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# Isoprenoidal glycerol dialkanol diethers: a series of novel archaeal lipids in marine sediments

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**ABSTRACT**

We report a new series of archaeal lipids, widespread in marine sediments and tentatively assigned as isoprenoid glycerol dialkanol diethers (GDDs). These lipids are structural analogues of isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) with one glycerol unit missing and with each biphytanyl moiety possessing a terminal hydroxyl group. The structural identification is based on molecular formulae determined from accurate mass measurement and interpretation of mass spectral fragmentation patterns. Acetylation of GDD-0 confirmed the presence of three hydroxyl groups, and ether cleavage and reduction of the products afforded two biphytanyl chains. Tests of different

protocols for both extraction and acid hydrolysis indicate that GDDs are not formed during sample preparation. The co-existence of GDDs and GDGTs in 12 selected marine sediment samples of varying origin showed that the ring distribution in these two ether lipid pools is related and implies that the two compound classes share a common biological source. The presence of isoprenoid GDDs is possibly linked to the occurrence of biphytane diols in marine sediments, where both GDD and diol can be either biosynthetic intermediates or degradation products, and it also supports the recently proposed fossil lipid recycling by benthic archaea (Takano, Y., Chikaraishi, Y., Ogawa, O.N., Nomaki, H., Morono, Y., Inagaki, F., Kitazato, H., Hinrichs, K.-U., Ohkouchi, N., 2010. Sedimentary membrane lipids recycled by deep-sea benthic archaea. Nature Geoscience 3, 858–861). GDD core lipid was detected in a culture of *Methanothermococcus thermolithotrophicus*, suggestive of a potential biological function of these compounds, but no intact polar lipids (IPLs) containing GDDs as core lipids have been detected to date.

## 1. Introduction

During the last decade, isoprenoidal glycerol dialkyl glycerol tetraethers (GDGTs) have been recognized as widespread archaeal lipids in marine and terrestrial environments (Schouten et al., 2000, 2002; Lipp et al., 2008), while the other archaeal lipid type, glycerol dialkyl diethers, is usually abundant in hydrocarbon seeps at the ocean's seafloor (e.g., Hinrichs et al., 1999; Pancost et al., 2001). These ether lipids have attracted increasing interest from different fields of geobiological research largely because they can be used as proxies to trace the activity of Archaea in modern and

48 ancient environments. The development of liquid chromatography mass spectrometry
49 (LC-MS) has facilitated identification and quantification of archaeal lipids (e.g.,
50 Hopmans et al., 2000; Sturt et al., 2004) and opportunities for exploring novel types of
51 archaeal lipids have become available as a result.

52 Previous studies of archaeal cultures, such as methanogens (Tornabene and
53 Langworthy, 1979; Koga et al., 1993), have found both diether- and tetraether-based
54 lipids as major membrane constituents. In general, three categories of archaeal lipids are
55 found in environmental samples: IPLs, glycerol ether core lipids (e.g., GDGT core lipids)
56 and degraded fossil products such as polyisoprenoidal hydrocarbons. Archaeal lipids in
57 their intact form, mainly glyco- and phospholipids, contain one or two labile polar head
58 groups attached to the glycerol ether core. The polar head groups of IPLs, especially
59 phospholipids, can be rapidly hydrolyzed after cell decay (e.g., White et al., 1979), which
60 makes IPLs suitable proxies for live prokaryotes. However, their applicability to low-
61 activity subseafloor sediment remains controversial (cf. Lipp and Hinrichs, 2009;
62 Schouten et al., 2010) and will require further validation. IPLs can be detected with LC-
63 MS in lipid extracts of biomass from cultures, marine particulate organic matter and
64 sediments (e.g., Sturt et al., 2004; Schouten et al., 2008; Lipp et al., 2008; Rossel et al.,
65 2010). GDGT core lipids are thought to represent a predominantly fossil signal and are
66 ubiquitous in various depositional environments where they occur with high structural
67 diversity (Schouten et al., 2000). They are relatively stable and are able to survive in
68 sediments over tens of millions of years (e.g., Kuypers et al., 2001), so that they can serve
69 as biomarkers of past archaeal activity and components of molecular proxy ratios, such as
70 the paleo sea surface temperature indicator $TEX_{86}$ (e.g., Schouten et al., 2002). Smaller

fossil derivatives of archaeal lipids amenable to analysis by gas chromatography (GC), such as biphytanediols (Schouten et al., 1998; Saito and Suzuki, 2010) and biphytanic diacids (Meunier-Christmann, 1988; Birgel et al., 2008) have been found in both recent sediments and ancient rock samples. However, it is not clear whether these biphytane derivatives are products of isoprenoidal GDGT degradation or intermediates in lipid biosynthesis. In addition, a series of $C_{80}$, $C_{81}$ and $C_{82}$ isoprenoid biphytanyl tetraacids were identified in crude oil (Lutnaes et al., 2006, 2007) and the authors assumed that they were of recent archaeal origin.

Our knowledge of the biosynthesis of archaeal lipids is limited although a pathway, based on both IPL analysis and genetic studies, has been proposed (Koga and Morii, 2007). Expanding knowledge of the distribution of archaeal lipid derivatives in nature should promote a better understanding of the activity of Archaea, the origin and fate of archaeal lipids in different geologic settings and the limitations of related molecular proxies. Modification of the analytical protocol revealed numerous compounds that presumably have been overlooked in previous studies (e.g., Hopmans et al., 2000). Here we report the identification of a novel series of lipids, i.e., so-called isoprenoidal glycerol dialkanol diethers (GDDs). We discuss their distribution in marine sediments as well as the evidence pertaining to their source and potential biosynthetic significance.

## 2. Materials and methods

### *2.1. Materials and sample preparation*

Twelve globally distributed marine subsurface sediments from various geological settings including open ocean samples with low total organic carbon (TOC) content and

94 continent margin samples with high TOC content (Table 1) were immediately frozen
95 after collection and stored at -80°C until extraction and analysis. More detailed
96 information on sampling sites is given by Liu et al. (2011). Samples were freeze-dried
97 and extracted using the modified Bligh and Dyer protocol as described by Sturt et al.
98 (2004). In addition, one archaeal culture, *Methanothermococcus thermolithotrophicus*
99 strain (DSM 2095), grown at 85°C in enamel-protected fermentors with stirring (400 rpm)
100 and continuous gassing ($H_2/CO_2$, 80:20) and harvested in the exponential growth phase,
101 was provided by M. Baumgartner and K. Stetter (University of Regensburg, Germany).
102 Using an ultrasonic probe (HD 2200, Bandelin electronic GmbH & Co. KG, Germany),
103 0.5 g freeze-dried biomass of *Methanothermococcus thermolithotrophicus* was extracted
104 (4 x) with dichloromethane (DCM): methanol (MeOH) [20 ml; 1:1, v/v] for 15 min. In
105 order to test the existence of IPL precursors of GDD an aliquot (1%) of the total lipid
106 extract (TLE) of *Methanothermococcus thermolithotrophicus* was spiked with 200 ng of
107 a $C_{46}$ GDGT standard (Huguet et al., 2006) before splitting it into two aliquots for direct
108 lipid analysis and for treatment with 6 M HCl/MeOH [1 ml; 1:9, v/v] at 70°C for 3 h to
109 hydrolyze the IPLs to core lipids.
110
111 *2.2. Analysis of GDGTs and GDDs*
112 One aliquot of each sample was dissolved in 200 µl hexane/isopropanol (99:1, v/v)
113 for HPLC-MS. Separation was performed with a Prevail Cyano column (2.1×150 mm,
114 3 µm; Grace, Germany) maintained at 35°C in an Agilent 1200 series HPLC instrument.
115 Using a flow rate of 0.25 ml $min^{-1}$, the gradient of the mobile phase was first held for
116 5 min with 100% eluent A [*n*-hexane/isopropanol, 99:1 (v/v)], followed by a linear

gradient to 90% A and 10% B [*n*-hexane/isopropanol, 90:10 (v/v)] in 20 min, followed by a linear gradient to 100% B at 35 min, and finally holding at 100% B for 5 min. After analysis the column was equilibrated with 100% A at 0.6 ml $min^{-1}$ for 5 min. Detection was achieved with an Agilent 6130 MSD single quadrupole mass spectrometer, coupled to an Agilent 1200 series HPLC instrument via a multimode ion source in atmospheric pressure chemical ionization (APCI) mode. APCI settings were: nebulizer pressure 60 psi, vaporizer temperature 250°C, drying gas ($N_2$) flow 6 l $min^{-1}$ and drying gas temperature 200°C, capillary voltage 2 kV, and corona current 5 μA. With Chemstation software the detector was set for selective ion monitoring (SIM) of $[M+H]^+$ ions (*m/z* 1302, 1300, 1298, 1296, 1292, 1246, 1244, 1242, 1240 and 1236, fragmentor voltage 190 V).

In addition, small amounts of GDD-0 (acyclic GDD) and GDD-cren (GDD corresponding to crenarchaeol) were isolated from the combined extracts of the twelve sediments by collecting fractions manually through a flow splitter installed between the HPLC instrument and detector. The LC method used for isolating small amounts of GDD was as described above for GDD analysis. In order to record accurate molecular masses in full scan and $MS^2$ spectra, isolated GDDs were injected again into an Agilent 1200 series HPLC system coupled to an Agilent 6520 quadrupole time-of-flight (qTOF) mass spectrometer through an APCI interface. The APCI source temperature was 350°C with a gas ($N_2$) flow of 4 l $min^{-1}$. The qTOF parameters for auto MS/MS scanning mode with $MS^1$ range *m/z* 500-2000 and $MS^2$ *m/z* 100-2000 were: capillary voltage 1 kV, corona current 5 μA, fragmentor voltage 150 V, skimmer voltage 65 V and octapole voltage 750 V. The qTOF system was tuned and calibrated with Agilent commercial tuning mix to reach a mass accuracy better than 2 ppm.

Relative ring distributions of GDGTs and GDDs were determined using peak areas of the $[M+H]^+$ ions of compounds containing 0, 1, 2, 3, and 5 rings. Because proper standards were unavailable, we assumed an identical response factor for GDDs, GDGTs and $C_{46}$ GDGT when we calculated their absolute concentrations in the culture extract, and the ratio of GDDs vs. GDGTs was estimated directly from the peak area of isoprenoid GDGTs and GDDs according to (GDDs) / (GDGTs + GDDs) x 100%. The resulting ratio (cf. Table 1) probably does not represent the relative abundance of GDDs properly but is useful for comparison of different samples.

*2.3. Analysis of acetylated GDD*

For determination of the number of OH groups in GDD, an aliquot of purified GDD-0 was transferred into a 2 ml vial and dried with $N_2$; 1 ml acetic anhydride and pyridine (1:1,v:v) was added, and the mixture was kept at 50°C for 1 h. Acetylated GDD-0 was analyzed with APCI-MS by infusing the sample directly into the ion source with a syringe pump set at 0.2 ml $min^{-1}$.

*2.4. Analysis of alkyl moieties released by ether cleavage*

One aliquot of isolated GDD-cren was transferred to a 2-mL vial and dried with $N_2$, then 0.5 ml $BBr_3$ (1 M in DCM; Aldrich) was added. The reaction was performed at 60°C for 2 h. After evaporating the solvent and residual $BBr_3$ with $N_2$, 0.5 ml superhydride ($LiEt_3BH$, in THF, Aldrich) were added to reduce the bromides to hydrocarbons at 60°C for 2 h. Adding a few drops of water quenched the reaction. The hydrocarbons were extracted (3 x) with *n*-hexane and the extracts combined for GC-MS.

163 In order to analyze potential hydroxylated compounds, one aliquot of the ether
164 cleavage/reduction products was transferred into a 2-ml vial, dried with a flow of $N_2$ and
165 mixed with 100 µl BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) and 100 µl
166 pyridine at 70°C for 1 h. GC-MS was performed with an Agilent 5975C inert XL MSD
167 system equipped with an Agilent DB-5HT column (30 m x 250 µm x 0.25 µm).
168 Separation was achieved using an oven temperature program of 60°C (1 min) to 150°C at
169 10°C $min^{-1}$ and then to 320°C (held 36 min) at 5°C $min^{-1}$.
170
171 *2.5. Tests for production of GDDs from GDGTs*
172 To test potential production of GDD via degradation of GDGT during sample
173 extraction and preparation, three experiments were conducted. In the first, 1 µg of
174 purified GDGT-0 was subjected to acid hydrolysis as described above for the *M.*
175 *thermolithotrophicus* culture (experiment A). For the second, pre-extracted sediment,
176 which was extracted with accelerated solvent extraction and subsequently checked for the
177 absence of extractable lipids, was spiked with another aliquot of 1 µg of purified GDGT-
178 0 before extraction with the Bligh and Dyer method (experiment B).
179 The third experiment evaluated the influence of three different extraction
180 protocols on the yield of GDGT and GDD (experiment C). Freeze-dried and
181 homogenized sediment (M76/1 GeoB 12806-2) was separated into six aliquots of 1.5 g
182 dry wt. Homogenization was achieved using a freezer mill (CryoMill, Retsch GmbH,
183 Haan, Germany) cooled with liquid $N_2$ (cf. Lipp et al., 2008). Each aliquot was spiked
184 with 1 µg $C_{46}$ GDGT standard (Huguet et al., 2006). The first two aliquots were extracted
185 with the Bligh and Dyer protocol in four steps, twice with phosphate buffer and twice

with trichloroacetic acid (TCA) buffer (cf. Sturt et al., 2004); the second two aliquots were extracted with only phosphate buffer (4 x), and the last two aliquots were extracted using an accelerated solvent extractor (ASE 200, DIONEX) with a mixture of DCM:MeOH (9:1, v/v) and three cycles of 5 min each at 100°C and $7.6\times10^6$ Pa (cf. Leider et al., 2010).

## 3. Results and discussion

### *3.1. Identification of isoprenoidal GDDs*

A novel series of compounds structurally related to the known isoprenoidal GDGTs with 56 Da lower molecular mass was recognized in extracts of marine sediment samples (Table 1). The components eluted several minutes after GDGT during normal phase HPLC (Fig. 1). For instance, the analogues of GDGT-0 ($[M+H]^+$, *m/z* 1302) and crenarchaeol ($[M+H]^+$, m/z 1292) show molecular ions $[M+H]^+$ at *m/z* 1246 and 1236, respectively. Their $MS^2$ spectra gave dominant fragment ions at *m/z* 669 and 663, respectively, representing the fragments of a glycerol moiety plus one ether-bound biphytanediol (with one free OH; no ring for *m/z* 669 and three rings for *m/z* 663). Consequently, the neutral losses of 577 Da and 573 Da correspond to biphytanediols with 0 and 2 rings (Fig. 2).

Accurate mass determination of the two compounds via time-of-flight mass spectrometry with less than 2 ppm of mass uncertainty showed a $[M+H]^+$ at *m/z* 1246.2950 and 1236.2166, respectively, affording calculated formulae $C_{83}H_{168}O_5$ and $C_{83}H_{158}O_5$. Based on this, their elution pattern relative to GDGTs and their $MS^2$ spectra, these lipids were tentatively identified as isoprenoid GDDs.

209 Three additional observations support this structural assignment. Firstly, the
210 acetylated GDD-0 gave a molecular ion at *m*/*z* 1372, which can be interpreted as
211 acetylation of three OH groups with an added mass of 126 Da. Secondly, identical
212 compounds were released by ether cleavage/reduction from GDD-cren and crenarchaeol,
213 i.e., mixtures of biphytanes with two and three rings. Theoretically, biphytane mono-ols
214 might be released by ether cleavage of GDD, but no alcohols were detected in the
215 BSTFA-derivatized sample. This confirms that C-O bonds of hydroxyl functions are
216 susceptible to attack by $BBr_3$ with subsequent reduction of the bromides to biphytane
217 hydrocarbons by superhydride. Thirdly, the degradation tests on GDGT-0 showed that
218 after 72 h acid hydrolysis at 70°C only small amounts of GDD-0 had been generated
219 from GDGT-0 (Table 1). This detection of GDD-0 as a degradation product of GDGT-0
220 provides further evidence for the proposed structure of GDDs.
221
222 *3.2. Occurrence of GDDs and GDGTs*
223 Twelve marine sediment samples from six sites (Table 1) were analyzed for the
224 occurrence of isoprenoidal GDDs and GDGTs. In all of them GDDs coexisted with the
225 corresponding GDGTs. The ratio of signal responses of total GDDs vs. sum of total
226 GDGTs and GDDs ranged from 3.5% to 17% (Table 1), suggesting a substantial
227 contribution of GDDs to the overall pool of sedimentary archaeal lipids. The ring
228 distribution of GDDs and GDGTs with identical numbers of rings is illustrated as a cross
229 plot in Fig. 3 in order to expose potential relationships of the two compound classes.
230 Since GDDs contain only one glycerol, no regioisomers with parallel and antiparallel
231 glycerol units (cf. Sinninghe Damsté et al., 2002) exist (Fig. 3e). However, two isomers

232 related to the two options of coupling the dicylic and tricyclic biphytanol moieties to
233 glycerol are probably present but were not chromatographically resolved.
234 In general, the data plots of compounds with one, two and three rings scatter
235 within a deviation of a few % along the 1:1 line (Fig. 3b-d), but GDD-0 shows a trend to
236 lower relative abundance than GDGT-0 (Fig. 3a) while GDD-cren is relatively more
237 abundant than the corresponding GDGT (Fig. 3e). This phenomenon is analogous to the
238 relationship between biphytanediols and GDGTs (Schouten et al., 1998), where, in a
239 selection of marine sediments, the tricyclic biphytane diol was present in higher relative
240 abundance than the tricyclic biphytanes released by ether cleavage. This observation
241 likely indicates that isoprenoid GDDs share common sources and/or formation pathways
242 with biphytanediols.
243 *Methanothermococcus thermolithotrophicus* contained both the core lipids of
244 GDD-0 and GDGT-0 in abundance of 22 ng GDD-0 and 240 ng GDGT-0 $g^{-1}$ biomass,
245 respectively. After acid hydrolysis of the TLE their abundance increased to 140 ng GDD-
246 0 and 12000 ng GDGT-0 $g^{-1}$ biomass. Although no IPLs of GDD were found in either the
247 marine sediments or the archaeal culture, the increase in GDD abundance in acid-
248 hydrolyzed TLE may reflect the presence of GDD IPLs.
249
250 *3.4. Source of GDDs*
251 By analogy with previously identified biphytanediols (Schouten et al., 1998), the
252 coexistence of GDDs and GDGTs with similar ring distribution in the biphytanyl chains
253 suggests that these compounds have closely related if not identical biological sources.
254 However, for interpreting the environmental occurrence of GDDs, knowledge of their

255 formation pathway is crucial. Three principal scenarios need to be considered and are
256 briefly discussed: (i) production during sample preparation; (ii) diagenetic production in
257 sediments; (iii) production as intermediates in GDGT biosynthesis or as products of cell
258 metabolism.

259 Firstly, GDD production during sample preparation cannot be entirely excluded
260 but the experimental evidence argues against this as a major mechanism. Different
261 extraction protocols (experiment C) provided different yields of GDDs and GDGTs
262 (Fig. 4) but the effect of TCA or elevated temperature and pressure during ASE
263 extraction did not result in an increased relative yield of GDD within the uncertainty of
264 quantification. Likewise, in the experiment in which purified GDGT-0 was added to
265 extracted sediment, no production of GDD-0 occurred during Bligh and Dyer extraction
266 (experiment B). Finally, no GDD-0 was detected within 24 h of subjecting GDGT-0 to
267 acid hydrolysis (experiment A). The production of 0.2% GDD-0 after 72 h of acid
268 hydrolysis is low compared to the relative abundance of GDDs in sediments (Table 1).
269 These experiments strongly suggest that GDDs were already present in the sediments and
270 not formed during sample preparation.

271 Secondly, the formation of small quantities of GDD during prolonged acid
272 hydrolysis shows that, in principle, diagenetic production of these compounds in
273 sediments is a conceivable mechanism over long timescales. A diagenetic link of GDGTs
274 and GDDs is consistent with the general relationship of their ring distributions (Fig. 3).
275 Diagenetic processes could also involve biotic mechanisms such as the recently proposed
276 recycling of exogenous GDGTs by benthic archaea (Takano et al., 2010; cf. Liu et al.,
277 2011). The conclusions drawn by Takano et al. (2010) were based on selective uptake of

278 stable isotope label in the glycerol moiety of GDGTs during incubation. The mechanism
279 would require an intermediate to which newly synthesized glycerol is added; GDDs
280 fulfill the criteria as intermediate in such a scenario.
281 The detection of GDD-0 as a free core lipid in the TLE of *Methanothermococcus*
282 *thermolithotrophicus* was probably not due to its artificial production according to the
283 result of 'experiment C'. Consequently, even though GDD IPLs were not found, a
284 biological function as normal functional lipids or intermediates in GDGT synthesis
285 should not be ruled out. Assuming that GDDs represent components of archaeal
286 membranes or biosynthetic intermediates, their existence may provide new insights into
287 the biosynthetic pathway leading to ether lipids. According to the proposed pathway, the
288 biphytane skeleton should be formed via head-to-head condensation of two archaeol units
289 (Nemoto et al., 2003). This pathway seems inconsistent with a biological function of
290 GDDs in archaeal membranes. An alternative, currently unknown biosynthetic pathway,
291 which can fuse biphytanediol and glycerol units with ether bonds, may be responsible for
292 this.
293
294 **4. Conclusions**
295 A series of novel archaeal lipids was tentatively assigned as glycerol dibiphytanol
296 diethers, in which a primary OH group was located on the terminal (ω) carbon. Future
297 nuclear magnetic resonance (NMR) analysis of isolated compounds should provide
298 confirmation of the structures reported here for GDDs with additional information on
299 their stereochemistry. The coexistence of GDDs with corresponding GDGTs, their
300 widespread distribution and remarkable abundance in marine sediments qualify these

compounds as important archaeal lipid components in sediments and important targets in future studies. Diagenesis of GDGTs, especially biodegradation by benthic archaea is a possible source of GDDs in sediments but also a biological origin should not be excluded at this point as GDD core lipid was also found in a culture of *Methanothermococcus thermolithotrophicus*. However, so far, no IPL derivatives of GDD have been detected.

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418

**Figure captions**

**Fig. 1**. HPLC-APCI-MS chromatogram (SIM mode, shown as extracted ion chromatogram EIC), showing GDDs and corresponding GDGTs in the TLE of marine sediment sample M76/1 GeoB 12806-2. GDGTs and corresponding GDDs are labeled in the same color. Molecular structures of possible GDD isomers were not shown.

**Fig. 2**. $MS^2$ (qTOF) mass spectra showing dominant fragment ions for GDD-0 and GDD-cren.

**Fig. 3.** GDGT vs. GDD plots showing ring distribution of GDGTs and GDDs in 12 marine sediment samples.

**Fig. 4.** Yield of GDGTs and GDDs with three different extraction protocols. Error bars were calculated from two replicate samples for each extraction protocol. Concentrations of GDGTs and GDDs (ng/g dry sediment) in the TLE of each sample are based on duplicated analysis. 'B&D +$PO_4$' and 'B&D +$PO_4$ +TCA' represent the Bligh and Dyer extraction with only phosphate buffer and with both phosphate and TCA buffers, respectively. 'ASE' stands for extraction performed by accelerated solvent extraction.

**Table 1**

The ratio of total GDDs vs. the sum of total GDGTs and GDDs in 12 marine sediment samples[a] and other samples. Since appropriate standards were not available for GDD quantification, the ratio is based on detector responses and may therefore not represent the relative abundance of GDDs properly. However, the ratio is useful for comparison of different samples.

| Sample | | GDDs/(GDGTs+GDDs) (%) |
|---|---|---|
| M 76/1 | GeoB 12806-2 | 4.5 |
| Namibia Margin | GeoB 12807-2 | 9.3 |
| ODP Leg 201 | 1229D 4H4 | 17.0 |
| Peru Margin | 1229A 22H1 | 11.1 |
| ODP Leg 201 | 1226B 10H3 | 8.6 |
| Equatorial Pacific | 1226E 20H3 | 8.8 |
| ODP Leg 204 | 1250D 6H5 | 11.3 |
| Hydrate Ridge | 1250D 12H5 | 9.9 |
| IODP Exp. 311 | 1327C 10H5 | 10.9 |
| Cascadia Margin | 1327C 13X6 | 10.7 |
| ODP Leg 160 | 966C 5H02 | 3.5 |
| Mediterranean | 966C 7H04 | 7.6 |
| Methanothermococcus | TLE | 8.6 |
| thermolithotrophicus | hydrolyzed TLE | 1.1 |
| GDGT-0 + extracted sediment + B&D[b] | | n.d.[c] |
| GDGT-0 + acid hydrolysis at 70°C for 24 h | | n.d.[c] |
| GDGT-0 + acid hydrolysis at 70°C for 72 h | | 0.2 |

[a] Detailed sampling data of marine sediment samples is given by Liu et al. (2011); [b]B&D, Bligh and Dyer extraction; [c] n.d., GDDs not detected .

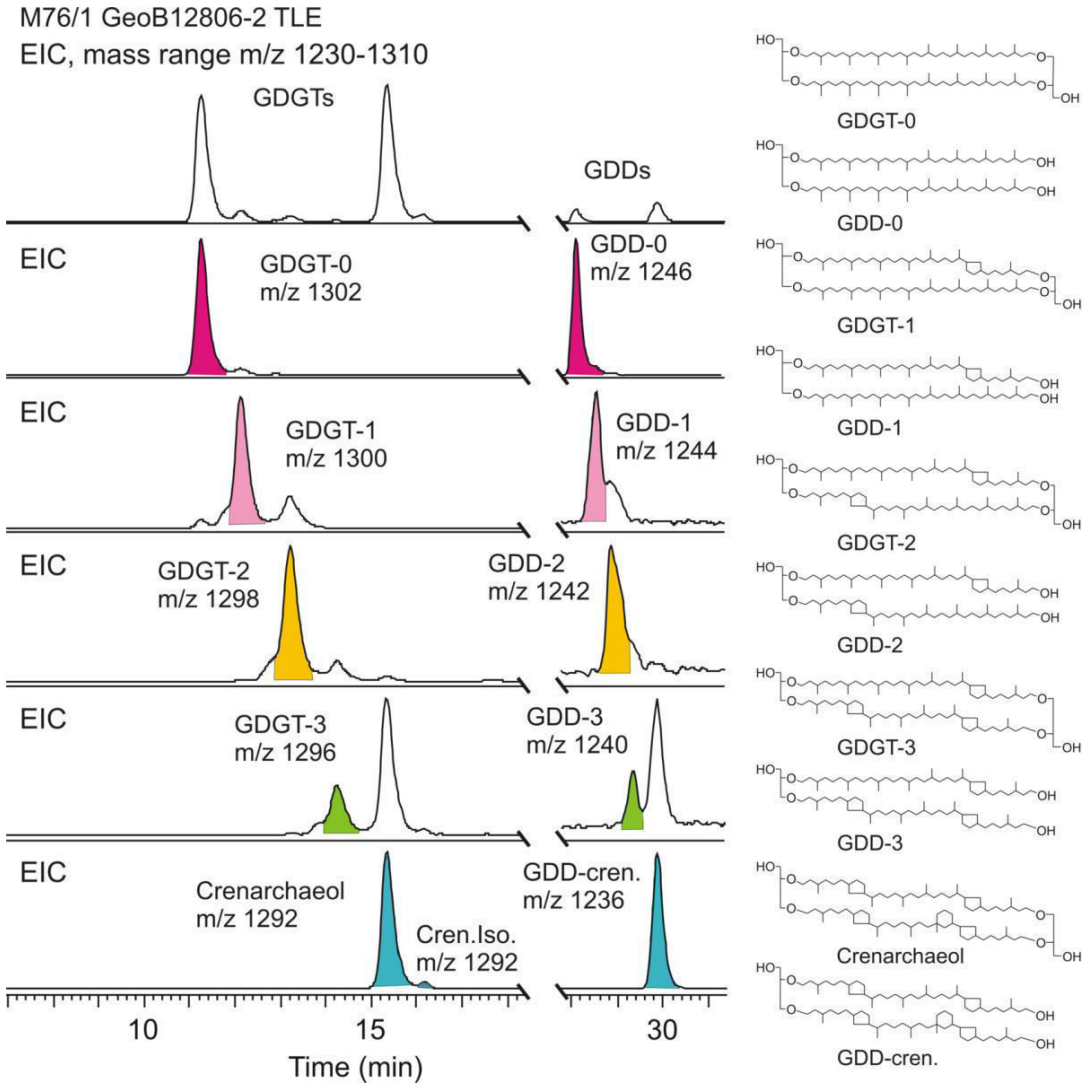
M76/1 GeoB12806-2 TLE
EIC, mass range m/z 1230-1310
GDGTs
GDDs
EIC
GDGT-0
m/z 1302
GDD-0
m/z 1246
EIC
GDGT-1
m/z 1300
GDD-1
m/z 1244
EIC
GDGT-2
m/z 1298
GDD-2
m/z 1242
EIC
GDGT-3
m/z 1296
GDD-3
m/z 1240
EIC
Crenarchaeol
m/z 1292
Cren.Iso.
m/z 1292
GDD-cren.
m/z 1236
10
15
30
Time (min)
GDGT-0
GDD-0
GDGT-1
GDD-1
GDGT-2
GDD-2
GDGT-3
GDD-3
Crenarchaeol
GDD-cren.

Figure 1.

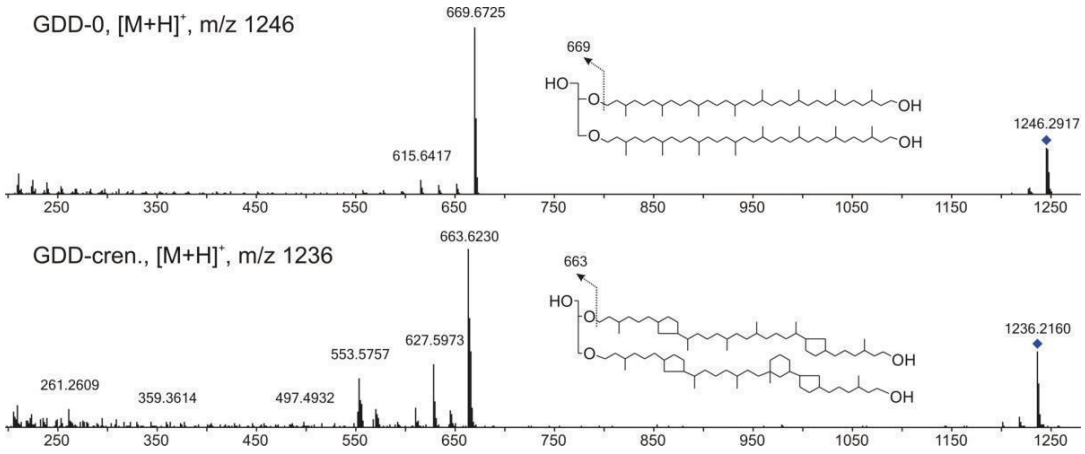
GDD-0, [M+H]+, m/z 1246
669.6725
669
HO
O
OH
O
OH
1246.2917
615.6417
250
350
450
550
650
750
850
950
1050
1150
1250
GDD-cren., [M+H]+, m/z 1236
663.6230
663
HO
O
OH
O
OH
1236.2160
627.5973
553.5757
261.2609
359.3614
497.4932
250
350
450
550
650
750
850
950
1050
1150
1250

Figure 2.

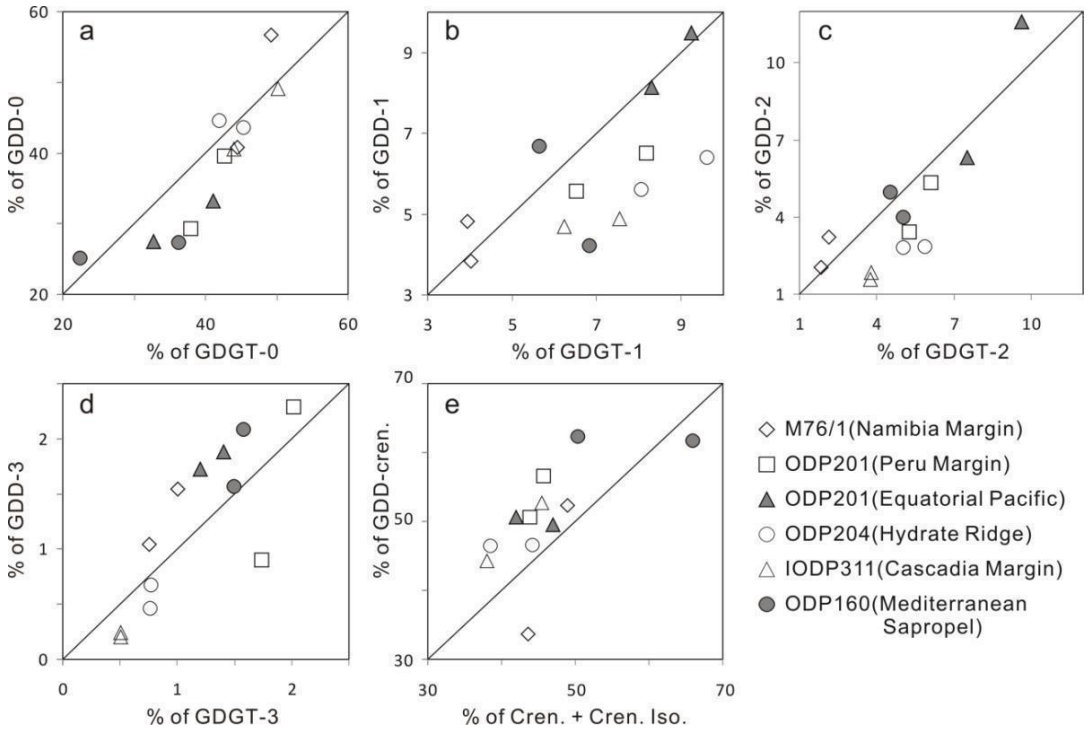
a
% of GDD-0
% of GDGT-0
b
% of GDD-1
% of GDGT-1
c
% of GDD-2
% of GDGT-2
d
% of GDD-3
% of GDGT-3
e
% of GDD-cren.
% of Cren. + Cren. Iso.
M76/1(Namibia Margin)
ODP201(Peru Margin)
ODP201(Equatorial Pacific)
ODP204(Hydrate Ridge)
IODP311(Cascadia Margin)
ODP160(Mediterranean Sapropel)

Figure 3.

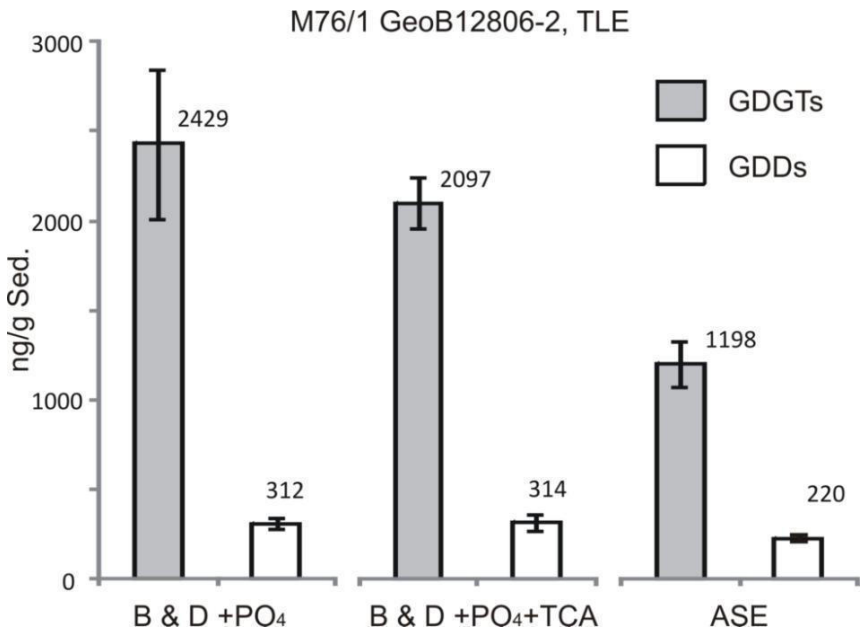
M76/1 GeoB12806-2, TLE
3000
2000
1000
0
ng/g Sed.
GDGTs
GDDs
2429
312
2097
314
1198
220
B & D +PO4
B & D +PO4+TCA
ASE

Figure 4.