Olefin dimerization has been observed for the $(C_5Me_5)Ta(olefin)Cl_2$ system[16] but not for the Ti complex $(C_5Me_5)_2Ti(ethylene)$.[2] Full reactivity of **2** will be reported elsewhere.

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**Supplementary Material Available:** Experimental details and spectral data for all compounds, crystal data, and lists of positional and thermal parameters (11 pages); listing of observed and calculated structure factors for **2** (13 pages). Ordering information is given on any current masthead page.

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## Stereochemistry of the Biosynthesis of *sn*-2,3-*O*-Diphytanyl Glycerol, Membrane Lipid of Archaebacteria *Halobacterium halobium*

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One of the most striking and characteristic differences of archaebacteria from other evolutionary diverged eubacteria and eukaryotes is the stereostructure of a unit lipid of the cell membrane, *sn*-2,3-*O*-dialkylated glycerol, having, when present, a polar head group on the *sn*-C-1 position.[1,2] Eubacteria and eukaryotic cells mostly contain antipodal *sn*-1,2-*O*-diacyl glycerol as a major lipid. Biochemical pathway concerning to this intriguing stereochemical divergence has yet to be uncovered. This paper deals with the cryptic stereochemistry of glycerol incorporation into the archaebacterial lipid studied by tracing stereospecifically deuteriated glycerol and demonstrates for the first time that stereochemical inversion takes place at the C-2 position of glycerol.

Biosynthetic studies on the lipid and related metabolite have been reported recently by using two classes of archaebacterial strains, i.e., halophilic *Halobacterium cutirubrum*[3] and extreme acidothermophile *Sulfolobus* sp. (*Caldariella acidophila*).[4] The latter actually contains an interesting 72-membered ring structure of biphytanyl diglycerol tetraether as a principal membrane lipid which can also be classified in the *sn*-2,3-*O*-dialkylated glycerol family.[5] In either case, glycerol was reported to be incorporated efficiently into the membrane lipid,[3,4] and all the hydrogens of glycerol except hydroxyl groups were reported to be retained in the biosynthesis of the lipid in *Sulfolobus* sp.[3] If, as emphasized previously,[2,4,6] formation of the ether linkages might take place between glycerol or its derivative and prenyl pyrophosphate,

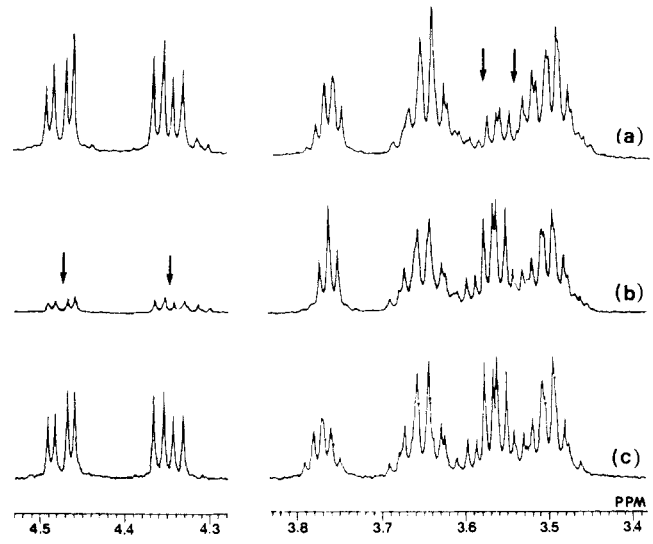


**Figure 1.** $^1H$ NMR spectra (500 MHz, $CDCl_3$ solvent, TMS reference) of benzoylated lipids: (a) the lipid obtained by feeding of (*S*)-[1,1-$^2H_2$]glycerol, (b) the lipid obtained by feeding of (*R*)-[1,1-$^2H_2$]glycerol, and (c) the unlabeled control.

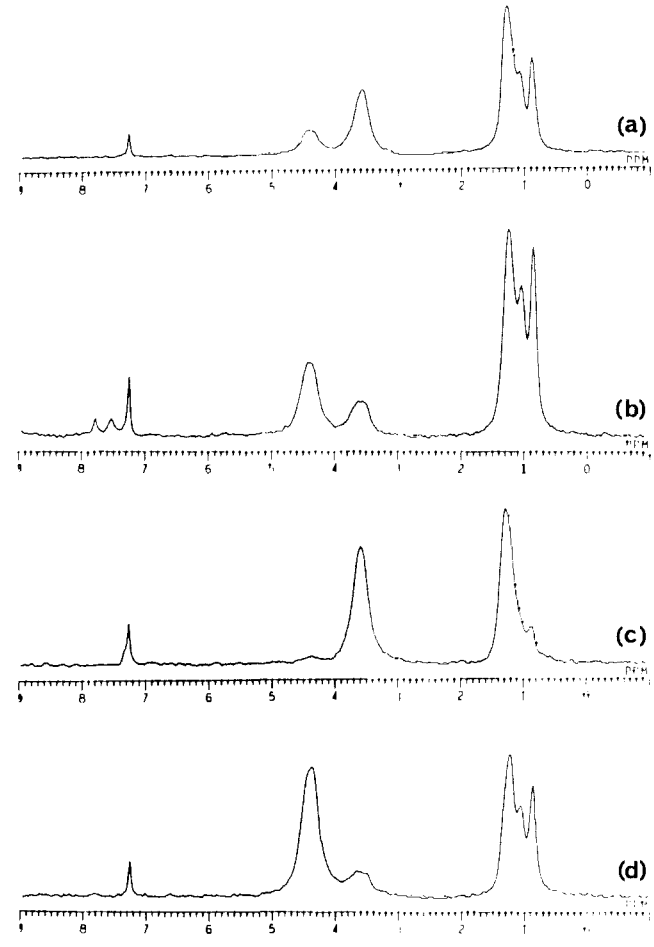


**Figure 2.** $^2H$ NMR spectra (61.48 MHz, $CHCl_3$ solvent, natural abundance $^2H$ signal of $CHCl_3$ was used for a chemical shift standard, $\delta$ = 7.26 ppm) of benzoylated lipids obtained by feeding of (a) racemic [1,1-$^2H_2$]glycerol, (b) (*R*)-[1,1-$^2H_2$]glycerol, (c) (*S*)-[1,1-$^2H_2$]glycerol, and (d) D-[6,6-$^2H_2$]glucose.

stereochemical inversion would not occur at the C-2 position of glycerol. Alternatively, antipodal stereochemistry might arise from stereochemically opposite phosphorylation or other activation of glycerol to the case of eubacteria or eukaryotes.

Separate feeding of chemically synthesized (*RS*)-[1,1-$^2H_2$]-glycerol, (*R*)-[1,1-$^2H_2$]glycerol, and (*S*)-[1,1-$^2H_2$]glycerol to the

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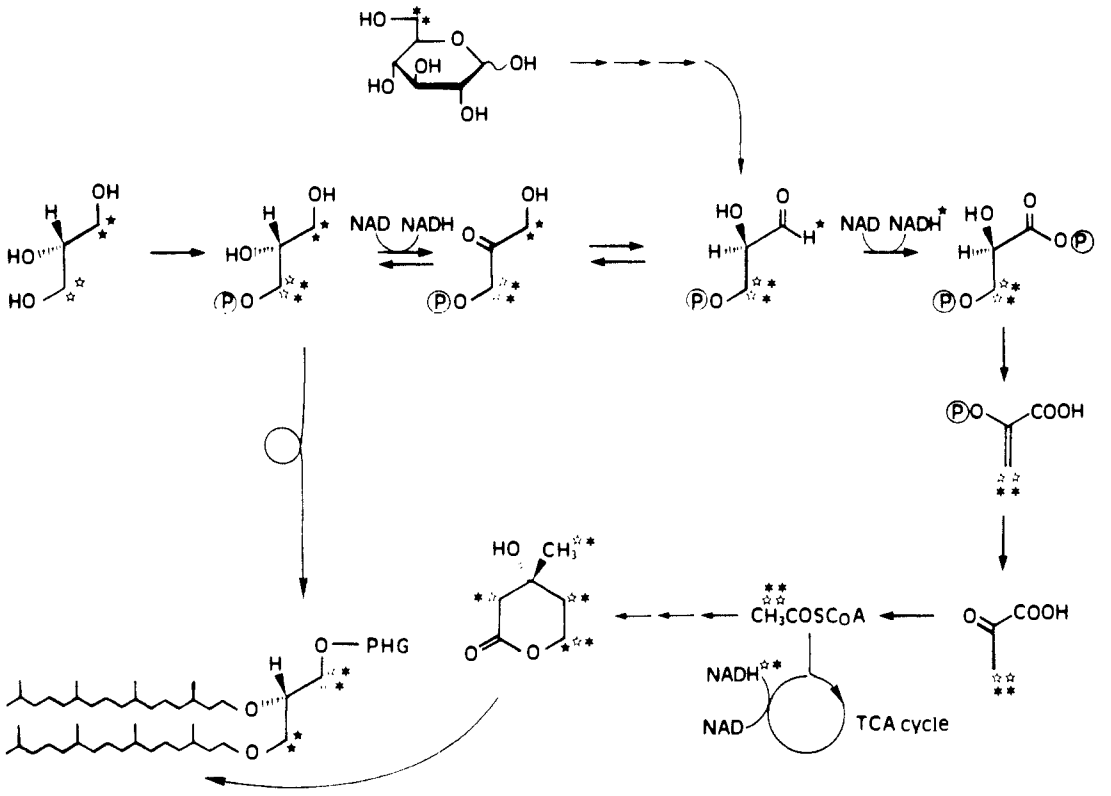
**Scheme I.** Biosynthetic Pathway of 2,3-Di-*O*-phytanylglycerol Lipid in *Halobacterium halobium*[a]




[a] Symbols ★, ☆, and * represent deuterium used for labeling experiments. PHG denotes *p*olar *h*ead *g*roup in the cell membrane. Symbols of deuterium in the phytanyl chains are omitted for clearity.

culture of *Halobacterium halobium* IAM 13167, followed by isolation of the lipid by known procedures,[2,3] yielded three isotopically labeled samples of *sn*-2,3-*O*-diphytanyl glycerol, which were then benzoylated to differentiate the NMR chemical shifts of the C-1 and C-3 methylene groups of the glycerol moiety. The $^1H$ and $^2H$ NMR spectra of these labeled lipid were recorded at 500 MHz and at 61.48 MHz, respectively,[7] and pertinent spectra are shown in Figures 1 and 2.

Signals marked by arrows in Figure 1 indicated significant decrease of intensity compared with those of unlabeled lipid, and this clearly visualized efficient and stereospecific incorporation of deuterium atoms into the C-1 position of the lipid from (*R*)-[1,1-$^2H_2$]glycerol and into the C-3 position from (*S*)-[1,1-$^2H_2$]glycerol, respectively. Enrichment ratio was estimated on the basis of relative decrease of a signal in question to an integration intensity of a benzoyl proton as an undeuteriated intramolecular control. Thus, the C-1 position of the lipid obtained by the feeding of (*R*)-[1,1-$^2H_2$]glycerol was about 79% enriched, and, in the case of (*S*)-isomer, enrichment at C-3 was approximately 68%. Also noted by these $^1H$ NMR studies was that no specific exchange of a single deuterium took place from methylene groups in both cases.

This labeling pattern was further supported by the $^2H$ NMR spectra. Deuterium signals due to labeling from (*RS*)-[1,1-$^2H_2$]glycerol were observed at 4.40 and 3.55 ppm (intensity ratio ca. 1:3), suggesting that both methylene groups of the glycerol moiety were incorporated intact. The observed higher intensity of the latter signal suggested deuterium incorporation into the oxymethylene moiety of the phytanyl chains. This argument was supported by the fact that phytanyl iodide obtained by treatment of the biosynthesized lipid with HI showed a deuterium signal for the iodomethyl group at 3.20 ppm.[8] Provided that the mevalonate pathway in *H. halobium* is similar to that of eubacteria and other cells, the oxymethylene moiety of the phytanyl chain is derived from the carboxyl group of acetate. Therefore, this deuterium incorporation may be ascribable to the reduction process in the mevalonate pathway by the deuteriated NADH formed presumably from deuteriated acetyl CoA in the TCA cycle. Labeling of the methyl groups and other portions of the phytanyl moieties were also indicated by the signals at 0.86, 1.10, and 1.25 ppm.

The labeling pattern from (*R*)-[1,1-$^2H_2$]glycerol was rather similar to that from racemic glycerol. A significant difference that the signal intensities at 4.40 and 3.60 ppm were reversed suggested stereospecific incorporation of deuterium into the C-1 methylene group of glycerol of the lipid and a lower level of deuteriated NADH in the nicotinamide cofactor pool. The fact that methyl groups of the phytanyl chains were efficiently labeled clearly demonstrates that the *sn*-C-3 of glycerol is metabolized to the methyl group of acetyl CoA as in eubacteria and other organisms as shown in Scheme I.

Complementary results were obtained by labeling from (*S*)-[1,1-$^2H_2$]glycerol. Thus, in the glycerol moiety, deuterium was stereospecifically incorporated into the C-3 position (3.60 ppm). The methyl signals were not enriched in this case. The very weak signal may be due to partial scrambling. This result further supports the proposal that *sn*-C-3 carbon of glycerol is metabolized to the methyl group of acetyl CoA accompanied by a loss of the *sn*-C-1 carbon by decarboxylation. Labeling of other positions of the phytanyl chains may again be explained by formation of deuteriated NADH, among others, at the oxidation step of D-glyceraldehyde-3-phosphate into D-glycerate 1,3-bisphosphate.

To compare the stereochemical outcome of glycolysis in *H. halobium* with that of eubacteria and eukaryotes, similar feeding experiments with D-[6,6-$^2H_2$]glucose were also undertaken. The efficient incorporation of deuterium into the benzoyloxy methylene moiety was indicated by the observed signal at 4.40 ppm in the $^2H$ NMR spectrum and by diminished signals at 4.35 and 4.47 ppm in the $^1H$ NMR spectrum, and the phytanyl chains were labeled similarly to the (*R*)-[1,1-$^2H_2$]glycerol feeding. These observations argue that the glucose–glycerol and acetate–meva-

(7) The $^1H$ NMR signals of the glycerol moiety of the benzoylated lipid were unambiguously assigned by spin decoupling experiments as well as by homonuclear COSY spectroscopy.

(8) Also observed were intense signals at 0.86, 1.07, and 1.27 ppm.

lonate pathways in *H. halobium* are identical with those in other organisms.

As discussed above, the cryptic stereochemistry involved in the incorporation of glycerol into the membrane lipid of *H. halobium* was firmly established. In contrast to eubacteria and eukaryotes, it is the *sn*-C-1 methylene group of 2,3-di-*O*-phytanyl glycerol that is derived from the *sn*-C-3 carbon of glycerol and the C-6 carbon of D-glucose. Although stereochemically feasible, a pathway including reduction of D-glyceraldehyde-3-phosphate can be ruled out, since the present as well as previous studies clearly showed that no hydrogen was lost from either hydroxymethyl group of glycerol.[3,4] Furthermore, the ether-forming reaction is also different from that of *O*-alkyl dihydroxyacetone phosphate in mammalian cells,[9,10] in which a hydrogen of the *sn*-C-1 methylene group is stereospecifically replaced. Consequently, the most likely mechanism of formation of *sn*-2,3-*O*-diphytanyl glycerol in *H. halobium* apparently includes a unique stereochemical inversion at the C-2 carbon of glycerol as shown in Scheme I.

**Acknowledgment.** Proton and deuterium NMR spectra were recorded through the courtesy of Dr. M. Uramoto, Institute of Physical and Chemical Research, and Professor H. Seto and Dr. K. Furihata, the University of Tokyo, to whom we are grateful.

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## $[S_4Cd_{17}(SPh)_{28}]^{2-}$, the First Member of a Third Series of Tetrahedral $[S_wM_x(SR)_y]^{z-}$ Clusters

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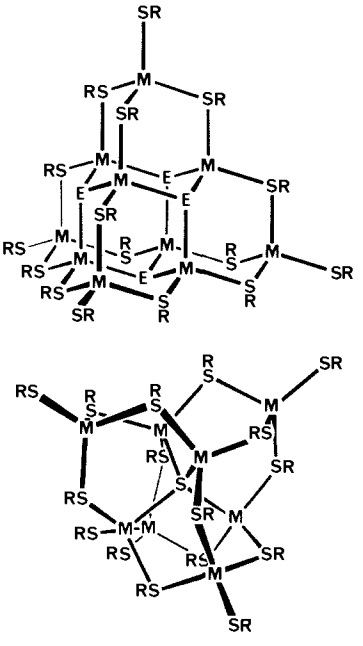
The largest $[S_wM_x(SR)_y]^{z-}$ molecular aggregate structure reported to date is $[E_4M_{10}(SPh)_{16}]^{4-}$ (E = S, Se; M = Zn, Cd) (**1**).[1] With four fused adamantanoid cages, **1** is structurally congruent



with a tetrahedral fragment of the nonmolecular cubic (sphalerite) metal chalcogenide lattice, and therefore it is possible to conceive

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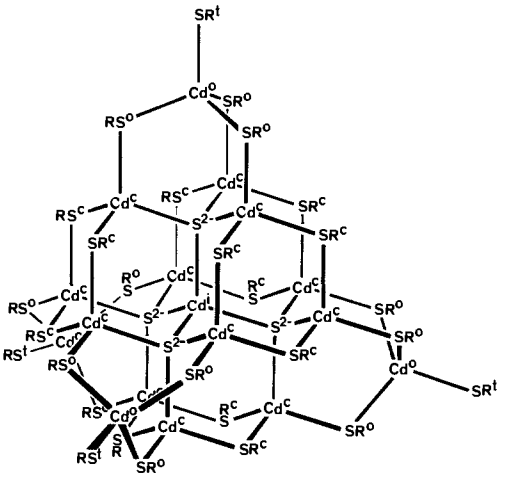

**Figure 1.** The idealized structure of **6**. The $Cd^i(S^{2-})_4$ center is connected to the $(Cd^c)_{12}$ cuboctahedron, bridged by a $(\mu\text{-}SR^c)_{12}$ tetratruncated tetrahedron, and with four tripodal $(\mu\text{-}SR^o)_3Cd^o(SR^t)$ caps.

from **1** an infinite series of stereochemically legitimate (but still hypothetical) macromolecules, based on enlarged tetrahedral cores.[2] Subsequent members of this infinite series, denoted $\mathbf{2}^{[n]}$ (where $n$ is the number of layers of M atoms), are $[E_{13}M_{20}(SR)_{22}]^{8-}$ ($\mathbf{2}^{[4]}$) and $[E_{28}M_{35}(SR)_{28}]^{14-}$ ($\mathbf{2}^{[5]}$). At $n = 7$, $[E_{80}M_{84}(SR)_{40}]^{32-}$, the size (edge length of tetrahedral core $\approx 28$ Å) reaches that of the smallest characterized colloidal particles of CdS, which appear to be an irregular fragment of the cubic lattice.[3] The size regime in which the electronic characteristics of CdS change from bonds to bands is 20–100 Å.[3a] We report here the formation and structures of additional and larger $[S_wM_x(SR)_y]^{z-}$ macromolecules, which are the prototypes of two additional extended series of aggregates which could also intersect the size regime of colloidal metal chalcogenides and relate to their geometrical and electronic structures and reactivities.

A distinctly different macromolecular structure, **3**, is also known. The connectivity pattern comprised of a central ligand, a bitetrahedron of four inner and four outer metal atoms, and 12 doubly bridging thiolate ligands, occurs in $[ClZn_8(SPh)_{16}]^{-}$[4] and in $\{SCd_8(SBu^s)_{12}\}(CN)_{4/2}$, **4**, where the aggregates are linked three dimensionally in the crystal by CN ligands as interaggregate bridges between outer metal atoms.[5,6] A second infinite series of macromolecules, $\mathbf{5}^{[n]}$,[7] can be conceived with **4** as the first member. These have tetracapped tetrahedral-core topology, with barrelanoid cages at the core/cap interfaces: the compositions of the next two members of the series are $[S_{16}M_{26}(SR)_{28}]^{8-}$ ($\mathbf{5}^{[2]}$) and $[S_{50}M_{60}(SR)_{40}]^{20-}$ ($\mathbf{5}^{[3]}$).

The $^{113}Cd$ NMR spectra of various reaction mixtures designed to synthesize cadmium complexes in series **2** and **5** have revealed the formation of at least three new polycadmium products. Recrystallization of the mixed products of reaction of PhSH, $Et_3N$, $Cd^{2+}$, $Na_2S$, and $Me_4NCl$ in methanol/acetonitrile[8] yields

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(6) Cubic space group $Pn3$, $a = 15.928$ (2) Å, 1196 unique reflections (Mo K$\alpha$), $R = 0.033$. The crystal structure is the $Cu_2O$ (or $Cd(CN)_2$) type, with interpenetrating unconnected lattices each composed of tetrahedral $\{SCd_8(SBu^s)_{12}\}$ clusters linked at the four outer Cd atoms by linear CN ligands.
(7) The size index $n$ is here the number of layers of $MS_4$ tetrahedra in the core: the total number of M atoms is $(n + 1)(n + 2)(5n + 3)/6$.
(8) Preparation of **6** was by treatment of a solution of PhSH (80 mmol) and $Et_3N$ (80 mmol) in acetonitrile with $Cd(NO_3)_2$ (50 mmol) in acetonitrile and $Na_2S$ (20 mmol) in methanol, with alternating additions of the latter two reagents, followed by addition of $Me_4NCl$ (18 mmol) in methanol. The colorless crystalline precipitate was recrystallized from hot $CH_3CN$ to yield **6** as colorless block crystals, which gave satisfactory elemental analysis: yield $\approx$ 20%.

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