Archaea *Membrane Lipids*

Determination of the Configuration of an *Archaea* Membrane Lipid Containing Cyclopentane Rings by Total Synthesis**

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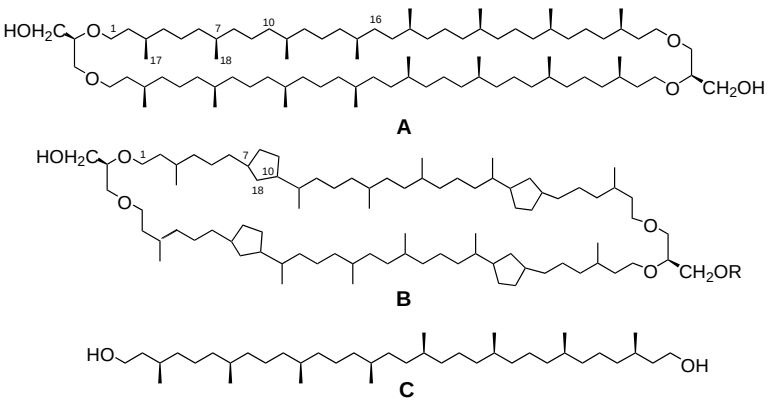
Dedicated to Professor Kurt Mislow on the occasion of his 80th birthday

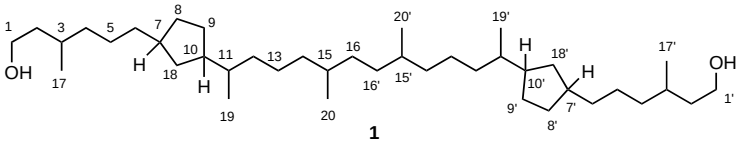
The *Archaea* are microorganisms that proliferate under extreme environmental conditions, such as high acidity, high temperature and/or high salt concentration.[1] *Archaea* are classified into three phenotypes on the basis of their living habitats: methanogens, halophiles, and thermoacidophiles. Among the distinctive features of the thermoacidophiles are their membranes. These contain lipids consisting of mixtures of macrocyclic, 72-membered tetraethers composed of saturated isoprenoid chains linked to glycerol or higher sugars.[2] Furthermore, compounds with up to eight five-membered rings were isolated. Typical tetraethers are compounds **A** and **B**.[3] Note that there is a relationship between **A** and **B** in that the five-membered rings of **B** can formally be generated by connecting CH_3 and CH_2 groups of **A**, for example C18 and C10.

During the last decade syntheses of ethers related to *archaea* membranes have been reported by several groups.[4]



In fact, the configuration of the macrocyclic tetraether **A** has been determined by total synthesis of the diol **C** and comparison with a sample of **C** obtained by degradation of natural **A**.[5] In the meantime, a synthesis of **A** has also been carried out.[6]

In contrast, the configurations of the compounds containing five-membered rings are largely unknown and, as a consequence, stereoselective synthesis of a lipid containing cyclopentane units has not been undertaken. De Rosa et al. obtained various pure diols with five-membered rings, among them **1**, by degradation of natural etherlipids and determined



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their constitution by mass spectrometry and NMR spectroscopy.[7] Important features, revealed by ^{13}C NMR spectroscopy, are the C_2 symmetry of the structure of **1** and the *trans* configuration of the rings which was deduced by comparison of the ^{13}C NMR spectroscopy data with those of *cis*- and *trans*-1,3-dimethylcyclopentane.[8] Herein we report the determination of the complete relative and absolute configuration of the diol **1** by a stereoselective synthesis.

Before embarking on the considerable effort of the synthesis of **1**, it appeared wise to exclude possible configurations by comparing naturally derived C_{40} diol **1** with C_{20} model compounds. Exploiting the C_2 symmetry of **1**, the diastereomers **2a–d** (Scheme 1) were chosen as model compounds. The methylated center at C3 was assumed to be analogous to the all-methylated chain **C**, but all possible configurations in the 1,3-*trans*-disubstituted ring and the α-methyl group were considered.

Our route to these compounds relies on lactones (*S*,*S*)-**3** and (*R*,*R*)-**3** as starting materials, available enantiomerically pure on a 100 g scale by asymmetric allylic substitution.[9] The CH_3 group corresponding to C19 was introduced by formation of the enolate (lithium diisopropylamide (LDA), THF, −78 °C) and alkylation with methyl iodide which proceeded with diastereoselectivity of **4a**:**4b** = 6:1; both lactones can be obtained in pure form by column chromatography.[10]

Compounds **2a–d** were synthesized by analogous routes. As a representative example, the synthesis of alcohol **2a** is described in Scheme 2. Bromide **5** was prepared from citronellol (98% *ee*), and transformed into an organocopper reagent which was coupled with lactone (+)-**4a** by an S_N2' reaction.[11] Reduction of the resulting acid **6** with $LiAlH_4$ gave the corresponding alcohol which was transformed into the tosylate **7**. Chain elongation by cross coupling tosylate **7** with 3-methylbutylmagnesium bromide, catalyzed by Li_2CuCl_4,[12] furnished the unsaturated precursor of **8** in high

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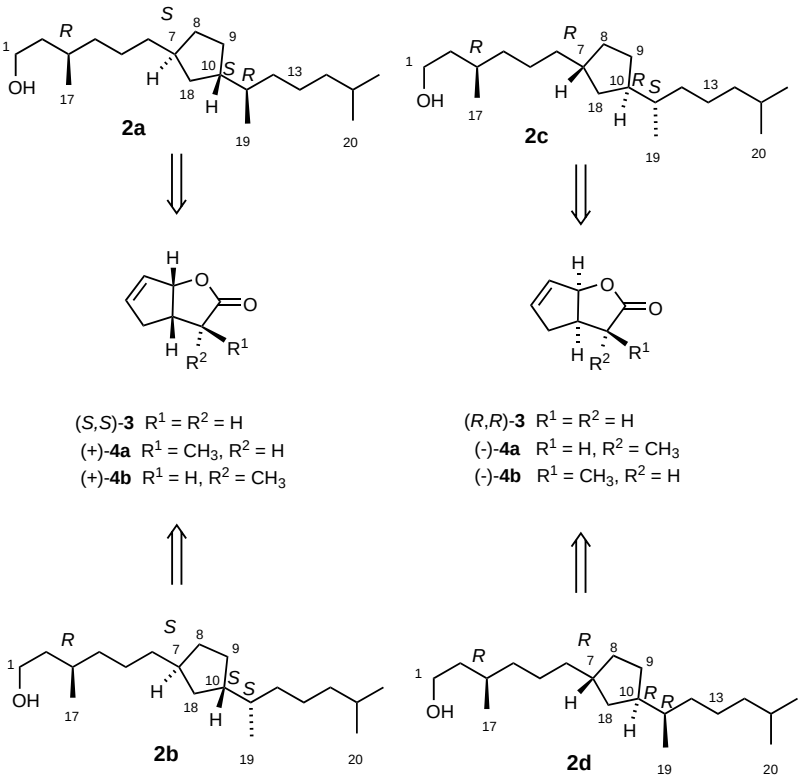
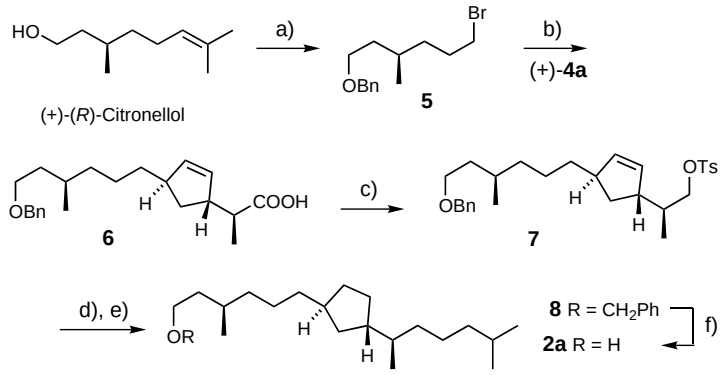


Scheme 1. Model compounds **2a–d** and starting materials.



Scheme 2. Synthesis of model compound **2a**. a) 1. BnBr, NaH, DME, 0 °C→reflux, 4 h; 2. O_3, $NaBH_4$, $MeOH/CH_2Cl_2$ (1:1), −78 °C→RT, ≈12 h; 3. CBr_4, Ph_3P, Et_2O, RT, ≈12 h, 70% (3 steps); b) 1. Mg, THF, 65 °C; 2. CuBr·SMe_2, THF/SMe_2 (5:1), −78 °C; 3. (+)-**4a**, −78 °C→RT, ≈12 h, 80%; c) 1. $LiAlH_4$, THF, 65 °C; 2. TsCl, pyridine, 0 °C, 77% (2 steps); d) 3-methylbutyl magnesium bromide (3 equiv), 10 mol% Li_2CuCl_4, THF, −78 °C→0 °C, ≈12 h, 85%; e) $TsNHNH_2$, DME, NaOAc, H_2O, reflux, 2 h; f) $Pd(OH)_2/C$, H_2, AcOEt/MeOH (1:1), 1.1 atm, RT, ≈12 h, 88% (2 steps). Bn = benzyl, Ts = *p*-toluenesulfonyl, DME = dimethoxyethane.

yield. The double bond was reduced with diimine without the isomerization[13] which had occurred in a variety of transition-metal catalyzed hydrogenations. Finally, hydrogenation of benzyl ether **8** furnished the desired model compound **2a** in 32% overall yield from (+)-(*R*)-citronellol.

NMR spectra of isomers **2a–d** were recorded and the chemical shift assignments established by COSY, HMQC, HMBC, and DEPT experiments. Data were compared with those of diol **1** which was prepared by degradation of a mixture of tetraether lipids extracted from *Archaea Sulfolobus acidocaldarius*.[14] In Figure 1 the differences in ^{13}C chemical shift values are plotted for the cyclopentane ring and adjacent CH(Me) units. From these data, configurations **2b** and **2d** can be excluded.

The ^{13}C chemical shift values of the diastereomers **2a** and **2c** differ at most by 0.18 ppm. Thus, a decision between the two corresponding configurations for **1** on the basis of this NMR spectroscopy data is not possible. Nevertheless, these compounds were useful because it was found that the diastereomeric alcohols **2a–d** all display significantly different optical rotations (Figure 1). On the basis of this observation it was expected that comparison of the optical rotations of **1**, obtained by degradation and by total synthesis, would allow conclusive establishment of configuration. Accordingly, a synthesis of diol **1** was carried out.[15]

The synthesis of **1** starting from **7** involved chain elongation by a hydroisoprene unit followed by dimerization (Scheme 3). For chain elongation, a copper catalyzed cross-coupling of tosylate **7** (see Scheme 2) with a Grignard reagent prepared from bromide **9** was carried out. After removal of the protecting group, alcohol **10** was obtained and transformed into the corresponding bromide. Copper-catalyzed dimerization, reduction of the double bonds, and deprotection gave the diol **1** in 32% yield from **7**.

Samples of synthetic C_{40} diol **1** and **1** derived from *archaea* lipid displayed identical ^{13}C NMR spectra. All the resonance signals were assigned by 2D NMR spectroscopic techniques. Optical rotations of $[\alpha]_{436}^{20} = +14.7$ ($c = 1.00$, $CHCl_3$) for the synthetic diol **1** and $[\alpha]_{436}^{20} = +15.5$ ($c = 1.00$, $CHCl_3$) for the natural derived diol **1** were found. Thus, there is agreement within the range of precision of the measurement of optical rotation. Considering also the large differences in the optical rotations of the model compounds **2a–d** the proposed configuration of diol **1** is strongly supported.

In conclusion, the absolute and relative configuration of an *archaea* membrane lipid containing five-membered rings was determined for the first time. This was accomplished by

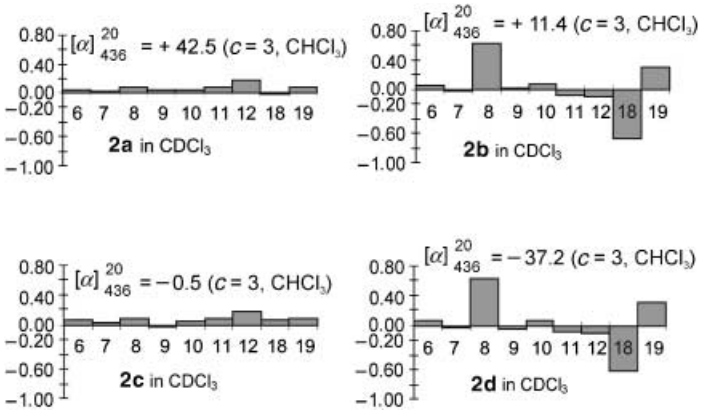
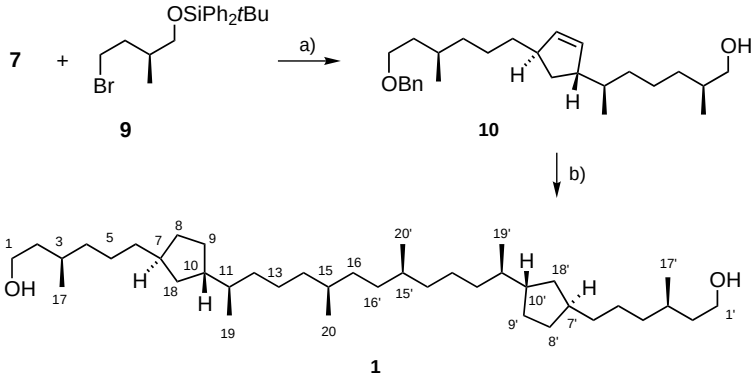


Figure 1. Differences in ^{13}C NMR chemical shifts of samples of diol **1** obtained by degradation of *Archaea* membrane lipids and of synthetic compound **2a–d** (125 MHz, $CDCl_3$). The *x* axis gives the number of the carbon atom and *y* axis the $\Delta\delta$ (δ(**2**)−δ(**1**)). In addition the optical rotations of **2a–d** are given.



Scheme 3. Synthesis of diol **1**. a) 1. **9**, Mg, THF, 65 °C; 2. 5 mol% Li_2CuCl_4, THF, −70 °C; 3. **7**, −70 °C→RT, ≈12 h; 4. Bu_4NF, THF, 85% (2 steps); b) 1. CBr_4, Ph_3P, Et_2O, RT, ≈12 h; 2. Mg (0.5 equiv), THF, 65 °C, 5 mol% Li_2CuCl_4, THF, 65 °C; 3. PtO_2, EtOAc, 2 h; 4. $Pd(OH)_2/C$, H_2, 4 bar, 38% (4 steps). Ts = *p*-toluenesulfonyl, DME = dimethoxyethane.

synthesis of the four model compounds **2a–d** and the diol **1** and comparison of their NMR spectroscopic and optical rotation data with those of diol **1** derived from natural *archaea* lipids.

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Keywords: *archaea* membrane lipids · asymmetric synthesis · configuration determination · cross-coupling · natural products

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