Peptide and Probe: A two-pronged approach to investigating menaquinone binding peptides

Eilidh J. Matheson, Roy A. M. van Beekveld, Sarah Gothard, Paolo Innocenti, Nathaniel I. Martin, Markus Weingarth, Stephen A. Cochrane
School of Chemistry and Chemical Engineering, Queen's University, Belfast, UK, BT7 1NN, Biological Chemistry Group, Institute of Biology, Leiden University Sylviusweg 72, 2333 BE Leiden, NL, NMR Spectroscopy, Bijvoet Centre for Biomolecular Research, Department of Chemistry, Utrecht University, Utrecht, NL
ematheson01@qub.ac.uk, s.cochrane@qub.ac.uk

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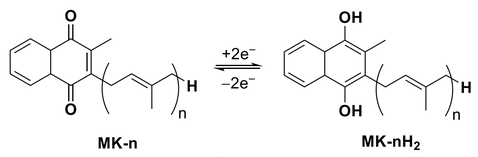
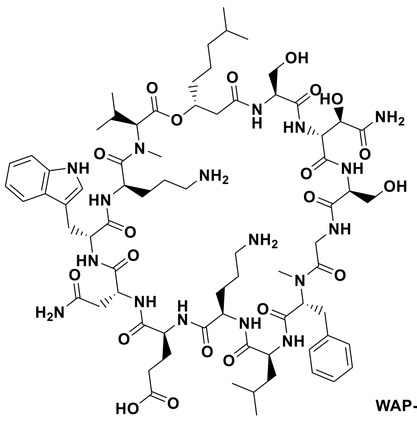
Background

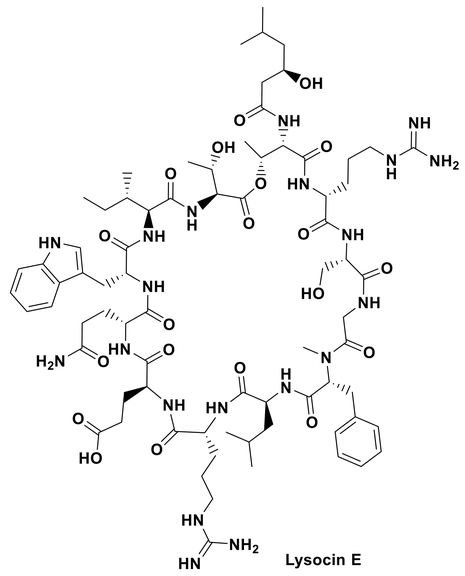
In 2015, lysocin E was first reported. A broad spectrum depsipeptide with good activity against Gram positive bacteria.[1] Its novel mode of action involves binding to menaquinone (MK), an essential membrane bound enzyme cofactor involved in the electron transport chain. Since lysocin E, a number of other MK binding antimicrobial peptides (MBAMPs) have been reported, including WAP and MBA2.[2,3] It is thought that MK acts as an anchoring point for the MBAMPs into cell membrane, causing cell lysis. Beyond this, not much is known about this novel mode of action. Our lab has previously developed chemistry to selectively modify terminal isoprene units in simple polyprenols.[4] We sought to further apply this ω-modification chemistry to create a library of highly valuable MK-derived probes. These probes could then be utilised to better understand MBAMPs and their mode of action.



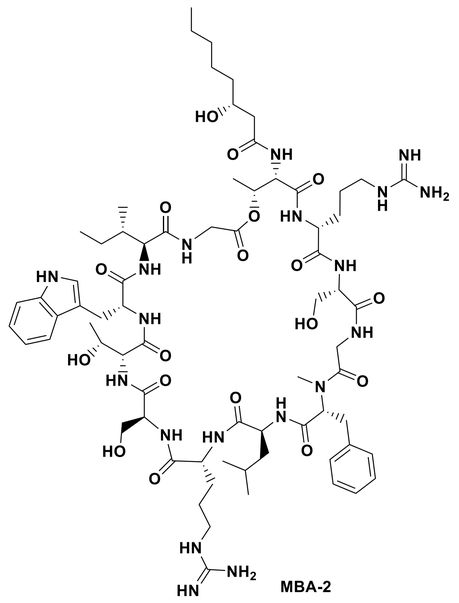
MK-n MK-nH$_2$



WAP-8294A2

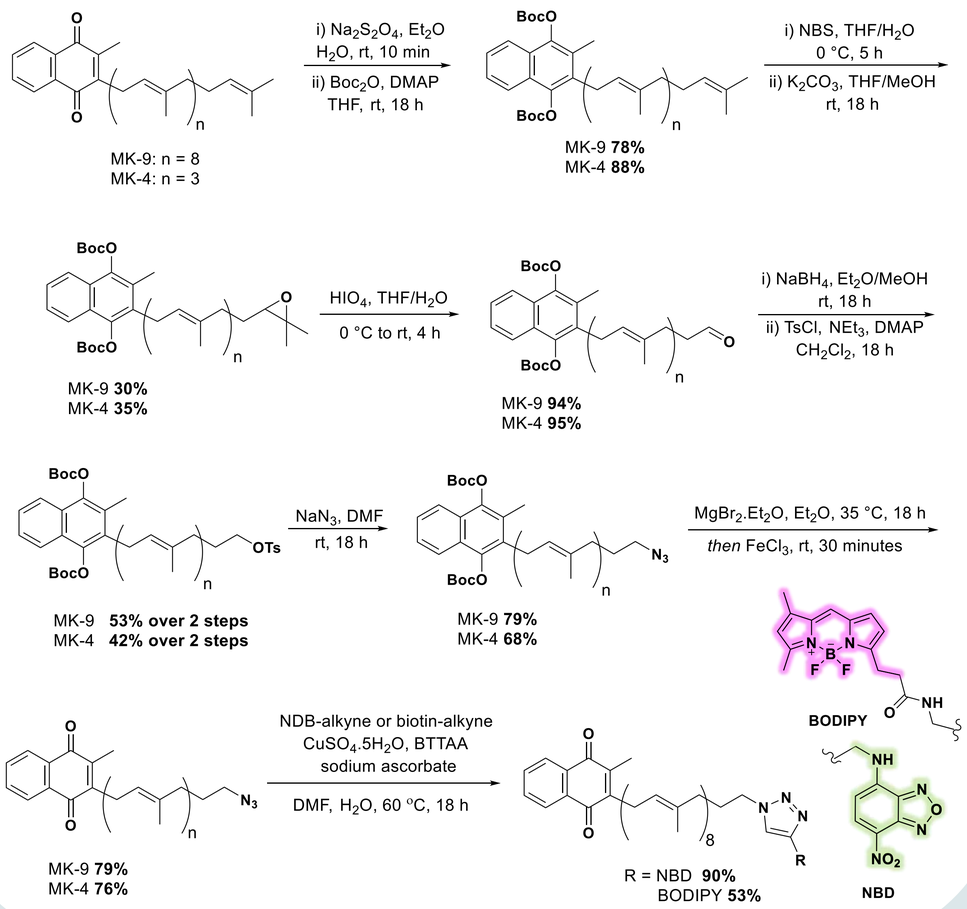


Lysocin E

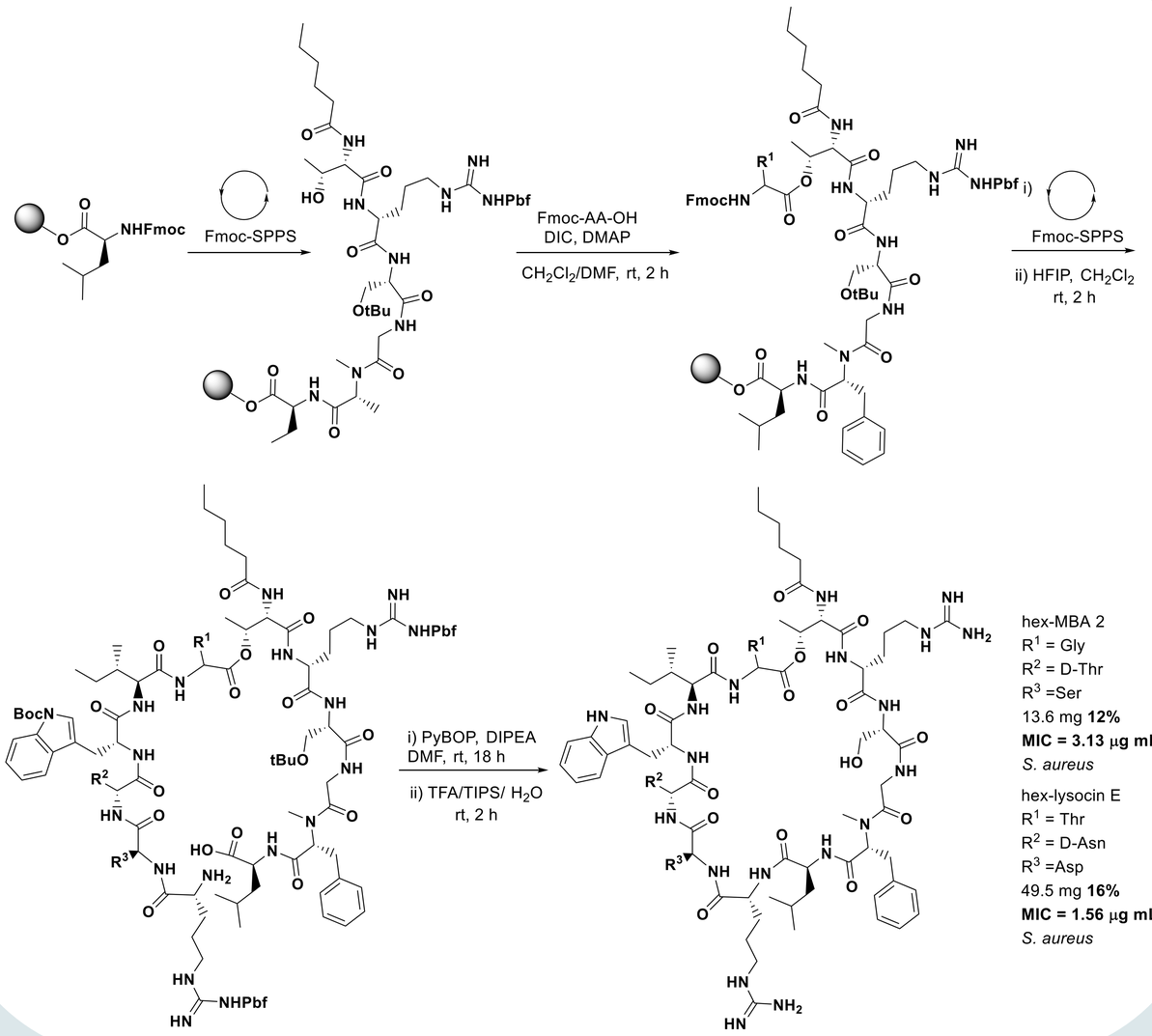


MBA-2

MK Probe Synthesis

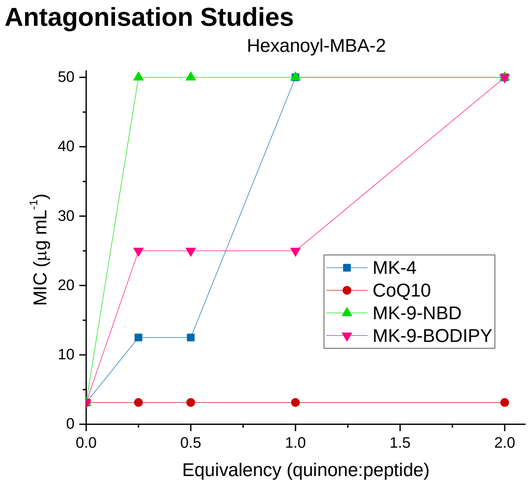


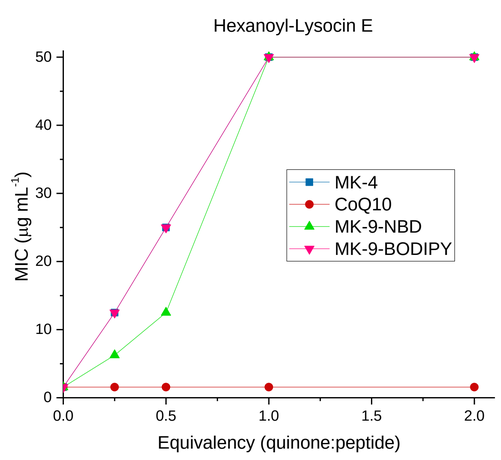
MBAMP Synthesis



Do ω-modifications affect binding?

Antagonisation Studies

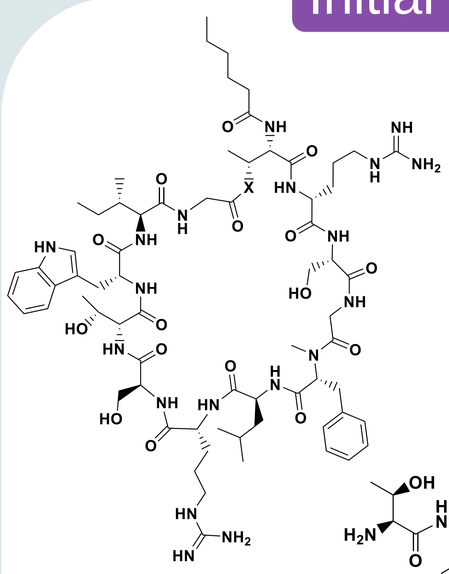




ITC Results

Quinone	Hexanoyl-Lysocin E		Hexanoyl-MBA-2	
	K_D (nM)	ΔH (kcal mol^{-1})	K_D (nM)	ΔH (kcal mol^{-1})
MK-4	59.2	– 8.49	56.9	– 5.63
MK-9	80.3	– 8.99	61.1	– 5.02
MK-9-NBD	101.4	– 5.02	84.3	– 8.37
MK-9-BODIPY			106.1	– 9.47
CoQ_{10}	0	0	0	0

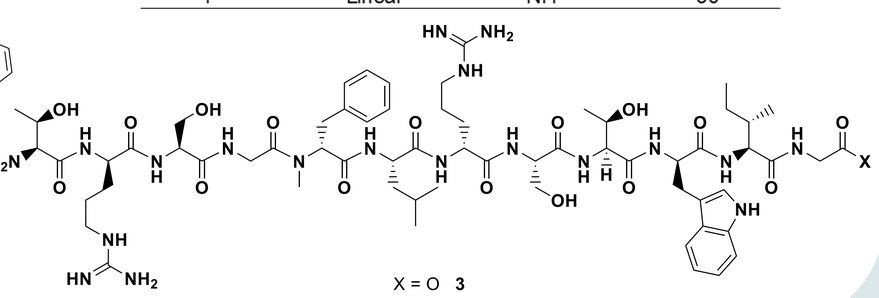
Initial SAR MBA-2 Studies



X = O **1** (hexanoyl-MBA-2)
= NH **2**

- Previous SAR work showed N-methyl group necessary
- Tolerance for lipid tail substitution
- Conserved GXLXXXW throughout MBAMPs
- Relative but not absolute stereochemistry necessary
- What about structure?

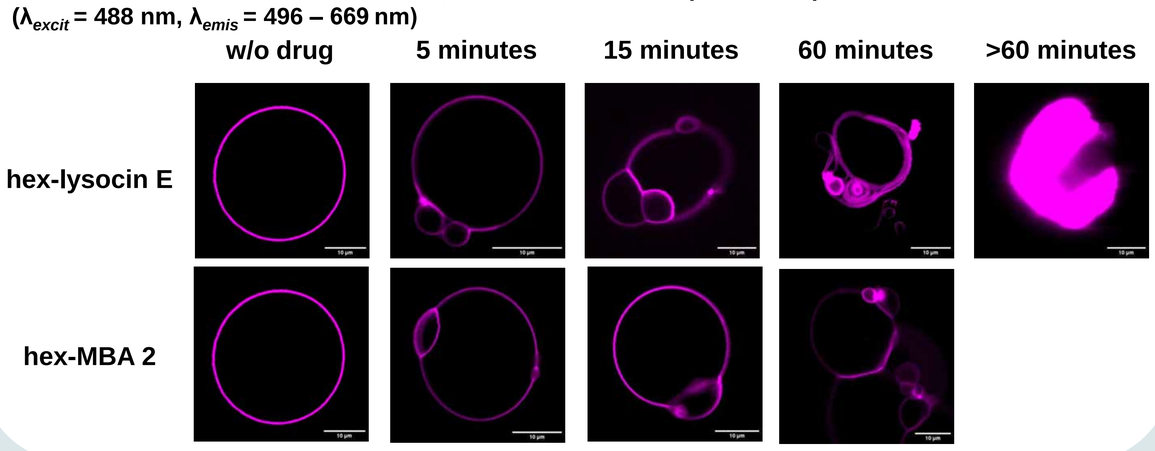
Compound	Cyclic or Linear	X	MIC (μg mL^{-1})
1	Cyclic	O	3.13
2	Cyclic	NH	>50
3	Linear	O	>50
4	Linear	NH	50



X = O **3**
= NH **4**

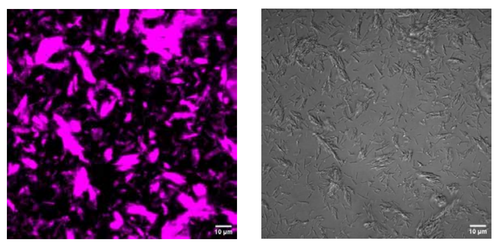
Model Membrane Studies

Giant unilamellar vesicles: 98% DOPC, 2% MK-9, 0.1% MK-9(ω-BODIPY)
(λ_{excit} = 488 nm, λ_{emis} = 496 – 669 nm)



Cellular Uptake Studies

***Mycobacterium smegmatis* labelled with MK-9(ω-BODIPY)**



λ_{excit} = 488 nm, λ_{emis} = 496 – 669 nm Bright-field

Uptake conditions: 7H9 broth, 0.1% v/v TWEEN 80, 0.2% v/v glycerol solution (50% w/v Aq.) and 0.2% w/v glucose, 10 μM MK-9(ω-BODIPY), 20 h

Conclusions

- ω-modification chemistry to achieve MK probes
- ω-modification does not significantly affect binding
- MBAMPs cause MK localisation, causing membrane perturbation and vesiculation
- SAR work: cyclisation necessary and conformational flexibility important for activity
- *M. Smegmatis* will uptake MK-9(ω-BODIPY)

Ongoing:

- Continued whole cell uptake studies
- ssNMR to determine binding conformation

References

1. H. Hamamoto *et al.*, *Nat. Chem. Bio.,* 2015, **11**, 127 – 133.
2. H. Itoh *et al.*, *J. Org. Chem.,* 2018, **83 (**13), 6924 – 6935.
3. L. Li, B. Koirala, Y. Hernandez *et al., Nat. Microbiol.,* 2022, **7**, 120 – 131.
4. R. V. K. Cochrane, F. M. Alexander, C. Boland, S. K. Fetics, M. Caffrey and S. A. Cochrane, *Chem. Commun.*, 2020, **56**, 8603 –8 606.

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