 

# Observation of the Pathway from Lysine to the Isoprenoidal Lipid of Halophilic Archaea, *Halobacterium halobium* and *Natrinema pallidum*, Using Regiospecifically Deuterated Lysine

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We examined the incorporation of lysine into archaeal isoprenoidal lipids of halophilic archaea, *Natrinema pallidum* and *Halobacterium halobium* using two regiospecifically deuterium-labeled derivatives, [3,3-$^2H_2$] and [6,6-$^2H_2$]lysines. The two deuterated lysines were synthesized, and the incorporation of deuterium to the lipid core was defined by $^2H$ NMR. The results revealed that lysine is degraded to crotonoyl-CoA by the decarboxylation of carboxylate in the metabolism of halophilic archaea, much like the metabolism of lysine in aerobic bacteria; the process converts lysine to isoprenoidal lipids via the mevalonate pathway through glutaryl-CoA, crotonoyl-CoA, and acetoacetyl-CoA.

Archaea (archaebacteria) live in relatively hostile environments, such as those characterized by low pH (2–3), high temperature (~110 °C), completely anaerobic conditions, or high salt concentration. They can now be clearly distinguished from eucarya and eubacteria from the 16S ribosomal RNA sequences, the cell-wall structure, and the membrane lipid patterns.[1,2] Many investigators regard the archaea as the evolutionarily oldest among living organisms based on their molecular biological characteristics, while others insists archaea evolved from existing microorganisms as a way of adapting to their living environments. Among archaea, halophilic archaea live in an environment such as a medium with nearly saturated NaCl concentration. The existence of an unusual ether lipid in archaea is one of the clearest distinctions between archaea and other organisms. Distinct from the lipids of eucarya or eubacteria, the lipids of halophilic archaea characteristically consist of saturated $C_{20}$ (and $C_{25}$) isoprenoids bonded with glycerol by an ether-linkage (**1** and **2**), as shown in Fig. 1.[3]

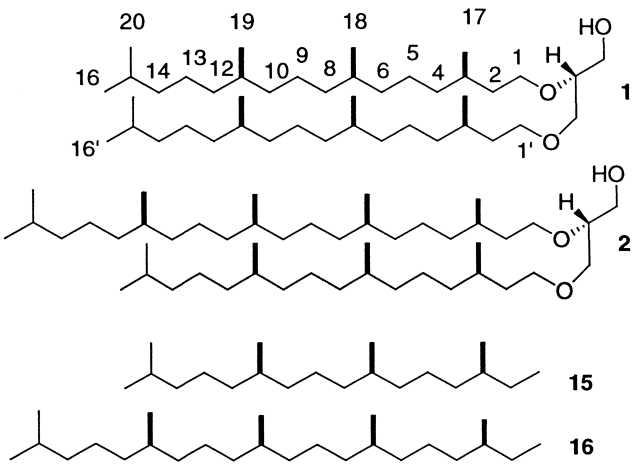


Fig. 1. Structure of liquid core and related hydrocation fragment in halophilic archaea.

The entire outer sphere of archaea consists of isoprenoid-based biomembrane lipids. The isoprenoidal diether lipid core is completely absent in membrane lipids of eubacteria and eucarya. Therefore, the biosynthesis of isoprenoidal lipid in archaea has intrigued many scientists, who have investigated its structural analysis in biosynthetic studies.[3] Kates et al. have shown the incorporation of radiolabeled mevalonate in diether lipid,[4] and Kakinuma et al. recently reported the incorporation of [$^2H_9$]mevalonate in halophilic archaea.[5] These results suggest that isoprenoid was biosynthesized from acetate to mevalonate, a ubiquitous biosynthetic precursor of various types of isoprenoid. They also suggest that a mevalonate-independent pathway,[6] from which many parts of bacterial secondary metabolites and plant secondary metabolites (included in terpenoids and pigments, respectively) have been shown to be biosynthesized, was not involved in these archaea. However, Smith et al. pointed out that $^{13}C$-labeled acetate was not incorporated in the branched methyl and methine carbon of the isoprenoidal lipid in *Halobacterium salinarium* NRC34001.[7] Our recent incorporation experiments for the halophilic archaea *Natrinema pallidum*, using [3-($^2H_3$)methyl]-mevalonolactone and [$^{13}C$]labeled acetate,[8] suggested that some metabolic pathways from amino acid to mevalonate, in keeping with Smith's proposal,[7] cooperate with the usual pathway from acetate to mevalonate.

Smith's result and our result show a strong correlation of lysine metabolism and the mevalonate pathway. In typical microorganisms, it has been shown that lysine is degraded to cro-

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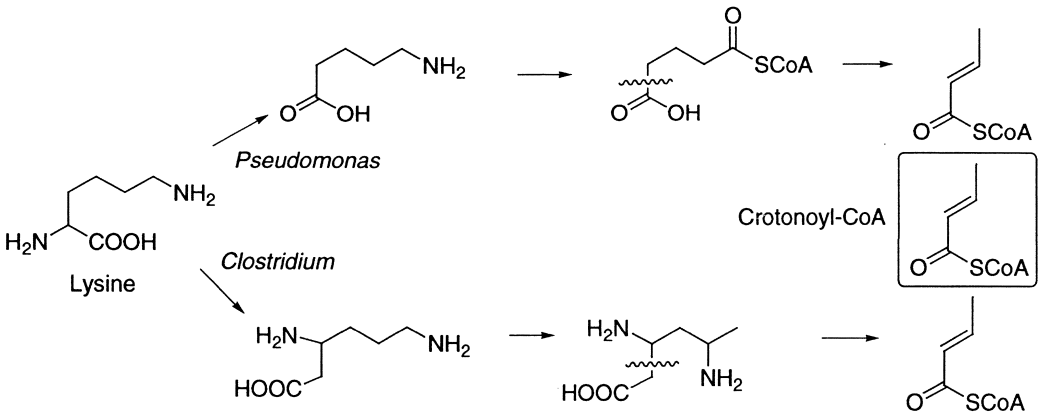
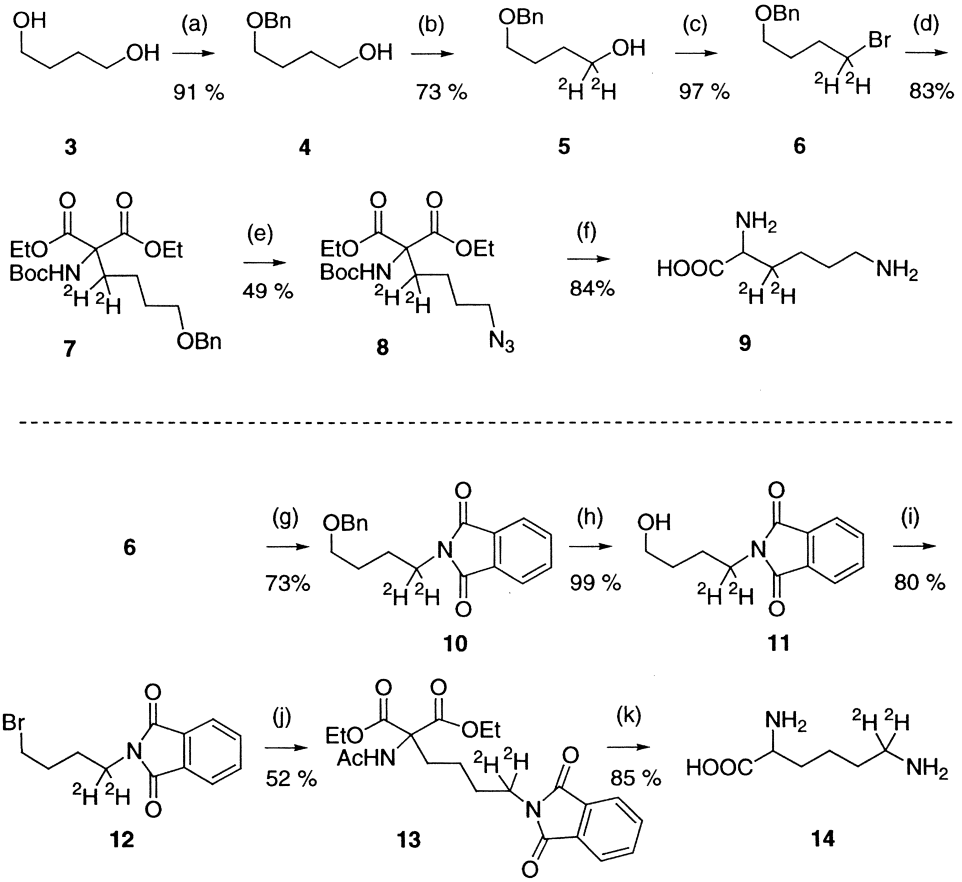



Scheme 1. The two metabolic pathway of lysine in microorganisms.




(a) (1) PhCHO, $H^+$ ; (2) $LiAlH_4$ - $AlCl_3$ ; (3) Jones oxidation. (b) (1) MeOH , $H^+$ ; (2) $LiAl^2H_4$; (c) $CBr_4$, $PPh_3$ ; (d) diethyl N-Boc-aminomalonate, NaOEt. (e) (1) $H_2$/ Pd-C; (2)TsCl ; (3) $NaN_3$. (f) (1) $H_2$/ Pd-C (2) HCl; (g) K-phthalimide (h) $H_2$/ Pd-C ; (i) $CBr_4$, $PPh_3$ ; (j) diethyl N-Ac-aminomalonate, NaOEt; (k) HCl

Scheme 2. Synthesis of [3,3-$^2H_2$] and [6,6-$^2H_2$]Lysines.

tonoyl-CoA by a rearrangement of the amino group with lysine 2,3-aminomutase (*Clostridium*),[9] or by the decarboxylation of carboxylate (*Pseudomonas*), as shown in Scheme 1.[10] In archaea, however, it is unclear which pathway exists, or if both exist, which is predominant. In order to eradicate this ambiguity, we investigated the metabolic pathways using regiospecifically deuterium-labeled lysine. We herein report on our results.

### Synthesis of [3,3-$^2H_2$] and [6,6-$^2H_2$]Lysines

The fatty acid content in halophilic archaea is very low; however, researchers have observed the existence of fatty acid

synthase (FAS) activity and the inhibition of its activity in high salt concentration.[3,11] This may indicate that a part of the intermediates in fatty acid biosynthesis would be converted to isoprenoid biosynthesis in archaea. Initially, we thought that our observation and Smith's result could be explained by the link of the known pathway for a microorganism in lysine metabolism to the intermediate of fatty acid biosynthesis. Thus, one of the pathways between the above-mentioned two paths of lysine degradation would be relatively active in halophilic archaea. The intermediate in the lysine-degradation pathway, crotonoyl-CoA, is converted to mevalonate and isoprenoid through the mevalonate pathway, which is the main pathway in the biosynthesis of the isoprenoidal lipid core in halophilic archaea.

If the predominant catabolism of lysine in the archaea was via lysine 2,3-aminomutase, the hydrogen at C-3 of lysine was finally lost at the crotonoyl-CoA, and C-6 of lysine was converted to the terminal methyl group. If, on the other hand, the predominant catabolism was via decarboxylation at C-1 of lysine, the hydrogen at C-6 of lysine was finally lost at the crotonoyl-CoA, and C-3 of lysine was converted to the terminal methyl group and C-2 of crotonoyl-CoA. To discriminate between the two, [3,3-$^2H_2$]lysine and [6,6-$^2H_2$]lysine were synthesized. Several reports on the synthesis of 3- and 6-labeled lysine have appeared in the literature recently.[12] Among them, Aberhart and Gould et al. synthesized [3,3-$^2H_2$]lysine and stereoselectively 3-deuterated lysine, and used them as a probe in work concerning the mechanistic implication of the stereochemistry of lysine 2,3-aminomutase from *Clostridium* and the biosynthesis of streptothricin.[13] Their method appears to be convenient for large-scale preparations. According to their concept concerning the preparation, the $C_4$ units for [3,3-$^2H_2$]lysine and [6,6-$^2H_2$]lysine were synthesized and coupled with protected aminomalonate,[14] as shown in Scheme 2. The $C_4$ unit for synthesizing the two-labeled amino acid, the same $C_4$ component **4**, which was easily prepared from an inexpensive starting material, 1,4-butanediol (**3**), was selected and prepared. In the synthesis of [3,3-$^2H_2$]lysine, **4** was converted to bromide **6** and coupled with diethyl *t*-butoxycarbonylaminomalonate to yield amide **7**. The terminal protection group at **7** was deprotected and converted to azide **8**. Finally, catalytic hydrogenation and acid hydrolysis of **8** gave [3,3-$^2H_2$]lysine **9**. Next, in the synthesis of [6,6-$^2H_2$]lysine **14**, **4** was converted to phtalimide **10**, and the benzyl-protecting group of **10** was deprotected to give **11** and further converted to bromide **12**. Then, **12** was coupled with diethyl acetamidomalonate to give **13**, which was finally deprotected to give **14**. The isotopic enrichment of the product was $> 95\%$, as determined from its $^1H$ NMR spectrum.

### Incorporation of [3,3-$^2H_2$] and [6,6-$^2H_2$]Lysines

Incorporation experiments of *Halobacterium halobium* (which contains **1** as a lipid-core component) and *Natrinema pallidum*[15] (which contains **1** and the $C_{20}$–$C_{25}$ diether **2**) were performed as reported previously.[8] In brief, *Halobacterium halobium* JCM 9120 and *Natrinema pallidum* IAM 13147 were incubated for 6 days at 37 °C in 300 mL of a medium containing 250 mg of **9** or **14**. Lipids were extracted, then hydrolyzed with methanolic HCl. A mixture of diethers **1** and **2** was separated from the remaining mixture of the other hydrolyzate.

The resulting lipid core **1** and **2** were identified by $^1H$ and $^{13}C$ NMR, respectively. Because of the low incorporation of deuterium, no significant difference was caused by deuterium incorporation on the $^1H$ and $^{13}C$ NMR spectra. The determination of the position of deuterium was examined by $^2H$ NMR (61.4 MHz, $CDCl_3$ standard (7.26 ppm)). The $^2H$ NMR spectra of each lipid obtained from a feeding experiment along

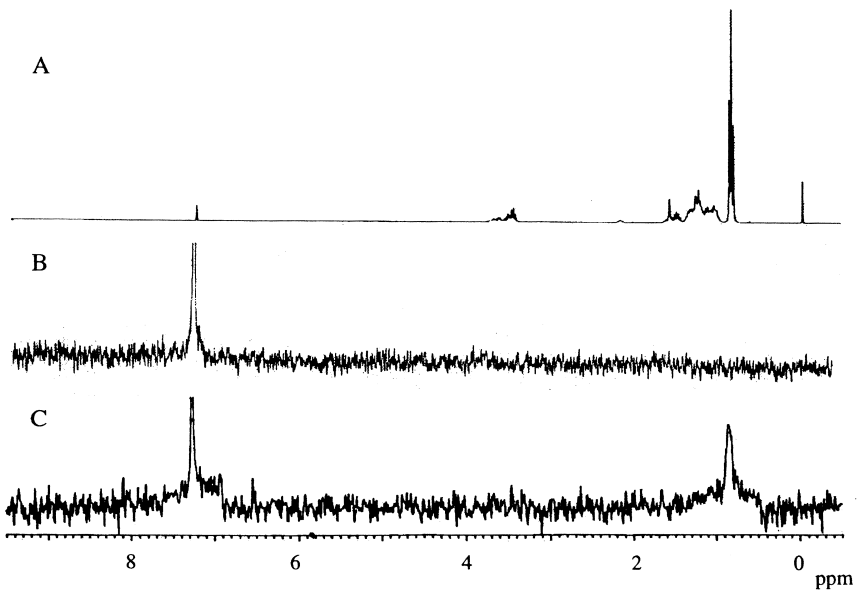
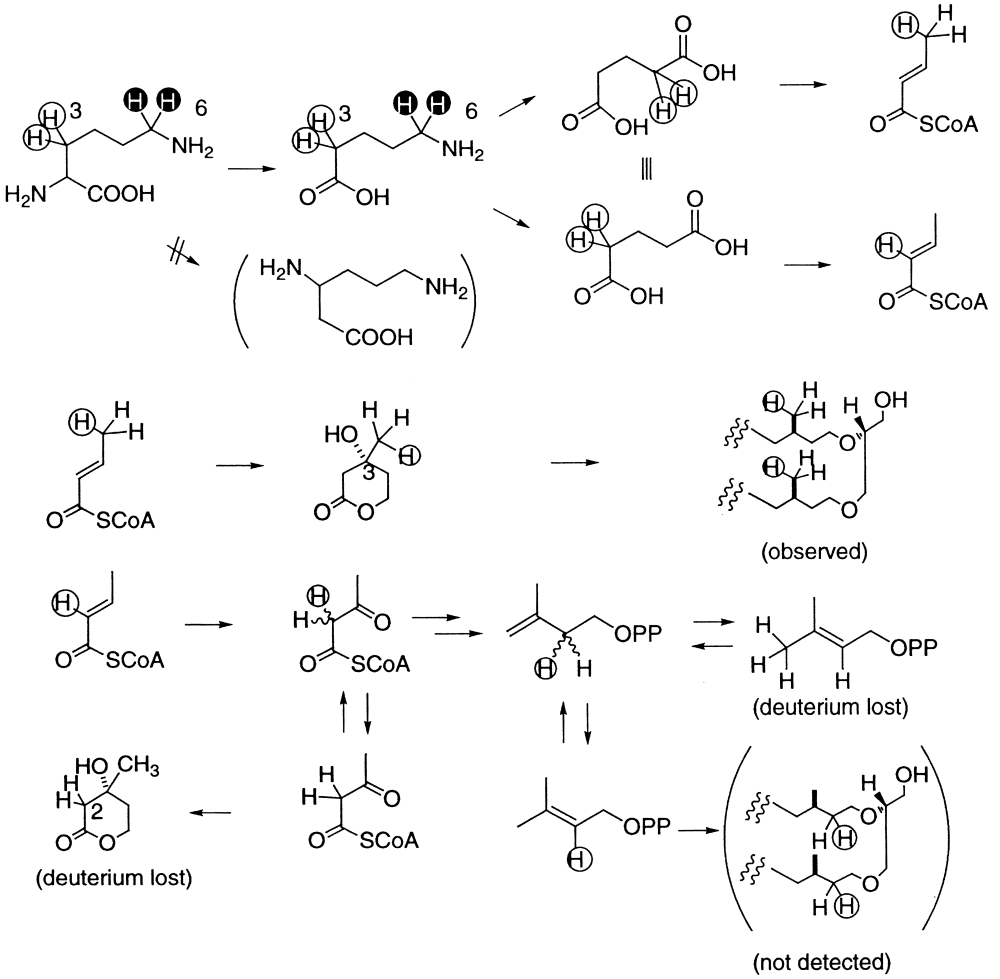



Fig. 2. $^1H$ NMR spectrum and $^2H$ NMR spectra of **1** and **2** from *Natrinema pallidum*: (A) ($^1H$) non-labeled standard; (B) ($^2H$) the sample derived from [6,6-$^2H_2$]-lysine-incubation experiment; (C) ($^2H$) the sample derived from [3,3-$^2H_2$]lysine-incubasion experiment. Similar result was obtained from **1** of *Halobacterium halobium*.




Scheme 3. Proposed pathway from lysine to mevalonate in halophilic archaea.

with the $^{1}$H NMR of non-labeled lipid diether are shown in Fig. 2. No significant $^{2}$H was incorporated in the lipids afforded by these bacteria cultivated in a **14**-containing medium (Fig. 2, B). On the other hand, a significant $^{2}$H signal at the non-substituted methyl region ($\delta$ 0.81) was observed in the NMR spectrum of the lipids obtained by incubation using a medium containing **9** (Fig. 2, C). The signal corresponded to hydrogens at branched methyl in isoprenoidal lipids. No other deuterium signal was observed in the $^{2}$H NMR spectrum. Further, the lipid core was degraded by HI and reduced to hydrocarbons **15** and **16**; then, GC-MS analysis was performed. The mass spectra of **15** and **16** showed a slight incorporation of deuterium from a sample in the feeding experiment of **9** (ca. 3% deuterium incorporation). No incorporation of deuterium was observed in the hydrocarbon fraction from a sample in a feeding experiment of **14**. The result was that the incorporation of deuterium occurred in the isoprenoidal portion.

These results indicated that the C-3 carbon (and hydrogen) in lysine was converted to a branched methyl carbon in isoprenoidal lipid, showing the presence of an efficient route, as suggested by Smith et al.,[7] for the conversion of lysine to the acetate unit at the branched methyl and methine carbon. This conclusion is based on the logic that if lysine was degraded to crotonoyl-CoA by rearranging an amino group, then the C-3 hydrogens in lysine would be lost and the C-6 hydrogens would be retained via crotonoyl-CoA through lysine metabolism, as shown in Scheme 2.

Our observation and Smith's proposed pathway can be explained as a known lysine metabolic pathway in aerobic bacteria that has not been shown to exist in archaea, however, as shown in Scheme 3. In these archaea, lysine would be converted to "free" glutaric acid, like in the catabolism of lysine in *Pseudomonas* by the action of glutarate semialdehyde dehydrogenase.[16] Then, because of the $C_2$ symmetry of the molecule, two labeling patterns of glutaric acid must be obtained from [3,3-$^{2}H_2$]lysine **9**. [2,2-$^{2}H_2$]glutaric acid, which is presumably formed from **9**, was converted to crotonoyl-CoA, acetoacetyl-CoA, and mevalonate, and deuterium was incorporated to the C-3 methyl hydrogens of mevalonate. [4,4-$^{2}H_2$]glutaric acid, which is also formed from **9**, was converted similarly, and deuterium was incorporated to the C-2 methylene hydrogens of mevalonate. If these conversions were to occur, the deuterium at C-3 of lysine would be converted to hydrogens connected with the branched methyl carbon via the C-3 methyl of mevalonate, and C-2, C-6, C-10, and C-14 (methylene) carbon via the C-2 of mevalonate. However, a part of the deuterium at the C-2 of mevalonate would be lost because of the low p$K_a$ of the methylene hydrogens in acetoacetyl-CoA. Furthermore, the C-2 hydrogen in mevalonate may be lost during isomerization at IPP and DMAPP, regardless of the stereo-

chemical ambiguity at the methylene hydrogen at this position.[17] Thus, because of the low level of incorporation of deuterium, the latter could not be detected and the former deuterium was only observed by $^2$H NMR. Therefore, our result suggests that lysine is efficiently converted to glutarate, crotonoyl-CoA, acetoacetyl-CoA, and mevalonate in halophilic archaea. The results also suggest that the lysine metabolism in halophilic archaea resembles that in *Pseudomonas*.

Because of the low incorporation level of lipid diether from **9**, all of Smith's proposed pathway, the $C_2$ unit of amino acid is efficiently converted to the isoprenoidal lipid core at the branched methyl and methyne region, could not explain our lysine-incorporation experiments. A search for other carbon sources (such as the degradation of leucine or valine) is still needed to clarify the unique path for the efficient biosynthesis of isoprenoidal lipid in halophilic archaea.

Recently, the complete genome sequence of one halophilic archea was published; however, only one gene with enzyme coding related to lysine metabolism could be observed from the sequence similarity.[18] Additional experiments are needed to clarify the metabolic pathway of the unique microorganism, and further investigations of other species of archaea (e.g., thermophilic archaea) and differences in the biosynthetic pathway are currently underway.

## Experimental

Infrared spectra were obtained with a Perkin Elmer 1600 FT-IR spectrometer. $^1$H NMR spectra were recorded with a JEOL EX-90 spectrometer. $^{13}$C NMR spectra were recorded with a JEOL EX-90 spectrometer. $^2$H NMR spectra were recorded with a JEOL GX-400 spectrometer. For the $^1$H spectra and $^{13}$C NMR, tetramethylsilane (0 ppm) was used as an internal standard at $CDCl_3$. For the $^2$H NMR spectra, the natural abundance of the deuterium signal of $CDCl_3$ solvent (7.26 ppm) was used as an internal standard. HR-EI-MS spectra were recorded with a JEOL-D300 spectrometer. GC-MS were recorded with a Shimadzu QP-5000 spectrometer. Chromatographic separations were carried out with Merck Kieselgel 60, 70–230 mesh columns.

**Synthesis of [3,3-$^2H_2$]Lysine. 4-Benzyloxybutan-1-ol (4).** 1,4-Butanediol **3** (12.0 g, 110 mmol) and freshly distilled benzaldehyde (10.0 g, 110 mmol) in 70 mL of benzene was added to 100 mg of p TsOH and refluxed while removing water that evolved during the reaction procedure. The reflux was continued for 12 h, the mixture was cooled, and 50 mL of aq $Na_2CO_3$ was added. The organic layer was separated and the water layer was extracted with 50 mL of ether. The combined organic layer was dried over $NaSO_4$. After filtration, the organic solution was evaporated. Then, 18.7 g of the crude 7-membered ring ketal was obtained. A part of the product (12.5 g) was dissolved into the mixture of $CH_2Cl_2$–ether (60 mL–60 mL). After three portions of $LiAlH_4$ (3.0 g) were added to the mixture with stirring, it was slowly heated to 40 °C (oil bath). $AlCl_3$ (9.0 g) in ether (60 mL) was added during 30 min, and the boiling was continued for 2 h. The mixture was cooled, the excess of $LiAlH_4$ was decomposed with ethyl acetate (30 mL), and $Al(OH)_3$ was precipitated by the addition of water. After dilution with ether (100 mL), the organic layer was filtered and the residue was washed with ether. The combined organic layer was washed with water, dried, and concentrated. The residue was purified by silica-gel column chromatography (300 g, hexane : EtOAc = 2 : 1) to give 12.0 g of **4** (90.7%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.71 (4H, m), 2.31 (1H, broad, -OH), 3.50 (2H, t, $J$ = 7.2 Hz), 3.61 (2H, m), 4.59 and 4.61 (2H, d, $J$ = 11.0 Hz), 7.30 (5H, aromatic). IR ($CHCl_3$) 3619, 3422, 3011, 2940, 2866, 1602, .1496, 1464, 1362, 1234 cm$^{-1}$. HRMS Calcd for $C_{11}H_{16}O_2$: 180.1150. Found: 180.1138.

**[1,1-$^2H_2$]-4-Benzyloxy-butan-1-ol (5).** To a solution of **4** (2.8 g, 15.6 mmol) in acetone (25 mL) was added Jones Reagent (0.3 mL, 0.81 mmol) in a dropwise manner, and the solution was stirred for 30 min. Excess reagent was quenched by the addition of *i*PrOH, and the mixture was stirred for several minutes. The mixture was evaporated to ca. 10 mL, and then ether (50 mL) was added. The carboxylate was extracted with 1 M NaOH (50 mL), and the water layer was acidified with conc. HCl. The acidified water layer was extracted with ether (50 mL × 2). The combined organic phase was washed with brine and dried over anhydrous $Na_2SO_4$. After filtration, the organic solution was evaporated to dryness. Thirty milliliters of anhydrous methanol and 3 drops of conc. $H_2SO_4$ were added to the residue (2.9 g), and the mixture was refluxed for 3 h. The mixture was evaporated to ca. 30 mL and diluted with ether (30 mL), and then $Na_2CO_3$ solution was added to it. The organic layer was separated, and the water layer was extracted with ether (30 mL × 2). The combined organic layer was dried over $Na_2SO_4$, then filtrated, and evaporated to dryness. The residue was purified by silica-gel column chromatography (100 g, hexane : EtOAc = 4 : 1) to give 2.56 g of ester. To a mixture of ester in 60 mL of THF was added 600 mg of $LiAl^2H_4$ with three portions and the mixture was stirred for 1 h. The mixture was diluted with ether (60 mL), and a small amount of water was added to quench any excess reagent and to precipitate $Al(OH)_3$; then $Na_2SO_4$ was added. The mixture was filtrated, evaporated, and the residue was purified by silica-gel column chromatography (200 g, hexane : EtOAc = 2 : 1) to give 2.05 g of **5** (72.5%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.69 (2H, m), 2.31 (1H, broad, -OH), 3.50 (2H, t, $J$ = 7.2 Hz), 4.59 and 4.61 (2H, d, $J$ = 11.0 Hz), 7.30 (5H, aromatic). IR ($CHCl_3$) 3619, 3422, 3011, 2940, 2866, 1602, 1496, 1464, 1362, 1234 cm$^{-1}$. HRMS Calcd for $C_{11}H_{14}O_2{}^2H_2$: 182.1274. Found: 182.1314.

**[4,4-$^2H_2$]-4-Benzyloxy-1-bromobutane (6).** To a solution of **5** (3.38 g, 18.5 mmol) in $CH_2Cl_2$ (140 mL) was added triphenylphosphine (10.99 g, 41.7 mmol) and $CBr_4$ (13.89 g, 41.8 mmol); the mixture was stirred at rt for 3 h, and then evaporated. The residue was purified by silica-gel column chromatography (90 g, hexane : EtOAc = 10 : 1) to give 4.41 g of **6** (97.3%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.90 (4H, m), 3.50 (2H, t, $J$ = 7.0 Hz), 4.49 (2H, s), 7.30 (5H, aromatic). IR ($CHCl_3$) 3011, 2940, 2866, 1602, 1496, 1454, 1362, 1234 cm$^{-1}$. Anal. Calcd for $C_{11}H_{13}BrO^2H_2$: C, 53.89; H + $^2$H, 6.16%. Found: C, 53.57; H + $^2$H, 6.54%.

**Diethyl [1,1-$^2H_2$]-4-Benzyloxybutyl-*N*-*t*-butoxycarbonylaminomalonate (7).** To a solution of sodium ethoxide (0.87 mmol/L, 7.4 mL, 6.44 mmol) in 10 mL of EtOH was added diethyl *t*-butoxycarbonylaminomalonate (1.70 g, 5.86 mmol); and the solution was stirred at rt for 30 min. Then, bromide **6** (1.43 g, 5.82 mmol) in 10 mL of EtOH was added, and the mixture was refluxed for 3 h. After removing the precipitate, the mixture was diluted with 20 mL of ethyl acetate and water was added. The organic layer was separated, and the water layer was reextracted with ethyl acetate (20 mL). The combined organic layer was washed, dried, filtered, and evaporated. The residue was purified by silica-gel column chromatography (50 g, hexane : EtOAc = 10 : 1 to 2 : 1) to give 2.18 g of **7** (83.2%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.22 (6H, t, $J$ = 6.8 Hz), 1.42 (6H, s), 3.46 (2H, t, $J$ = 6.3 Hz), 4.21 (4H, q, $J$ = 6.8 Hz), 4.46 (2H, s), 5.90 (1H, broad,

-NH), 7.30 (5H, aromatic). IR ($CHCl_3$) 3011, 2940, 2866, 1602, 1496, 1454, 1362, 1234 $cm^{-1}$. Anal. Calcd for $C_{23}H_{33}NO_7{}^2H_2$: C, 62.85; H + $^2$H, 8.03; N, 3.19%. Found: C, 62.91; H + $^2$H, 7.95; N, 3.60%.

**Diethyl [1,1-$^2H_2$]-4-Azidobutyl-*N*-*t*-butoxycarbonylaminomalonate (8).** To a solution of **7** (4.58 g, 10.4 mmol) in 50 mL of AcOH was added 100 mg of Pd–C, and the mixture was stirred vigorously under a $H_2$ atmosphere for 4 h. The mixture was filtered and evaporated to give 3.58 g of the alcohol (98.4%). The product was converted to azide with no further purification. To a solution of the above product (3.58 g, 10.3 mmol) in 30 mL of pyridine was added TsCl (2.5 g, 13.0 mmol); and the mixture was stirred at rt for 1 h, and then worked up and purified by silica-gel column chromatography (50 g, hexane : EtOAc = 10 : 1 to 2 : 1) to give 2.86 g of tosylate. Then, the tosylate was dissolved into 20 mL of DMF, and sodium azide (715 mg, 11 mmol) was added to the mixture. The mixture was stirred at 100 °C (bath temp) for 2 h and cooled to rt. The usual work-up and chromatography (50 g, hexane : EtOAc, 4 : 1 to 2 : 1) were done, to give 1.96 g of **8** (50.2%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.25 (6H, t, $J$ = 6.8 Hz), 1.44 (6H, s), 3.29 (2H, t, $J$ = 6.7 Hz), 4.21 (4H, q, $J$ = 6.8 Hz), 4.46 (2H, s), 5.90 (1H, broad, -NH). IR ($CHCl_3$) 3454, 3011, 2940, 2866, 1744, 1725, 1496, 1362, 1055 $cm^{-1}$. Anal. Calcd for $C_{16}H_{26}N_4O_6{}^2H_2$ C, 51.32; H + $^2$H, 7.54; N, 14.96%. Found: C, 51.82; H + $^2$H, 8.01; N, 15.06%.

**[3,3-$^2H_2$]Lysine (9).** To a solution of **8** (1.96 g, 5.23 mmol) in 20 mL of AcOH was added 100 mg of Pd–C, and the mixture was stirred vigorously for 12 h under a $H_2$ atmosphere, and then filtered, evaporated, and dissolved into a THF-1M HCl mixture (20 mL–5 mL). The mixture was stirred for 2 h at 100 °C (bath temp) and evaporated. It was then dissolved into 10 mL of 6 M HCl and stirred at 100 °C for 2 h. The filtrate was evaporated to ca. 10 mL and applied to IR120B ($H^+$) ion-exchange column chromatography (r 15 mm × 70 mm, 35 mL). The column was washed with 3 volumes of water, and the product was eluted with 1 M aq $NH_3$. The eluent was evaporated and dried under reduced pressure to give 653 mg of the product (84.4%).

**Synthesis of [6,6-$^2H_2$]Lysine. *N*-(4-Benzyloxy-[1,1-$^2H_2$]butyl)phthalimide (10).** To a solution of **6** (4.41 g, 18.0 mmol) in *N*,*N*-dimethylformamide (50 mL) was added potassium *N*-phthalimide (5.40 g, 0.81 mmol); and the resulting solution was refluxed at 120 °C (bath temp) for 2 h. The mixture was diluted with ether (50 mL), and sat. NaCl (30 mL) was added. The organic phase was separated, the water phase was reextracted with ether (50 mL × 3), and the combined organic phase was washed with brine, and then dried over anhydrous $Na_2SO_4$. The residue was purified by silica-gel column chromatography (90 g, hexane : EtOAc = 7 : 1) to give 4.12 g of **10** (73.3%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.71 (4H, m), 3.70 (2H, t, $J$ = 7.0 Hz), 4.49 (2H, s), 7.30 (5H, aromatic) 7.77 (4H, aromatic). $^{13}$C NMR (22 MHz, $CDCl_3$) $\delta$ 40.24, 67.81, 73.09, 73.42, 112.20, 127.75, 128.43, 144.49. IR ($CHCl_3$) 3028, 2940, 2866, 1770, 1711, 1602,1496,1454, 1394,1234 $cm^{-1}$. HRMS Calcd for $C_{12}H_{10}O_3{}^2H_2$ ((M−$C_7H_7$ (Bn)$^+$): 220.0941. Found: 220.0916.

***N*-(4-Hydorxy-[1,1-$^2H_2$]butyl)phthalimide (11).** To a solution of **10** (4.12 g, 13.2 mmol) in 50 mL of ethanol was added 200 mg of 10% Pd–C. The mixture was stirred for 30 min in a $H_2$ atmosphere for 20 h. The mixture was filtered, and the filtrate was evaporated to give 2.89 g of **11** (98.5%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.71 (4H, m), 3.70 (2H, t, $J$ = 7.0 Hz), 4.49 (2H, s), 7.77 (4H, aromatic). IR ($CHCl_3$) 3624, 3465, 3028, 2940, 2866, 1770, 1711, 1602, 1468, 1454, 1394 $cm^{-1}$. HRMS Calcd for $C_{12}H_{11}NO_3{}^2H_2$: 221.1019. Found: 221.0968.

***N*-(4-Bromo-[1,1-$^2H_2$]butyl)phthalimide (12).** To a solution of **11** (2.88 g, 13.1 mmol) in $CH_2Cl_2$ (130 mL) were added triphenylphosphine (9.64 g, 36.6 mmol) and $CBr_4$ (12.17 g, 36.6 mmol); the mixture was stirred at rt for 2.5 h, and then evaporated. The residue was purified by silica-gel column chromatography (80 g, hexane : EtOAc = 10 : 1) to give 2.98 g of **12** (80.1%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.90 (4H, m), 3.45 (2H, m), 7.77 (4H, aromatic), 7.30 (5H, aromatic). IR ($CHCl_3$) 3028, 2940, 2866, 1770, 1711, 1602, 1468, 1454, 1394 $cm^{-1}$. HRMS Calcd for $C_{12}H_{11}BrNO_2{}^2H_2$: 283.0175. Found: 283.0200.

**Diethyl *N*-Acetamido-([4,4-$^2H_2$]-4-phthalimidobutyl)malonate (13).** To a solution of sodium ethoxide (0.87 mmol/L, 5.3 mL, 6.0 mmol) in 10 mL of EtOH was added diethyl acetamidomalonate (0.62 g, 5.7 mmol); the solution was warmed to 70 °C in an oil bath and stirred for 30 min. Then, **12** (1.01 g, 3.55 mmol) in 10 mL of hot EtOH was added, and the mixture was refluxed for 12 h. After removing the precipitate, the mixture was diluted with 20 mL of ethyl acetate and water was added. The organic layer was separated, and the water layer was reextracted with ethyl acetate (20 mL). The combined organic layer was washed, dried, filtered, and evaporated. The residue was purified by silica-gel column chromatography (50 g, hexane : EtOAc = 10 : 1 to 2 : 1) to give 0.77 g of **13** (51.6%). $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.25 (6H, t, $J$ = 6.8 Hz), 1.71 (2H, m), 2.03 (3H, s), 2.37 (2H, m), 4.21 (4H, q, $J$ = 6.8 Hz), 6.75 (1H, broad, -NH), 7.77 (4H, aromatic). IR ($CHCl_3$) 3414, 3028, 2940, 2866, 1736, 1706, 1678, 1602, 1468, 1454, 1394 $cm^{-1}$. Anal. Calcd for $C_{21}H_{24}N_2O_7{}^2H_2$: C, 59.99; H + $^2$H, 6.23; N, 6.66%. Found: C, 59.91; H + $^2$H, 6.25; N, 6.55%.

**[6,6-$^2H_2$]Lysine (14).** **13** (1.50 g, 3.57 mmol) was suspended in 15 mL of 6 M HCl, and the mixture was refluxed for 12 h. The mixture was diluted with 30 mL of water, and precipitated phthalimide was filtered under reduced pressure. The filtrate was evaporated to ca. 10 mL and applied to an IR-120B ($H^+$) ion-exchange column chromatography (r 15 mm × 70 mm, 35 mL). The column was washed with 3 volumes of water, and the product was eluted with 1 M aq. $NH_3$. The eluent was evaporated and dried under reduced pressure to give 450 mg of the product (85.1%). IR (KBr) 3500–2500, 1627, 1586, 1418 $cm^{-1}$.

**Bacterial Culture and Lipid Extraction.** *Natrinema pallidum* (formerly *Halobacterium halobium*) IAM 13147 was obtained from the Institute of Applied Microbiology, University of Tokyo, and *Halobacterium halobium* JCM 9120 was obtained from Riken. The cultivation and isolation of lipid diether were carried out according to our previous report. Three hundred milliliters of the medium were incubated for each experiment, and 250 mg of deuterium-labeled lysine was applied to the medium. Cells were harvested by centrifugation to yield typically 2 g of wet cells. Lipids were extracted, and the non-polar contaminants were separated by acetone-precipitation. After methanolysis of the polar lipid fraction, the residue was chromatographed over silica gel with hexane–diethyl ether (4 : 1) to give, typically, about 4 to 6 mg of lipid (**8** and **9**, as a mixture in the case of *Natrinema pallidum*; **8**, as a single compound in the case of *Halobacterium halobium*).

**Degradation of Lipid-Core and Conversion to Hydrocarbon by HI Cleavage and $LiAlH_4$ Reduction.** HI (1 mL) and Lipid (1 mg) were refluxed for 12 h at 100 °C (bath temp). The mixture was cooled to rt, and 5 mL of water was added to it. The iodide was extracted with hexane (10 mL × 2), and the hexane layer was dried with $Na_2SO_4$. After filtration, the hexane layer was evapo-

rated, and then 1 mL of THF and 20 mg of $LiAlH_4$ were added. After the mixture was stirred for 30 min, a small amount of water was added and the mixture was diluted with hexane. The mixture was filtrated, and hexane was removed under a stream of nitrogen. The products were analyzed by GC-MS.

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