Combining Quaternary Ammonium Compounds and Peptides- Its Application to Antimicrobials and Beyond

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What are Quaternary Ammonium Compounds?

Also known as **quats**- a tetravalent nitrogen bonded to four alkyl/aryl substituents

- **Permanent positive charge**

Broad spectrum antimicrobial activity

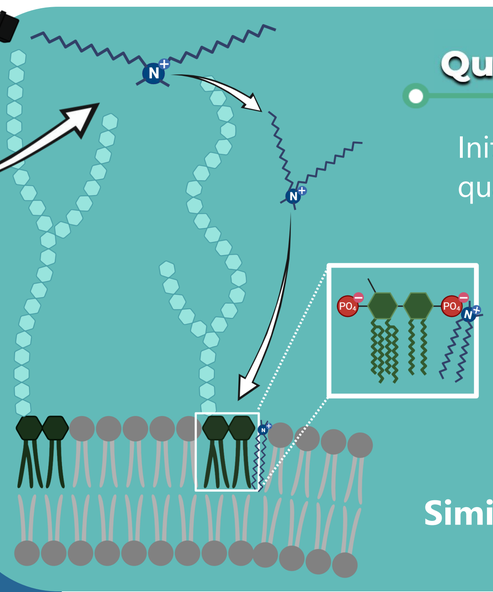
- In disinfectants, antiseptics, preservatives

Quats' Mode of Action involve...

Initial **electrostatic interaction** between quats and negatively charged bacterial membranes

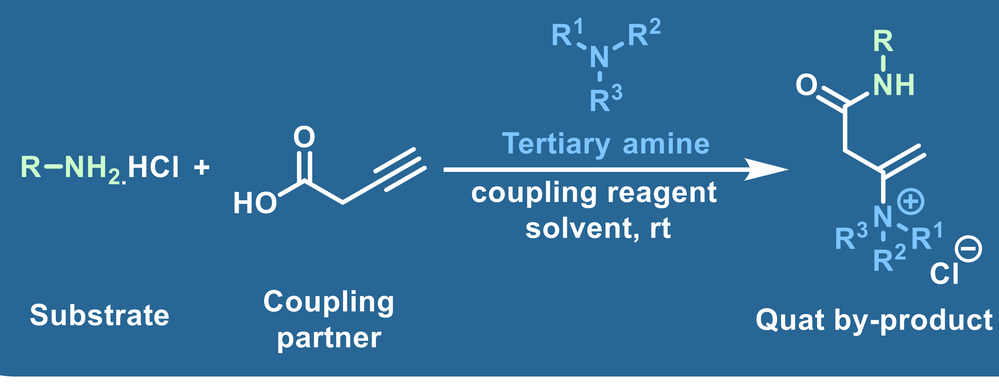
- Penetration of quat hydrophobic tails into the hydrophobic membrane core leads to:
  - Loss of membrane integrity
  - Leakage of cellular contents
  - Cell death

Similar MOA to cationic antimicrobial peptides



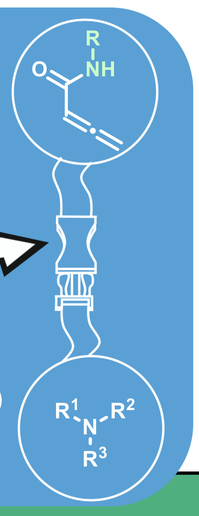
Serendipitous Finding...

While developing allenamide handles for peptides,[1] we observed quat by-products resulting from the **aza−Michael addition of tertiary amines** to allenamides.



Why are We Interested?

1. **Interesting chemistry**- a new way to prepare quats
2. Aza-Michael reaction is a **"click" reaction**
   - No alkylating agents!
3. Could be adapted for **solid-phase peptide synthesis (SPPS)**
   - Given our established method to introduce allenamides during SPPS[1]

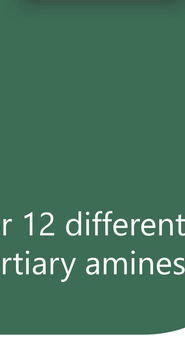


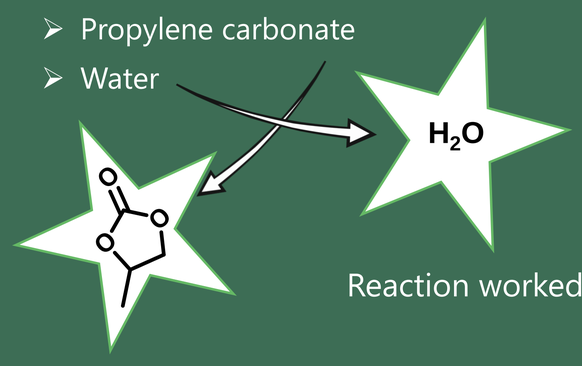
The Research

1 Reaction Optimization And Scope

Solvent was critical

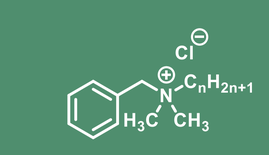
- **Green solvents** were best performing
  - Propylene carbonate
  - Water



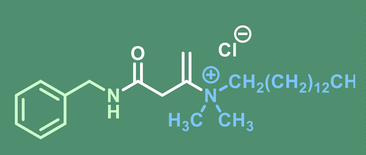


Reaction worked for 12 different tertiary amines

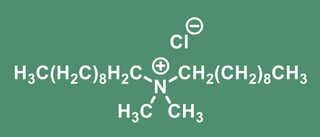
2 Preparation of Quat Mimics



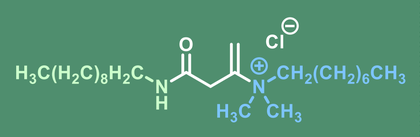
BAC



BAC mimic



DDAC



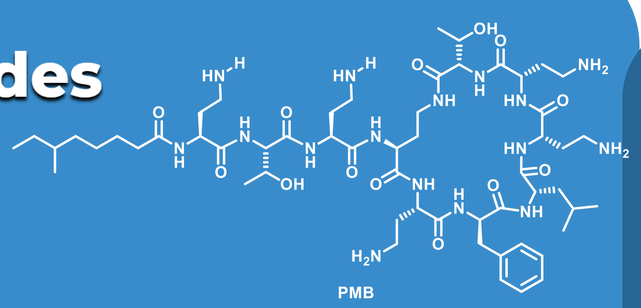
DDAC mimic

Bacteria	Minimum inhibitory concentrations (μM)			
	BAC	BAC mimic	DDAC	DDAC mimic
E. coli	32	(32)	16	(32)
S. aureus	8	(4)	4	(8)
C. albicans	16	(8)	8	(8)

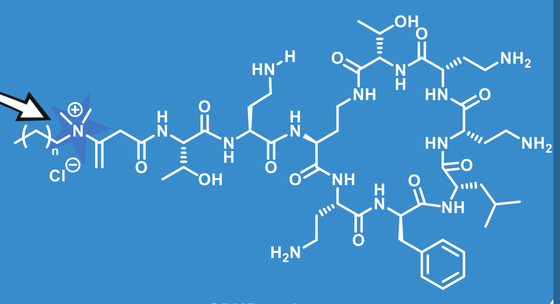
BAC and DDAC- two of the most common quat disinfectants

3 Application to Antimicrobial Peptides

Previous literature showed **incorporation of quats to polymyxin B (PMB) broadens activity towards Gram-positive bacteria**[2]
-may infer activity against resistant strains



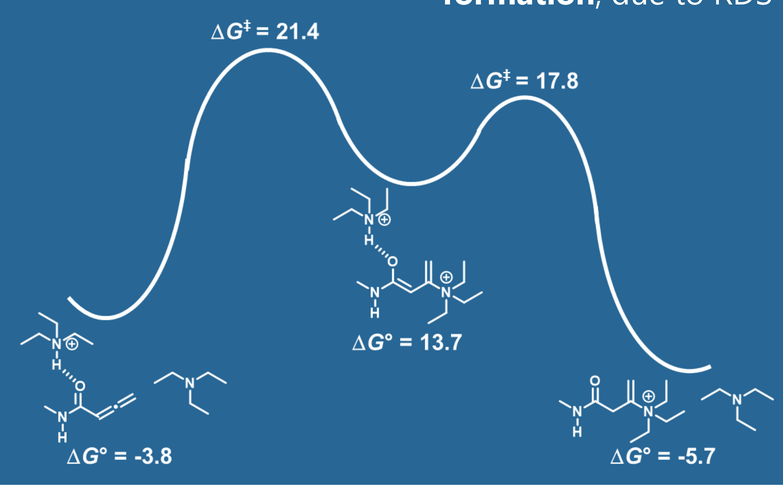
∴ **Quaternization was adapted for Fmoc-SPPS**



Bacteria	Minimum inhibitory concentrations (μM)			
	PMB	QPMB1 (n=4)	QPMB2 (n=8)	QPMB3 (n=12)
E. coli	0.125	2	(0.25)	1
S. aureus	>64	>64	32	(2)

4 Computational Chemistry Corroborates...

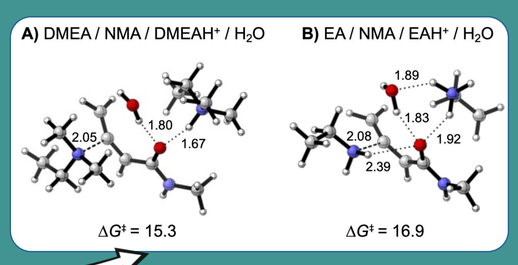
Aza-Michael addition of 3° amines to allenamide **uniquely favours product formation**, due to RDS



5 Reaction is selective in the presence of peptide-related functional groups

Side chains of Asn, Met, Ser, Trp, Tyr and **Lys**

Experimental selectivity of 3° amines over 1° amines supported by DFT



6 Future Work

- Application as **prodrug**- in progress
- More PMB analogues
- More QAC disinfectant analogues
- Test against resistance strains

References:

1. Cameron, A. J.; Harris, P. W. R.; Brimble, M. A. On-Resin Preparation of Allenamidyl Peptides: A Versatile Chemoselective Conjugation and Intramolecular Cyclisation Tool. *Angewandte Chemie International Edition* **2020**, *59* (41), 18054–18061. https://doi.org/10.1002/anie.202004656.
2. Ongwae, G. M.; Morrison, K. R.; Allen, R. A.; Kim, S.; Im, W.; Wuest, W. M.; Pires, M. M. Broadening Activity of Polymyxin by Quaternary Ammonium Grafting. *ACS Infect. Dis.* **2020**. https://doi.org/10.1021/acsinfecdis.0c00037

Also check out:

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