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ARTICLE

Chemical and enzymatic modification of sophorolipids

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The significance of renewable resources within the chemical industry is constantly increasing. In the pursuit of sustainability, they serve as alternatives for fossil resources whose supply is limited and who have a major impact on the environment. Due to their complex structure and divergent biological activities, sophorolipids are interesting renewable resources. Unfortunately, industrial applications of natural sophorolipids are limited because of their high production cost. Therefore, chemical and enzymatic modifications provide an excellent tool to shift the application area of sophorolipids to high-added value sectors, in particular for the pharmaceutical sector. This review will give an overview of the modifications performed so far and their possible applications.

Introduction

Sophorolipids are one of the most important classes of glycolipid biosurfactants. They consist of sophorose as hydrophilic carbohydrate head and a hydrophobic lipid tail, which gives them an amphiphilic character. Sophorolipids lower the surface tension of water from 72.8 to 30-40 mN/m and have a critical micelle concentration of 40-100 mg/L.[1] They possess self-assembly properties due to the formation of nanostructures with supramolecular chirality.[2] Their emulsifying properties can be applied for the recovery of oil and hydrocarbons and for the decontamination of soil and water.[3] The most common natural derivatives are diacetylated sophorolipid lactone **1** and sophorolipid acid **2** (Scheme 1).

Interest in sophorolipids exponentially increased over the last 20 years, reaching over 200 citations in 2013 and 2014 (Figure 1). Several reviews have been written on sophorolipids lately, with their focus on the microbial production of sophorolipids and application of these natural compounds.[1, 4] To date, natural sophorolipids are commercialized by different companies worldwide. The Japanese company Saraya and the Belgian company Ecover apply sophorolipids in their laundry, dishwashing and cleaning products while the former French company Soliance, now part of the Swiss company Givaudan, and the Korean company MG Intobio offer respectively the products Sopholiance S and Sopholine for cosmetic appplications.[5] Other companies working on the industrial production of sophorolipids are Evonik, DSM and Croda.[5-6] Despite these positive examples, application of natural sophorolipids is limited because of their high production cost. Van Bogaert *et al.* reported a production cost of 2 to 5 €/kg, but an up-to-date production price is not communicated by the companies.[1]

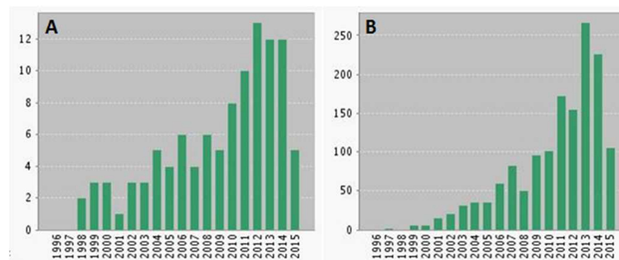
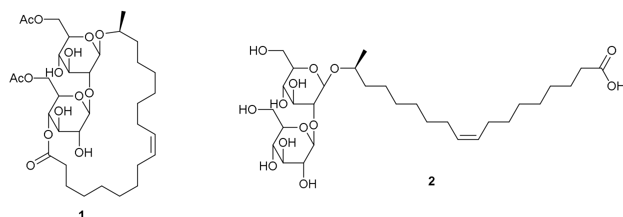


Figure 1. Number of publications (A) and citations (B) per year on sophorolipids



Scheme 1. Diacetylated sophorolipid lactone **1** and sophorolipid acid **2**

Modification of natural sophorolipids creates opportunities for their application in other high-added value sectors, in particular the pharmaceutical sector. Multiple modifications of natural sophorolipids have already been described, but were never combined in one review. In this review, it is aimed to provide an overview of all the chemical and enzymatic modifications performed on sophorolipids in the development of innovative high-added value compounds, in particular for the pharmaceutical sector.

Production of natural sophorolipids

Sophorolipids are produced by a selected number of yeast species.[1, 4a, 7] They were first described by Gorin *et al*. in the early 1960s as an extracellular glycolipid produced by *Candida apicola*, formerly known as *Torulopsis apicola*. Other producing strains comprise *Rhodotorula bogoriensis* (formerly known as *Candida bogoriensis*),

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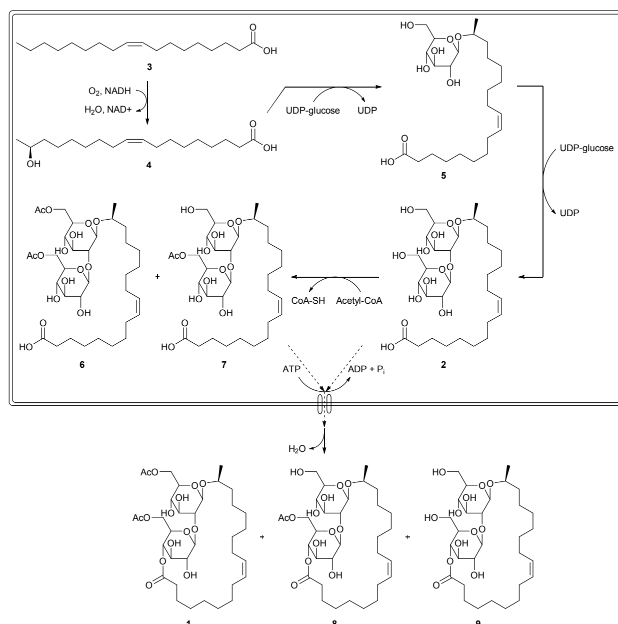
Starmerella bombicola (formerly known as *Torulopsis bombicola* and *Candida bombicola*) and *Wickerhamiella domericqiae*. *Starmerella bombicola* is the best known and preferred producing strain with a production yield of more than 400 g/L. The sophorolipid biosynthesis starts with glucose as the hydrophilic carbon source and fatty acids, fatty acid methyl esters, triglycerides or alkanes as the hydrophobic carbon source (Scheme 2). All hydrophobic carbon sources are converted into fatty acids, which contain in general 16 or 18 carbon atoms with one or more double bonds. When no hydrophobic carbon source is present in the fermentation medium, fatty acids can be formed de novo via the acetyl-CoA pathway. The fatty acids are subsequently oxidized at the terminal (ω) or subterminal (ω-1) position by a cytochrome P450 monooxygenase enzyme. The resulting hydroxylated fatty acids are β-glycosidically linked to a first glucose molecule at the 1'-position by a glycosyltransferase I. A second glucose molecule is linked to the 2'-position of the first one by a glycosyltransferase II, yielding sophorolipid acid 2. Subsequently, the carbohydrate head can be acetylated at the 6'- and/or 6"-position by an acetyl-CoA dependent acetyl transferase. After excretion in the fermentation medium, lactonization can occur at the 4"-position by an extracellular esterase.[8] The major microbial product is the diacetylated, ω-1 hydroxylated, mono-unsaturated sophorolipid lactone **1**.

Most sophorolipid fermentations are run at a temperature between 25 to 30°C and a pH of 3.5. Sophorolipid synthesis starts in the stationary phase under nitrogen-limiting conditions and is very dependent on good aeration conditions. The highest yields are obtained when both a hydrophilic and a hydrophobic carbon source are present, but fermentation on only one type of carbon source is possible. After the fermentation, sophorolipids are extracted with ethyl acetate and washed with hexane to remove residual fatty acids. On larger scale, physical separation methods such as centrifugation are applied for the isolation. Lactonic sophorolipids were also purified by crystallization.[9]



Scheme 2. Biosynthesis sophorolipid derivatives[1]

The biggest bottleneck for the sophorolipid production process is the variability in the composition of the fermentation product. To optimize chemical modification procedures, one single compound is preferred as starting compound. If this is not the case, problems can arise along the modification pathway. For example, in the synthesis of sophorolipid alkyl esters via alkaline hydrolysis of di-acetylated sophorolipid lactone, the products are precipitated in water after reaction. In some cases, precipitation does not occur, possibly due to the presence of a considerable fraction of sophorolipid acids or residual fatty acids. Therefore, it is of utmost importance to have access to selective organisms for the fermentation process or suitable purification procedures to obtain the desired sophorolipid compounds in high purity.[5, 10]

Physiological activity

Biodegradability and toxicity

The biodegradability of sophorolipids was determined via biological oxygen demand studies and the manometric respirometry method (OECD 301C and 301F method).[4a, 11] They can be classified as readily biodegradable since 61% of the product was degraded after only 8 days of cultivation. The aquatic toxicity is subdivided in acute and chronic toxicity. The acute toxicity is determined as the EC_{50} after 48 h on *Daphnia magna*, *Tetrahymena terhmophila* and *Pseudokirchneriella subcaptitata* and proved to be ten times higher as compared to conventional surfactants.[4a, 12] The chronic toxicity was determined via a reproduction test on *Daphnia magna*. The no-observed-effect concentration (NOEC) was 11.3 mg/L, which is also ten times higher as compared to conventional surfactants.[12] The cytotoxicity was determined by the MTT method with human epidermal keratinocytes, displaying a lower cytoxicity for sophorolipids than for surfactin, arthrofactin, pluronic L31, sodium dodecyl sulfate and polyoxyethylene lauryl ether.[11] The cytotoxicity was also evaluated on normal Chang liver cells, displaying an IC_{50} value of 81.9 mg/mL for crude sophorolipids and a higher cell viability for acidic sophorolipids compared to lactonic ones.[13] Sophorolipids are not irritating to skin and eyes, have an oral safety level which is greater than or equal to 5mL/kg body weight and cause no allergic reactions.[14]

Dermatological activity

Sophorolipids inhibit elastase activity, the enzyme which is responsible for the degradation of elastin fibres, at a concentration of 5% (w/v) and radical effects towards the hydroxy-radical at a concentration of 0.083% (w/v).[14] Diacetylated sophorolipid lactones stimulate the metabolism of skin dermis fibroblast cells and hereby the *in vitro* neosynthesis of collagen.[15] Therefore, they possess restructuring and tissue repairing activity which is higher as compared to crude sophorolipid mixtures. Besides moisturizing and skin-conditioning properties, sophorolipid lactones proved active to eliminate dandruff.[16] Moreover, sophorolipids proved to possess desquamating, depigmenting and melanogenesis inhibiting activity.[17] The desquamation is achieved by detachment of the corneocytes and offers opportunities for the use of sophorolipids against acne and as an anti-wrinkle product. Sophorolipids induce the secretion of leptin by adipocytes, hereby inducing the lipolysis of adipocytes and reducing the subcutaneous deposition of fat.[18]

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Antimicrobial activity

Diacetylated sophorolipid lactones synthesized by *Torulopsis bombicola* inhibited the growth of *Candida*, *Pichia*, *Debaryomyces*, *Saccharomycopsis* and *Lodderomyces* yeasts on *n*-alkanes.[19] Natural sophorolipids also inhibited the growth of *Candida albicans*, *Candida Antarctica* and *Candida tropicalis* with respectively 30, 32 and 25% at a concentration of 5 mg/mL, while the inhibition with sophorolipid acids was respectively 24, 45 and 40%.[20] Kulakovskaya *et al*. determined the minimum inhibitory concentrations (MIC) of sophorolipids against *Filobasidiella neoformans*, *Candida tropicalis* and *Candida albicans* as respectively 1, 15 and 15 mg/mL.[21] The antibacterial activity of sophorolipids against *Rhodococcus erythropolis*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Moraxella* sp., *Pseudomonas putida*, *Enterobacter aerogenes* and *Escherichia coli* was evaluated, displaying a higher activity against Gram-positive bacteria than Gram-negative bacteria.[22] The activity was expressed as minimum lethal doses (MLD_{50}) and was 98 µg/mL for *R. erythropolis*, *B. subtilis*, *S. agalactiae* and *Moraxella* sp., and >6250 µg/mL for *S. epidermis*, *P. putida*, *E. aerogenes* and *E. coli*. Kulakovskaya *et al*. determined the MIC values of sophorolipis against *E. coli*, *Streptococcus salivarius* and *Micrococcus luteus* as respectively 32, 23 and 23 mg/mL.[21] More antimicrobial activities against other species are described in a patent by Gross and Shah.[23] Overall, sophorolipid lactones perform better than sophorolipid acids, with the lowest MIC values of 10µg/mL for sophorolipid lactones against *Bacillus subtilis* and *Rhodococcus rhodochrous*. Sleiman *et al*. evaluated MIC values of different sophorolipid derivatives in sucrose and ethanol vehicles against *Escherichia coli* and *Staphylococcus aureus*.[24] No significant inhibitory activity was observed for most derivatives at a concentration of 512 µg/mL and against *S. aureus* at a concentration of 218 µg/mL for two derivatives in an ethanol vehicle. The antibacterial activity of lauryl alcohol based sophorolipids was also evaluated against *E. coli* and *S. aureus*, respectively displaying a zero percent survival at 3000 µg/mL after 2h and 600 µg/mL after 4h.[25] Both crude and acidic sophorolipids inhibited the growth of *Cupriavidus necator* and *Bacillus subtilis* at a concentration of 5% (v/v).[26] They also disrupted biofilms of *B. subtilis* and a mixed culture of *B. subtilis* and *S. aureus* at a concentration of 5% (v/v). Synergistic effects were observed for administration of sophorolipids with the antibiotics tetracycline and cefaclor against respectively *S. aureus* and *E. coli*.[27] Sophorolipids inhibited the motility of the harmful algae *Alexandrium tamarense*, *Heterosigma akashiwo* and *Cochlodinium polykrikoides* at concentrations of 10-20 µg/mL.[28] At these concentrations, sophorolipids have low adverse effects on the non-harmful microalgae *Platymonas helgolandica* var. *tsingtaoensis*, *Isochrysis galbana* and *Nitzschia closterium* f. *minutissima*.[29] The EC_{50} for the zooplankton *Strombidium* sp., *Calanus sinicus* and *Neomysis awatschensis* was respectively 20, 50 and 150 µg/mL after 96h. The EC_{50} for the zooplankton *Artemia salina* was 600 µg/mL after 24h. The fish *Lateolabrax japonicas* and *Paralichthys olivaceus* displayed an EC_{50} of respectively 60 and 110 µg/mL and the relative clearance rate of the mussel *Mytilus edulis* decreased to 80% at a sophorolipid concentration of 20µg/mL. Synergistic effects were observed for the combination of sophorolipids and loess on the motility inhibition and removal of the harmful algae *C. polykrikoides* and *A. tamarense*.[30] It can be concluded that sophorolipids display no significant antibacterial activity against clinically relevant bacteria, but are effective in the inhibition of harmful algae in concentrations which are not detrimental to most of the tested organisms.

Anticancer activity

Sophorolipids induced differentiation of human acute promyelocytic leukemia cell line HL60.[31] At a concentration of 10 µg/mL, the proliferation of the HL60 cells and the protein kinase C activity was inhibited after 2 days and differentiation into monocytes took place. For two other leukemic cell lines, the human myelogenous leukemia cell line K562 and the human basophilic leukemia cell line KU812, differentiation into megakaryocytes was also induced at a sophorolipid concentration of 15 µg/mL. Chen *et al*. evaluated the anticancer activity of diacetylated sophorolipid lactone on liver cancer cell line H7402, lung cancer cell line A549 and leukemia cell lines HL60 and K562 via an MTT assay.[32] The cell proliferation of all four cell lines was inhibited at concentrations ranging from 0 to 62.5 µg/mL after 2 days with a 0% cell viability at concentrations exceeding 62.5 µg/mL. The mechanism of anticancer activity on the liver cancer cell line H7402 was identified as apoptosis.[33] The condensation of chromatin, nuclear fragmentation and appearance of apoptotic bodies was observed. At a sophorolipid concentration of 50 µg/mL, cell viability of both liver cancer cell line H7402 and lung cancer cell line A549 was completely inhibited while only a little decrease was observed for the normal liver cell lines HL7702 and Chang liver. The anticancer activity of diacetylated sophorolipid lactone and sophorolipid acid against human pancreatic cancer cells was also evaluated.[34] A decreasing cytotoxicity of respectively 40.3 to 3.4% and 49 to 0% was observed with increasing concentration from 500 to 2000 µg/mL. No cytotoxicity was observed against healthy peripheral blood mononuclear cells. Shao *et al*. described the anticancer activity of different sophorolipid derivatives against the esophageal cancer cell lines KYSE109 and KYSE450.[35] Diacetylated sophorolipid lactone displayed the highest activity with complete inhibition of both cell lines at a concentration of 30 µg/mL. The anticancer activity depended on the acetylation and unsaturation degree of the derivatives. No inhibition was observed for acidic sophorolipids. Evaluation of the anticancer activity on MDA-MB-321 breast cancer cell line demonstrated a higher activity for sophorolipid lactones compared to sophorolipid acids, with IC_{50} values of respectively 15-20 and 80 µg/mL.[36] Sophorolipids also induce differentiation in LN-229 glioma cell lines.[37] Morphological changes were detetected at concentrations of 10 µg/mL and 400 ng/mL for respectively oleic acid and linolenic acid based sophorolipids.

Immunoregulatory activity

Sophorolipids possess a pro-inflammatory activity by activating macrophages to induce the release of cytokines.[17] They also promote wound healing due to the proliferative effect of these cytokines on the fibroblasts, the phagocytosis of bacteria, cells and cellular fragments which could obstruct the wound by macrophages and their fibrinolytic activity. Sophorolipids can reduce septic shock related mortality by inhibition of the nitrogen oxide production of

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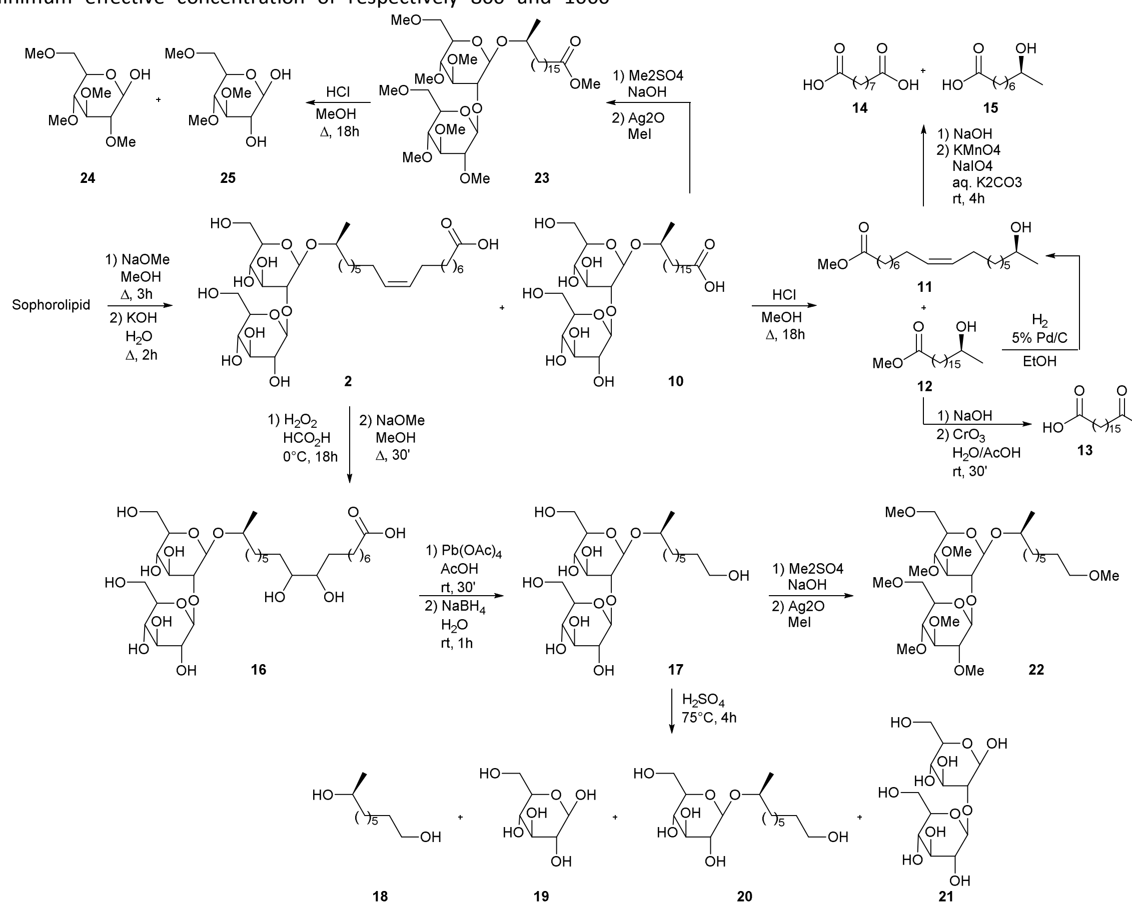
macrophages and modulation of the inflammatory response.[38] Experiments were performed in *in vivo* rat models with intravenous and intraperitoneal administration of Nembutal to induce septic shock. The survival rate after 36h with and without addition of 5mg/kg natural sophorolipid mixture was respectively 81.8 and 47.8% for the intervenous administration and 67 and 53% for the intraperitoneal administration. Macrophages which were *in vitro* cultured with lipopolysaccharides showed reduced nitrogen oxide production and induced expression of several cytokines. The viability of these macrophages also increased from 56 to 66% and from 23 to 36% after respectively 36 and 48h.[39] Moreover, sophorolipids at a concentration of 100µg/mL decreased the IgE production in U266 myeloma cells with 63%.[40] The effect of dosing was evaluated in the *in vivo* rat models.[41] When a single dose was administered, survival increased with 28 and 42% after respectively 24 and 72h while survival increased with 39 and 26% for sequential dosing after respectively 24 and 72h. Increased mortality was observed for treatment with purified diacetylated sophorolipids lactone. Sophorolipids also reduced asthma severity in an *in vivo* asthma model with mice, which was demonstrated on the basis of decreased leukocytic infiltrate in the lungs and decreased levels of ovalbumin specific IgE in the bronchoalveolar lavage fluid.[42]

Spermicidal and antiviral activity

Sophorolipids are active as spermicidal and antiviral agents.[43] Natural mixture sophorolipids and sophorolipid lactones displayed a minimum effective concentration of respectively 800 and 1000 µg/mL after 30 seconds of incubation. Sophorolipids at a concentration of 300 µg/mL immobilized spermatozoa completely and irreversibly after 2 minutes. Immobilization occurred faster than death and the mechanism of action is most likely membrane perturbation and disruption. Sophorolipids also displayed some degree of anti-HIV activity, with sophorolipid acids being more potent than sophorolipid lactones. Natural mixture sophorolipids and sophorolipid lactones exerted high cytotoxicity against human vaginal cells with a 50% effective concentration of around 15 µg/mL, while sophorolipid acids displayed a 50% effective concentration higher than 100 µg/mL. Natural mixture sophorolipids and sophorolipid lactones also induced the production of proinflammatory cytokines by vaginal epithelial cells. Sophorolipids also displayed anti-herpes virus activity for which the Epstein-Bar virus was used as a model organism.[44] The EC_{50} values for diacetylated sophorolipid lactone and sophorolipid acid were respectively 25.8 and 49.2 µM.

Modifications in the context of structural characterization

The first modifications of sophorolipids produced by *Torulopsis magnoliae* are described by Gorin *et al.* in the context of the characterization of this class of biosurfactants (Scheme 3).[45] In a first step, the crude sophorolipid product was transformed into unsaturated sophorolipid acid **2** and saturated sophorolipid acid **10**



Scheme 3. Sophorolipid modifications in the context of the structure determination[43]

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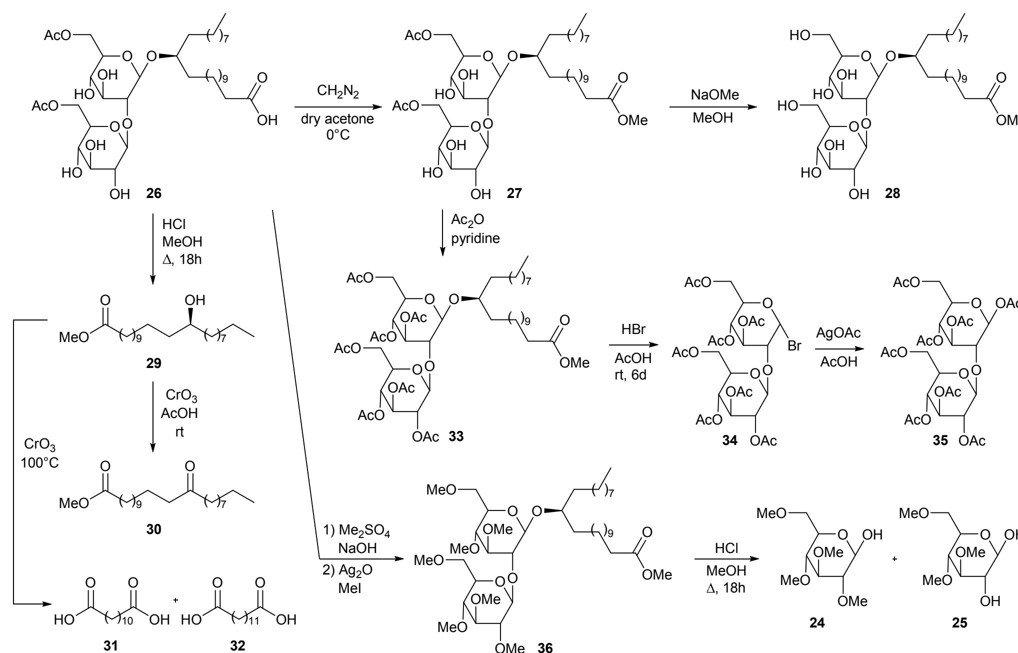
through removal of the acetyl groups with sodium methoxide. Acid methanolysis of sophorolipid acids **2** and **10** resulted in the cleavage of fatty acid methyl esters **11** and **12** which were separated via fractional crystallization. The saturated methyl ester **12** was oxidized with chromium trioxide into 17-oxo-stearic acid **13**, which enabled the determination of the hydroxyl position. The unsaturated methyl ester **11** was oxidized with potassium permanganate and sodium periodate into azelaic acid **14** and 8-hydroxy-pelargonic acid **15**, which enabled the determination of the double bond. Unsaturated sophorolipid acid **2**, on the other hand, was oxidized with performic acid followed by alkaline hydrolysis into the *threo*-9,10-dihydroxy sophorolipid **16**. Subsequent oxidation with lead(IV) acetate and reduction with sodium borohydride yielded sophorolipid alcohol **17**. Partial acid hydrolysis furnished a mixture of 1,8-*L*-nonanediol **18**, glucose **19**, 1,8-*L*-nonanediol-β-*D*-glucopyranoside **20** and sophorose **21**. The latter two were isolated by chromatography. Sophorolipid alcohol **17** and saturated sophorolipid acid **10** were methylated into respectively sophorolipid ether **22** and sophorolipid methyl ester **23**. Both compounds were hydrolyzed into 2,3,4,6-tetra-*O*-methyl-*D*-glucose **24** and 3,4,6-tri-*O*-methyl-*D*-glucose **25** which confirmed the 1,2-glycosidic link between the two glucose units.

Similar modifications were performed by Tulloch *et al*. for the characterization of diacetylated sophorolipid **26** which is produced by *Candida bogoriensis* (Scheme 4).[46] In a first step, sophorolipid **26** was transformed into diacetylated sophorolipid methyl ester **27** with diazomethane. Alkaline hydrolysis furnished the corresponding methyl ester **28**. Acid methanolysis of sophorolipid acid **26** resulted in the cleavage of fatty acid methyl ester **29**. Oxidation with chromium oxide at room temperature or 100°C yielded respectively methyl 13-oxodocosanoate **30** or dodecanedioic acid **31** and tridecanedioic acid **32**, which enabled the determination of the hydroxyl position. Diacetylated sophorolipid **17** was acetylated with acetic anhydride towards the peracetylated sophorolipid **33**. Bromination towards α-acetobromosophorose **34** was followed by the synthesis of β-octaacetyl sophorose **35**. Diacetylated sophorolipid **36** was further methylated towards sophorolipid methyl ester **36** and subsequently hydrolyzed into 2,3,4,6-tetra-*O*-methyl-*D*-glucose **24** and 3,4,6-tri-*O*-methyl-*D*-glucose **25** which confirmed the 1,2-glycosidic link between the two glucose units.

Modifications starting from diacetylated sophorolipid lactone **37** are described by Tulloch *et al.* (Scheme 5).[47] Diacetylated sophorolipid lactone **37** proved to be the major fermentation product and was obtained in pure form after crystallization and hydrogenation of the fermentation product followed by subsequent chromatography. Tetraphenylurethane sophorolipid **38** was synthesized via reaction with phenyl isocyanate. Acetylation of diacetylated sophorolipid lactone **37** was performed with acetic anhydride towards the hexaacetylated sophorolipid lactone **39** followed by brominolysis towards α-bromosophorose hexaacetate derivative **40**. Subsequent treatment with silver acetate gave the corresponding β-bromosophorose hexaacetate derivative **41**. The α-bromosophorose hexaacetate **40** was converted into β-methoxysophorose hexaacetate **42** followed by deactylation towards β-methoxysophorose **43** and acetylation towards β-methoxysophorose heptaacetate **44**. The latter compound was also prepared via brominolysis of β-sophorose octaacetate **35** towards β-bromosophorose heptaacetate **34** and subsequent reaction with methanol in the presence of silver carbonate. A third method for the synthesis of β-methoxysophorose heptaacetate **44** comprises the Koenigs-Knorr reaction of 3,4,6-tri-*O*-acetyl-β-*D*-glucopyranoside **45** and α-acetobromoglucose **46** in the presence of mercuric cyanide in nitromethane.

The brominolysis reaction of hexaacetylated sophorolipid lactone **39** also resulted in the cleavage of the linkage between the two glucose units and reversion of the anomeric centre. Treatment of the mother liquor after isolation of α-bromosophorose hexaacetate **40** with silver acetate and subsequent chromatography resulted in the isolation of hexaacetylated α-sophorolipid lactone **47** and β-glucose tetraacetate derivative **48** (Scheme 6). The structure of the



Scheme 4. Modifications for the structural determination of diacetylated sophorolipid **26**[44]

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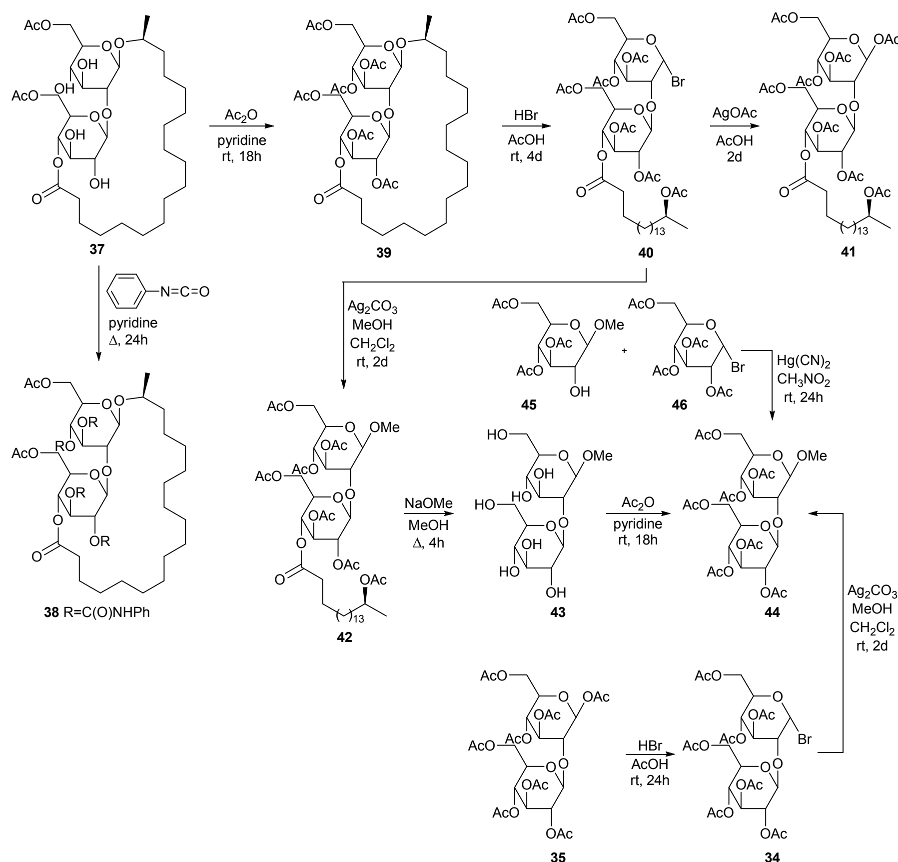
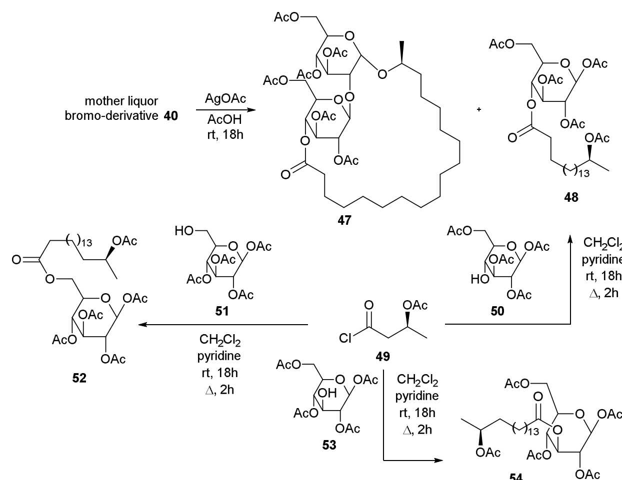


Scheme 5. Modifications of diacetylated sophorolipid lactone **37**[45]

latter compound was confirmed upon reaction of the acid chloride **49** synthesized from 17-*L*-acetoxyoctadecanoic acid and 1,2,3,6-tetra-*O*-acetyl-β-*D*-glucopyranose **50**. In this context, acid chloride **49** was also coupled with 1,2,3,4-tetra-*O*-acetyl-β-*D*-glucopyranose **51** and 1,2,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose **53** towards the β-glucose tetraacetate derivatives **52** and **54** respectively. Isolation and characterization of β-glucose tetraacetate derivative **48** demonstrated that the fatty acid tail is linked to the C4"-position in the diacetylated sophorolipid lactone **37**.



Scheme 6. Isolation of brominolysis side-products[44]

Next to diacetylated sophorolipid lactone **37**, a second major fermentation product was present, i.e. diacetylated sophorolipid acid **55**. Treatment of sophorolipid acid **55** with diazomethane on the one hand, and treatment of sophorolipid lactone **37** with very weak sodium methoxide on the other hand both resulted in the synthesis of diacetylated sophorolipid methyl ester **56** (Scheme 7). Subsequent deacetylation gave sophorolipid methyl ester **57**, which was also prepared by deacetylation of sophorolipid lactone **37** and by treatment of sophorolipid acid **55** with a methanolic hydrogen chloride solution. The syntheses of sophorolipid methyl esters **56** and **57** confirmed that the structure of diacetylated sophorolipid acid **55** is similar to that of diacetylated sophorolipid lactone **37**. Sophorolipid methyl ester **57** was acetylated towards heptaacetylated sophorolipid methyl ester **58** followed by brominolysis towards α-acetobromosophorose **34**. When the reaction time was reduced to seven hours, heptaacetylated α-sophorolipid methyl ester **59** was obtained, which confirmed that brominolysis reaction conditions induced reversion of the anomeric centre. Deacetylation with sodium methoxide gave α-sophorolipid methyl ester **60**.

The unexpected production of diacetylated sophorolipid octadecyl ester 61 via fermentation of octadecanol by *Torulopsis bombicola* and subsequent modifications is described by Tulloch and Spencer (Scheme 8).[48] Alkaline hydrolysis with sodium hydroxide followed by reaction with diazomethane in methanol yielded sophorolipid methyl ester **57**. Methanolysis with sodium

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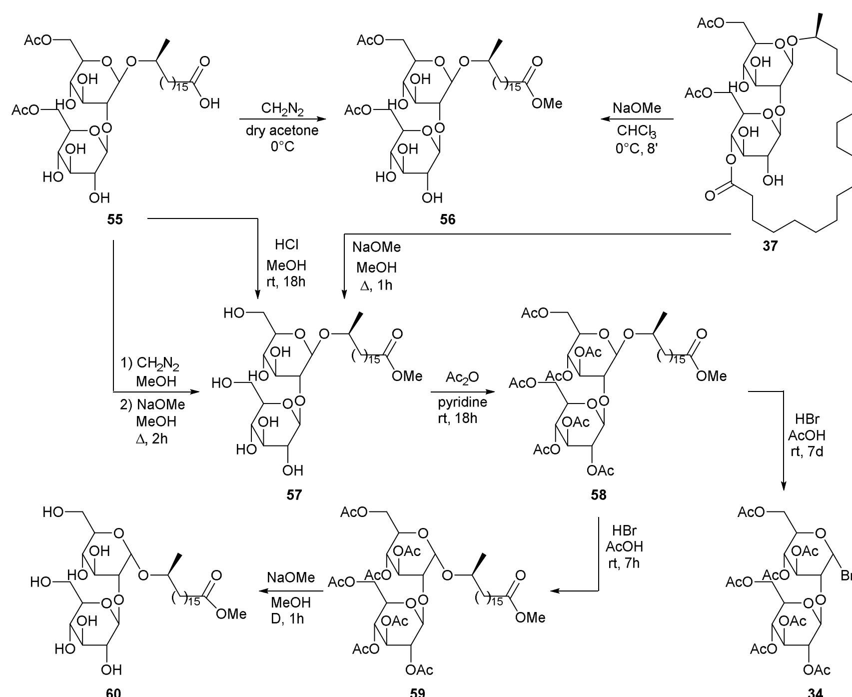
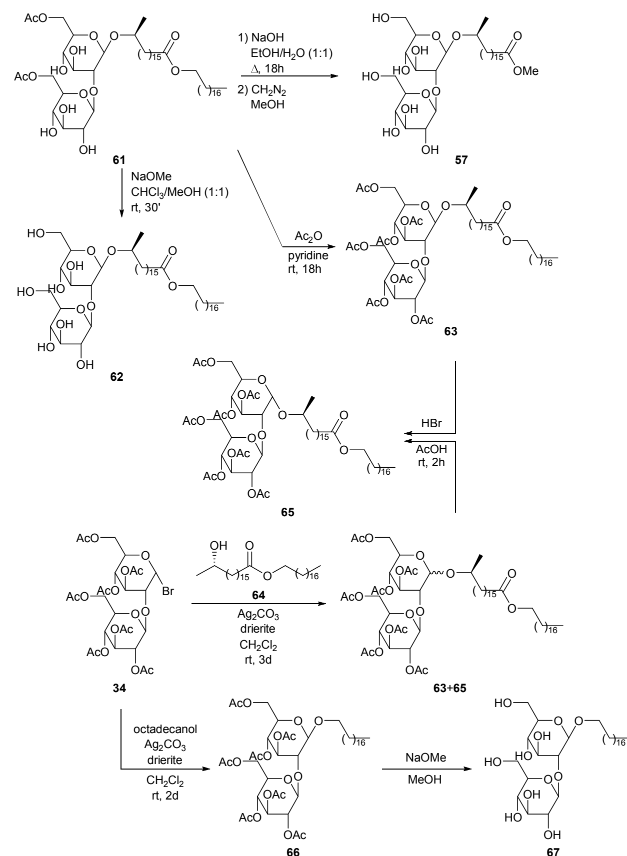


Scheme 5. Modifications of diacetylated sophorolipid acid **55**[45]

methoxide gave sophorolipid octadecyl ester **62** and acetylation resulted in the synthesis of heptaacetylated sophorolipid octadecyl ester **63**. To confirm the structure of diacetylated sophorolipid octadecyl ester **61**, synthesis of heptaacetylated sophorolipid octadecyl ester **63** via the Koenigs-Knorr reaction was attempted. Octadecyl 17-*L*-hydroxyoctadecanoate **64** was synthesized from 17-*L*-formyloxyoctadecanoyl chloride and octadecanol and subsequently reacted with α-acetobromosophorose **34**. A mixture of α and β anomers **65** and **63** was obtained which was completely converted into heptaacetylated α-sophorolipid octadecyl ester **65** upon reaction with hydrogen bromide. Synthesis of the heptaacetylated derivative 66 of the desired fermentation product, i.e. an octadecyl β-sophoroside, was performed via the Koenigs-Knorr reaction of octadecanol with α-acetobromosophorose **34**. Deacetylation gave octadecyl β-sophoroside **67**.

The first total synthesis of sophorolipid lactone **9** was described by Fürstner *et al.*[49] In a first step, two major building blocks were synthesized (Scheme 9). The ring-opening reaction of (*S*)-propenoxide with Grignard reagent **68** yielded the enantiomerically pure alcohol **69**. *D*-glucal **70** was protected with *p*-methoxybenzyl chloride and the resulting tri-*O*-PMB ether **71** was subsequently treated with dimethyldioxirane to yield epoxide **72**. Alcohol **59** was reacted with epoxide **72** into the first building block **73**. On the other hand, *D*-glucose and *p*-methoxybenzaldehyde dimethylacetal **74** were coupled via a transacetalization reaction to yield 4,6-*O*-*p*-methoxybenzylidene acetal **75**. Peracetylation towards acetal **76** was followed by selective deprotection of the anomeric centre yielding reducing sugar **77**. Reaction with bromine delivered the second building block **78**. The two building blocks were coupled via a glycosylation reaction under modified Koenigs-Knorr conditions which yielded the desired sophorose glycoside **79** (Scheme 10). Deacetylation into diol **80** was followed by protection with *p*-methoxybenzyl chloride. The resulting PMB



Scheme 6. Modifications of diacetylated sophorolipid octadecyl ester **61**[46]

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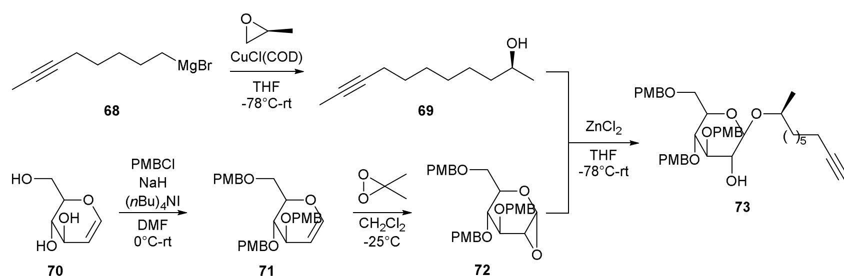
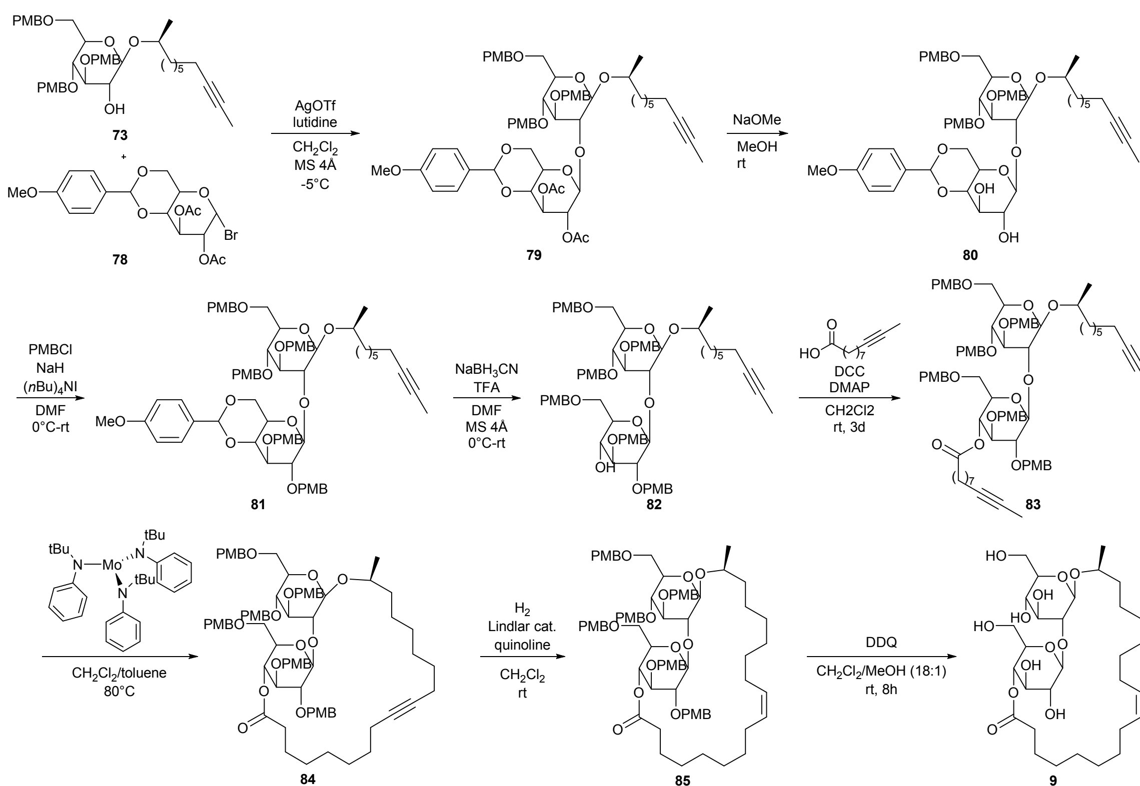


Scheme 9. Synthesis of building blocks **73** and **78**[46]



Scheme 10. First total synthesis of sophorolipid lactone **9**[47]

ether derivative **81** was treated with sodium cyanoborohydride to remove the acetal protecting group. The *p*-methoxybenzylated sophorolipid **82** was esterified with 9-undecynoic acid towards diyne **83**. A ring-closing metathesis reaction was performed which was catalyzed by a molybdenum catalyst to yield cycloalkyne **84**. Protected sophorolipid lactone **85** was obtained via Lindlar hydrogenation and subsequent deprotection delivered the desired sophorolipid lactone **9**.

Modifications towards new derivatives

Regioselective reactions at the sugar head group

Regioselective acylations of the sophorolipid head group through the use of enzymes is described by Bisht *et al.* (Scheme 11).[50] First, the crude sophorolipid fermentation product needed to be transformed into a single pure compound to enable the synthesis of well-defined sophorolipid analogues. Therefore, the starting

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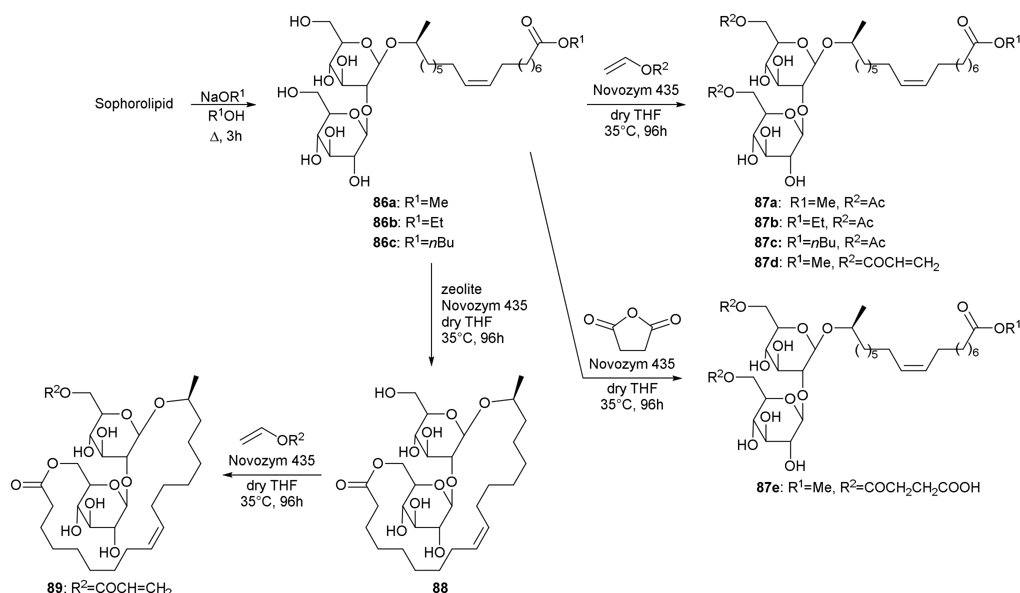


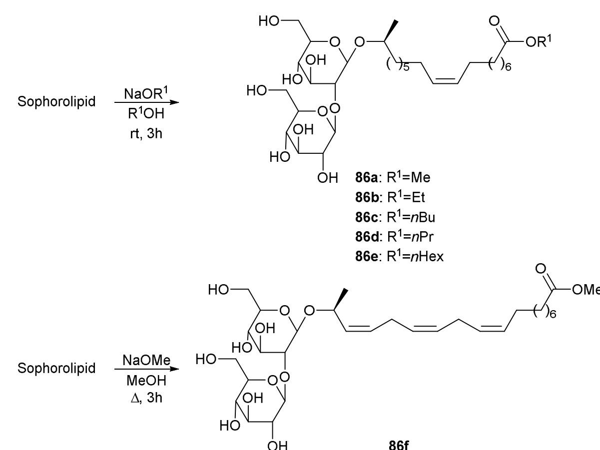
Scheme 7. Regioselective acylation of sophorolipid esters **86** at both the 6'- and 6"-position[48]

product was subjected to alkaline hydrolysis with sodium alkoxides, yielding sophorolipid esters **86**. Transformation of the starting product into sophorolipid acid was not useful, since this acid is only soluble in water and polar aprotic solvents which are not suitable for transesterification reactions with lipase enzymes. The sophorolipid esters **86** were subjected to lipase-catalyzed esterifications with an excess of vinyl acetate, vinyl acrylate or succinic anhydride. Multiple enzymes were evaluated and the highest conversion was obtained with Novozym 435. With this enzyme, selective acylations at both the 6'- and 6"-position of the sugar head were obtained, yielding acylated sophorolipid esters **87**. Acylation of only one of these positions was attempted through variation of the ratio sophorolipid ester/acylating agent. With a ratio of 1:1 or less, 1,6"-sophorolipid lactone **88** was formed which is an unnatural analogue of the 1,4"-sophorolipid lactone. This lactone was subsequently used for the regioselective esterification at the 6'-position of the sugar head, yielding the monoacrylated sophorolipid lactone **89**. Sophorolipid methyl ester **86a** displayed a cytotoxicity of 63±5% against human pancreatic cells at a concentration ranging from 500 to 2000 µg/mL.[34] Sophorolipid ethyl esters **86b** and **87b** displayed a lower cytotoxicity of respectively 23.6% at 1000 µg/mL and 42.6% at 500 µg/mL. No cytotoxicity was observed against healthy peripheral blood mononuclear cells. Sophorolipid ethyl ester **87b** also reduced septic shock related mortality in *in vivo* rat models with 23% and possessed high spermicidal and anti-HIV activity comparable to those of nonoxynol-9.[41, 43b] Unfortunately, high cytotoxicity was exerted on human vaginal cells.

More sophorolipid alkyl esters **86** were synthesized by Zhang *et al*. in order to evaluate the relationship between the length of the alkyl ester chain and the interfacial properties of the derivatives (Scheme 12).[51] The surface tension was measured for all the derivatives. Critical micelle concentration (CMC) and the minimum surface tension decreased for increasing alkyl ester chain length. Adsorption at solid/liquid interfaces was studied as well, demonstrating adsorption on alumina but much less on silica. Hydrogen bonding was proposed to be the primary driving force for adsorption on alumina and the maximum adsorption density suggested bilayer formation at higher concentrations.

The transesterification of an α-linolenic acid based sophorolipid mixture into sophorolipid methyl ester **86f** was described by Gupta & Prabhune (Scheme 12).[52] Their minimum inhibitory concentrations against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were determined and proved to be respectively 20, 10 and 10 µg/mL for the fermentation product and respectively >20, 20 and 20 µg/mL for sophorolipid methyl ester **86f**.[53]

Selective enzyme-catalyzed deacetylation of sophorolipid lactone **1** at the 6'-position was performed by Peng *et al*. with the lipase from *Candida rugosa* (Scheme 13).[54] This mono-acetylated sophorolipid lactone **8** was subsequently hydrolysed into the non-acetylated sophorolipid lactone **9** with the cutinase from *Humicola*



Scheme 8. Alkaline hydrolysis towards sophorolipid esters **86**[49-50]

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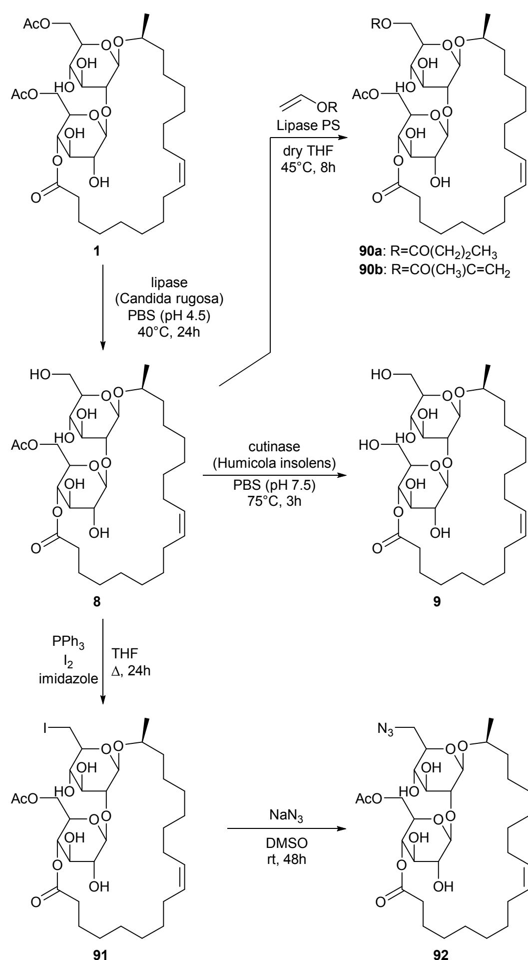
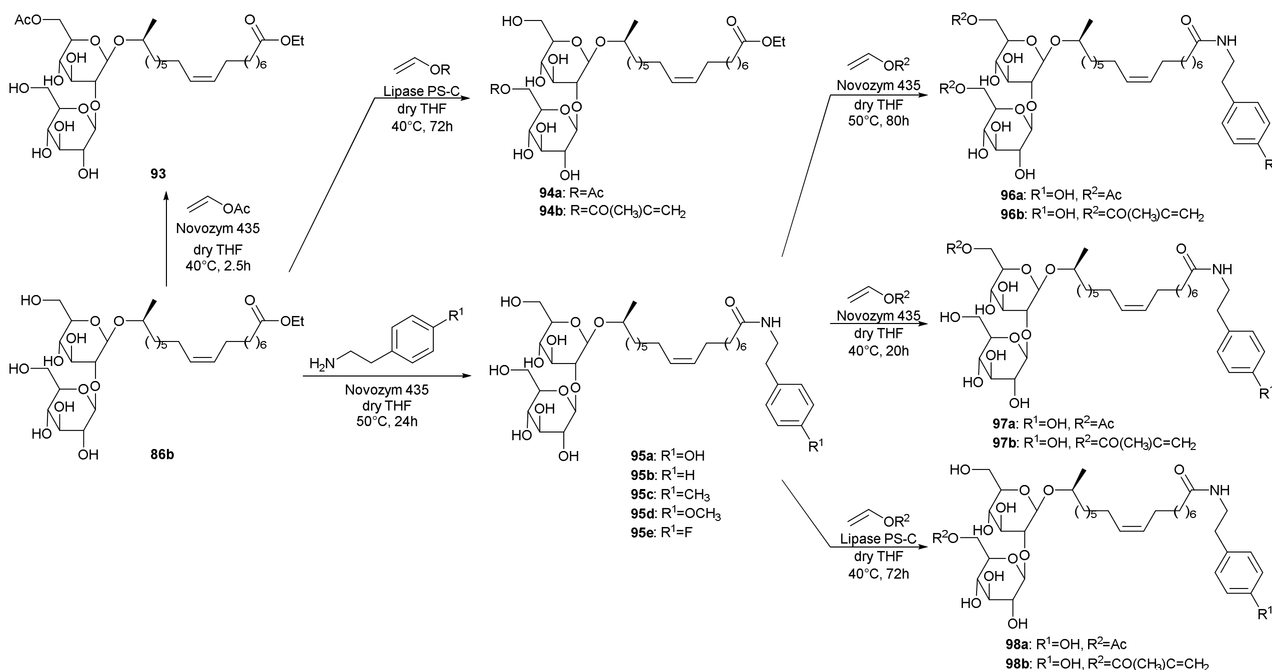


Scheme 13. Regioselective functionalization of sophorolipid lactone **1**[52]

insolens. Methacrylate and butyrate groups were introduced at the 6'-position through an enzyme-catalyzed acylation of the mono-acetylated sophorolipid lactone **9** with the respective vinyl acylates. An azide group was introduced in two steps. First, a iodination was performed followed by reaction with sodium azide. Methacrylate and azide groups offer the opportunity to introduce bioactive groups through click reactions.

More enzyme-mediated regioselective acylations of sophorolipids at the 6'- and/or 6"-position of the sugar head are described by Singh *et al.* (Scheme 14).[55] Selective acylation at the 6'-position was accomplished through reaction of sophorolipid ethyl ester **86b** with excess vinyl acetate and Novozym 435, yielding sophorolipid monoacetate **93**. A small amount of the corresponding sophorolipid diacetate was formed as well. When Lipase PS is used as catalyst, selective acylation at the 6"-postion is obtained. Vinyl acetate and vinyl methacrylate were used respectively for the synthesis of sophorolipid monoacetates **94a** and **94b**. Enzymatic catalysis was also evaluated for the amidation of sophorolipid ethyl ester **86b** with a number of primary amines. Different enzymes were evaluated, of which only Novozym 435 was successful for the formation of sophorolipid amides **95** in high yield. The regioselective acylations with vinyl acetate and vinyl methacrylate at the 6'- and/or 6"-postions of the sugar head were extended to the sophorolipid amines, yielding sophorolipid diacetate 96 and sophorolipid monoacetates **97** and **98**. Also a one-pot synthesis of compound **96b** was successfully accomplished.

The modification of 2-dodecyl sophorolipid **99**, prepared by fermentation with 2-dodecanol as substrate, was performed by Recke *et al.* (Scheme 15).[56] The 2-dodecyl sophorolipid **99** was transformed into the 2-dodecyl glucolipid **100** via the selective removal of a glucose unit with the β-glucuronidase enzyme. Subsequently, this glucolipid was acylated at the 6'-position with sebacic acid towards glucolipid **101**. The free carboxylic acid function offers the opportunity for further derivatization towards polyesters. The 2-dodecyl sophorolipid **99** was also acylated at the



Scheme 14. Regioselective acylation and enzyme-catalyzed amidation of sophorolipid esters **86**[53]

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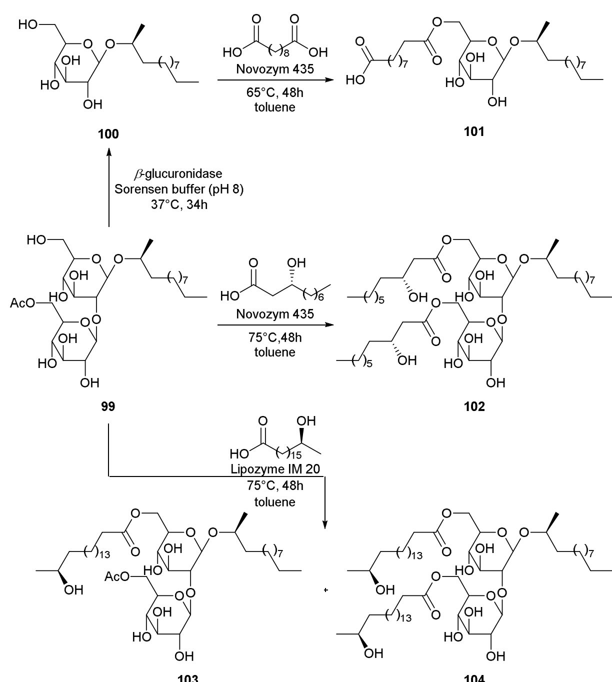


Scheme 9. Acylations at the 6'- and 6”-position of sophorolipid **99** and glucolipid **100**[54]

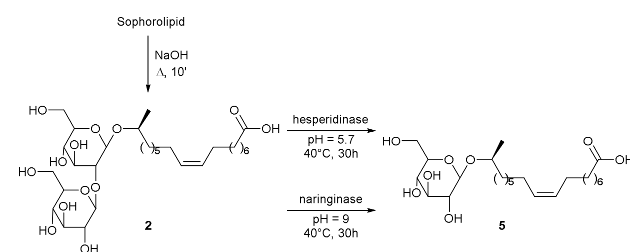
6’- and 6”-position with the unusual fatty acids (*R*)-3-hydroxy decanoic acid and (*S*)-17-hydroxy stearic acid which are obtained via hydrolysis of respectively rhamnolipids and sophorolipids. Acylation with (*R*)-3-hydroxy decanoic acid resulted in the synthesis of one major product, i.e. the diacylated sophorolipid **102**. On the other hand, acylation with (*S*)-17-hydroxy stearic acid was not complete after 48 hours, resulting in the formation of both the mono-acylated sophorolipid **103** and the di-acylated sophorolipid **104**. Longer reaction times were however not evaluated to obtain full conversion. The emulsion stability, CMC values and reduction of the air-water surface tension (γ) and water-*n*-hexadecane interfacial tension (σ) of the derivatives were evaluated. A good stabilization of w/o emulsions was achieved with all derivatives. This was however not the case for o/w emulsions. CMC, γ- and σ-values were respectively 150-200 mg/L, 27-50 mN/m and 3-7 mN/m. Antimicrobial activities of the derivatives were also evaluated. All derivatives showed strong growth inhibition against the Gram-positive bacteria *Bacillus megaterium* and *Bacillus subtilis*, and against the fungus *Candida magnolia*. Only di-acylated sophorolipid **102** and the mixture of mono-acylated sophorolipid **103** and di-acylated sophorolipid **104** showed inhibition against the Gram-positive bacteria *Staphylococcus capitis*. No growth inhibition was observed against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, the fungi *Eurotium repens*, *Mycotypha microspora* and *Ustilago maydis*, and against the alga *Chlorella fusca*. Finally, the anti-tumor promoting activity of the derivatives was evaluated via a short-term *in vitro* assay for Epstein-Barr virus activation in Raji cells induced by TPA using heptyl-galactosyl-glyceride as a reference compound. Concentrations were expressed as mol ratio of compound compared to TPA. At 1000 mol ratio/TPA, only 10-15% activation was observed for the derivatives. All compounds displayed a cell viability of 70% at this concentration, which corresponds to a weak cytotoxicity against the Raji cells.

The enzymatic conversion of crude sophorolipid fermentation product into glucolipid **5** is described by Rau *et al*. (Scheme 16).[57] In a first step, the crude fermentation product was transformed via alkaline hydrolysis into deacetylated sophorolipid acid **2**. Different glycosidases were evaluated for the specific release of one glucose molecule and the best results were obtained with the hesperidinase and naringinase enzyme. A comparison was made for the interfacial activities of glucolipid **5** and sophorolipid lactone both at the air-water and water-*n*-hexadecane interface. In both cases, the interfacial activity of the sophorolipid lactone could not be matched by glucolipid **5**.

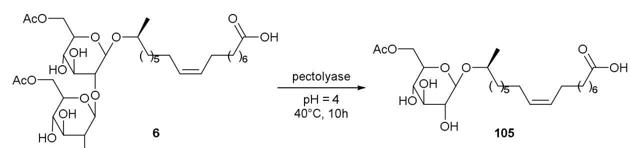
A similar deglycosylation of diacetylated sophorolipid acid **6** is described by Imura *et al*. (Scheme 17).[58] For this acetylated substrate, no activity was observed with hesperidinase or naringinase as was the case for the non-acetylated compound. Selective cleavage of the β-1,2-glycosidic bond was obtained with invertase, pectinase solution, pectinase and pectolyase. The highest activity for the conversion towards acetylated glucolipid acid 105 was obtained with pectolyase. Evaluation of the surface active properties of sophorolipid acid **2**, glucolipid acid **5**, diacetylated sophorolipid acid 6 and acetylated glucolipid acid **105** showed little variation in CMC value for the different compounds.

Non-selective reactions at the sugar head

Modifications at the head group of stearic acid based sophorolipids are described by Zerkowski *et al.* (Scheme 18).[59] The crude sophorolipid fermentation product is subjected to alkaline hydrolysis, yielding the sophorolipid free acid and the sophorolipid ethyl ester **106a**. The sophorolipid free acid is transformed into the sophorolipid benzyl ester 106b through esterification with benzyl alcohol. Head group modified sophorolipid esters **107** were obtained through coupling of protected amino acids possessing a *para*-aminobenzoic acid linker (Paba) with the carbohydrate hydroxyl groups. The presence of a Paba-linker should avoid decoupling of the amino acids under alkaline conditions and an excess of sophorolipid ester is used to favor mono-acylation. Two major isomers are obtained for each derivative, most probably the



Scheme 10. Deglycosylation of sophorolipid acid **2**[55]



Scheme 11. Deglycosylation of diacetylated sophorolipid **6**[56]

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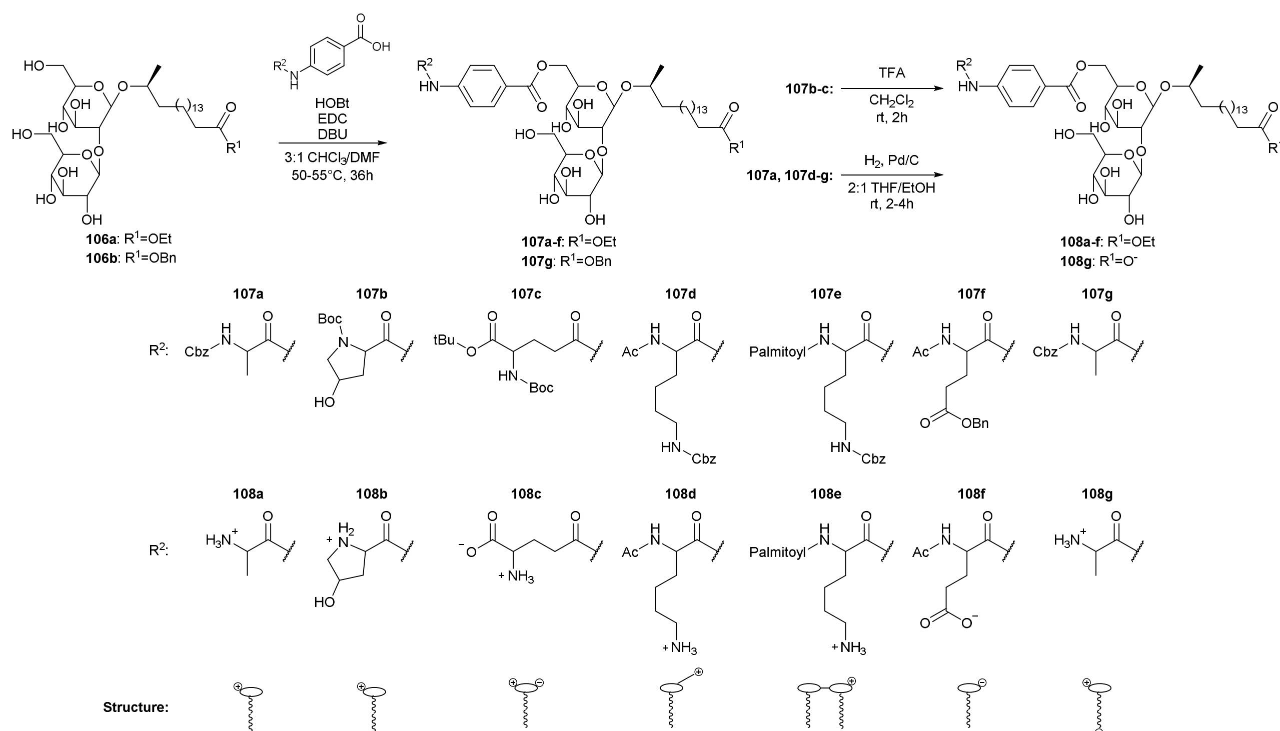
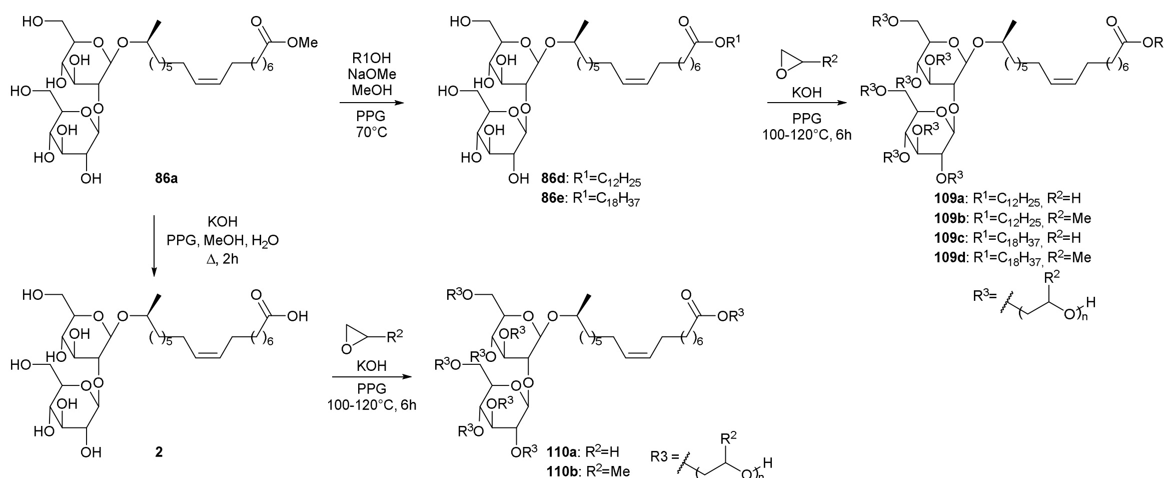


Scheme 18. Modification at the head group with amino acids towards charged sophorolipid derivatives[57]

6' and 6" adducts. Acidolytic or hydrogenolytic deprotection yields the water-soluble sophorolipids **108** with charged head groups. CMC values were determined for the derivatives at different pH-values and were 6-24 µM for comounds **108a-e** and 51-110 µM for compounds **108f-g**. The results show a significant impact of the modified head groups on the surface tension-lowering properties.

A patent was published by Inoue *et al.* on the synthesis of hydroxyalkyl-etherified sophorolipid esters (Scheme 19).[60] The crude sophorolipid fermentation product was subjected to methanolysis to produce sophorolipid methyl ester **86a**. After transformation towards sophorolipid esters **86d** and **86e** or sophorolipid acid **2**, the hydroxyalkyl-etherified sophorolipid esters **109** and **110** were produced through reaction with alkylene oxides in the presence of an alkali catalyst. These hydroxyalkyl-etherified sophorolipid esters were incorporated in cosmetic compositions to prepare powdered compressed cosmetic materials such as face powder, cheek rouge, highlight or eye shadow or stick-shaped cosmetic materials such as stick-shaped rouge, lip cream, stick-shaped eye shadow or cosmetic pencil.[61] They were also applied in cosmetics as moisture-retaining and moisturizing agents, giving a less sticky and better moisturizing feeling than conventional moisturizers such as glycerine, sorbitol and ethylene glycol.[62] The hydroxyalkyl-etherified sophorolipid esters were formulated as moisturizers in hand cream, cleansing milk, skin lotion, facial pack and lip stick.



Scheme 19. Synthesis of hydroxy-alkyl etherified sophorolipids **109** and **110**[58]

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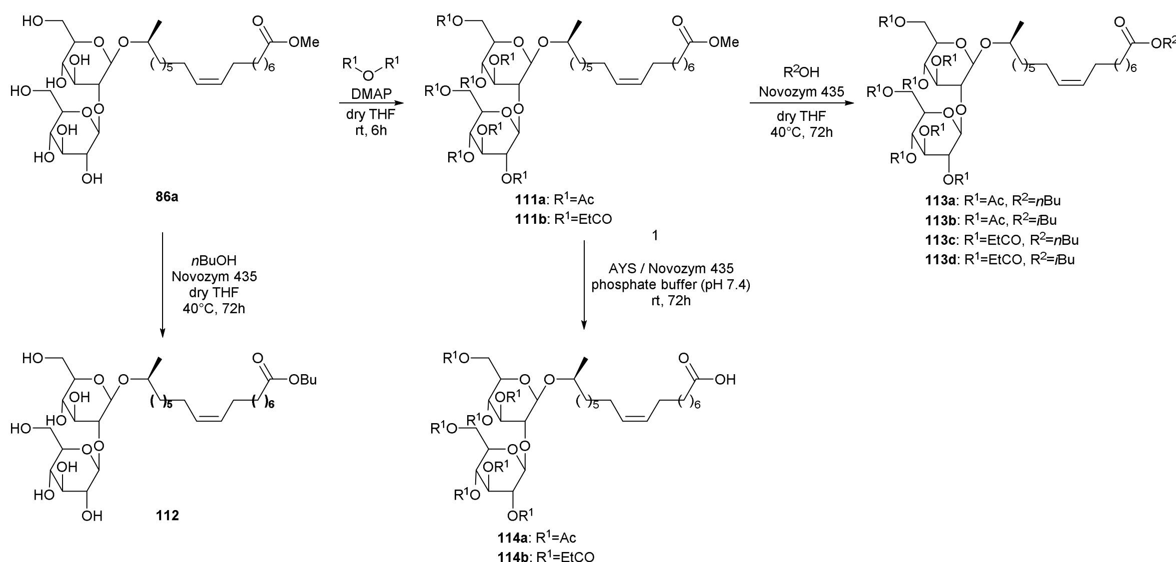
Modifications at the end of the lipid tail

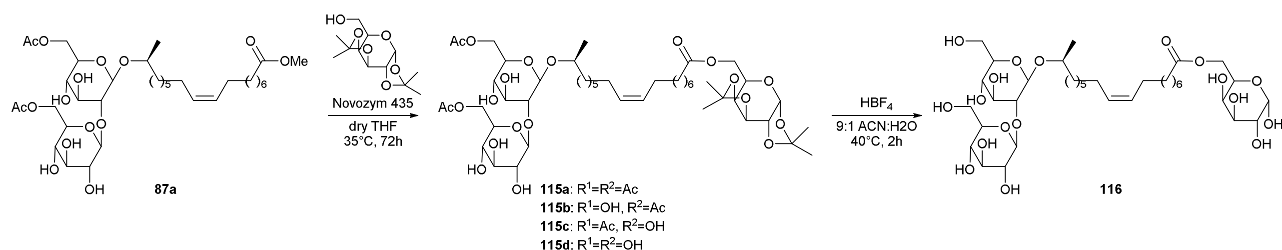
Enzyme-catalyzed transesterifications at the lipid tail are described by Carr & Bisht (Scheme 20).[63] Sophorolipid methyl ester 86a was transformed into the corresponding peracylated sophorolipid methyl esters **111** through reaction with acetic and propionic anhydride in the presence of a catalytic amount of DMAP. Different enzymes were evaluated for the transesterification in presence of *n*-butanol and *t*-butanol. Only Novozym 435 proved to be successful for the transesterification of both methyl ester 86a and peracylated methyl esters **111** respectively, into sophorolipid butyl ester **112** and peracylated sophorolipid esters **113**. Deacylation of the sugar head did not occur. It is suggested that the presence of the macrolactonic ring is necessary to fit the sugar head in the binding pocket of the lipase for deacylation to occur. A lipase catalyzed hydrolysis of the lipid tail was further performed with AYS and Novozym 435 in a phosphate buffer, yielding peracylated sophorolipid acids **114**.

Transesterification at the lipid tail was extended by Nuñez *et al*. for the coupling of a sugar monomer to the diacetylated sophorolipid methyl ester **87a** (Scheme 21).[64] A first attempt with glucose was not successful due to its poor solubility in organic solvents. Functionalization of the hydroxyl groups proved necessary where ketalization is favored for its selectivity. Galactose was used instead of glucose since the primary alcohol still has to be available for the transesterification. The transesterification reaction was performed with immobilized Novozym 435 and sophorolipid sugar esters **115** were formed which varried in the degree of acetylation. Deacetylation occured through the lipase-catalyzed transesterification of the acetylated primary alochols and the functionalized galactose unit. Deacetylation at the 6”-position resulted in the formation of 1,6”-sophorolipid lactone **88** (9%) and its 6’-acetylated analogue (14%) (Scheme 11). Acid hydrolysis with HBF_4 was performed for the deprotection of the acetyl and ketal groups towards sophorolipid galactopyranose ester **116**.

Coupling of sophorolipid acid **2** with different amino acids is described by Azim *et al*. (Scheme 22).[65] The crude fermentation product was transformed into sophorolipid acid **2** through alkaline hydrolysis with NaOH. Protected amino acid conjugated sophorolipids **117** and amino acid conjugated sophorolipid alkyl esters **118** were synthesized using DCC coupling. Deprotection of the different protecting groups yielded amino acid conjugated sophorolipids **119**. Deprotection of benzyl ester groups in compounds **117a-d** was performed by hydrogenation on Pd/C which simultaneously resulted in the reduction of the double bond. Deprotection of *t*-butyl groups in compound **117e** was accomplished under acid conditions. The antimicrobial, anti-HIV and spermicidal activity were evaluated for amino acid conjugated sophorolipid alkyl esters **118** and amino acid conjugated sophorolipids **119**. All tested derivatives possessed antimicrobial activity against Gram-positive and Gram-negative organisms. The best results were obtained for leucine conjugated sophorolipid **119c**, with MIC values of 1 and 2 μg/mL against respectively



Scheme 12. Enzyme-catalyzed transesterifications at the lipid tail[61]



Scheme 13. Transesterification towards sophorolipid galactopyranose ester **116**[62]

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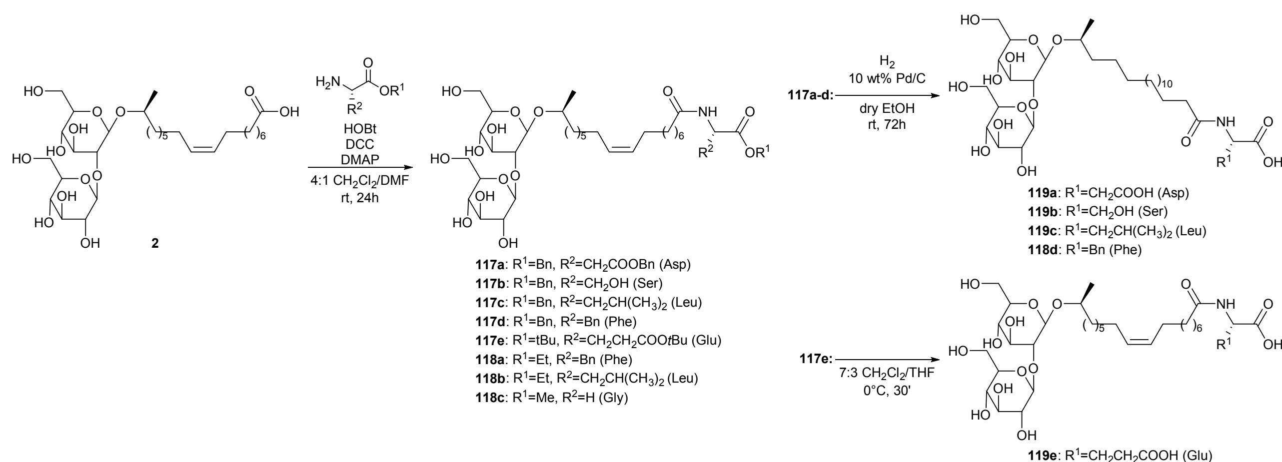


Scheme 22. Synthesis of amino acid conjugated sophorolipids[63]

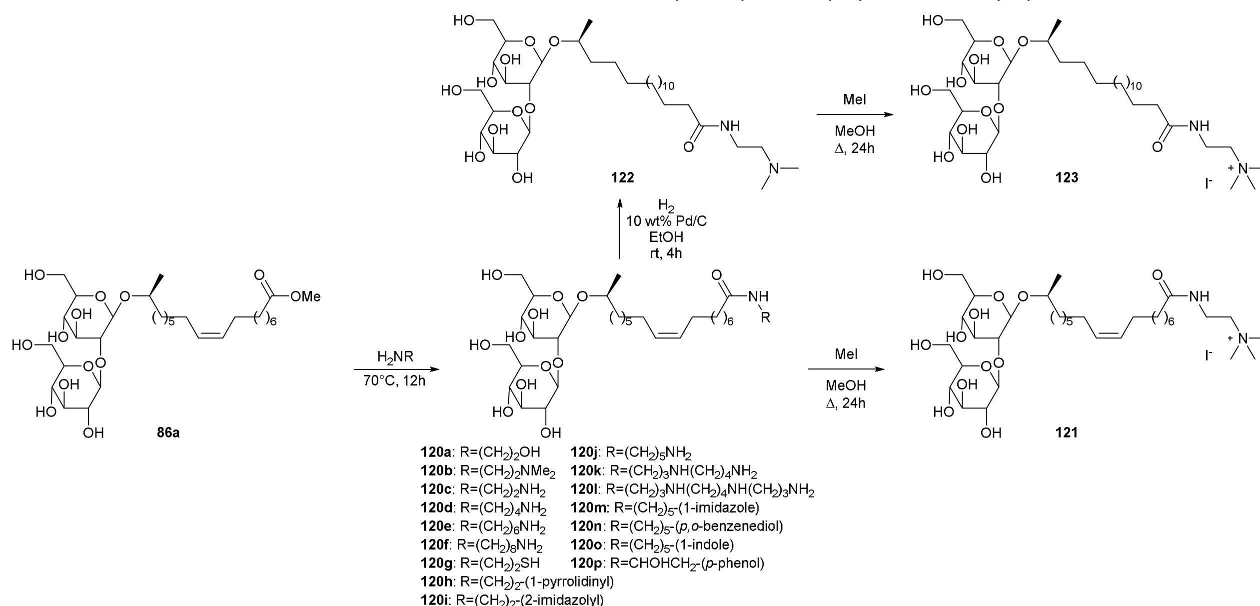
Moraxella sp. and *S. sanguinis*. Also the leucine conjugated sophorolipid ethyl ester **118b** feautured high activity, especially against *Moraxella* sp. with a MIC value of 830 µg/mL. Monoacetylated ethyl ester sophorolipid (MAEE) was also included in the antimicrobial screening and showed higher activity against all the organisms except for leucine conjugated sophorolipid ethyl ester **118b** against *Moraxella* sp. All tested derivatives displayed virus inactivation with EC_{50} below 200 µg/mL. The esterified derivatives **118** are more potent than the corresponding non-esterified compounds **119**. Leucine conjugated sophorolipid **118c** was the most potent and displayed even higher anti-HIV activity than the commercial spermicide nonoxynol-9 (EC_{50}≈65 µg/ml). None of the tested derivatives displayed significant spermicidal activity. Nonoxyl-9 and MAEE were used as positive controls.

Synthesis of sophorolipid amides **120** is described in a patent published by Schofield *et al*. (Scheme 23).[66] Non-biogenic sophorolipid amides **120a-120i** and biogenic sophorolipid amides **120j-120p** were synthesized from sophorolipid methyl ester **86a** through amidation with the corresponding amines. Sophorolipid *N′*,*N′*-dimethylethylamide **120b** was quaternized with methyl iodide to the sophorolipid quaternary ammonium salt **121**. This compound was also hydrogenated to the saturated sophorolipid *N′*,*N′*-dimethylethylamide **122** and subsequently quaternized to the saturated sophorolipid quaternary ammonium salt **123**. Antimicrobial activities were evaluated against a wide variety of micro-organisms.

Hydrogenation of diacetylated sophorolipid lactone **1** and sophorolipid acid **2** was also performed, yielding respectively the saturated diacetylated sophorolipid lactone **37** and the saturated sophorolipid acid **10** (Scheme 24).[66] Antimicrobial activities were evaluated against a wide variety of micro-organisms.

Sophorolipid polymerization

The sophorolipid lactone **89** was used by Bisht *et al*. for the synthesis of polymers with amphiphilic properties (Scheme 25).[67] Homopolymerisation was performed with AIBN as initiator, yielding sophorolipid homopolymer **124**. This polymer is soluble in DMF and



Scheme 23. Synthesis of non-biogenic and biogenic sophorolipid amides[64]

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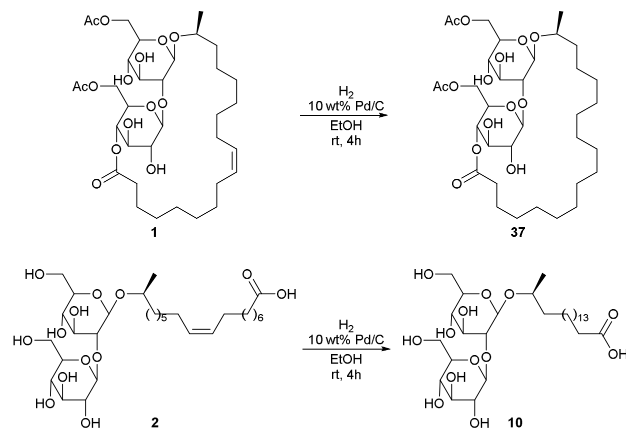
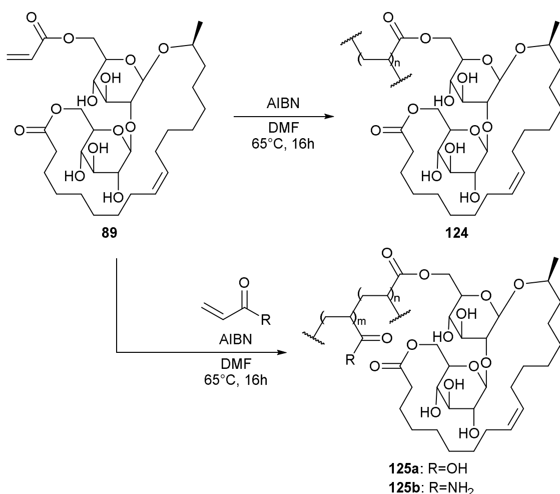


Scheme 24. Hydrogenation of diacetylated sophorolipid lactone **1** and sophorolipid acid **2**[64]

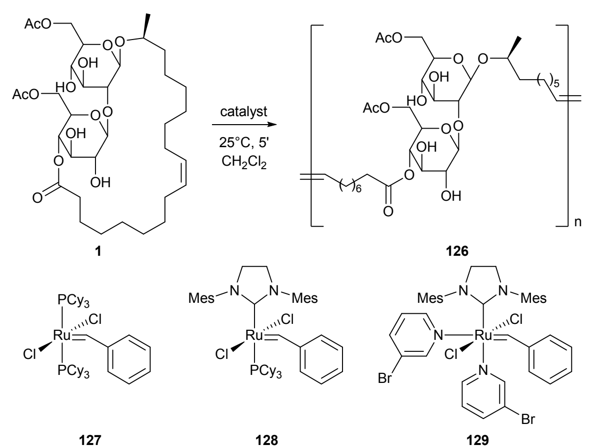


Scheme 25. Homo- and copolymerization of sophorolipid lactone **89**[65]

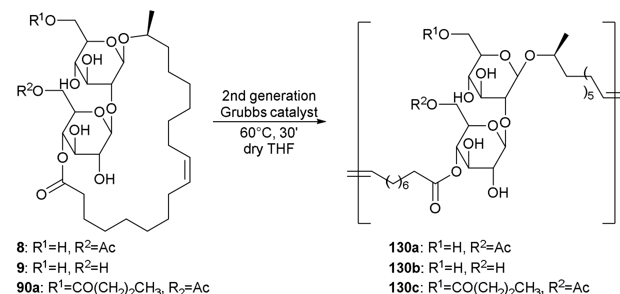
DMSO but not in water. To increase the hydrophilic character of the polymer, copolymers **125** were produced with acrylic acid and acrylamide. The copolymer composition was controlled by the monomer feed ratio. Copolymers **125a** and **125b** were synthesized with respectivily 3, 5, 10 and 50 mol% and 0.5, 1, 2, 3, 5 and 50 mol% sophorolipid lactone **89** in the feed. Copolymer **125b** is soluble in water with less than 3 mol% sophorolipid lactone **89** in the feed.

A ring-opening methathesis polymerization (ROMP) for the synthesis of a poly(sophorolipid) **126** from sophorolipid lactone **1** was first described by Gao *et al*. (Scheme 26).[68] The presence of degradable links in the polymer structure makes these polymers promising bioresorbable materials. Three different ruthenium-based Grubbs catalysts were evaluated and the highest yields were obtained with catalyst **128**. The solid-state properties of this poly(sophorolipid) were evaluated by Zini *et al*.[69] Both *cis* and *trans* double bonds are present in the poly(sophorolipid), respectively in 90% and 10%. Thermogravimetric analysis demonstrated the presence of two main degradation steps. The first step around 350°C may be associated with the degradation of the hydrophilic sugar moieties, the second step around 450°C can be attributed to the thermal degradation of the hydrophobic fatty acid chain. The combined results of differential scanning calorimetry (DSC), X-ray diffraction (XRD) and temperature modulated DSC (TMDSC) suggest that the poly(sophorolipid) possesses crystalline domains which melt at 123°C and has a glass transition at 61°C. A kinetic study on the polymerization mechanism was performed by Peng *et al*., categorizing it as an enthalpy driven ROMP.[70] The effect of catalyst loading, monomer concentration and solvent choice were evaluated for polymerization with Grubbs catalysts **128** and **129**.

The structural diversity of the poly(sophorolipid) was expanded by Peng *et al*. through the incorporation of modified sophorolipid lactone monomers in the polymer product (Scheme 27).[54] The di-acetylated, mono-acetylated, non-acetylated and butyrated sophorolipid lactones **1**, **8**, **9** and **90a** were homopolymerized according the optimal conditions which were determined in the kinetic study. TGA analysis showed that all polymers are stable up to 200°C and DSC analysis was used to analyze their thermal behavior. No melting transitions were observed for the mono- and non-acetylated sophorolipid polymers, probably due to the absence of a crystalline phase caused by a restricted chain mobility. These polymers show significant potential as substrates for bone tissue engineering due to their solid-sate properties. Biological properties relevant for tissue engineering were determined for all polymers except for the non-acetylated sophorolipid polymer **130b** due to stability problems. Cytotoxicity on human mesenchymal stem cells was evaluated via LDH and cell proliferation assays and a similar



Scheme 14. ROMP towards poly(sophorolipid) **126**[66]



Scheme 15. Homopolymerization of sophorolipid lactone derivatives[52]

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cytocompatibility was observed as the control tissue culture polystyrene (TCPS). All three polymers also displayed similar cell adhesion and spreading as the control CTPS. The capacity of the three polymers to support the differentiation of the mesenchymal stem cells to an osteoblast cell phenotype was evaluated, giving the best results for butyrated sophorolipid polymer **130c** compared to the control TCPS. Finally, degradation of the polymers in an osteogenesis medium was determined. All three polymers underwent an appreciable molecular weight loss in fourteen days and the degradation rate was dependent on the substitution at the sugar moiety.

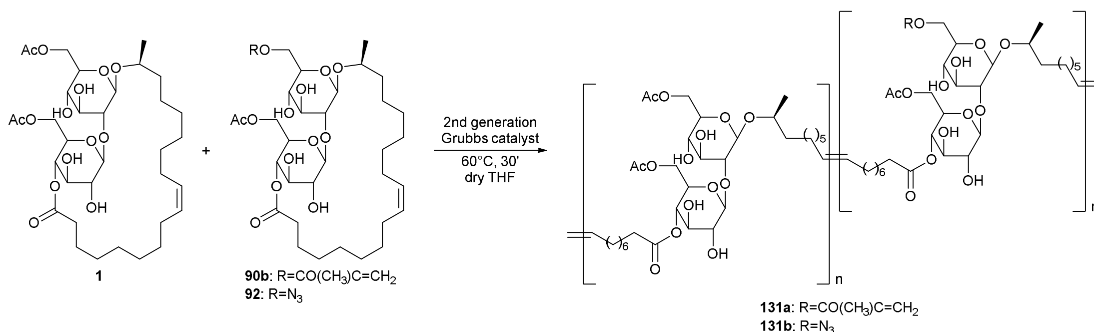
Copolymers of diacetylated sophorolipid lactone **1** and methacrylated or azidated sophorolipid compounds were prepared with control of the presence of the clickable functional groups (Scheme 28).[54] Functionalization of the methacrylate containing copolymer **131a** via a thiol-ene reaction was performed with mercaptoethanol as a model compound. Similarly, functionalization of the azide containing copolymer **131b** was peformed with 3-butyn-1-ol via the azide-alkyne cycloaddition reaction. Copolymers containing both methacrylate and azide groups were also prepared.

Ring-opening polymerization of sophorolipid lactones with lipases was explored by Hu & Ju (Scheme 29).[71] The starting material for the polymerization was a mixture of ω-1 C18:1 diacetylated sophorolipid **1**, ω-1 C16:0 diacetylated sophorolipid **132** and ω C16:0 diacetylated sophorolipid **133**. Ethyl acetate, pyridine, isopropyl ether, toluene, cyclohexane and hexane were evaluated as solvents for polymerization with porcine pancreatic lipase (PPL), giving the highest conversion in isopropyl ether and toluene. An evaluation of the reaction mechanism revealed that the diacetylated sophorolipid lactones were selectively deacetylated at the 6'-position prior to the ring-opening polymerization. This results in linkage of the monomers via both the 6'-position and the 4"-position at the sugar moiety. Four different lipases were used to evaluate the conversion efficiency in both acetonitrile and isopropyl ether, namely PPL, immobilized *Mucor miehei* lipase (MML), lyophilized *Candida antarctica* lipase (CAL-B) and lyophilized Pseudomonas sp. Lipase (PSL). The highest conversion was obtained with MML in isopropyl ether, but CAL-B proved to be the least solvent sensitive. Substrate selectivity dependended on the temperature, with an increasing conversion at 60°C but decreasing conversion at 50°C for larger ring sizes. The overall polymerization proceeded the fastest at 60°C.

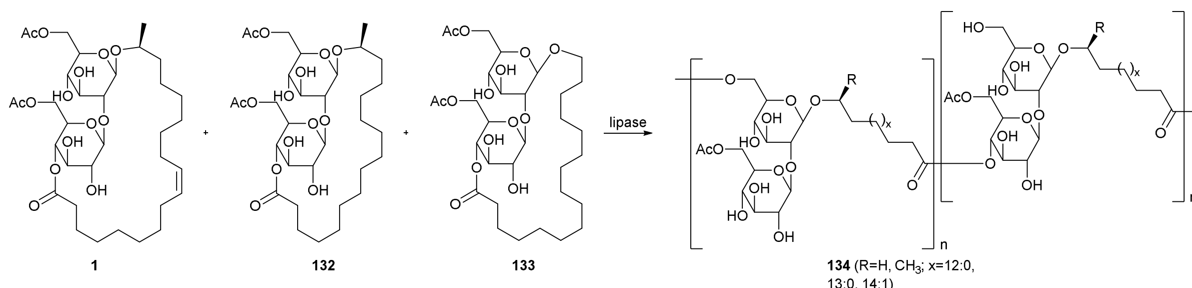
Modifications towards short-chained sophorolipids

The synthesis of short-chained sophorolipids was described by Develter and Fleurackers, and by Van Bogaert *et al*. (Scheme 30).[72] The esters dodecyl pentanoate, pentyl dodecanoate, decyl heptanoate, dodecyl malonate, myristyl malonate and dodecyl glutarate were used for the synthesis of sophorolipid esters **135** by *Candida bombicola*. These sophorolipid esters were subjected to alkaline hydrolysis, yielding short-chained sophorolipids **136**. An alternative strategy for the production of short-chained derivatives implied the ozonolysis reaction of sophorolipid acid **2** in water towards C9 sophorolipid acid **137**. The mixture of this short-chained sophorolipid acid and azelaic acid reduced the surface tension of water to 30 mN/m and dynamic surface tension measurements indicated that the reduction of the surface tension was faster compared to crude sophorolipids after fermentation. The mixture proved to be a better wetting agent than Simulsol AS48 and a comparable hydrotrope as Simulsol AS48 and crude sophorolipids.

The ozonolysis strategy was further exploited by Delbeke *et al*. for the synthesis of sophorolipid amines and sophorolipid quaternary ammonium salts (Scheme 31).[73] Diacetylated sophorolipid lactone **1** was transformed into peracetylated sophorolipid methyl ester **111a**



Scheme 16. Synthesis of copolymers with methacrylate and azide groups[52]



Scheme 17. Enzymatic ring-opening polymerization of sophorolipid lactones[69]

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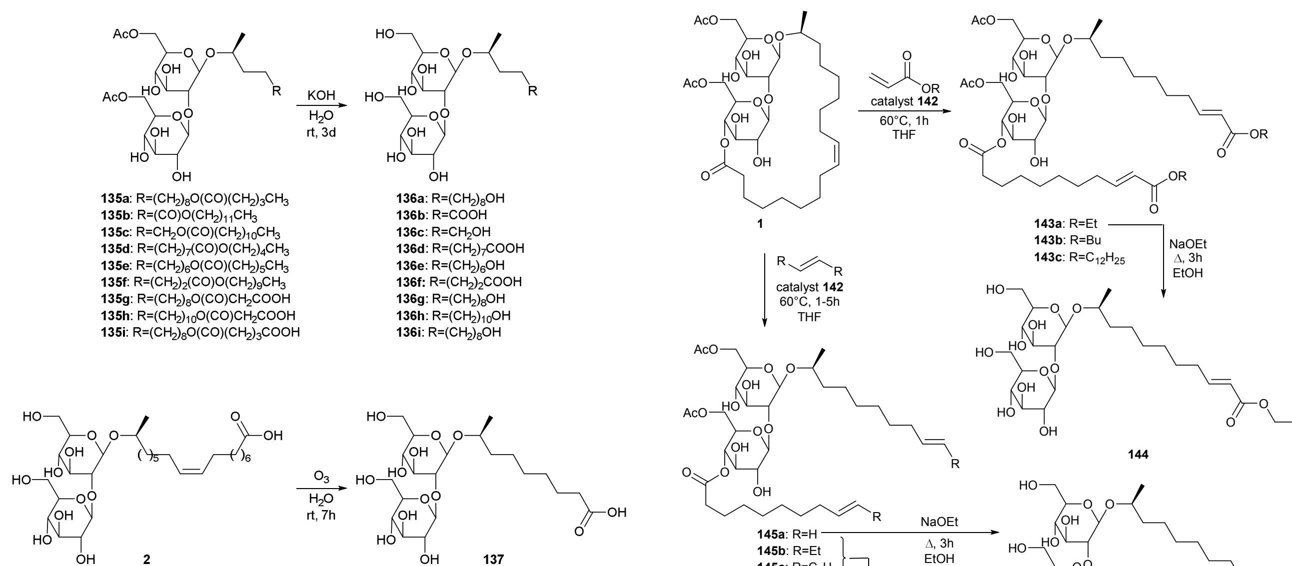


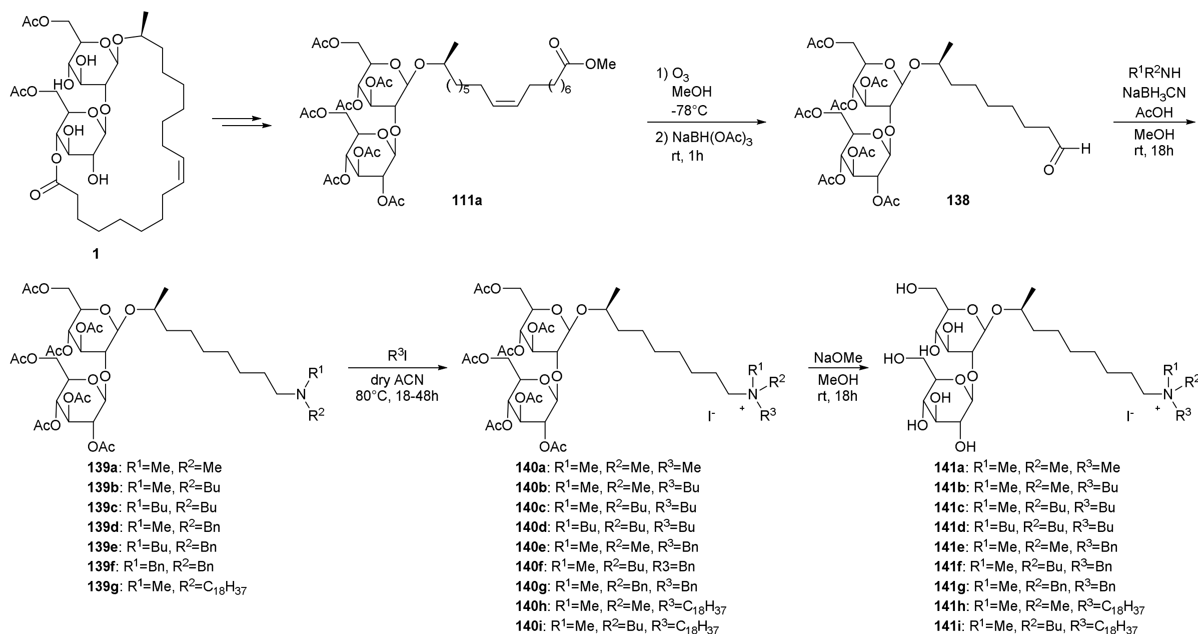
Scheme 30. Synthesis of short-chained sophorolipids[70]

according to procedures described earlier. A subsequent ozonolysis reaction with reductive work-up yielded sophorolipid aldehyde **138**. Reductive amination with a variety of secondary amines furnished sophorolipid amines **139**, which were subsequently quaternized and deprotected to yield sophorolipid quaternary ammonium salts **140** and **141**. Eleven of the sophorolipid quaternary ammonium salts possessed antimicrobial activities against Gram-postive organisms. The best results were obtained for the derivatives with an octadecyl chain on the nitrogen atom.

Ring-opening cross-metathesis of sophorolipid lactone **1** was performed by Peng *et al*. for the synthesis of short-chained sophorolipid derivatives (Scheme 32).[74] The cross-metathesis reactions with *n*-acrylates, trans-3-hexene, 1-hexene and ethylene were catalyzed with a second generation Grubbs catalyst **142**.

Scheme 32. Ring-opening cross metathesis towards short-chain sophorolipids[72]

Cross-metathesis with *n*-acrylates resulted in the synthesis of three gemini type sophorolipids **143**. Subsequent ethanolysis reaction yielded the corresponding ethyl ester **144**. Cross-methathesis with trans-3-hexene, 1-hexene and ethylene followed by ethanolysis and



Scheme 18. Synthesis of sophorolipid amines **139** and sophorolipid quaternary ammonium salts **140** and **141**[71]

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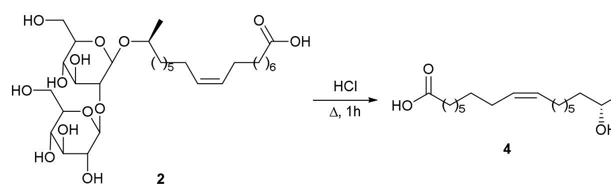
optional hydrogenation yielded short-chain alkyl sophorolipids **146** and **147**. Hydrogenation of unsaturated alkyl sophorolipid **146** was not performed since this terminal double bond is valuable for further functionalization. Critical micelle concentrations were determined via the Wilhelmy plate method and *n*-dodecyl-*β-D*-maltoside was used as a reference compound for comparison. Both the CMC and minimum surface tension (γ_{min}) decrease with increasing hydrophobicity and are thus dependent on the chain length. The maximum surface adsorption density (‘$_{m}$) and minimum surface coverage area per surfactant (A_{min}) were also calculated. Increasing the chain length results in a higher packing density and lower minimum surface area, especially from alkyl sophorolipid **146** to alkyl sophorolipid **147a**. Sophorolipid ethyl ester **144** displayed the highest packing density and lowest minimum surface area despite its hydrophilic character. It is thought that the ester moiety induces additional lateral attraction which reduces the distance between the molecules at the air-water interface. Comparison of the values for *n*-dodecyl-*β-D*-maltoside and alkyl sophorolipid **147a** demonstrates that sophorose occupies a larger effective area than maltose at the air-water interface. The influence of the chain length of alkyl sophorolipids on the CMC proved to be smaller than that of alkyl glucoside and maltoside sugar-based surfactants. This is likely due to the higher effective area occupied by the sophorose group.

Degradation of sophorolipids in smaller building blocks

Deacetylated sophorolipid acid **2** was converted into the ω-1 hydroxy fatty acid **4** by Rau *et al*. via acid hydrolysis (Scheme 33)[57a]. The ω-1 hydroxy fatty acid **4** is a rare fatty acid which is difficult to prepare via organic synthesis. This fatty acid could be used as a building block for the synthesis of pharmaceutically valuable products and for polyester- and macrocyclic lactones.

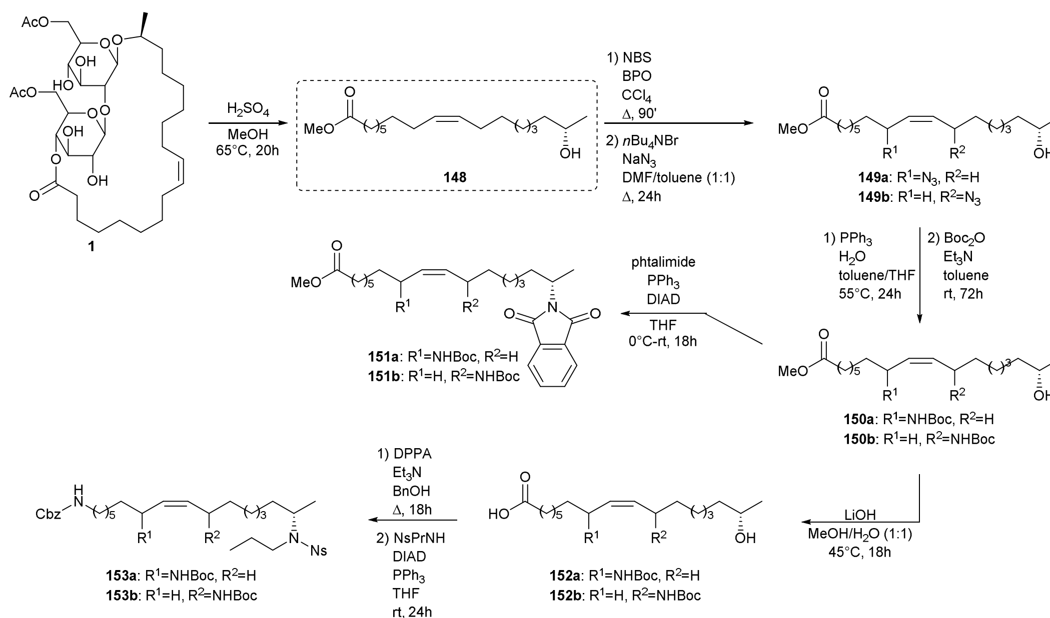
Zerkowski and Solaiman described the synthesis of polyfunctional fatty amines starting from sophorolipids (Scheme 34).[75] In a first step, sophorolipid lactone **1** is subjected to acid hydrolysis to yield methyl 17-hydroxy oleate **148**. An allylic substitution was used to introduce an amine function at the allylic position of the double



Scheme 19. Degradation towards 17-hydroxy oleic acid **4**[55a]

bond. Methyl 17-hydroxy oleate **148** was reacted with *N*-bromosuccinimide and sodium azide towards azide **149** and was subsequently subjected to a Staudinger reaction. Protection of the amine function towards Boc-protected amine **150** was performed without intermediate isolation. Amine **148** was further subjected to a Mitsonobu reaction with phtalimide to yield diamine **151**. After conversion of the ester function of amine **150** into a carboxylic acid function, a Curtius rearrangement with diphenylphosphorylazide and a Mitsonobu reaction were performed to yield triamine **153**. Further modifications of methyl 17-hydroxy oleate **148** comprised a Mitsonobu reaction towards subterminal amine **154** with phtalmide and the synthesis of azide **155** followed by a Staudinger reaction and protection of the amine function towards Boc-protected amine **156** (Scheme 35). Conversion of the ester function into a carboxylic acid function and a subsequent Curtius rearrangement yielded diamine **157**. Finally, methyl 17-hydroxy oleate **148** was converted into 17-hydroxy oleic acid **4** and was subsequently subjected to a Curtius rearrangement to yield amine **158** and isocyanate **159**. The latter was converted into urea derivative **160** with mono-Boc piperazine. Amine **158** was transformed into azide **161** and subsequently subjected to a Staudinger reaction with protection of the amine function towards Ns-protected amine **162**. Amine **158** was also reacted with *N*-bromosuccinimide and sodium azide towards azide **163** and additionally subjected to a Staudinger reaction and protection of the amine function towards Fmoc-protected amine **164**.

A similar strategy was adopted by Zerkowski and Solaiman for the



Scheme 20. Synthesis of polyfunctional fatty amines from sophorolipids (part 1)[73]

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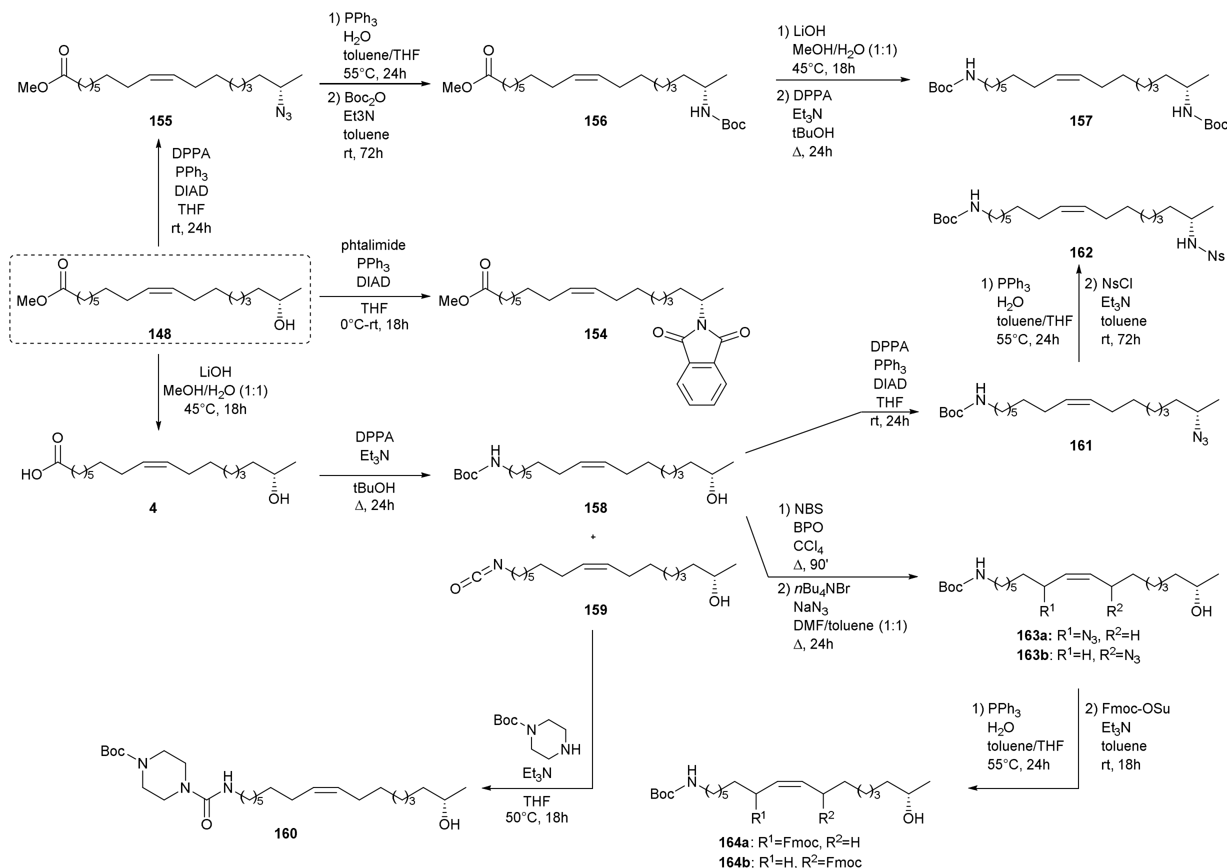
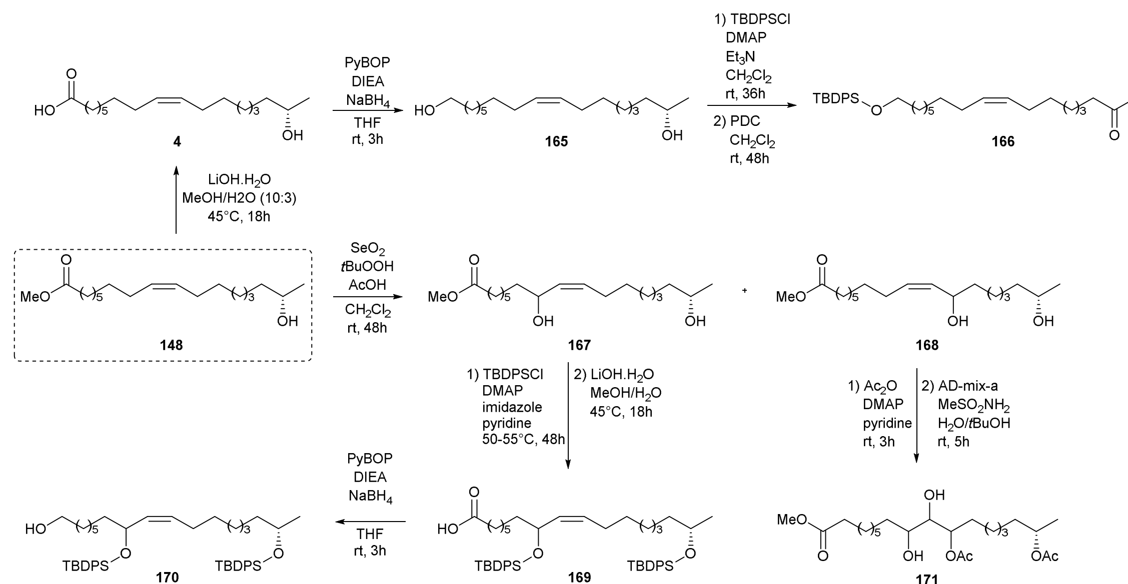


Scheme 21. Synthesis of polyfunctional fatty amines from sophorolipids (part 2)[73]

synthesis of polyhydroxy fatty acids (Scheme 36).[76] Methyl 17-hydroxy oleate **148** was converted into 17-hydroxy oleic acid **4** and subsequently reduced towards diol **165** via an intermediate activated ester. The primary alcohol was selectively protected with *t*-butyldiphenylsilyl chloride after which the secondary alcohol was oxidized with pyridinium dichromate towards keton **166**. Allylic hydroxylation with selenium dioxide of methyl 17-hydroxy oleate **148** yielded two isomers **167** and **168**. The former was protected with *t*-butyldiphenylsilyl chloride, converted to carboxylic acid **169** and reduced to the primary alcohol **170**. The latter was protected with acetic anhydride and subjected to the Sharpless' asymmetric dihydroxylation towards methyl ester **171**. Protection of methyl



Scheme 22. Synthesis of polyhydroxy compounds from sophorolipids (part 1)[74]

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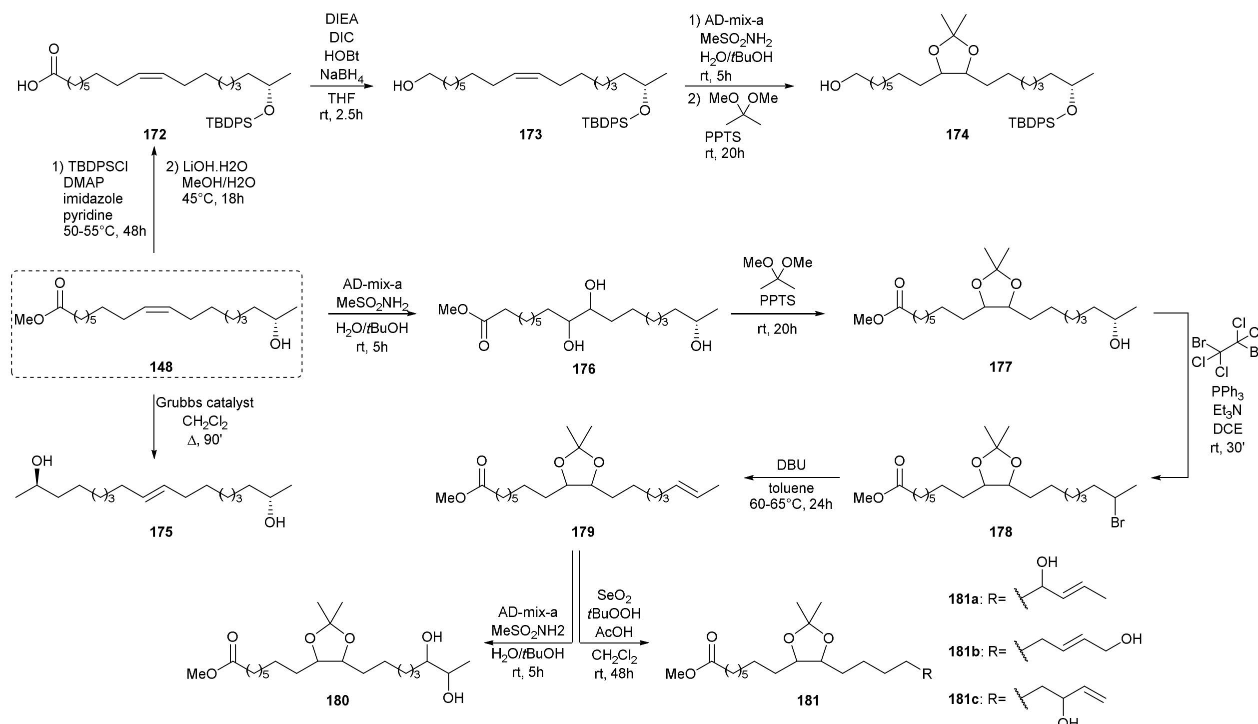
17-hydroxy oleate **148** with *t*-butyldiphenylsilyl chloride followed by hydrolysis yielded carboxylic acid **172**, which was subsequently reduced to alcohol **173** (Scheme 37). Sharpless' asymmetric dihydroxylation and protection with 2,2-dimethoxypropane yielded alcohol **174**. Olefin metathesis of methyl 17-hydroxy oleate **148** with a Grubbs' first generation catalyst yielded resulted in diol **175**. Methyl 17-hydroxy oleate **148** was also directly subjected to Sharpless' asymmetric dihydroxylation towards methyl ester **176** and subsequently protected with 2,2-dimethoxypropane towards methyl ester **177**. Treatment with 1,2-dibromotetrachloroethane resulted in bromide **178**. A new double bond was formed upon elimination with DBU towards methyl ester **179**. Sharpless' asymmetric dihydroxylation yielded methyl ester **180** whereas allylic hydroxylation with selenium dioxide resulted in three isomeric products **181**.

Sophorolipids were applied by Hoffmann *et al*. in the synthesis of the natural products Ebracteatoside C, Zizybeoside I and phenetyl glucoside (Scheme 38).[77] The crude sophorolipid fermentation product was transformed into the sophorolipid methyl ester **86a**. After protection of the carbohydrate hydroxyl groups and reduction of the double bond, sophorolipid methyl esters **58** and **183** were transformed into anomeric sophorose bromides **34** and **184**. The acetylated sophorose bromide **34** was then treated under Koenigs-Knorr conditions with benzyl alcohol or 2-phenetylethanol to yield Zizybeoside **186** or phenetyl glycoside **187** after deprotection under Zemplén conditions. Sophorose bromides **34** and **184** were hydrolyzed and subsequently reacted with trichloroacetonitrile to form the trichloroacetimidates **189**. Glycosylation of the acetate protected derivative **189a** selectively led to α-anomer **190a**. Benzoate protected derivative **189b** was glycosylated with $Pd(CH_3CN)_4(BF_4)_2$ to selectively form the enriched β-anomer **190b** and was subsequently hydrolyzed to furnish Ebracteatoside C **191**.

Sophorolipid based nanoparticles

The application of sophorolipids for the formation of magnetic cobalt nanoparticles is described by Kasture et al. (Scheme 40).[78] Sophorolipids have the advantage over oleic acid that they are better water soluble which resulted in the formation of more stable nanoparticles. The cobalt nanoparticles were synthesized by reduction of Co2+ with sodium borohydride using the sophorolipid acid **2** as capping agent. The nanoparticles were obtained from aqueous dispersions as a stable powder via simple centrifugation or magnetic separation and could easily be redispersed in water. Transmission electron microscopy (TEM) revealed that well separated, polydisperse particles were obtained with an average particle size of around 50 nm. The binding of the sophorolipids to the cobalt nanoparticle surface via the carboxylic acid function and the double bond was confirmed by Fourier transform infrared spectroscopy (FTIR). Room-temperature magnetization measurements were performed to demonstrate the good magnetic features of the sophorolipid-capped nanoparticles.

Kasture *et al*. also described the formation of sophorolipid based silver nanoparticles (Scheme 41).[79] The sophorolipid acids **2** and **192** were used as reducing and capping agents in the synthesis of the nanoparticles with silver nitrate under alkaline conditions at different temperatures. The differences in size and dispersion for oleic acid sophorolipid-capped nanoparticles (OA) and linolenic acid sophorolipid-capped nanoparticles (LA) at different temperatures were evaluated. Particle size distribution was determined via both transmission electron microscopy (TEM) and dynamic light scattering measurements (DLS). It was observed that particle size decreased with increasing temperature and longer time is needed at low temperatures to obtain complete particle formation and growth. The nanoparticles were isolated as a stable powder via centrifugation and air-drying which could easily be redispersed in water.[80] Just like for the cobalt nanoparticles, binding of the



Scheme 23. Synthesis of polyhydroxy compounds from sophorolipids (part 2)[74]

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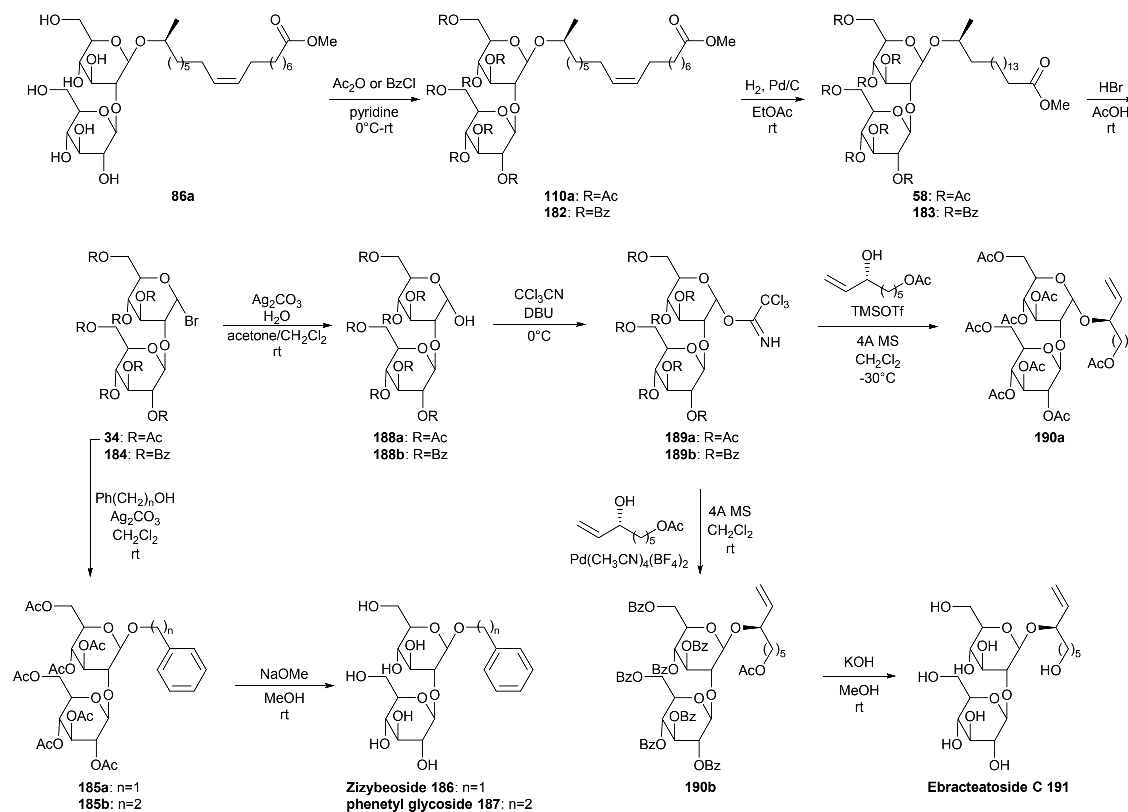
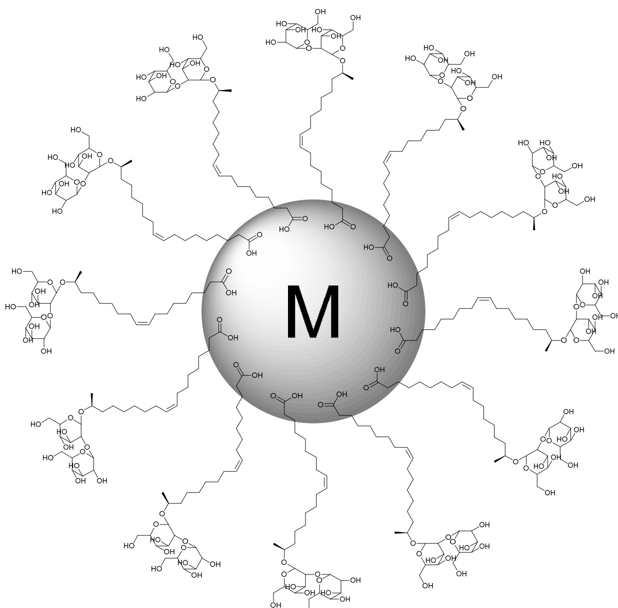


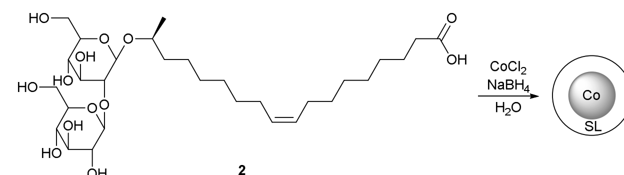
Scheme 38. Synthesis of natural products from sophorolipids[75]

sophorolipids to the silver nanoparticle surface occurs via the carboxylic acid function and the double bond. Continuous flow synthesis of sophorolipid-capped silver nanoparticles is described by Kumar *et al.*[81] Stearic acid based sophorolipid **10** was used as reducing and capping agent for the continuous flow experiments



Scheme 39. Sophorolipids as nanoparticle capping agents

since the resulting nanoparticles were formed faster and featured a better size uniformity than those synthesized with oleic acid based sophorolipid **2**. The influence of the flow rate on the average particle size and particle size distribution were evaluated. Higher flow rates led to better mixing but reduced residence time, which resulted in an incomplete reaction towards large polydisperse particles. At lower flow rates, the reaction was complete and small monodisperse spherical nanoparticles were obtained. The synthesis of sophorolipid-capped silver nanoparticles via segmented flow in a microreactor was performed by Kumar *et al.*[82] Both liquid-liquid and gas-liquid segmented flows were applied with respectively kerosene and air as inert phase. The particle size was much smaller for gas-liquid flow than for liquid-liquid flow and the particle size distribution could be controlled by the choice of inert phase. Singh *et al*. investigated the antibacterial activities of these sophorolipid-capped silver nanoparticles.[80] For sophorolipids as such, good inhibition was obtained against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* while there was only a faint inhibition against the Gram-negative bacterium *Pseudomonas aeruginosa*. In case of the sophorolipid-capped nanoparticles,



Scheme 40. Sophorolipid-capped cobalt nanoparticles[76]

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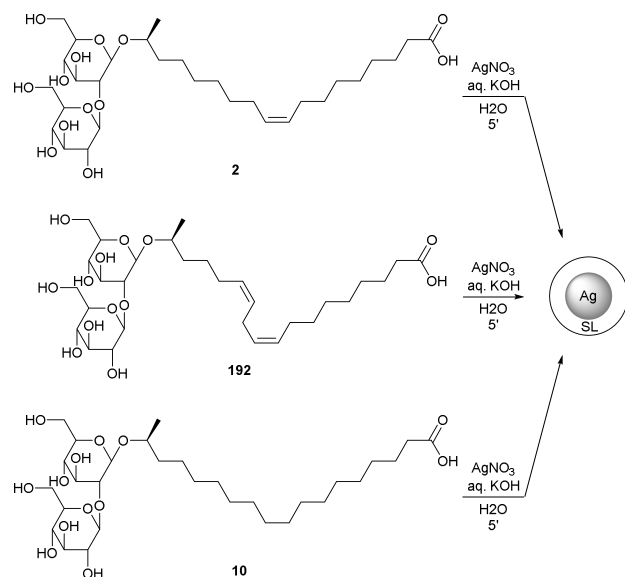


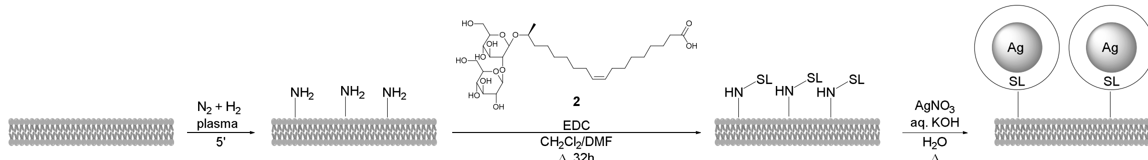
Scheme 24. Sophorolipid-capped silver nanoparticles[77, 79]

Bacillus subtilis and *Staphylococcus aureus* were exposed to concentrations ranging from 20 to 100 µg/mL. The cell survival of *Bacillus subtilis* dropped to 0.4% after one hour, but no survival was observed for *Pseudomonas aeruginosa*. Sophorolipid-capped silver nanoparticles are thus more effective against Gram-negative bacteria. Silver proved to be the cause of the observed antibacterial effects via the induction of pore formation in the bacterial cell membrane through the formation of reactive oxygen species.

The same methodology was applied by D'Britto *et al*. for the synthesis of silver nanoparticle studded polyethylene scaffolds (Scheme 42).[83] The polymer scaffold was first treated with *in situ* generated ammonia plasma to introduce amine groups. Sophorolipid **2** was covalently attached to the polymer surface via an amidation reaction followed by formation of silver nanoparticles at the polymer surface with silver nitrate. The size of these silver nanoparticles was approximately 60 to 70 nm. The antimicrobial activity of the modified polyethylene scaffolds against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* was evaluated. Within six hours of exposure, the silver nanoparticle studded polyethylene scaffolds displayed a broad activity against both the Gram-positive and Gram-negative bacteria. The adhesion and proliferation of mammalian cells on the polymer scaffolds was also evaluated. This revealed that the growth of the mammalian cells was encouraged by the silver nanoparticle studded polyethylene scaffolds and that the cells exhibited good viability. These modified polymer scaffolds are good candidates for tissue-engineering and bio-implant applications.

Dhar *et al*. described the use of sophorolipids for the synthesis of biocompatible gold nanoparticles (Scheme 43).[84] Gellan gum **193** was used as the primary reducing and capping agent in the formation of the gold nanoparticles with chloroauric acid, followed by conjugation of the nanoparticles with sophorolipid acid **2**. The nanoparticles were further loaded with the anticancer drug doxorubicin hydrochloride **194**. The average particle size was determined via transmission electron microscopy and amounted to 13 and 17 nm for respectively the gellan gum capped nanoparticles and the sophorolipid conjugated nanoparticles. After fluorescent labeling, the efficient cellular uptake of the nanoparticles by human glioma cell line LN-229 was demonstrated. The *in vitro* cytotoxicity of both sophorolipid-conjugated nanoparticles and doxorubicin chloride loaded nanoparticles were evaluated and compared to free doxorubicin chloride 194 against human glioma cell line LN-229 and human glioma stem cell line HNHC-2. The doxorubicin loaded nanoparticles performed better then both the sophorolipid-conjugated nanoparticles and the free doxorubicin chloride **194** against both cell lines. This synergistic effect could be explained by the better cell penetration of the doxorubicin loaded nanoparticles compared to free doxorubicin chloride **194**.

The formation of sophorolipid-capped iron oxide nanoparticles was executed by Baccile *et al*. (Scheme 44).[85] The magnetic iron nanoparticles were synthesized with iron (II) chloride and iron (III) chloride in the presence of ammonia at room temperature and 80°C. Both a two-step and one-step procedure were applied which resulted in nanoparticles with respectively a maghemite (γ-Fe_2O_3) or two-line ferrihydrite ($Fe_5HO_8{\cdot}4H_2O$) structure. Particle size distribution was determined via both transmission electron microscopy (TEM) and dynamic light scattering measurements (DLS). A very broad particle size distribution was obtained for the one-way synthesis pathway at room temperature via TEM, while monodisperse nanoparticles were obtained in all other cases. A narrow size distribution was obtained for all samples via DLS measurement in water and all average sizes are systematically higher than those measured by TEM. When DLS measurements were performed in an ethanol/water mixture (8:2), particle sizes increased compared to those measured in water, indicating the aggregation of the nanoparticles into large aggregates. The much higher increase of the average particle size for the nanoparticles synthesized at room temperature indicated that sophorolipids were more loosely bonded to the nanoparticle surface. FTIR analysis revealed that the sophorolipids are mainly attached to the nanoparticle surface via the carboxylic acid function. Adsorption studies were performed for the sophorolipid-capped iron oxide nanoparticles with two lectines, namely Concanavalin A and lectin from *Bandeiraea simplicifolia*. No specific interaction occurred with the lectin from *Bandeiraea simplicifolia*, but affinity for Concanavalin A was observed. Sophorolipid-capped iron oxide nanoparticles thus proved to be interesting as selective targets for sugar/protein interactions.



Scheme 42. Synthesis of silver nanoparticle studded polyethylene scaffolds[81]

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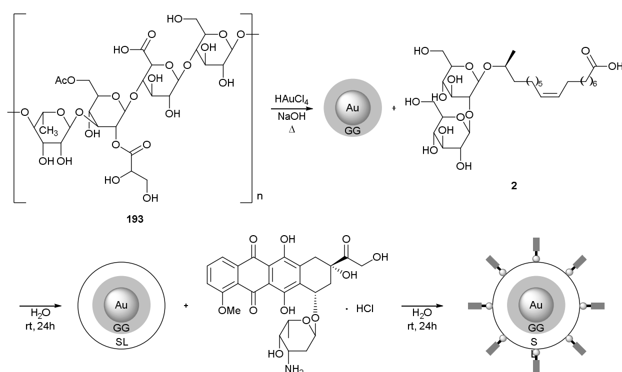
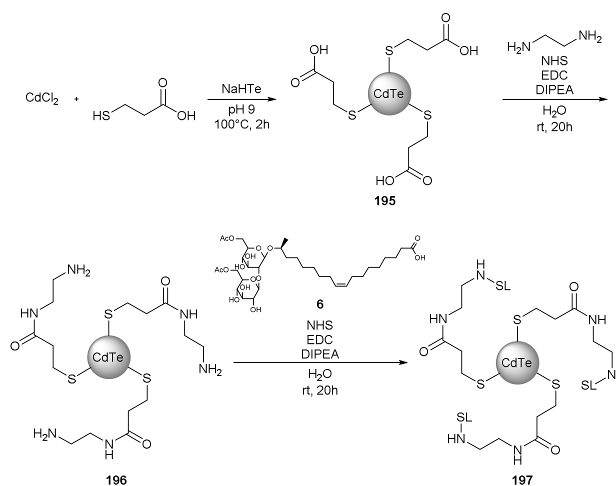


Scheme 25. Sophorolipid-conjugated gellan gum capped gold nanoparticles[82]

Singh *et al*. described the synthesis of sophorolipid self-assembled vesicular mesostructures via laser irradiation and their subsequent loading with iron oxide nanoparticles (Scheme 45).[86] Laser irradiated sophorolipids proved to be highly fluorescent and in the case of diacetylated sophorolipid acid, uniform spherical microstructures were obtained. Upon irradiation, sheet-like structures are formed initially which are converted into fully developed spherical mesostructures after one hour. By adjusting the irradiation conditions, nanoparticles of approximately 100 nm could be obtained. After drying, the sophorolipid mesostructures could easily be redispersed in water. When Fe_3O_4 nanoparticles were added during the synthesis, sophorolipid mesostructures embedded with iron oxide nanoparticles were obtained. An MTT-assay on a HeLa derived cell line revealed that the sophorolipid mesostructures displayed no cytotoxicity against eukaryotic cells. It was also demonstrated that the sophorolipid mesostructures loaded with iron oxide nanoparticles could serve as effective hyperthermia agents.

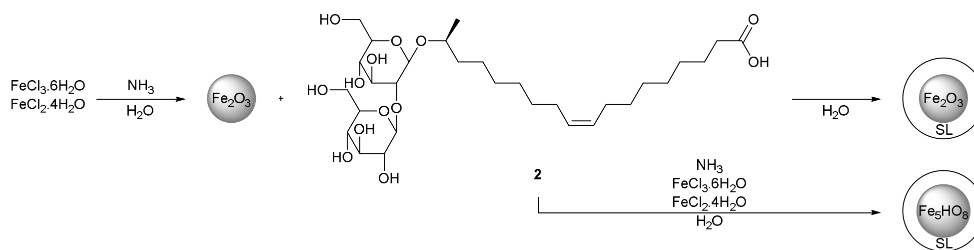
Sophorolipid conjugated cadmium telluride quantum dots (CdTe QD) were synthesized by Singh *et al*. (Scheme 46).[87] In a first step, 3-mercaptopropionic acid was used as the capping agent in the



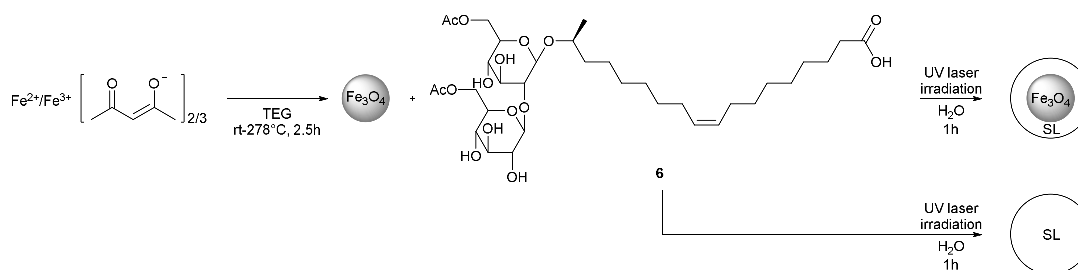
Scheme 46. Synthesis of sophorolipid conjugated cadmium telluride quantum dots[85]

synthesis of the CdTe QDs. Terminal amine groups were introduced via an EDC-mediated amidation reaction with ethylenediamine prior to the coupling of diacetylated sophorolipid acid **6**. The obtained nanoparticles were nearly monodispersed with an average particle size of 5 nm for both CdTe QDs **195** and sophorolipid conjugated CdTe QDs **197** via TEM analysis. DLS measurements indicated average particle sizes of 8 and 118 nm for respectively CdTe QDs **194** and sophorolipid conjugated CdTe QDs **197**. Bioimaging studies revealed that sophorolipid conjugated CdTe QDs **197** were taken up in the cytosol of a cancer cell line. Cytotoxicity studies demonstrated a specific toxicity against the cancer cell line compared to a control cell line.

Basak *et al*. described the synthesis of sophorolipid functionalized zinc oxide nanoparticles (Scheme 47).[88] Diacetylated sophorolipid acid **6** was used for the nanoparticle synthesis in combination with zinc nitrate hexahydrate under alkaline conditions. The average particle size was calculated via the Debye-Scherrer formula and was



Scheme 26. Sophorolipid-capped iron oxide nanoparticles[83]



Scheme 45. Synthesis of sophorolipid mesostructures wether or not loaded with iron oxide nanoparticles[84]

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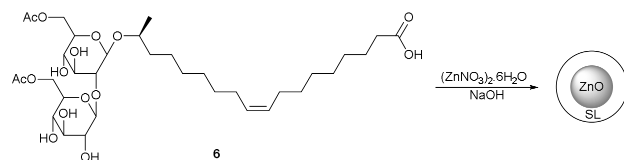

Scheme 27. Sophorolipid-capped zinc oxide nanoparticles[86]

found to be 6.55 and 6.02 nm for respectively naked and sophorolipid-capped zinc oxide nanoparticles. The antimicrobial activities of the zinc oxide nanoparticles were evaluated against the Gram-negative bacterium *Salmonella enterica* and the fungus *Candida albicans*. The sophorolipid-capped zinc oxide nanoparticles proved to exhibit a significant inhibitory effect on the growth of both strains at a concentration of 5 mg/mL. Higher activities were observed for the sophorolipid-capped zinc oxide nanoparticles than for the naked zinc oxide nanoparticles, probably due to the better penetration of the former ones in the pathogenic cells. The mechanism of cell damage on *S. enterica* consisted of cell elongation followed by cell wall disruption. In the case of *C. albicans*, complete cell rupture occurred while cell elongation was not detected. A higher antimicrobial activity was observed against *S. enterica* compared to *C. albicans*.

Conclusions and perspectives

Sophorolipids are interesting renewable resources but to date, applications have only been commercialized for sectors in which they can hardly be competitive in terms of production cost. It was demonstrated that sophorolipids feature divergent biological activities which creates opportunities for application in high-added value sectors, in particular the pharmaceutical sector. Modification of these renewable feedstocks is an excellent strategy to improve their biological activity, but the purity of the sophorolipids is essential for the development of good synthesis pathways. The enzymatic sophorolipid modifications mostly enable selective transformations at the sugar head, whereas chemical modifications open up a whole new range of possible sophorolipid analogues. Natural sophorolipids were used for the synthesis of polymers, surface-active compounds, nanoparticles and as source of smaller, non-commercial building blocks. Future research should focus on optimizing fermentation processes towards pure natural sophorolipids in combination with developing selective modification pathways to obtain sophorolipid derivatives in high yields for high added value applications because competitiveness with the traditional base chemical industry will remain an issue.

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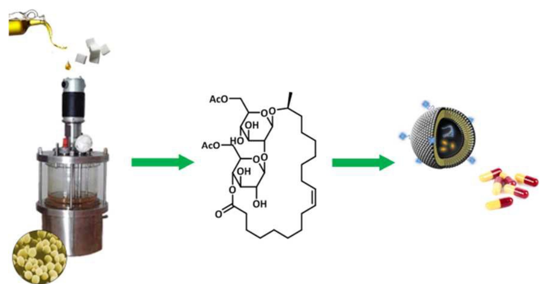
This review focuses on the chemical and enzymatic modification of sophorolipid biosurfactants into new derivatives.



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