General synthetic methods

Chemicals were obtained from Acros Organics (Geel, Belgium) or Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and were used without further purification. $^{34}S_8$ (>99.5% enriched) was obtained from Campro Scientific GmbH (Berlin, Germany). Solvents were purified by distillation and dried according to standard methods. Reactions, apart from those performed in water, were carried out under argon atmosphere in vacuum-heated flasks with dried solvents. Thin-layer chromatography (SiO_2, TLC) was performed on 0.20 mm Macherey-Nagel silica gel plates (Polygram SIL G/UV 254). Column chromatography was peformed with Merck silica gel 60 (0.040 - 0.063 mm) using standard flash chromatographic methods. The NMR spectra were recorded on Bruker DPX 300, DPX 400 or DPX 500, and were referenced against $CHCl_3$ (7.26 ppm) for ^{1}H-NMR and $CHCl_3$ (77.01 ppm) for ^{13}C-NMR. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Infrared spectra were recorded on a Bruker-Alpha spectrometer. GC/MS analyses for the synthetic compounds and for the identification of bacterial volatiles were obtained on a HP6890 gas chromatograph fitted with a HP5-MS fused silica capillary column (25 m, 0.25 mm i. d., 0.25 µm film) connected to a HP5973 mass selective detector. Conditions were as follows: inlet pressure: 77.1 kPa, He 23.3 mL min^{-1}; injection volume: 1 µL; injector: 250 °C operated in split mode (20:1); transfer line: 300 °C; electron energy: 70 eV. The GC was programmed as follows: 50 °C (5 min), increasing at 10 °C min^{-1} to 320 °C, carrier gas (He): 1.0 mL min^{-1}. Retention indices were determined from a homologous series of *n*-alkanes (C_8H_{18} – $C_{32}H_{66}$)

Synthesis of K^{34}SCN

To a solution of KCN (0.362 g, 5.56 mmol, 1.0 equiv.) in water (22 mL) was added elemental $^{34}S_8$ (0.189 g, 5.56 mmol, 1.0 equiv.) and stirred under reflux until the sulfur was consumed (48 h). After cooling to room temperature the mixture was filtrated and concentrated in vacuo to give K^{34}SCN (0.546 g, 5.52 mmol, 99%) as a colorless solid. ^{13}C-NMR (75 MHz, D_2O): δ = 136.1 (C_q) ppm.

Synthesis of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl methanesulfonate (18)

(2,2-Dimethyl-1,3-dioxolan-4-yl)methanol **17** (7.977 g, 50.0 mmol, 1.0 equiv.) was dissolved in dichloromethane (25 mL) and triethylamine (8.3 mL, 60.0 mmol, 1.2 equiv.) was added. The solution was cooled to 0°C. Over a period of 2 h mesyl chloride (6.70 g, 59.0 mmol, 1.18 equiv.) was added and the solution was stirred over night at room temperature. The mixture was washed with saturated sodium bicarbonate (3 x 20 mL) and water (3 x 20 mL). After drying, filtrating and evaporation of solvents the product **18** was obtained as a yellow oil in sufficient quality for the next reaction without further purification (7.98 g, 38.0 mmol, 76%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.37 (dq, *J* = 6.4, 5.3 Hz, 1H), 4.21 (d, *J* = 5.3 Hz, 2H), 4.09 (dd, *J* = 8.8, 6.5 Hz, 1H), 3.82 (dd, *J* = 8.7, 5.5 Hz, 1H), 3.06 (s, 3H), 1.43 (s, 3H), 1.36 (s, 3H) ppm.^{13}C-NMR (400 MHz, $CDCl_3$): δ = 110.7, 73.7, 69.6, 66.3, 38.1, 27.1, 25.6 ppm.

Synthesis of (^{34}S)-2,2-dimethyl-4-(thiocyanatomethyl)-1,3-dioxolane (19)

To a solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl methanesulfonate **18** (2.00 g, 9.5 mmol, 1.0 equiv.) in acetonitrile (100 mL) was added K^{34}SCN (1.05 g. 10.0 mmol, 1.05 equiv.) and the mixture was allowed to stir for 6d under reflux. The mixture was filtrated and concentrated to give a yellow oil. The product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate = 3:1, R_f = 0.3) to yield the target compound as colourless oil (0.91 g, 5.2 mmol, 52%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.41 (quin, *J* = 5.8 Hz, 1H), 4.16 (dd, *J* = 8.8, 6.1 Hz, 1H), 3.81 (dd, *J* = 8.8, 5.2 Hz, 1H), 3.10 (dd, *J* = 5.8, 1.2 Hz, 2H), 1.44 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C-NMR (300 MHz, $CDCl_3$): δ = 112.4 (C_q), 111.0 (C_q), 74.5 (CH), 68.3 (CH_2), 37.4 (CH_2), 27.3 (CH_3), 25.7 (CH_3) ppm. IR (ATR): ν = 2987, 2934, 2155, 2091, 1373, 1213, 1150, 1112, 1059, 871, 841, 556 cm^{-1}.

The pure enantiomers (*R*)- and (*S*)-**19** were obtained analogously from commercially available (*S*)- and (*R*)-**17**. (*R*)-**19**: $[\alpha]_D^{24}$ = –16.1 (*c* 0.60, $CHCl_3$), (*S*)-**19**: $[\alpha]_D^{24}$ = +11.4 (*c* 0.75, $CHCl_3$).

Synthesis of (^{34}S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanethiol (20)
A suspension of lithium aluminium hydride (0.34 g, 9.0 mmol, 2.0 equiv.) in dry diethylether (100mL) was cooled to 0 °C. (^{34}S)-2,2-Dimethyl-4-(thiocyanatomethyl)-1,3-dioxolane **19** (0.79 g, 4.5 mmol, 1.0 equiv.) in Et_2O (5 mL) was added slowly. The mixture was stirred for 5 min, followed by quenching with 0.5 M HCl until no more gas was generated. The mixture was extracted with Et_2O, washed with brine and dried over $MgSO_4$. Filtration and concentration gave the title compound **20** (0.50 g, 3.33 mmol, 74%) as a colorless oil which was pure without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.20 (quin, *J* = 6.0, 1H), 4.10 (dd, *J* = 8.2, 6.2 Hz, 1H), 3.76 (dd, *J* = 8.4, 6.0 Hz, 1H), 2.73 (ddd, *J* = 13.7, 8.1, 5.7 Hz, 1H), 2.60 (ddd, *J* = 13.6, 9.2, 6.7 Hz, 1H), 1.43 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C-NMR (300 MHz, $CDCl_3$): δ = 109.8 (C_q), 77.1 (CH), 68.4 (CH_2), 27.8 (CH_2), 27.0 (CH_3), 25.6 (CH_3) ppm. IR (ATR): ν = 3423, 2984, 2929, 2880, 1370, 1211, 1182, 1152, 1131, 1092, 911, 818, 729 cm^{-1}.
The pure enantiomers (*R*)- and (*S*)-**20** were prepared analogously. (*R*)-**20**: $[\alpha]_D^{24}$ = –38.6 (*c* 0.30, $CHCl_3$), (*S*)-**20**: $[\alpha]_D^{24}$ = +29.8 (*c* 0.40, $CHCl_3$).

Synthesis of (^{34}S)-2,3-dihydroxypropane-1-sulfonic acid (6)
To a solution of (^{34}S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanethiol **20** (0.49 g, 3.3 mmol, 1.0 equiv.) in methanol (10 mL) was added H_2O_2 (2.33 mL, 35% in H_2O, 21.3 mmol, 7.0 equiv.). The mixture was stirred over night at room temperature. The residue was concentrated to give the product as a colorless oil (0.485 g, 2.9 mmol, 88%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.91 (m, 1H), 4.63 (dd, *J* = 10.2, 5.2 Hz, 1H), 4.43 (ddd, *J* = 10.2, 2.6, 0.5 Hz, 1H), 3.46 (dd, *J* = 13.8, 7.1 Hz, 1H), 3.34 (ddd, *J* = 13.9, 2.7, 0.6 Hz, 1H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 76.3 (CH_2), 68.5 (CH), 52.7 (CH_2) ppm. IR (ATR): ν = 3343, 1721, 1321, 1205, 1149, 1016, 938, 885, 742, 665, 571, 462 cm^{-1}. The pure enantiomers (*R*)- and (*S*)-**6** were prepared analogously. (*R*)-**6**: $[\alpha]_D^{24}$ = +10.6 (*c* 0.58, MeOH), (*S*)-**6**: $[\alpha]_D^{24}$ = – 8.3 (*c* 0.53, MeOH).

Bacterial strains, growth conditions and feeding experiments. *Phaeobacter inhibens* DSM 17395 was obtained form the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Other strains of the *Rhodobacteraceae* investigated in this study were isolated from carapaces of *Cancer pagurus* specimens, collected on October 15th, 2014, along the northern coast of the island of Helgoland (North Sea). Four intermoult males of *C. pagurus* were caught in cages in a depth of 6.5 meters (N 54°12.084" E 007°52.973" and N 54°12.097" E 007°52.971"). After capture, the animals were kept separately in cleaned storage tanks on wet ground (seawater 15°C, 35‰ salinity). Animals were washed three times with sterile filtered, autoclaved artificial seawater[S1] to remove loosely attached bacteria. The specimens were directly used for the sampling procedure. Swab samples (© COPAN,Brescia, Italy <2 mm in Diameter) were taken from the carapaces and spread out on half concentrated marine agar plates 2216 (Difco, Detroit, Michigan). Plates were incubated at 15°C and single-grown colonies were selected and transferred at least three times until considered as pure. The 16S rRNA genes of the isolates were amplified using the primers GM3f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′).[S2,S3] Obtained PCR products were sequenced by Macrogen (Seoul, Korea) using primer GM3f. Sequences of at least 776 bp length were obtained and compared with those in GenBank at the NCBI database using the BLAST tool (http://www.ncbi.nlm.nih.gov/) to determine the phylogenetic affiliation of the isolates. Sequences were deposited at GenBank under the accession numbers KY513433-KY513437 (Table S1).
For the experiments performed in this study a preculture of each strain was grown in MB2216 medium at 28°C and shaking with 160 rpm over two days. A culture flask with 50 mL MB2216 was inoculated with 1 mL of the preculture and grown over night at 28°C, 160 rpm. At an OD = 0.5 and the culture flask was connected to a CLSA[S4,S5] for collection of volatiles. The volatiles were trapped on charcoal filters and after 24 h the filter was extracted with CH_2Cl_2 (50 μL) followed by GC/MS analysis of the obtained extract.
For the feeding experiments (*rac*)-(^{34}S)DHPS was added (feeding of 4 mg per day) over a period of 3 days during which the volatiles were continuously collected. For the individual enantiomers of (*R*)- and (*S*)-(^{34}S)DHPS and for (^{34}S)cysteine only one portion (4 mg) was

added and the volatiles were subsequently collected for 24 h. Extraction of the charcoal filter with CH_2Cl_2 was followed by GC/MS analysis.
Incorporation of labelling into TDA was followed by HPLC-MS analysis that was performed on a Thermo LTQ XL system with a C_{18} column (Hypersil GOLD, 3 μm, 150 × 2.1 mm). MS conditions were as follows: source type: HESI, capillary temperature: 275 °C, source heater temperature: 40 °C, sheath gas flow: 15 a. u., aux gas flow: 10 a. u., negative ionisation mode, source voltage: 4 kV.

Table S1. Strains of marine bacteria investigated in this study.

Strain	**Accession no. of the 16S rRNA gene**	**Next classified relative (percent similarity of the 16S rRNA gene)**
Phaeobacter inhibens DSM 17395	KC176241	not applicable
CP32	KY513437	*Paracoccus marcusii* MH1 (100%)
CP127	KY513434	*Ruegeria faecimaris* HD-28 (99%)
CP137	KY513436	*Paracoccus yeei* G1212 (100%)
CP152	KY513435	*Roseobacter litoralis* Och 149 (99%)
CP176	KY513433	*Sulfitobacter litoralis* (97%)

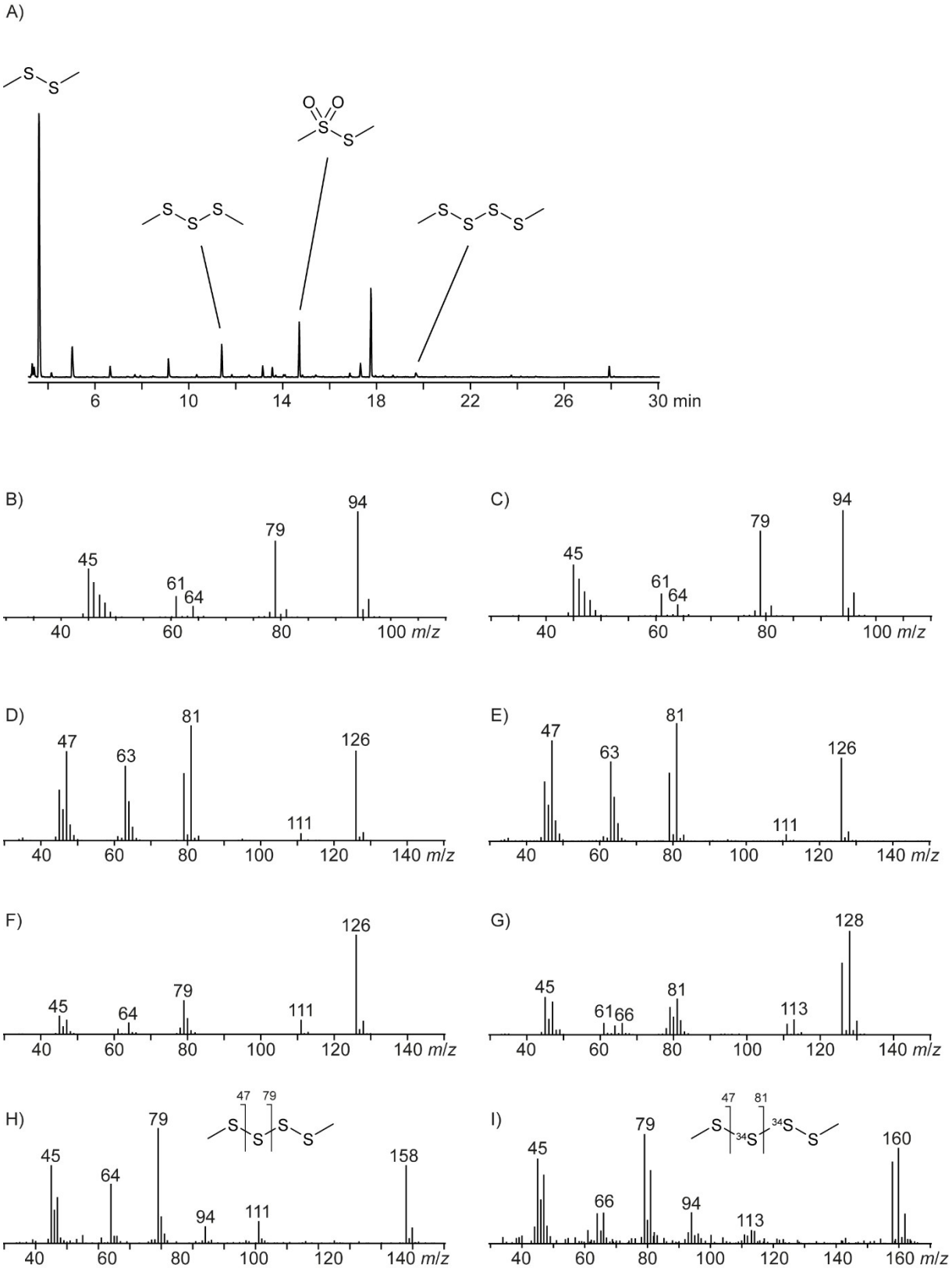

Figure S1. Sulfur volatiles from *P. inhibens*. A) Total ion chromatogram of a CLSA headspace extract from *P. inhibens*. EI mass spectra of B) dimethyl disulfide (DMDS), C) DMDS after feeding of (*rac*)-(^{34}S)DHPS, D) *S*-methyl methanethiosulfonate, E) *S*-methyl methanethiosulfonate after feeding of (*rac*)-(^{34}S)DHPS, F) dimethyl trisulfide (DMTS), G) DMTS after feeding of (*rac*)-(^{34}S)DHPS, H) dimethyl tetrasulfide, and I) dimethyl tetrasulfide after feeding of (*rac*)-(^{34}S)DHPS.

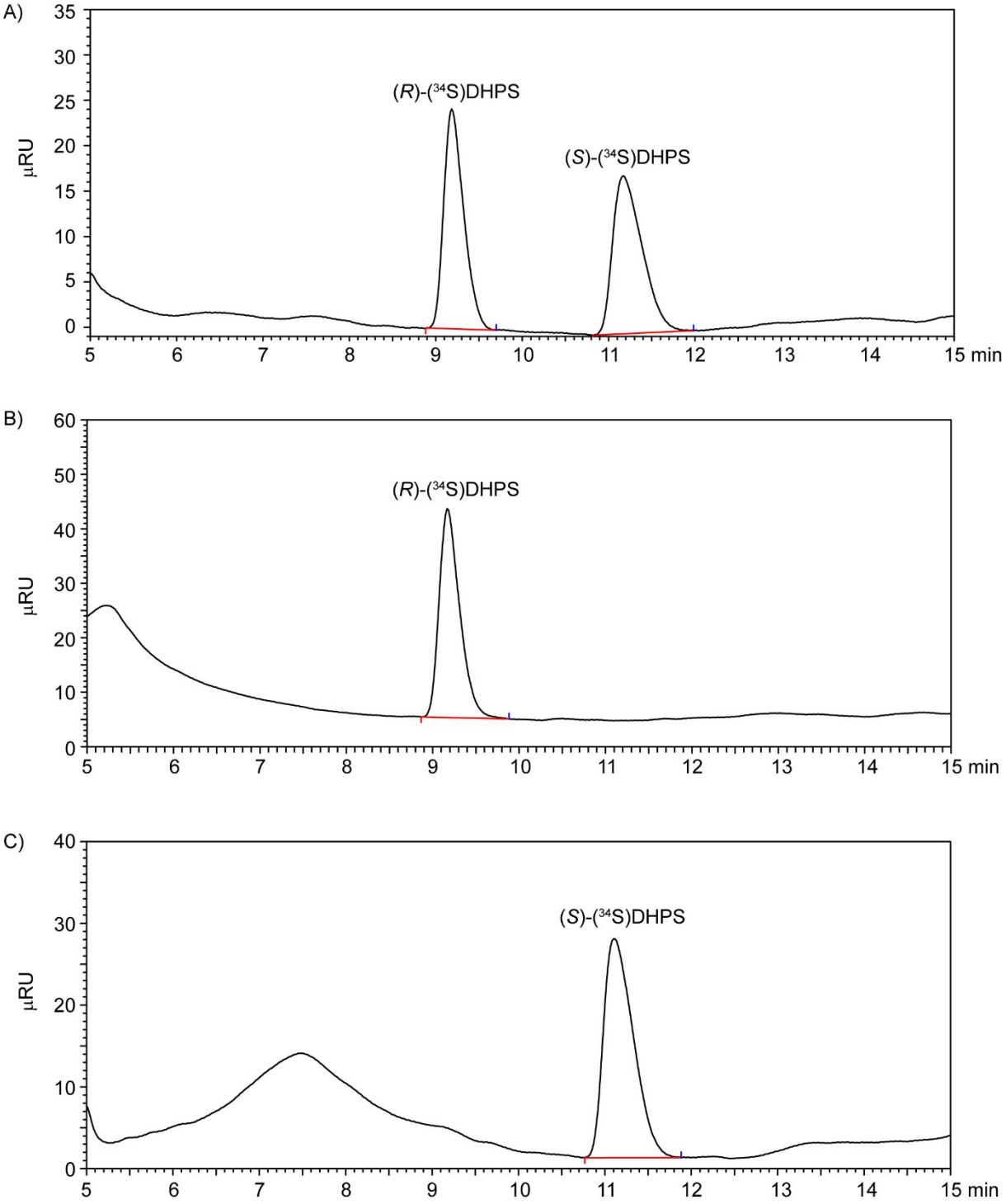

Figure S2. HPLC analysis of A) synthetic (*rac*)-(^{34}S)DHPS, B) (*S*)-(^{34}S)DHPS, and C) (*R*)-(^{34}S)DHPS.

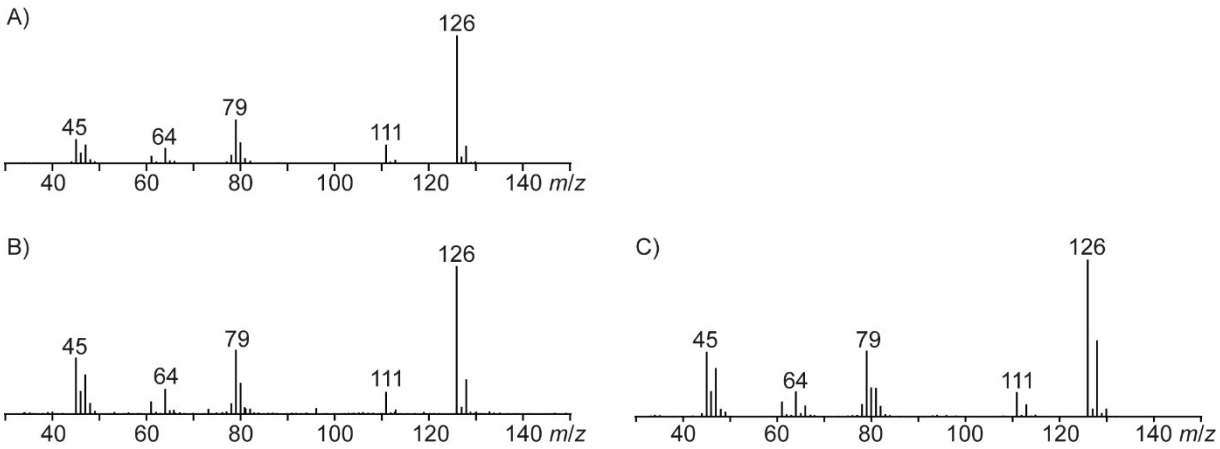

Figure S3. Feeding experiments with (*R*)- and (*S*)-(^{34}S)DHPS. EI mass spectra of A) unlabelled DMTS, B) DMTS after feeding of (*R*)-(^{34}S)DHPS, and C) DMTS after feeding of (*S*)-(^{34}S)DHPS.

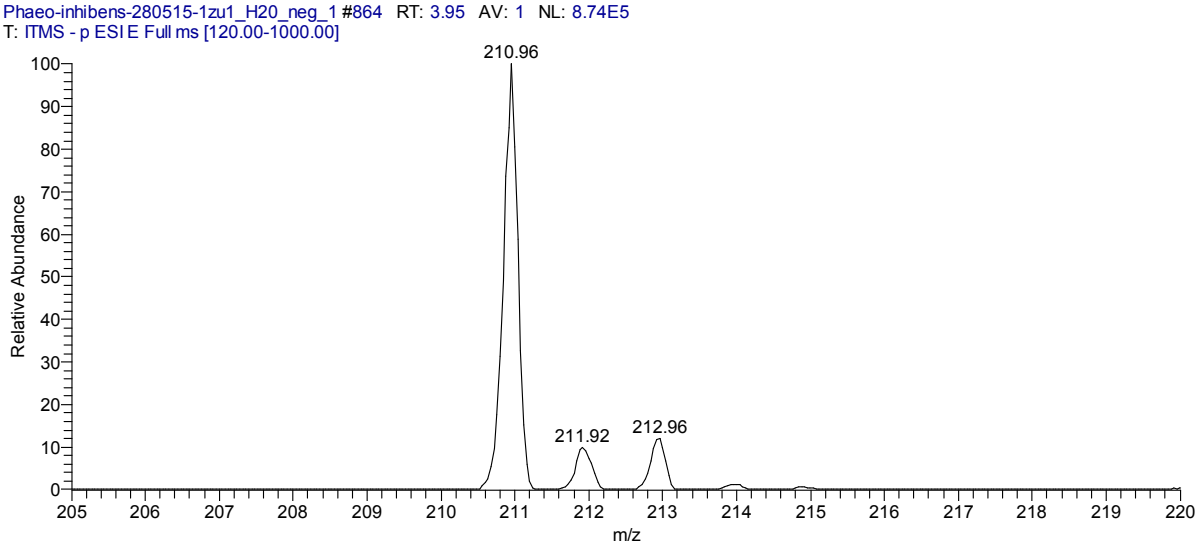

Figure S4A. ESI mass spectrum of unlabelled TDA from *P. inhibens* showing the molecular ion $[M+H]^+$ and its natural isotope pattern.

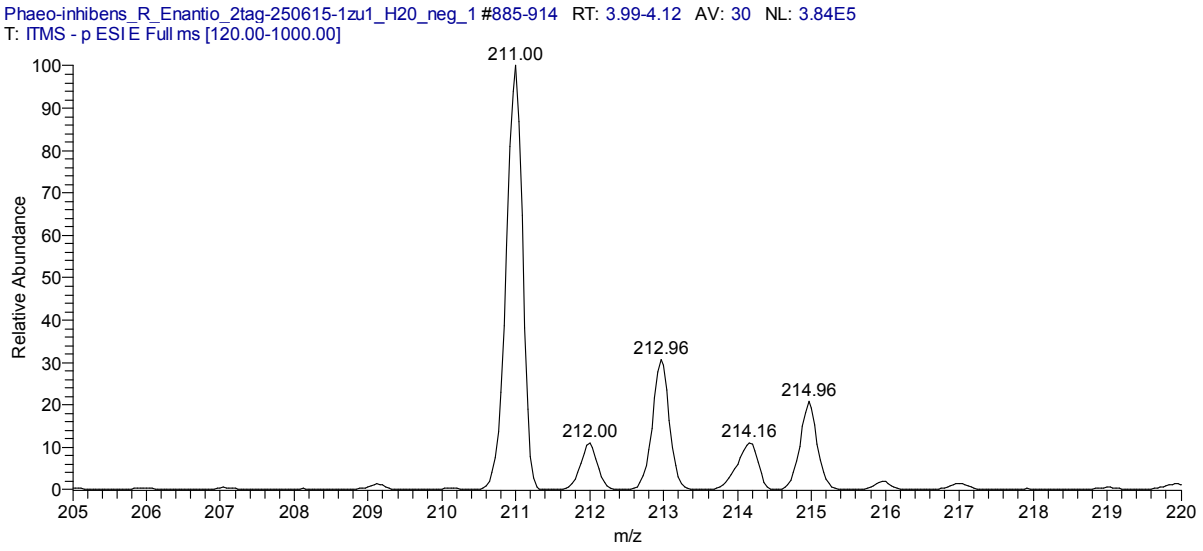

Figure S4B. ESI mass spectrum of labelled TDA obtained after feeding of (*R*)-(^{34}S)DHPS to *P. inhibens*. The isotope pattern of the molecular ion shows increased signals indicating the incorporation of ^{34}S-labelling.

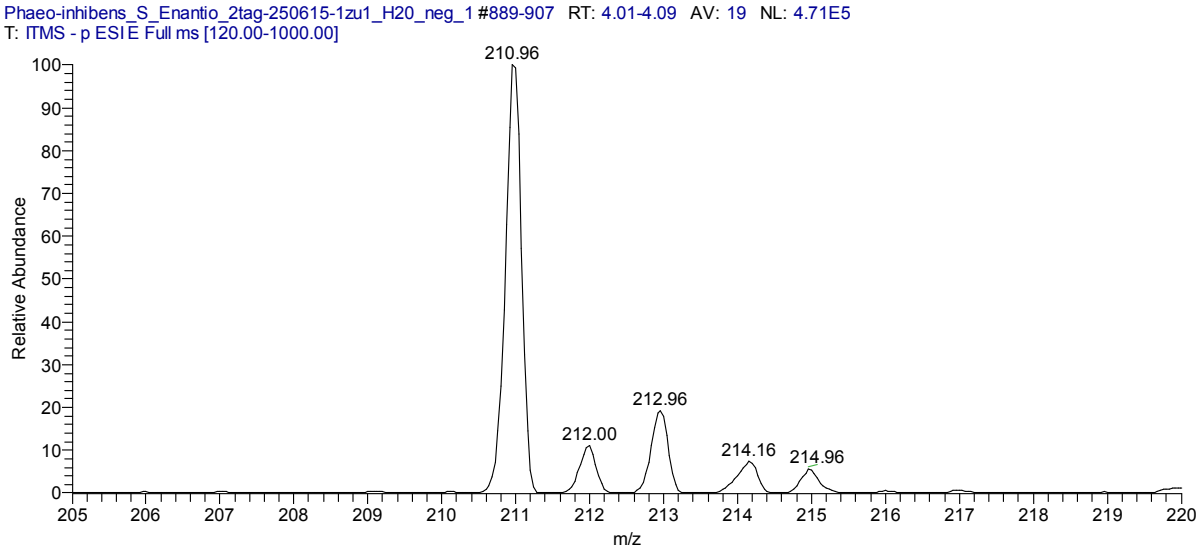

Figure S4C. ESI mass spectrum of labelled TDA obtained after feeding of (*S*)-(^{34}S)DHPS to P. inhibens. The isotope pattern of the molecular ion shows increased signals indicating the incorporation of ^{34}S-labelling.

Table S2. Genes for DHPS degradation encoded in *P. inhibens* DSM 17395.

accession no.	gene name[a]	identity[b]	function	ref.
WP_014879373	*slcD*	73%	sulfolactate dehydrogenase	[S6]
WP_014879374	*comE*	64%	sulfopyruvate decarboxylase subunit β	[S6]
WP_014879375	*comD*	84%	sulfopyruvate decarboxylase subunit α	[S6]
WP_014879376			universal stress protein	
WP_014879377	*hpsP*	46%	DHPS dehydrogenase	[S7]
WP_014879378	*hpsO*	49%	DHPS dehydrogenase	[S7]
WP_014879379	*hpsN*	54%	DHPS dehydrogenase	[S7]
WP_014879380			transporter	
WP_014879381			transporter	
WP_014879382			transporter	
WP_014879383	*hpsR*	38%	LacI type transcriptional regulator	[S7]
WP_014880786	*cuyA*	78%	L-cysteate sulfo-lyase	[S8]
WP_014881050			GntR family transcriptional regulator	
WP_014881051	*xsc*	45%	sulfoacetaldehyde acetyltransferase	[S9]
WP_014881052	*tauE*	40%	sulfite exporter	[S10]
WP_014881053			acetate kinase	
WP_014881054			phosphate acetyltransferase	

[a] Names of genes previously shown to be involved in DHPS degradation. [b] Amino acid sequence identity between the enzyme products of these genes and the corresponding closest homolog in *P. inhibens*.

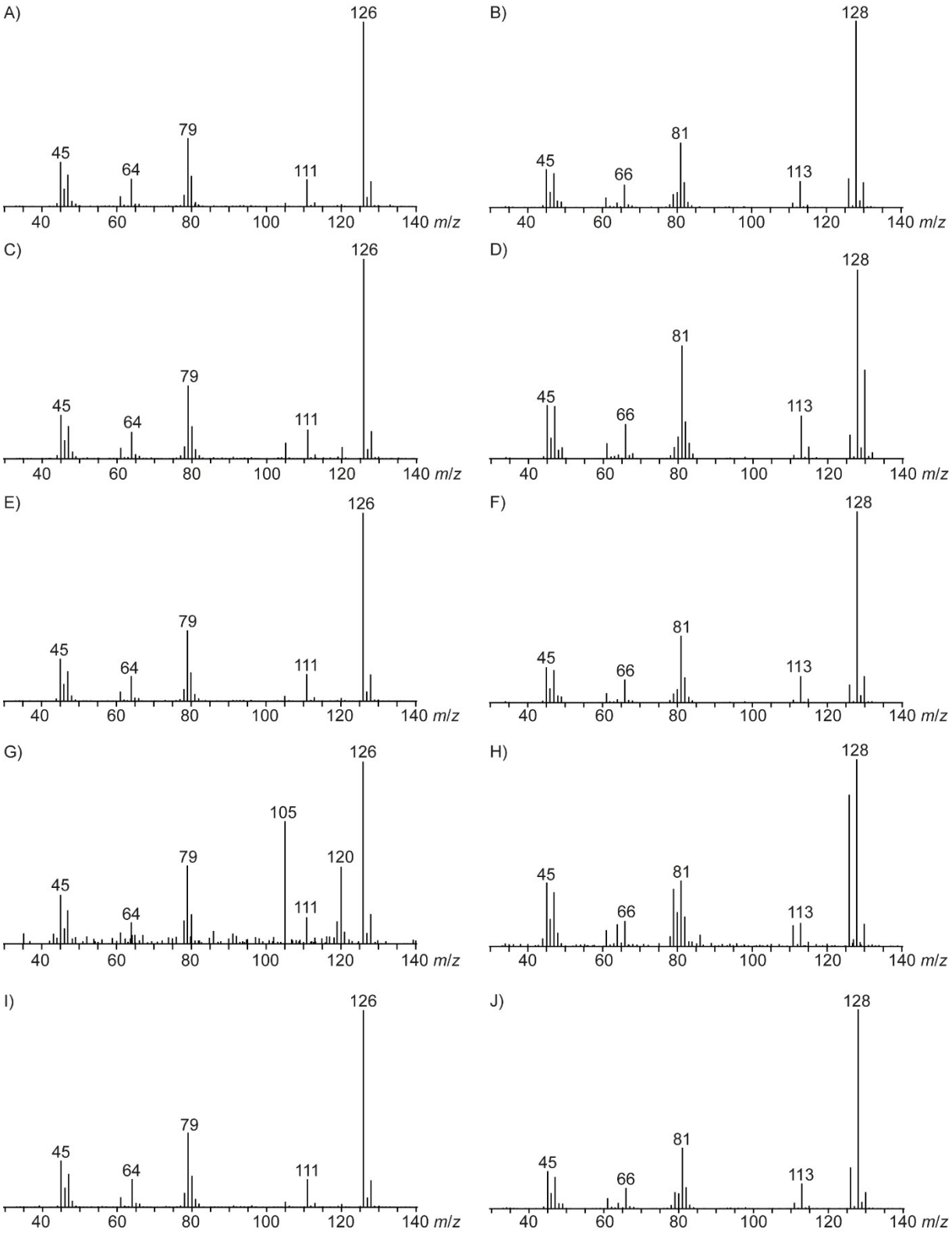

Figure S5. Feeding experiments with new isolates of marine bacteria from the crustacean *Cancer pagurus*. Mass spectra of DMTS in the headspace extracts of strain CP32 (A + B), strain CP127 (C + D), strain CP137 (E + F), strain CP152 (G + H), and strain CP176 (I + J). Left spectra are from feeding experiments with (*rac*)-(^{34}S)DHPS, right spectra from feeding experiments with (^{34}S)cysteine.

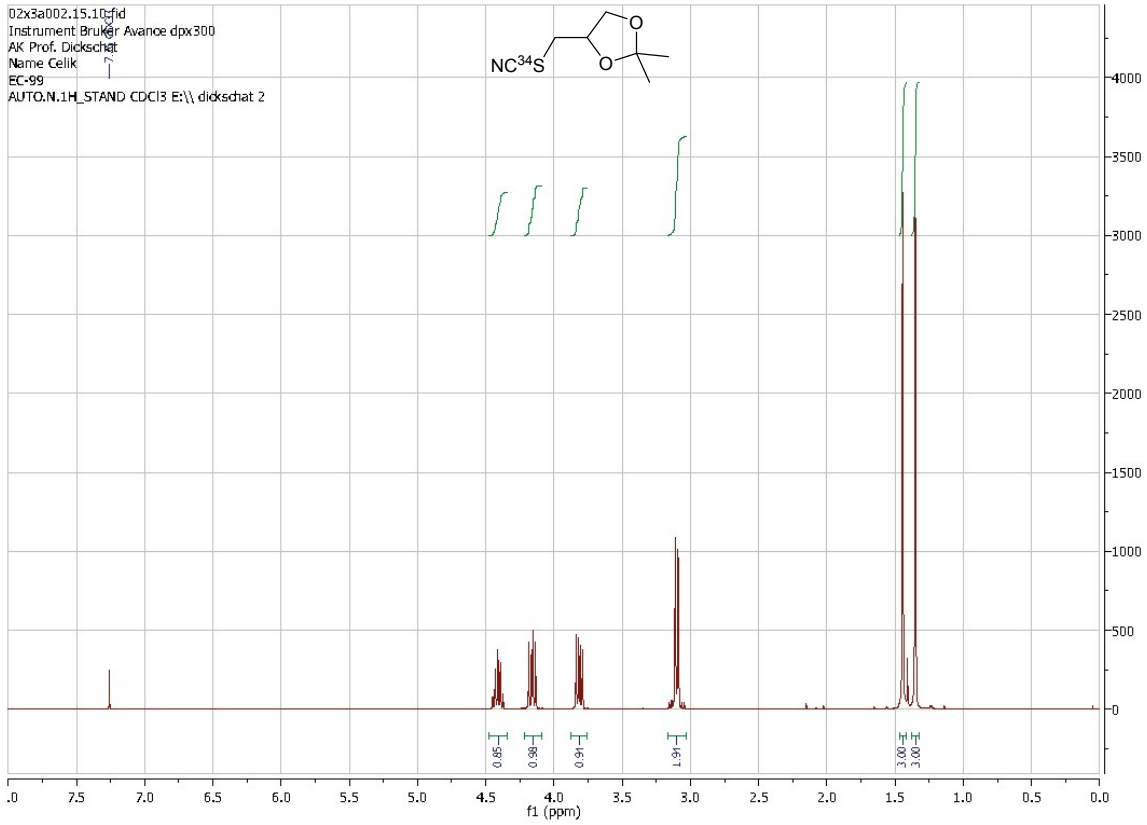

Figure S6. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **19**.

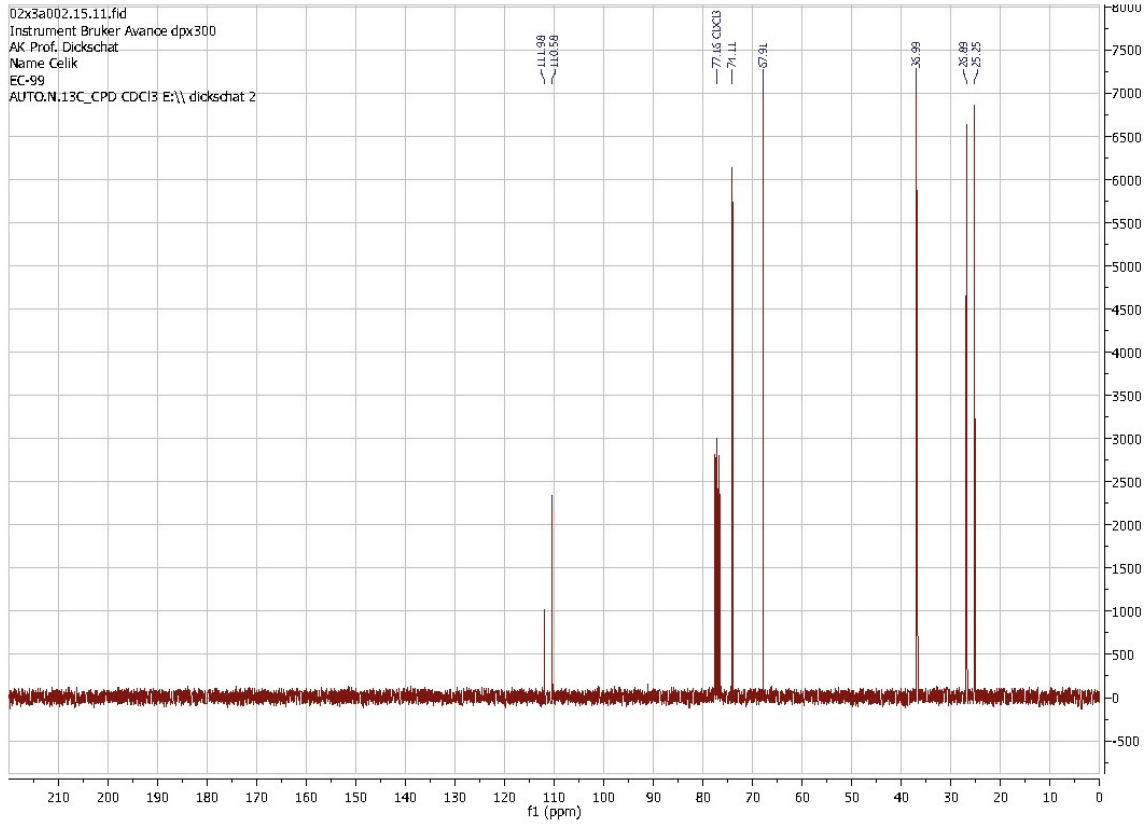

Figure S7. ^{13}C-NMR ($CDCl_3$, 75 MHz) of **19**.

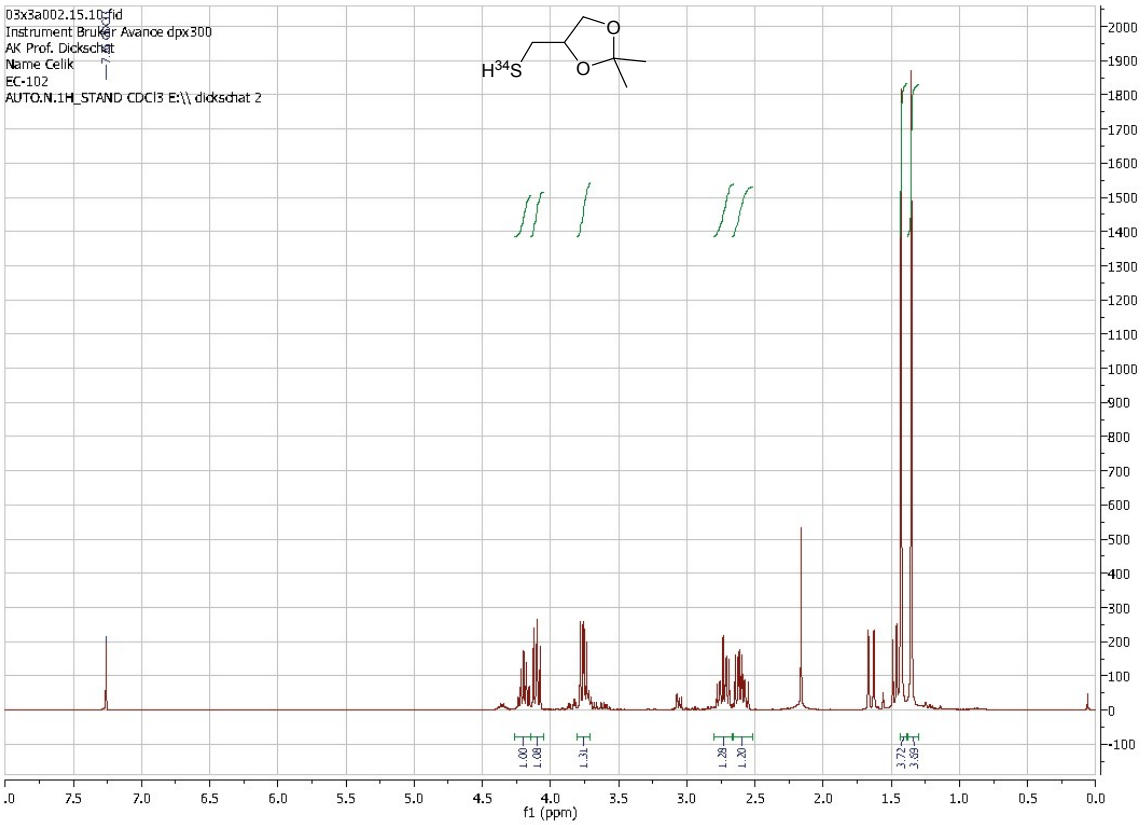

Figure S8. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **20**.

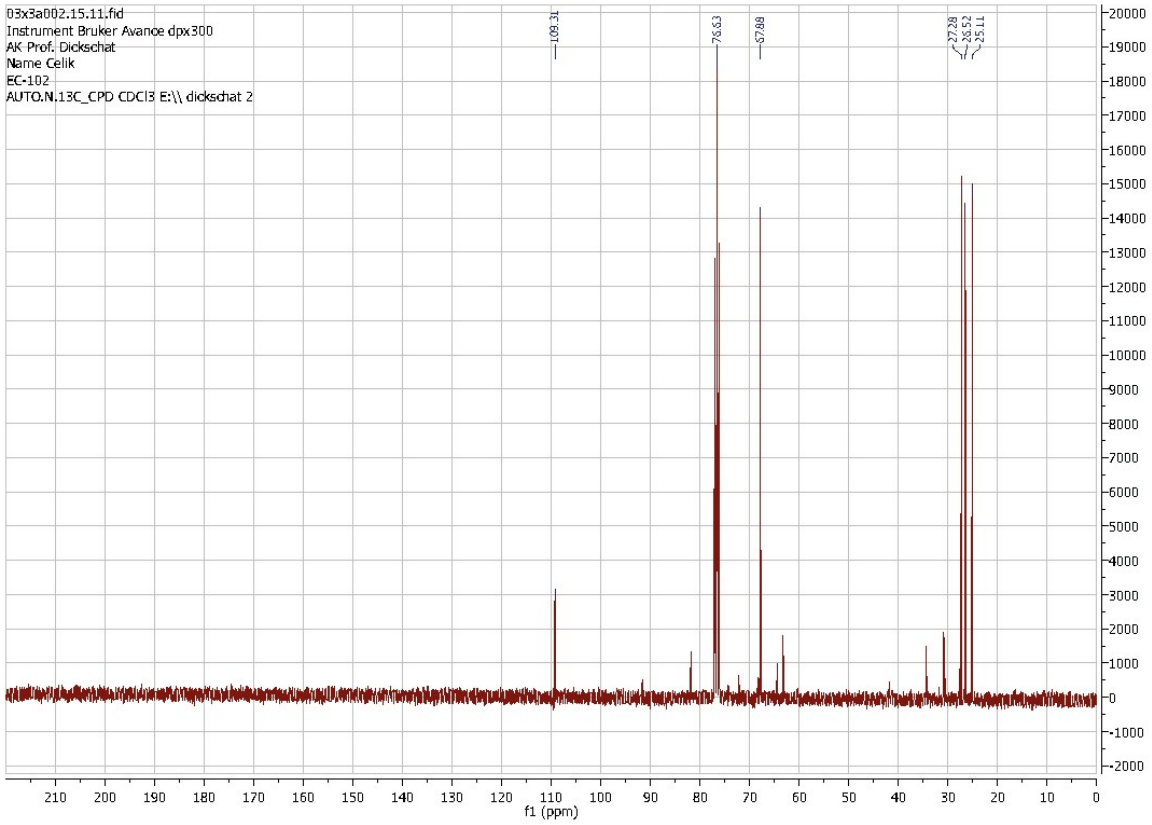

Figure S9. ^{13}C-NMR ($CDCl_3$, 75 MHz) of **20**.

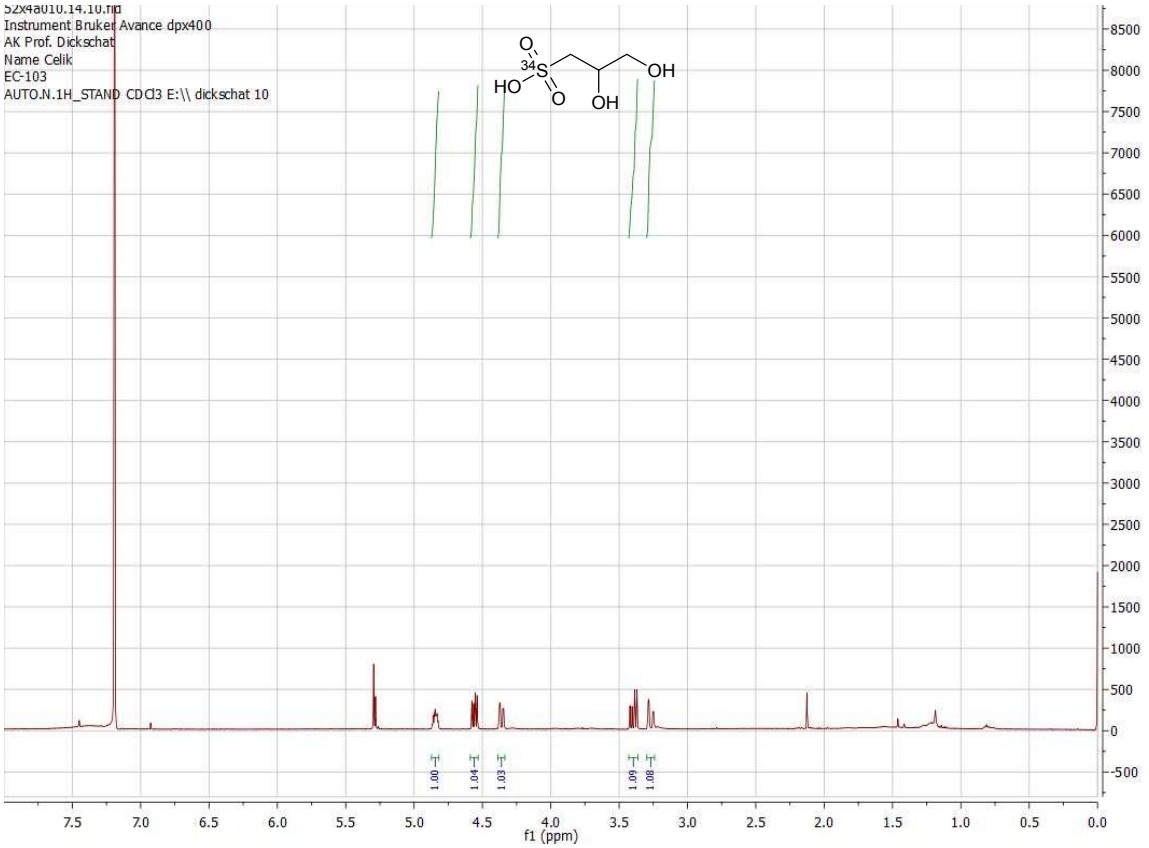

Figure S10. ^{1}H-NMR ($CDCl_3$, 400 MHz) of **6**.

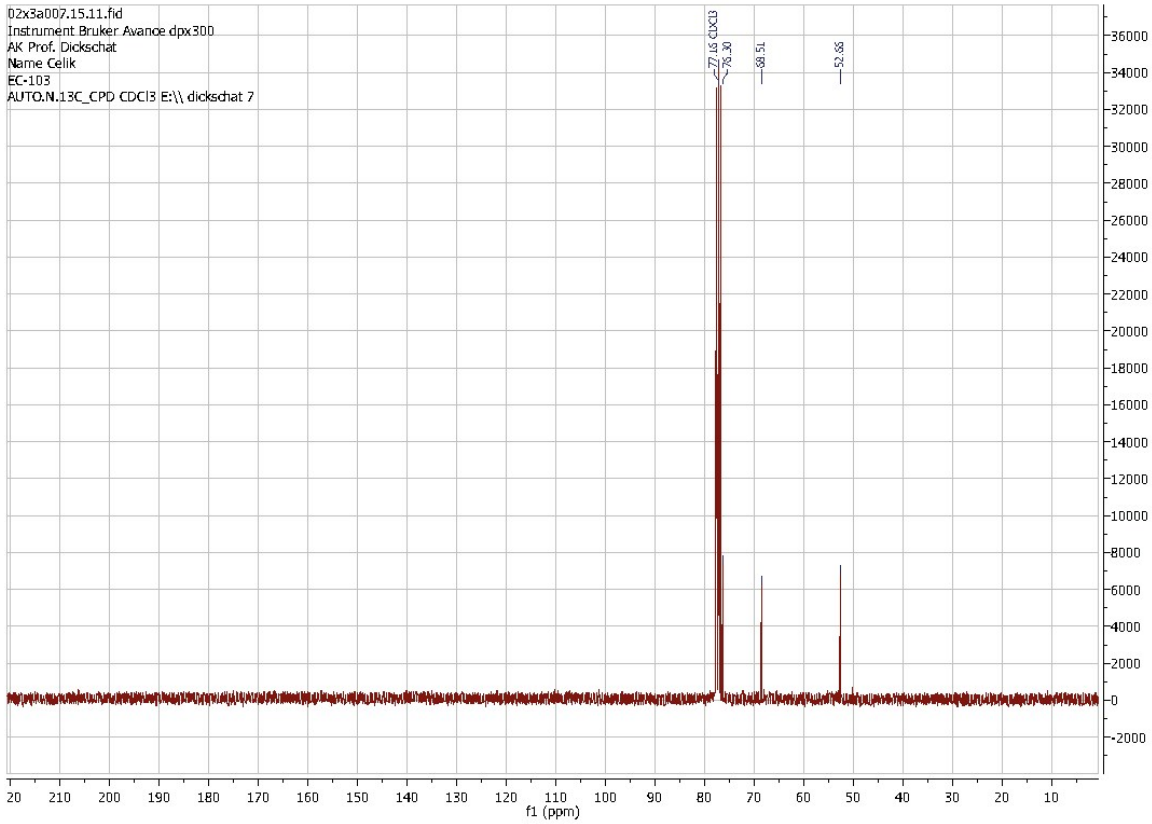

Figure S11. ^{13}C-NMR ($CDCl_3$, 75 MHz) of **6**.

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