21. Figures for 1980–1982 are from *Arizona Copper Smelter Handbook* (Arizona Department of Health Services, Phoenix, 1983). Figures for 1983 were obtained from the publisher (personal communication).
22. Kennecott Minerals Company, Salt Lake City, Utah.
23. Hurley smelter: Kennecott Minerals Company, Salt Lake City, Utah. Hidalgo smelter: Air Quality Bureau, New Mexico Department of Health and Environment, Santa Fe). No data are available for emissions from the Chino Mines smelter in Hurley, N.M., in 1980 or in June or July 1981. The totals for New Mexico in 1980 and 1981 are based on an estimate, assuming that emissions during those months for which data are lacking were equal to the average monthly figures during the period of record.
24. We thank M. Kim for technical assistance.

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Stereostructure of the Archaebacterial C_{40} Diol

Abstract. *The stereostructure of the archaebacterial C_{40} diol has been established as (3R,7R,11R,15S,18S,22R,26R,30R)-3,7,11,15,18,22,26,30-octamethyldotriacontane-1,32-diol by stereorational total synthesis. This provides the final evidence necessary to establish the structure of an archaebacterial membrane substance that is a 72-membered-ring tetraether with 18 stereocenters.*

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The archaebacteria (*1*, *2*) [metabacteria (*3*)] include the methanogens, the extreme halophiles, extreme thermophiles, and some thermoacidophiles. It has been demonstrated that the archaebacteria are no more closely related to the eubacteria than to the eukaryotes, which suggests an evolutionary divergence for the archaebacteria. Several features set archaebacteria apart from the prokaryotes. For example, archaebacteria often do not have cell walls, and when they do the cell walls are not based on a muramic acid peptoglycan structure (*4*); and there are numerous differences in the structures of the *t*-RNA (*5*) and the ribosomal proteins of the two species (*6*). The only molecular feature that seems to be common to all of the archaebacterial species is the nature of their lipids (*7*), which are isopranyl glycerol ethers instead of the fatty acid glycerol esters found in all other species (*8–18*). These unusual lipids have interesting implications in lipid bilayer theory (*7*, *19*) and have been used as molecular fossils (*20*) in addition to being used to identify archaebacteria. The alkyl chains usually consist of phytane or biphytane [two phytanes linked together by a 4′–4 linkage (*21*)] units (*8–18*).

A common lipid constituent in the thermoacidophiles and methanogens is the diglyceryl tetraether **1** (Fig. 1), a natural product having a 72-membered ring with 18 stereocenters (*8–18*). The C_{40} diol **2** (Fig. 1), which is a structural component of **1**, has been detected in geologic sediment (*22*) and was recently isolated from a kerogen (*23*) and from a nonpolymerized sediment (*24*). The gross structure of **1** was determined correctly for a sample isolated from *Thermoplasma acidophilum* (*9*) after two earlier misassignments (*15*, *16*). The absolute stereochemistry at the glycerol stereocenters was also determined (*18*), but until now nothing was known about the stereochemistry at the 16 stereocenters bearing methyl groups. We have carried out a stereorational total synthesis of (3*R*,7*R*,11*R*,15*S*,18*S*,22*R*,26*R*,30*R*)-3,7,11,15,18,22,26,30-octamethyldotriacontane-1,32-diol and have shown it to be identical by high-resolution ^{13}C–nuclear magnetic resonance (NMR) spectroscopy, with a natural form of **2** obtained by degradation of **1** isolated from *Methanobacterium thermoautotrophicum*. The synthesis has provided the evidence necessary to completely define the structure of **1**.

The strategy we used in our synthesis is based on technology used for a synthesis of the vitamin E sidechain (*25*). Optically active β-hydroxy acid **3** (98.7 percent enantiomeric excess) is prepared by the Evans protocol (*26*) from valinol oxazolidone. Reduction of **3** with $LiAlH_4$ provides diol **4**, which is selectively protected to give the *t*-butyldimethylsilyl (*t*-$BuMe_2Si$) ether **5** (*27*). The corresponding propionate ester **6** is subjected to the Ireland variant of the Claisen rearrangement (*28*) to obtain **7**. Hydrogenation of **7** with Adams's catalyst gives the protected hydroxy acid **8**. It was determined that the Claisen rearrangement of **6** to **7** had given 95.5 percent (2*R*, 6*R*) and 4.5 percent (2*S*, 6*R*) by conversion of **8** with $LiAlH_4$ to alcohol **9**, which was assayed by Mosher's method (*29*). Treatment of *p*-toluenesulfonate (Ts) **10** with KCN in the presence of 18-crown-6 (*30*) affords nitrile **11**, which is reduced with diisobutylaluminum hydride to give the ten-carbon aldehyde **12**.

Reduction of aldehyde **12** with $LiAlH_4$ gives alcohol **13**, which is converted to the β-(methoxyethoxy)methyl (MEM) ether **14** (*32*). Removal of the *t*-$BuMe_2Si$ group with HF yields alcohol **15**, which is converted to methanesulfonate **16** by reaction with methanesulfonyl chloride. Reaction of **16** with lithium thiophenoxide gives **17**, which is oxidized with *m*-chloroperoxybenzoic acid to provide sulfone **18**.

Mesylate **19**, prepared from alcohol **13**, is treated with tetra-*n*-butylammonium iodide in tetrahydrofuran to obtain iodide **20**. Alkylation of the dianion of sulfone **18** with iodide **20** affords sulfone **21**, which is reduced by Danheiser's method (*32*) to obtain, after treatment of the crude product with HF in acetonitrile, alcohol **22**. Treatment of the corresponding methanesulfonate **23** with tetra-*n*-butyl-ammonium bromide in tetrahydrofuran gives bromide **24**, which is converted into the corresponding Grignard reagent; oxidation of the latter intermediate with silver nitrate gives the

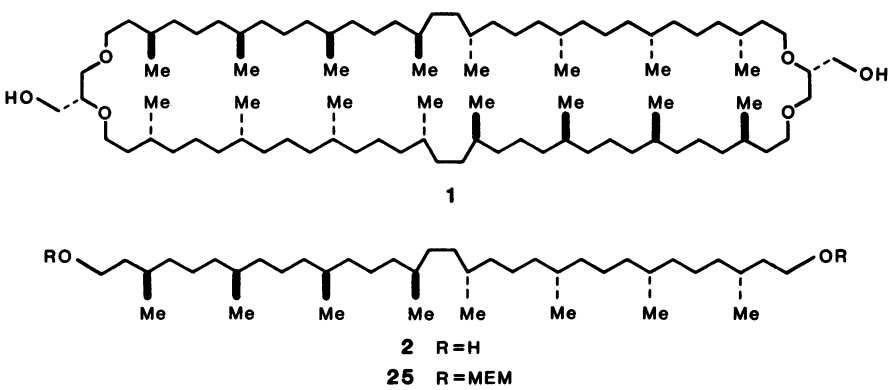


Fig. 1. Structures of a 72-membered-ring diglyceryl tetraether **1**, a C_{40} diol **2** that is its structural component, and the synthetic precursor to the diol **25**.

coupled product **25** (Fig. 1). Treatment of **25** with HBr in aqueous methanol yields diol **2**.

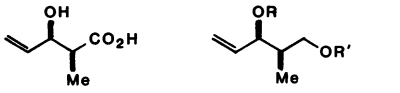
A sample of a biosynthetic form of **2** was obtained from *M. thermoautotrophicum*. A procedure similar to that described for diether **26** (*33*) was used to isolate tetraether **1** from freeze-dried cells. The tetraether was degraded (*11*) to yield **2**, which was purified by preparative thin-layer chromatography on silica gel (ethyl acetate:hexane, 3:7 by volume).

Because the C_{10} building block we used was a mixture of stereoisomers in the ratio of 94.83:4.47:0.66:0.03 (an enantiomeric ratio of 99.3:0.7 from the Evans alkylation and a diastereomeric ratio of 95.5:4.5 from the Claisen rearrangement, our sample of synthetic **2** should have been a mixture of diastereomers in the ratio 73.5:9.4:9.4:2.2:2.2. The major diastereomer has C_2 symmetry and should show only 20 resonances in its ^{13}C-NMR spectrum, whereas the minor isomers, which lack this symmetry, could show up to 40 resonances each. In fact, the spectrum of synthetic **2** consisted of a major set of 19 lines (*34*), the chemical shifts of which were identical with those of the resonances observed in the spectrum of **2** isolated from *M. thermoautotrophicum*.

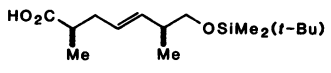
To test whether such stereoisomers can be distinguished by ^{13}C NMR, we measured the spectrum of a 1:1 mixture of (3*R*)- and (3*S*)-dihydrophytol, obtained by catalytic hydrogenation of commercial phytol. This mixture showed ten coincident resonances and ten pairs with chemical shift differences of 0.007 to 0.073 part per million (ppm) (*35*). Similar effects were seen for synthetic squalane. The resonances of several atoms in the squalane chain, including the four central carbons, appeared as a closely spaced group of peaks because the sample is a complex mixture of stereoisomers. Individual peaks within a group were separated by up to 0.1 ppm (a typical value was 0.02 ppm), and the stereocenter could affect carbons up to six bonds away (*36*).

Synthetic **2** showed $[\alpha]_D$ +1.9 ± 0.15° (*c*, 0.97 g/100 ml in $CHCl_3$), compared to $[\alpha]_D$ +4.8 ± 0.63° (*c*, 0.30 g/100 ml in $CHCl_3$) for the natural material. The archaebacterial C_{40} diol is thus the (3*R*,7*R*,11*R*,15*S*,18*S*,22*R*,26*R*,30*R*) diastereomer. Since the absolute configuration at the glycerol stereocenters of **1** has been established as *sn*-2,3-*sn*-2,3 (*18*) the full stereostructure of that compound is now known.

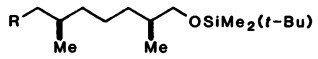
The elucidation of the full stereostructure of **1** may have biosynthetic implications. If the absolute configurations of all 16 methyl-bearing stereocenters were established by double bond reduction, it is likely that they would be the same, given the apparently primitive nature of the organisms. However, the nature of the



3 **4** R=R'=H
5 R=H, R'=SiMe$_2$(*t*-Bu)
6 R=EtCO, R'=SiMe$_2$(*t*-Bu)

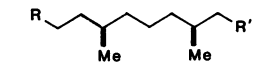


7
8 4,5 dihydro-**7**

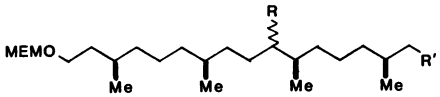


9 R=OH
10 R=OTs
11 R=CN
12 R=CHO

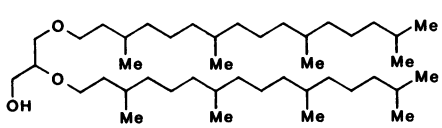
biosynthetic step that forms the 4′–4 linkage between C-16 and C-17 in **2** is not known, although it has been postulated that tetraether **1** may be formed from diether **26** (*37*), a common component of archaebacterial lipids, or that condensation may occur in the polar diether lipids rather than in the free diethers (*18*). In either case, the stereocenters at C-3, C-7, C-11, C-22, C-26, and C-30 in **2** would have the *R* configuration (*8*), whereas the stereochemistry at C-15 and C-18 would be established in the 4′–4 coupling and might be different.



13 R=OH, R'=OSiMe$_2$(*t*-Bu)
14 R=OMEM, R'=OSiMe$_2$(*t*-Bu)
15 R=OMEM, R'=HO
16 R=OMEM, R'=MeSO$_2$O
17 R=OMEM, R'=PhS
18 R=OMEM, R'=PhSO$_2$
19 R=MeSO$_2$O, R'=OSiMe$_2$(*t*-Bu)
20 R=I, R'=OSiMe$_2$(*t*-Bu)



21 R=PhSO$_2$, R'=OSiMe$_2$(*t*-Bu)
22 R=H, R'=HO
23 R=H, R'=MeSO$_2$O
24 R=H, R'=Br



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solution at 125.76 gauss (Bruker WP-500). Spectral parameters were chosen to achieve a digital resolution of 0.33 hertz (Hz); the natural resolution (width at half-height) was 0.6 Hz. Carbon type was determined by spin-polarization transfer experiments. Both synthetic and natural 2 and a 1:1 mixture of both gave signals (δ scale, in parts per million) of 19.680 (CH_3), 19.748 (CH_3), 19.777 (CH_3), 19.797 (CH_3), 24.356 (CH_2), 24.452 (CH_2), 24.473 (CH_2), 29.494 (CH), 32.789 (CH), 32.796 (CH), 33.045 (CH), 34.297 (CH_2), 37.301 (CH_2), 37.373 (CH_2), 37.396 (CH_2), 37.473 (CH_2), 37.542 (CH_2), 39.944 (CH_2), and 61.268 (CH_2). The intensity of the peak at 37.396 ppm suggests that it arises from the resonance of two carbons.

35. A 1:1 mixture of (3*R*)- and (3*S*)-dihydrophytol in $CDCl_3$ showed 28 signals at 125.76 gauss. The two peaks at 32.858 ppm and 37.515 ppm appear to result from the overlap of one resonance common to two isomers and a resonance unique to one isomer. If it is assumed that these two peaks do arise from such overlap, the following 30 resonances may be assigned (δ scale, in parts per million): 19.681 and 19.746, 19.774 and 19.847, 19.823, 22.701, 22.797, 24.436 and 24.443, 24.535 and 24.557, 24.873, 28.046, 29.566 and 29.586, 32.858, 32.858 and 32.871, 37.354, 37.389 and 37.451, 37.515 and 37.529, 37.560 and 37.570, 39.437, 40.017 and 40.102, and 61.281. A single number refers to a peak that results from the coincident resonance of corresponding carbons in the isomers, and a pair of numbers refers to two peaks, each approximately half the height of the taller ones, that result from the separate resonances of corresponding carbons.
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Chronology of Guitarrero Cave, Peru

Abstract. *Dating by accelerator mass spectrometry of wooden artifacts, cord, and charcoal samples from Guitarrero Cave, Peru, supports the antiquity of South America's earliest textiles and other perishable remains. The new dates are consistent with those obtained from disintegration counters and leave little doubt about the integrity of the lower Preceramic layers and their early cultivars. Reevaluation of the mode of deposition suggests that most of the remains resulted from short-term use of the cave in the eighth millennium B.C., with a possible brief human visit as early as 12,560 years ago.*

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At Guitarrero Cave, in a high Peruvian mountain valley, unusually dry conditions permitted recovery of a wide range of artifacts, including textiles, wood, bone, and domesticated plants (*1*), that were previously unknown for such an early period in South America (10,000 years ago). However, conventional radiocarbon age determinations made by three laboratories (1968 to 1973) left the Guitarrero stratigraphy and chronology unresolved (*2–4*). Accelerator mass spectrometry (AMS) makes possible the direct dating of minute samples of rare organic artifacts and cultivars, removing all question of their association with charcoal samples. Our analysis shows that the principal use of Guitarrero Cave, from which most of the remains resulted, occurred between 9,500 and 10,000 years ago.

The new dates were obtained by means of a tandem electrostatic accelerator as described (*5*). All samples were treated with acid to remove carbonates and with alkali to remove humic acids. Cellulose was extracted from wood and textiles by bleaching them with sodium chlorite. Excellent preservation allowed the use of small samples; for example, 60 to 70 mg of cord yielded 15 to 20 mg of purified carbon for combustion.

Charcoal from woody plants is the traditionally perferred material for radiocarbon dating. The Guitarrero charcoal samples, presumably from dispersed hearths, yielded a straightforward chronology when they were first tested by Geochron (Table 1). Samples GX 1778 and GX 1779 were out of apparent stratigraphic order, but their dates were within 2 standard deviations of counting error. Only the date of 12,560 years ago, in precedence of generally accepted dates for North American artifacts, was controversial. Dates determined from a verification series of Complex I charcoal conducted at the Smithsonian Institution laboratory were considerably younger than GX 1859 and equal to or younger than GX 1779 (Table 1). These results were consistent with both the previously determined dates and the stratigraphy of Complex I. The dates on Complex I, all internally consistent, were weighed equally when the site was described in 1980 (*1*).

The Smithsonian dates on Complex II, in grid square B1/A2 (Fig. 1), form a consistent series of five determinations (Table 1). However, the Smithsonian date on Complex IIa in grid square B1/A2 (9,580 years ago) is nearly 1,000 years more recent than the Geochron date of 10,535 years ago, whereas the Smithsonian date on Complex IIa in grid square C6 is essentially identical to the two Geochron dates. The Smithsonian date on Complex IIe overlaps the Geochron dates at 2 SD of counting error. As with the Geochron dates, there was only one stratigraphic inconsistency—that between the dates for samples SI 1502 and SI 1499 and those for Complex I. Because the relation between Complex IIa in grid square C6 and in B1/A2, which is more than 6 m distant (see Fig. 1), is based on interpolation, the discrepancy in apparent age is not extraordinary.

Despite the long chronology based on the Geochron dates (12,560 to 7,575 years ago), the shorter Smithsonian series (10,240 to 8,175 years ago), and Lynch's attempt to reconcile the dates (*1*), Vescelius (*4*) proposed as few as two brief occupations, one 10,000 years ago and another around 7,900 years ago, each lasting for perhaps a single generation. Mixture of charcoal from the two brief Preceramic occupations and intrusion of modern organics from the Christian era, when the cave was reused, would explain all discordant dates. The new dates from Oxford support the proposal of a brief occupation rather than steady use over several millennia, but they do not show contamination of Complex II with modern artifacts. Further, they do not support Patterson's reorganization of the stratigraphy nor his assumption of technological progression from unifacial to bifacial industries (*3*).

The Oxford dates from grid squares B1/A2 and B2N1/2 are all on charcoal and are uniform from top to bottom. The pooled mean age of these seven samples is 9,425 ± 55 years, all dates being effectively the same as judged from the procedures of Ward and Wilson to determine the mean, test statistic, and variance (*6*). The agreement with the five Smithsonian results on Complex I and the lower part of Complex II is excellent. Only sample SI 1501 is seriously divergent. However, as with Geochron sample GX 7575, SI 1501 might be contaminated with Ceramic age charcoal from mixed Complex IV. Both of these conventional dates came from samples composed of several pieces of charcoal, one of which might have been recent.

The accelerator dates support the antiquity of the Guitarrero artifacts (*1*). Moreover, a wood dowel from a Preceramic context in grid square B6 that