Total Synthesis of the Alleged Structure of Crenarchaeol Enables Structure Revision**

Mira Holzheimer, Jaap S. Sinninghe Damsté, Stefan Schouten, Remco W. A. Havenith, Ana V. Cunha, and Adriaan J. Minnaard*

Abstract: Crenarchaeol is a glycerol dialkyl glycerol tetraether lipid produced exclusively in Archaea of the phylum Thaumarchaeota. This membrane-spanning lipid is undoubtedly the structurally most sophisticated of all known archaeal lipids and an iconic molecule in organic geochemistry. The 66-membered macrocycle possesses a unique chemical structure featuring 22 mostly remote stereocenters, and a cyclohexane ring connected by a single bond to a cyclopentane ring. Herein we report the first total synthesis of the proposed structure of crenarchaeol. Comparison with natural crenarchaeol allowed us to propose a revised structure of crenarchaeol, wherein one of the 22 stereocenters is inverted.

Introduction

In 1990, Woese proposed to classify all living organisms in three domains of life: Archaea, Bacteria and Eukarya.[1] Before that, “archaeabacteria” were considered to belong to the Bacteria. Based on differences in their genome and lipidome, Archaea were ultimately recognized as separate, third domain.[2] For a long time, Archaea were primarily associated with extreme habitats such as high temperature, extreme pH, and hypersaline environments.[3] Growing interest over the years, however, led to the discovery of meso- and extremophilic Archaea in virtually any habitat on Earth.[4] The cell membrane of Archaea is built up of diether or membrane-spanning tetraether lipids containing isoprenoid chains, contrary to the straight chain fatty acid glycerol ester lipids found in Bacteria and Eukarya.[5] Apart from the difference in lipid linkage, the stereochemistry of the glycerol backbone in archaeal isoprenoidal glycerol dialkyl glycerol tetraether lipids (GDGTs) is opposite to bacterial or eukaryotic glycerolipids, raising questions on the evolution of archaeal and bacterial/eukaryotic lipid membranes.[6] The lipid composition of Archaea varies, depending on the species and environmental factors, and this is considered an adaptation to their habitat.[7] The ether-linkages provide chemical stability against hydrolysis, and the presence of methyl-branches and cyclopentane moieties, which are formed by internal cyclization of the biphytanol chain,[8] leads to decreased membrane permeability, allowing growth at extreme pH, salinity, and temperature.[9] One archaeal GDGT—named crenarchaeol—stands out from all other archaeal membrane lipids due to its unique chemical structure (Figure 1). Crenarchaeol is produced by a specific lineage of...
Archaea, the Thaumarchaeota, \[10\] and was first isolated from surface sediments of the Arabian Sea. After extensive GC–MS and NMR analysis, the structure and stereochemistry of this unique GDGT was proposed, a considerable achievement given the fact that the molecular complexity originates merely from its unusual hydrocarbon framework.\[11\] It contains four 1,3-trans-substituted cyclopentane moieties. One of these is connected by a single bond to a cyclohexane ring, a structural feature rarely found in natural products.\[12\] This feature of crenarchaeol is likely formed by further internal cyclization of the bicyclic biphytanyl moiety.\[86\] Crenarchaeol contains a total of 22 stereocenters, most of which are remote, including an all-carbon quaternary stereocenter. Recently, a parallel glycerol configuration of sedimentary crenarchaeol was inferred from chemical derivatization experiments.\[13\] Montenegro et al. confirmed the structure of the bicyclic biphytanyl moiety in archaeal GDGTs by total synthesis,\[14\] yet to date, there is no proof of structure for the tricyclic biphytanyl moiety of crenarchaeol and no total synthesis. The 5–6 ring motif of crenarchaeol is particularly interesting due to its complexity and uniqueness in nature. In order to ultimately confirm the structure and stereochemistry of crenarchaeol, we embarked on its total synthesis.

Results and Discussion

Our retrosynthetic analysis of crenarchaeol made use of the inherent symmetry of the bicyclic biphytanyl chain of the molecule (Scheme 1). It started with the disconnection of the central C–C-bond of the bicyclic biphytanyl moiety by intramolecular alkene metathesis and ether bond disconnection of 1. This led to two key intermediates, termed Fragment A and B, and protected glycerol building block 2. Fragment A can be further simplified via dithiane disconnections to arrive at building blocks 4 and 6, both carrying a methyl-branched stereocenter, and cyclopentane building block 5. In turn, 5 can be traced back to hydroxyketone 7, which is accessed from commercially available (S)-carvone via ring contraction. Syntheses of archaeal cis-\[15\] and trans-substituted\[14\] cyclopentane containing lipids have been previously reported. As we planned to build the macrocycle by alkylation of a suitably functionalized glycerol building block and ring-closing metathesis, we required differentially protected lipid chains containing the trans-substituted cyclopentane and the methyl-branches. Based on the stereochemical assessment of the bicyclic biphytane moiety in crenarchaeol\[17\] and its subsequent confirmation provided by Helmchen et al.,\[22\] we planned the synthesis of the desired stereoisomer.

Retrosynthesis of Fragment B commenced with the C–C-bond disconnection of 8 arriving at dithiane 10 and iodide 9, the latter originating from Fragment A. Further simplification of 10 by asymmetric Cu-catalyzed Grignard alkylations and a Wittig olefination delivered diacetate 11. The 5–6 ring motif of 11 was disconnected at the C–C-bond joining the two carbocycles.\[16\] We realized that for this challenging transformation an advanced intermolecular Pd-catalyzed asymmetric allylic alkylation could be instrumental, inspired by the work of Trost.\[17\] By this, we arrived at building blocks 13 and 14.
readily accessible from pimelic acid and cyclopentadiene, respectively.

**Synthesis of Fragment A**

The synthesis of Fragment A was initiated by the preparation of known β-hydroxyketone 7 from (S)-carvone (Scheme 2). Via a four-step sequence involving a hydrolytic ring contraction,[18] 7 was obtained as single diastereomer, as confirmed by NOESY. Notably, this sequence proved robust and scalable and allowed multigram synthesis of 7 (see Supporting Information). After acetal protection of 7, the hydroxyl group of 15 was removed by Barton-McCombie deoxygenation, providing 16 in excellent yield. Notably, acetal protection was necessary to avoid elimination of the β-hydroxyl group in the synthesis of the xanthate intermediate. Initially, we envisioned to stereoselectively install the methyl stereocenter adjacent to the 5-membered ring by means of Cu- or Co-catalyzed asymmetric hydroboration.[19] No published method to perform the asymmetric hydroboration of the 1,1-disubstituted terminal alkene of 16 delivered 17 in acceptable yield and stereoselectivity, however. Thus, we resorted to non-stereoselective hydroboration-oxidation of 16 followed by diastereomer separation, giving 17 in 43% yield as single stereoisomer. The stereochemistry of the methyl-branched center in 17 was determined by amidation of its corresponding acid with phenylglycine methyl ester, followed by 1H NMR analysis (See Supporting Information).[20]

In addition, the efficiency of the synthesis was further increased by “recycling” of the undesired epi-17 by iodination and elimination, giving alkene 16 in 77% yield over two steps. After silyl protection of 17, the acetal moiety of 18 was removed. Optimization of the reaction conditions, to minimize epimerization, resulted in treatment of 17 in acetone with FeCl₃ adsorbed to silica,[21] giving 19 in quantitative yield with 3% epimerization. Ketone 19 was converted to the corresponding terminal alkene by enol-triflation and Pd-catalyzed triflate reduction, delivering 20 in 80% yield over two steps. Hydroboration-oxidation of 20 gave alcohol 21 in 87% yield, which was converted to the corresponding bromide 5 in excellent yield. With 5 in hand, the stage was set for the first dithiane alkylation. [22] After optimization of the lithiation conditions of 4 (prepared using known methods, see Supporting Information), the alkylation proceeded in high yield (87%) giving 22. Desilylation followed by Appel iodination delivered iodide 9, which serves as intermediate in the synthesis of both Fragment A and B. In turn, after identification of the optimal lithiation conditions, deprotonation of dithiane 6 with n-BuLi at 0°C followed by addition of 9 produced bis-dithiane 3 in 68% yield. With the carbon skeleton of Fragment A constructed, the dithiane moieties and the benzyl ether of 3 were removed by Raney-nickel.

**Scheme 2.** Synthesis of Fragment A.
reduction in good yield, thus concluding the synthesis of Fragment A.

Synthesis of Fragment B

Next, the considerably more complex Fragment B was to be constructed. The synthesis started with the preparation of two building blocks 14 and 27 (Scheme 3). The synthesis of cyclopentene 14 started from meso-diacetate 24, accessible in two steps from cyclopentadiene. Diacetate 24 was subjected to enzymatic desymmetrization in excellent yield and ee, followed by silyl protection giving 14. Cyclohexanone 27 was prepared according to the method developed by the Stoltz laboratory from allyl cyclohexanone 13, which was protected and subjected to hydroboration/oxidation to deliver 26. Omission of the protection of the ketone in 13 led to the formation of the corresponding hemiacetal. Benzylation and acetal hydrolysis provided the desired cyclohexanone 27 in 92% yield over two steps.

With acetate 14 in hand, we chose to investigate the key step—the intermolecular Pd-catalyzed Tsuji–Trost alkylation—with 2,2-dimethylcyclohexanone 28 as model substrate (Table 1). We started by screening ligands L1–L4 (Scheme 3) in combination with Pd(dba)$_2$CHCl$_2$ in order to achieve good...
The hydroxyl moiety of 35 was then removed by a Barton-McCombie deoxygenation reaction in excellent yield. After Pd-catalyzed hydrogenolysis of the benzyl ether in 36, alcohol 37 was oxidized to the corresponding aldehyde and subjected to a Wittig olefination delivering α,β-unsaturated thioester 39. The last methyl-branched stereocenter of Fragment B was then introduced in an excellent dr of 20:1 (see Supporting Information for details) by copper-catalyzed asymmetric conjugate addition of methylmagnesium bromide producing 40 in 87% yield. With the last stereocenter of the biphynate core of crenarchaeol set, the dithiane moiety of 10 was installed, after MOM deprotection of 40, through thioester reduction and treatment with 1,3-propanediol in the presence of BF₃·OEt₂. Dithiane 10 was obtained in 82% over the three steps. Notably, dithiane synthesis in presence of the MOM ether resulted in a complex mixture of 10 and various trans-acetalization products. With 10 in hand, the last dithiane alkylation was performed, in presence of the free hydroxyl group. After optimization of the lithiation conditions, the reaction of lithiated 10 with iodide 9 smoothly provided the coupling product 8 in 67% yield, containing the entire carbon-skeleton of Fragment B. The synthesis of Fragment B was concluded by a two-step sequence, involving removal of the dithianes with Raney-nickel, followed by Pd-catalyzed hydrogenolysis of the remaining benzyl ether.

Endgame—Completion of the Total Synthesis of the Proposed Structure of Crenarchaeol

After the successful stereoselective synthesis of both Fragment A and B, the macrocycle of crenarchaeol was assembled (Scheme 4). The endgame of the synthesis started with the O-alkylation of protected glycerol 2 with mesylate 41 prepared from Fragment A. During the reaction using sodium hydride in DMF, partial cleavage of the TBDPS ether was observed. Therefore, after O-alkylation, the silyl ether was reintroduced, giving alkylation product 42 in 62% yield. The trityl ether was then removed delivering 43, the substrate for the next ether synthesis, in 94% yield. The double O-alkylation of 43 with bis-mesylate 44 came about after considerable experimentation, by reaction with KOtBu as the base in toluene in the presence of TBAB as phase-transfer catalyst. After deacylation of the crude double alkylation product, the desired diol 45 was obtained in a poor yield of 27% over the two steps. There are multiple factors complicating this reaction. It is a double O-alkylation of a bis-mesylate. The sheer size and flexibility of this electrophile plays a role in the reaction rate as we expect that the site of alkylation is not always exposed for reaction with the weak alkoxide nucleophile. In addition, small amounts of elimination products were observed. Consequently, given the difficulty of this step, we continued with the synthesis. In order to perform the final ring closing, the substrate 45 was oxidized to the corresponding aldehyde and subjected to an aldol reaction with cyanide to give cyanohydrin 46 (Scheme 5).

### Table 1: Optimization of the Pd-catalyzed allylic alkylation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ligand Base Solvent</th>
<th>Conversion (%)</th>
<th>dr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L1 LHMDS THF</td>
<td>80%</td>
<td>25.75</td>
</tr>
<tr>
<td>2</td>
<td>L2 LHMDS THF</td>
<td>40%</td>
<td>51.49</td>
</tr>
<tr>
<td>3</td>
<td>L3 LHMDS THF</td>
<td>40%</td>
<td>81.19</td>
</tr>
<tr>
<td>4</td>
<td>L4 LHMDS THF</td>
<td>40% (27%)</td>
<td>86.14</td>
</tr>
<tr>
<td>5</td>
<td>L5 LHMDS PhCH₃</td>
<td>40%</td>
<td>85.15</td>
</tr>
<tr>
<td>6</td>
<td>L4 LHMDS DME</td>
<td>42%</td>
<td>94.6</td>
</tr>
<tr>
<td>7</td>
<td>L4 NaHMDS DME</td>
<td>10–15%</td>
<td>92.8</td>
</tr>
<tr>
<td>8</td>
<td>L4 LDA DME</td>
<td>41%</td>
<td>92.8</td>
</tr>
<tr>
<td>9</td>
<td>L4 LHMDS DME</td>
<td>full (53%)</td>
<td></td>
</tr>
</tbody>
</table>

[a] See Supporting Information for details. [b] Determined by 1H NMR. [c] Isolated yield. [d] Determined by 13C NMR of the crude product. [e] 1.6 equiv. of LHMDS and 3 equiv. LiCl were used.
closure of the macrocycle, 45 was converted to bis-alkene 1 by oxidation and Wittig reaction. The 66-membered macrocycle was closed by means of ring-closing metathesis with Grubbs 2nd generation catalyst, a method often used for the construction of large rings.[28] This provided 46 in 65% yield, given the size of the produced macrocycle a more than satisfactory result. In the final step, the double bond as well as the benzyl ethers were removed by hydrogenolysis with palladium on carbon in low yield of 34%, which could be partially attributed to the scale of the reaction. This concluded the synthesis of this structurally complex lipid and provided 1.2 mg of synthetic crenarchaeol. With both synthetic crenarchaeol and the tricyclic intermediate Fragment B in hand we sought to investigate the chemical structure of natural crenarchaeol. For this purpose, we re-isolated natural crenarchaeol in a laborious procedure (see Supporting Information) and made a comparison of their NMR spectra. Furthermore, we performed chemical derivatization in combination with GC–MS analysis.

**Comparison of Natural Crenarchaeol and Fragment B by GC–MS**

The Bligh Dyer extract of the thermophilic Thaumarchaeota “Ca. Nitrosotenuis uzonensis” (dominated by crenarchaeol and its cis-cyclopentyl isomer,[29] see Figure 2A) has previously been treated with HI. This cleaves the ether bonds to produce a mixture of biphytane diiodides.[29] Reduction of the iodides with H₂/PtO₂ led to the corresponding hydrocarbons I–III, which were analyzed by GC–MS.[29] This showed a ratio of bi- and tricyclic biphytanes of approximately 1:1 (Figure 2B). As a direct comparison of the configuration of the tricyclic biphytane unit within synthetic and natural crenarchaeol was considered complicated, we subjected also Fragment B to this derivatization (Figure 2A).[29,30] This enabled a precise comparison by GC–MS. Treatment of fragment B with HI followed by reduction yielded biphytane IV which appeared, as expected, as a single peak in the gas chromatogram (Figure 2C), but much to our surprise with a significantly different retention time than the supposedly identical II derived from natural crenarchaeol. The mismatch in chemical structure was confirmed by co-injection, showing retention time differences of IV and II or III of approximately 1.5 and 2 min, respectively (Figure 2D).

Next, we turned our attention to the mass spectra of II–IV (see Supporting Information). The fragmentation patterns of natural II and III were equivalent to their previously reported mass spectra,[29,31] and featured the characteristic fragment m/z 262, originating from bond cleavage adjacent to the quaternary stereocenter. This fragment was also clearly visible in the mass spectrum of synthetic IV.

Furthermore, the remaining fragmentation patterns of II/III and IV are also virtually identical, providing strong evidence that the overall chemical connectivity of II/III and synthetic IV is identical. Thus, we concluded that II and IV are stereoisomers.
Comparison of Fragment B with Isolated Natural Crenarchaeol by NMR

In order to elucidate the exact structural difference between synthetic Fragment B and the tricyclic biphytanyl moiety of natural crenarchaeol, we compared their NMR spectra. The $^1$H and $^{13}$C signals of natural crenarchaeol and Fragment B were assigned by thorough 2D NMR analysis. In addition, the $^{13}$C signals of synthetic crenarchaeol were assigned based on the NMR analysis of Fragment B.

The comparison of selected $^{13}$C NMR signals of Fragment B and synthetic crenarchaeol with those of natural crenarchaeol is shown in Table 2 (see Supporting Information for a table with all signal assignments).

The carbon numbering is shown in Figure 3, and significant differences in $^{13}$C NMR shifts between Fragment B and natural crenarchaeol are marked in orange ($\Delta \delta = 0.25$–1 ppm) and red ($\Delta \delta > 1$ ppm). Upon comparison of the $^{13}$C NMR signals of Fragment B with those of natural crenarchaeol, it becomes clear that the majority of the chemical shifts of Fragment B are in very good agreement ($\Delta \delta < 0.25$ ppm) with those of the tricyclic biphytane of crenarchaeol. In particular, the $^{13}$C chemical shift differences of the three cyclopentane rings (which are not connected to the cyclohexane ring) and their alkyl substituents are virtually identical (see Supporting Information).

Table 2: Comparison of $^{13}$C NMR values of natural crenarchaeol with Fragment B and synthetic nominal crenarchaeol.

<table>
<thead>
<tr>
<th>Carbon number[a]</th>
<th>$^{13}$C shift natural crenarchaeol (ppm)</th>
<th>$^{13}$C shift Fragment B (ppm)[b]</th>
<th>$\Delta \delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A1’</td>
<td>70.23, 70.26</td>
<td>61.42 (70.28, 70.25)</td>
<td>-8.81, -8.84</td>
</tr>
<tr>
<td>A2, A2’</td>
<td>36.72, 36.75</td>
<td>40.14, 40.15 (36.74)</td>
<td>+3.42, +3.40</td>
</tr>
<tr>
<td>A11’</td>
<td>39.38</td>
<td>39.01 (39.10)</td>
<td>-0.37</td>
</tr>
<tr>
<td>A12’</td>
<td>32.27</td>
<td>31.80 (31.81)</td>
<td>-0.47</td>
</tr>
<tr>
<td>A13’</td>
<td>22.40</td>
<td>22.10 (22.12)</td>
<td>-0.30</td>
</tr>
<tr>
<td>A14’</td>
<td>44.13</td>
<td>38.17 (38.30)</td>
<td>-5.96</td>
</tr>
<tr>
<td>A15’</td>
<td>33.20</td>
<td>32.92 (32.93)</td>
<td>-0.28</td>
</tr>
<tr>
<td>A16</td>
<td>30.12</td>
<td>30.50 (30.51)</td>
<td>+0.38</td>
</tr>
<tr>
<td>A16’</td>
<td>37.80</td>
<td>33.51 (33.46)</td>
<td>-4.29</td>
</tr>
<tr>
<td>A20’</td>
<td>22.55</td>
<td>30.12 (30.13)</td>
<td>+7.57</td>
</tr>
</tbody>
</table>

[a] Assignments of $^{13}$C NMR chemical shifts of crenarchaeol and Fragment B. Signals are reported relative to the solvent residual signal (CDCl$_3$, $\delta = 77.16$ ppm). [b] Corresponding signals of synthetic nominal crenarchaeol are shown in brackets.
around the all-carbon quaternary stereocenter elicit large differences in chemical shift at positions A14’ (δ = -5.96 ppm), A16’ (δ = -4.29 ppm) and A20’ (δ = +7.57 ppm), indicating a difference in structure around these positions. It is noteworthy that the $^{13}$C signals of the remaining stereocenters of the 5-6-ring system (A10’ and A7’) in Fragment B show no significant difference. In particular the good agreement of A10’ is indicative for the ascribed stereochemistry of the single bond connecting the 5- and 6-membered ring. It is expected that a difference in stereochemistry on A11’ would translate to a significant $^{13}$C chemical shift difference in A10’. This indicates that, on these positions, the chemical structure of natural crenarchaeol matches that of Fragment B. The chemical shifts of synthetic nominal crenarchaeol (chemical shifts in brackets in Table 2) show the same pattern of chemical shift differences. It should be highlighted that there is no significant $^{13}$C chemical shift difference between Fragment B and the tricyclic biphytane of synthetic crenarchaeol (except for the terminal carbons A1/A1’ and A2/A2’) excluding an influence of the macrocyclic structure on the chemical shifts.

Besides the good agreement of most of the $^{13}$C NMR chemical shifts of crenarchaeol and Fragment B, the $^1$H NMR chemical shifts of A7’, A10’ and A11’ correlate well (Table 3, see Supporting Information for all assignments). At position A19’ (axial) and A20’, only minor $^1$H shift differences were observed. Only three positions show significant chemical shift differences: the equatorial proton of A14’ (δ = 0.27 ppm), A16’ (δ = 0.47 ppm) and the equatorial proton of A19’ (δ = 0.15 ppm). This provides further evidence that the difference in structure of natural and synthetic crenarchaeol is located around these positions.

Since the relative and absolute stereochemistry of Fragment B is known, the methyl substituent A20’ of Fragment B is assigned to be equatorial due to the 1,3-cis relationship of the methyl and cyclopentyl substituents on the cyclohexane ring. As a result of the deshielding 𝜏-gauche effect, the $^{13}$C NMR chemical shift of axial substituents in cyclohexanes is more upfield relative to equatorial substituents. In Fragment B the $^{13}$C signal of methyl group A20’ resonates at 30.12 ppm, while the methyl group A20’ of natural crenarchaeol is shifted more upfield at 22.55 ppm. This strongly suggests that the methyl group A20’ in natural crenarchaeol is in axial position in contrast to the initially proposed structure. To further support this, we considered the $^{13}$C chemical shifts of A16’. In Fragment B, the carbon atom A16’ of the alkyl side-chain of the cyclohexyl ring is axially oriented. The $^{13}$C signal resonates at 33.51 ppm, whereas in crenarchaeol the $^{13}$C signal of A16’ is shifted downfield to 37.80 ppm. Thus, the downfield shift of A16’ in natural crenarchaeol strongly suggests equatorial substitution of the alkyl chain substituent on the cyclohexyl ring.

Further support comes from the computationally calculated $^{13}$C shift values for A16’ and A20’. First, MD simulations in chloroform were carried out on fragment B and its isomer to determine the lowest energy conformations. Subsequently, the energies of the conformers from the MD trajectory were evaluated using DFT calculations and the chemical shifts calculated (See Supporting Information for the protocol and the calculated shifts). The DFT prediction is in good agreement with the upfield shift of methyl group A20’ in natural crenarchaeol and the expected downfield shift of methylene A16’.

All in all this combined data provides overwhelming evidence for an inverted stereochemistry of crenarchaeol at A15’ compared to Fragment B. On the basis of the evidence from chemical derivatization, NMR studies, and computation, we therefore propose a revised structure of crenarchaeol (Figure 4), in which the stereochemistry of the all-carbon quaternary stereocenter is inverted compared to the original proposal.

**Conclusion**

The first total synthesis of the originally proposed structure of the thaumarchaeotal GDGT crenarchaeol has been achieved. The synthesis involved the stereoselective construction of a unique 5-6 ring motif as well as a late-stage 66-membered macrocyclization by means of RCM. The structure determination of crenarchaeol has a considerable history. Due to the very complex structure, including 22 stereocenters, as well as the highly aliphatic character and its lack of rigidity, NMR-based structural studies have been heavily complicated. Furthermore, since this lipidic molecule does not have the tendency to crystallize, X-ray diffraction was not possible. The synthesis of the proposed structure of crenarchaeol and the key intermediate Fragment B enabled direct comparison with natural crenarchaeol by chemical derivatization and GC–MS analysis. This revealed a mismatch of the chemical structure of the tricyclic biphytane chain. Subsequently, detailed NMR analysis including computational simulation of $^{13}$C chemical shifts, of Fragment B and synthetic crenarchaeol.

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**Table 3: Comparison of $^1$H NMR values of natural crenarchaeol and Fragment B.**

<table>
<thead>
<tr>
<th>Carbon number$^a$</th>
<th>$^1$H shift crenarchaeol (ppm)</th>
<th>$^1$H shift Fragment B (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A7’</td>
<td>1.79</td>
<td>1.78</td>
</tr>
<tr>
<td>A10’</td>
<td>1.47</td>
<td>1.46</td>
</tr>
<tr>
<td>A11’</td>
<td>1.17</td>
<td>1.12</td>
</tr>
<tr>
<td>A14’</td>
<td>1.15</td>
<td>1.42</td>
</tr>
<tr>
<td>A16’</td>
<td>1.31</td>
<td>1.78</td>
</tr>
<tr>
<td>A19’</td>
<td>ax.: 0.70; eq.: 1.39</td>
<td>ax.: 0.64; eq.: 1.52</td>
</tr>
<tr>
<td>A20’</td>
<td>0.84</td>
<td>0.79</td>
</tr>
</tbody>
</table>

$^a$ Assignments of $^1$H NMR chemical shifts of crenarchaeol$^{[11]}$ and Fragment B. Signals are reported relative to the solvent residual signal (CDCl$_3$, δ = 77.16 ppm).
archaeal, and comparison with natural crenarchaeol isolated from sea surface sediments was performed. Ultimately, from the spectroscopic data of fragment B, synthetic and natural crenarchaeol, we were able to revise the originally proposed structure beyond reasonable doubt. Through this extensive analysis we identified the inversion of just one out of the 22 stereocenters of crenarchaeol, namely the quaternary stereocenter embedded in the cyclohexane ring.

Total synthesis not only comprises the access to complex molecules, but serves also as a breeding ground for new synthetic methodology as well as probing current synthetic methods. Mistakes in the proposed structure of a natural product are by no means a rare occurrence.[33] The architectural and stereochemical complexity of a new unknown structure, in combination with very small amounts of isolated material often make assignments extremely difficult, in particular in a case such as crenarchaeol, which features almost no heteroatom functionalities and is highly flexible. By using the information gathered from the synthetic epimer of natural crenarchaeol, we were able to reassign the structure without the need to repeat the entire, very complex, synthesis.

The correction of the structure of crenarchaeol has important implications for the study of its role in archaeal membranes. The current hypothesis is that the presence of crenarchaeol regulates membrane fluidity and packing, an important adaptation to temperature and pressure changes in the environment. As the stereochemistry of the quaternary center in crenarchaeol has a significant influence on its conformation, and thus membrane packing, we expect that an explanation (supported by for instance molecular dynamics simulations) for its role in membrane behavior is now within reach.

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Conflict of Interest

The authors declare no conflict of interest.

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