

Sophorolipid derivatives: Chemical derivatization towards green surfactants for medicinal applications

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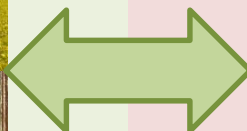
³Bio Base Europe Pilot Plant, Belgium

⁴Laboratory for Chemical Technology (LCT), Ghent University



Introduction

Renewable resources



Fossil resources



Regained in a short period of time

Sustainable natural resources

Low ecological footprint

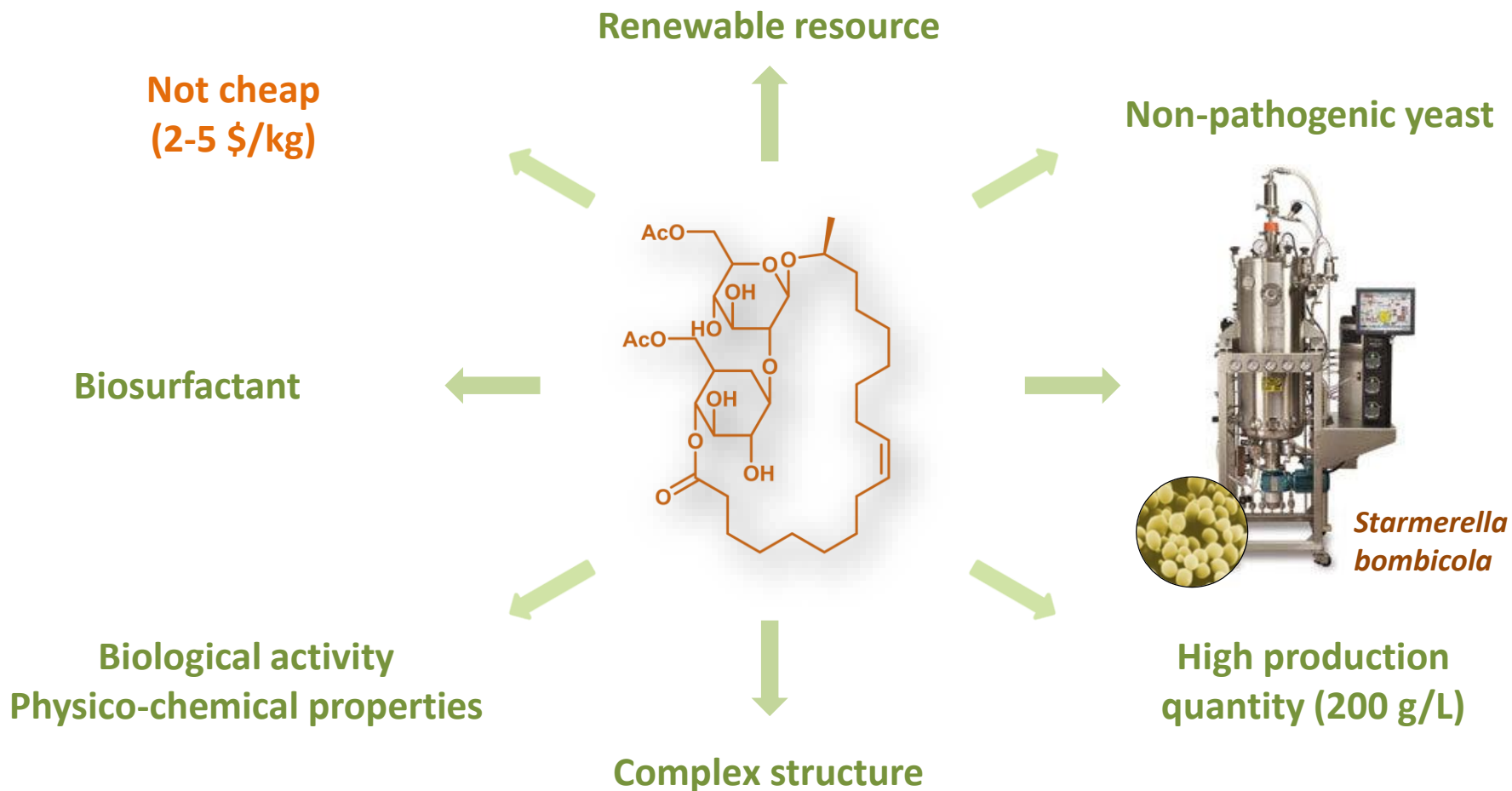
Formation over millions of years

Limited availability

Negative impact on environment

What are Sophorolipids?

Sophorolipid = sophorose head + fatty acid tail



Building blocks for chemical derivatization

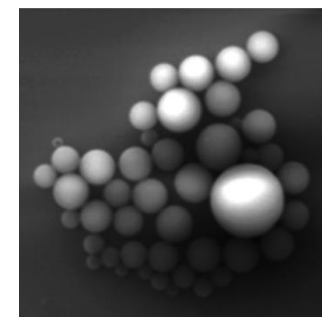
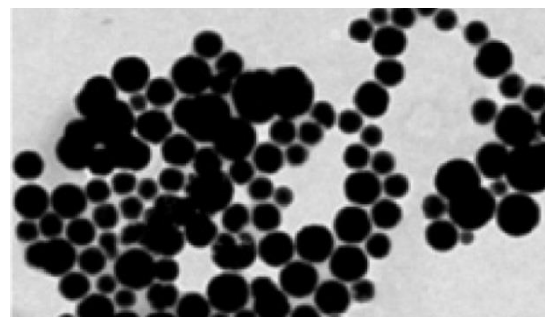
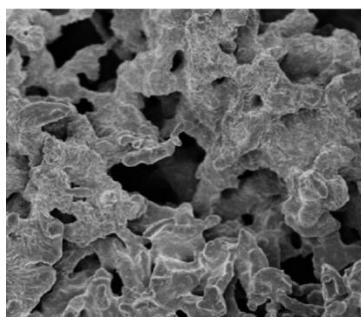
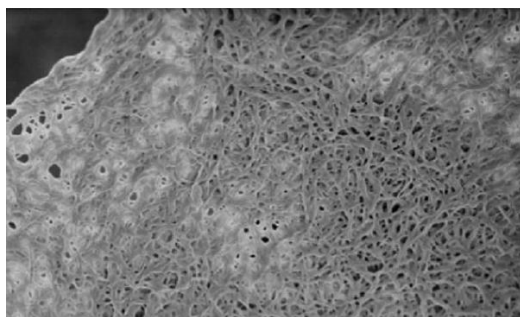
Why study Sophorolipids?

Biological activity:

- Dermatological
- Antimicrobial
- Anticancer
- Immunoregulatory
- Spermicidal and antiviral

Self-assembly properties:

- Nanostructures with supramolecular chirality
- Capping of nanoparticles



Commercial Sophorolipid applications

Commercialized by different companies

ECOVER[®]
Givaudan[®]



SARAYA



Natural detergent made from natural cleansing agent
Sophorolipid and other food-based ingredients

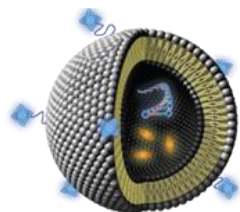
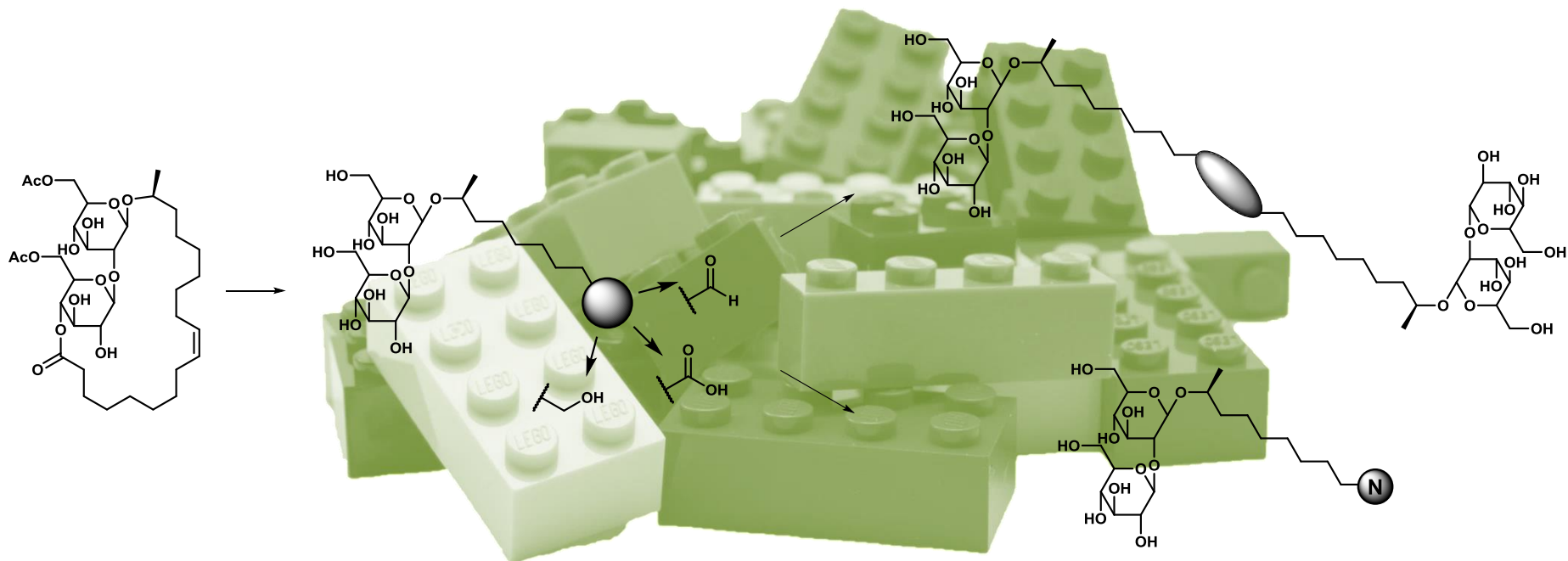


Happy Elephant
safe and sustainable



Objectives

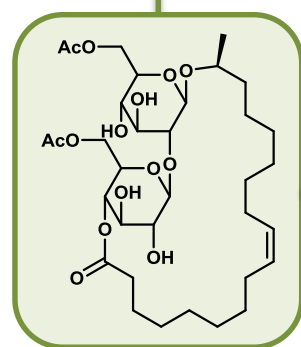
Synthesis of innovative sophorolipid derivatives



- High added-value products
- Pharmaceutical sector
- Focus on property evaluation

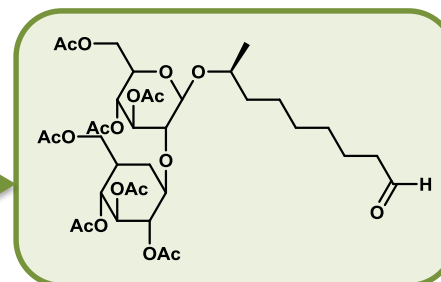
State of the art

Development of a synthetic pathway towards sophorolipid intermediates

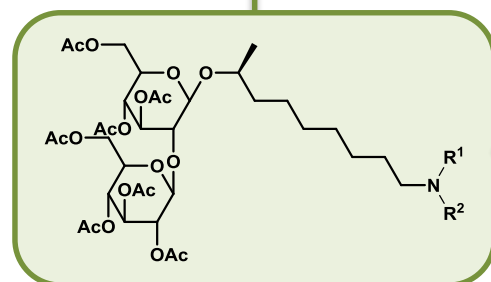


3 steps

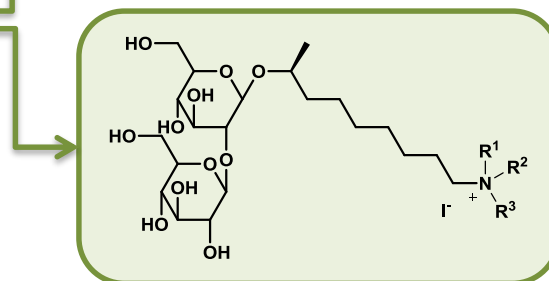
Sophorolipid aldehyde



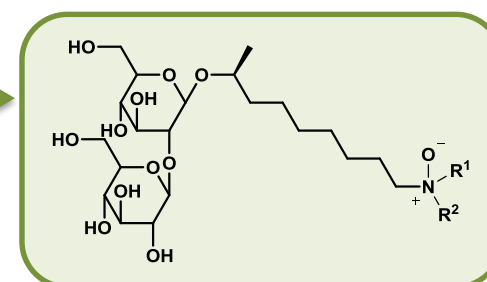
Synthesis of new sophorolipid derivatives



Sophorolipid amines



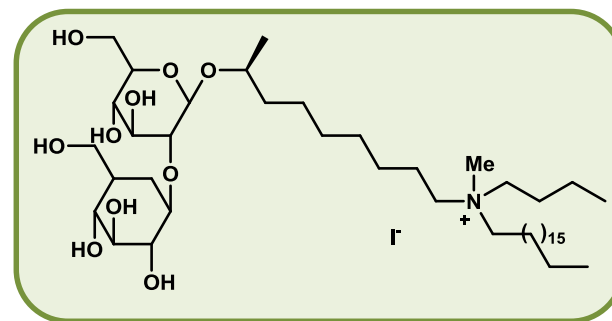
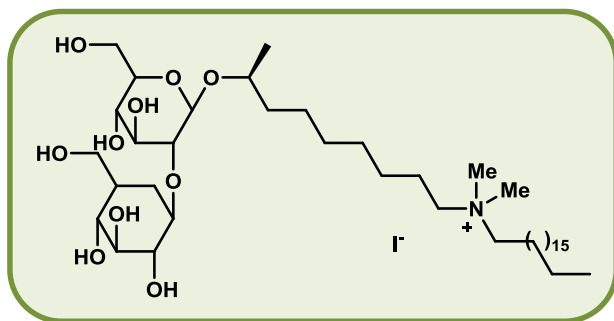
Quaternary ammonium sophorolipids



Sophorolipid amine oxides

State of the art

Most interesting derivatives: Quaternary ammonium sophorolipids with octadecyl chain



- **Antimicrobial activity** against *S. aureus*, *E. faecium*, *B. subtilis* and *K. pneumoniae* (MIC 5 µg/mL)
- **Good transfection efficiency** (vectors for gene delivery)
- **Positive influence of carbohydrate head** was demonstrated

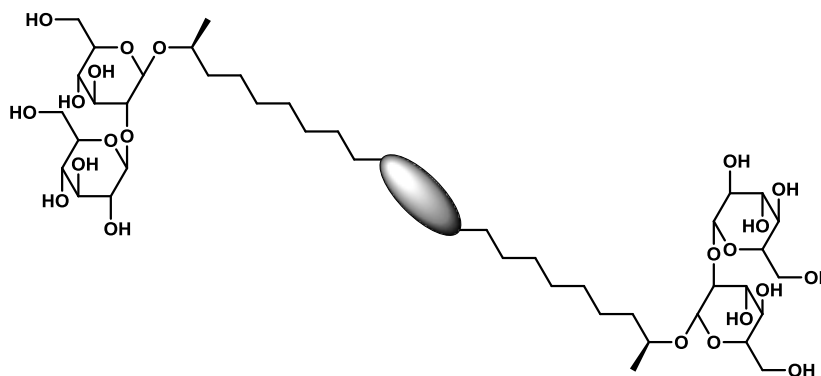
E.I.P. Delbeke, B.I. Roman, G.B. Marin, K.M. Van Geem, C.V. Stevens, *Green Chem.*, 2015, **17**, 3373-3377

E.I.P. Delbeke, O. Lozach, T. Le Gall, M. Berchel, T. Montier, P.-A. Jaffrès, K.M. Van Geem, C.V. Stevens, *Org. Biomol. Chem.*, 2016, **14**, 3744-3751

E.I.P. Delbeke, S.L.K.W. Roelants, N. Matthijs, B. Everaert, W. Soetaert, T. Coenye, K.M. Van Geem, C.V. Stevens, *Ind. Eng. Chem. Res.*, 2016, **55**, 7273-7281

New Sophorolipid derivatives

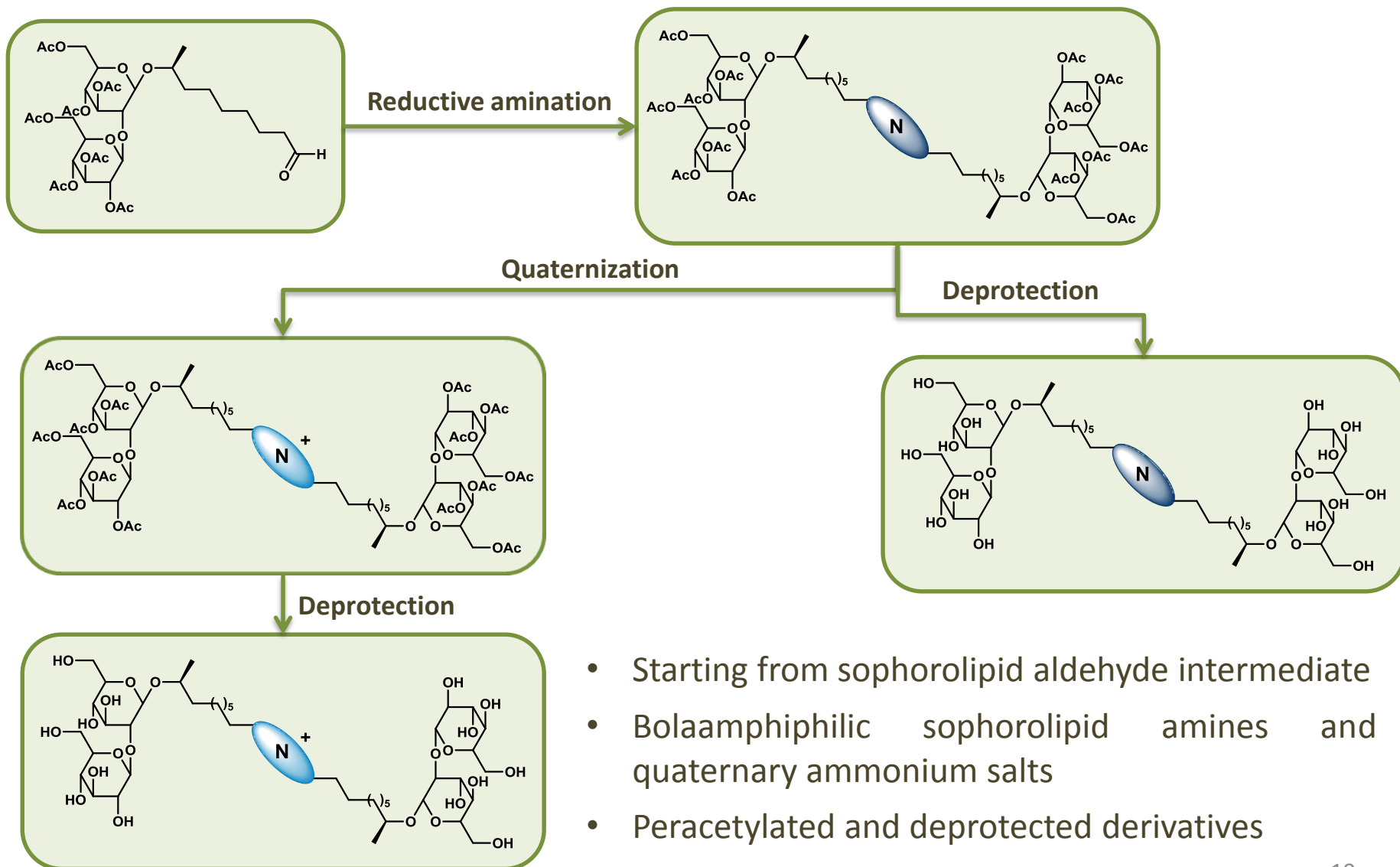
Synthesis of bolaamphiphilic sophorolipids



- **Bolaamphiphiles:** two hydrophilic parts linked by hydrophobic linker
- Tetraether lipid membranes of archaeobacteria
- Formation of stable nanostructures
- **Applicability:** Stable vesicles for drug delivery
Membrane-spanning linkers for biosensors

...

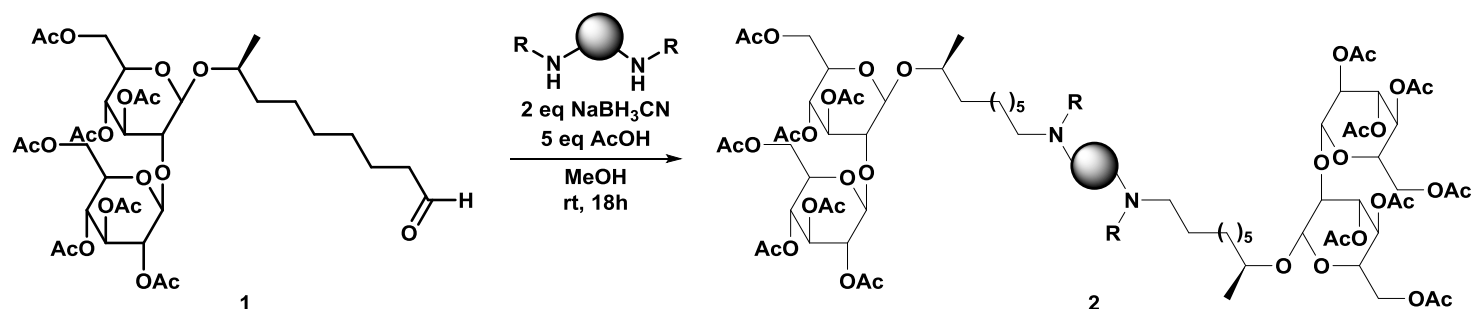
Synthesis overview



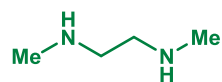
- Starting from sophorolipid aldehyde intermediate
- Bolaamphiphilic sophorolipid amines and quaternary ammonium salts
- Peracetylated and deprotected derivatives

Reductive amination

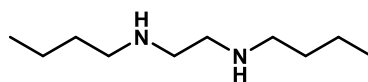
Towards bolaamphiphilic sophorolipid amines



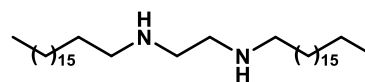
- Reductive amination with diamines
- Set of 8 secondary diamines **3** was selected
- Only **3a** and **3b** are commercially available



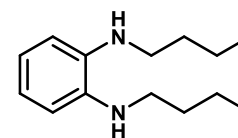
3a



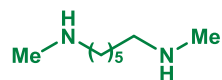
3c



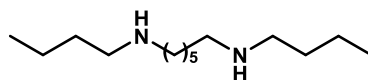
3e



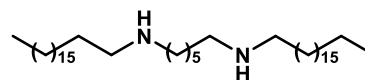
3g



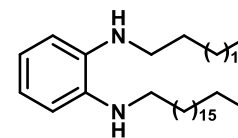
3b



3d

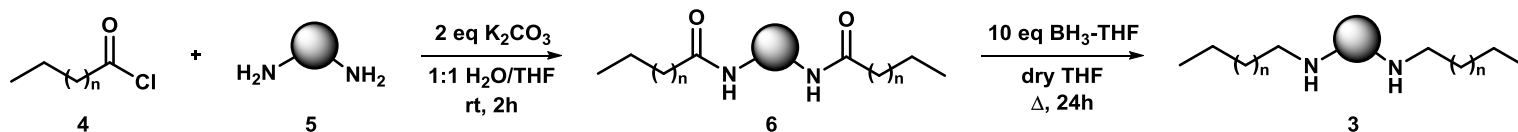


3f

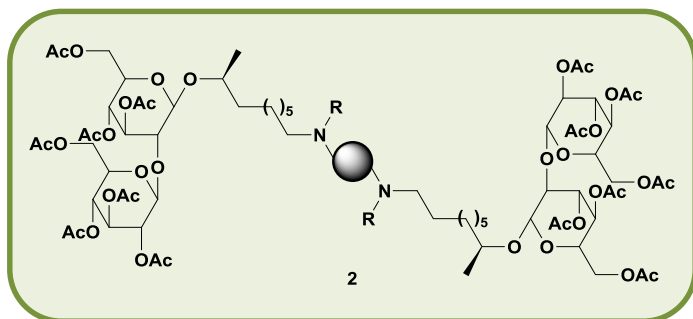


3h

Reductive amination: results



- Non-commercial diamines synthesized *via* Schotten-Baumann reaction and reduction
- Results reductive amination:

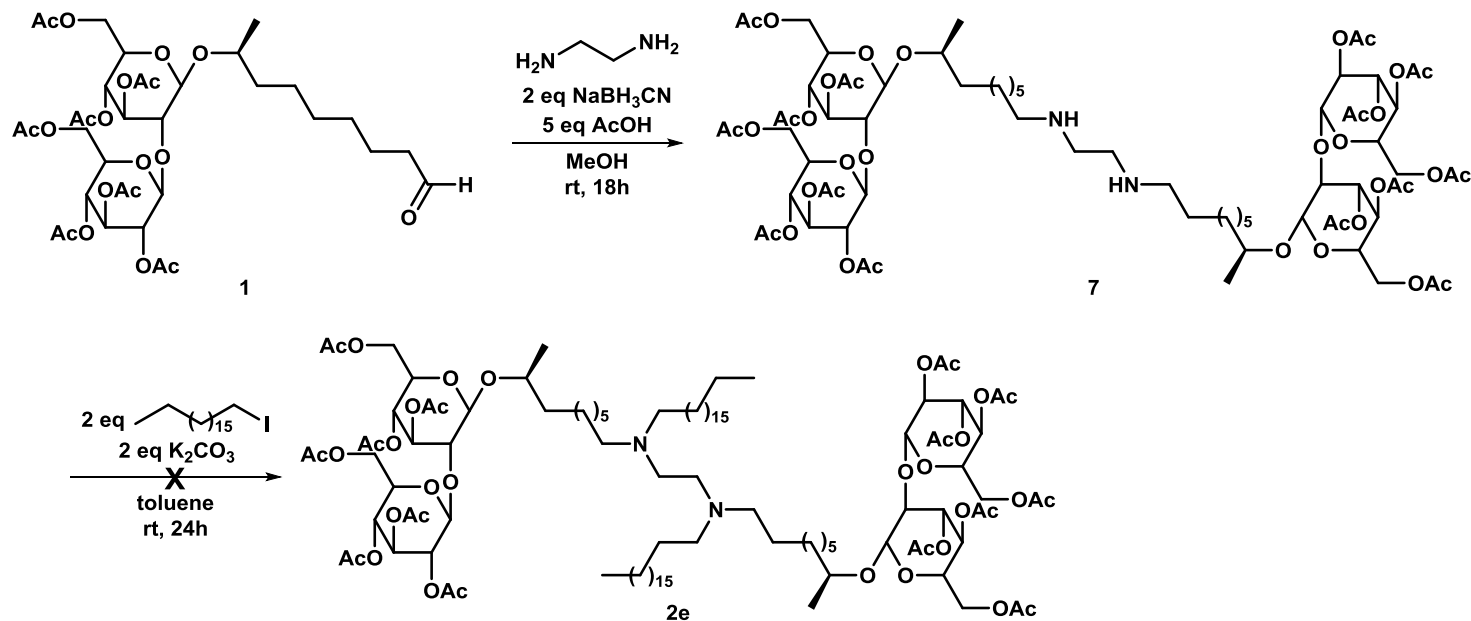


- Uncomplete conversion towards **2g**
- No reaction with **2e**, **2f** and **2h**

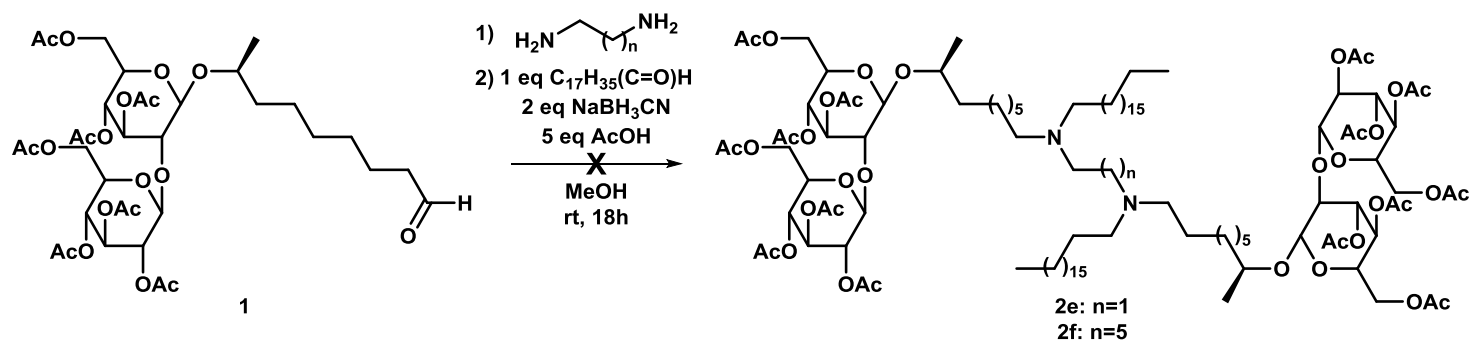
2	Diamine	Yield (%)
2a		31
2b		31
2c		41
2d		27
2e		/
2f		/
2g		/
2h		/

Alternative procedures

- Reductive amination with primary diamine and alkylation with alkyl iodide

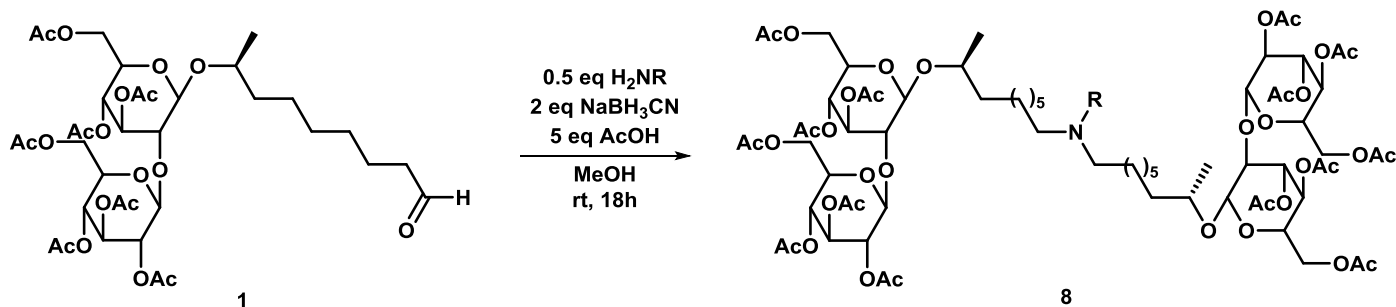


- Tandem reductive amination with primary diamine and aldehyde



2nd type of bolaamphiphilic sophorolipids

- Reductive amination with primary amines



8	Amine	Yield (%)
8a	Methylamine	55
8b	Butylamine	15
8c	Benzylamine	39
8d	Octadecylamine	18

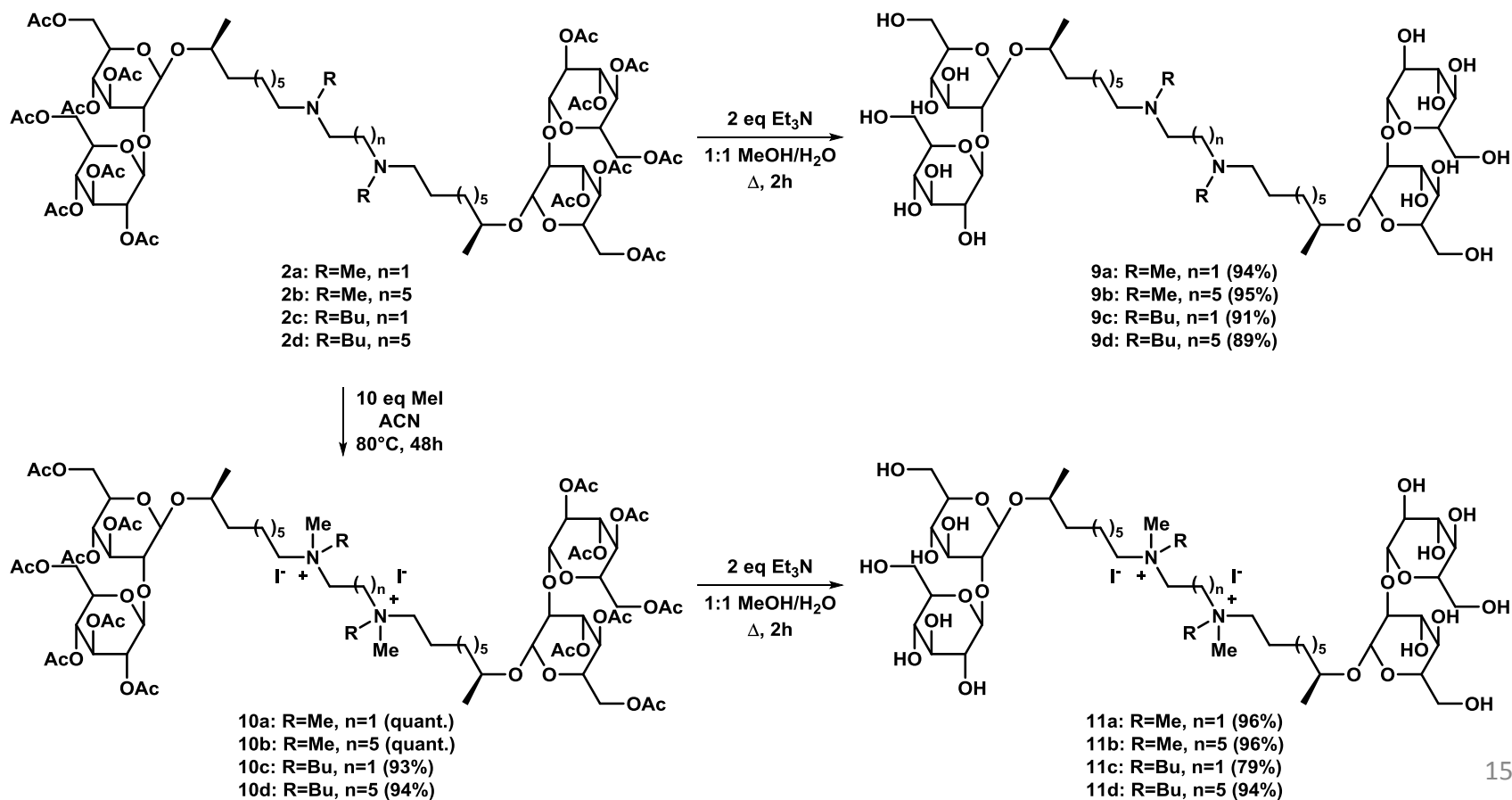


Total set of 8 different peracetylated bolaamphiphilic sophorolipid amines

Modification of *N,N'*-dialkyl bolaamphiphiles

Towards deprotected and quaternary ammonium derivatives

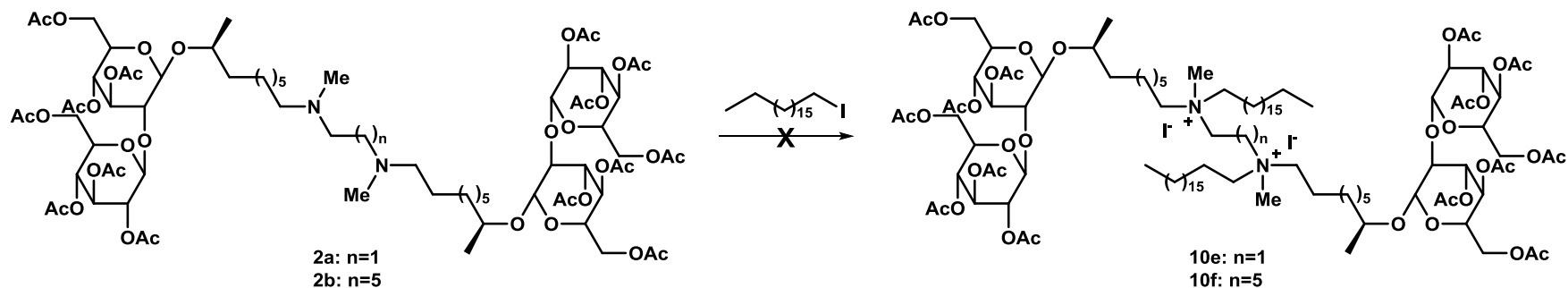
- Quaternization with methyl iodide
- Deprotection with triethylamine



Alternative procedure

Alternative procedure for the introduction of an octadecyl chain

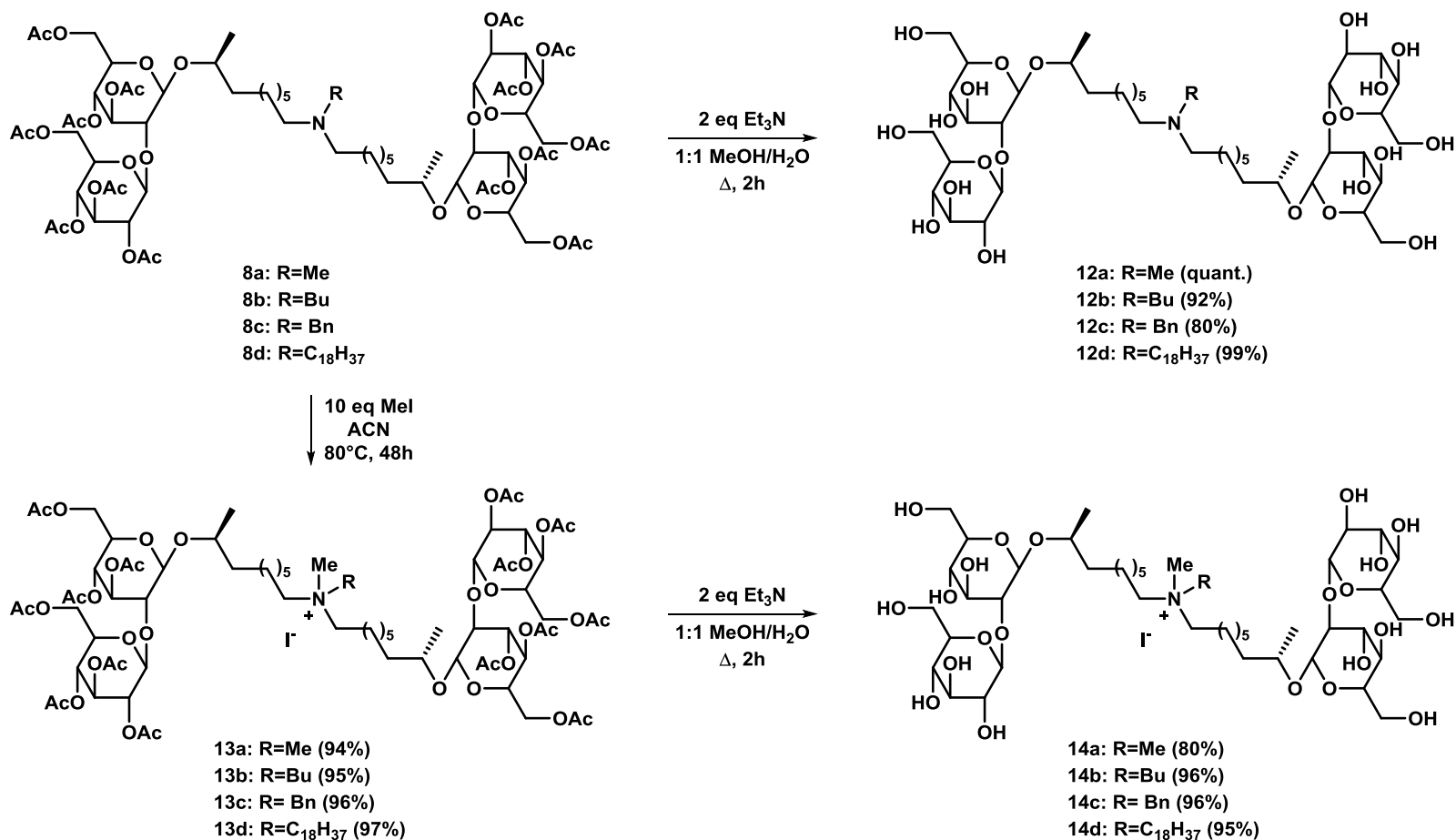
- Quaternization with octadecyl iodide



No quaternization in acetonitrile or toluene after 72h

Modification of *N*-alkyl bolaamphiphiles

- Same procedure for *N*-alkyl bolaamphiphilic sophorolipid amines



Total set of 32 different bolaamphiphilic sophorolipid derivatives

Antimicrobial evaluation

- Determination of MIC and MBC values
- Gram-positive strains *Staphylococcus aureus* ATCC 6538
 Staphylococcus aureus Mu50
- Gram-negative strains *Escherichia coli* LMG 8063
 Klebsiella pneumoniae LMG 2095
 Pseudomonas aeruginosa PAO1



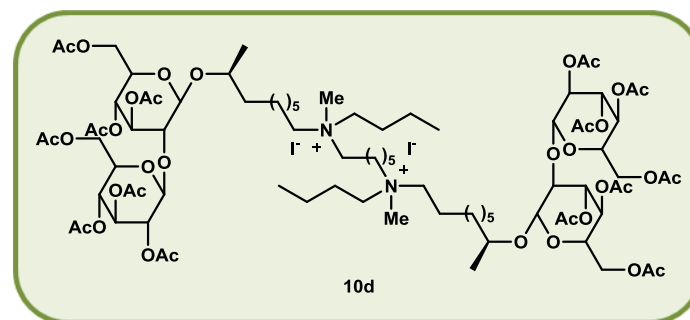
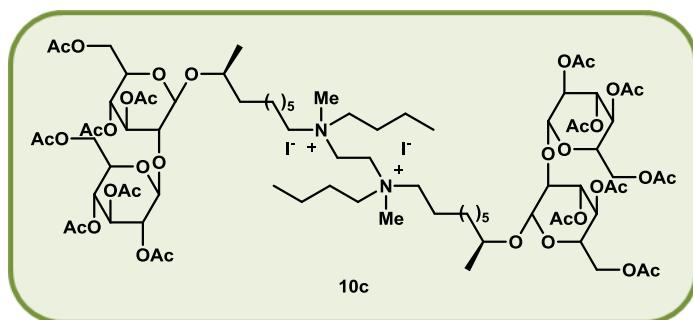
➔ Set of 9 bolaamphiphilic sophorolipids active against Gram-positive strains

Laboratory of Pharmaceutical Microbiology (Ghent University, Prof. T. Coenye)

Antimicrobial evaluation

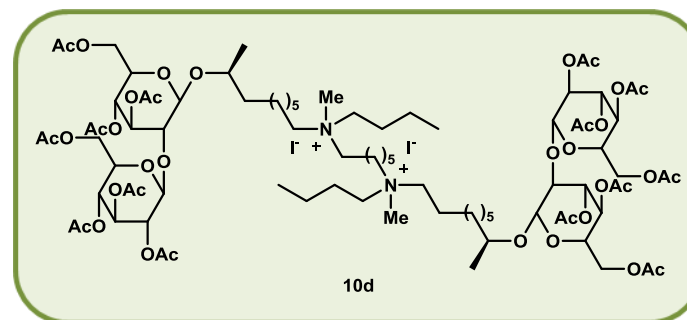
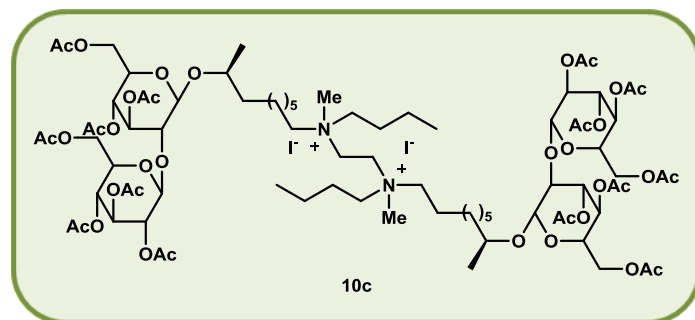
($\mu\text{g/mL}$)		2b	10a	10b	10c	10d	13a	13b	13c	14d
<i>S. Aureus</i> ATCC 6538	MIC	625	312	156	39	78	625	625	625	156
	MBC	>2500	625	625	312	312	2500	1250	>2500	312
<i>S. Aureus</i> Mu50	MIC	2500	1250	625	156	312	312	625	312	312
	MBC	2500	2500	625	312	1250	2500	2500	1250	>2500

(μM)		2b	10a	10b	10c	10d	13a	13b	13c	14d
<i>S. Aureus</i> ATCC 6538	MIC	375	165	80	20	38	369	360	353	116
	MBC	>1501	330	321	158	153	1476	720	>1412	233
<i>S. Aureus</i> Mu50	MIC	1501	660	321	79	153	185	360	176	233
	MBC	1501	1320	321	158	614	1476	1440	706	>1861



Conclusions

- Synthesis of a new class of sophorolipid derivatives was accomplished
- Activity against *S. aureus* ATCC 6538 and *S. aureus* Mu50



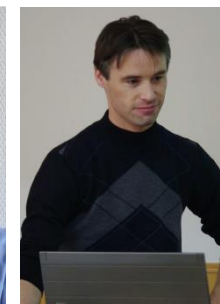
➔ 10-fold lower compared to quaternary ammonium sophorolipids

- Evaluation of transfection efficiency and self-assembly in progress

Acknowledgements



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- Prof. dr. ir. K.M. Van Geem
- Prof. dr. ir. G.B. Marin
- InBio Research Group
- Prof. T. Coenye



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