Total Synthesis and Semi-synthesis of Fluorinated Analogues of the Antifungal Cyclic Lipopeptide Iturin A

Periklis Karamanis[a,b*], Matthew Kiernan[a,b], Jimmy Muldoon[a], Paul Evans[a,b], Cormac D. Murphy[b,c], Marina Rubini[a,b]

[a] School of Chemistry, University College Dublin, Belfield, Dublin, Ireland, [b] BiOrbic Bioeconomy SFI Research Centre, University College Dublin, Belfield, Dublin, Ireland [c] School of Biomolecular and Biomedical Science, University College Dublin, Belfield, Dublin, Ireland

periklis.karamanis@ucdconnect.ie

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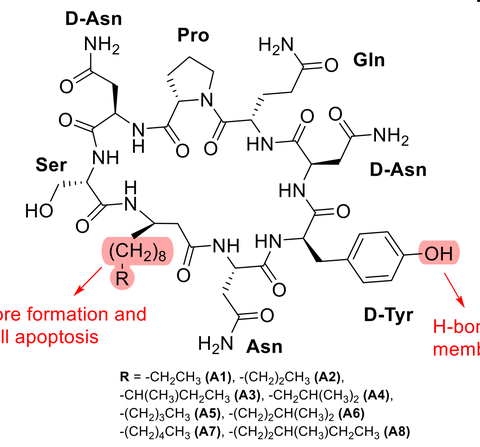
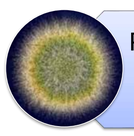
Introduction

Iturin A

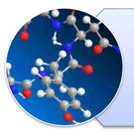


R = $-CH_2CH_3$ **(A1)**, $-(CH_2)_2CH_3$ **(A2)**, $-CH(CH_3)CH_2CH_3$ **(A3)**, $-CH_2CH(CH_3)_2$ **(A4)**, $-(CH_2)_3CH_3$ **(A5)**, $-(CH_2)_2CH(CH_3)_2$ **(A6)** $-(CH_2)_4CH_3$ **(A7)**, $-(CH_2)_2CH(CH_3)CH_2CH_3$ **(A8)**

Cyclic lipopeptide produced by *Bacillus subtilis.*



Potent **antifungal agent** against several fungal pathogens, including *Fusarium graminearum and Candida albicans.*[1]



Its **structure** consists of seven α-amino acids and a β-amino acid with varying alkyl side chains (iturin A1-A8).[1]

Fluorine in Peptide Engineering

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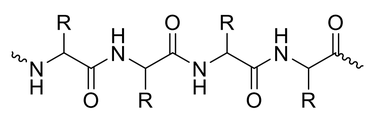

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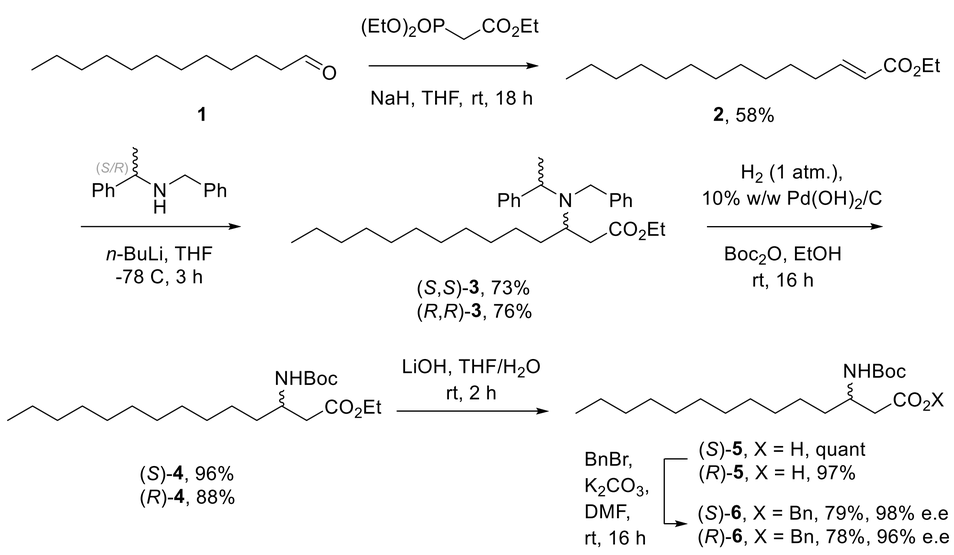
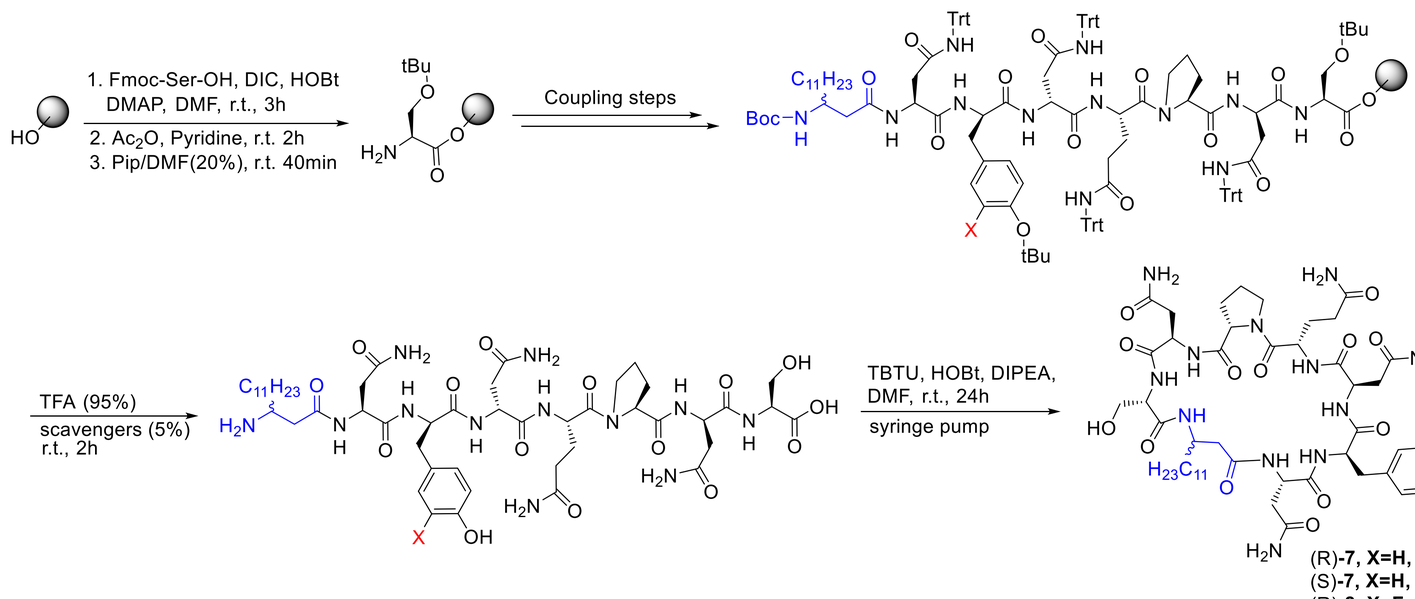
- Enhanced hydrogen bond formation capacity.
- Enhanced metabolic stability.
- Isostere of C-H and C-OH moieties.
- New characterization techniques available (such as ^{19}F NMR).[2,3]

Synthesis and characterization of iturin A analogues

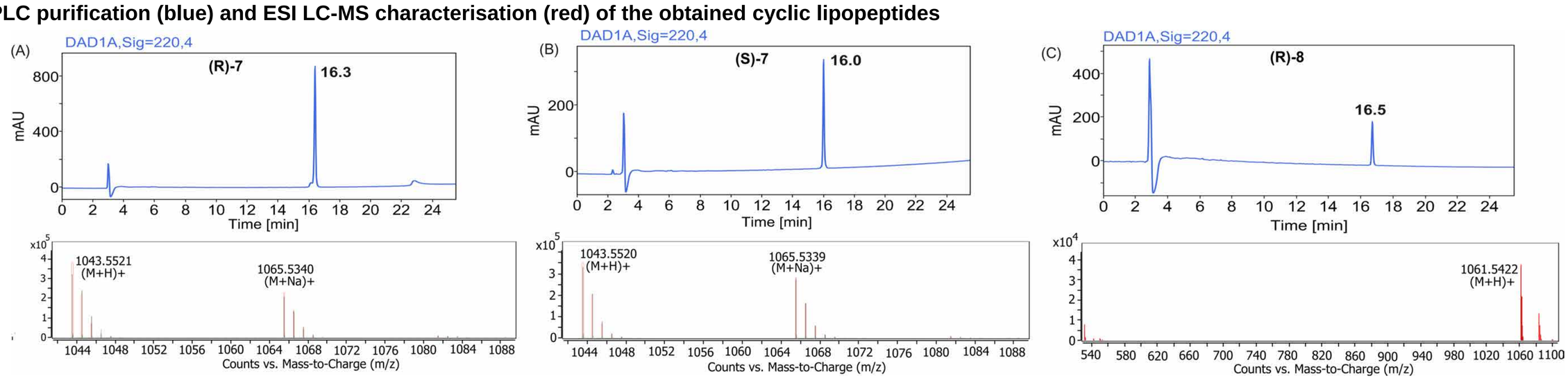
1. Synthesis of the β-amino fatty acid



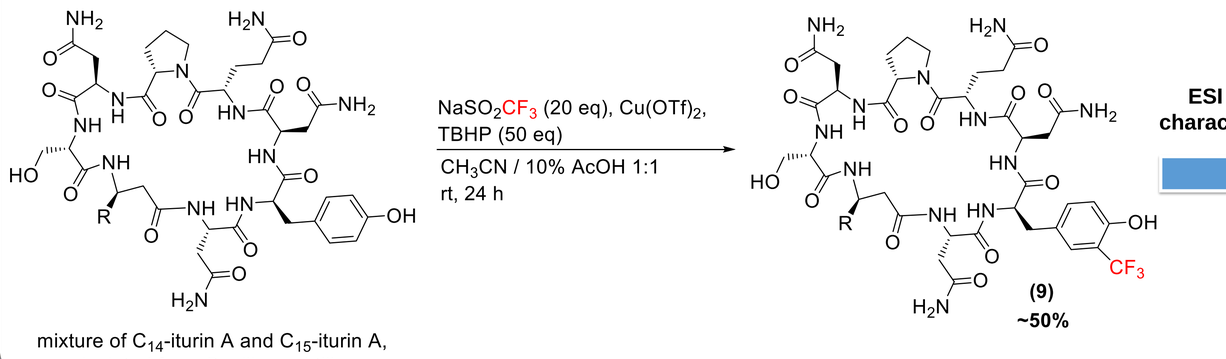
2. Solid phase peptide synthesis and cyclisation reactions



3. HPLC purification (blue) and ESI LC-MS characterisation (red) of the obtained cyclic lipopeptides

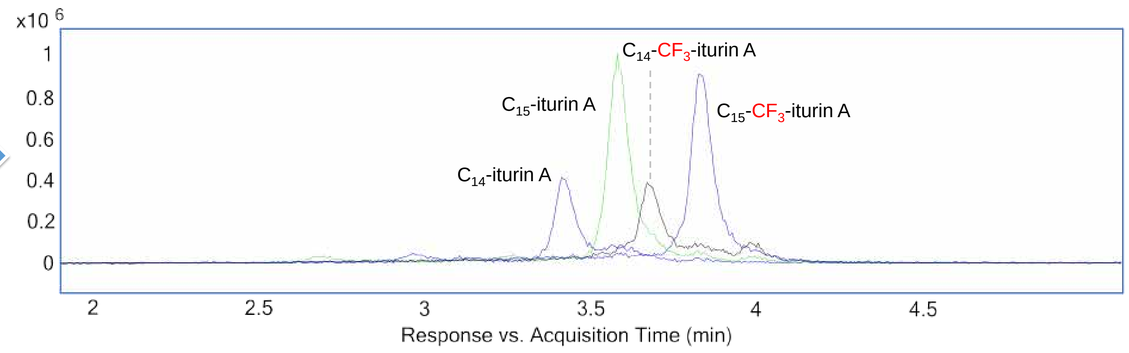


Late-stage trifluoromethylation of natural iturin A



mixture of C_{14}-iturin A and C_{15}-iturin A, isolated from *Bacillus subtilis*

ESI LC-MS characterisation



Antifungal assays

Compound	Minimum inhibitory concentration (μg/mL)	
	Fusarium graminearum	*Candida albicans*
Amphotericin B	<7.8	<7.8
Commercial iturin A	31.2	62.5
(R)-7	15.6	62.5
(S)-7	>1000	>1000
(R)-8	15.6	62.5

Concentration range tested: 7.8 – 1000 μg/mL

Conclusions and outlook

- A novel synthetic route to obtain iturin A analogues has been developed.[4]
- The importance of the stereochemistry of the β-amino fatty acid on the bioactivity of the natural lipopeptide has been elucidated, as the non natural epimer is significantly less bioactive.
- The obtained monofluorinated iturin A analogue displays identical bioactivity to the natural compound and can be a useful ^{19}F NMR probe.
- A high yielding semi-synthetic method to obtain trifluoromethylated iturin A analogues has been shown.

Future work:

- Antifungal assays to assess the bioactivity of the trifluoromethylated iturin A analogues, when compared to the natural lipopeptide.
- ^{19}F NMR studies to investigate the mode of binding of iturin A to the fungal membrane.

References

(1) D. A. Yaraguppi, Z. K. Bagewadi, N. R. Patil, N. Mantri, *Biomolecules* **2023**, *13*, 1515.
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(4) P. Karamanis, J. Muldoon, C. D. Murphy, M. Rubini, *Journal of Peptide Science* **2024**, *30*, e3569.

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