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Water-soluble longibramide E analogs: synthesis and antibacterial activity

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Introduction/Aim

The overuse and misuse of antibiotics have significantly contributed to the emergence of antibiotic-resistant bacteria, making infections harder to treat, and increasing the risk of spread. To address this challenge, the development of new classes of antibacterial drugs is crucial. In this context, peptaibols are promising membrane-active peptides since their antibacterial action does not involve specific cellular targets, reducing the likelihood of bacterial resistance development. Recently, Zhang *et al.* 2022 [1] isolated a series of new 11-residue peptaibols, denominated longibramides, from *Trichoderma longibrachiatum*. Among them, longibramide E sparked us particular interest due to its activity against *S. aureus* MRSA T144 [1]. However, a major drawback to the exploitation and application of peptaibols is their poor water solubility and longibramide E offers no exception to this limitation. Our current study demonstrated that the removal of the acetylation in the *N*-terminus and the replacement of the relatively expensive *C*-terminal leucinol moiety by a leucine amide markedly improve peptide hydrophilicity. Aiming at the further improvement of peptide water solubility, other Lys-containing analogs have been synthesized. Furthermore, based on the growing body of evidence that acylation of the *N*-terminus with a fatty acid increases peptide antimicrobial properties [2], a longibramide E analog featuring a *n*-octanoyl in the *N*-terminus was synthesized. Both manual and automated solid phase synthesis were used to compare synthesis method efficiency for this class of metabolites, whose antibacterial activity was determined against susceptible strains and multidrug-resistant clinical isolates of Gram-positive and Gram-negative bacteria.

Methodology

Peptides **1**, **3**, **6-9** were synthesized using Symphony® X (Gyros Protein Technologies) at a 100 µmol scale, using a low loading rink amide AM resin (0.29 mmol/g), and following a Fmoc/*t*Bu orthogonal protection scheme. Deprotection of the resins was carried out with a solution of 20% piperidine in DMF and coupling steps were conducted in the presence of HCTU and NMM. To optimize the outcome of these synthesis, different conditions were tested (**Table 1**). Coupling steps were followed by a capping step, with a solution of Ac_2O/Lut/DMF (5:6:89), for 5 min at room temperature. For longibramide E and peptides **3** and **4**, *N*-terminal acetylation was achieved using Ac_2O/DIEA/DMF (1.3:4.8:93.9). The rink amide (peptides **1-3** and **6-9**) and L-Leucinol-2-chlorotrityl (longibramide E and peptide **4** and **5**) resins were cleaved with a solution of TFA/TIS/H_2O (95:2.5:2.5) and TFA/DCM/TIS (50:47.5:2.5) for 2 hours at room temperature, respectively.

Table 1. Methods used to synthesize the target peptides.

Method	Coupling		Deprotection		Capping
	Time (min)	T (ºC)	Time (min)	T (ºC)	
A - Manual	60	RT	20	RT	☒
B – Automated RT	2 × 60	RT	2 x 2.5	RT	☑
C – Automated IR	2 × 10	75	2 x 2.5	RT	☑

Results

1. Comparing synthesis methodologies

Table 2. Sequences produced in this study.

Peptide	Sequence	Method	Purity (%)
Longibramide E	Ac-Aib-Asn-Ile-Ile-**Aib**-Hyp-Leu-Leu-Aib-Pro-Lol	A	99.9
1	Aib-Asn-Ile-Ile-**Aib**-Hyp-Leu-Leu-Aib-Pro-**Leu-NH$_2$**	A/B/C	91.3
2	Aib-Asn-Ile-Ile-Hyp-Leu-Leu-Aib-Pro-**Leu-NH$_2$**	A	95.7
3	Ac-Aib-Asn-Ile-Ile-Aib-Hyp-Leu-Leu-Aib-Pro-**Leu-NH$_2$**	B	98.3
4	Ac-Aib-Asn-Ile-Ile-Hyp-Leu-Leu-Aib-Pro-Lol	A	99.5
5	Aib-Asn-Ile-Ile-Aib-Hyp-Leu-Leu-Aib-Pro-Lol	C	90.5
6	Aib-Asn-Ile-Ile-Aib-Hyp-**Lys-Lys**-Aib-Pro-**Leu-NH$_2$**	B	90.6
7	**Lys-Lys-Lys-Ala**-Aib-Asn-Ile-Ile-Aib-Hyp-Leu-Leu-Aib-Pro-**Leu-NH$_2$**	C	97.3
8	Aib-Asn-Ile-Ile-Aib-Hyp-Leu-Leu-Aib-Pro-Leu-**Ala-Lys-Lys-Lys-NH$_2$**	B	98.9
9	***n*Oct-Lys-Lys-Lys-Ala**-Aib-Asn-Ile-Ile-Aib-Hyp-Leu-Leu-Aib-Pro-**Leu-NH$_2$**	C	91.3

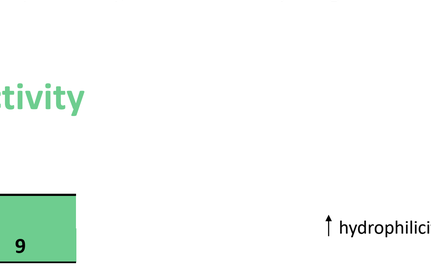

Figure 1: HPLC chromatogram of the crude peptide **1** synthesized manually without capping (Method A), automatically at room temperature (Method B), and automatically using infrared heating (Method C).

2. Assessment of antibacterial activity

Table 3: Activity of the peptides under study against Gram-positive and Gram-negative bacteria.

Bacterial strains	MIC in µg/mL (µM)									
	Longibramide E	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
S. aureus (ATCC 29213)	> 256 (215)	128 (110)[a]	1024 (950)[a]	> 256 (213)	> 256 (231)	256 (223)[a]	> 1024 (859)	64 (40)[a]	512 (316)[b]	4 (2)[b]
HJS-SA007 [1]	> 256 (215)	64 (55)[a]	1024 (950)[a]	> 256 (213)	> 256 (231)	256 (223)[a]	> 1024 (859)	32 (20)[c]	256 (158)[b]	4 (2)[b]
Sa3-SA3 [2]	> 256 (215)	128 (110)[b]	-	-	-	256 (223)[a]	-	32 (20)[b]	> 256 (158)	4 (2)[a]
E. coli (ATCC 25922)	> 256 (215)	512 (440)[b]	> 1024 (950)	> 256 (213)	> 256 (231)	512 (445)[a]	> 1024 (859)	8 (5)[a]	128 (79)[a]	8 (4)[a]
Ec1-SA1 [3]	> 256 (215)	> 256 (220)	> 1024 (950)	> 256 (213)	> 256 (231)	512 (445)[b]	> 1024 (859)	32 (20)[a]	> 256 (158)	8 (4)[a]
HSJ-EC004 [4]	> 256 (215)	> 256 (220)	-	-	-	> 256 (223)	-	16 (10)[a]	256 (158)[a]	4 (2)[a]

Antimicrobial resistance pattern: [1] CIP; CLI; ERY; GEN; OXA; LEV; MOX. [2] VAN; AMC; AMP; CXI; IMI; CIP; OXA. [3] AZT; CTZ; CTA; TET; AMP; TRS; CIP; GEN. [4] AMC; AMP; CXA; CXM; CIP; PIT; LEV. [a] MBC = MIC. [b] MBC = 2 × MIC. [c] MBC = 16 × MIC. AMC: amoxicillin/clavulanic acid; AMP: ampicillin; AZT: aztreonam; CTZ: ceftazidime; CIP: ciprofloxacin; CLI: clindamycin; CTA: cefotaxime; CXA: cefuroxime axetil; CXM: cefuroxime sodium; ERY: erythromycin; CXI: Cefoxitin; GEN: gentamicin; IMI: imipenem; LEV: levofloxacin; MOX: moxifloxacin; OXA: oxacillin; TRS: Trimethoprim/Sulfamethoxazole; TET: tetracycline; PIT: piperacillin/Tazobactam; VAN: vancomycin.

Conclusions

- The use of infrared (IR) heating markedly improved the synthesis of this class of metabolites, as the coupling of Aib to Hyp was significantly more efficient.
- The Aib coupled to Hyp is crucial for the antibacterial activity (peptide **1** vs **2**), contrarily to the substitution of the expensive *C*-terminal alcohol by a leucine amide (**1** vs **5**).
- The incorporation of **Lys-Lys-Lys-Ala-** in the *N*-terminus (**7**) markedly improved peptide water solubility and enhanced antibacterial activity.
- The addition of ***n*-octanoyl** (**9**) further potentiated the antibacterial activity.
- Peptide **9** may be a promising molecule to tackle infections caused by resistant Gram-positive and Gram-negative bacteria.

References

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