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SUPPLEMENTARY MATERIAL

Origin of arsenolipids in sediments from Great Salt Lake

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Sampling was performed either from a boat using a box-core (for deep water samples), or by hand (for shallow wetland samples), taking the 0-2 cm surface sediment using acid-cleaned polycarbonate core rings. Samples were transferred to acid-cleaned mason jars and placed on ice until returned to the laboratory where they were frozen (-80 °C) prior to lipid extraction. Full collection and sample preparation details have been reported in Boyd et al. (2017), where corresponding chemical and microbiological data for these samples are also reported. Sample size depended on expected biomass in each sample (Table 1). The lipids were extracted from the sediments by a modified Bligh and Dyer extraction using a mix of DCM/MeOH/aqueous buffer (2 x phosphate buffer at pH 7.2; and 2 x TCA at pH 2.0; 5/10/4, v/v/v). The exact procedures are described in Sturt et al. (2004).

Extracted samples were lyophilized, stored at -20 °C, and analyzed later for arsenolipids in our laboratory in Graz, after re-dissolving in absolute EtOH (300 µL), by using HPLC-elemental mass spectrometry (inductively coupled plasma mass spectrometry, ICPMS), and HPLC-electrospray ionization high resolution mass spectrometry (HR-ESMS). Separation of species was performed by HPLC (Agilent 1100 series for ICPMS determinations) equipped with an Asahipak reversed-phase column (ODP-50, 4.6 x 125 mm, 5 µm; 0.5 mL min^{-1} flow; 20 µL injection volume; 40 °C column temperature) under gradient elution conditions: 30-100 % aqueous ethanol (EtOH, ≥ 99.9 %; obtained from VWR, Vienna, Austria) containing 0.1 % formic acid (≥ 98 %, v/v; obtained from Carl Roth, Karlsruhe, Germany). The HPLC outflow was split whereby 20 % of total flow was introduced into the ICPMS (Agilent 7500ce) for determination of arsenic-containing species. The outflow from the HPLC reaching the ICPMS was supported over a T-piece by an aqueous internal standard containing 1 % formic acid (v/v) and In, Ge, and Te (1000 mg L^{-1}, obtained from Carl Roth) at concentrations of 20 µg L^{-1} each, delivered at a flow of 0.5 mL min^{-1}. Carbon compensation was performed as described by Raber et al. (2010) by introducing aqueous EtOH (20 %, v/v) directly into the spray chamber at a flow of 0.3 mL min^{-1} to ensure a constant carbon load reaching the plasma.

HR-ESMS measurements were performed using a Q-Exactive Hybrid Quadrupole-Orbitrap MS following HPLC with a Dionex Ultimate 3000 series HPLC-system (Thermo Fisher Sci., Erlangen, Germany). The injection volume was 5 µL; chromatographic conditions were the same as for HPLC-ICPMS. The HR-MS was fitted with a HESI-II atmospheric pressure electrospray ionization source, using nitrogen as nebulizer and drying gas. Measurements were performed in positive ionization mode using the following settings: spray voltage 3.2 kV, capillary temperature 270 °C, sheath gas flow rate 52.5 instrument units (IU), auxiliary gas temperature 438 °C, auxiliary gas flow rate 14 IU, automatic gain control target $1e^6$, maximum injection time 100 ms, and the resolution was 70000 (FWHM). The mass range m/z 300-1500 was scanned and data dependent fragmentation of the five most intense ions (isolation window was 0.4 m/z) at two normalized collision energies (NCE: 30, soft; 50, hard) was performed with subsequent recording of fragment ions.

Besides accurate mass and MS/MS determinations, we used two reference materials for the identification and confirmation of proposed arsenolipid compounds in this study. The certified reference material NMIJ 7405-a (Trace Elements and Arsenic Compounds in Seaweed - Hijiki)

from the Natural Metrology Institute of Japan (Tsukuba, Ibaraki, Japan) was previously characterized for its arsenolipid content including As-hydrocarbons and various arsenosugar phospholipids (Glabonjat et al. 2014). Our in-house reference material *Dunaliella tertiolecta*, a marine microalga cultured and harvested in our laboratory in Graz (Glabonjat et al. 2017), was also previously characterized for water- and lipid-soluble arsenicals including the unusual phytyl 5-dimethylarsinoyl-2-*O*-methyl-ribofuranoside **12** (Glabonjat et al. 2017; Glabonjat et al. 2018). The two reference materials (10 mg dry mass) were weighed directly into polypropylene tubes (2 mL; Greiner Bio-One Kremsmuenster, Austria) and extracted with 1 mL of a mixture of dichloromethane (≥ 99.9 %; obtained from VWR) and EtOH (2+1, v/v) for 1 h on a head-over-head rotating cross at room temperature. After centrifugation of the mixtures (10 min, 5000 G), the supernatants were transferred to new vials, evaporated to dryness (50 mbar, 25 °C) on a Christ RVC 2-33 CD plus (Martin Christ GmbH, Osterode am Hartz, Germany), and stored at -20 °C until analysis, which was usually within 24 hours. Dry lipid extracts of NMIJ 7405-a Hijiki and *D. tertiolecta* were re-dissolved directly before HPLC-ICPMS/HR-ESMS measurements in pure EtOH (300 µL) and analyzed together with GSL-samples. Quantification of arsenolipids was based on external calibration against six arsenolipid standards, namely AsFA362, AsFA388, AsFA418, AsHC332, AsHC360, and AsHC444. These standards were synthesized in-house according to Taleshi et al. (2014) and standard solutions of these species, each at 7.5 mg As L^{-1}, were prepared in EtOH.

An aliquot of the total lipid extract was analyzed for total intact polar microbial lipids on a Bruker maXis Plus ultra-high-resolution quadrupole time-of-flight mass spectrometer (QTOF) coupled to a Dionex Ultimate 3000RS ultra-high-performance liquid chromatography system with an electrospray ionization source. Separation of intact lipids followed the protocol by Woermer et al. (2013) using a Water Acquity UPLC BEH Amide column (2.1 x 150 mm, 1.7 µm), 40 °C column temperature and a gradient program of 99 % A (A: acetonitrile/dichloromethane, 75+25, incl. 0.01 % formic acid and 0.01 % ammonium hydroxide, v/v) held for 2.5 min at a flow of 0.4 mL min^{-1}, ramping to 5 % B at 4 min (B: methanol/water, 50+50, incl. 0.4 % formic acid and 0.4 % ammonium hydroxide, v/v), 25 % B at 22.5 min and 40 % B at 26.5 min, held for 1 min and equilibrated to initial conditions for 8 min. Identification of compounds occurred via accurate masses, retention times and comparison to authentic standards and previously published data (e.g. Sturt et al. (2004); Schubotz et al. (2018)). Compounds of interest were quantified using manual peak integration

and comparing the peak areas of the monoisotopic mass of the parent ions to an internal standard C16-PAF (Avanti Polar Lipids, Berlin, Germany). Response factors were considered when commercially available standards were available (1G-DAG, 2G-DAG, SQ-DAG, PG-DAG and DGTS, Avanti Polar Lipids). For the detected archaeal lipids, no commercial standards were available therefore, their abundances could not be corrected. The range of response factors for non-archaeal lipids relative to C16-PAF was 0.08 (for the anionic SQ-DAG) to 2.2 (for the zwitterionic DGTS); we assume the maximum error for archaeal lipids to be in a similar range.

Table S1. Proposed fragments originating from the major naturally occurring isoprenoidal arsenoriboside compounds present in extracts from GSL sediments. The m/z values are given for $[M+H]^+$ fragment ions.

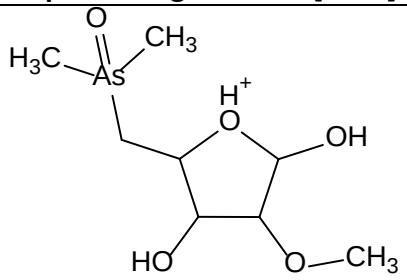
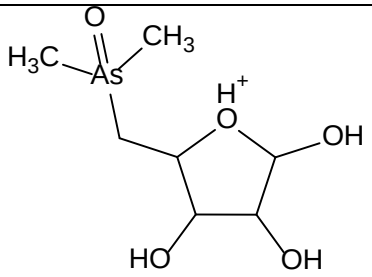
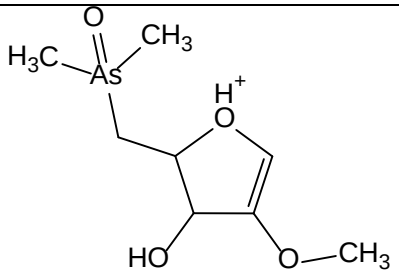
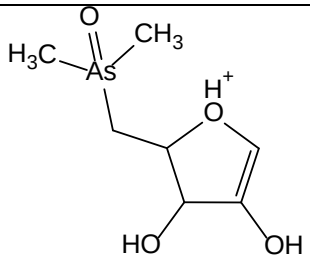
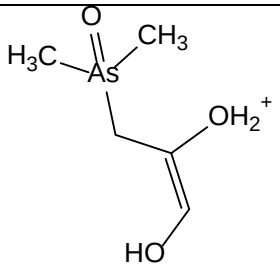
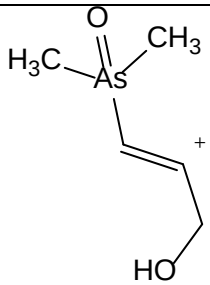
Elemental composition	Calculated mass $[M+H]^+$	Proposed fragment ion $[M+H]^+$
$C_8H_{17}AsO_5$	269.0364	
$C_7H_{15}AsO_5$	255.0208	
$C_8H_{15}AsO_4$	251.0259	
$C_7H_{13}AsO_4$	237.0102	
$C_5H_{11}AsO_3$	194.9997	
$C_5H_9AsO_2$	176.9891	

Table S1 continued.

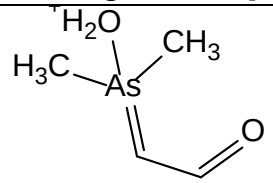
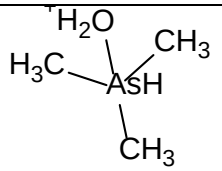
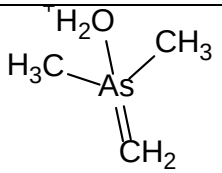
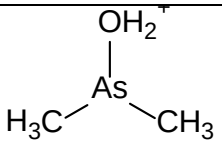
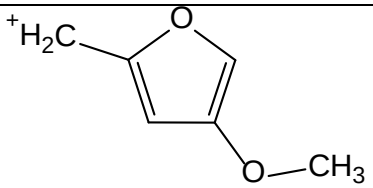
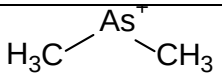
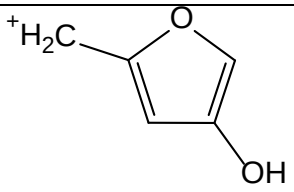
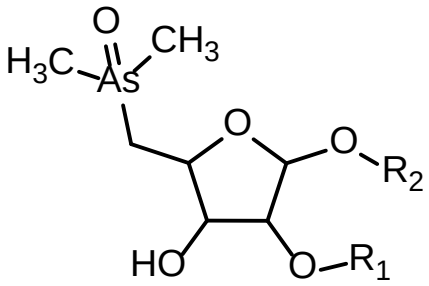
Elemental composition	Calculated mass $[M+H]^+$	Proposed fragment ion $[M+H]^+$
$C_4H_9AsO_2$	164.9891	
$C_3H_{11}AsO$	139.0099	
C_3H_9AsO	136.9942	
C_2H_7AsO	122.9786	
$C_6H_7O_2$	111.0441	
C_2H_5As	104.9680	
$C_5H_5O_2$	97.0284	

Table S2. Mass spectral data for all 29 arsenolipids identified in Great Salt Lake sediment extracts in this study.



Compound	Elemental composition	R_1	R_2	Calculated mass $[M+H]^+$	Measured mass $[M+H]^+$	Mass difference [ppm]
1 (AsHC332)	$C_{17}H_{37}AsO$	-	-	333.2133	333.2133	+0.1
2 (AsHC346)	$C_{18}H_{39}AsO$	-	-	347.2290	347.2290	+0.1
3 (AsHC360)	$C_{19}H_{41}AsO$	-	-	361.2446	361.2449	+0.9
4 (AsHC388)	$C_{21}H_{45}AsO$	-	-	389.2759	389.2760	+0.2
5 (AsHC404)	$C_{23}H_{37}AsO$	-	-	405.2133	405.2137	+0.8
6 (AsHC406)	$C_{23}H_{39}AsO$	-	-	407.2290	407.2293	+0.9
7 (AsHC408)	$C_{23}H_{41}AsO$	-	-	409.2446	409.2450	+1.0
8 (AsHC410)	$C_{23}H_{43}AsO$	-	-	411.2603	411.2605	+0.6
9	$C_{28}H_{55}AsO_6$	CH_3	$C_{20}H_{39}O$	563.3287	563.3293	+1.0
10	$C_{28}H_{53}AsO_6$	CH_3	$C_{20}H_{37}O$	561.3131	561.3135	+0.8
11	$C_{28}H_{57}AsO_5$	CH_3	$C_{20}H_{41}$	549.3494	549.3499	+0.8
12	$C_{28}H_{55}AsO_5$	CH_3	$C_{20}H_{39}$	547.3338	547.3340	+0.3
13	$C_{27}H_{53}AsO_6$	H	$C_{20}H_{39}O$	549.3131	549.3127	-0.7
14		CH_3	$C_{19}H_{37}O$		549.3136	+0.9
15	$C_{27}H_{55}AsO_5$	CH_3	$C_{19}H_{39}$	535.3338	535.3344	+1.0
16		H	$C_{20}H_{41}$		535.3335	-0.6
17	$C_{27}H_{53}AsO_5$	H	$C_{20}H_{39}$	533.3181	533.3185	+0.7
18	$C_{26}H_{53}AsO_6$	CH_3	$C_{18}H_{37}O$	537.3131	537.3136	+1.0
19	$C_{26}H_{51}AsO_6$	H	$C_{19}H_{37}O$	535.2974	535.2968	-1.3
20	$C_{26}H_{53}AsO_5$	CH_3	$C_{18}H_{37}$	521.3181	521.3187	+1.1
21	$C_{26}H_{51}AsO_5$	CH_3	$C_{18}H_{35}$	519.3025	519.3027	+0.4
22	$C_{25}H_{51}AsO_5$	CH_3	$C_{17}H_{35}$	507.3025	507.3030	+1.0
23		H	$C_{18}H_{37}$		507.3029	+0.8
24	$C_{24}H_{49}AsO_5$	CH_3	$C_{16}H_{33}$	493.2868	493.2878	+1.8
25		H	$C_{17}H_{35}$		493.2876	+1.5
26	$C_{23}H_{47}AsO_5$	CH_3	$C_{15}H_{31}$	479.2712	479.2718	+1.2
27		H	$C_{16}H_{33}$		479.2722	+1.9
28	$C_{22}H_{45}AsO_5$	CH_3	$C_{14}H_{29}$	465.2555	465.2564	+1.8
29		H	$C_{15}H_{31}$		465.2559	+0.9

Table S3. Intact polar membrane lipid concentrations (μg g^{-1}) of chloroplast lipids (1G-DAG – monoglycosyl diacylglycerol, 2G-DAG – diglycosyl diacylglycerol, SQ-DAG – sulfoquinovosyl diacylgylcerol, PG-DAG - phosphatidylglycerol diacylglycerol), betaine lipids and dominant classes of archaeal lipids (IP – intact polar, i.e. containing different types of polar head groups, C20 – C20 phytyl chain, C25 – C25 isoprenoid chain, CL – archaeal cardiolipin, GDGT – glycerol dialkyl glycerol tetraethers) found in lipid extracts of surface sediments from the four Great Salt Lake (GSL) sites. Presented also are percent abundance of C25 archaeal lipids over summed C20 and C25 archaeal lipids, and percent abundance of total archaeal lipids over summed total archaeal lipids, chloroplast and betaine lipids.

	GSL1 North Basin	**GSL2 Gunnison Island**	**GSL3 South Shore**	**GSL4 Farmington Bay**
Chloroplast lipids	**8.7**	**42.8**	**81.4**	**756**
1G-DAG	1.5	11.5	4.4	89.0
2G-DAG	0.4	1.5	5.0	31.2
SQ-DAG	4.0	20.6	45.3	360
PG-DAG	2.9	9.1	26.7	276
Betaine lipids	**0.5**	**0.9**	**9.9**	**65.1**
Archaeal lipids	**1.1**	**0.3**	**0.3**	**0.3**
C20 IP-archaeol + CL	0.88	0.26	0.22	0.20
C25 IP-archaeol + CL	0.11	0.04	0.05	0.04
IP-GDGT	0.13	0.03	0.003	0.04
% C25 archaeal lipids	9.7	11.5	18.0	15.4
% archaeal lipids	11.4	0.8	0.3	0.04

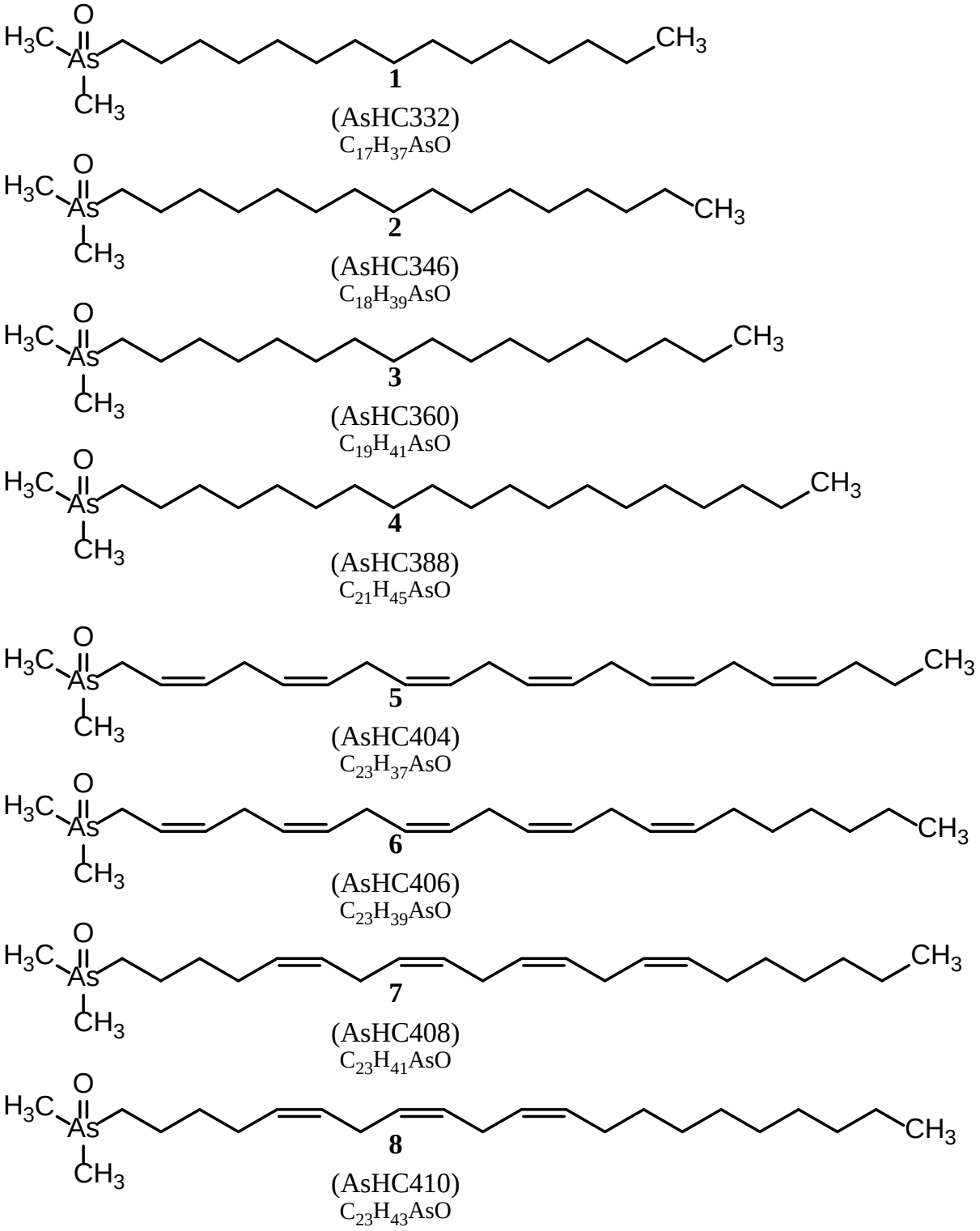


Figure S1. Arsenic containing hydrocarbons (AsHCs) found in Great Salt Lake sediment extracts. For unsaturated AsHCs we show only one possible isomer of the lipophilic side-chain.

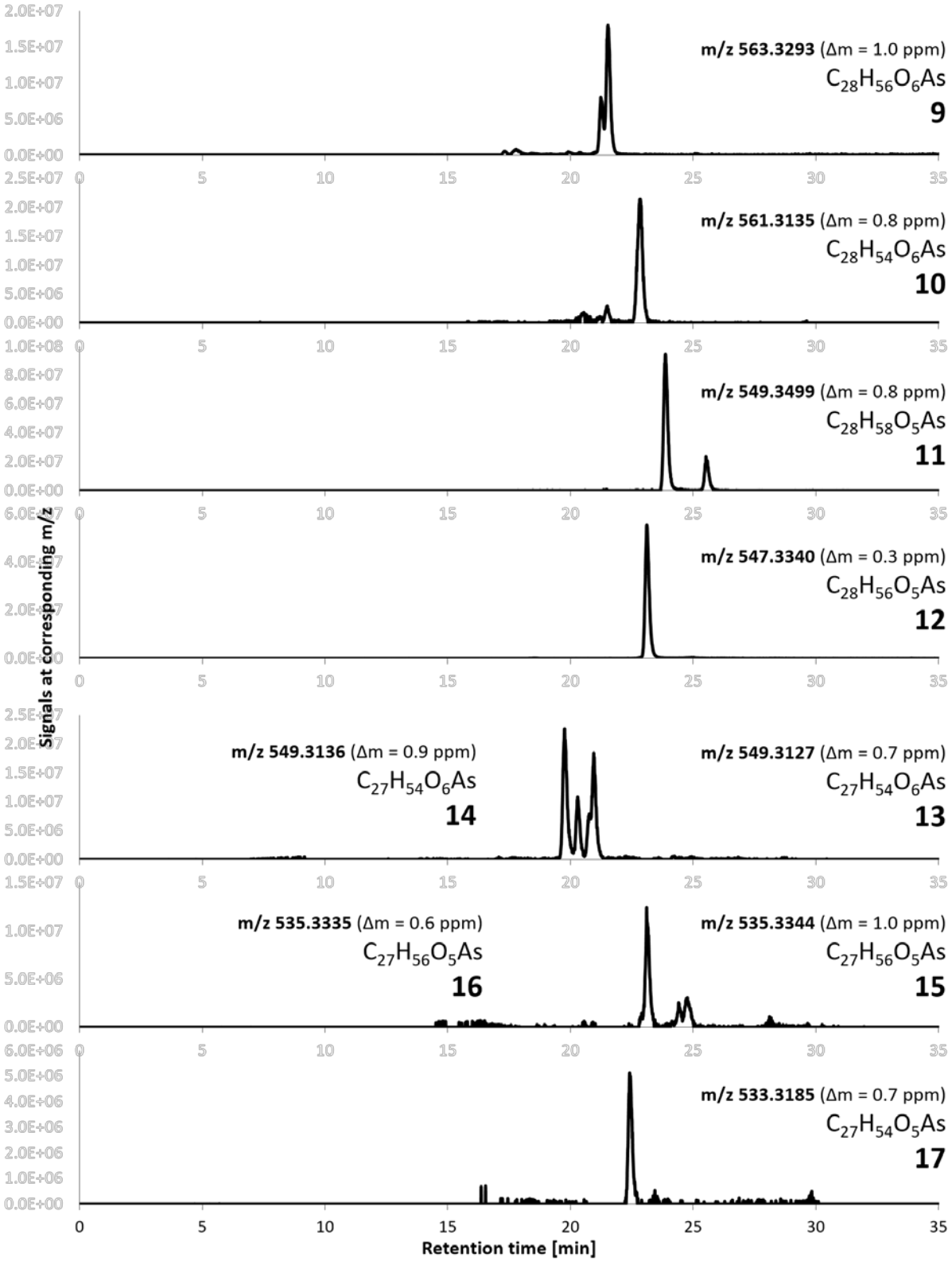
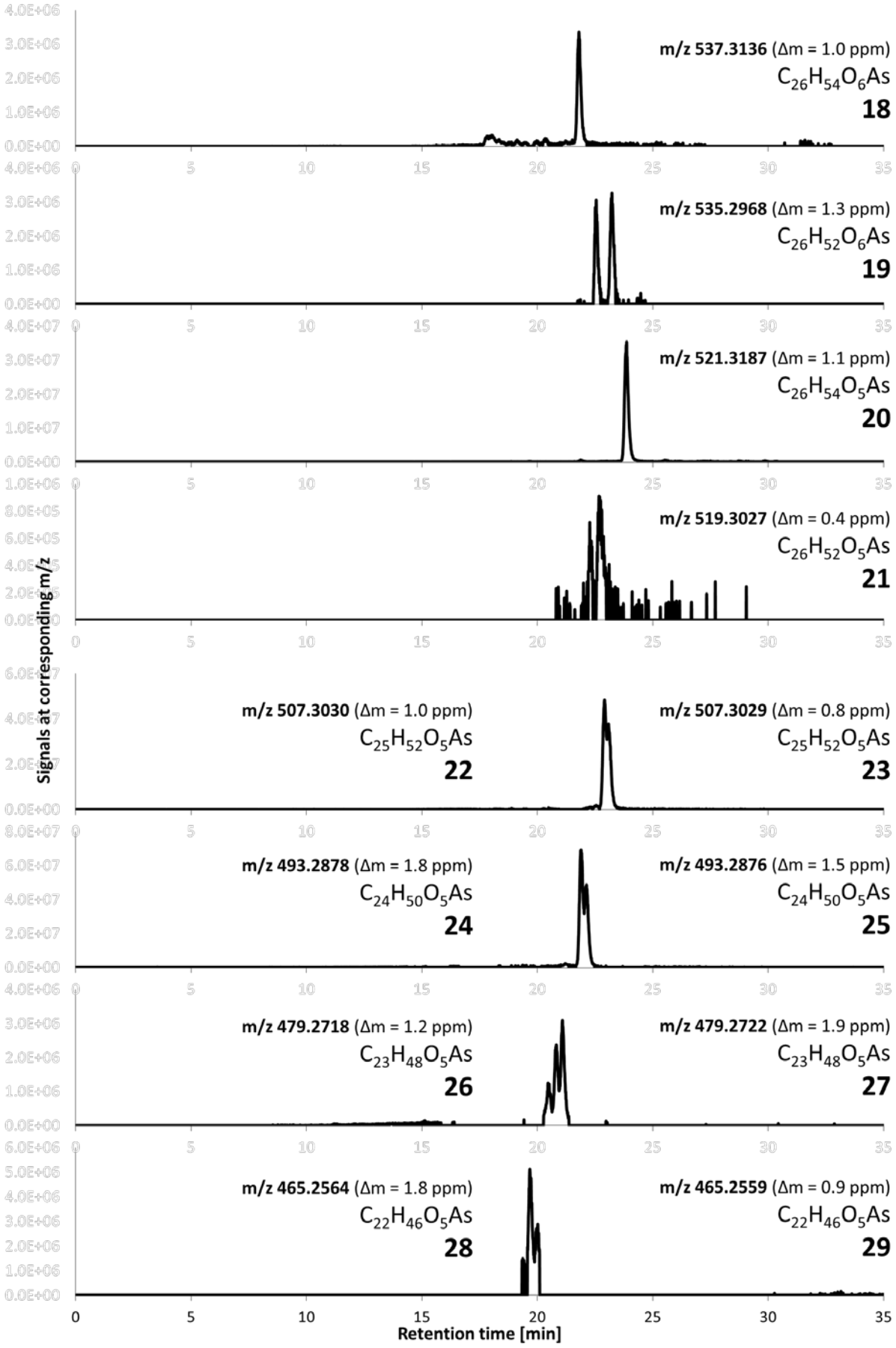


Figure S2. RP-HPLC-HR-ESMS chromatograms of all 29 arsenolipids found in GSL sediment extracts. HPLC conditions: Asahipak ODP-50 (4.6 x 125 mm, 5 µm); 40 °C; 5 µL injection volume; flow rate 0.5 mL min^{-1}; mobile min, 30 % B; 1-30 min, 30-100 % B; 30-32 min, 100 % B; 32-32.1 min, 100-30 % B; 32.1-40 min, 30 % B.



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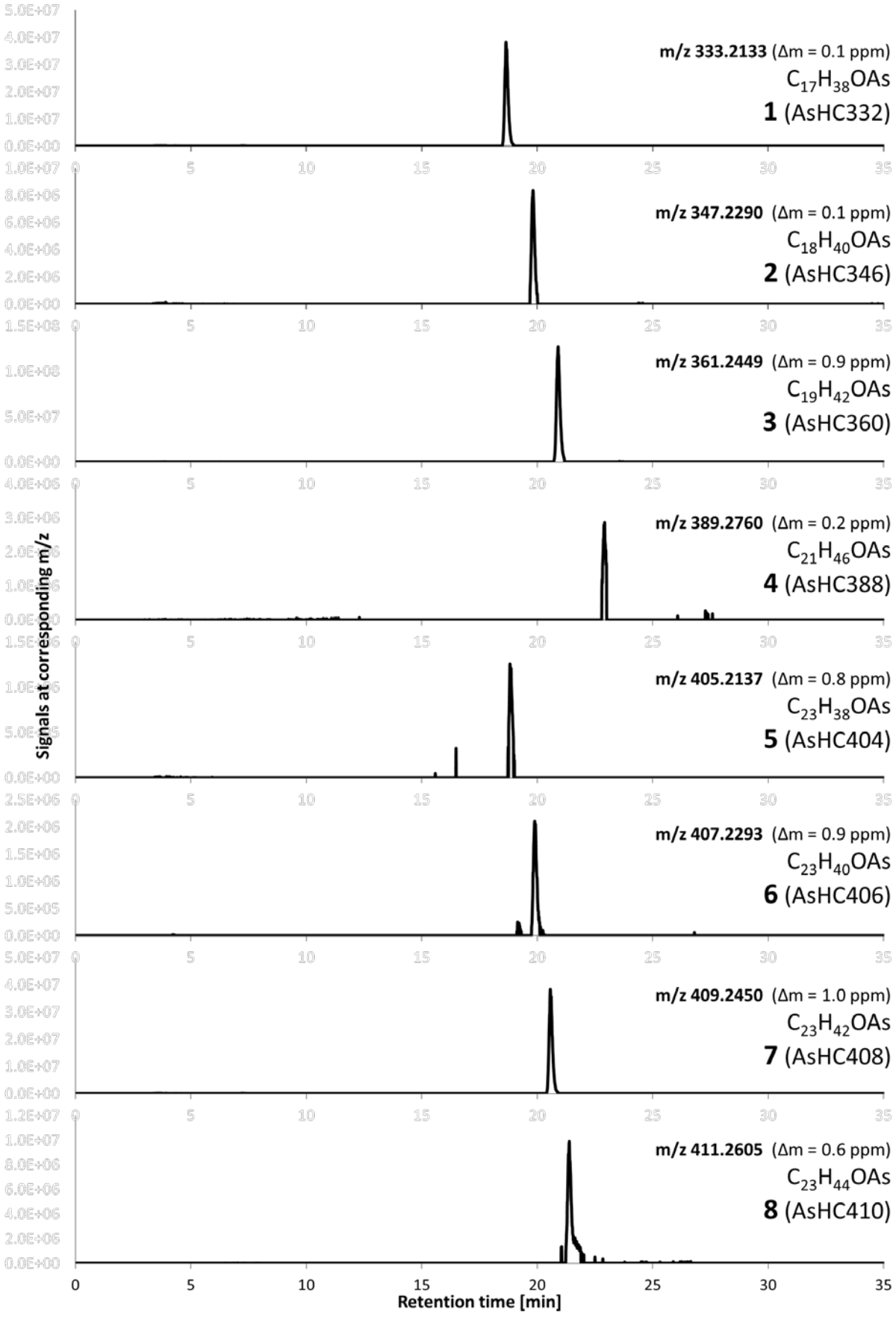

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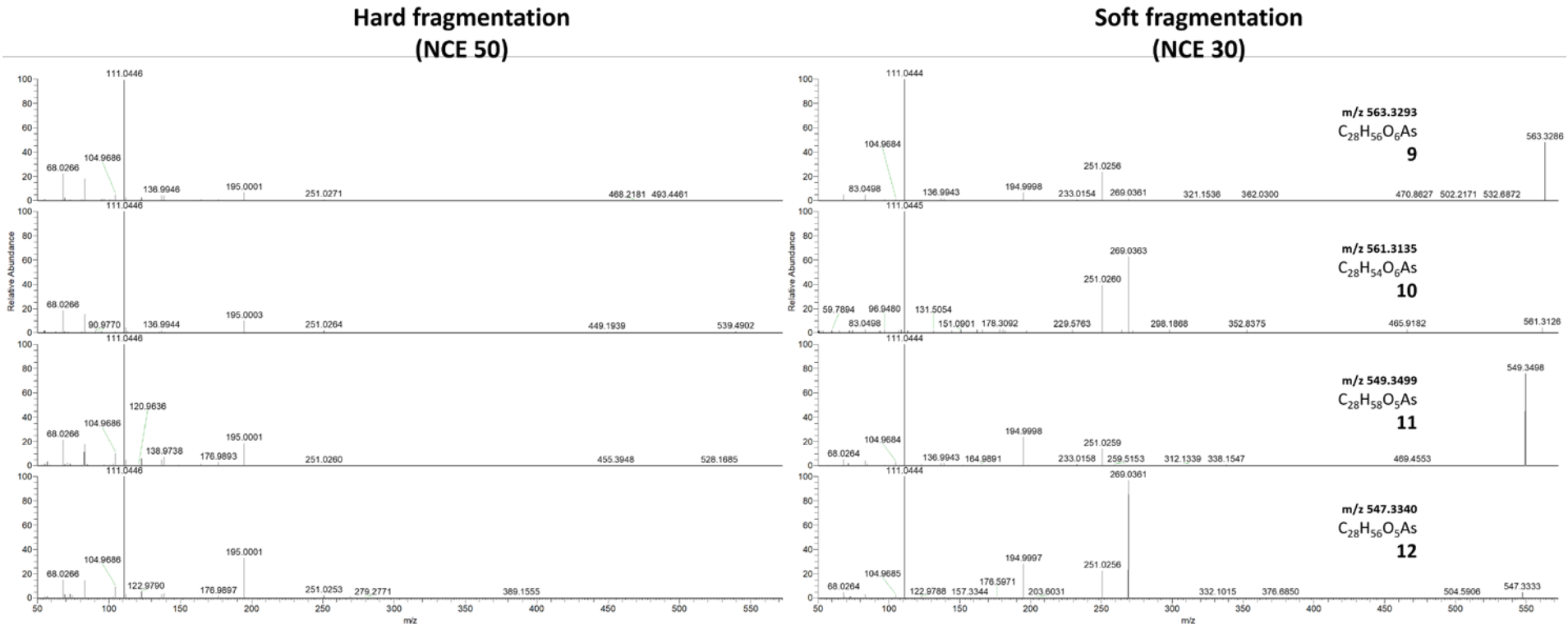
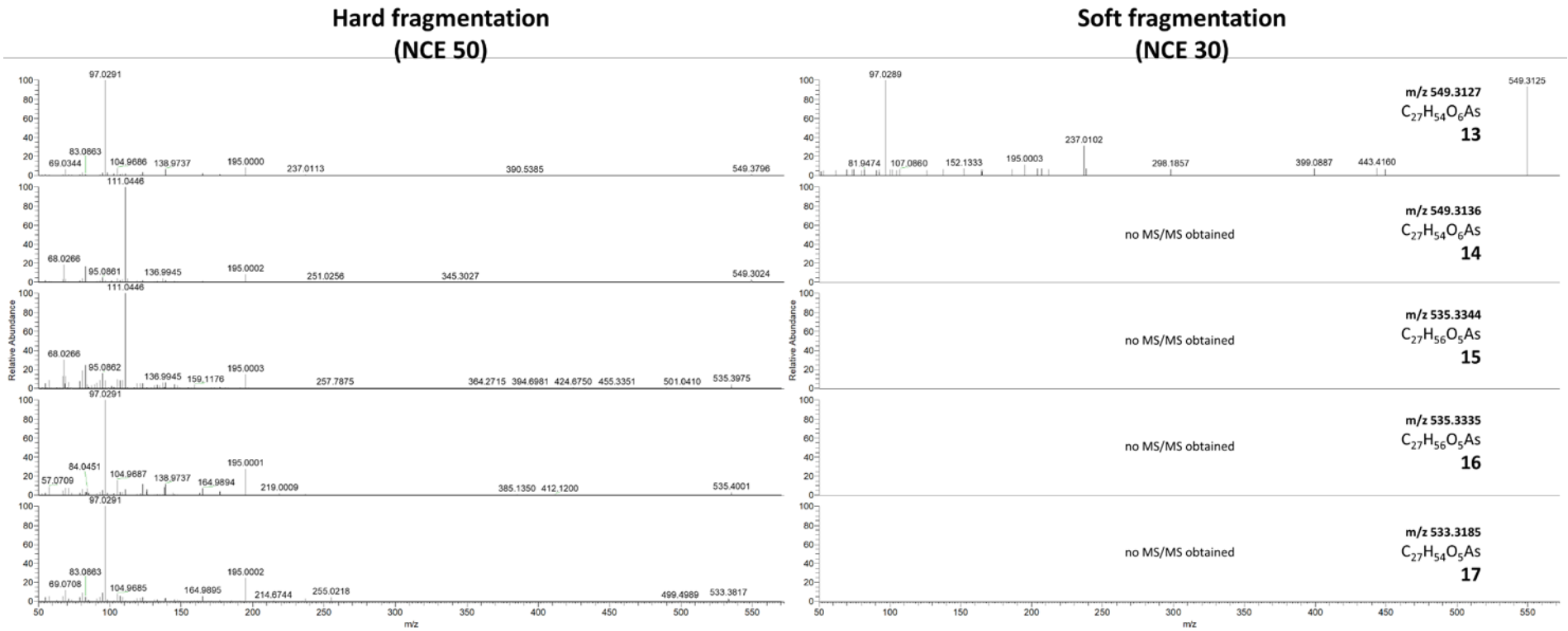


Figure S3. Tandem mass spectra of all naturally occurring arsenolipids including the phytyl 2-*O*-methylriboside (**12**) present in Great Salt Lake sediment samples. The given m/z values are for $[M+H]^+$ precursor ions used for fragmentation at a collision energies of 50 NCE (hard fragmentation) and 30 NCE (soft fragmentation). For some of the $[M+H]^+$ ions, the initial concentrations were too low to obtain MS/MS spectra.



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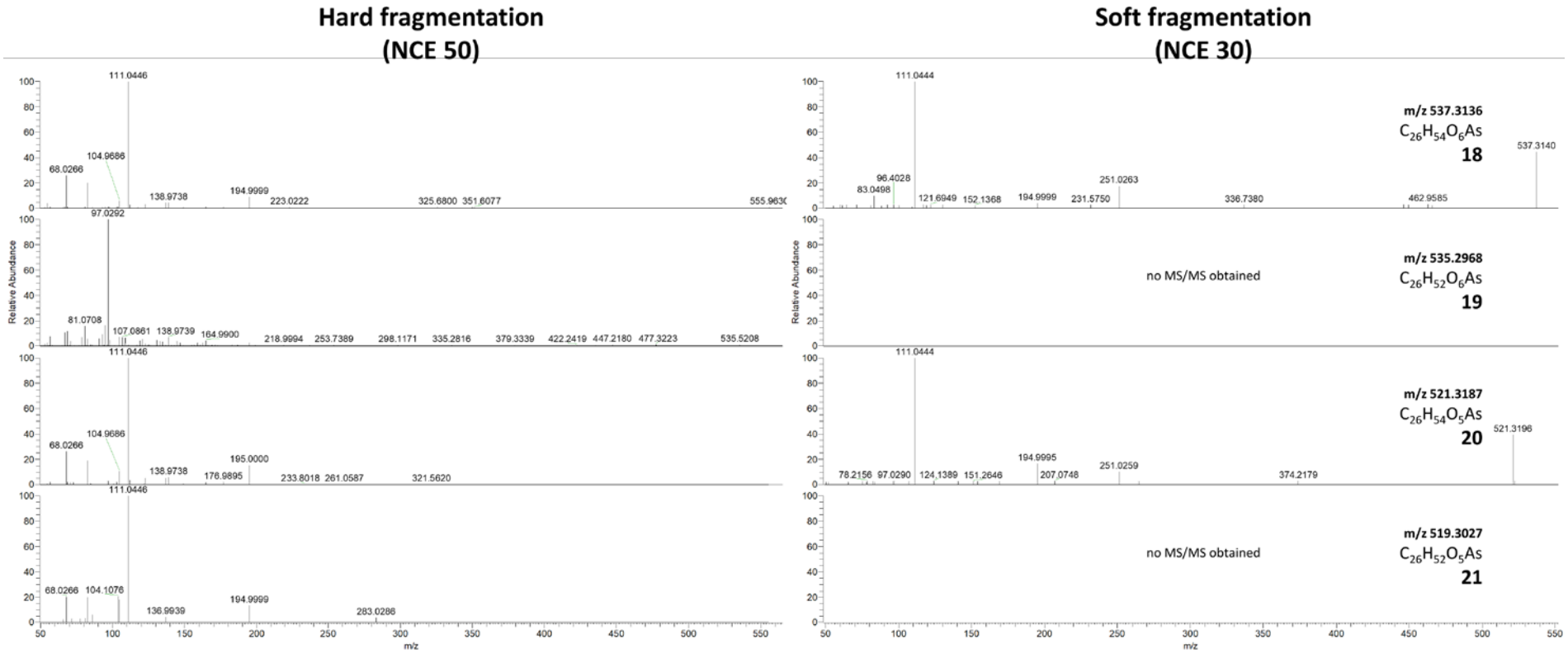

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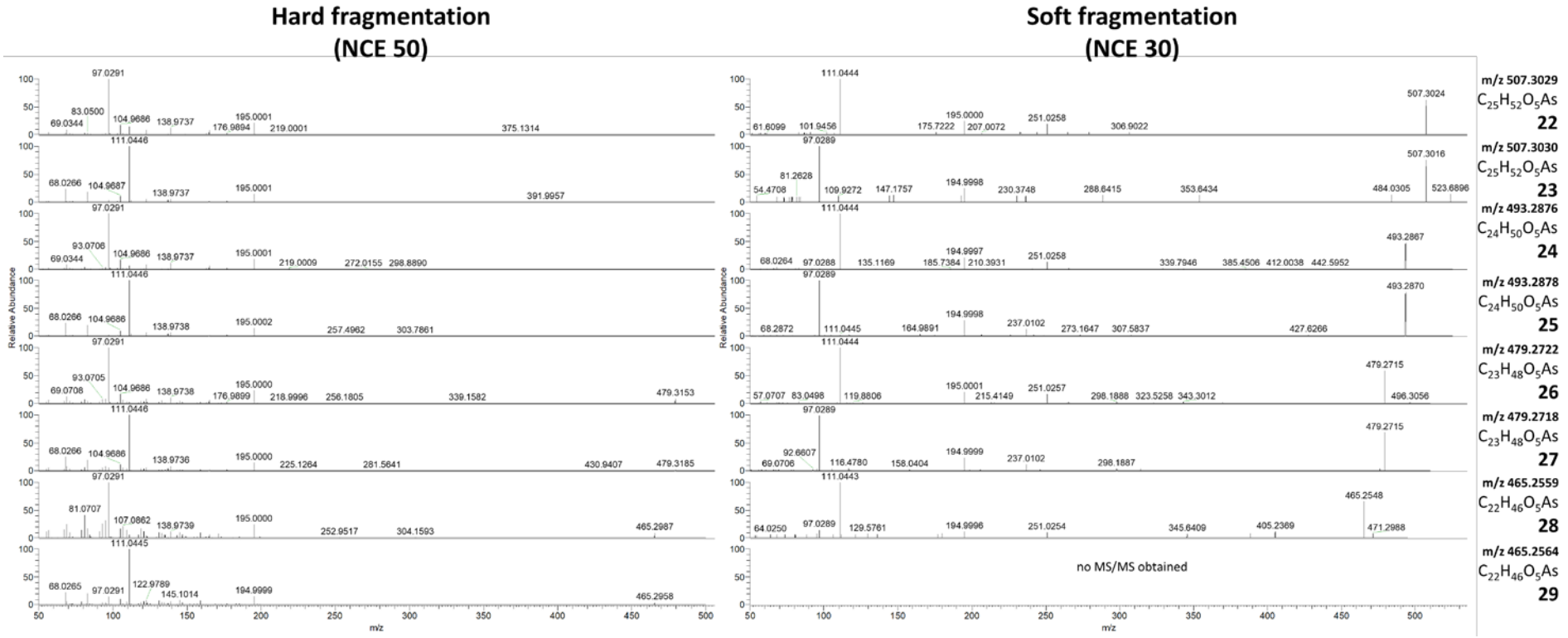

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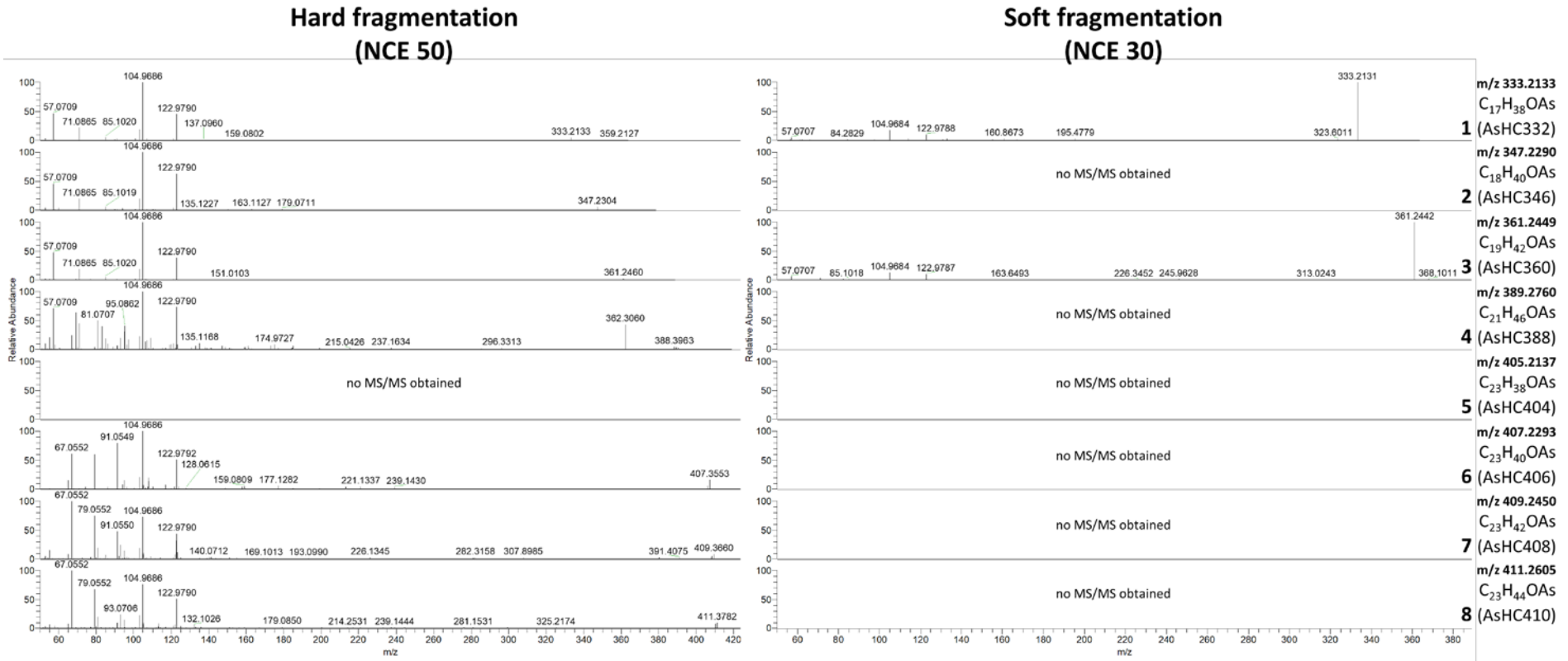

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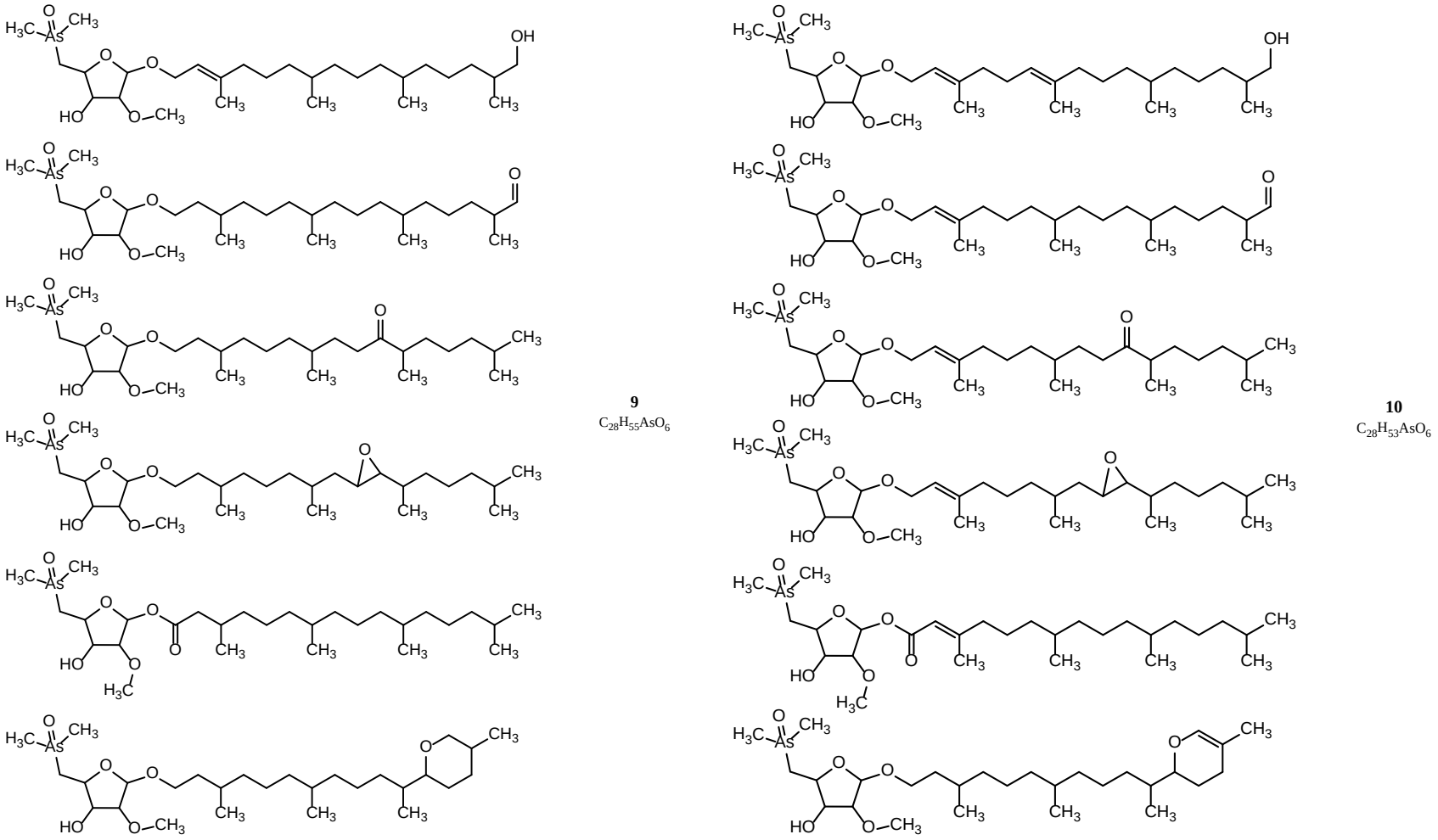

9
$C_{28}H_{55}AsO_6$

10
$C_{28}H_{53}AsO_6$

Figure S4. Possible structures of arsenic containing ribosyl lipids **9** and **10** found in Great Salt Lake sediments (the lipophilic side chain modifications presented here are also applicable to compounds **13**, **14**, **18**, and **19**). For alcohols, aldehydes, ketones, epoxides, esters, and ring-systems we show only one possible isomer of the lipophilic side-chain.

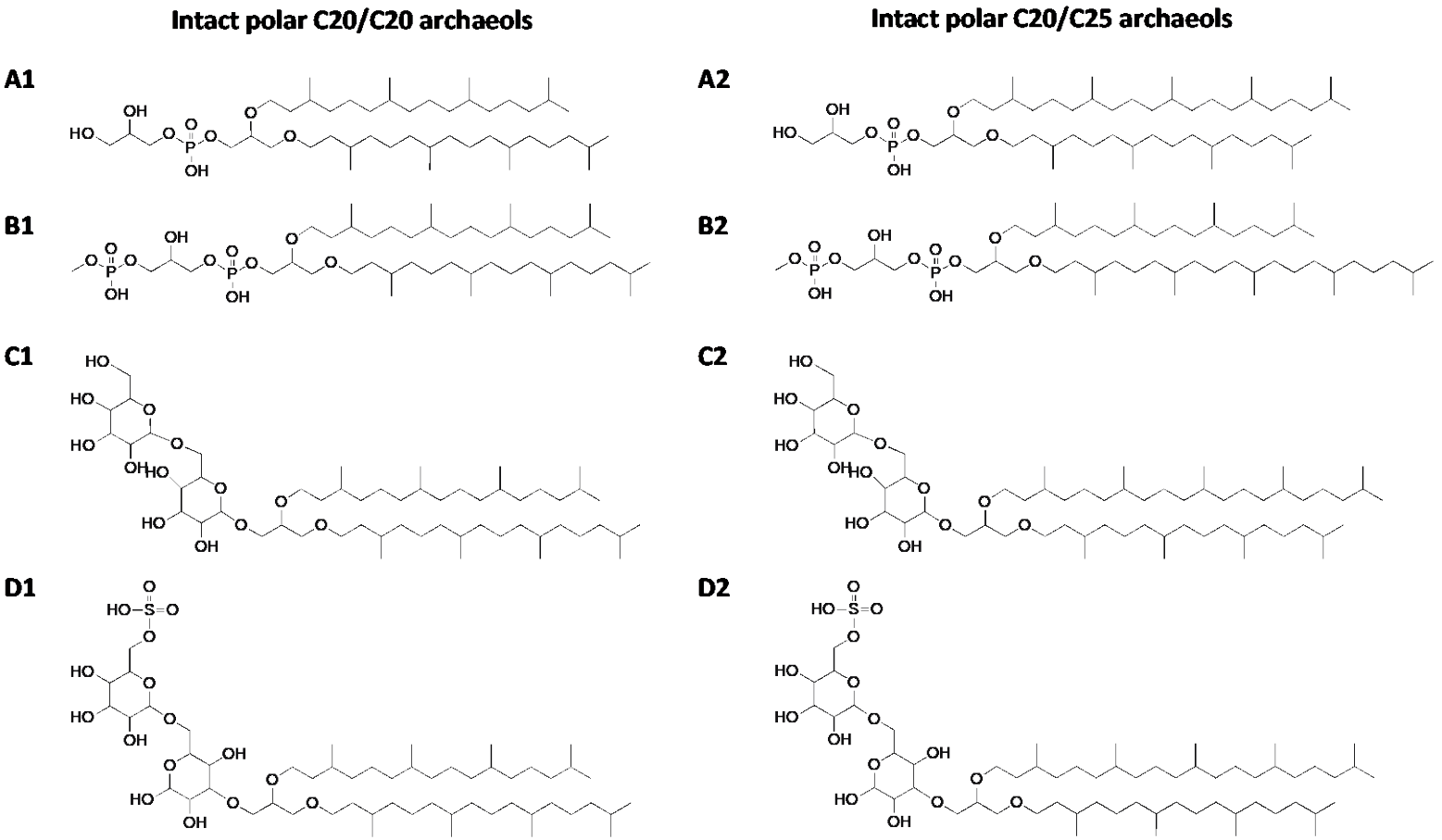

Figure S5. Examples of detected archaeal lipids in the Great Salt Lake sediments with C20 and C25 isoprenoidal hydrocarbon chains, characteristic for halophilic archaea. (A1) C20/C20 archaeol and (A2) C20/C25 extended archaeol with phosphatidyl glycerol head group; (B1) C20/C20 archaeol and (B2) C20/C25 extended archaeol with phosphatidyl glycerol methyl phosphate head group; (C1) C20/C20 archaeol and (C2) C20/C25 extended archaeol with diglycosyl head group; (D1) C20/C20 archaeol and (D2) C20/C25 extended archaeol with sulfated diglycosyl head group.

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