and the resins were washed three times with fresh DMF. The filtrates and washings were collected in a 50-mL volumetric flask. This solution was diluted to a suitable degree (usually by a factor of 50) and its UV spectrum was measured in order to determine quantitatively the concentration of the fulvene−piperidine adduct obtained from the Fmoc removal. The overall efficiency of the coupling/deprotection step could hence be established; the results are listed in Table 1. It is apparent that the acylation efficiencies were nearly 100% when the quantity of amino acid derivatives was around 2.5 molar excess in relation to the free amino groups on the peptide-linked resin. In step 3 of the synthesis of loloatin C, however, a higher excess of amino acid derivative and coupling reagent had to be used to optimize the coupling step. A likely reason for the lower yield (89.5%) in this step is the steric interaction occurring between the bulky 2-indolyl group in the Trp and the phenyl group in D-Phe.

Formation of diketopiperazine did not intervene during removal of the Fmoc group of D-Phe, even though this is usually a problem when the peptide is linked to the resin by an ester linkage.[15] In our case, the less reactive amide linkage reduces the possibility of formation of the diketopiperazine. In addition, the amide linkage to the polystyrene AM-RAM had a sterically bulky environment, and so attack of the free terminal amino group at this point is inhibited.

Removal of the allyl group with $Pd(PPh_3)_4$/HOAc/NMM (Scheme 1) was effected after removal of the Fmoc protecting group. As it was not easy to monitor this reaction, a longer reaction time and a fivefold excess of reagent were employed to ensure complete deprotection. Whilst alkylation of free terminal amino groups by the (π-allyl)Pd complex formed during the deprotection is possible, the use of a large amount of the nucleophilic scavenger NMM/HOAc used in this process apparently inhibites this reaction; no *N*-alkylation was observed to take place.

The final step was cyclization. Bromophenol blue was also employed to monitor the course of the reaction in this step. Unfortunately, the color change was not as clear as in previous steps, because the additive (HOAt) became deep red on admixture with disopropylethylamine, thus partly obscuring the endpoint of the cyclization. HOAt nevertheless had to be used as it enhances the coupling efficiency, and is claimed to reduce epimerization.[16]

Analytical HPLC performed on the crude loloatin products indicated the presence of relatively small amounts of contaminants, and it was relatively straightforward to purify the loloatins; usually only one preparative HPLC run was required to obtain the pure peptide. Table 2 gives the relative purities and overall yields of loloatins A, B, and C; overall yields were between 30 and 40%. In comparison, use of a peptide synthesizer to prepare all-L-loloatin A, a synthesis in which the cheaper all-L-amino acids were used, provided an overall yield of just 10%. All the compounds were characterized by FAB-MS and HRMS (Table 2). ^1H, ^1H-^1H COSY, TOCSY, and NOESY data were also acquired, and confirmed the correct sequences. All the spin systems in each of the loloatins have been assigned (Table 3). The data were in reasonable agreement with those recorded for the material isolated from the natural source.[1]

Table 2. Synthesis of loloatins A, B, and C

	Purity[a] (%)	Overall yield (%)	FAB-MS $[M + H]^+$	HR-MS $[M + 2H]^{++}$
Loloatin A	97.0	31	1273.7	637.31613
Loloatin B	98.2	35	1296.9	648.82399
Loloatin C	99.6	37	1335.9	668.33002

[a] Purity determined by HPLC. Eluent: 0.1% TFA in water (component A) and methanol (component B); method: isocratic (25% A/75% B); flow: 1.0 mL/min; wavelength: 224 nm; column: Xterra-C_{18}, 4.6 × 250mm.

Table 1. Acylation efficiencies in each coupling step in synthesis of loloatins

Coupling step	Loloatin A		Loloatin B		Loloatin C	
	Mol ratio of free amino groups to coupling reagent	Yield (%)	Mol ratio of free amino groups to coupling reagent	Yield (%)	Mol ratio of free amino groups to coupling reagent	Yield (%)
1	1:2.47	100	1:2.45	98.5	1:1.70	93
2	1:2.47	98.5	1:3.12	100	1:1.85	96
3	1:3.09	96	1:3.12	99	1:2.38	89.5
4	1:3.09	97	1:3.43	99	1:2.66	100
5	1:3.39	100	1:3.48	98	1:2.66	100
6	1:3.39	99	1:3.54	100	1:2.66	93
7	1:3.39	97	1:3.54	97	1:2.66	97
8	1:3.48	100	1:3.65	98	1:2.76	100
9	1:3.48	100	1:3.74	100	1:2.76	98
10	1:3.48	96	1:3.74	99	1:2.82	100

Table 3. ^{1}H NMR spectroscopic data (500 MHz; [D_6]DMSO) for loloatins A, B, and C

	Loloatin A: Phe^6 Tyr^{10}	Loloatin B: Phe^6 Trp^{10}	Loloatin C: Trp^6 Trp^{10}
Val^1			
NH	7.58(d)	7.64(d)	7.67(d)
αCH	4.64(m)	4.66(m)	4.69(m)
βCH	2.06(m)	2.13(m)	2.14(m)
γCH_3	0.99(d)	1.00(d)	1.05(d)
$\gamma\ CH_3$	1.01(d)	1.04(d)	1.05(d)
Orn^2			
NH	8.98(d)	8.99(d)	9.00(d)
αCH	5.38(m)	5.39(m)	5.42(m)
βCH_2	1.78, 1.90(m)	1.78, 1.90(m)	1.79, 1.92(m)
γCH_2	1.76(m)	1.76(m)	1.82(m)
δCH_2	2.84, 3.00	2.86, 3.0	2.86, 3.00
δNH	7.55(bs)	7.52(bs)	7.53(brs)
Leu^3			
NH	8.02(brs)	8.00(brs)	8.08(brs)
αCH	4.64(m)	4.63(m)	4.66(m)
βCH_2	1.34, 1.49(m)	1.33, 1.47(m)	1.37, 1.52(m)
γCH	1.55(m)	1.60(m)	1.59(m)
δCH_3	1.00(d)	1.04(d)	1.03(d)
δCH_3	1.09(d)	1.04(d)	1.03(d)
d-Tyr^4			
NH	9.31	9.31(brs)	9.31(brs)
αCH	4.32(m)	4.33(m)	4.33(m)
βCH_2	2.79, 2.92(m)	2.79, 2.92(m)	2.80, 2.95(m)
o-CH	7.08(d)	7.05(d)	7.07(d)
m-CH	6.72(d)	6.72(d)	6.70(d)
p-COH	Not observed	Not observed	Not observed
Pro^5			
αCH	4.19	4.15	4.14
βCH_2	1.3, 1.52(m)	1.31, 1.55(m)	1.53, 1.32(m)
γCH_2	1.16, 0.50(m)	1.18, 0.51(m)	1.14, 0.44(m)
$\delta\ CH_2$	3.40, 2.34(m)	3.4, 2.34(m)	3.40, 2.27(m)
Phe^6/Trp^6			
NH	7.26(d)	7.28(d)	7.33(d)
αCH	4.58(m)	4.58(m)	4.60(m)
βCH_2	2.30(m)	2.32(m)	2.54, 2.40(m)
o-CH/C	7.18	7.15(m)	
m-CH/CH	7.18	7.15	7.87(m)
p-CH/CH	7.10	7.06	7.04
/CH			7.09
/CH			7.34
/NH			10.72(sh)
/CH			7.11(d)
d-Phe^7			
NH	9.16(d)	9.16(d)	9.31(brs)
αCH	5.66(m)	5.68(m)	5.73(m)
βCH_2	2.81, 3.06(m)	2.83, 3.12(m)	2.90(m)
o-CH	7.18(m)	7.18(m)	7.16(m)
m-CH	7.18	7.18(m)	7.16(m)
p-CH	7.09	7.10(d)	7.09(m)
Asn^8			
NH	9.10(d)	9.13(d)	9.14(d)
αCH	4.56(m)	4.57(m)	4.62(m)
βCH_2	3.06(m)	3.26(m)	3.12(m)
NH_2	8.15, 7.55(bs)	8.15, 7.55(brs)	8.18, 7.56(brs)
Asp^9			
NH	8.45(d)	8.45(d)	8.45(d)
αCH	4.32(m)	4.34(m)	4.37(m)
βCH_2	2.42, 2.47(m)	2.35, 2.43(m)	2.49, 2.39(m)
Tyr^{10}/Trp^{10}			
NH	8.65(d)	8.75(d)	8.75(d)
αCH	4.39(m)	4.56(m)	4.61(m)
βCH_2	3.00(m)	3.26(m)	3.27(m)
o-CH/C	7.04		
m-CH/CH	6.74	7.58	7.85
p-COH/CH	Not observed	7.08	7.04
/CH		7.18	7.09
/CH		7.44	7.42
/NH		10.91(sh)	10.91(sh)
/CH		7.14(d)	7.13(d)

Conclusion

In summary, we have developed an efficient, on-resin, solid-phase synthesis of loloatins A, B, and C, the key features of which are the use of the new DMF/dichloroethane solvent combination for the peptide coupling steps, and the use of monitoring to provide optimal yields in the coupling and deprotection steps.

Experimental Section

General Remarks: *N*,*N*-Dimethylformamide (Millipore, peptide synthesis grade) was dried over 4 Å molecular sieves. Chloroform and dichloromethane were freshly distilled from CaH_2 and stored over 4 Å molecular sieves; piperidine was dried over KOH and distilled immediately prior to use; 1,2-dichloroethane (DCE) was distilled from phosphorus pentoxide under nitrogen. Other commercial reagents and solvents were used as received. Sources of reagents for peptide synthesis were as follows: Fmoc-protected amino acids, 1.0 M HOBt and 1.0 M DCCI in *N*-methylpyrrolidone solution, Applied Biosystem; DCC, $Pd(PPh_3)_4$, HBTU, Aldrich; PyBOP, TBTU, Nova Biochem; TentaGel S RAM resin, Polystyrene AM RAM resin, RAPP Polymere. All the resins used in the synthesis were dried at room temp. in vacuo for several hours. Melting points were determined with an IA 9100 MK2 Electrothermal, UV spectra were recorded with a Milton ROY 3000 Spectrometer, and optical rotation values were determined with a Perkin−Elmer 241 Polarimeter. Reversed-phase HPLC was carried out with a Waters 717/600/486 chromatograph with Waters Nucleosil C_{18} columns with gradients of acetonitrile/0.1% TFA in water, or methanol/0.1% TFA in water. Mass spectra were obtained in FAB mode with a Finnigan mat TSQ 7000 machine. NMR spectra were recorded with a Varian Unity INOVA 500 MHz instrument. Because of the exact matches of the molecular weights determined by FAB-MS and HR-MS with the calculated values, elemental analyses were not carried out.

Procedure for Synthesis of Natural Loloatins: The resin-linked linear decapeptides were assembled manually in the C-to-N direction by iterative coupling reactions. The synthesis was carried out on a $1.5-2.5 \times 10^{-4}$ mol scale, starting with 0.2−0.3 g polystyrene AM-RAM resin (capacity: 0.78 mmol/g) in a small tube equipped with sintered glass frit and take-off head. The resin was first treated with 25% piperidine in DMF (v/v) to release the free amino group. Coupling reactions were carried out in 20% *N*,*N*-dimethylformamide (DMF) in 1,2-dichloroethane (DCE) (v/v) medium with a threefold excess of PyBOP/DIEA and a threefold excess of the Fmoc amino acid derivative. The mixture was agitated by passage of 99.99% nitrogen through the frit. Usually, the coupling reaction was allowed to run for 2−3 h. Bromophenol blue in DMF solution (1−2 drops) was added to monitor the coupling process. A change in the color of the resin from dark blue to its original color signified the completion of the reaction. Solvent was removed by filtration through the frit under reduced pressure, and the resin was washed five times with DMF to remove excess starting materials and by-products. Removal of the terminal Fmoc group was effected with two treatments with 25% piperidine in DMF, with reaction times of 25 and 5 min, respectively. After removal of solvent, washing some five times with DMF was again employed to ensure that all of the residual piperidine had been completely removed. The resin-linked linear decapeptide was dried thoroughly under vacuum. A solvent mixture of chloroform, *N*-methylmorpholine (NMM), and

acetic acid (HOAc) (75:4:2, 15 mL) was then added to swell the resin, with N_2 bubbling. After 10 min, a threefold excess of $Pd(PPh_3)_4$ was added to initiate the removal of allyl group; the mixture was left for 12 h to ensure that deallylation was complete. The resin was washed with DCM and DMF, and then treated with HATU/HOAt/DIEA (fivefold molar excess) for at least 24 h to cyclize the peptide. The resin was then washed with DMF and DCM, and treated twice with a mixture of TFA/Et_3SiH/DCE (18:1:1) at room temperature for 1.5 h. The solutions were separated from the resin by filtration, and the combined filtrate was concentrated under vacuum at room temperature. The residual peptides were purified by reversed-phase HPLC (55 min gradient 50−99% methanol). Fractions containing the products were collected and submitted to freeze-drying to give the pure product. FAB-MS and NMR data were acquired to confirm the structure of target compounds.

Loloatin A [Cyclic (L-valyl-L-ornithyl-L-leucyl-D-tyrosyl-L-prolyl-L-phenylalanyl-D-phenylalanyl-L-asparaginyl-L-aspartyl-L-tyrosyl)]: The quantity of the free amino group determined from 0.2247 g of crude polystyrene AM-RAM resin (capacity: 0.78 mmol/g) was 0.1285 mmol. Pure compound obtained by reversed-phase HPLC purification: white solid; total synthesis yield: 67.1 mg (30.9%); m.p. 230.4−235.5 °C (ref.[1] 229−232 °C). $[\alpha]_D^{25} = -87.9$ (ethanol, $c = 0.182$) [ref.[1] −88 (ethanol)]. UV (ethanol): λ_{max} (ε) = 204.7 (46186.95), 224 (shoulder, 22211.34), 278.4 (3114.1). FAB-MS: m/z = 1273.8 [calcd. for M + H ($C_{65}H_{85}N_{12}O_{15}$) 1273.45]. HRMS: m/z = 637.31613 for $[M + 2H]^{++}$. ^{1}H NMR: see Table 3.

Loloatin B [Cyclic (L-valyl-L-ornithyl-L-leucyl-D-tyrosyl-L-prolyl-L-phenylalanyl-D-phenylalanyl-L-asparaginyl-L-aspartyl-L-tryptophanyl)]: The quantity of free amino group determined from 0.2178 g crude polystyrene AM-RAM resin (capacity: 0.78 mmol/g) was 0.1228 mmol. Pure compound obtained by reversed-phase HPLC purification: slightly yellowish solid; total synthesis yield: 55.2 mg (34.6%); m.p. 229.4−236.5 °C (ref.[1] 229−233 °C). $[\alpha]_D^{25} = -81.9$ (ethanol, $c = 0.072$) [ref.[1] −80 (ethanol)]. UV (ethanol): λ_{max} (ε) = 204.3 (47588.5), 220 (shoulder, 29666.82), 280.2 (4637.3). FAB-MS: m/z = 1296.9 [calcd. for M + H ($C_{67}H_{86}N_{13}O_{14}$) 1297.50]. HRMS: m/z = 648.82399 for $[M + 2H]^{++}$. ^{1}H NMR: see Table 3.

Loloatin C [Cyclic (L-valyl-L-ornithyl-L-leucyl-D-tyrosyl-L-prolyl-L-tryptphanyl-D-phenylalanyl-L-asparaginyl-L-aspartyl-L-tryptophanyl)]: The quantity of free amino group determined from 0.253 g crude polystyrene AM-RAM resin (capacity: 0.78 mmol/g) was 0.1973 mmol; pure compound obtained by reversed-phase HPLC purification: yellowish solid; total synthesis yield: 96.5 mg (36.6%); m.p. 241.6−246.5 °C (ref.[1] 239−243 °C). $[\alpha]_D^{25} = -79.1$ (ethanol, $c = 0.196$) [ref.[1] −76 (ethanol)]. UV (ethanol): λ_{max} (ε) = 205.8 (537289.7), 221.6 (55092.4), 280.9 (9443.4). FAB-MS: m/z = 1336.9 [calcd. for M + H ($C_{69}H_{87}N_{14}O_{14}$) 1336.54]. HRMS: m/z = 668.33002 for $[M + 2H]^{++}$. ^{1}H NMR: see Table 3.

Acknowledgments

We express our thanks to Bayer AG for their generous financial support of this work at the Hong Kong University of Science and Technology. Support from the Hong Kong University of Science and Technology (Grant HKUST DAG01/02.SC10) is also gratefully acknowledged.

[1] J. M. Gerard, P. Haden, M. T. Kelly, R. J. Anderson, *J. Nat. Prod.* **1999**, *62*, 80−85.
[2] E. Katz, A. L. Demain, *Bacteriol. Rev.* **1977**, *41*, 449−474.
[3] W. Danders, M. A. Marahiel, M. Krause, M. Kosui, T. Kato, N. Izumiya, H. Kleinkauf, *Antimicrob. Agents Chemother.* **1982**, *22*, 785−590.
[4] A. Bohg, H.-J. Ristow, *Eur. J. Biochem.* **1986**, *160*, 587−591; A. Bohg, H.-J. Ristow, *Eur. J. Biochem.* **1987**, *170*, 253−258.
[5] F. J. Aranda, B. de Kruijff, *Biochim. Biophys. Acta* **1988**, *937*, 195−203.
[6] A. P. Tonge, P. Murray-Rust, W. A. Gibbons, L. K. McLachlan, *J. Comput. Chem.* **1988**, *9*, 522−38.
[7] B. A. Wallace, "Gramicidin and Related Ion Channel-Forming Peptides" in *Novartis Foundation Symposium 225*, Wiley, New York **1999**, pp. 23−32 and references therein.
[8] E. J. Prenner, A. H. Lewis, R. N. Elhaney, *Biochem. Biophys. Acta* **1999**, *1462*, 201−221.
[9] E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, B. J. Williams, *J. Chem. Soc., Chem. Commun.* **1978**, 537; E. Atherton, C. J. Logan, R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1* **1981**, 538.
[10] M. Quibell, T. Johnson, "Difficult Peptides" in *Fmoc Solid Phase Peptide Synthesis: A Practical Approach* (Eds.: W. C. Chan, P. D. White), Oxford University Press, Oxford, **2000**, pp.115−183; S. L. Mellor, D. A. Wellings, J.-A. Fefrentz, M. Paris, J. Martinez, N. J. Ede, A. M. Bray, D. J. Evans, G. B. Bloomberg, "Synthesis of Modified Peptides" in *Fmoc Solid Phase Peptide Synthesis: A Practical Approach* (Eds.: W. C. Chan, P. D. White), Oxford University Press, Oxford, New York, **2000**, pp.115−183.
[11] N. Nishino, M. Xu, H. Mihara, T. Fujimoto, Y. Ueno, H. Kumagai, *Tetrahedron Lett.* **1992**, *33*, 1479−1482; N. Nishino, M. Xu, H. Mihara, T. Fujimoto, M. Ohba, Y. Ueno, K. Kumagai, *J. Chem. Soc., Chem. Commun.* **1992**, 180−181; G. Osapray, A. Profit, J. W. Taylor, *Tetrahedron Lett.* **1990**, *31*, 6121−6124.
[12] A. Kapurniotu, J. W. Taylor, *Tetrahedron Lett.* **1993**, *34*, 7031−7034; S. A. Kates, N. A. Sole, C. R. Johnson, D. Hudson, G. Barany, F. Albericio, *Tetrahedron Lett.* **1993**, *34*, 1549−1552; P. Rovero, L. Quarara, G. Fabbri, *Tetrahedron Lett.* **1991**, *32*, 2639−2642.
[13] J. Coste, D. Le-Nguyen, B. Castro, *Tetrahedron Lett.* **1990**, *31*, 205−208.
[14] H. R. Chen, R. K. Haynes, J. Scherkenbeck, unpublished work.
[15] J. M. Stewart, J. D. Young, *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chemical Company, Rockford, Illinois, **1984**, pp. 30−41.
[16] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

Received January 9, 2002
[O02010]