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Quaternary ammonium sophorolipids as renewable based antimicrobial products

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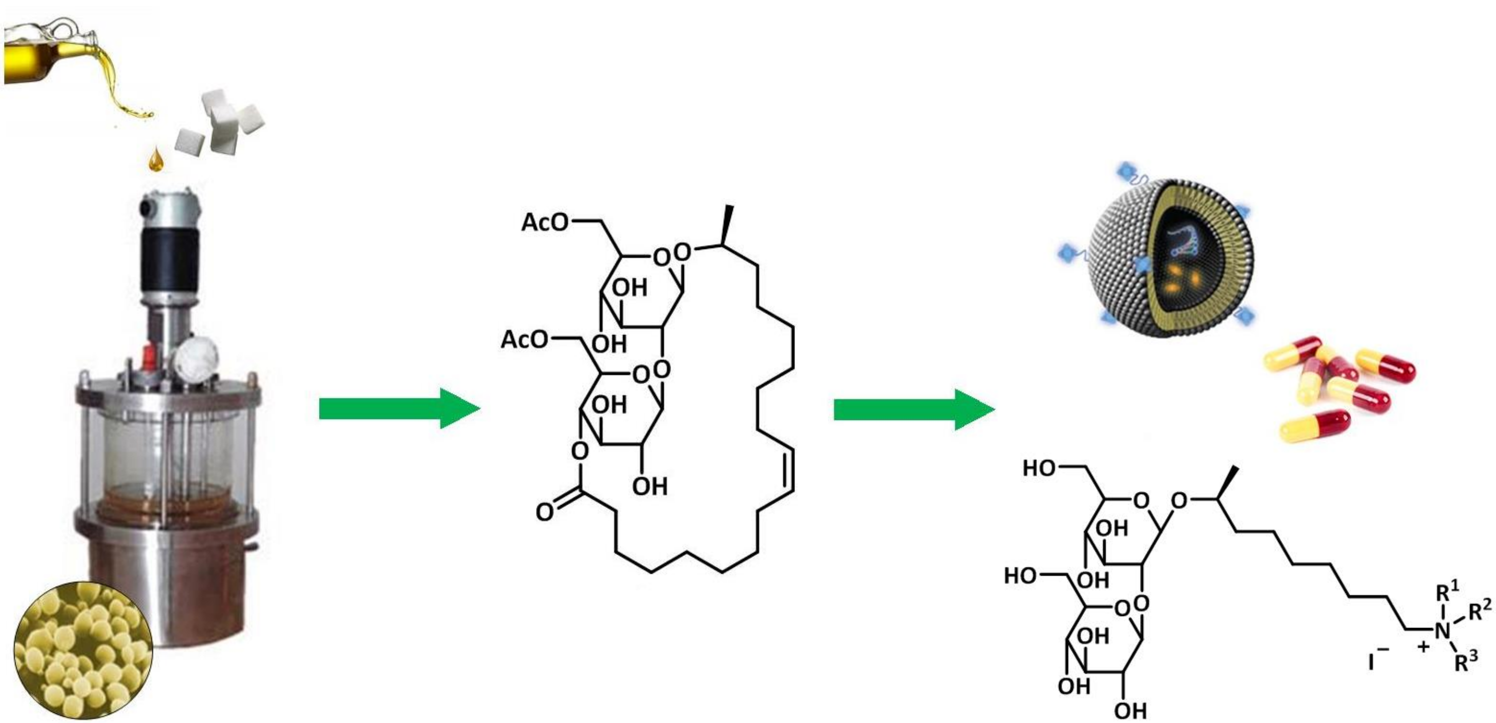
Quaternary ammonium sophorolipids as renewable based antimicrobial products

Graphical Abstract



Abstract:
In the European chemical industry, there is a strong drive to shift from fossil to renewable resources in the pursuit of sustainability. Sophorolipids, a class of biosurfactants, are interesting renewable resources, since they combine a complex structure with divergent biological and physico-chemical properties. The microbially produced lactonic sophorolipids were used for the production of a broad range of innovative sophorolipid amines and sophorolipid quaternary ammonium salts. These sophorolipid quaternary ammonium salts were evaluated for their antimicrobial activity against Gram-negative and Gram-positive bacterial test strains. Minimum inhibitory concentration (MIC) values were determined for the active compounds. Values of 5-8 µM were obtained for the derivatives containing an octadecyl chain attached to the nitrogen atom, compared to values of 10-52 µM for the antibiotic gentamicin sulfate. These results show great promise for modified sophorolipids in the medical sector, for example for the inhibition of biofilm formation.

Keywords: sophorolipids; biosurfactants; derivatization; antimicrobial activity

Introduction

Renewable resources:

- Slow transition to bio-based economy
- Used for production of only 8% of chemicals (Europe)

Fossil resources:

- Limited availability
- Negative impact on environment

General approach: **Renewable Resources** → **Base Chemicals** → **Products**

Drawback:

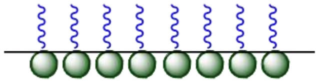
- High cost
- Long reaction sequence

→ **Renewable resources with complex structure for the synthesis of high added value products**

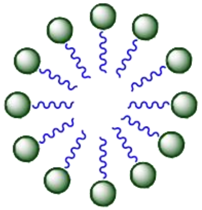
Introduction

Surfactant = Surface Active Compound

- Amphiphilic molecules which contain a **hydrophilic** and a **hydrophobic** moiety
- Reduces interfacial tension between liquids, solids and gases by arrangement at the interface (**a**)
- Formation of micelles (**b**) at a defined concentration
- Used as detergents, wetting agents, emulsifiers, ...
- **Biosurfactants:** surface-active compounds produced by living cells, e.g. Sophorolipids



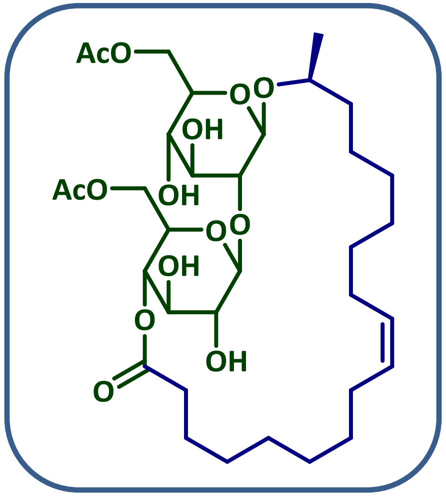
(**a**)



(**b**)

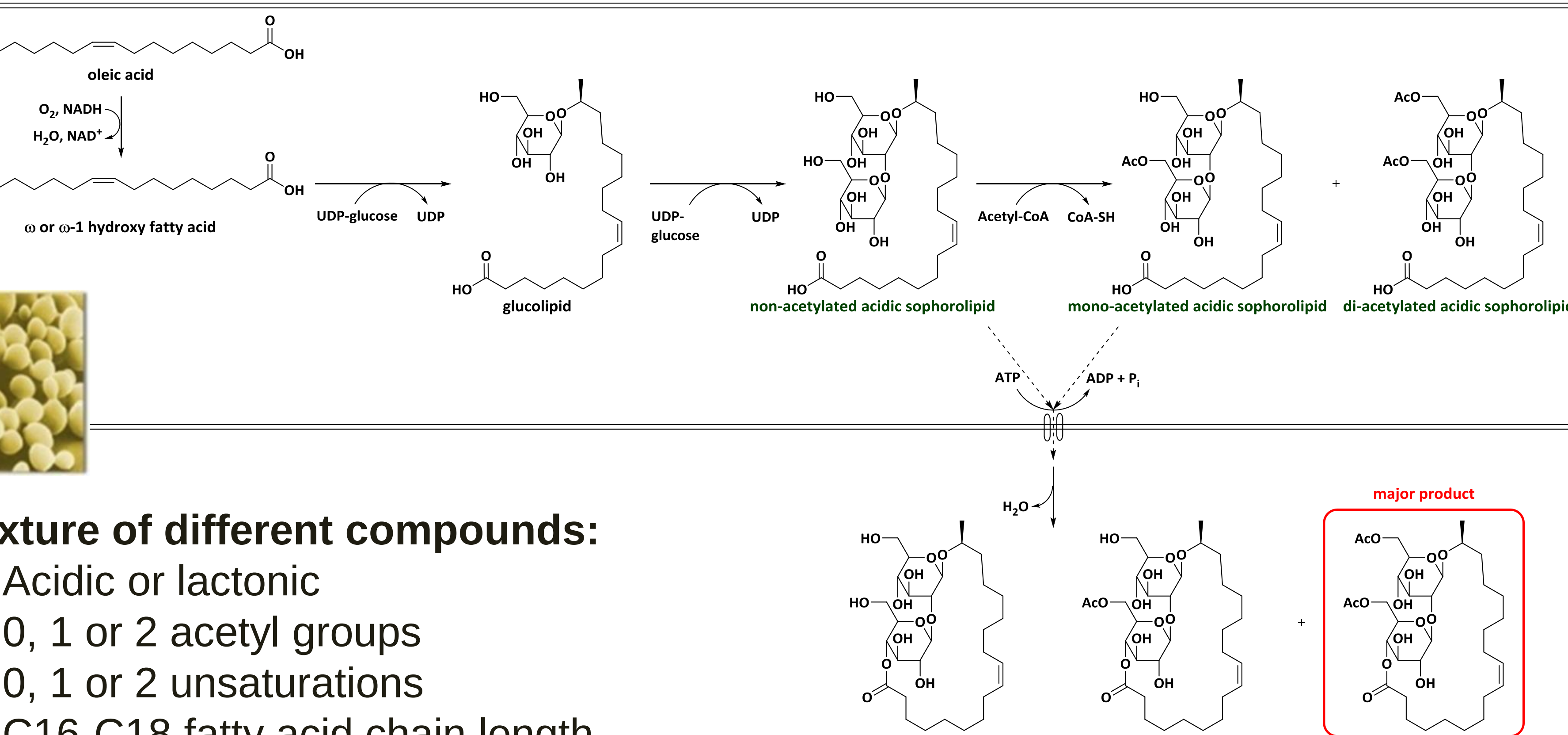
Sophorolipids:

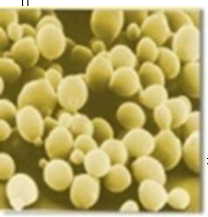
- Sophorose as **hydrophilic** carbohydrate head
- **Hydrophobic** lipid tail



Introduction

Microbial production of sophorolipids by *Starmerella bombicola*

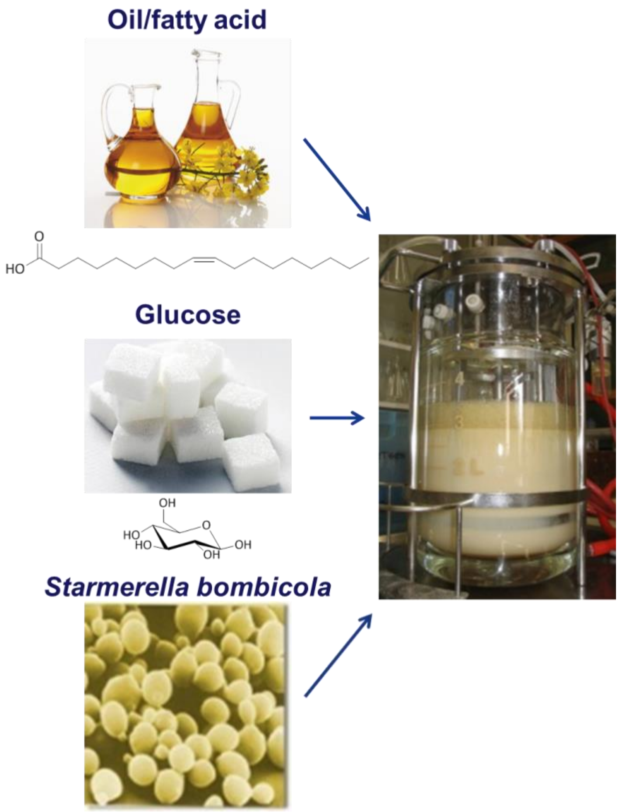




Mixture of different compounds:

- Acidic or lactonic
- 0, 1 or 2 acetyl groups
- 0, 1 or 2 unsaturations
- C16-C18 fatty acid chain length
- ω or ω-1 hydroxylation

Introduction



Advantages:

- Low eco-toxicity and high biodegradability
- Renewable resources as feedstock
- Surface-active properties linked to biological activities
- Non-pathogenic yeast
- High production: 400 g/L

Disadvantages:

- Higher price compared to chemical surfactants
- Microbial production is restricted to few derivatives

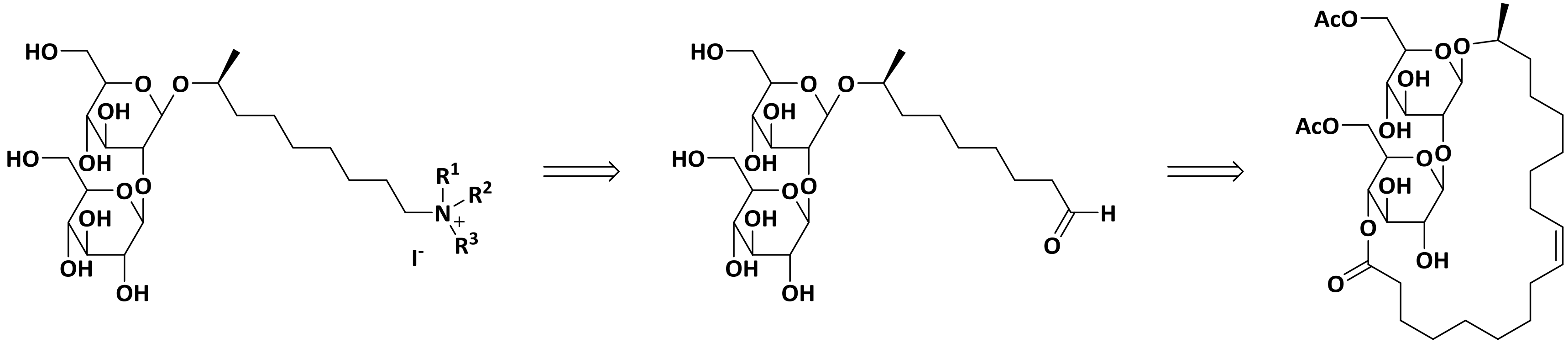
→ Application range is limited to detergents

Introduction

Chemical modification of microbial product

- **Goal:** Synthesis of short-chained derivatives with nitrogen functionality to increase the applicability of sophorolipids for medical applications

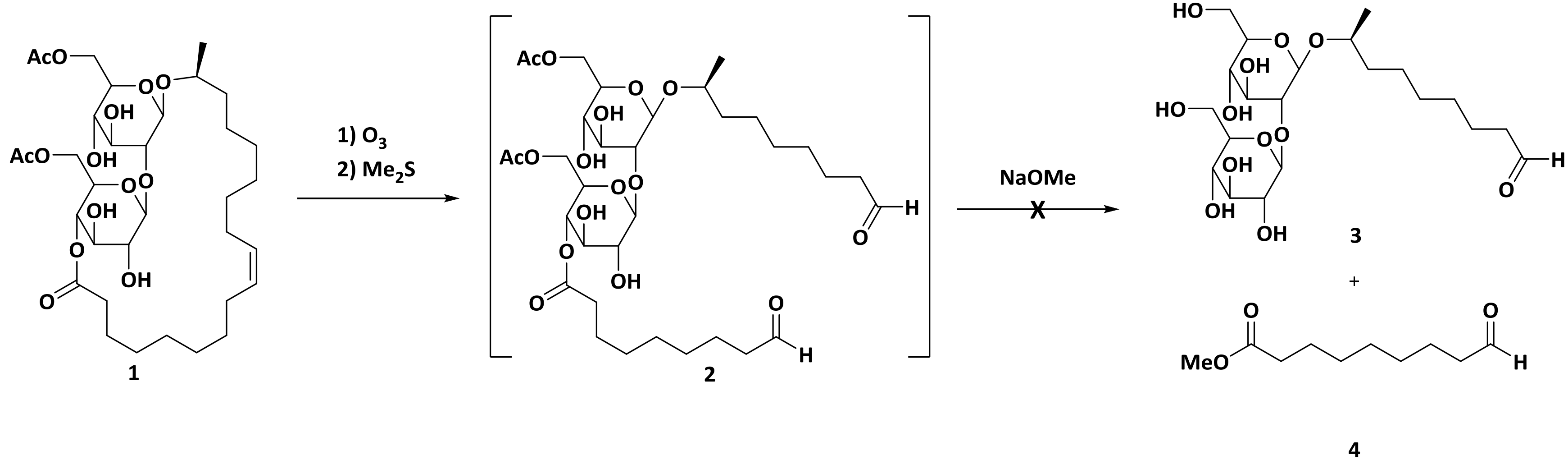
- Synthesis of aldehyde intermediate
- Modification towards quaternary ammonium salts



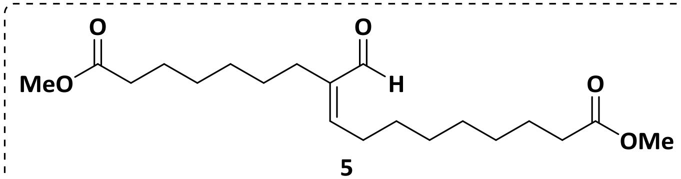
Results published as: E. I. P. Delbeke, B. I. Roman, G. B. Marin, K. M. Van Geem and C. V. Stevens, *Green Chem.*, 2015, **17**, 3373-3377.

Results and discussion

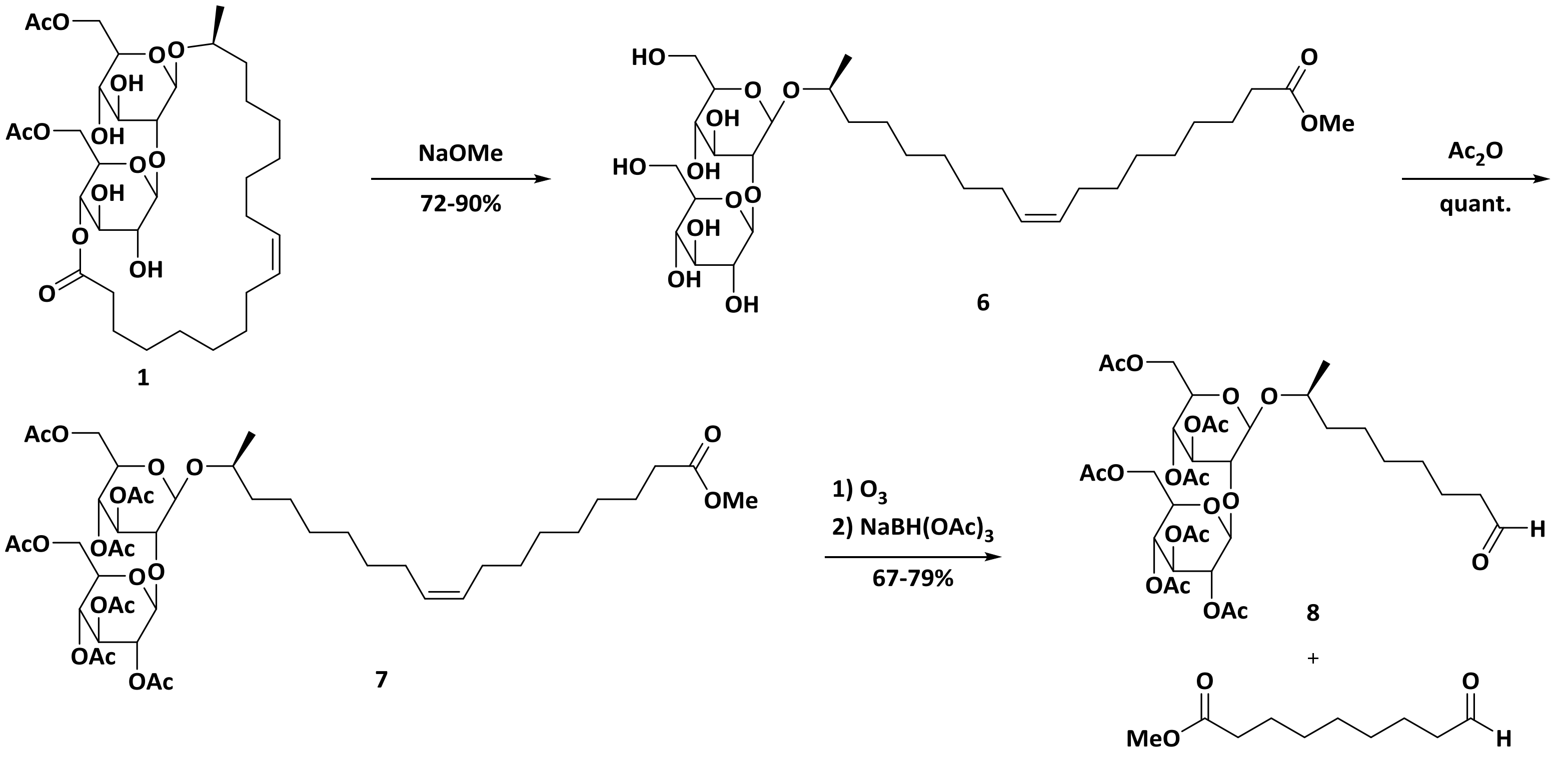
1. Synthesis of aldehyde intermediate



- Ozonolysis to intermediate sophorolipid dialdehyde **2**
- Transesterification to sophorolipid aldehyde **3** failed
- Only aldolcondensation to aldehyde **5** observed



Results and discussion



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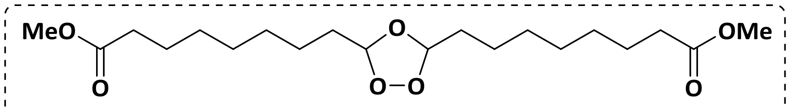
Stable ozonide intermediate **9** formed for work-up with Me_2S



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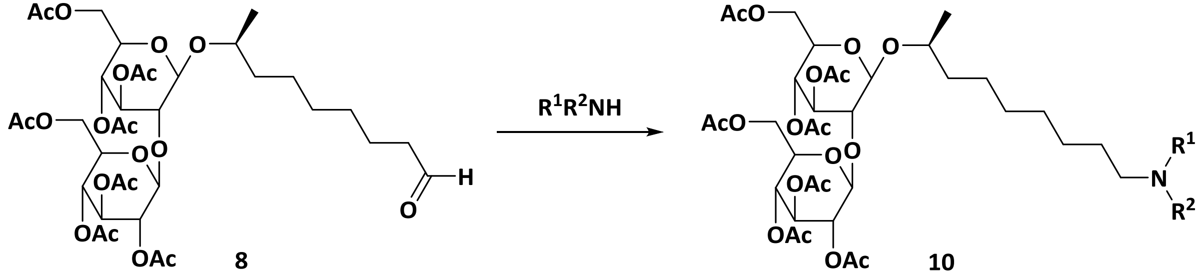
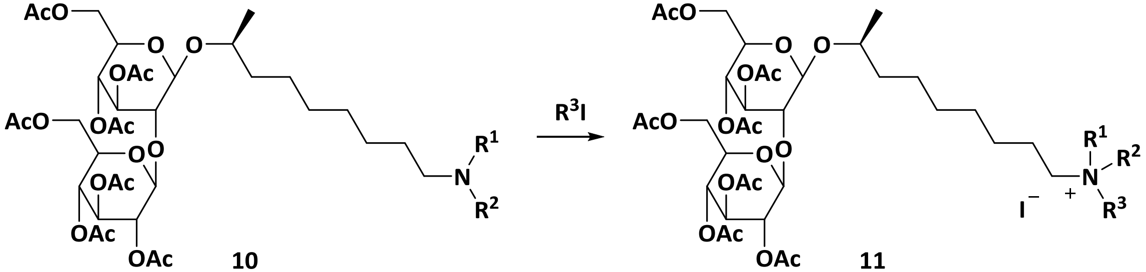
Results and discussion

2. Synthesis of quaternary ammonium salt library



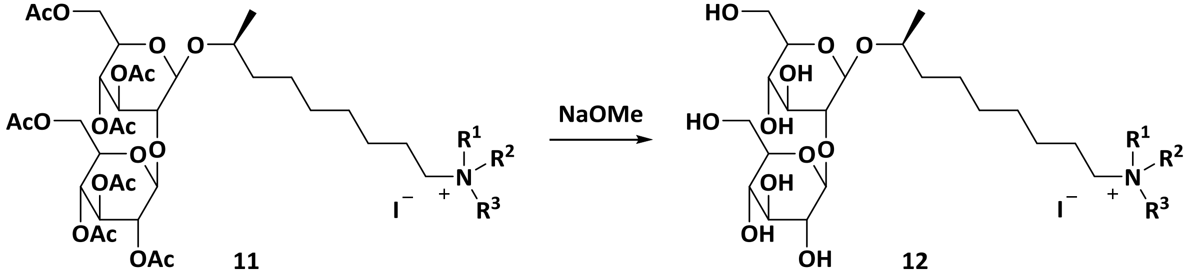
Entry	R¹R²NH		Yield (%)
1	Dimethylamine	**10a**	38
2	*N*-Methylbutylamine	**10b**	52
3	Dibutylamine	**10c**	53
4	*N*-Methylbenzylamine	**10d**	40
5	*N*-Butylbenzylamine	**10e**	49
6	Dibenzylamine	**10f**	48
7	*N*-Methyloctadecylamine	**10g**	39

Results and discussion



Entry		R^3I		Yield (%)
1	**10a**	Methyl iodide	**11a**	91
2	**10b**	Methyl iodide	**11b**	89
3	**10c**	Methyl iodide	**11c**	96
4	**10c**	Butyl iodide	**11d**	94
5	**10d**	Methyl iodide	**11e**	Quant.
6	**10e**	Methyl iodide	**11f**	89
7	**10f**	Methyl iodide	**11g**	Quant.
8	**10g**	Methyl iodide	**11h**	98
9	**10g**	Butyl iodide	**11i**	Quant.

Results and discussion



Entry			Yield (%)
1	**11a**	**12a**	Quant.
2	**11b**	**11b**	88
3	**11c**	**11c**	Quant.
4	**11d**	**12d**	Quant.
5	**11e**	**12e**	Quant.
6	**11f**	**12f**	Quant.
7	**11g**	**12g**	99
8	**11h**	**12h**	97
9	**11i**	**12i**	66

Results and discussion

3. Antimicrobial testing

Gram-positive strains:
- *Staphylococcus aureus*
- *Bacillus subtilis*

Gram-negative strains:
- *Escherichia coli*
- *Klebsiella pneumoniae*

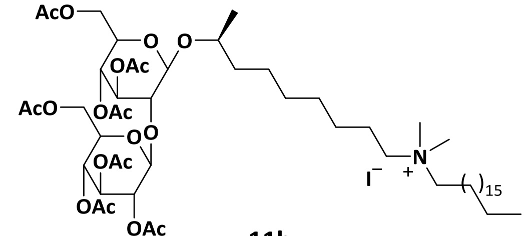
- Significant growth inhibition against **Gram-positive** strains for some sophorolipid analogues
  - 8 peracetylated quaternary ammonium sophorolipids
  - 3 deprotected quaternary ammonium sophorolipids
- Determination of Minimum Inhibitory Concentration (MIC) for the active compounds against 4 Gram-positive strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecium* and *Streptococcus pneumoniae*
- Antibiotic gentamicin sulfate was used as control

Results and discussion

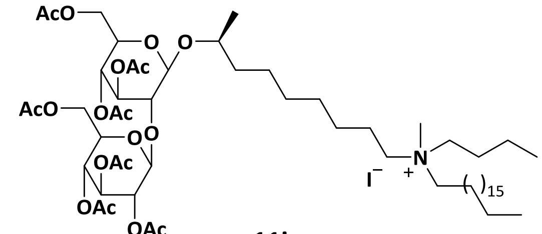
Minimum inhibitory concentration (MIC) values

(µg/mL)	11h	11i	12h	12i	Gentamicin sulfate
S. aureus	10	10	5	5	5
E. faecium	10	10	5	5	10
B. subtilis	10	10	5	5	5
S. pneumoniae	10	10	5	5	25

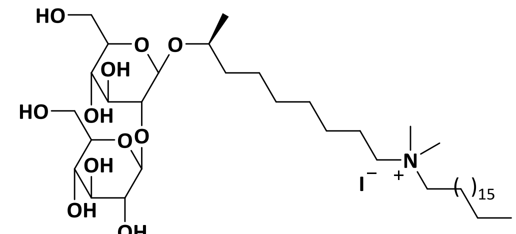
(µM)	11h	11i	12h	12i	Gentamicin sulfate
S. aureus	8	8	6	5	10
E. faecium	8	8	6	5	21
B. subtilis	8	8	6	5	10
S. pneumoniae	8	8	6	5	52



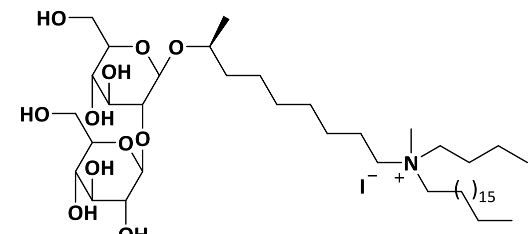
11h



11i



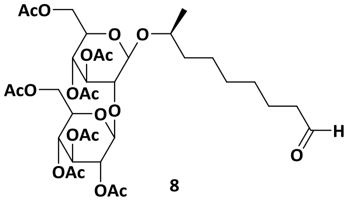
12h



12i

Conclusions

- **Synthesis of new-to-nature sophorolipids accomplished**



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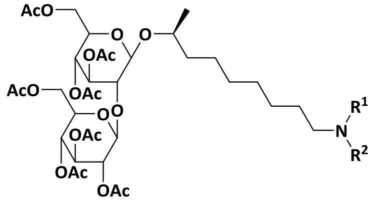
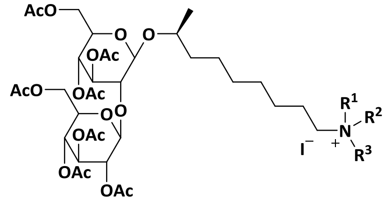
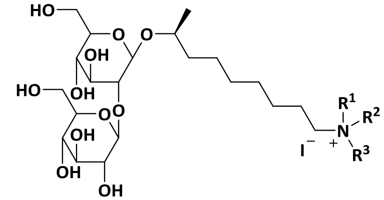


10a: R^1=R^2=Me
10b: R^1=Me, R^2=Bu
10c: R^1=R^2=Bu
10d: R^1=Me, R^2=Bn
10e: R^1=R^2=Bn
10f: R^1=Bu, R^2=Bn
10g: R^1=Me, R^2=$C_{18}H_{37}$

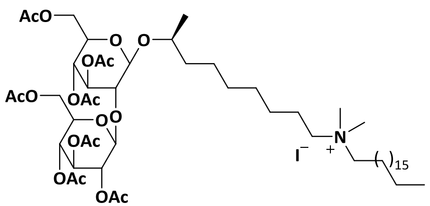


11a: R^1=R^2=R^3=Me
11b: R^1=R^2=Me, R^3=Bu
11c: R^1=Me, R^2=R^3=Bu
11d: R^1=R^2=R^3=Bu
11e: R^1=R^2=Me, R^3=Bn
11f: R^1=Me, R^2=Bu, R^3=Bn
11g: R^1=Me, R^2=R^3=Bn
11h: R^1=R^2=Me, R^3=$C_{18}H_{37}$
11i: R^1=Me, R^2=Bu, R^3=$C_{18}H_{37}$



12a: R^1=R^2=R^3=Me
12b: R^1=R^2=Me, R^3=Bu
12c: R^1=Me, R^2=R^3=Bu
12d: R^1=R^2=R^3=Bu
12e: R^1=R^2=Me, R^3=Bn
12f: R^1=Me, R^2=Bu, R^3=Bn
12g: R^1=Me, R^2=R^3=Bn
12h: R^1=R^2=Me, R^3=$C_{18}H_{37}$
12i: R^1=Me, R^2=Bu, R^3=$C_{18}H_{37}$

- **Four derivatives with high antibacterial activity**



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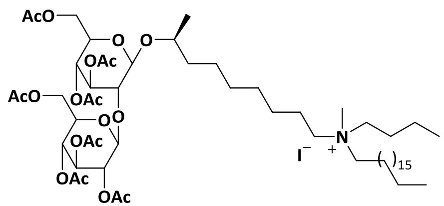

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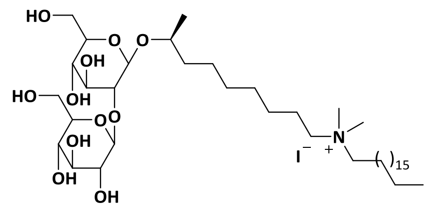

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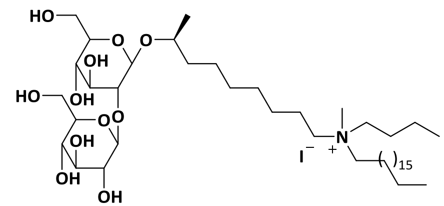

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