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Supporting Information

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Diana Koteska,[a] Pia Marter, [b] Sixing Huang, [b] Silke Pradella, [b] Jörn Petersen,[b] and Stefan Schulz*[a]

Abstract: Volatiles released by the apicomplexan alga *Chromera velia* CCAP1602/1 and their associated bacteria were investigated. A metagenome analysis allowed the identification of the most abundant heterotrophic bacteria of the phycosphere, but the isolation of additional strains showed that metagenomics underestimated the complexity of the algal microbiome, However, the culture-independent approach revealed the presence of a planctomycete that likely represents a novel bacterial family. We analysed algal and bacterial volatiles by open-system-stripping analysis (OSSA) on Tenax TA desorption tubes, followed by thermodesorption, cryofocusing and GC/MS-analysis. The analyses of the alga and the abundant bacterial strains *Sphingopyxis litoris* A01A-101, *Algihabitans albus* A01A-324, "*Coraliitalea coralii*" A01A-333 and *Litoreibacter* sp. A01A-347 revealed sulfur- and nitrogen-containing compounds, ketones, alcohols, aldehydes, aromatic compounds, amides, one lactone, as well as typical algal products, apocarotenoids. The compounds were identified by gas chromatographic retention indices, comparison of mass spectra and syntheses of reference compounds. A major algal metabolite was 3,4,4-trimethylcyclopent-2-en-1-one, an apocarotenoid indicating the presence of carotenoids related to capsanthin, not reported from algae so far. A low overlap in volatiles bouquets between *C. velia* and the bacteria was found and the xenic algal culture almost exclusively released algal components.

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Results

Table S3. VOCs identified in headspace extracts of *Sphingopyxis litoris* A01A-101.

Compound	RI (exp)[a]	RI (lit)[b]	Identification[c]	Rep 1	Rep 2	Rep 3
Pentan-3-one (**69**)	738	707	ms, ri	x	xx	xx
3-Methylpentan-2-one (**63**)	765	771[d]	ms, ri	xx		xx
5-Methylhexan-3-one (**72**)	831	858[d]	ms, ri	xx	xx	
4-Methylhexan-3-one (**78**)	831	870[d]	ms, ri	xx	xx	
3-Methylpyridine (**44**)	860	860	ms, ri	xx	xx	xx
Hexanenitrile (**39**)	878	879	ms, ri	x	xx	xx
5-Methylhex-4-en-3-one (**80**)	895		ms	xx	xx	xx
2,5-Dimethylpyridine (**45**)	932	928	ms, ri	xx	xx	xx
(*E*)-4-Methylhex-4-en-3-one (**81**)	932	934	ms, ri	xx	xxx	xx
3,5-Dimethylpyridine (**46**)	978	980	ms, ri		xx	xx
Heptanenitrile (**40**)	981	985	ms, ri	xx	xx	xx
2,3,6-Trimethylpyridine (**48**)	1008	1009	ms, ri		xx	xx
4-Cyanocyclohexene (**52**)	1018	1028	ms, ri, syn	xx		
N-Isobutylacetamide (**33**)	1036	1027	ms, ri		xx	xx
2,3,5-Trimethylpyridine (**47**)	1057	1060	ms, ri	xx	xx	xx
Octanenitrile (**41**)	1083	1082	ms, ri		xx	xx
Phorone (**82**)	1122	1092	ms, ri		xx	xx
Unknown compound M [134]	1134				xx	xx
N-(3-Methylpentyl)acetamide (**34**)	1136		ms		xx	xx
N-Isopentylacetamide (**35**)	1143	1150	ms, ri		xx	xx
2-Acetyl-5-methylpyridine (**49**)	1160		ms		xx	xx
2,5-Diisopropylpyrazine	1189	1200	ms, ri			xxx
N-Isopentylpropionamide	1215		ms			xx
Cyclohexyl isothiocyanate	1235	1231	ms, ri	xx		
2-Isopropyl-5-*sec*-butylpyrazine	1273	1279	ms, ri			xx
Unknown compound M [208]	1346		ms	xxx	xxx	xxx
N-(2-Phenylethyl)acetamide (**37**)	1517	1512	ms, ri		x	xx

[a]The retention indices are averaged values of the measurements of all used replicates. [b] Retention indices were from NIST Chemistry WebBook or our own database. [c] The compounds were identified by comparison of the mass spectrum to a database spectrum (ms), comparison of the retention index to a published retention index on the same or similar GC fused silica capillary column (ri) or comparison to a synthetic or commercially (syn) available reference compound. [d] GC retention indices were calculated according to an empirical method.[S1] exp = experimental. lit = literature. Rep = replicate. The amounts of the compounds are given as 0–2 % (x), 2–20 % (xx), 20–100 % (xxx) relative to the largest peak area in the total ion chromatogram.

Table S4. VOCs identified in the headspace extracts of *Algihabitans albus* A01A-324.

Compound	RI (exp)[a]	RI (lit)[b]	Identification[c]	Rep 1	Rep 2	Rep 3
4-Cyanocyclohexene (**52**)	1018	1028	ms, ri, syn	xx		
N-Isobutylacetamide (**33**)	1035	1027	ms, ri			xx
N-Isobutylpropionamide	1107		ms			xx
N-(2-methylbutyl)acetamide (**34**)	1136		ms		xx	xx
N-Isopentylacetamide (**35**)	1144	1150	ms, ri		xx	xx
Tropone (**91**)	1159	1182	ms, ri	xx	xx	xx
2,5-Diisopropylpyrazine	1190	1200	ms, ri			xx
Tropolone	1204		ms	xx		x
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)pent-1-en-3-one	1524	1503	ms, ri	xx		

[a]The retention indices are averaged values of the measurements of all used replicates. [b] Retention indices were from NIST Chemistry WebBook or our own database. [c] The compounds were identified by comparison of the mass spectrum to a database spectrum (ms), comparison of the retention index to a published retention index on the same or similar GC fused silica capillary column (ri) or comparison to a synthetic or commercially (syn) available reference compound. [d] GC retention indices were calculated according to an empirical method developed by Schulz et al..[S2] exp = experimental. lit = literature, Rep = replicate. The amounts of the compounds are given as 0–2 % (x), 2–20 % (xx), 20–100 % (xxx) relative to the largest peak area in the total ion chromatogram.

Table S5*.* VOCs identified in the headspace extracts *Coraliitalea coralii* A01A-333.

Compound	RI (exp)[a]	RI lit[b]	Identification[c]	Rep 1	Rep 2	Rep 3
Hexane-3,4-dione (**92**)	801	800	ms, ri	xx	xx	
2-Hydroxypentan-3-one (**95**)	812	821	ms, ri	xx	xxx	xxx
4-Thiapentan-2-one (**31**)	838	863	ms, ri		xx	x
2,4,5-Trimethyloxazole (**50**)	844	846	ms, ri		xx	xx
5-Methylhexan-2-one (**55**)	854	862[d]	ms, ri	xx	xx	xx
(*E*)-Hex-2-en-1-ol	875	874	ms, ri	xx		
S-methyl 3-methylbutanethioate (**32**)	937	938	ms, ri	xx	xx	
6-Methylheptan-2-one (**56**)	954	963[d]	ms, ri	xx	xx	xx
5-Methylheptan-2-one (**64**)	964	972[d]	ms, ri	xxx	xxx	xx
Dimethyl trisulfide (**29**)	966	965	ms, ri	xxx	xxx	xx
3-Pyridinecarbonitrile (**43**)	1007	1007	ms, ri		xx	xx
Cyclohept-4-en-1-one (**90**)	1008	1004[S3]	ms, ri		xx	xx
Unknown compound M [126]	1024				xx	xx
7-Methyloctan-2-one (**57**)	1056	1063[d]	ms, ri	xx	xxx	xx
2,3,5,6-Tetramethylpyrazine	1087	1087	ms, ri		xxx	

cis-Hexahydrophthalid-isomer	1091					xx
Unknown compound M [172] a1	1100			xxx	xx	
2-Phenylethanol (**8**)	1114	1114	ms, ri	xxx	xxx	xxx
2,3,5-Trithiahexane (**30**)	1125	1130	ms, ri	xx	xx	
1-Phenylpropan-2-one (**9**)	1131	1124	ms, ri	xxx	xxx	xxx
N-Isopentylacetamide (**35**)	1140	1150	ms, ri		xx	xx
8-Methylnonan-3-one (**73**)	1151	1158[d]	ms, ri	xx	xx	
8-Methylnonan-2-one (**58**)	1158	1163[d]	ms, ri	xx	xxx	xx
Tropone (**91**)	1158	1182	ms, ri		xx	xx
7-Methylnonan-2-one (**66**)	1164	1172[d]	ms, ri	xxx	xxx	xxx
Methyl 2-phenylacetate	1166	1165	ms, ri			xx
Unknown compound M [172] a2	1172			xxx	xx	x
Unknown compound b1	1179			xx	xx	xx
Unknown compound c1	1190			xx	xx	xx
Dimethyl tetrasulfide	1213	1215	ms, ri	xxx		
β-Phenylethyl isocyanate (**11**)	1224	1225	ms, ri		xx	xx
Cyclohexyl isothiocyanate	1231	1231	ms, ri	xx		
Unknown compound b2	1249		ms, ri	xx		
Unknown compound c2	1252		ms, ri	xx		
9-Methyldecan-3-one	1254	1260[d]	ms, ri		xx	
9-Methyldecan-2-one (**59**)	1258	1264[d]	ms, ri	xxx	xxx	xx
2-Aminoacetophenone (**15**)	1302	1302	ms, ri	xxx	xxx	xxx
Unknown compound M [196]	1330				xx	
10-Methyl-3-undecanone (**74**)	1355	1358[d]	ms, ri	xxx	xxx	xx
Unknown compound M [166]	1357			xx		
10-Methylundecan-2-one (**60**)	1359	1366[d]	ms, ri	xx	xxx	xx
9-Methylundecan-2-one (**68**)	1366	1373[d]	ms, ri	xxx	xxx	xxx
Unknown compound M [198]	1416			xx	xx	xx
(*Z*)-Geranylacetone (**88**)	1436	1434	ms, ri	xx	xx	xx
(*E*)-Geranylacetone (**85**)	1455	1458	ms, ri	xx	xxx	xxx
11-Methyldodecan-3-one (**75**)	1461	1458[d]	ms, ri	xxx	xxx	xx
11-Methyldodecan-2-one	1461	1461[d]	ms, ri			xx
N-(2-Phenylethyl)formamide (**36**)	1485	1487	ms, ri		xx	xx
Unknown compound M [196]	1486			xx		
N-(2-Phenylethyl)acetamide (**37**)	1515	1512	ms, ri		xxx	xxx

(3*E*,5*Z*)-6,10-dimethylundeca-3,5,9-trien-2-one (**89**)	1534	1535	ms, ri		xx	xx
Unknown compound M [218]	1540				xx	xx
12-Methyltridecan-3-one (**76**)	1556	1558[d]	ms, ri	xx	xxx	xx
12-Methyltridecan-2-one (**61**)	1562	1565[d]	ms, ri	xxx	xxx	xxx
11-Methyltridecan-2-one (**67**)	1570	1572[d]	ms, ri	xxx	xxx	xxx
12-Methyltridecan-2-ol (**24**)	1575	1575[d]	ms, ri		xx	xx
(3*E*,5*E*)-6,10-dimethylundeca-3,5,9-trien-2-one (**86**)	1588	1589	ms, ri		xx	xx
N-(2-Phenylethyl)propanamide (**38**)	1591		ms		xx	xx
Tetradecan-3-one (**71**)	1592	1585	ms, ri		xx	xx
Unknown compound M [226]	1611			xx	xx	
Unknown compound	1613				xx	xx
Unknown compound M [178]	1646			xx		
13-Methyltetradecan-3-one (**77**)	1657	1658[d]	ms, ri	xx	xx	xx
13-Methyltetradecan-2-one (**62**)	1663	1664[d]	ms, ri	xxx	xxx	xx
12-Methyltetradecan-3-one (**79**)	1664	1670[d]	ms, ri	xx	xxx	
2-Pentadecanone (**54**)	1699	1699	ms, ri		xx	xx
14-Methylpentadecan-2-one	1764	1765[d]	ms, ri			xx
13-Methylpentadecan-2-one (**68**)	1772	1776[d]	ms, ri		xx	xx
Farnesyl acetone (**87**)	1896	1895	ms, ri	xx	xxx	xxx

[a]The retention indices are averaged values of the measurements of all used replicates. [b] Retention indices were from NIST Chemistry WebBook or our own database. [c] The compounds were identified by comparison of the mass spectrum to a database spectrum (ms), comparison of the retention index to a published retention index on the same or similar GC fused silica capillary column (ri) or comparison to a synthetic or commercially (syn) available reference compound. [d] GC retention indices were calculated according to an empirical method developed by Schulz et al..[S2] exp = experimental. lit = literature, Rep = Replicate.a1/a2, b1/2, c1/c2 = isomers. The amounts of the compounds are given as 0–2 % (x), 2–20 % (xx), 20–100 % (xxx) relative to the largest peak area in the total ion chromatogram.

Table S6. VOCs identified in the headspace extracts of *Litoreibacter* sp. A01A-347.

Compound	RI (exp)[a]	RI (lit)[b]	Identification[c]	Rep 1	Rep 2	Rep 3
4-Methylpentan-2-one	757	762[d]	ms, ri	xx		
5-Methylhexan-2-one (**55**)	854	862[d]	ms, ri	xx		xx
Hexane-2,5-dione (**93**)	938	931	ms, ri	xx	xx	
6-Methylheptan-2-one (**56**)	957	963[d]	ms, ri			xx
4-Cyanocyclohexene (**52**)	1018	1028	ms, ri, syn	xxx		xx
1-Phenylpropan-2-one (**9**)	1130	1124	ms, ri	xxx	xxx	xxx
(*E*)-3-Nonen-2-one (**83**)	1141	1140	ms, ri, syn	xxx	xx	xxx

Nonane-2,4-dione (**94**)	1176	1177	ms, ri, syn	xx	xx	xx
1-Phenylbutan-2--one (**10**)	1228	1227	ms, ri, syn	xx	xx	xx
Unknown compound M [168]	1288				xx	xx
Unknown compound M [168]	1293				xx	xx
Unknown compound M [182]	1311				xx	xxx
Unknown compound M [166] d1	1394			xx	xx	xx
(3*E*,5*E*)-Undeca-3,5-dien-2-one (**84**)	1402	1402	ms, ri, syn		xx	xx
1-Phenylhexan-2-one	1411	1412	ms, ri, syn	xx		
Unknown compound M [166] d2	1434				xx	xx
1-Phenylhexan-1-one (**13**)	1463	1460	ms, ri	xx	x	xx
Unknown compound M [196]	1473					xxx
Unknown compound M [222] e1	1507				xx	xx
Unknown compound M [198]	1511			xx	xx	xx
Unknown compound M [210]	1513				xx	xxx
Unknown compound M [210]	1520			xx	xx	xx
Unknown compound M [222] e2	1524				xx	xx
Unknown compound	1558				xx	xx
Unknown compound M [210]	1582			xx	xx	xxx
Unknown compound M [208] f1	1595				xx	xx
Unknown compound M [222] e3	1604			xx		
Unknown compound M [208] f2	1641				xx	xx
Unknown compound M [220] g1	1646				xx	xx
Unknown compound M [220] g2	1679				xx	xx

[a]The retention indices are averaged values of the measurements of all used replicates. [b] Retention indices were from NIST Chemistry WebBook or our own database. [c] The compounds were identified by comparison of the mass spectrum to a database spectrum (ms), comparison of the retention index to a published retention index on the same or similar GC fused silica capillary column (ri) or comparison to a synthetic or commercially (syn) available reference compound. [d] GC retention indices were calculated according to an empirical method developed by Schulz et al..[S2] exp = experimental. lit = literature, Rep = replicate, d1/d2, e1/e2/e3, f1/f2, g1/g2 = isomers. The amounts of the compounds are given as 0–2 % (x), 2–20 % (xx), 20–100 % (xxx) relative to the largest peak area in the total ion chromatogram.

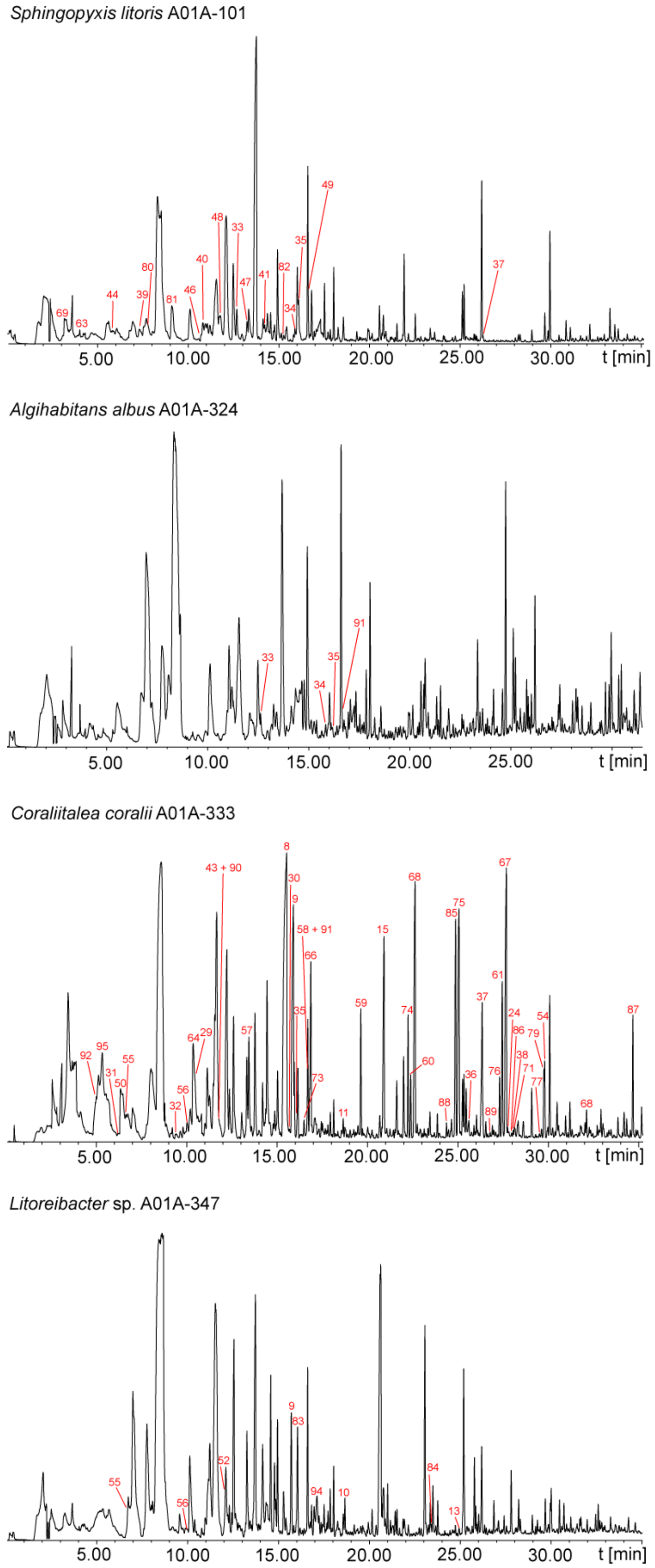


Figure S1. Total ion chromatograms of the headspace analyses of the investigated bacteria in this study. The numbers in the chromatograms refer to the identified compounds from the Tables S3-S6 and the main text.

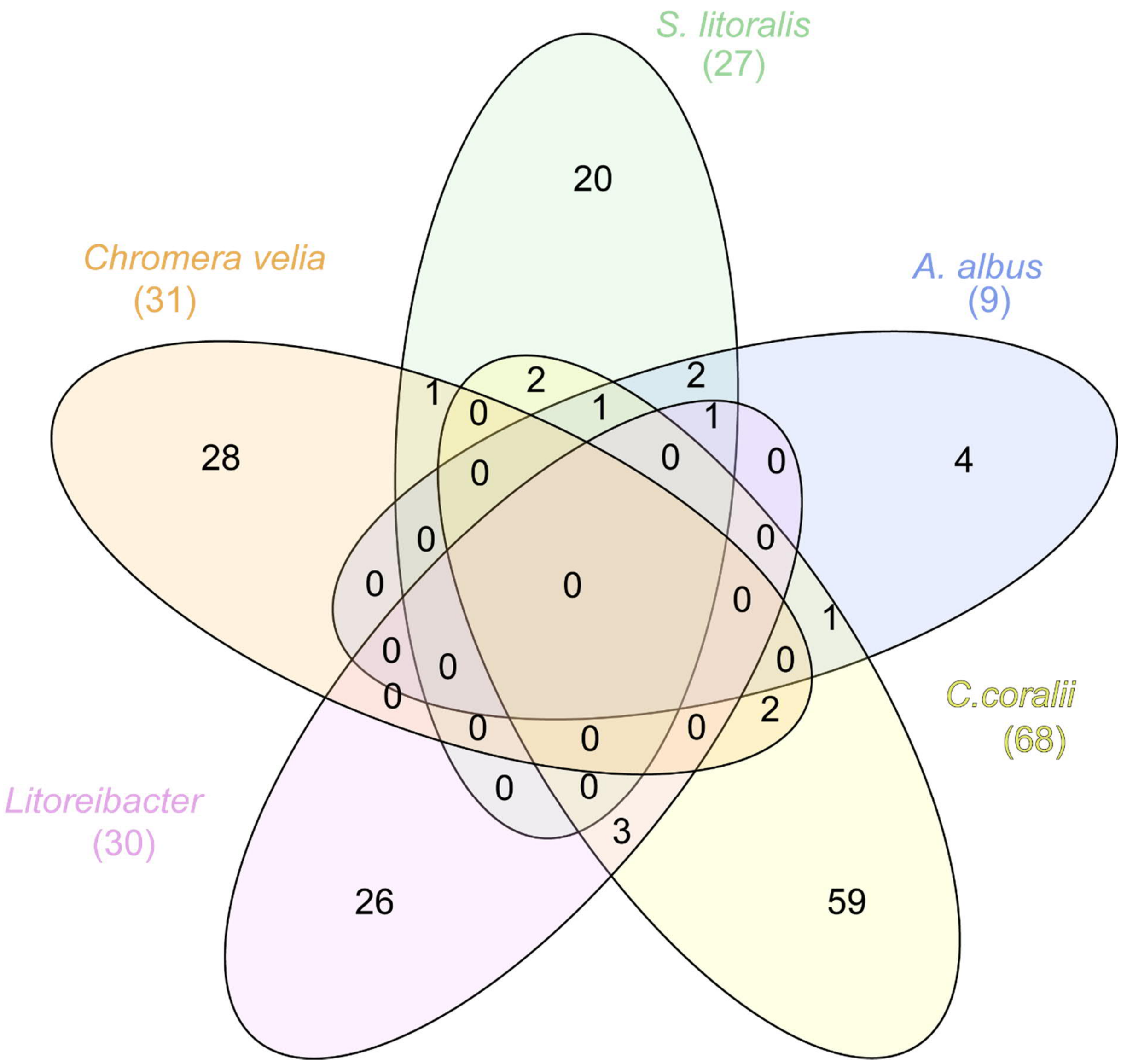


Figure S2. Venn diagram of the of the compounds listed in Table 1 and Tables S3-S6. The diagram shows the low overlap in compounds produced between *Chromera velia* and the bacteria and within the bacteria.

Experimental procedures

General experimental procedures: The chemicals were purchased from Sigma Aldrich (Germany), TCI (Germany) or from abcr GmbH (Germany) and used without further purification. The solvents were purified by distillation and dried according to the usual standard laboratory methods. Reactions with air- and moisture-sensitive compounds were carried out in vacuum-heated flasks under a nitrogen atmosphere. Solutions at 0 °C were obtained with an ice-water bath. Thin-layer chromatography (TLC) was carried out on silica gel coated films of the type Polygram® SIL G/UV254 (Macherey-Nagel, layer thickness 0.2 mm). In addition to UV detection (254 nm), common staining reagents such as molybdophosphoric acid or potassium permanganate were. Flash column chromatography was carried out on silica gel 60 Å (grain size 35 – 70 µm) from Fisher Scientific. The NMR spectra were recorded with the models Avance II 300 (300 MHz for ^{1}H, 75 MHz for ^{13}C) and Avance III 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) from Bruker at room temperature. Tetramethylsilane served as the internal standard. The chemical shifts are given in ppm relative to tetramethylsilane as standard. The coupling constants *J* are given in Hertz (Hz).

Algal culture

The non-axenic alga *Chromera velia* CCAP1602/1 was obtained from the Culture Collection of Algae and Protozoa (CCAP) at the SAMS Limited Scottish Marine Institute (Oban, Argyll, Scotland, UK; www.ccap.ac.uk). For maintenance and isolation of the heterotrophic bacteria the chromerid alga was grown in enriched seawater medium L1 (as indicated by the CCAP; https://www.ccap.ac.uk) with a vitamin mix (thiamine [B_1], cobalamin [B_{12}], biotin [B_7]) at daylight and room temperature using vented tissue culture flasks (Thermo Fisher Scientific 178905).

Isolation of bacteria from *Chromera velia* CCAP1602/1: Bacteria associated with CCAP1602/1 were isolated from the algal culture as described.[S4] In brief, 1 ml of the algal culture was centrifuged at 12.000 x g for 10 s. The supernatant was removed and the cell pellet was resuspended in 100 µl of L1 medium and shortly vortexed. The cell suspension was diluted 10^{-3} and 2 x 10^{-4} in L1 medium and 200 µl of each dilution were spread onto agar plates (140 x 140 mm) containing the following media: (I) MA [marine broth (BD Difco 2216, Michigan) amended with 1.5% (w/v) agar (BD 214010, France]; (II) L1-PY [L1 medium amended with 0.05% (w/v) peptone (BD 211677, France), 0.01% (w/v) yeast extract (BD 212750, France) and 1.5% (w/v) agar]; (III) L1-LNHM (L1 medium amended with 0.0001% (w/v) NH_4Cl, 0.001% (w/v) D-glucose, D-ribose, sodium succinate, sodium pyruvate, glycerol, *N*-acetyl-glucosamine and 0.002 % (v/v) ethanol according to the low nutrient heterotrophic medium LNHM[S5] and 1.5% (w/v) agar]. The plates were incubated in the dark at 20° for 4 to 12 weeks. Bacterial colonies representing different colony morphotypes were picked using a stereomicroscope. The isolates were passaged on the respective agar media (see above), until pure cultures were obtained. Isolated strains were preserved with the MicrobankTM System (PL.170, Pro-Lab Diagnostics, U.K.) at -80 °C.

Identification of the bacterial isolates: Bacterial isolates were identified by partial 16S rRNA gene sequencing. Therefore, the genomic DNA of the isolates was prepared with the DNeasy Blood & Tissue Kit (69504 Qiagen, Germany) according to the manufacturer's protocol for Gram positive bacteria. PCR-amplification of 16S rRNA genes from the genomic DNA of the bacteria was done in 1 x Phusion Green HF buffer containing each deoxynucleoside triphosphate at a concentration of 200 µM, 0.5 µM of the primers 27f 5'-GAGTTTGATCCTGGCTCAG-3' and 1525r 5'-AGAAAGGAGGTGATCCAGCC-3'),[S6] as well as 0.5 µl of the Phusion Hot Start II High Fidelity DNA Polymerase (F-537, Thermo Fisher Scientific, USA). The reactions were adjusted to a final volume of 50 µl with nuclease free water (R0581, Thermo Fisher Scientific). The thermal cycling steps were: (I) initial denaturing at 98 °C for 30 s; (II) 31 cycles of denaturing at 98 °C for 10s, annealing at 57 °C for 10 s and extension at 72 °C for 45 s followed by (III) a final extension at 72°C for 3 min. Purified PCR-amplificates were Sanger-sequenced with the primers 27f, 1525r, 519r (5'-G(T/A)ATTACCGCGGC(T/G)GCTG-3') and 803f (5'-ATTAGATACCCTGGTAG-3')[S6] by the DNA sequencing services of the LGC genomics GmbH (Berlin, Germany). The bacterial isolates were identified by comparison of their 16S rRNA gene sequences to publicly available 16S rRNA gene reference data bases, i.e. the EzBioCloud "Identify" Service[S7] (https://www.ezbiocloud.net/identify) and the NCBI Blastn suite using the megablast algorithm against the non-redundant nucleotide collection (nr/nt) and the database of 16S ribosomal sequences of Bacteria and Archaea (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

Strains and cultivation conditions: All bacterial strains (Table S1) were incubated at 28 °C on Marine Broth agar plates (MB, Carl Roth). 50 mL preparatory primary liquid cultures were obtained with these plated cultures. Liquid cultures for OSSA were inoculated with 1 mL of a preculture in 100 mL fresh MB media in 250 mL Erlenmeyer flasks. All liquid cultures were cultivated at 28 °C in a shaker.
The cultivation conditions of the xenic alga *C. velia* CCAP1602/1 were optimized by the company CellDEG GmbH (Berlin, Germany) with respect to the medium, light regime, temperature and CO_2-concentration in a series of pre-experiments analogous to former experiments with cyanobacteria.[S8] Final high-density cultivation of the alga was conducted by CellDEG in HD100 cultivators on the HDC 9.100 platform with 100 rpm (Sartorius orbital shaker) in 100 mL ¼ HD 4087 medium (N, 16 mM; K, 5 mM; P; 1 mM; S, 1.5 mM; Mg, 1.5 mM; Ca, 0.125 mM; Fe-EDTA, 0.03 mM; B, 12.5 µM; Mn; 5 µM, Zn, 1.25 µM; Mo, 0.75 µM; Ni, 0.025 µM; Co, 0.025 µM; Cu, 0.025 µM) comprising the vitamin mix of L1 medium. *C. velia* was cultivated at a temperature of 28 °C with a low light setup (photon flux density: 30 µmol m^{-2} s^{-1}, light source: Valoya RX400, light spectrum: AP673L) with a day-night regime of 16 hours light and 8 hours darkness. The CO_2-concentration in the gas supply unit of the HD100 cultivator was constantly 5%. The cultivation was finished after 19 days at an OD of 1.2. The culture was then transferred into 50 mL Falcon tubes.

GC/MS analyses: The GC/MS analyses of synthetic samples were performed on an Agilent 8860 gas chromatograph coupled to an Agilent 5977B mass selective detector. The measurements were carried out in a pulsed split mode at the following temperature programme: 50 °C (5 min. isothermal) start temperature, 20 °C/min heating rate, 320 °C (5 min isothermal) final temperature. The analyses of the headspace extracts of the natural samples were performed on an Agilent 7890B gas chromatograph with a 5977A mass selective detector. The measurements were carried out at the following temperature programme: 50 °C (5 min. isothermal) start temperature, 5 °C/min heating rate, 320 °C (10 min isothermal) final temperature. Gas chromatographic separation was performed on fused-silica capillary columns HP-5MS (30 m × 0.25 mm ID × 0.25 µm film, Agilent Technologies). Helium was used as carrier gas with a volume flow of 1.2 mL/min and ionisation was carried out by electron impact ionisation at 70 eV for both instruments. The GC/MS instrument for the headspace analysis was equipped with a thermal desorption unit (TDU 2, Gerstel GmbH & Co.KG, Germany), a PTV inlet with a cooled injection system (CIS 4, Gerstel GmbH & Co.KG, Germany) and a multipurpose sampler (MPS 2 XL, Gerstel GmbH & Co.KG, Germany). The analytes were desorbed from Tenax TA desorption tubes (Gerstel GmbH & Co.KG, Germany) under the following temperature programme: initial temperature: 30 °C (delay time: 0.80 min, initial time: 0.10 min), 60 °C/min heating rate, 280 °C (5 min isothermal) final temperature. The analytes were cryofocussed in the CIS under the following temperature programme: initial temperature: –100 °C (equilibration time: 0.50 min, initial time: 0.01 min), 12 °C/s heating rate, 300 °C (3 min isothermal) final temperature. The analytes were desorbed in a splitless mode and the PTV inlet was in a solvent vent mode (vent flow: 40 mL/min, vent pressure: 7.70 psi until 0.01 min, purge flow to split vent: 50 mL/min at 0.76 min (45 sec. splitless time, method 1) or 50 mL/min at 1.01 min (60 sec. splitless time, method 2). The TDU transfer temperature was set at 300 °C with a fixed transfer temperature mode. The TDU was cooled with a UPC Plus (Gerstel GmbH & Co.KG, Germany) equipped with ethanol and the CIS was cooled with liquid nitrogen. Gas chromatographic retention indices (RI) were determined from a homologous series of n-alkanes (C8–C40). The mass numbers *m*/*z* are given in amu and the relative intensities in %.

Collection of headspace volatiles: Inoculated algal or bacterial liquid cultures (100 mL) of were placed in 250 mL Erlenmeyer flasks and analysed by OSSA at room temperature. The gas phases of the samples were adsorbed on Tenax TA desorption tubes using a pump (MB-21E, Senior Flextronics, USA). The analytes were desorbed by thermodesorption, trapped by cryofoccusing and analysed by GC/MS. Three replicates of each bacterial strain were used for the headspace extraction and GC/MS analysis. One replicate of each bacterial strain was analysed with method 1. The other two replicates were analysed with method 2. Headspace extraction and GC/MS analysis were executed for two replicates of the algal strain with method 1.

Synthetic procedures

3,4,4-Trimethylcyclopent-2-en-1-one (1)

According to the published procedure of Vacas et al.[S9], polyphosphoric acid (25.0 g) was heated to 95 °C and isobutyl (*E*)-but-2-enoate (**97**, 5.00 g, 5.60 mL, 32.5 mmol, 1.00 eq.) was added over 4 h under an atmosphere of nitrogen. The reaction mixture was stirred for 3 h at 95 °C, cooled to room temperature and poured into water (50 mL) while stirring. The layers were separated and the aqueous layer was extracted three times with diethyl ether. The combined organic layers were successively washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel [pentane/Et_2O (10:1)] to give the product **1** (845 mg, 6.82 mmol, 21%) as a clear, colourless oil.

R_f: 0.04 (pentane/Et_2O 10:1); R_I: 1050; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.81 (q, *J* = 1.4 Hz, 1 H), 2.31 (s, 2 H), 2.05–2.03 (m, 3 H), 1.22 (s, 6 H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 207.8 (C=O), 185.6 (Cq), 129.3 (CH), 51.5 (CH_2), 42.9 (Cq), 26.7 (2 x CH_3), 14.1 (CH_3) ppm; MS (EI, 70 eV): *m*/*z* (%) = 124 (35) $[M]^+$, 110 (8), 109 (100), 82 (6), 81 (34), 79 (17), 53 (7), 41 (8), 40 (4), 39 (10).

2-Hydroxy-2,6,6-trimethylcyclohexan-1-one (2)

A solution of *m*CPBA (77%, 587 mg, 2.62 mmol, 2.00 eq.) in $CHCl_3$ (7.0 mL) was slowly added to a solution of freshly distilled β-cyclocitral (**98**, 200 mg, 0.21 mL, 1.31 mmol, 1.00 eq.) in $CHCl_3$ (0.7 mL) at reflux under an atmosphere of nitrogen. The solution was stirred for 6 h at reflux temperature, then cooled to room temperature and successively washed with aq. $NaHSO_3$ solution, 10% aq. NaOH solution and H_2O. The solvent was removed under reduced pressure and the resulting oil was hydrolysed with a 1% methanolic NaOH solution at room temperature for 2 h and then heated to reflux for 1.5 h. Methanol was evaporated under reduced pressure, the crude product was dissolved in $CHCl_3$, washed with brine and dried over $MgSO_4$. Purification by column chromatography on silica gel [pentane/diethyl ether (10:1)] gave the product **2** (162 mg, 1.04 mmol, 79%) as a clear, slightly yellow oil.[S10]

R_f: 0.25 (pentane/Et_2O 10:1); R_I: 1109; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.92 (br.s, 1 H), 2.14–2.05 (m, 1 H), 1.92–1.55 (m, 5 H), 1.41 (s, 3 H), 1.22 (s, 3 H), 1.15 (s, 3 H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 218.8 (C=O), 75.9 (Cq), 44.3 (Cq), 40.7 (2 x CH_2), 27.3 (CH_3), 27.2 (CH_3), 25.7 (CH_3), 18.9 (CH_2) ppm; MS (EI, 70 eV): *m*/*z* (%) = 156 (3) $[M]^+$, 128 (42), 110 (42), 95 (61), 85 (22), 84 (18), 71 (100), 58 (35), 55 (15), 43 (40), 41 (15).

7,9,9-Trimethyl-1,4-dioxaspiro[4.5]dec-7-ene (100)

As described by Babler et al.[S11], a solution of α-isophorone (**99**, 2.76 g, 3.00 mL, 20.0 mmol, 1.00 eq.), glycol (3.72 g, 3.40 mL, 60.0 mmol, 3.00 eq.) and *p*-toluenesulfonic acid monohydrate (114 mg, 3.00 mol%) in toluene (50 mL) was refluxed for 7 h while removing water and glycol with a Dean-Stark trap and then stirred at room temperature for 15 h. The reaction was quenched with sat. $NaHCO_3$, the layers were separated and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over

$MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel [pentane/EtOAc (80:1)] to give the product **100** (2.34 g, 12.8 mmol, 64%) as a clear, colourless oil.

R_f: 0.26 (pentane/EtOAc 80:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.19–5.14 (m, 1 H), 3.97–3.94 (m, 4 H), 2.16–2.12 (m, 2 H), 1.69–1.66 (m, 3 H), 1.62–1.59 (m, 2 H), 1.05 (s, 6 H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 131.3 (CH), 128.0 (Cq), 109.2 (Cq), 64.1 (2 x CH_2), 43.6 (CH_2), 39.8 (CH_2), 34.2 (Cq), 30.4 (2 x CH_3), 23.4 (CH_3) ppm; MS (EI, 70 eV): *m*/*z* (%) = 182 (38) $[M]^+$, 167 (23), 96 (51), 95 (15), 87 (31), 86 (100), 81 (33), 79 (12), 67 (11), 41 (12).

3,5,5-Trimethylcyclohex-3-en-1-one (101)

The β-ketal **100** (498 mg, 2.73 mmol, 1.00 eq.) was dissolved in acetic acid and water (4:1; 13.7 mL) and stirred at room temperature for 4 h. The reaction mixture was then carefully poured into a cold $NaHCO_3$ solution (205 mL) and diluted with brine (137 mL). The aqueous layer was extracted three times with diethyl ether, the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. A product mixture of **101** and α-isophorone (**99**, 356 mg, 2.58 mmol, 95%) was obtained as a clear, slightly yellow oil in the ratio of 5:1 as determined by NMR-analysis.[S11]

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.46–5.39 (m, 1 H), 2.74–2.70 (m, 2 H), 2.32 (s, 2 H), 1.71 (dt, *J* = 1.5, 0.9 Hz, 3 H), 1.04 (s, 6 H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 210.5 (C=O), 132.5 (CH), 129.0 (Cq), 53.2 (CH_2), 43.7 (CH_2), 36.4 (Cq), 29.6 (2 x CH_3), 22.6 (CH_3) ppm; MS (EI, 70 eV): *m*/*z* (%) = 138 (59) $[M]^+$, 123 (58), 96 (89), 95 (87), 81 (100), 79 (27), 67 (40), 53 (20), 41 (30), 39 (34).

3,5,5-Trimethylcyclohex-3-en-1-ol

According to the published procedure of Rossini et al.[S12], a solution of β-isophorone (**101**, 314 mg, 2.27 mmol, 1.00 eq.) in dry diethyl ether (1.0 mL) was slowly added to $LiAlH_4$ (57.3 mg, 1.51 mmol, 0.67 eq.) in dry diethyl ether (3.6 mL) under an atmosphere of nitrogen. The mixture was refluxed for 1 h, cooled to 0 °C and then water and 5% HCl solution were added successively. The organic layer was washed five times with brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. A product mixture of 3,5,5-trimethylcyclohex-3-en-1-ol and the isomer 3,5,5-trimethylcyclohex-2-en-1-ol (266 mg, 1.89 mmol, 84%) was obtained as a clear, colourless oil in the ratio of 5:1 as determined by NMR-analysis.

R_l: 1064; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.12–5.08 (m, 1 H), 3.99 (dddd, *J* = 11.6, 9.4, 5.6, 3.7 Hz, 1 H), 2.27–2.16 (m, 1 H), 1.92–1.66 (m, 3 H), 1.66–1.64 (m, 3 H), 1.54 (br. s, 1 H), 1.00 (s, 3 H), 0.98 (s, 3 H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 131.7 (CH), 128.6 (Cq), 66.3 (CH), 46.1 (CH_2), 39.8 (CH_2), 34.1 (Cq), 31.4 (CH_3), 29.5 (CH_3), 23.3 (CH_3) ppm; MS (EI, 70 eV): *m*/*z* (%) = 140 (11) $[M]^+$, 125 (18), 122 (14), 107 (100), 96 (14), 91 (18), 81 (26), 79 (19), 55 (21), 41 (18), 39 (14).

3,5,5-Trimethylcyclohex-3-en-1-yl acetate (5)

A solution of the alcohol mixture of 3,5,5-trimethylcyclohex-3-en-1-ol and 3,5,5-trimethylcyclohex-2-en-1-ol (50.3 mg, 0.36 mmol, 1.00 eq.), TMEDA (41.8 mg, 50.0 µL, 0.36 mmol, 1.00 eq.) and acetic anhydride (73.5 mg, 70.0 µL, 0.72 mmol, 2.00 eq.) was stirred for 50 min at room temperature. The reaction was quenched by the addition of water, the aqueous layer was extracted three times with diethyl ether and the combined organic layers were dried over $MgSO_4$. Purification by column chromatography on silica gel [pentane/diethyl ether (10:1)] gave a product mixture of **5** and the isomer 3,5,5-trimethylcyclohex-2-en-1-yl acetate (49.0 mg, 0.27 mmol, 75%) in the ratio of 5:1 as determined by GC/MS analysis as a clear, colourless oil.[S13]

R_f: 0.12 (pentane/Et_2O 10:1); R_I: 1192; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.14–5.11 (m, 1 H), 5.06 (dddd, J = 11.6, 9.4, 5.8, 3.7 Hz, 1 H), 2.31–2.24 (m, 1 H), 2.17 (s, 3 H), 2.04 (s, 3 H), 1.93 (dddd, J = 16.5, 9.2, 2.4, 1.2 Hz, 1 H), 1.76–1.67 (m, 2 H), 1.01 (s, 6 H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 170.8 (C=O), 131.7 (CH), 128.2 (Cq), 69.4 (CH), 41.8 (CH_2), 35.8 (CH_2), 33.7 (Cq), 31.1 (CH_3), 29.4 (CH_3), 23.2 (CH_3). 21.5 (CH_3) ppm; MS (EI, 70 eV): m/z (%) = 182 (missing) $[M]^+$, 123 (4), 122 (28), 108 (9), 107 (100), 105 (7), 91 (16), 81 (4), 79 (5), 43 (9), 41 (3).

5-Hydroxy-3,4-dimethylfuran-2(5*H*)-one (103)

Under an atmosphere of nitrogen, $LiAlH_4$ (331 mg, 8.72 mmol, 1.10 eq.) was suspended in dry THF (16 mL) and a solution of *t*-butanol (1.93 mg, 26.2 mmol, 3.30 eq.) in dry diethyl ether (8.0 mL) was added. This suspension was added dropwise over 30 min to a solution of 3,4-dimethylfuran-2,5-dione (**102**, 1.00 g, 7.93 mmol, 1.00 eq.) in dry diethyl ether (30 mL) at -10 °C and stirred for 1 h at this temperature. The solution was warmed to room temperature and stirred for 19 h, then cooled to 0 °C and quenched with 2 M H_2SO_4 (30 mL). The aqueous layer was extracted three times with diethyl ether and the combined organic layers were dried over Na_2SO_4. The crude product was purified by column chromatography on silica gel [pentane/EtOAc (5:1)] to give the product **103** (684 mg, 5.33 mmol, 67%) as a white solid.[S14]

R_f: 0.06 (pentane/EtOAc 5:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.91 (d, J = 4.6 Hz, 1 H), 5.21 (br. s, 1 H), 2.04–1.97 (m, 3 H), 1.84–1.78 (m, 3 H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 173.5 (C=O), 156.5 (Cq), 125.8 (Cq), 98.7 (CH), 11.4 (CH_3), 8.3 (CH_3) ppm.

3,4-Dimethyl-5-pentylfuran-2(5*H*)-one (27)

According to a procedure of Surmont et al.[S15], pentylmagnesium bromide (2 M in Et_2O, 0.47 mL, 0.94 mmol, 2.40 eq.) was added to a solution of 5-hydroxy-3,4-dimethylfuran-2(5*H*)-one (**104**, 50.0 mg, 0.39 mmol, 1.00 eq.) in dry and degassed THF (1.7 mL) under an atmosphere of nitrogen. The solution was stirred for 21.5 h at room temperature and quenched with 1 M HCl. The organic layer was extracted with diethyl ether three times and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel [pentane/Et_2O (5:1)] to give the product **27** (51.2 mg, 0.28 mmol, 72%) as a clear, slightly yellow oil.

R_f: 0.35 (pentane/ Et_2O 5:1); R_I: 1525, ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.78–4.66 (m, 1 H), 1.94 (quin, J = 1.1 Hz, 3 H), 1.92–1.83 (m, 1 H), 1.81 (dq, J = 2.1, 1.1 Hz, 3 H), 1.53–1.21 (m, 7 H), 0.95–0.83 (m, 3 H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 175.1 (C=O), 159.6 (Cq), 123.8 (Cq), 83.6 (CH), 32.5 (CH_2), 31.9 (CH_2), 24.5 (CH_2), 22.8 (CH_2), 14.3 (CH_3). 12.4 (CH_3), 8.8 (CH_3) ppm; MS (EI, 70 eV): m/z (%) = 182 (16) $[M]^+$, 153 (8), 126 (11), 112 (27), 111 (46), 99 (5), 84 (8), 83 (100), 55 (24), 43 (8).

(3*E*,5*E*)-Undeca-3,5-dien-2-one (84)

According to a procedure of Gao et al.[S16], a solution of the phosphonate **104** (693 mg, 0.69 mL, 3.57 mmol, 1.50 eq.) in dry THF (10 mL) was cooled to 0 °C and *n*-BuLi (1.6 M in hexane, 2.20 mL, 3.57 mmol, 1.50 eq.) was slowly added and the solution was stirred for 20 min under an atmosphere of nitrogen. A solution of (*E*)-oct-2-enal (**105**, 300 mg, 0.36 mL, 2.38 mmol, 1.00 eq.) in dry THF (5.0 mL) was slowly added. The reaction mixture was warmed to room temperature, stirred overnight and quenched by the addition of sat. NH_4Cl solution. The aqueous layer was extracted three times with diethyl ether, the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel [pentane/diethyl ether (40:1)] to give the ketone **84** (196 mg, 1.17 mmol, 49%) as a clear, colourless liquid.

R_f: 0.24 (pentane/Et_2O 40:1); R_I: 1402; ^1H-NMR (300 MHz, $CDCl_3$): δ = 7.16–7.04 (m, 1 H), 6.22–6.15 (m, 2 H), 6.06 (d, J = 15.6 Hz, 1 H), 2.26 (s, 3 H), 2.23–2.13 (m, 2 H), 1.53–1.38 (m, 2 H), 1.38–1.21 (m, 4 H), 0.91 (t, J = 7.0 Hz, 3 H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 198.9 (C=O), 145.8 (CH), 144.0 (CH), 128.8 (CH), 128.7 (CH), 33.1 (CH_2), 31.3 (CH_2), 28.3 (CH_2), 27.1 (CH_3), 22.4 (CH_2), 14.0 (CH_3); MS (EI, 70 eV): m/z (%) = 166 (9) $[M]^+$, 97 (5), 96 (9), 95 (100), 81 (24), 79 (5), 67 (8), 53 (4), 43 (16), 41 (5).

Mass spectra

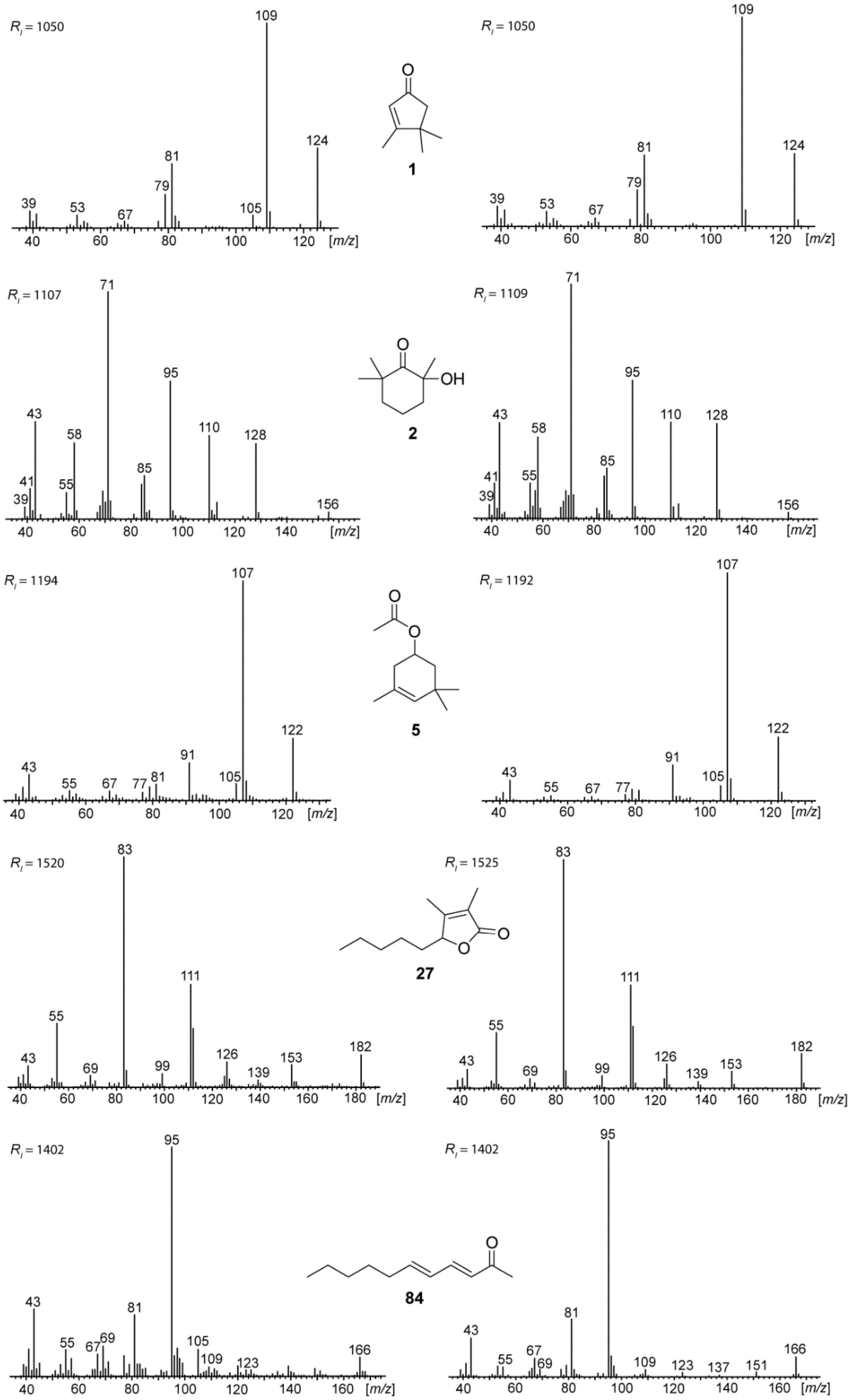
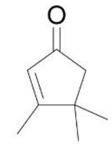


Figure S3. Comparison of the mass spectra and linear gas chromatographic retention indices (RI) of the natural(left) and the synthetic compounds (right). Mass spectra will be made publicly available via the open access data base MACE after publiucation of this article.[S17]

NMR Spectra



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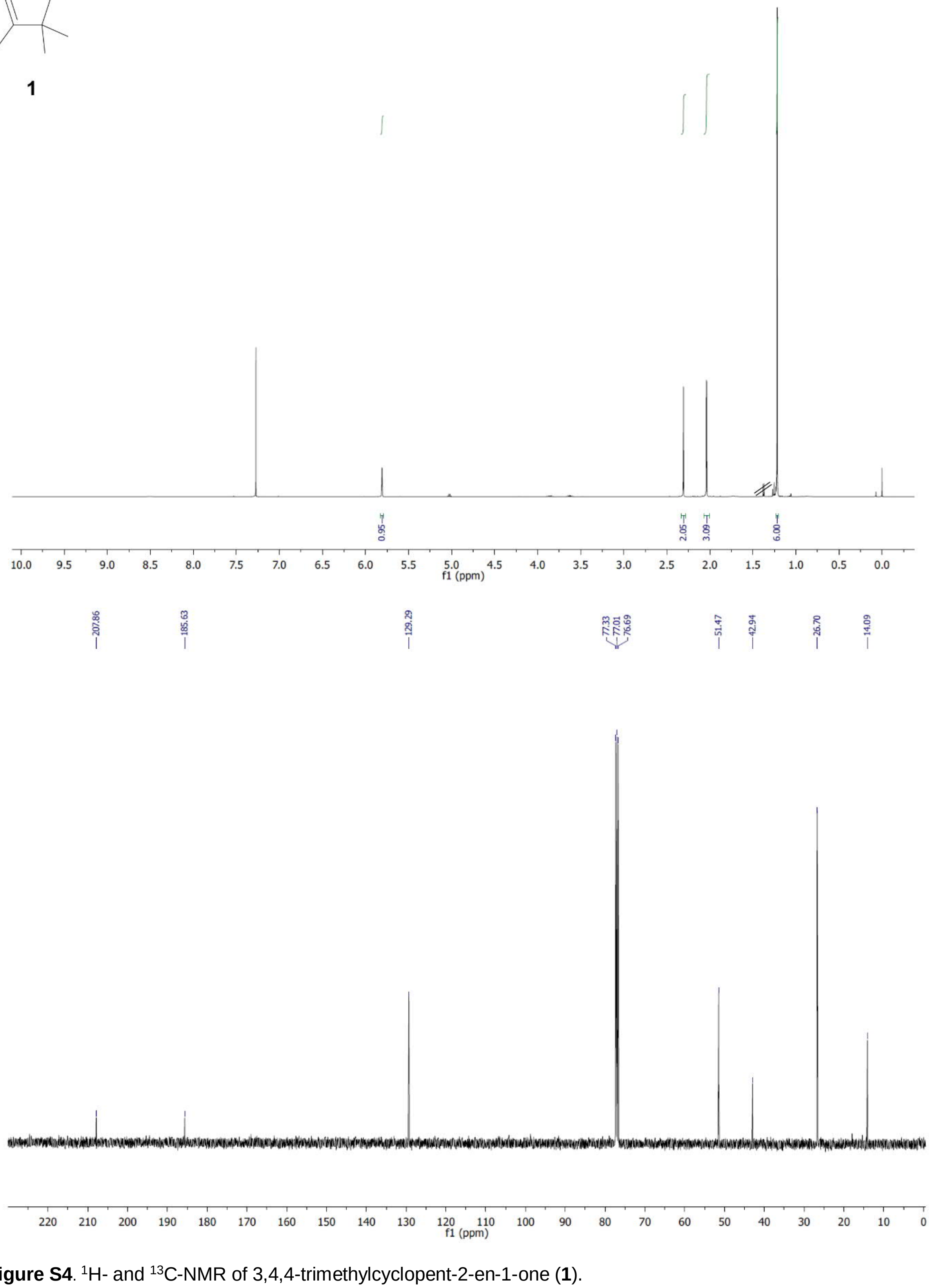
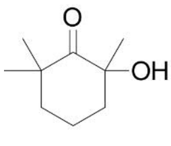
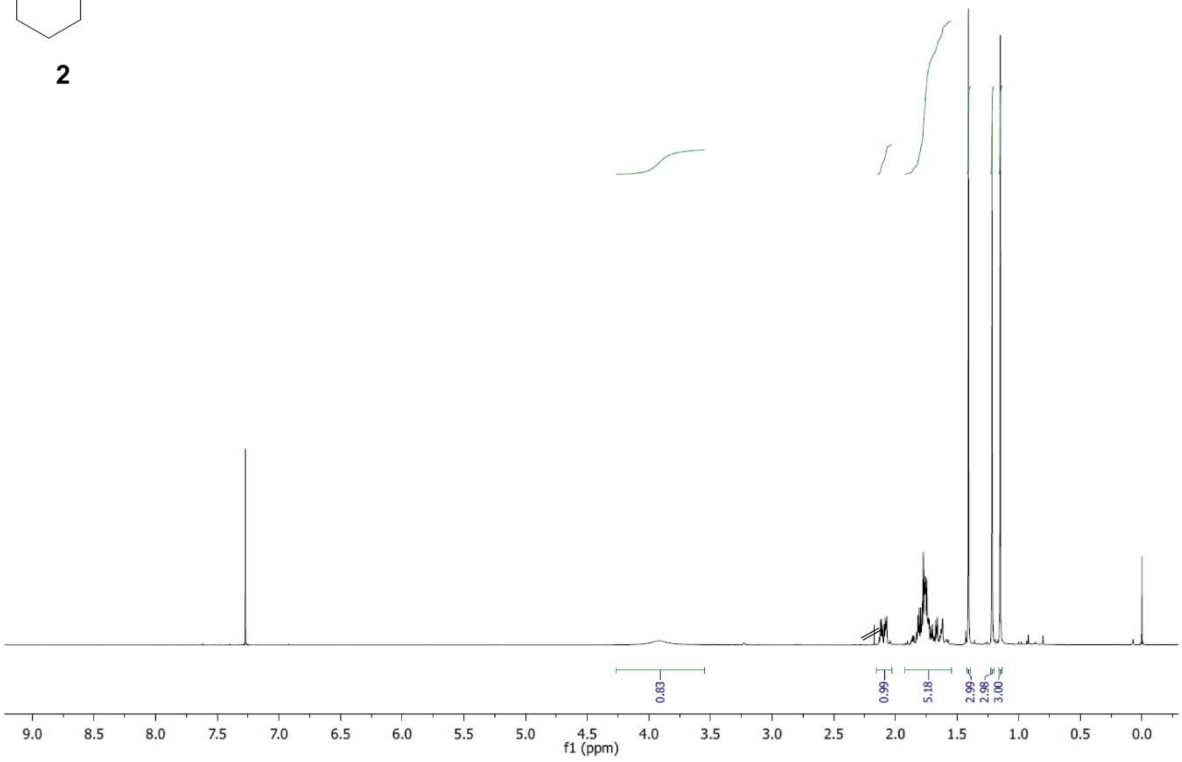


Figure S4. ^{1}H- and ^{13}C-NMR of 3,4,4-trimethylcyclopent-2-en-1-one (**1**).



2



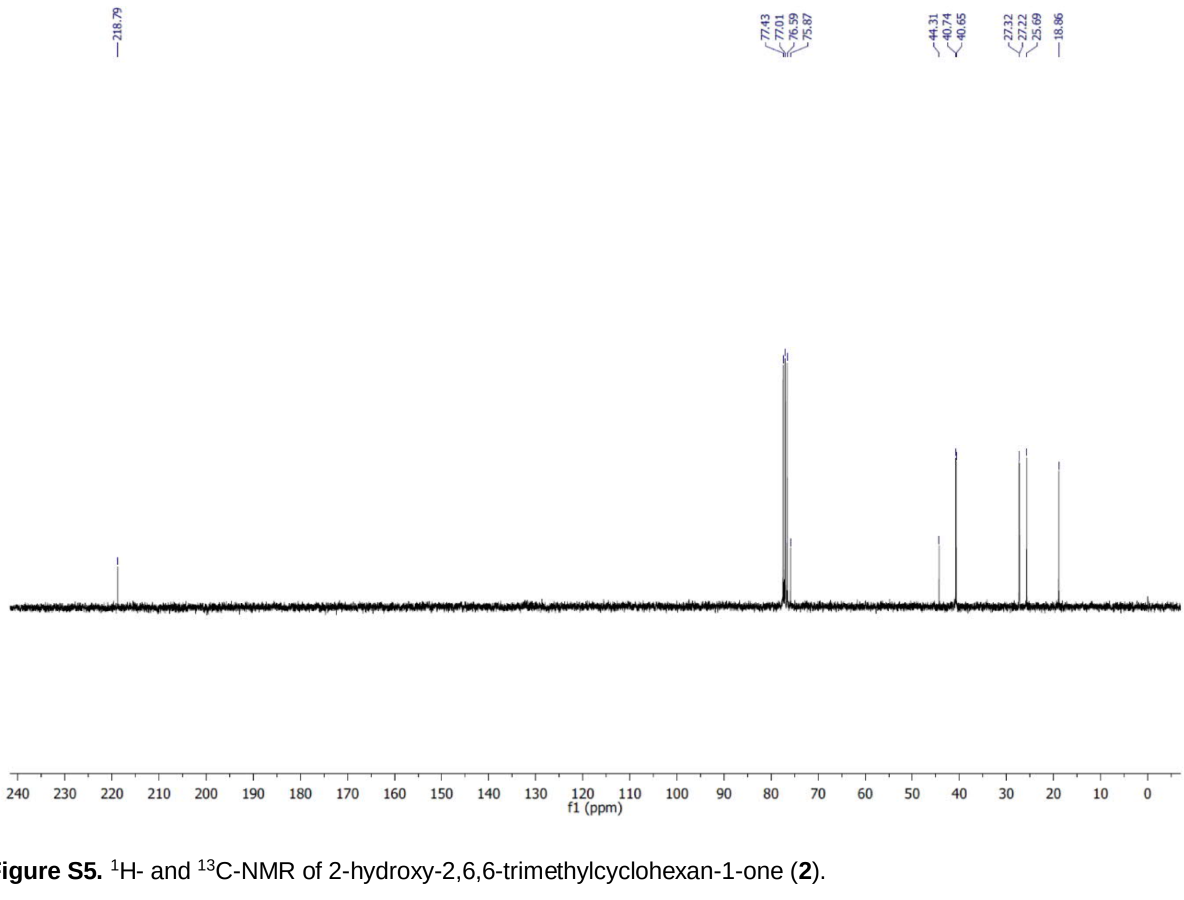
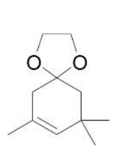
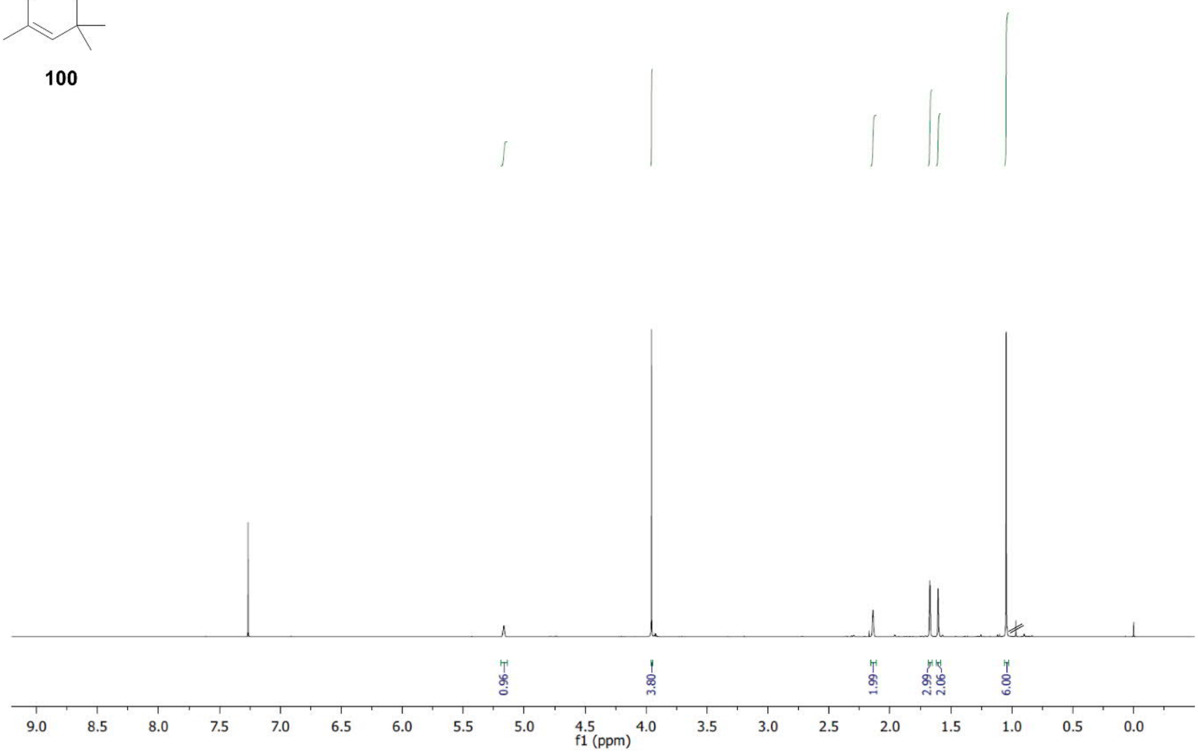


Figure S5. ^{1}H- and ^{13}C-NMR of 2-hydroxy-2,6,6-trimethylcyclohexan-1-one (**2**).



100



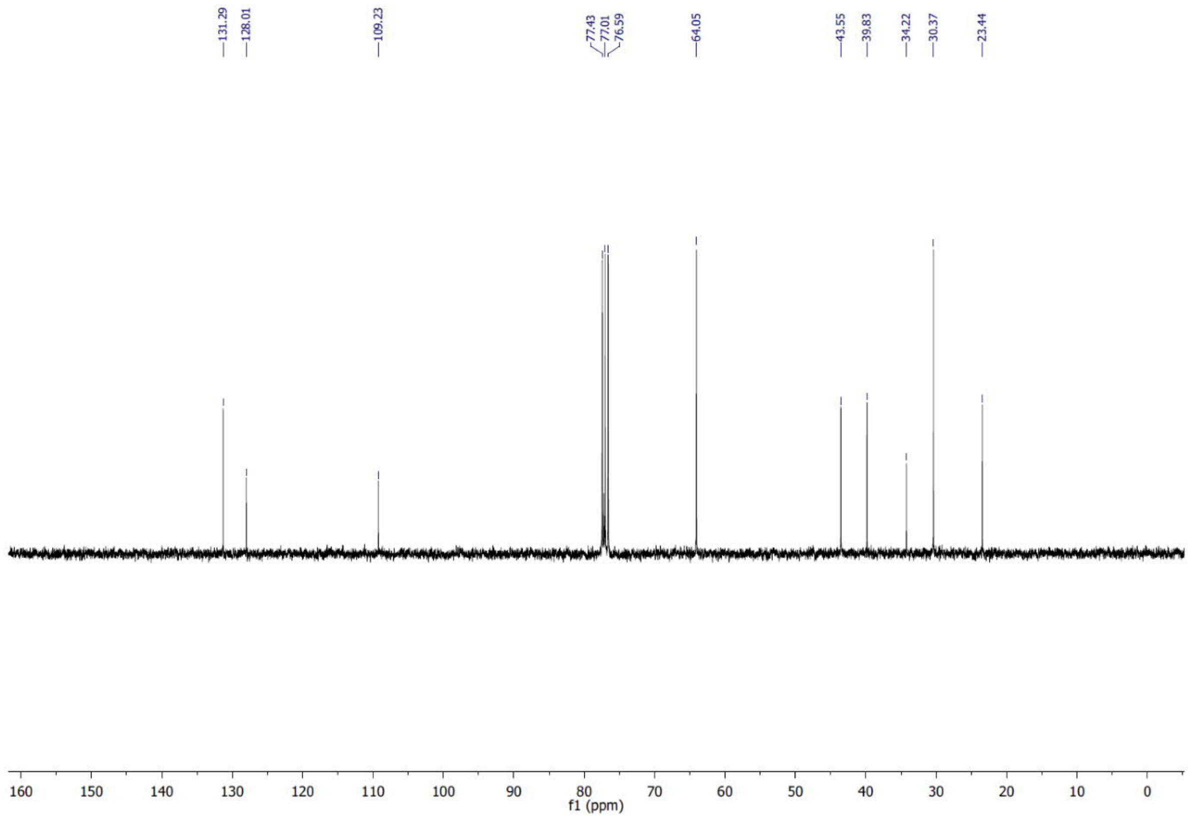
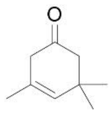
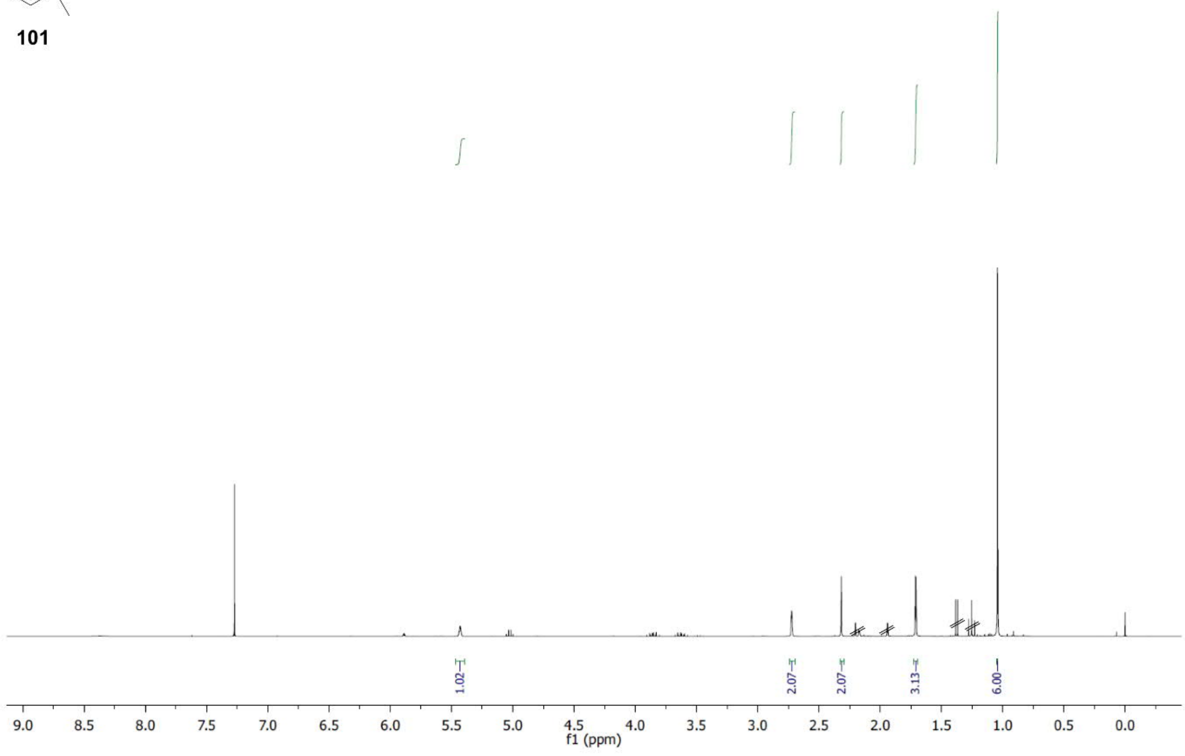


Figure S6. ^{1}H- and ^{13}C-NMR of 7,9,9-trimethyl-1,4-dioxaspiro[4.5]dec-7-ene (**100**).



101



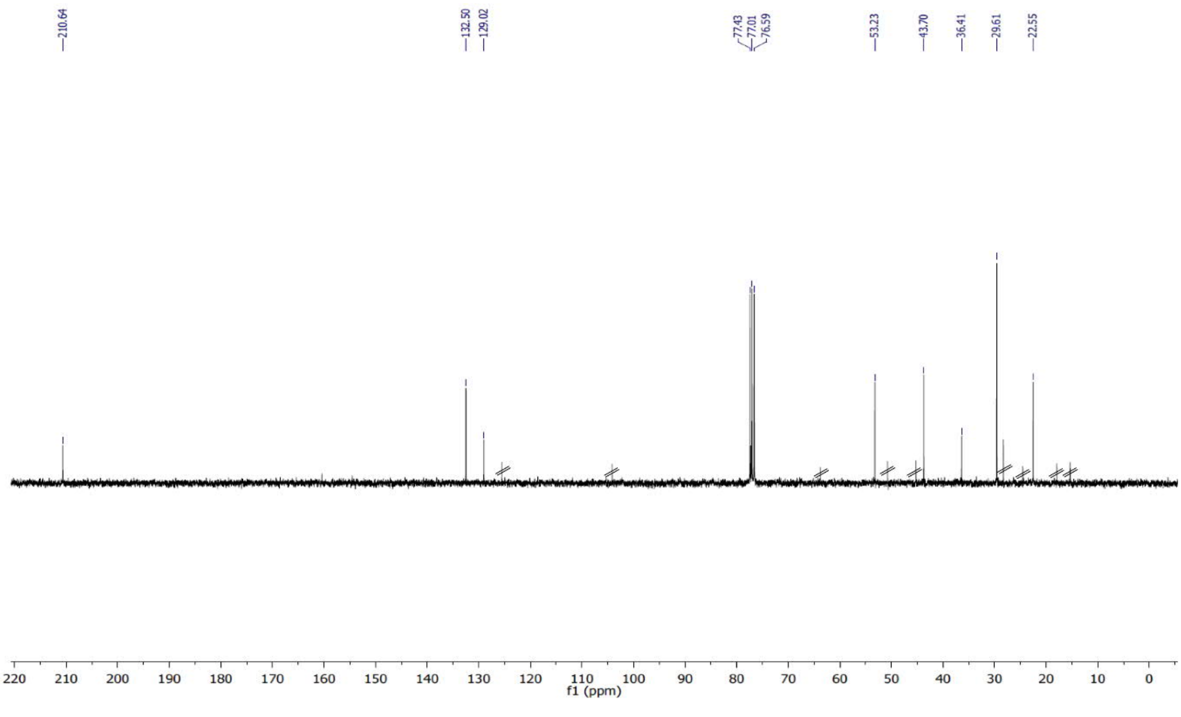
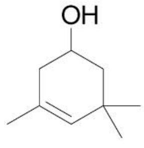
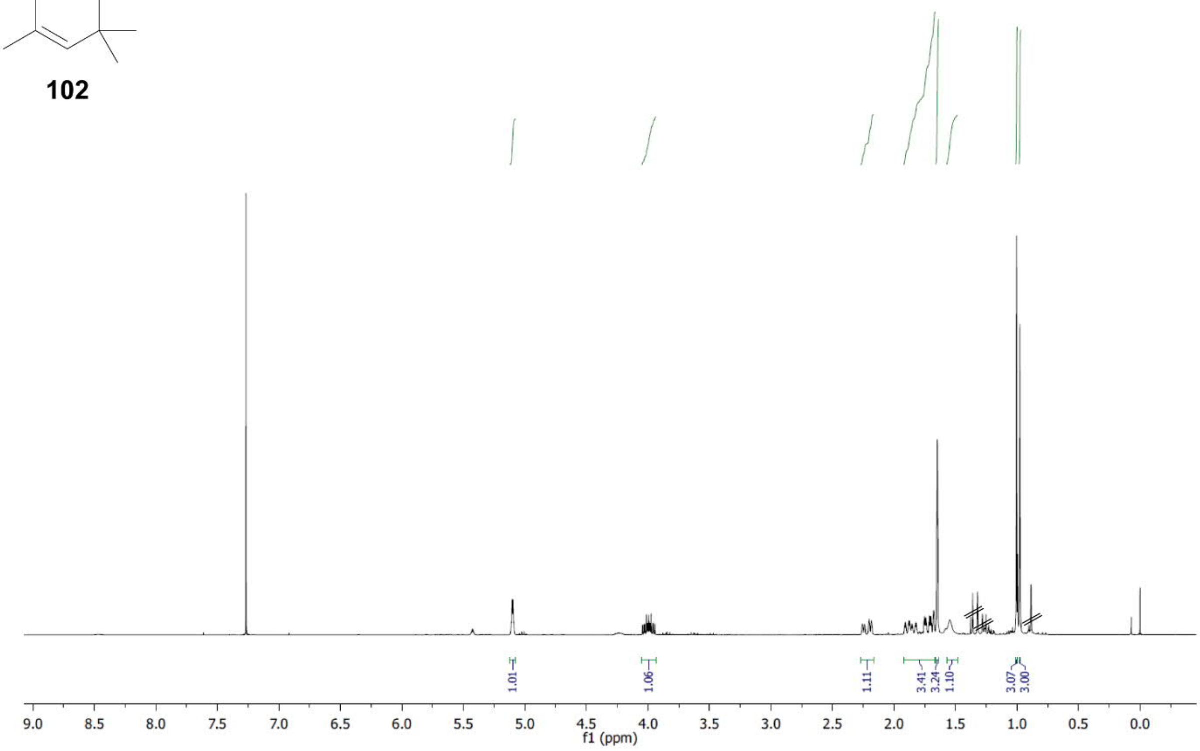


Figure S7. ^{1}H- and ^{13}C-NMR of 3,5,5-trimethylcyclohex-3-en-1-one (**101**).



102



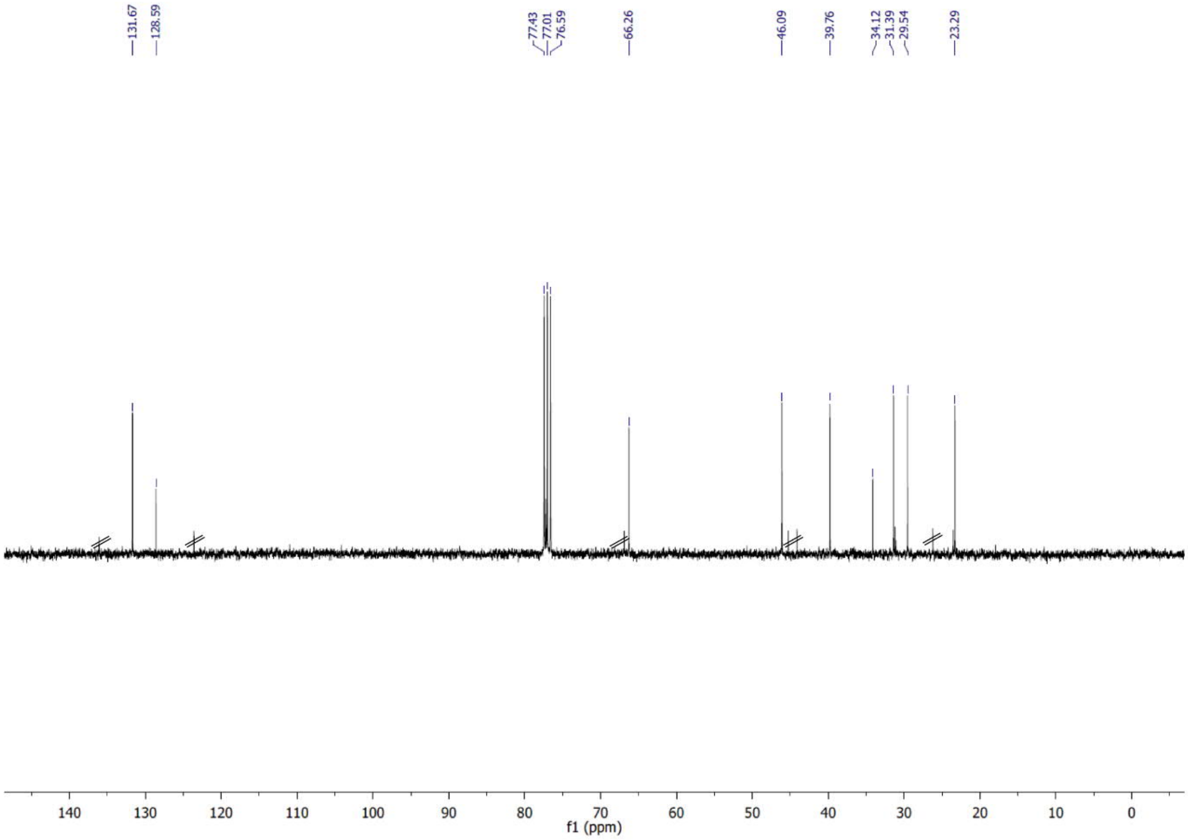
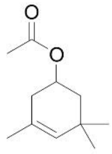


Figure S8. ^{1}H- and ^{13}C-NMR of 3,5,5-trimethylcyclohex-3-en-1-ol (**102**).



5

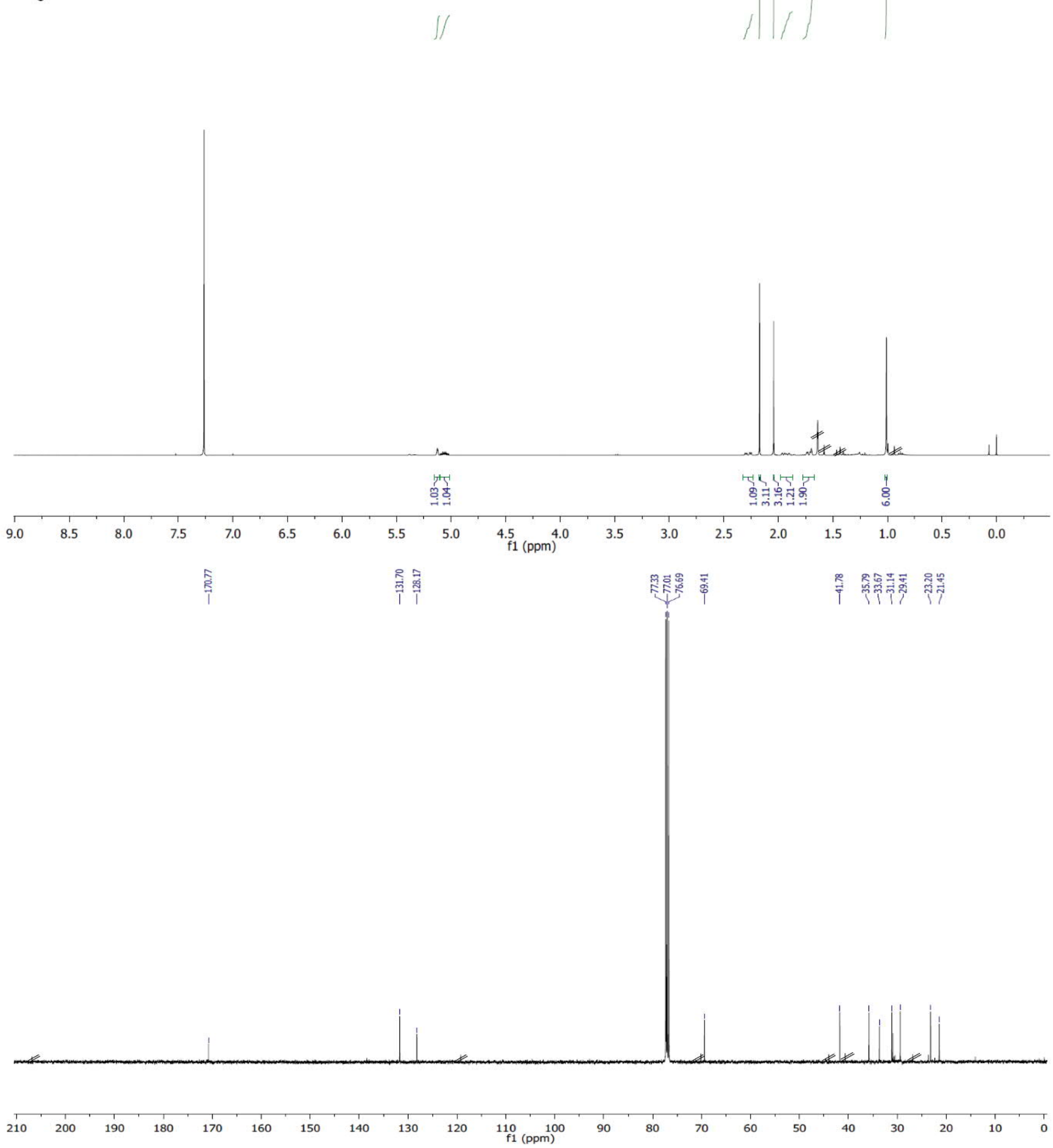


Figure S9. ^{1}H- and ^{13}C-NMR of 3,5,5-trimethylcyclohex-3-en-1-yl acetate (**5**).

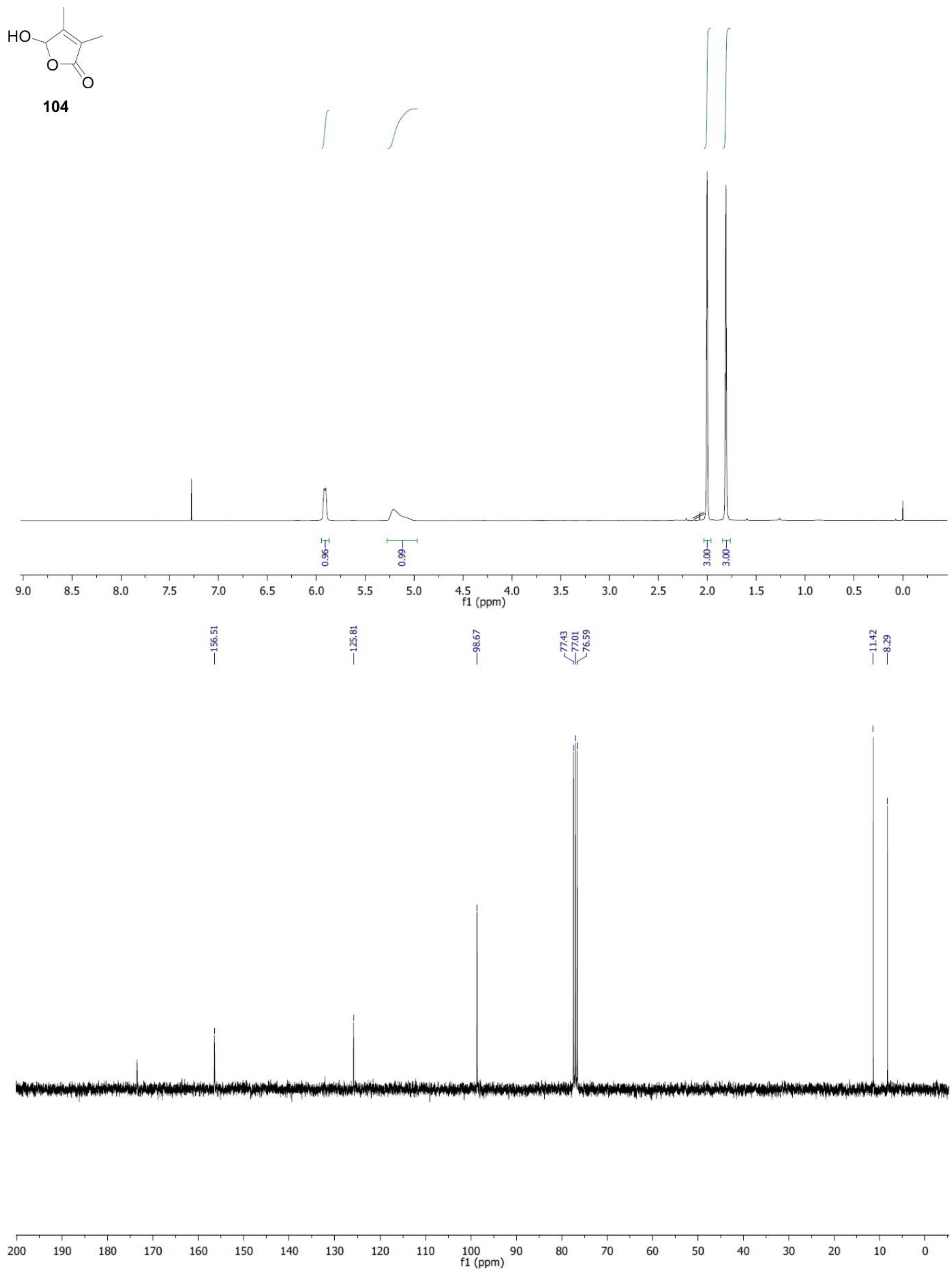
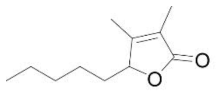
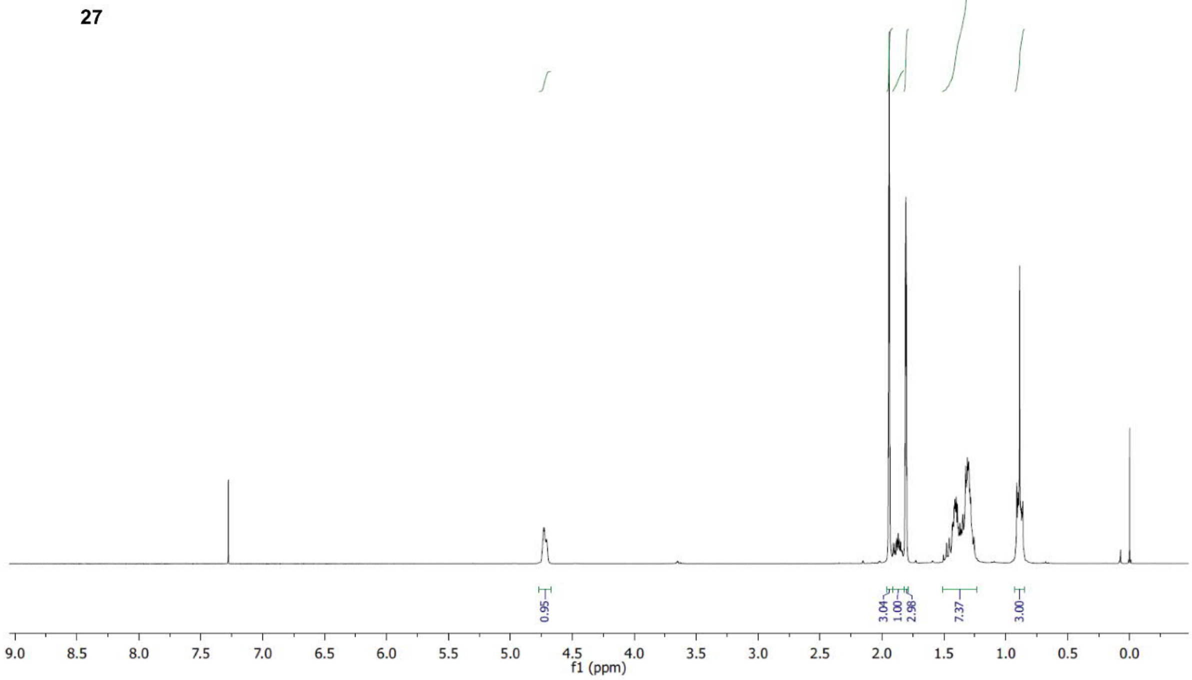


Figure S10. ^{1}H- and ^{13}C-NMR of 5-hydroxy-3,4-dimethylfuran-2(5*H*)-one (**104**).



27



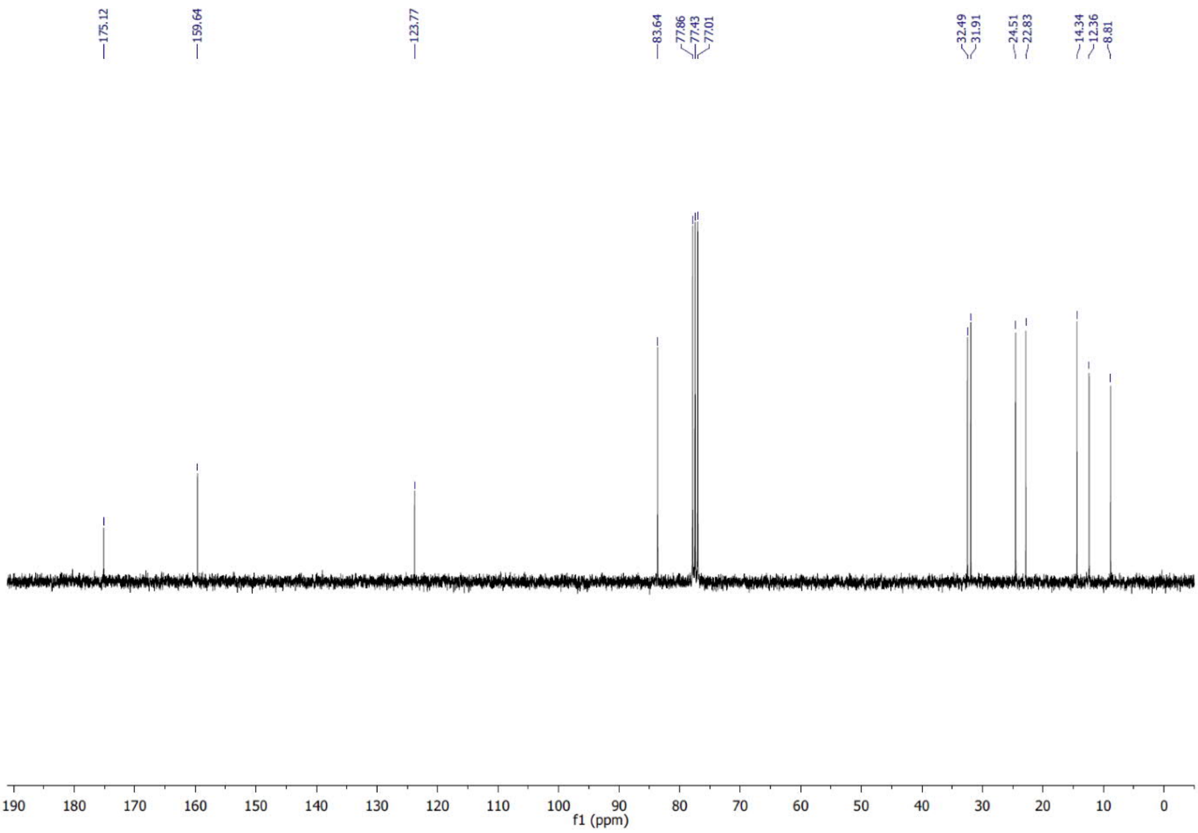
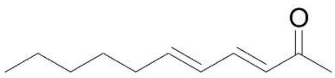


Figure S2. ^{1}H- and ^{13}C-NMR of 3,4-dimethyl-5-pentyl-5*H*-furan-2-one (**27**).



84

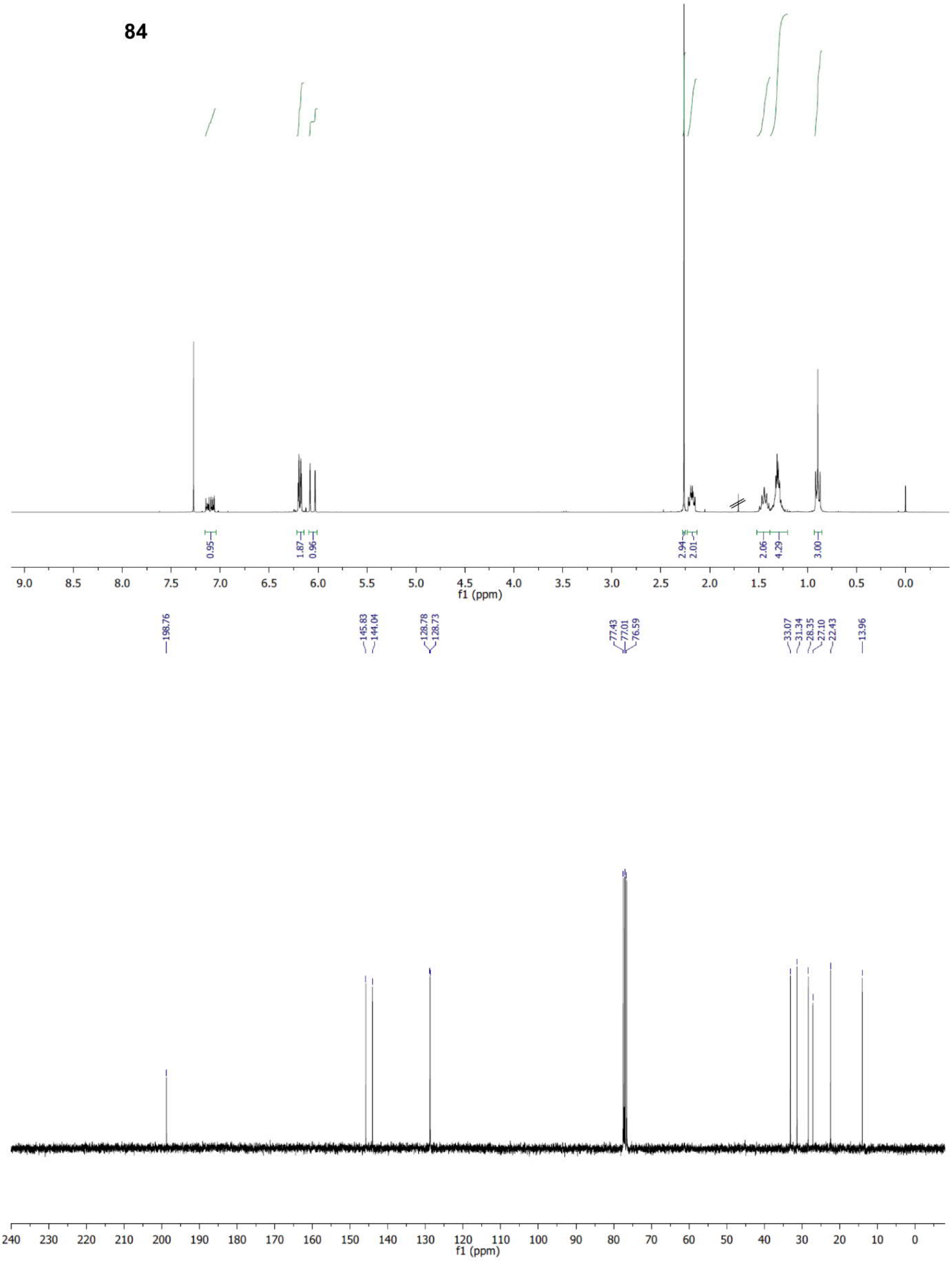


Figure S3. [1]H- and [13]C-NMR of (3*E*,5*E*)-undeca-3,5-dien-2-one (**84**).

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