Synthesis of octapeptin C4 and biological profiling against NDM-1 and polymyxin-resistant bacteria

Bernd Becker, Mark S. Butler, Karl A. Hansford, Alejandra Gallardo-Godoy, Alysha G. Elliott, Johnny X. Huang, David J. Edwards, Mark A. T. Blaskovich*, and Matthew A. Cooper*

Institute of Molecular Bioscience, University of Queensland, Brisbane, 4072, Australia

Abstract

The first synthesis of octapeptin C4 was achieved using a combination of solid phase synthesis and off-resin cyclisation. Octapeptin C4 displayed antibiotic activity against multi-drug resistant, NDM-1 and polymyxin-resistant Gram-negative bacteria, with moderate activity against Staphylococcus aureus. The linear analogue of octapeptin C4 was also prepared, which showed reduced activity.

Graphical Abstract

Keywords

Lipopeptide; Colistin; Drug resistance; Antibiotic; Natural product

The rapid spread of multi-drug resistant (MDR) bacteria is recognised as one of the most serious threats to human health today.1–4 In particular, Gram-negative bacteria such as Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii and Pseudomonas aeruginosa form the core of the dangerous ESKAPE pathogens highlighted by the Infectious Diseases Society of America (IDSA).5 Infections caused by these pathogens are difficult to treat due to the bacteria’s propensity to assimilate and share multiple functional resistance pathways, coupled with their intrinsic protection from antibiotics due to the outer membrane, the presence of multiple efflux systems and the ability to form biofilms. The bacterial-derived polymyxin class of antibiotics consists of the cyclic lipopeptides

*Joint corresponding authors. Tel.: +61-3-3346-2044/2994; m.blaskovich@uq.edu.au, m.cooper@uq.edu.au.

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A. Supplementary Material

Supplementary data associated with this article can be found, in the online version, at: ##.
polymyxin B and colistin (also termed polymyxin E and generally administered as colistimethate, a polymethanesulfonic acid prodrug). These ‘forgotten’ antibiotics were first identified in the late 1940s, but until recently were only used sparingly due to potential nephrotoxic and neurotoxic side effects.6–8 Today they are seeing a resurgence in use as ‘antibiotics of last resort’ to treat these MDR Gram-negative infections.

Octapeptins are 8-residue peptides, consisting of a cyclic heptapeptide core with a lipophilic acyl monopeptide tail (Fig. 1).9–11 They are similar in structure to the polymyxins, sharing the same 2,4-diaminobutyric acid (Dab)-rich cyclic core but with two fewer amino acids in the tail and an inversion in stereochemistry in the exocyclic Dab residue. They possess potent, broad spectrum activity against Gram-negative bacteria, but weak activity against Gram-positive bacteria and fungi.12 Notably, mixtures of natural product octapeptins were reported13,14 to display activity against polymyxin-resistant strains of E. coli and showed a low propensity for resistance generation.15 Most of the octapeptins were isolated and investigated as mixtures in the 1970s and 1980s and were grouped into four sub-classes, octapeptins A, B, C, and D, based variations in amino acid (AA) sequence at positions AA4, AA5 and AA8. Individual members of each class differ in the N-terminal lipophilic acid component, having straight chain, iso and anteiso fatty acids and C8-C10 chain lengths.16 The absolute configuration of the 3-hydroxy and anteiso methyl groups were both assigned as R using optical rotations of fatty acids derived from a mixture of octapeptins A1, A2, A3 and/or B1, B2, B3.17 Interestingly, the stereochemistry of AA4 and AA5 in the recently reported octapeptin B5 (battacin) was reversed compared to other members of the same subclass, featuring L-Phe4 and D-Leu5 instead of D-Phe4 and L-Leu5.18 The structure of octapeptin B5 was recently confirmed by synthesis.19 Several reports have described synthetic modifications of the octapeptins, replacing the 3-hydroxy fatty acid with simple, long-chain aliphatic acids20 and non-selective poly-acylation and poly-alkylation of the Dab groups.21

Given their structural similarity to the polymyxins and our work on the structure-activity and structure-toxicity relationships of this class,22 we wished to determine if a synthetic, pure octapeptin would retain the intriguing antimicrobial activity seen for the natural product mixtures. We thus embarked upon the total synthesis of octapeptin C4, as a representative of this class of lipopeptide antibiotics, and evaluated its biological activity against a panel of polymyxin-sensitive and -resistant strains and for mammalian cell cytotoxicity. A non-cyclised linear analogue of octapeptin C4 was also synthesised and assayed.

Octapeptin C4 was synthesised using manual Fmoc-based solid phase chemistry according to the procedure outlined in Scheme 1 and the Supplementary data). A completely protected linear peptide was prepared on a 2-chlorotrityl resin using Boc side chain protection on Dab residues 1,3,6 and 7, and either ivDde (1a) or Boc (1b) protection on Dab-2. Sequential resin cleavage of (1b) followed by global deprotection with TFA gave linearised octapeptin C4 (2b). Alternatively, the Dab-2 ivDde protecting group of (1a) was selectively removed using 4% hydrazine; subsequent cleavage of the resin-bound intermediate was effected with 20% hexafluorooisopropanol (HFIP) in DCM without the risk of premature Boc cleavage.23 Cyclisation of (2a) between the deprotected Dab-2 side chain and unmasked C-terminal carboxyl group was achieved using diphenylphosphoryl azide (DPPA)/NaHCO3 in DMF.
under high dilution conditions, (Scheme 1). Final deprotection with TFA and HPLC purification provided octapeptin C4, with the structure confirmed using HRMS, MS/MS fragmentation and NMR (Supplementary Data, Fig. S1-S13).

Octapeptin C4 and its linear analogue 2b were screened against a representative panel of sensitive and resistant Gram-negative ESKAPE strains, against the Gram-positive *S. aureus*, and counter-screened for mammalian cell cytotoxicity against the kidney cell lines HK2 (human kidney 2) and hRPTEC (primary human renal proximal tubule epithelial cells) (Table 1). Octapeptin C4 displayed activity against all Gram-negative pathogens, albeit with less potency compared to polymyxin B, while the linear analogue 2b displayed weaker activity, demonstrating the importance of cyclisation. More importantly, octapeptin C4 was active (MIC 2 μg/mL) against two polymyxin-resistant (PmxR) *P. aeruginosa* strains, in which polymyxin B activity was reduced to 32 and 128 μg/mL and the linear analogue 2b was ≥32 μg/mL. Octapeptin C4 showed four-fold greater cytotoxicity than polymyxin B against the HK-2 human kidney cell line (CC₅₀ = 31 μM vs 135 μM), with a similar level of toxicity against primary proximal tubule hRPTEC cells (CC₅₀ = 39 μM), which may be more predictive of nephrotoxic potential. The linear analogue 2b showed similar toxicity as polymyxin B to HK2 cells and intermediate toxicity to hRPTEC cells (CC₅₀ = 139 μM and 71 μM, respectively).

In conclusion, a discrete component of the naturally-occurring mixtures of octapeptins, octapeptin C4, has been chemically synthesised for the first time, and remained active against polymyxin-resistant strains of *P. aeruginosa*. Work is ongoing to investigate the mechanistic differences between octapeptins and polymyxins in their action against MDR bacteria, and to synthesise analogues with a broad spectrum Gram-negative activity against polymyxin-resistant and -sensitive strains with improved toxicity profiles.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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primary cultures of human proximal tubular cells. Pharmacol Res Perspect. 2015; 3 doi: 10.1002/prp1002.1148
Fig. 1.
Structures of the naturally occurring octapeptins with amino acids are numbered from the $N$-terminus.
Scheme 1.
Solid phase synthesis and off-resin cyclisation of octapeptin C4. Reagents: i) amino acid, HCTU, 2,4,6-collidine, DMF; ii) 30% piperidine in DMF; iii) (R)-3-hydroxydecanoic acid, HCTU, 2,4,6-collidine, DMF; iv) 4% hydrazine hydrate in DMF; v) 20% HFIP in DCM; vi) TFA/H$_2$O/Pr$_3$SiH; vii) DPPA, NaHCO$_3$, DMF, 0.008 M.
### Table 1
Activity and cytotoxicity of octapeptin C4, linear octapeptin C4 (2a) and polymyxin B (PMXB)

| Bacteria/cell lines<sup>a</sup> | Octapeptin C4 (μg/mL) | Linear 2b (μg/mL) | PMX B (μg/mL) |
|---------------------------------|-----------------------|--------------------|---------------|
| **Gram-negative**               |                       |                    |               |
| *E. coli* ATCC 25922            | 4                     | 16                 | 0.25          |
| *K. pneumoniae* ATCC 13883      | 8                     | >32                | 0.25          |
| *K. pneumoniae* ATCC 700603 MDR | 8                     | 16                 | 0.25          |
| *K. pneumoniae* BAA-2146 NDM-1  | 8                     | >32                | 0.25          |
| *A. baumannii* ATCC 19606       | 4                     | >32                | 0.25          |
| *P. aeruginosa* ATCC 27853      | 2                     | 4                  | 0.5           |
| *P. aeruginosa* Pmx<sub>FADDI-PA070</sub> | 2                 | 32                 | 32            |
| *P. aeruginosa* Pmx<sub>PA9704</sub> | 2                 | >32                | 128           |
| **Gram-positive**               |                       |                    |               |
| *S. aureus* ATCC 25923 MSSA     | 16                    | 32                 | >64           |
| **Cytotoxicity (LDH assay)**    | CC<sub>50</sub> (μM) |                    |               |
| HK2 (10% FBS)                   | 31                    | 139                | 135           |
| hRPTEC (10% FBS)                | 39                    | 71                 | >300          |

<sup>a</sup>Bacterial strains and cell line sources are detailed in Supplementary Data (Table S2).