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Synthesis of Bacteria-Derived Lipopeptides and Applications in Antibiotic Discovery

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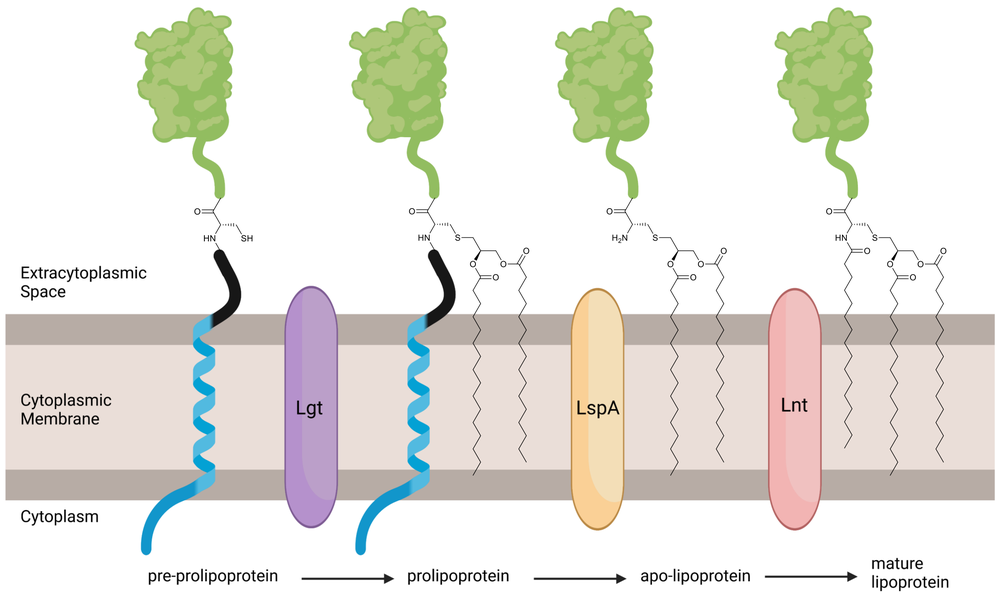
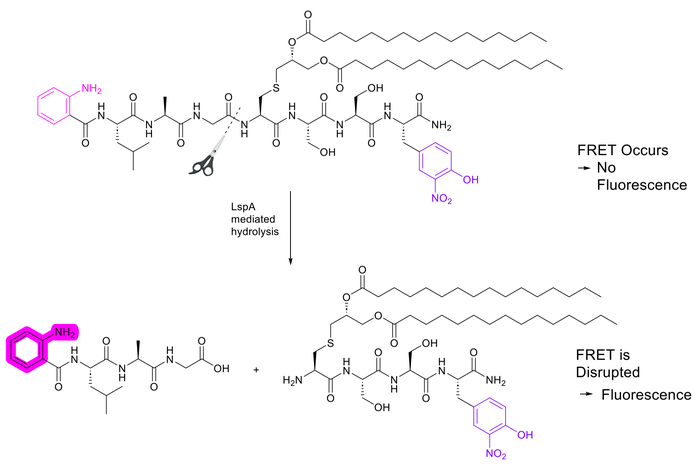
Introduction

Bacterial lipoprotein processing pathway (BLPP) in Gram-negative bacteria

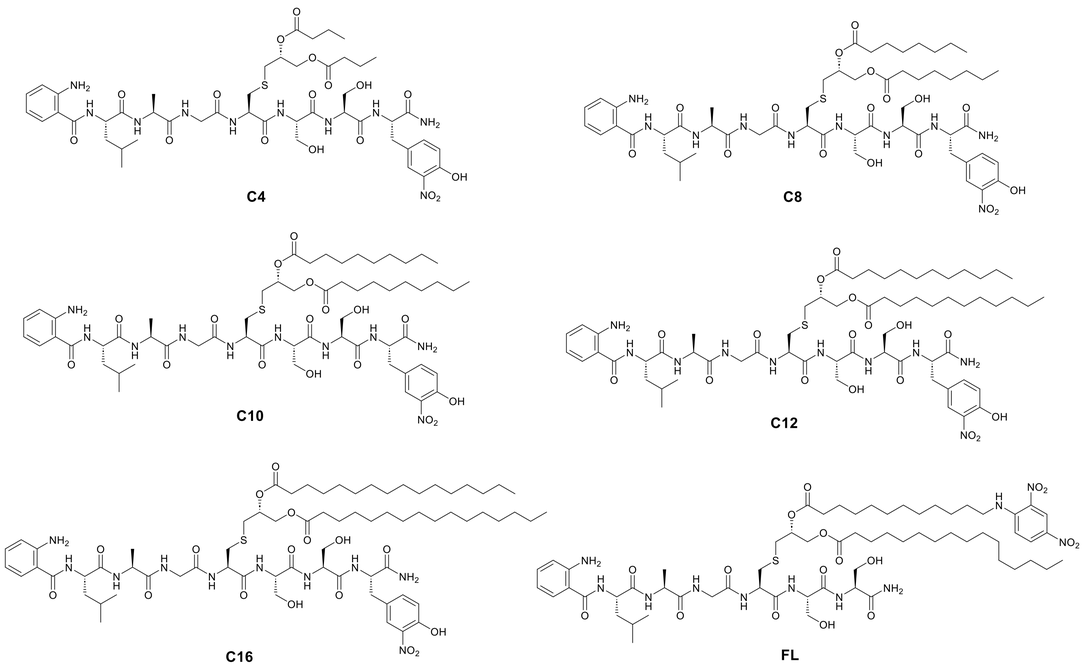


- The BLPP is a **post-translational modification** pathway of bacteria-specific enzymes.[1]
- Lipoproteins have **essential** functions in bacteria e.g. cell signalling, toxins, and for structural support.[2]
- Enzymes of the BLPP are potential **new antibiotic targets** therefore Inhibition of the BLPP offers novel opportunities for **antibiotic development**.
- Bacterial lipoproteins are **immunogenic** in humans and as potent TLR-2 agonists.[3]
- The first section of this work focuses on the **synthesis of fluorescent lipopeptide substrates** of LspA to probe the activity of this enzyme and to facilitate the discovery of **new inhibitors**.
- The second section focuses on a **convergent synthesis** methodology to access lipopeptides under mild **aqueous conditions**.

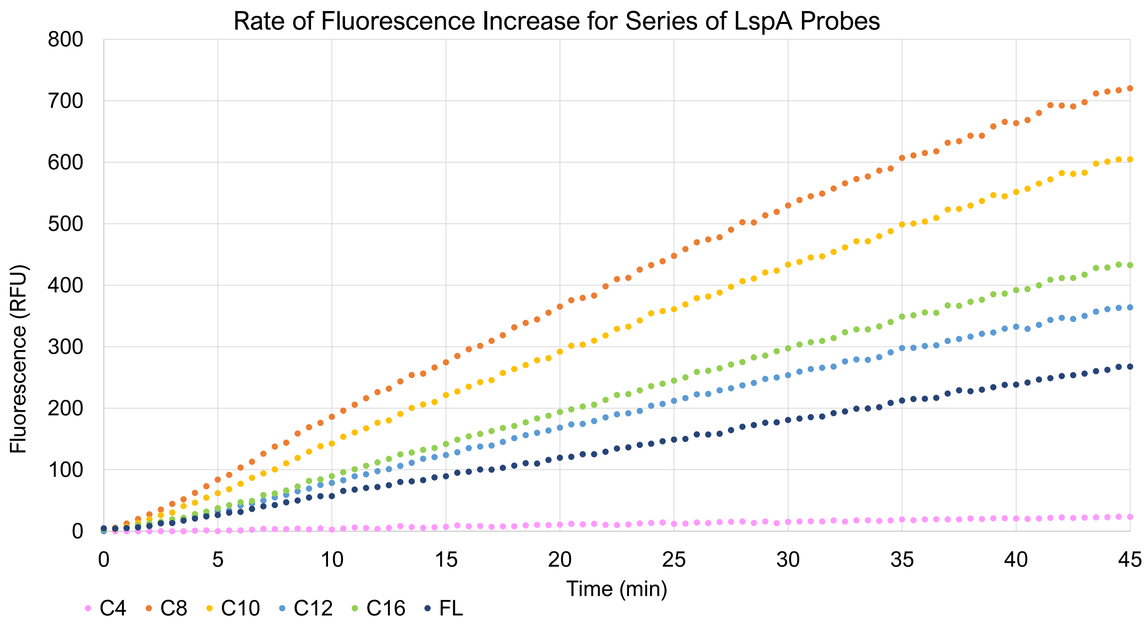
Lipopeptide Probe Synthesis



- Förster Resonance Energy Transfer (**FRET**) relies on the spectral overlap of a **donor** (pink) and an **acceptor** (purple) fluorescent moiety, and the **distance** between them.[4]
- Hydrolysis of the probe by LspA **disrupts FRET** resulting in an **increase in fluorescence** intensity corresponding to LspA activity.

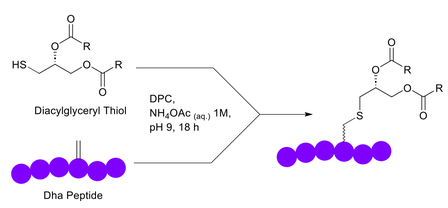


A range of lipopeptide probes for LspA with varying lipid lengths and structures were synthesised and tested as substrates for LspA.



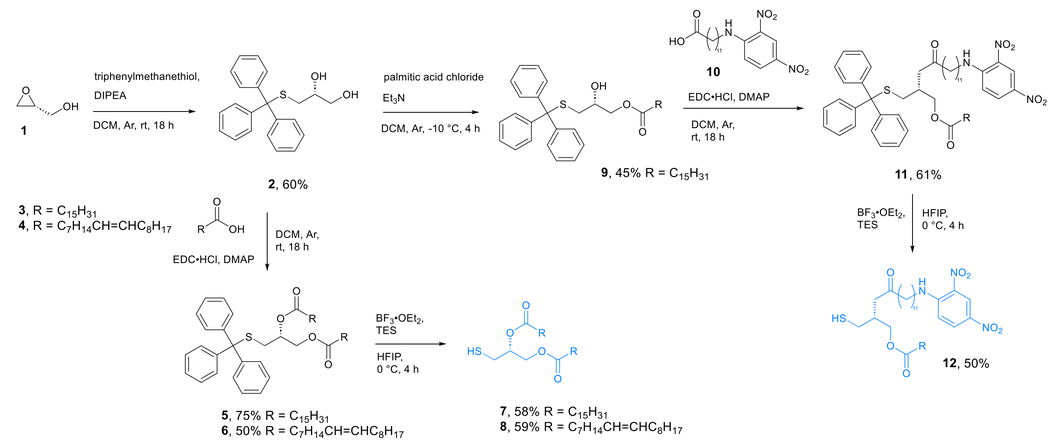
Peptide	C4	C8	C10	C12	C16	FL
Initial Rate (RFU/min)	0.606	19.4	16.0	9.4	10.7	6.41

Convergent Lipopeptide Synthesis

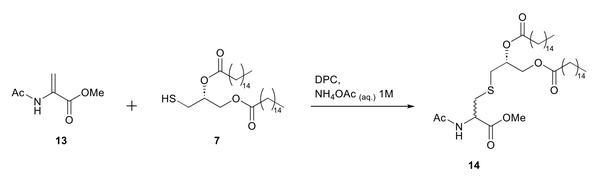


- ✓ Parallel assembly of starting materials
- ✓ Mild aqueous conditions
- ✓ Chemoselective late-stage addition

This methodology incorporates a thiol-Michael addition reaction between a Dha-containing peptide and a lipid-thiol that can be carried out under optimised aqueous conditions.



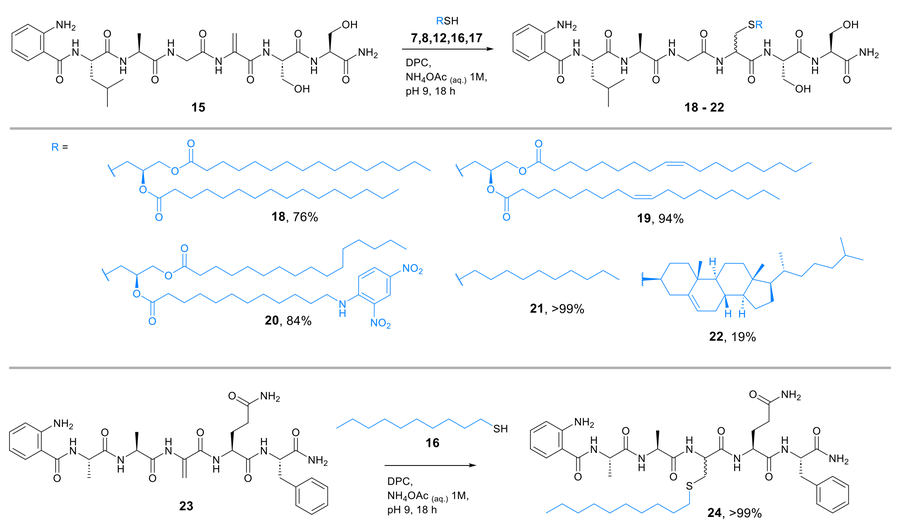
Three diacylglycerol lipid thiols were synthesised in good yield



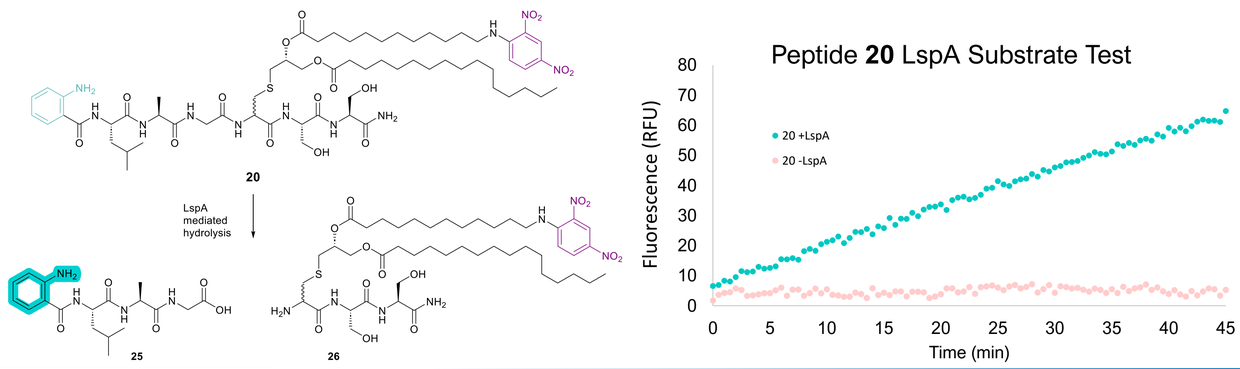
Conditions were optimised using a model reaction

Entry	Time (h)	pH	Consumption of Dha % (^{1}H NMR)
1	18	7	82%
2	18	4	69%
3	18	9	>99%
4	3	9	98%

- The conditions were then applied to a model peptide **15** with a series of lipids, showing the compatibility of this method with **saturated**, **unsaturated** and **complex lipids**.
- Lipid **16** was then reacted with a liraglutide-inspired peptide with excellent conversion.



Lipopeptide **20** was found to be a substrate and an effective activity probe for LspA



Conclusions and Future Work

A series of lipopeptide probes for LspA with varying lipid lengths were synthesised and the C8 analogue appeared to be the most sensitive followed by the C10 analogue. Future work includes enzyme kinetics experiments to characterise each probe.

A late-stage lipidation method was developed under mild aqueous conditions and demonstrated compatibility with a series of saturated, unsaturated, and complex lipids. The method was successfully used to make a novel probe for LspA.

References

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