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LETTERS

The synthesis of archaebacterial lipid analogues

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Abstract

An efficient and convenient route to two novel quasimacrocyclic archaebacterial lipid analogues is presented. The target compounds **2** and **3** are prepared in seven and four steps, respectively, from known starting materials, and are useful for the study of synthetic tethered bilayer membranes.

Keywords: lipids; membranes; membrane spanning lipids; archaebacteria; biosensors.

We recently reported a novel, generic biosensor based on an ion channel switch embedded in a synthetic lipid membrane tethered onto a planar gold electrode.[1] In order for the ion channel switch to function in a real environment such as whole blood or plasma, we required a stable, fluid bilayer membrane. The formation of such a membrane was achieved through the use of synthetic lipids based on the structural motifs found in archaebacterial lipids. Microorganisms belonging to the Archaea domain typically thrive in environments usually considered too hostile for conventional life such as areas of high temperature, high salt content and/or pH extremes.[2] The lipid components of membranes of the archaebacteria are essential for this extraordinary stability, and as such there have been numerous studies concerning the isolation, structural elucidation and synthesis of these lipidic compounds.[3] Additionally, these lipids are of interest for applications such as drug delivery, separations and photochemical energy conversion.[4]

Typically, archaebacterial lipids consist of a branched hydrocarbon (such as the isoprenoid phytanol) connected to a glycerol backbone via an ether linkage, in contrast to the lipids of eubacteria and eukaryoates which possess ester-linked straight chain fatty acids. Furthermore, archaebacterial membranes often contain macrocyclic lipids with ring sizes up to 72 atoms, such as the tetraether **1** obtained from the archaebacterium *Sulfolobus solfataricus.*[5]

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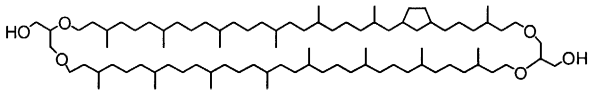

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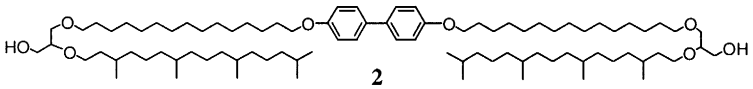

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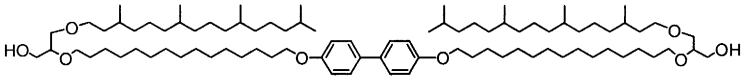
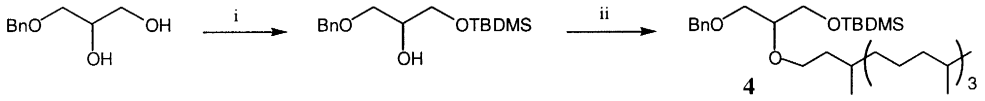


3

In this paper we report efficient synthetic routes for two novel quasimacrocyclic lipidic species (**2** and **3**), that have been used in the formation of tethered lipid membranes.[1]

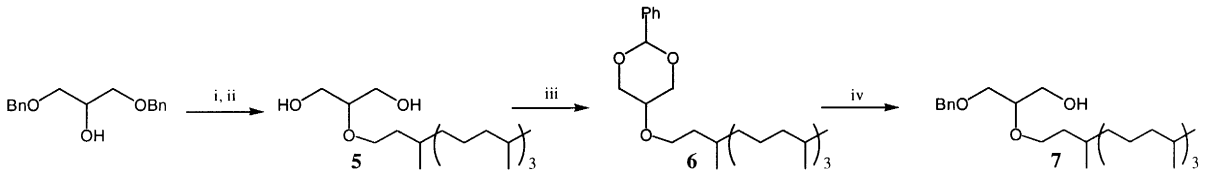
The compounds designed in this work (**2** and **3**) were modelled closely on macrocyclic archaebacterial lipids in that they contained phytanyl groups ether linked to glycerol moieties. The glycerol units were linked together by non-branched alkyl chains connected *via* a central biphenyl group, allowing the entire construct to span a bilayer membrane (~38 Å), whilst the phytanyl groups were required in order to ensure that the lipids remained in a liquid crystalline phase. The biphenyl group was employed so that the lipid could not readily adopt a U-shape (i.e. in one leaflet of a bilayer membrane) as has also been reported for other quasimacrocyclic lipid species.[6]

Central to the synthesis of both quasimacrocycles **2** and **3** was the preparation of a monophytanylated glycerol unit. Commercially available 1-*O*-benzyl glycerol was silylated at the primary hydroxyl (TBDMSCl, imidazole, DMF, 85%) and reacted with phytanyl bromide[7,8] (NaH, THF, 81%) to afford the differentially protected monophytanyl glycerol derivative (Scheme 1). While desilylation of **4** could be affected, the yields of the product were variable and so an alternative, more robust, route to this material was sought.



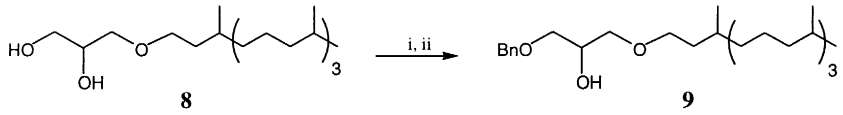
Scheme 1. (i) TBDMSCl, imidazole, DMF; (ii) Phytanyl bromide, NaH, THF

Commercially available 1,3-di-*O*-benzyl glycerol was reacted with phytanyl bromide (KOH, $PhCH_3$, 61%) and the product subsequently hydrogenolysed (Pd/C, H_2, MeOH, 97%) to afford the monophytanyl glycerol **5** as a colourless oil (Scheme 2). Condensation with benzaldehyde (cat. *p*-TSA, $PhCH_3$) afforded the six-membered ring ketal **6** which was smoothly reduced to the required 1-*O*-benzyl-2-*O*-phytanyl glycerol **7** by either DIBALH (CH_2Cl_2, $PhCH_3$, 69%) or in situ-generated borane (KBH_4, $BF_3 \cdot Et_2O$, 44%). The intermediate **6** could also be prepared by reaction of 2-phenyl-5-hydroxy-1,3-dioxane with phytanyl bromide; however, the low yield for the preparation of the starting dioxane[9] made this route unattractive.



Scheme 2. (i) Phytanyl bromide, KOH, $PhCH_3$; (ii) H_2, Pd/C, MeOH; (iii) PhCHO, *p*-TSA, $PhCH_3$; (iv) DIBALH, $PhCH_3/CH_2Cl_2$

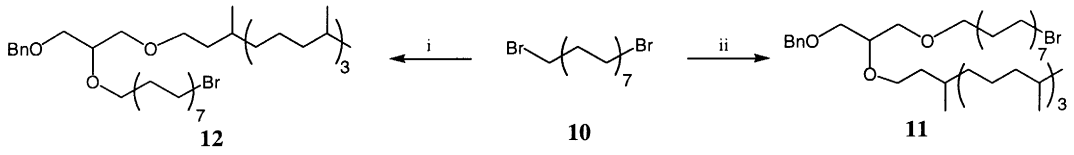
The alternative regioisomer of **7** was prepared by starting with known diol **8**.[7] Monobenzylation of **8** was achieved through alkylation (BnBr, CH_2Cl_2/THF, 68%) of the in situ-derived dibutylstannoxane derivative,[10] itself formed by treatment of **8** with dibutyltin oxide (Bu_2SnO, $PhCH_3$), to afford 1-*O*-phytanyl-2-*O*-benzyl-glycerol **9** (Scheme 3).



8 **9**

Scheme 3. (i) Bu_2SnO, $PhCH_3$; (ii) BnBr, CH_2Cl_2/THF

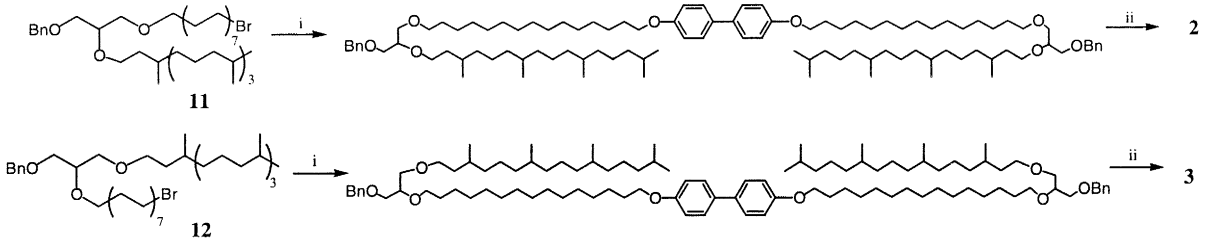
With efficient routes to two regioisomers of mono-phytanylated mono-benzylated glycerol, we next explored linking each unit via a membrane spanning tether. Thus, the known dibromide **10**[11] was reacted under basic conditions with either **7** or **9** to afford the bromolipid species **11** and **12**, respectively, in 52 and 70% yield (Scheme 4).



12 **10** **11**

Scheme 4. (i) **9**, KOH, $PhCH_3$; (ii) **7**, NaH, THF

Coupling of the bromolipid **11** was achieved by reaction with 4,4′-biphenol (NaH, DMF) to give the dibenzylated membrane spanning lipid in good yield (65%). Removal of the protecting groups under hydrogenolytic conditions (Pd/C, H_2, MeOH, 55%) afforded the desired membrane spanning lipid **2** as a wax. Reaction of the alternative bromolipid **12** under identical conditions afforded the isomeric membrane spanning lipid **3**, again in fair yield (45% for the dibenzylated compound, 95% yield for the debenzylation) (Scheme 5).



Scheme 5. (i) 4,4′-biphenol, NaH, DMF; (ii) H_2, Pd/C, MeOH/CH_2Cl_2

The synthetic procedures detailed above were carried out on a scale such that multigram quantities of these membrane spanning lipids were obtained. For instance, diol **2** was produced in >100 g quantities from **7** prepared by using the method shown in Scheme 2. All new materials prepared in this work have been fully characterised[12] and the product diols **2** and **3** have been subsequently coupled to disulfide containing species for attachment to gold and use in tethered membrane system.[1] Further studies on these and related species will be reported in due course.

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12. Spectroscopic data for key compounds prepared in this work. Compound **5**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.80–0.95 (15H, m, phytanyl C*H*$_3$s), 0.95–1.72 (24H, m, phytanyl C*H*$_2$s and C*H*s), 3.40–3.80 (7H, m, C*H*–O and C*H*$_2$–O); *m/z* (CI): 372.64 ($M+H^+$). Found: C, 73.6; H, 12.9; $C_{23}H_{48}O_3 \cdot 0.2H_2O$ requires: C, 73.4; H, 13.0%. Compound **6**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.81–0.93 (15H, m, phytanyl CH_3s), 0.93–1.69 (24H, m, phytanyl C*H*$_2$s and C*H*s), 3.51–3.70 (7H, m, C*H*$_2$–O), 4.36, 4.42 (1H, m, C*H*Ph, *cis*:*trans*, 1:1), 7.36, 7.45 (5H, m, CH*Ph*, *cis*:*trans*, 1:1); *m/z* (CI): 461.4 ($M+H^+$). Found: C, 78.2; H, 11.4; $C_{30}H_{52}O_3$ requires: C, 78.2; H, 11.4%. Compound **7**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.80–0.92 (15H, m, phytanyl C*H*$_3$s), 0.92–1.70 (24H, m, phytanyl C*H*$_2$s and C*H*s), 3.48–3.80 (7H, m, C*H*–O and C*H*$_2$–O), 4.55 (2H, m, *CH*$_2$Ph), 7.3 (5H, m, CH$_2$*Ph*); *m/z* (CI): 463.3 ($M+H^+$). Found: C, 78.0; H, 12.1; $C_{30}H_{54}O_3$ requires: C, 77.9; H, 11.8%. Compound **9**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.82–0.88 (15H, m, phytanyl C*H*$_3$s), 1.08–1.37 (24H, m, phytanyl C*H*$_2$s and C*H*s), 2.32 (1H, s(br), O*H*), 3.50 (6H, m, C*H*$_2$–O), 3.98 (1H, m, C*H*–O), 4.55 (s, 2H, O–C*H*$_2$–Ph), 7.32 (m, 5H, CH$_2$–*Ph*); *m/z* (EI) (HR) found: 462.4086; $C_{30}H_{54}O_3$ requires: 462.4072. Compound **11**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.80–0.90 (15H, m, phytanyl C*H*$_3$s), 0.90–1.62 (52H, m, phytanyl C*H*$_2$s and C*H*s), 3.39–3.66 (9H, m, C*H*–O and C*H*$_2$–O), 4.58 (2H, s, C*H*$_2$Ph), 7.35 (5H, m, CH$_2$*Ph*); *m/z* (CI): 753.4 ($M+H^+$). Found: C, 71.8; H, 11.6; $C_{45}H_{83}BrO_3$ requires: C, 71.9; H, 11.1%. Compound **12**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.82–0.88 (15H, m, phytanyl CH_3s), 1.18–1.59 (52H, m, phytanyl C*H*$_2$s, C*H*s and C15 chain C*H*$_2$s), 3.36–3.57 (9H, m, C*H*–O and C*H*$_2$–O), 4.55 (2H, s, C*H*$_2$–Ph), 7.32 (m, 5H, aromatic protons); *m/z* (EI) 752 (M^+). Found: C, 72.1; H, 11.2; $C_{45}H_{83}BrO_3$ requires: C, 71.9; H, 11.1%. Compound **2**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.80–0.95 (30H, m, phytanyl C*H*$_3$s), 0.95–1.70 (100H, m, phytanyl C*H*$_2$s and C*H*s), 3.40–3.80 (18H, m, C*H*–O and C*H*$_2$–O), 3.98 (4H, t, C*H*$_2$OAr), 6.93, 7.43 (8H, AA′XX′ multiplets, arom. –H); *m/z* (ES): 1370 ($M+Na^+$). Found: C, 78.3; H, 12.2; $C_{88}H_{162}O_8$ requires: C, 78.4; H, 12.1%. Compound **3**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.82–0.88 (30H, m, phytanyl C*H*$_3$s), 1.07–1.73 (100H, m, phytanyl C*H*$_2$s, C*H*s and C15 chain C*H*$_2$s), 3.47–3.62 (18H, m, C*H*–O and C*H*$_2$–O), 3.98 (4H, C*H*$_2$–O–Ar), 6.95, 7.46 (8H, AA′XX′ multiplets, arom. –H); *m/z* (ESI) 1348 (M^+). Found: C, 78.1; H, 12.1; $C_{88}H_{162}O_8$ requires: C,78.4; H, 12.1%.