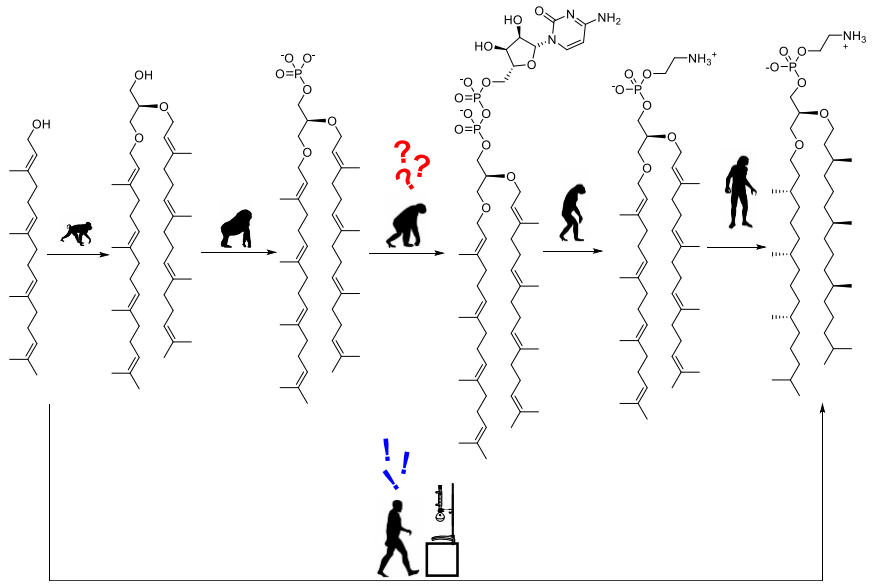
Chapter 7 A Missing Link in Archaeal Lipid Biosynthesis; a Contribution from Organic Synthesis



Abstract: The Archaea form a separate domain of Life known to occupy extreme ecological niches. Their extremophilicity is often associated with their characteristic membrane lipids, which require different biosynthetic pathways than eukaryotes and bacteria. Given that the in vivo identification of the involved enzymes is ambiguous, their function needs to be confirmed by in vitro experiments. However, development of the detection methods, assay conditions and isolation of the substrates are challenges on their own. At least with the substrates, organic synthesis can help. This chapter describes a chemical synthesis of an intermediate in the biosynthesis of archaeal lipids. This intermediate was essential for an in vitro assay, which revealed one of the missing links in biosynthesis of archaeal lipids. A second contribution of this chapter is a total synthesis of cycloarcheol and its *ß*-glucosyl analogue, which are important taxonomic tools in Archaea.

Parts of this chapter have been published.
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Introduction

Archaea form the third domain of life, comprising up to 20% of the biomass on Earth. Since 1977, when the domain Archaea was first described,[1] their evolutionary origin has been a topic of intense debate. Recently, Forterre[2] presented a hypothesis in which the Archaea and the Eukarya evolved from a common ancestor. He further hypothesized, that the ancestors of the Archaea escaped from their proto-eukaryotic predators by invading ecological niches with harsh environmental conditions. Surroundings like hydrothermal vents,[3] geysers[3-4] with temperatures over 120 °C, highly acidic (pH = 0)[5] or alkaline (pH >10)[6] springs, or lakes with salt[7] concentrations 10 times higher than sea water required a lot of adaptation. One of the features that distinguishes Archaea from Bacteria and Eukaryota is their cell envelope. This lacks a general cell wall polymer, and contains membrane phospholipids, which differ from bacterial and eukaryotic lipids in three aspects (Figure 1-I). First, in Archaea the hydrophobic part comprises of two terpenoid (mostly phytanyl) chains, while this part is composed of two fatty acid residues in bacterial and eukaryotic lipids.

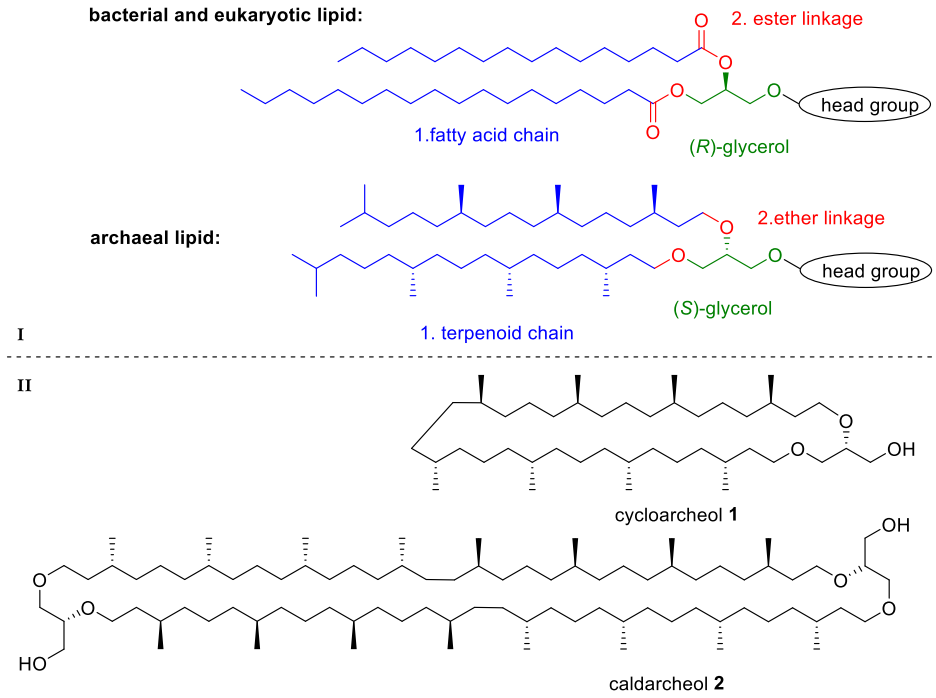

Figure 1. (I) Comparison of bacterial and eukaryotic lipids with archaeal lipids; (II) common archaeal lipid backbones.

Second, in Archaea the phytanyl chains are linked to the glycerol via an ether bond, while in Bacteria and Eukarya the fatty acids bind via an ester bond. Third, the stereogenic centre in the glycerol moiety of archaeal phospholipids has the opposite configuration compared to that in bacterial and eukaryotic lipids. Furthermore, Archaea display a greater variability of the lipid backbones compared to Bacteria and Eukarya. Structures like cycloarcheol (**1**) (Figure 1-II) and caldarcheol (**2**) are common. Despite substantial investigations on archaeal membrane lipid biosynthesis, several steps and their corresponding enzymes remain unknown.

The extremophilic nature of Archaea is often associated with their unique lipids. Driessen et al.[8] studied the stability of liposomes from lipid extracts of *Escherichia coli* (mesophilic Bacteria), *Bacillus stearothermophilus* (thermophilic Bacteria) and *Sulfolobus acidocaldarius* (thermophilic Archaea). The Archaea-derived liposomes (archeosomes) showed significantly higher stability at all studied temperatures. In the same study, the authors also reported that while the bacterial liposomes gradually released about 50% of their content (fluorescent dye) over 62 days, the archeosomes showed only 8-10% release over the same period of time. The higher stability of archeosomes already found application in bioelectronics[9], gene delivery[10] and vaccination.[11] A bottleneck for their wider application is their limited availability. Growing Archaea is technically more difficult than growing Bacteria or eukaryotic cells. Furthermore, the yields of lipids are low. Typically, 1 g of lyophilized archaeal cells affords only 0.11-54 mg of crude lipid extracts.[12]

Biosynthesis of archaeal membrane lipids

Complete genome sequencing[13] of *Archaeoglobus fulgidus* allowed a better understanding of the lipid metabolism in Archaea. Archaea species have a complete set of genes encoding fatty acid metabolism,[14] similar to the bacterial and eukaryotic metabolism. Nevertheless, the fatty acids in Archaea are not used for the synthesis of membrane lipids, but for posttranslational modification of proteins. As mentioned above, the lipophilic portion of archaeal membrane lipids is exclusively terpenoid-based. These terpenes are biosynthesized[15] in a pathway that is similar to the bacterial and eukaryotic pathways (Figure 2). Diphosphate **3** (coming from the mevalonic acid pathway) is isomerised to diphosphate **4.** A three- or fourfold extension of **4** affords geranylgeranyl diphosphate (**5**) or farnesylgeranyl diphosphate (**6**), which subsequently enters the lipid biosynthetic pathway.

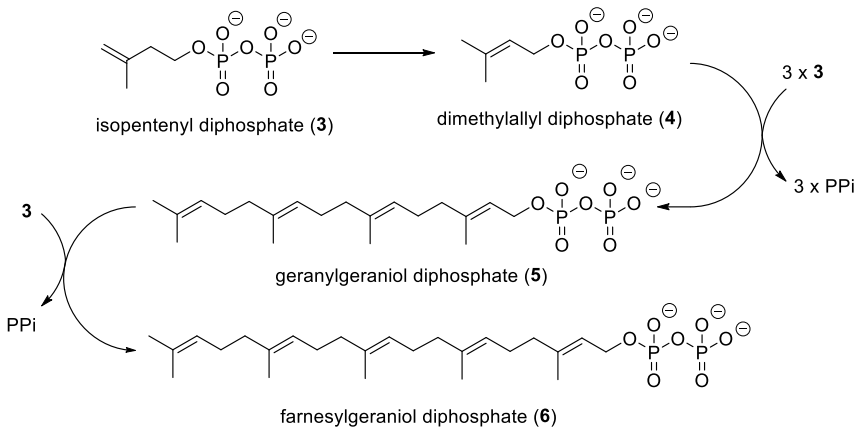

Figure 2. Biosynthesis of terpenes in Archaea.

The biosynthesis of the phospholipids starts with glycerol monophosphate **8** (Figure 3), which is obtained by the reduction of **7** (a product of glycolysis). Given that the absolute configuration of glycerol phosphate (**8**) is opposite in Bacteria and Eukarya, the corresponding reductase was considered unique for Archaea. Babinger et al.[16] recently showed that *Bacillus subtilis* produces a homologous enzyme. The biosynthesis of the phospholipids continues with the attachment of the terpenoid (here geranylgeranyl) chains to **8** (Figure 3). First, the primary hydroxyl group is prenylated in the cytosol. The significantly more lipophilic **9** is transferred to the cell membrane where the second prenylation takes place. Both prenylations proceed via a same S_N1 type mechanism. In the active site of the enzyme, the diphosphate of **5** is cleaved with the assistance of Mg^{2+}. Subsequently, the resulting allylic carbocation reacts with a nucleophilic hydroxyl group of the glycerol. With the bis-prenylated glycerol **10**, the biosynthesis of the archaeal phospholipids continues by attachment of the polar head group, which is achieved in two steps (Figure 3). The first step is an activation of **10** with cytidine triphosphate (CTP). Although an analogous step takes place in all three domains of life, the corresponding archaeal enzyme has been elusive until now. The identification of this enzyme is the topic of the first part of this chapter. The second step of the attachment of the phosphorous headgroup is the conversion of **11** to the final phospholipid **12**, **13** or **14**. With the headgroup attached, **12**, **13** or **14** need to undergo 1 or 2 more transformations, depending on the species. First, the reduction of the double bonds – a transformation that is common in all *Archaea* species. The reduction was studied by Nishimira and Eguchi,[17] who purified and characterized the corresponding enzyme in *Thermoplasma acidophilum*. This enzyme did not show any preference for a headgroup – phospholipids **12**, **13** and **14** were reduced in a similar rate.

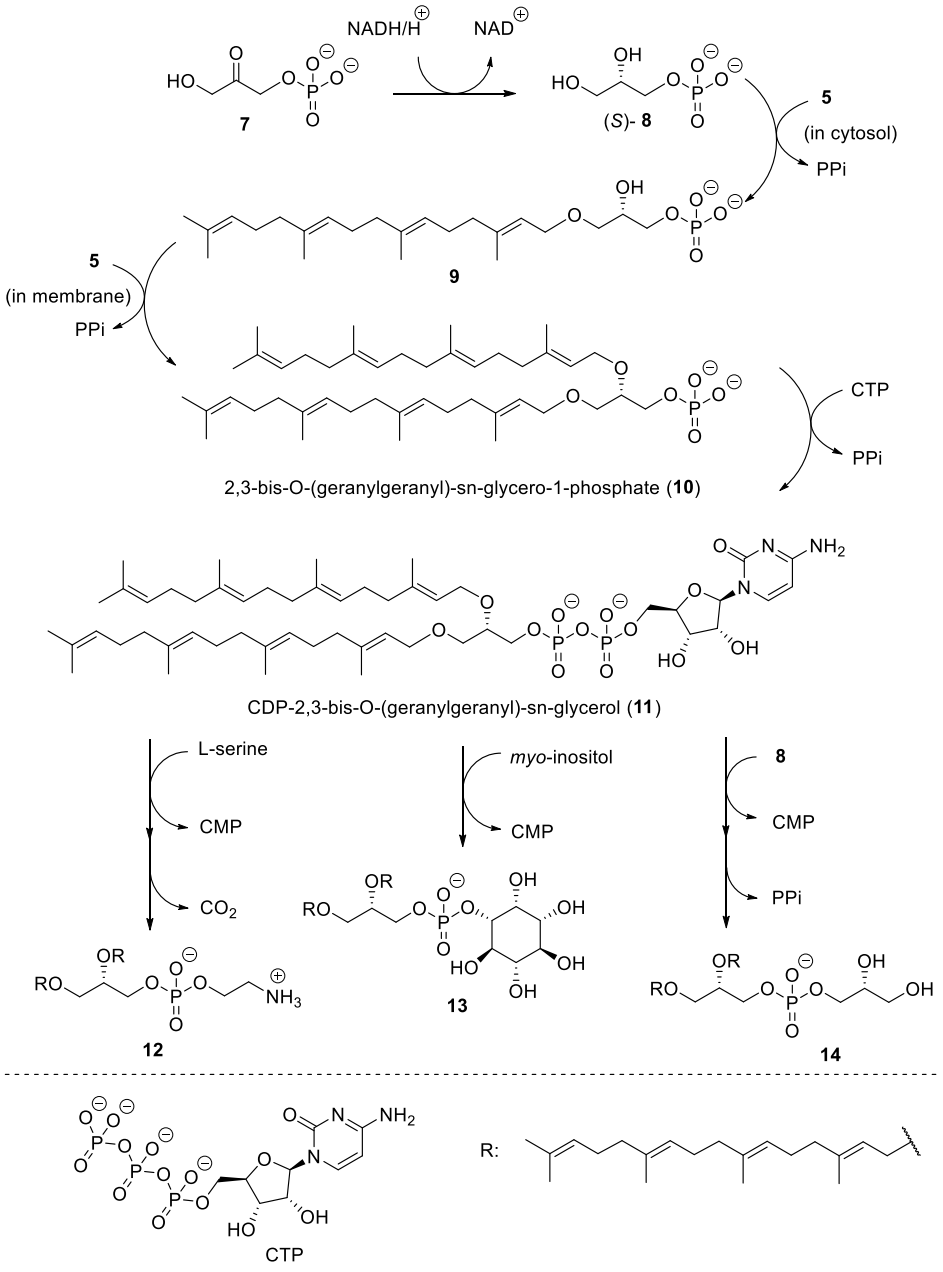

Figure 3. Biosynthesis of phospholipids in Archaea.

While for some of the Archaea, the biosynthesis ends with the reduction of the prenyl chains (from **15** to **16** in Figure 4-I), some other members further modify the chain by dimerization (**17** in Figure 4-I), cyclization (**18** in Figure 4-I), or dimerization followed by cyclization (**19** in Figure 4-I). The exact mechanism of these transformations remains unknown. A study by Eguchi et al.[18] suggests that the terminal double bonds are crucial for the dimerization. These findings are however

in contrast to the findings of Nemoto et al.,[19] who reported that the dimerization takes place at fully saturated precursors. Fitz and Arigoni[20] studied an analogous dimerization in *Butyrvibrio fibrisolvens* (a genus of Bacteria), which produces a membrane spanning diabolic acid (**21**) (Figure 4-II).

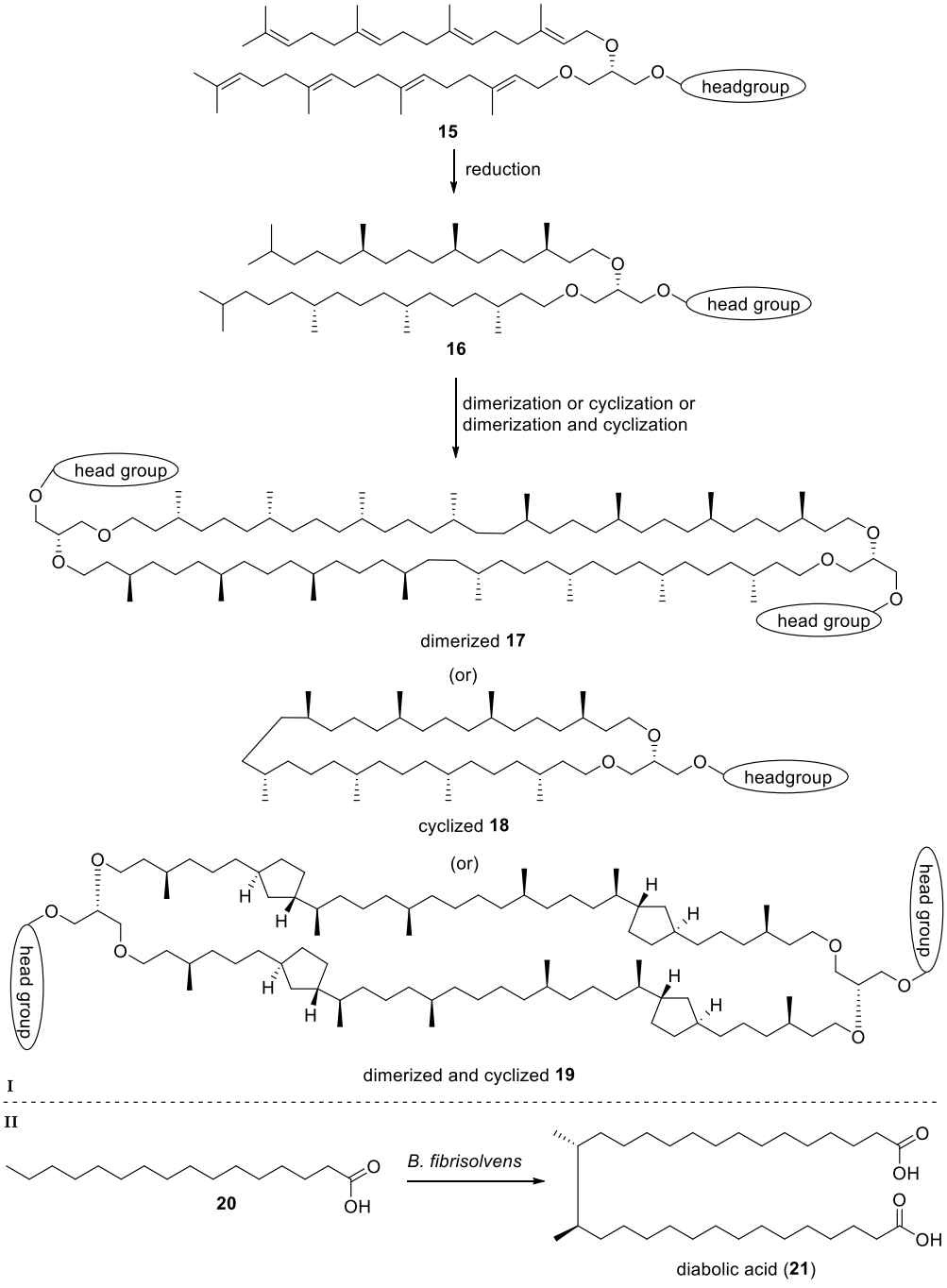

Figure 4. (I) Final steps in the biosynthetic pathway of membrane-spanning Archaea-lipids; (II) dimerization of palmitic acid in *Butyrivibrio fibrisolvens*.

The authors showed that diabolic acid is synthetized by dimerization of a fully saturated **20**.

Archaeal lipids as taxanomic markes

The second part of this chapter is dedicated to the total synthesis of the cycloarcheol lipid core **1** and its glycolipid analogue **22**. The lipids in Archaea fulfil also an important taxonomic function which highlights the necessity of their unambiguous structural determination. A total synthesis of the lipid is one of the options. Further comparison of the HPLC chromatograms and mass spectra of the synthetic and natural samples gives a high confidence in determining its presence. A total synthesis of cycloarcheol **1** (Figure 1) and its *ß*-glucosyl analogue **22** (Figure 5) is interesting in this context. Cycloarcheol was detected for the first time in 1983[21] in a deep sea hydrothermal vent.

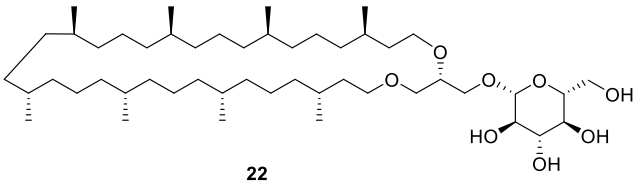

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Figure 5. *ß*-glucosyl analogue of **1**.

Results and discussion

Synthesis of 2,3-bis-O-(geranylgeranyl)-*sn*-glycero-1-phosphate

Retrosynthetic analysis **10** (Figure 6) suggests its preparation by phosphorylation of 2,3-bis-O-geranylgeranyl-*sn*-glycerol (**23**). **23** can be synthetized from a suitably protected glycerol derivative **24** and geranylgeranyl halide **25** via a Williamson ether synthesis. Another alternative would be ring-opening of protected enantiopure glycidol **26** (more details in chapter 3) with geranylgeraniol (**27**), followed by etherification of the formed secondary alcohol. Given that allyl halides are excellent partners in Williamson's reaction, this is the method of choice for the construction of the unsaturated derivatives. This strategy was already recognized by Morii, Nishihara and Koga.[22]In their synthesis, the authors used geranylgeranyl bromide and enantiopure benzylglycerol. The benzyl group was subsequently removed by Na/NH_3 (liq). Phosphorylation with dimethyl chlorophosphate in basic conditions and subsequent demethylation with TMSBr afforded **10** in <6% overall yield. The authors explained their low overall yield by instability of the intermediate

compounds. In an unrelated publication, Dannenmuller et al.[23] reported the synthesis and properties of archaeal membrane phospholipids analogues of **10**.

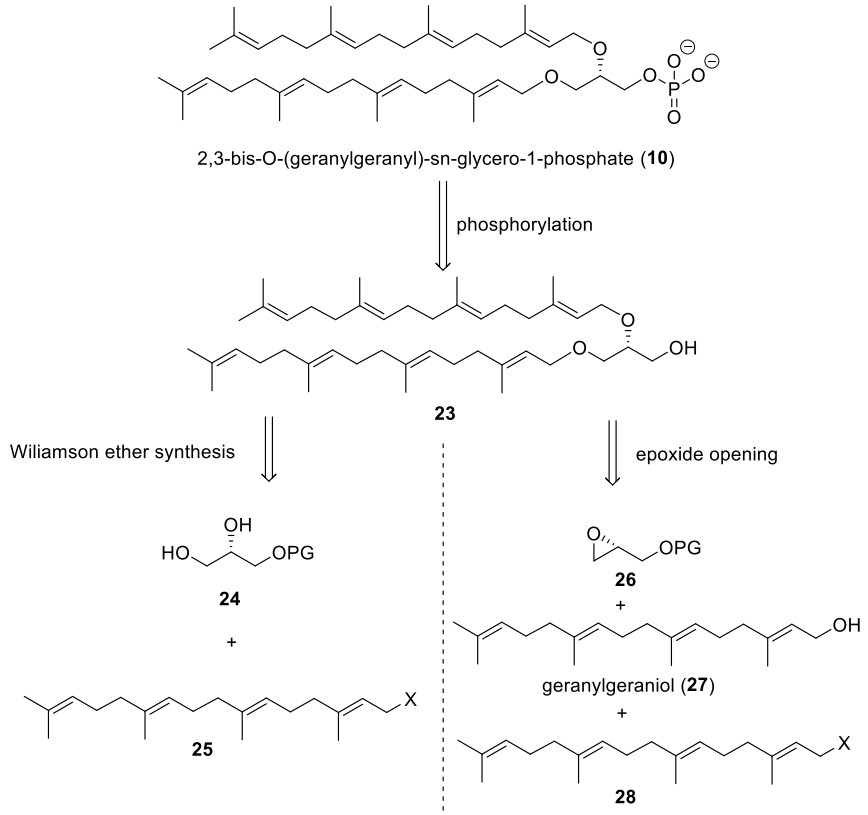
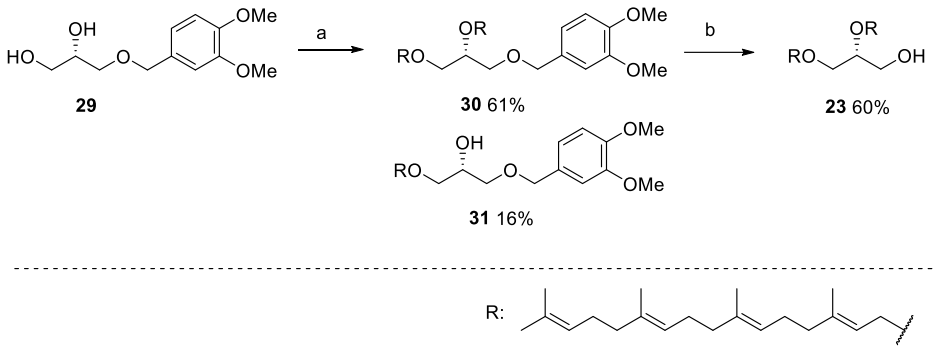


Figure 6. Retrosynthetic analysis of **10**.

The authors prepared bisgeranylgeranyl glycerol **23** via Wiliamson reaction of geranylgeranyl chloride and dimethoxybenzyl protected glycerol. Application of this protecting group is advantageous compared to the benzyl group because it can be removed using mild oxidative conditions. The reported conditions were applied to the synthesis with some minor modifications.

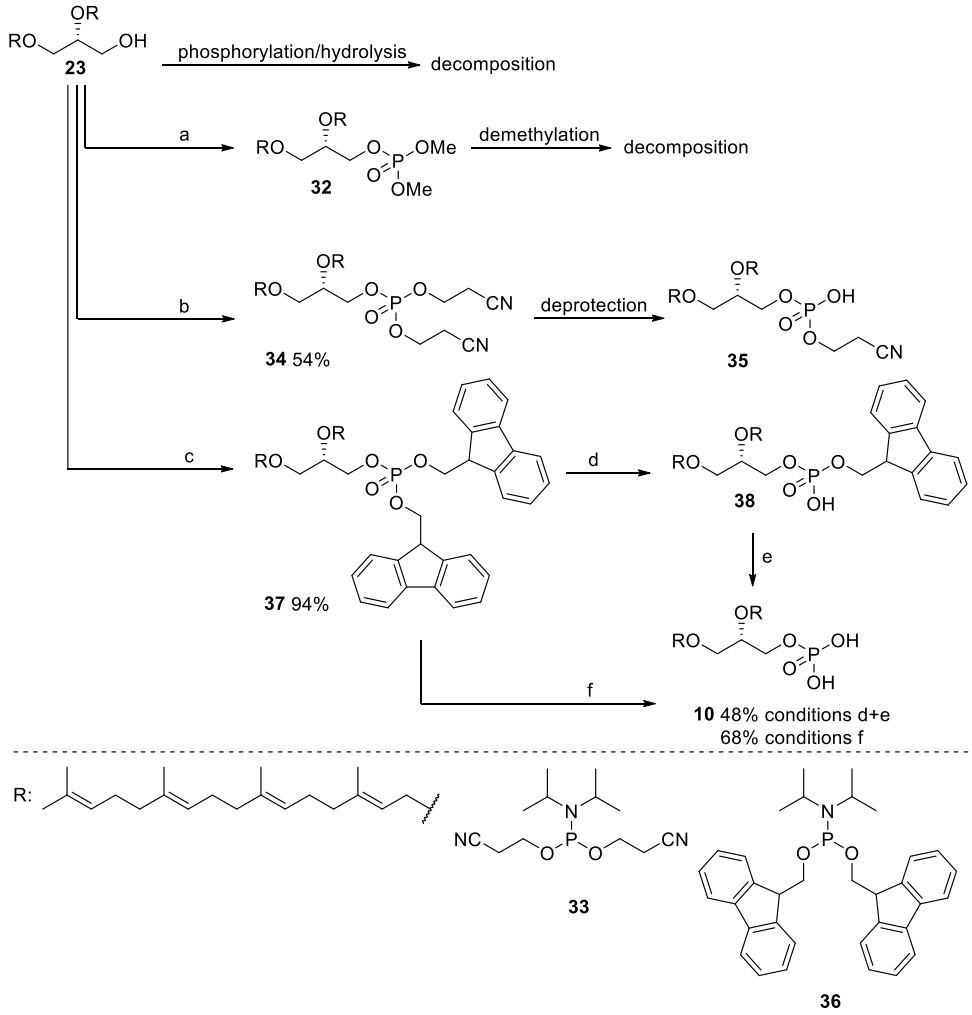
The glycerol derivative **29** was prepared in 2 steps from commercially available (*R*)-solketal.[24] The geranylgeranyl chloride as etherification partner was prepared by treatment of geranylgeraniol with N-chlorosuccinimide and Me_2S.[25] Reaction of both **29** and geranylgeranyl chloride (Scheme 1) in the presence of dimsyl sodium (sodium methylsulfinylmethylide) in DMSO afforded the desired diallylated **30** in 61% yield, together with monoallylated **31** in 16% yield. The yield of **30** is in perfect agreement to that reported by Dannenmuller et al.[23] (60%). Deprotection of **30** (Scheme 1) with DDQ in CH_2Cl_2/H_2O (40/1) afforded **23** in 60% isolated yield, again in a very good agreement with the literature (60%).[23]



Reagents and conditions: a) NaDMSO (2.1 equiv), DMSO, then geranylgeranyl chloride (2.0 equiv.), RT, 16 h; b) DDQ (2.0 equiv), CH_2Cl_2, H_2O, 0 °C

Scheme 1. Synthesis of bisgeranylgeranyl glycerol **13**.

The phosphorylation (Scheme 2) of **23** turned out to be a challenging step. Phosphorylation of **23** with $POCl_3$ (Scheme 2) and subsequent hydrolysis in the presence of $AgNO_3$[26] resulted only in decomposition of the starting material. The procedure reported by Morii, Nishihara and Koga[22] (Scheme 2) afforded dimethylphosphate **32**, but all attempted demethylations resulted in its decomposition. Phosphoramidites could be another viable option. First explored reagent **33** (scheme 2) underwent phosphoramidite coupling with **23** in the presence of tetrazole, and subsequent in situ oxidation with a solution of ^{t}BuOOH in decane afforded bisprotected **34** in 54% yield. However, all the explored deprotection methods of **34** resulted only in monodeprotected **35**. Next, phosphoramidite **36** was explored. The pKa value of the corresponding phosphate suggests a greater base-lability.[27] Synthesis of **36** was straightforward, but all attempts to purify the reagent resulted in its decomposition. Finally, a reaction of **23** with an excess of crude **36** in the presence of tetrazole and subsequent oxidation with ^{t}BuOOH, afforded bisprotected **37** in 94% yield. Reaction of **37** with excess Et_3N resulted again in monodeprotected **38**. Treatment of **38** with aqueous NaOH (1 M) resulted in the removal of the second fluorenylmethyl group, affording **10** in 48% yield. After further optimization, both protecting groups could be cleaved in 1 reaction. Stirring **37** in a 1 M aqueous NaOH in dioxane mixture followed by acidification and column chromatography on 130 Å Davisil silica gel afforded **10** in 68% yield. Overall, **10** was prepared in 4 steps and 23% overall yield, starting from **29**.



Reagents and conditions: a) $OP(OMe)_2Cl$, Et_3N CH_2Cl_2, 21 °C, 2h; b) **33**, tetrazole 4h, 21 °C then ^tBuOOH (2.0 equiv), -10 °C, 15 min; c) **36** (3.0 equiv), tetrazole (3.0 equiv), 24 h, 21 °C, then ^tBuOOH (4.0 equiv) -10 °C, 1 h; d) Et_3N (20 equiv), 21 °C, 18 h; e) 1 M aqueous NaOH; f) dioxane/1 M aqueous NaOH.

Scheme 2. Explored phosphorylations methods of **23**.

Identification of CDP-archaeol synthase

This paragraph summarizes the experiments of Dr. Samta Jain, and Dr. Antonella Caforio, from the department of Molecular Microbiology of the Rijksuniversiteit Groningen.

Based on the analogy between the biosynthesis of CDP-activated precursor **11** (Figure 3) in Archaea and Bacteria, bioinformatic analysis could identify a putative CDP-archaeol synthase in Archaea. The sequence of bacterial phosphatidate cytidylyltransferase[28] (CDP- diacylglycerol synthase) served as input for an NCBI-BLAST analysis. This resulted in a list of hypothetical proteins. Their sequences were aligned to an averaged hydropathy (hydrophobicity) profile. The alignment revealed common structural features of the hypothetical proteins – an extracellular N-terminus and 5 transmembrane helices. Although the bacterial enzymes are longer than the archaeal ones, the alignment of the family averaged hydropathy profile of the two showed a common pattern at the C-terminal region. Furthermore, analysis of the sequence of one of the protein loops revealed a consensus sequence between the archaeal and bacterial enzyme. This putative enzyme could be the CDP-archaeol synthase. The corresponding amino acid sequence was codon optimized for expression in *E. coli* and the C-terminus of the protein was equipped with an octahistidin tag. The enzyme was isolated after affinity chromatography. The predicted function of the enzyme was confirmed in two assays. Synthetic **10**, the natural substrate for the enzyme, was incubated in the presence of Mg^{2+} salts and cytidine triphosphate. LC/MS analysis of the reaction mixture confirmed the presence of CDP-archaeol **11**. When **10** was incubated with 2'-deoxycytidine 5'-triphosphate under identical conditions, LC/MS analysis confirmed the presence of deoxy-CDP-archaeol. Application of the other nucleosides did not lead to the corresponding products. In the second assay, **10** was incubated with a radiolabelled cytidine triphosphate ([5-T]CTP) under the same conditions. TLC analysis of the reaction mixture showed only a single radioactive spot.

With the identified enzyme CDP-archaeol synthase, the archaeal lipid biosynthesis could be reconstituted in vitro (Figure 7). After combining isopentenyl diphosphate (**3**), dihydroxyacetone phosphate (**7**), farnesyl diphosphate **38**, five enzymes catalyzing the steps of the biosynthesis and NADH, LC/MS analysis confirmed the presence of **11**.

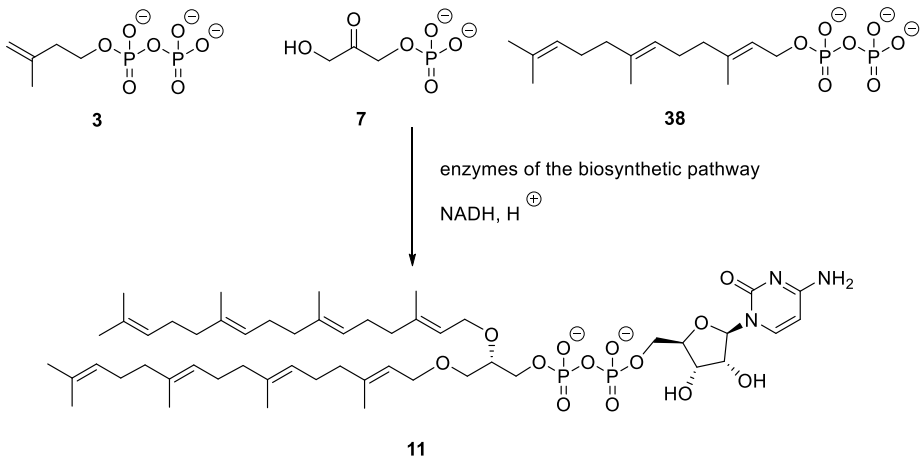


Figure 7. In vitro reconstitution of the biosynthesis of **11**.

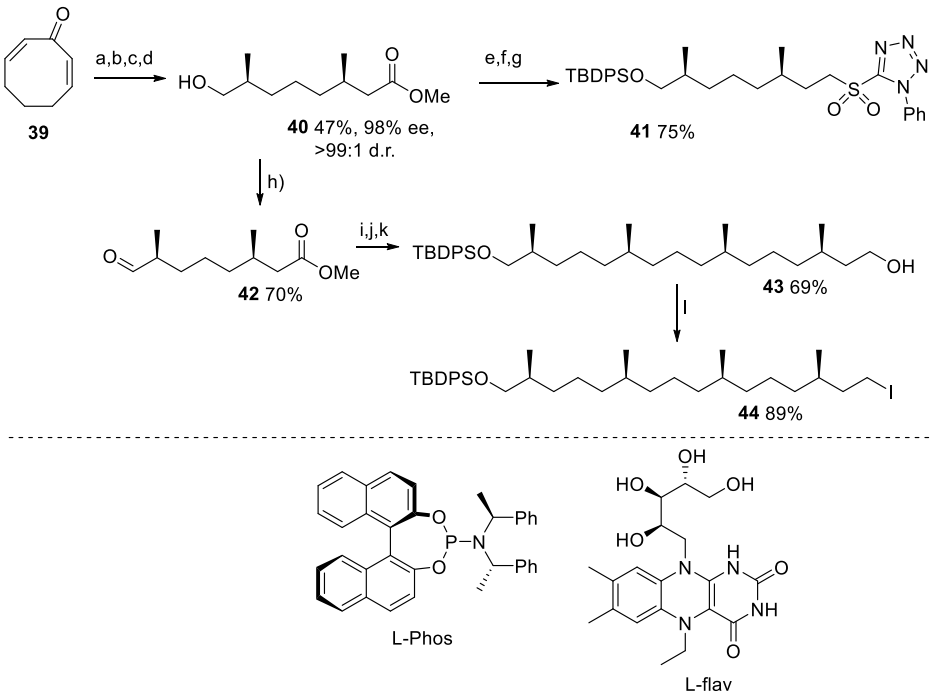
Catalytic alcoholysis of benzylglycidol as a key step in the synthesis of cyclo-archaeol and *β*-glucosyl-cyclo-archaeol

The following part of the chapter summarizes research performed together with Dr. Catalina Ferrer and Dr. Santiago Barroso.

Although bisgeranylgeranyl glycerol **10** and cycloarchaeol **1** (as their corresponding phosphates) are part of the same biosynthetic route, the synthetic challenges in **1** are considerably larger. Enzymatic reduction of the double bonds introduces 8 new stereogenic centers, making the synthesis of the hydrocarbon chain a challenge. A second, frequently underestimated hurdle is the construction of the ether bonds. While in the case of reactive, unsaturated allylic derivatives (as in the case of **10**) the Wiliamson synthesis is straightforward, in the case of the saturated alkylsulphonates or alkyl iodides, the competing elimination is a problem frequently resulting in low yields of the etherification. At least a partial solution can be alcoholysis of an enantiopure glycidyl ether catalysed by Jacobsen's catalyst.

A two-fold conjugate addition (Scheme 3) on cyclo-octadienone (**39**), followed by ozonolysis and esterification, afforded hydroxyl ester **40** with two methyl-branched stereogenic centres. One portion of **40** was converted in 3 steps to protected tetrazole **41**, the second portion of ester **40** was oxidized to aldehyde **42**. **41** and **42** were coupled in a Julia-Kocienski reaction. Hydrogenation using in situ generated diimide using the aforementioned flavine catalyst, afforded alcohol **43**. A part of **43** was converted to iodide **44**. The first ether bond was constructed (Scheme 4) by alcoholysis of (*R*)-benzylglycidol **45** with alcohol **43** using 8.0 mol% of the

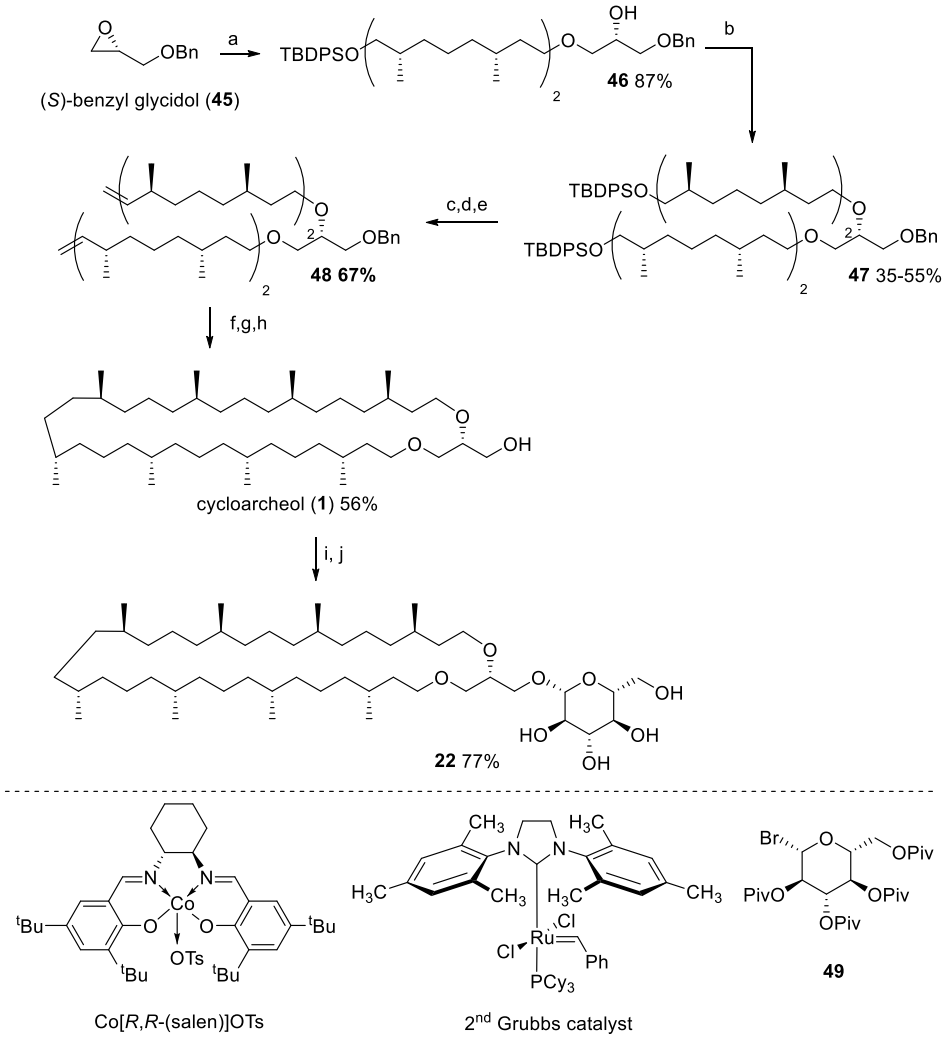
Co[*R*,*R*-(salen)]OTs, affording the desired ring-opened product **46** in 87% yield. The second ether bond was constructed via Williamson reaction. After testing a series of reaction conditions, best results were obtained when a mixture of **46** and the iodide **44** were treated with freshly ground KOH and catalytic $^{n}Bu_4NBr$ under solvent-free conditions. In a slow reaction, this provided the desired product **47** with yields varying from 35% to 55%. These values are in good agreement to the literature.[29]. The contrast between the two applied etherification methods is noteworthy. While the epoxide alcoholysis is a clean reaction with 87% yield, Wiliamson reaction affords the ether in significantly lower 35 to 55% yield, together with side products comming from the elimination.



Reagents and conditions: a) Me_2Zn (3.0 equiv), $Cu(OTf)_2$ (5.0 mol%), L-Phos (10 mol%), **39** added over 6 h, toluene, -25 °C, overnight; b) Me_2Zn (1.5 equiv), $Cu(OTf)_2$ (2.5 mol%), L-Phos (5.0 mol%), substrate added over 6 h, toluene, -25 °C, overnight then Et_3N (3.5 equiv), TMSCl (5.0 equiv), 2 h c) crude TMS enol ether dissolved in MeOH, CH_2Cl_2, O_3, -78 °C, then $NaBH_4$; d) p-toluenesulfonic acid (5.0 mol%), MeOH, reflux, 24 h; e; TBDPSCl (1.6 equiv), 1*H*-imidazole (2.0 equiv), DMF, rt, 16 h; f) DIBAL (5.0 equiv), THF, -78 °C, 2 h; g) 1-phenyl-1*H*-tetrazole-5-thiol (2.0 equiv), PPh_3 (1.5 equiv), DIAD (1.8 equiv), rt, overnight, then *m*CPBA (5.0 equiv), rt, overnight; h) TPAP (5.0 mol%), NMO (1.5 equiv), CH_2Cl_2, rt, overnight; i) LiHMDS (1.0 equiv), **41** (1.0 equiv), then **42** added, THF, -78 °C to rt, overnight; j) DIBAL (5.0 equiv), THF, -78 °C, 2 h; k) $NH_2NH_2.H_2O$ (20 equiv) added over 10 h, L-flav (2.0 equiv), EtOH, rt, 2 h; l) *N*,*N*,-dimethyl-*N*-(methansulfanylmethylene)ammonium iodide (1.5 equiv), 1*H*-imidazole (0.5 equiv), toluene, 85 °C, 16 h.

Scheme 3. Synthesis of methyl-branched precursors **43** and **44**.

However, the amounts of the building blocks were sufficient to complete the synthesis of **1** (Scheme 4). The deprotection, oxidation and a Wittig reaction sequence afforded bis-alkene **48**, which was cyclized by ring closing metathesis. The resulting double bond was reduced by hydrogenation over Pt/C catalyst because the flavin generated diimide did not result in a full conversion.



Reagents and conditions: a) **43** (0.55 equiv), Co[*R*,*R*-(salen)]OTs (4.5 mol%), O_2 (ballon), rt, 16 h, b) **44** (1.1 equiv), $^{n}Bu_4Br$ (0.5 equiv), KOH (2.7 equiv), 42 °C, 48 h; c) TBAF (4.0 equiv), THF, rt, overnight, d) Dess-Martin periodinane (2.5 equiv), CH_2Cl_2, rt, 1 h; e) Me_3PPh_3Br (4.5 equiv), KHMDS (4.2 equiv), THF, rt, 1 h; f) 2nd Grubbs catalyst (15 mol%), CH_2Cl_2 (0.002 M), reflux, 48 h; g) Pt/C (20 mol%), MeOH/CH_2Cl_2 (2/1), H_2 (ballon), rt, 16 h; Pd/C (Degussa type E101 NE/W, 25 mol%), H_2 (ballon), EtOAc, rt, 16h; i) **49** (3.5 equiv), AgOTf (3.5 equiv), tetramethylurea (4.5 equiv), toluene/CH_2Cl_2 (1/1), 0 °C; j NaOMe (30 equiv), MeOH, rt.

Scheme 4. Final steps of synthesis of **1** and **22**.

Final debenzylation afforded cycloarcheol **1**. The Koenigs–Knorr glycosylation followed by deprotection of the hydroxyl groups afforded the desired *ß*-glucosyl derivative **22**.

Detection of 1 and 22 in the deep sea samples

Both compounds were used to confirm their presence in hydrothermal vents. The analyzed sample was collected from the Rainbow hydrothermal vent (36°14'N) field located on the Mid-Atlantic Ridge. Samples were collected during a sampling campaign in 2008 using the remotely operated vehicle Jason. The sample is composed of material from the interior of a vent chimney collected at a depth of 2293 meters below the sea level. Analysis by GC-MS (in the case of **1**) and HPLC/ESI/MS (in the case of **22**) showed that synthetic and natural compounds co-eluted and that their mass spectra were identical. This suggest the presence of methanogenic *Archaea* in the Rainbow.

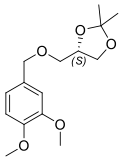
Conclusion

The chemical synthesis of unsaturated archaeatidic acid has been important in the identification of CDP-archaeol synthase, one of the missing links in the biosynthesis of archaeal membrane lipids. The synthetically challenging step was the phosphorylation of bisgeranylgeranyl-glycerol. This was achieved by the application of bisfluorenylmethyl substituted phosphoramidite, in situ oxidation, and subsequent deprotection under basic conditions.

In the second part of this chapter, a key step in the synthesis of cyclo-archaeol is described. As in chapter 3, the catalytic regioselective ring opening of a protected glycidol is successfully applied as an alternative for a Williamson ether synthesis with a glycerol derivative. A versatile method for the subsequent alkylation of the secondary hydroxyl group is still lacking, but the currently applied procedure is acceptable. The synthesis of cycloarchaeol and *ß*-glucosyl cycloarchaeol allowed to unambiguously establish their presence in a sample taken from a hydrothermal vent field.

Experimental part

(*S*)-4-(((3,4-dimethoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolane



A dry three-necked round-bottom flask equipped with a reflux condenser was charged with (*R*)-1,2-isopropylidene glycerol (744 mg, 4.0 mmol) and $^{n}Bu_4I$tetra-n-butylammonium iodide (147 mg, 0.4 mmol, 10 mol%,). Solids were degassed in three cycles before dry THF (12 ml) was added. To the obtained solution, KH (50% in paraffin, 370 mg, 4.6 mmol, 1.15 equiv) was added in small portions. The mixture was stirred for 10 min before 4-(chloromethyl)-1,2-dimethoxybenzene[30] (860 mg, 4.6 mmol, 1.15 equiv) was added in one portion. The so-obtained reaction mixture was immersed into a preheated oil bath (87 °C) and refluxed for 16 h. After removal from the oil bath and cooling down to rt, solid NH_4Cl (1 g) was added. The mixture was stirred for 15 min, filtered and the collected filtrate was evaporated to dryness. The yellow liquid residue was further purified by flash chromatography using 50% Et_2O in pentane. Fractions with an R_f = 0.37 (50% Et_2O in pentane) were collected and concentrated to afford 1.04 g of the desired compound as colourless thick liquid (92%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.82 (m, 3H), 4.49 (m, 2H), 4.27 (m, 1H), 4.03 (dd, *J* = 8.2, 6.5 Hz, 1H), 3.71 (dd, *J* = 8.2, 6.4 Hz, 1H), 3.51 (dd, *J* = 9.8, 5.8 Hz, 1H), 3.44 (dt, *J* = 12.3, 4.7 Hz, 1H), 1.40 (s, 3H), 1.34 (s, 3H).

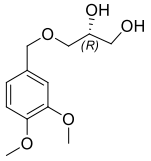
^{13}C NMR (100 MHz, $CDCl_3$, δ): 149.20, 148.83, 130.66 (-), 120.51, 111.21, 111.02 (-), 109.54 (-), 74.93 (-), 73.56, 70.96, 67.00, 56.06 (-), 55.99 (-), 26.95 (-), 25.54 (-).

α^{D} =+15.9 (c = 0.067, $CHCl_3$).

Anal. Calcd for $C_{15}H_{22}O_5$: C, 63.81; H, 7.85. Found: C, 63.51; H, 7.88%.

The spectroscopic data correspond to previously published[24]

(*R*)-3-((3,4-dimethoxybenzyl)oxy)propane-1,2-diol



The corresponding acetonide (950 mg, 3.4 mmol) was dissolved in CH_2Cl_2/MeOH (10 ml/10 ml). Amberlite IR120 (acid form, 100 mg) was added, and the mixture was stirred at RT until full conversion (36 h). The catalyst was filtered off and the filtrate was concentrated in vacuo.
The reaction afforded 815 mg of desired product (>99%) as colourless very thick liquid.

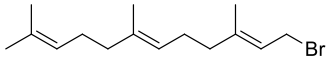
^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.85 (m, 3H), 4.47 (s, 2H), 3.87 (m, 7H), 3.69 (dd, *J* = 11.4, 3.9 Hz, 1H), 3.61 (dd, *J* = 11.4, 5.5 Hz, 1H), 3.57 – 3.48 (m, 2H), 2.31 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 149.02, 148.76, 130.18, 120.47, 111.16 (-), 110.94 (−), 73.45, 71.44, 70.71 (-), 64.02, 55.89 (-), 55.86 (-).

The spectral data corresponds to previously reported.[24]

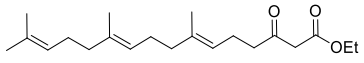
Geranylgeraniol prepared following the literature procedure

Farnesyl bromide



A dry flask was charged with farnesol (2.0 g, 8.9 mmol). Dry THF (30 ml) was added, and resulting solution was immersed into a -47 °C bath (ethanol, cryostat). After stirring for 10 min, freshly distilled MsCl (900 µl, 12 mmol, 1.3 equiv) was added via syringe over 5 min. Subsequently, Et_3N (2.5 ml, 18 mmol, 2.0 equiv) was added over another 5 min. After complete addition, the mixture was stirred for 45 min at –47 °C. To the resulting suspension, a solution of LiBr (3.0 g, 36 mmol, 4.0 equiv) in dry THF (10 ml) was added dropwise over 5 min. After complete addition, the reaction vessel was transferred to a 0 °C bath (ice/water) and stirred for 1 h. The reaction mixture was poured into chilled saturated $NaHCO_3$ solution. The organic layer was separated, the aqueous layer was extracted with cold Et_2O (a mixture of Et_2O with pieces of ice, 3 x 25 ml), the combined organic layers were washed with cold water, brine, dried over $MgSO_4$ and evaporated. The crude farnesyl bromide was obtained as a yellow liquid and used without further manipulation.
The reaction afforded 1.91 g of the desired product as a light yellow oil (75% yield) which was stored at -80 °C, and used within 1 day.

(6*E*,10*E*)-ethyl 7,11,15-trimethyl-3-oxohexadeca-6,10,14-trienoate



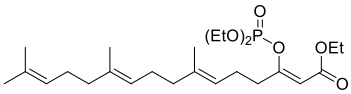
A dry Schlenk flask was charged with NaH (60% dispersion, 880 mg, 22 mmol, 3.3 equiv). The mineral oil was removed by three washings with pentane (3 x 10 ml). The resulting white solid was dried in vacuum. Dry THF (16 ml) was added and the resulting white suspension was cooled to 0 °C (ice/water bath). To this suspension, freshly distilled ethyl acetoacetate (2.6 ml, 3.0 equiv) was added dropwise over 5 min. After complete addition the suspension turned into a light yellow solution. To this solution was added a solution of ^{n}BuLi in hexanes (2.5 M, 8.3 ml, 3.1 equiv) over 15 min. The resulting orange solution was stirred for additional 15 min at 0 °C before a solution

of farnesyl bromide (from the previous experiment 1.91 g, 6.7 mmol) in dry THF (3.5 ml) was added dropwise over 5 min. The resulting solution was stirred at 0 °C for 15 min, during which formation of a precipitate was observed. The reaction was quenched by careful addition of chilled aqueous HCl (1 M, 10 ml, **EXOTHERMIC**). The mixture was transferred into a separatory funnel, where the organic layer was separated, the aqueous layer was extracted with Et_2O (3 x 10 ml), the combined organic layers were washed with brine, dried and evaporated.
The title compound was obtained after column chromatography using 10% Et_2O in pentane as 1.67 g of a light yellow liquid (55% starting from farnesol, lit 70%).

^{1}H NMR (400 MHz, CDCl3) δ 12.09 (s, 0.2H), 5.08 (d, *J* = 6.5 Hz, 3H), 4.19 (dt, *J* = 7.2, 5.3 Hz, 2H), 3.42 (s, 2H), 2.56 (t, *J* = 7.4 Hz, 2H), 2.38 – 2.17 (m, 3H), 2.05 - 1.98 (m, 8H), 1.68 (s, 3H), 1.64 – 1.58 (m, 9H), 1.28 (t, *J* = 7.1 Hz, 3H).

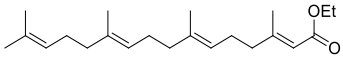
The spectral data corresponds to previously reported.[31]

Ethyl (2Z,6E,10E)-3-((diethoxyphosphoryl)oxy)-7,11,15-trimethylhexadeca-2,6,10,14-tetraenoate



A dry Schlenk flask was charged with NaH (60% dispersion, 228 mg, 1.15 equiv). The mineral oil was removed by three successive washings with hexane (3 x 5 ml) and the resulting white solid was suspended in dry Et_2O (21 ml). The suspension was immersed in an ice/water bath of 0 °C and a solution of (6*E*,10*E*)-ethyl 7,11,15-trimethyl-3-oxohexadeca-6,10,14-trienoate (1.65 g, 5.0 mmol) was added as a solution in dry Et_2O (7 ml) over 15 min. After the addition was complete, the resulting light yellow solution was stirred for 15 min at 0 °C and for 15 min at 21 RT. Then the solution was again cooled in the ice bath and neat $(EtO)_2P(O)Cl$ (1.1 ml, 7.5 mmol, 1.5 equiv) was added dropwise. The resulting reaction mixture was stirred for 15 min at 0 °C and subsequently quenched by addition of saturated aqueous NH_4Cl solution (15 ml). The organic layer was separated, and the aqueous layer was extracted with Et_2O (3 x 15 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (3 x 15 ml), brine (2 x 15 ml), dried over $MgSO_4$ and the solvent was removed in vacuo.
The resulting crude (2.08 g) was obtained as a yellow liquid and used without further purification in the following step.

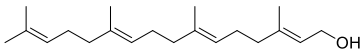
ethyl (2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenoate



A dry Schlenk flask was charged with CuI (1.7 g, 8.9 mmol, 1.8 equiv) which was suspended in dry Et_2O (5.5 ml) and cooled to 0 °C. The resulting suspension was treated with MeLi (1.6 M in Et_2O, 11.0 ml, 18 mmol, 3.6 equiv). The suspension turned yellow after initial addition of MeLi, after complete addition the CuI fully dissolved affording a nearly colourless solution.

The reaction vessel with Me_2CuLi was immersed in a cryostat at –78°C[32] and a solution of the phosphate (2.08 g, ca 5.0 mmol) in Et_2O (dry, 7 ml) was added dropwise via the cold wall of the Schlenk flask (in order to cool the solution of the phosphate). After complete addition, the colour changed to orange/red and the resulting solution was stirred at –78 °C. After 1 h the bath was allowed to warm to –47 °C and the reaction mixture was stirred at –47 °C for 2 h. After this time TLC showed full conversion of the phosphate and a new spot had appeared on TLC. MeI (630 µl) was added to quench the unreacted cuprate. After stirring for 10 min, the reaction mixture was carefully poured into a solution of NH_4Cl (24 ml) and NH_4OH (6 ml) (**can be exothermic with gas evolution**). The mixture was stirred until all solids dissolved. Layers were separated, the aqueous layer was extracted with Et_2O (3 x 20 ml) and the combined organic layers were washed with NH_4OH (10%, 2 x 40 ml), brine (2 x 40 ml), dried and evaporated.
The reaction afforded 1.15 g of a yellow liquid which was used without further purification.

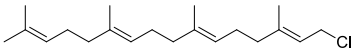
Geranylgeraniol



The ethyl ester from the previous step (1.15 g, 4.5 mmol) was dissolved in toluene (p.a. grade, 17 ml). This solution was cooled to –78 °C (N_2/acetone bath) and a solution of DIBAL (1M in hexane, 14.0 ml, 14 mmol, 3.0 equiv) in hexane (15 ml) was added dropwise. The mixture was stirred until complete consumption of the starting material (TLC). The reaction was quenched by careful addition of MeOH (3.0 ml, added over 10 min, **EXOTHERMIC, gas evolution**). When gas evolution ceased, the mixture was removed from the bath and stirred for 10 min at rt. The reaction mixture was poured into saturated NH_4Cl (50 ml)/HCl (50 ml) solution and stirred until clear separation of the layers took place (ca 30 min). The aqueous layer was extracted with Et_2O (3 x 50 ml). The combined organic layers were washed with water (2 x 50 ml) and brine (2 x 50 ml), dried over $MgSO_4$ and evaporated. The residual thick liquid was further purified by flash chromatography on silica using 30% Et_2O in pentane as the eluent. The reaction afforded 857 mg of geranylgeraniol (60% over three steps) with >99% double bond isomer purity according to GC analysis.

The spectral data corresponds to previously reported[31]

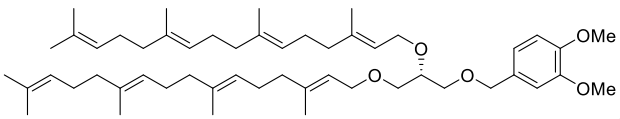
Geranylgeranyl chloride



N-chlorosuccinimide (1.2 g, 9.0 mmol, 1.3 equiv) was suspended in dry CH_2Cl_2 (15 ml). The suspension was cooled to –30 °C (acetone/liquid N_2 bath) and after stirring for 5 min, dimethyl sulfide (750 µl, 10 mmol, 1.5 equiv) was added. The reaction was stirred for 10 min at –30 °C and 10 min at 0 °C. Then the solution was cooled to –40 °C. A solution of geranylgeraniol (2.0 g, 6.9 mmol) in CH_2Cl_2 (dry, 5 ml) was added dropwise. The resulting suspension was allowed to warm during 150 min to 0 °C, turning into a cloudy solution at –15 °C. The reaction mixture was poured into pentane (150 ml), The organic layer was washed with water (2 x 50 ml), brine (50 ml), dried over $MgSO_4$ and evaporated.
The reaction afforded 1.94 g of geranylgeranyl chloride as a colourless liquid which was used without further purification.

Synthesis of 2,3-bisgeranylgeranyl-*sn*-glycerol (**23**), (scheme 1)

1-((3,4-dimethoxybenzyl)oxy)- 2,3-bisgeranylgeranyl-*sn*-glycerol (**30**)



A dry Schlenk flask was charged with NaH (60% dispersion in mineral oil, 265 mg, 6.6 mmol, 2.1 equiv). The mineral oil was removed by washing with pentane (3 x 5 ml) and the white solid was dried in high vacuum before suspending in DMSO (5 ml). The obtained suspension was immersed in a preheated oil bath (70 °C) and stirred for 40 min during which the suspension turned into a pale yellow solution. The flask was removed from the bath and allowed to cool to rt (21 °C). To this solution, a solution of (*R*)-3-((3,4-dimethoxybenzyl)oxy)propane-1,2-diol (595 mg, 3.2 mmol) in dry DMSO (5 ml) was added carefully. After the complete addition, the reaction mixture was stirred for 1 h at rt (21 °C). Then a mixture of geranylgeranyl chloride (1.94 g from the previous experiment, ca 6.3 mmol) in a small amount of DMSO (2 ml) was added. The resulting solution was stirred for 16 h before pouring into saturated aqueous NH_4Cl solution (20 ml). The aqueous layer was extracted with Et_2O (3 x 50 ml). The combined organic layers were washed with brine, dried and evaporated. The crude residue was further chromatographed using 30% Et_2O in pentane to afford the desired product and the product of the mono-alkylation.

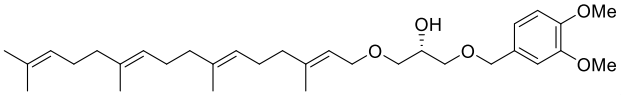
Dialkylated product 1.52 g (61%) as colourless liquid

^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.97 – 6.75 (m, 3H), 5.35 (dt, *J* = 13.5, 6.6 Hz, 2H), 5.10 (d, *J* = 5.8 Hz, 6H), 4.49 (s, 2H), 4.16 (d, *J* = 6.7 Hz, 2H), 4.01 (d, *J* = 6.7 Hz, 2H), 3.85 (s, 3H), 3.89 (s, 3H), 3.68 (dt, *J* = 10.0, 5.1 Hz, 1H), 3.62 – 3.46 (m, 5H), 2.16 – 1.90 (m, 23H), 1.68 (s, 6H), 1.65 (s, 26), 1.59 (s, 16H)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 149.06, 148.60, 140.20, 139.95, 135.43, 135.40, 135.06, 131.39, 131.10, 124.51 (-), 124.32 (-), 124.31 (-), 124.03 (-), 123.99 (-), 121.33 (-), 120.99 (-), 120.31 (-), 111.07 (-), 110.91 (-), 77.03(-), 73.40, 70.35, 70.19, 68.02, 66.95, 56.03 (-), 55.92 (-), 39.86, 39.84, 39.78, 26.89, 26.78, 26.53, 26.50, 25.85(-), 17.83 (-), 16.69(-) , 16.66 (-), 16.15 (-).

α^{D} =+5.2 (c = 1.0, $CHCl_3$)

NMR data correspond to those previously published[23].



Monoalkylated product 256 mg (16%) as colourless liquid.

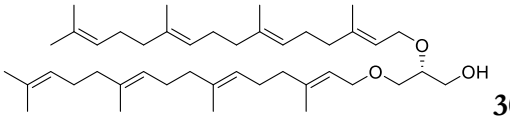
^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.84 (m, 3H), 5.34 (d, *J* = 7.1 Hz, 2H), 5.10 (s, 3H), 4.48 (d, *J* = 5.0 Hz, 2H), 4.01 (m, 3H), 3.85 (s, 3H), 3.89 (s, 3H), 3.76 – 3.40 (m, 5H), 2.05 (m, 12H), 1.66 (d, *J* = 8.3 Hz, 6H), 1.59 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 149.11, 148.76, 148.73, 140.72, 140.67, 135.49, 135.45, 135.05, 131.36, 130.62, 124.47 (-), 124.26 (-), 123.88 (-), 123.85 (-), 120.79 (-), 120.59 (-), 120.48 (-), 120.38 (-), 111.16 (-), 111.02 (-), 110.95 (-), 110.93 (-), 77.61 (-), 73.53, 73.48, 71.34, 71.22, 69.93, 69.70 (-), 67.94, 66.67, 63.01, 56.01 (-), 55.93 (-), 55.92 (-), 39.83, 39.80, 39.72, 26.86, 26.72, 26.43, 25.82 (-), 17.80 (-), 16.66 (-), 16.63 (-), 16.13 (-) 16.12 (-).

α^{D} =+9.2 (c = 1.0, $CHCl_3$).

HRMS-APCI (*m/z*): $[M + Na]^+$ calculated for $C_{32}H_{50}O_5Na$, 538.354; found, 538.355.

2,3-bisgeranylgeranyl-*sn*-glycerol (**23**)



30 (1.44 g, 1.8 mmol) was dissolved in CH_2Cl_2 (30 ml). To this solution, H_2O (0.75 ml) was added. The obtained biphasic solution was cooled in an ice bath (0 °C) and DDQ (830 mg, 3.6 mmol, 2.0 equiv) was added. The reaction was stirred for 4 h at 0 °C until TLC showed full conversion of the starting material. The crude reaction mixture was filtered over a small silica pad and washed with CH_2Cl_2 (200 ml). The washings were combined and evaporated. The obtained yellow liquid was further purified by column chromatography (30% Et_2O in pentane).
The reaction afforded 705 mg of the desired product (60%) as yellow thick liquid containing traces of unidentified co-eluting impurities.

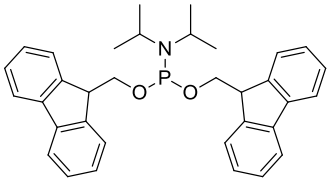
^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.35 (dt, *J* = 13.5, 6.7 Hz, 2H), 5.11 (t, *J* = 6.5 Hz, 6H), 4.26 – 4.06 (m, 2H), 4.02 (d, *J* = 6.7 Hz, 2H), 3.77 – 3.41 (m, 5H), 2.16 – 1.93 (m, 24H), 1.68 (s, 12H), 1.60 (s, 21H)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 140.68, 135.49, 135.47, 135.07, 131.37, 124.52 (-), 124.32 (-), 123.9 (-)3, 120.91 (-), 120.69 (-), 77.61 (-), 70.16, 68.08, 66.66, 63.21, 39.88, 39.86, 39.83, 39.75, 26.90, 26.77, 26.50, 26.46, 25.83 (-), 17.82 (-), 16.68 (-), 16.65 (-), 16.15 (-), 16.14 (-).

HRMS-APCI+ (*m*/*z*): $[M + Na]^+$ calculated for $C_{43}H_{72}O_3Na$, 659.536; found, 659.537.

Synthesis of 2,3-bis-O-(geranylgeranyl)-*sn*-glycero-phosphate (**10**) (Scheme 2)

bis((9H-fluoren-9-yl)methyl) diisopropylphosphoramidite



PCl_3 (7 ml, 80 mmol) was dissolved in pentane (700 ml). Via an addition funnel, a solution of diisopropylamine (distilled from CaH_2, 23 ml, 0.16 mol, 2.0 equiv) in pentane (100 ml) was added dropwise over 15 min. After this time, a significant amount of white precipitate had formed. The suspension was stirred for 2 h. The mixture was transferred to a separatory funnel. The pentane layer was washed with acetonitrile (the pentane layer stays on top, 5 x 100 ml of acetonitrile, after the washing the pentane layer was fully transparent). Pentane was subsequently evaporated. The reaction afforded 1,1-dichloro-N,N-diisopropylphosphinamine (6 g, 38%) as colourless liquid

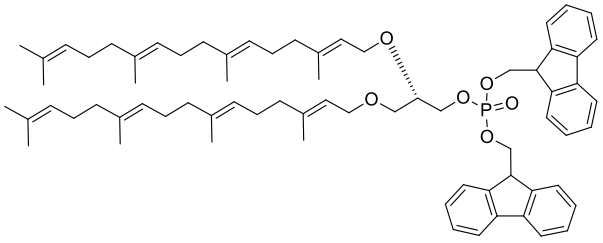
^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.02 – 3.83 (m, 1H), 1.28 (d, *J* = 6.8 Hz, 6H),

^{31}P NMR (400 MHz, $CDCl_3$, δ): 169.59.

From the obtained liquid, an aliquot was taken (2.0 g, 10 mmol). This was dissolved in dry THF (40 ml) and DIPEA (3.5 ml, 20 mmol, 2.0 equiv) was added. The solution was cooled in an ice bath and (9H-fluoren-9-yl)methanol (3.92 g, 20 mmol, 2.0 equiv) in dry THF (10 ml) was added. The solution was stirred for 10 h during which a white precipitate formed. The reaction was poured into aqueous phosphate buffer (1 M, pH = 7) and extracted with ethyl acetate (4 x 50 ml). The combined organic extracts were washed with the same phosphate buffer, brine, dried and evaporated. The crude product was used without further purification due to its sensitivity.

^{31}P NMR (162 MHz, $CDCl_3$) δ 148.01.

bis((9H-fluoren-9-yl)methyl) ((*S*)-2,3-bisgeranylgeranyl)oxy)propyl) phosphate **37**



(*R*)-2,3-bisgeranylgeranyl glycerol (66.0 mg, 0.1 mmol)was dissolved in CH_2Cl_2/CH_3CN (0.5 ml/0.5 ml) and **36** (154 mg, 0.3 mmol, 3.0 equiv) was added. The mixture was cooled to 0 °C and tetrazole (21 mg, 0.3 mmol, 3.0 equiv) was added. The mixture was allowed to gradually warm to rt (21 °C) and stirred overnight. When full conversion of starting material was observed (TLC), the mixture was cooled and a solution of ^{t}BuOOH (5 M in decane, 64µl, 0.3 mmol, 3.2 equiv) was added in one portion followed by stirring for 45 min. The mixture was subsequently poured into aqueous phosphate buffer (1 M, pH = 7) and extracted with Et_2O (4 x 20 ml). The combined extracts were washed with brine, dried and concentrated. The crude residue was purified by flash chromatography using (50% Et_2O in pentane). Fractions with an Rf = 0.4 (50% Et_2O in pentane) were collected to afford 102 mg (94%) of the desired compound.

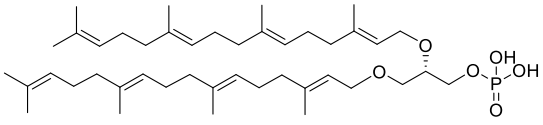
^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.71 (dd, *J* = 12.2, 4.3 Hz, 4H), 7.56 (m, 4H), 7.38 (m, 4H), 7.22 (m, 4H), 5.27 (q, *J* = 6.8 Hz, 2H), 5.09 (m, 6H), 4.28 (m, 4H), 4.14 (m, 3H), 3.97 (m, 5H), 3.59 (dd, *J* = 9.7, 4.9 Hz, 1H), 3.54 (m, 2H), 2.03 (m, 24H), 1.68 (s, 6H), 1.59 (dd, *J* = 11.2, 6.0 Hz, 24H)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 147.46, 147.40, 145.60, 144.69, 144.56, 139.59, 139.57, 139.21, 135.52 (-), 132.10 (-), 131.36 (-), 129.48 (-), 129.43 (-), 128.66

(-), 128.47 (-), 128.09 (-), 124.89 (-), 124.86 (-), 124.25 (-), 124.21 (-), 80.44 (-), 80.36 (-), 73.59, 73.53, 73.07, 72.22, 71.45, 71.40, 71.07, 52.24 (-), 52.16 (-), 44.00, 43.98, 43.87, 31.04, 30.93, 30.70, 30.65, 29.98 (-), 21.97 (-), 20.78 (-), 20.29 (-).

HRMS-APCI (*m/z*): $[M + Na]^+$ calculated for $C_{71}H_{93}O_6PNa$, 1095.660; found 1095.660.

2,3-bis-O-(geranylgeranyl)-*sn*-glycero-phosphate (**10**)



Bisprotected phosphoric ester **37** (102 mg, 0.10 mmol) was dissolved in acetonitrile (5.0 ml). To this solution, Et_3N (280 µmol, 2.0 mmol, 20 equiv) was added and the resulting mixture was stirred overnight. All volatiles were evaporated and the crude mono deprotected phosphoric ester (as assumed from the TLC) was suspended in aqueous NaOH (1 M, 5.0 ml) until full conversion of the monoprotected ester was observed (TLC, 3 h). The mixture was acidified with HCl (1 M) to pH = 1 and extracted with Et_2O (3 x 20 ml). The combined extracts were dried over $MgSO_4$ and concentrated. The crude residue was purified on a silica column using a carefully established gradient of 2% MeOH/$CHCl_3$ → 33% MeOH/$CHCl_3$.
Reaction afforded 34.3 mg (48%) of the desired compound as a colourless liquid.

One pot procedure for the deprotection:

Bisprotected phosphoric ester (102 mg, 0.10 mmol) was dissolved in dioxane (1 ml). To the stirred solution aqueous NaOH soulution (1 M, 1 ml) was added. The mixture was stirred until full conversion of the starting material (3 h). The mixture was acidified with HCl (1 M) to pH = 1 and extracted with Et_2O (3 x 20 ml). The combined extracts were dried over $MgSO_4$ and concentrated. The crude residue was purified on a 120 Å Davisil silica column using a carefully established gradient of 2% MeOH/$CHCl_3$ → 33% MeOH/$CHCl_3$.
The reaction afforded 48.6 mg (68%) of the desired compound as a colourless oil
NOTE: The final product was stored in the freezer (–20 °C).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.32 (m 2H), 5.09 (m, 6H), 4.15 (m, 2H), 4.00 (m, 4H), 3.57 (m, 3H), 2.01 (m, 24H), 1.77 – 1.49 (m, 30H).

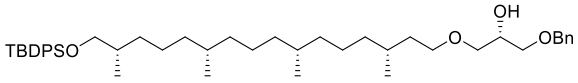
^{13}C NMR (100 MHz, $CDCl_3$, δ): (101 MHz, $CDCl_3$, the spectrum shows a considerable number of overlapping signals) δ 135.23, 134.85, 131.16, 124.38, 124.22, 123.88, 120.71, 39.75, 39.72, 26.76, 26.61, 25.67, 17.66, 16.53, 16.00, 15.97.

^{31}P NMR (162 MHz, $CDCl_3$) δ 1.37.

HRMS-ESI+ (*m*/*z*): $[M + H]^+$ calculated for $C_{43}H_{72}O_6P$, 715.507; found, 715.506.

Total synthesis of cycloarcheol **1** (Scheme 4)

(4*S*,9*R*,13*R*,17*S*,21*S*)-9,13,17,21,25,25-hexamethyl-1,24,24-triphenyl-2,6,23-trioxa-24-silahexacosan-4-ol (**46**)



To a roundbottom flask containing neat **13** (488 mg, 0.9 mmol) was added neat benzyl-(S)-glycidol (250 µl, 1.6 mmol, 1.65 equiv) and Co[*R*,*R*-(salen)]OTs (35 mg, 8.0 mol%). An atmosphere of dry oxygen was applied (balloon, 1 bar). The mixture was stirred for 16 h and then purified by silica gel chromatography using 20% Et_2O in hexane. The reaction afforded 533 mg of the desired product as colourless liquid (85%)

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.69 – 7.65 (m, 4H), 7.45 – 7.27 (m, 11H), 4.57 (s, 2H), 3.98 (br s, 1H), 3.59 – 3.40 (m, 8H), 2.47 (br d, *J* = 3.1 Hz, 1H), 1.70 – 1.47 (m, 4H), 1.46 – 1.14 (m, 20H), 1.06 (s, 9H), 0.92 (d, *J* = 6.7 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 5.9 Hz, 3H), 0.83 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 138.0, 135.6, 134.1, 129.4, 128.4, 127.7, 127.7, 127.5, 73.4, 71.8, 71.4, 70.0, 69.5, 68.9, 37.5, 37.4, 37.4, 36.6, 35.7, 33.5, 32.8, 32.8, 29.9, 26.9, 24.5, 24.4, 24.4, 19.8, 19.7, 19.3, 17.0.

HRMS-APCI (*m*/*z*): $[M + Na]^+$ calculated for $C_{46}H_{72}O_4SiNa$, 739.509; found: 739.509.

$[\alpha]_D$ -0.3 (c = 1.2, $CHCl_3$).

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