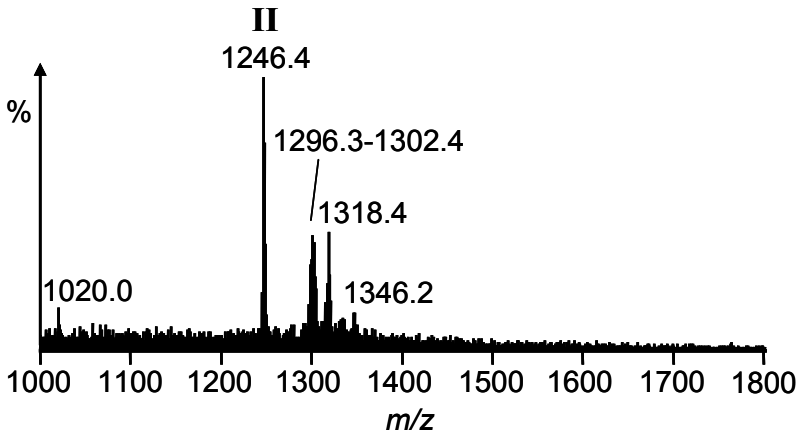
Further experimental details

Vena del Gesso sediments (approx. 5 g) were extracted *via* sonication (10 min), centrifugation (10 min, 2000 rpm) and filtration of the resultant supernatant using a three solvent program comprising methanol, followed by methanol:dichloromethane and finally dichloromethane. Following concentration *in vacuo*, each residue was fractioned into an apolar fraction and a polar fraction (containing ether lipids) on activated alumina as described in ref. 11. A polar lipid fraction from *M. thermautotrophicus* was obtained as outlined in ref. 11. To generate lipid acetylates, lipid fractions were treated in 1:1 acetic anhydride:pyridine at rt for 24 h before being reduced *in vacuo* (ref. 14).

Ion trap LC-MS/MS analysis of lipid extracts and their acetylates was performed using a HCTultra ETD II (Bruker Daltonics; Coventry, UK) mass spectrometer, operated using spectrometric conditions identical to those described in ref. 12. Reversed phase liquid chromatographic separations of lipid acetylates were achieved using 2 x Dionex Acclaim 120 C18 columns (2.1 x 100 mm; 2.2 μm), eluted isocratically with 80:20 methanol:chloroform for 70 min (60°C; 0.3 mL min^{-1}). The MS^3 spectrum of *m/z* 1246.4 was accumulated over 35 min during direct infusion of a solution of the *M. thermautotrophicus* lipid extract using an "Auto MS^n" feature of the spectrometer, set to select the base peak ion (i.e. the $[M+H]^+$ of **I**; *m/z* 1302.5) during the first tandem stage and, subsequently, for any ion formed from a 56 Da neutral loss. During the experiment, an isolation width of 3 *m/z* units, a fragmentation amplitude of 2.0 V and an accumulation time of 40 ms were employed.

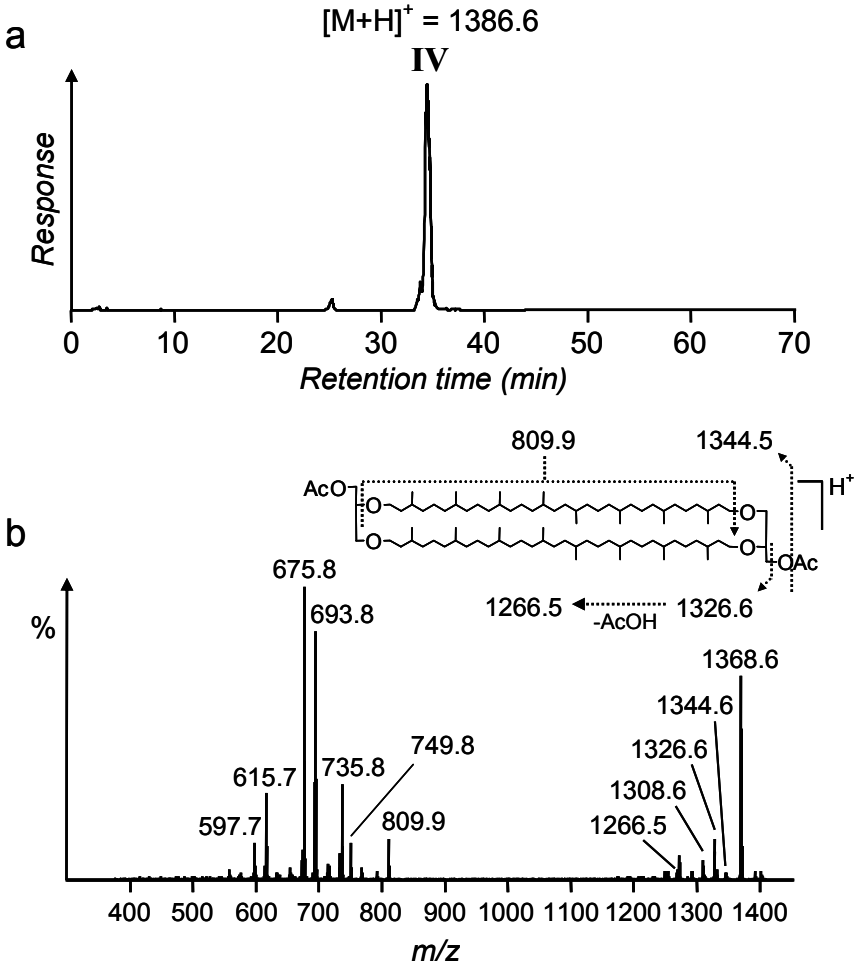
High mass accuracy LC-MS/MS was achieved using a 9.4 T Apex ultra quadrupole-hexapole-Fourier transform ion cyclotron resonance spectrometer (Bruker Daltonics), equipped with an atmospheric pressure chemical ionization source set at temperature 400°C, with drying gas temperature set to 220°C and nebulizer and drying gas flow rates set to 1 $Lmin^{-1}$ and 4 $Lmin^{-1}$, respectively. The Q1 resolution was held at 3 *m/z* units and the hexapole collision energy at -30 eV, with argon used as collision gas.

Supplementary Figure 1



Supp. Fig. 1 Quadrupole ion trap mass spectrum of the lipid fraction, enriched in **II**, prepared from VdG sediment.

Supplementary Figure 2



Supp. Fig. 2 a) Reversed phase LC-MS base peak chromatogram of the acetylated lipid core fraction from *M. thermautotrophicus*; b) Quadrupole ion trap MS/MS spectrum of **IV** (precursor ion $[M+H]^+$ *m/z* = 1386.6), highlighting the loss of isoprenoid chains as alcoholic (i.e. 1-ene-biphytan-32-ol; *m/z* 809.9) as opposed to fully unsaturated (i.e. 1,31-biphytadiene; no ion at *m/z* 827.9) elimination products.