Supporting Information

The Biosynthetic Gene Cluster for Sestermobaraenes—Discovery of a Geranylfarnesyl Diphosphate Synthase and a Multiproduct Sesterterpene Synthase from *Streptomyces mobaraensis*

Anwei Hou and Jeroen S. Dickschat*

anie_202010084_sm_misellaneous_information.pdf
Author Contributions

A.H. Investigation: Lead; Writing - Original Draft: Supporting; Writing - Review & Editing: Supporting.
Table of Contents

Strains, culture conditions, CLSA and GC/MS analysis S1
GC/MS analysis of CLSA extracts and enzyme products from SmTS1 S2
Type I terpene synthase homologs from *S. mobaraensis* S5
Phylogenetic tree for bacterial terpene synthase homologs S6
Amino acid sequences of SmTS1 – SmTS10 S7
Gene cloning and expression S9
Enzyme purification S11
Incubation experiments with unlabeled substrates S12
Compound purification procedure and analytical data for 6 – 12 S14
NMR data of sestermobaraene A (6) S16
NMR data of sestermobaraene B (7) S25
NMR data of sestermobaraene C (8) S34
NMR data of sestermobaraene D (9) S43
NMR data of sestermobaraene E (10) S52
NMR data of sestermobaraene F (11) S61
NMR data of sestermobaraol (12) S70
List of labeling experiments performed in this study S79
Enzymatic conversion 25 isotopomers of (13C)GFPP S80
Enzymatic conversion (7-13C)GPP and (E)- or (Z)-(4-13C,4-2H)IPP S105
Enzymatic conversion (R)- or (S)-(1-13C,1-2H)GPP and (2-13C)IPP S113
Enzymatic conversion (3-13C,2-2H)GGPP and IPP S116
Enzymatic conversion GPP, (Z)-(4-2H)IPP and (2-13C)IPP S118
Enzymatic conversion GPP and (3-13C,4-2H2)IPP S121
Determination of the absolute configurations of 6 – 12 S124
Synthesis of (5-13C)IPP S131
Synthesis of (3-13C,4-2H2)IPP S134
References S137
Strains and culture conditions
The strain *Streptomyces mobaraensis* NBRC 13819 (=NRRL B-3729) was obtained from the NRRL culture collection and cultured in SFM medium (2% mannitol, 2% soja flour, pH 7.2) or medium 65 GYM (0.4% glucose, 0.4% yeast extract, 1% malt extract, pH 7.2) at 28 °C. *Saccharomyces cerevisiae* FY834 was cultured in YPAD medium (1% yeast extract, 2% peptone, 2% glucose, 0.04% adenine sulphate) or SM-URA medium (0.17% yeast nitrogen base, 0.5% ammonium sulphate, 2% glucose, 0.077% nutritional supplement minus uracil). *E. coli* BL21 (DE3) was cultured in LB medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl). For agar plate cultures, 1.5% agar was added.

CLSA headspace extraction
The volatile organic compounds from an agar plate culture of *Streptomyces mobaraensis* were collected by a closed loop stripping apparatus (CLSA).[1] The emitted compounds were absorbed on charcoal for 24 hours and eluted with dichloromethane (30 μL). The obtained sample was immediately analyzed by GC/MS.

GC/MS
GC/MS analyses were performed on a 5977A GC/MSD system (Agilent, Santa Clara, CA, USA) with a 7890B GC and a 5977A mass selective detector. The GC was equipped with a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 μm film). Specific GC settings were 1) inlet pressure: 77.1 kPa, He at 23.3 mL min⁻¹, 2) injection volume: 2 μL, 3) temperature program: 5 min at 50 °C increasing at 5 °C min⁻¹ to 320 °C, 4) 60 s valve time, and 5) carrier gas: He at 1.2 mL min⁻¹. MS settings were 1) source: 230 °C, 2) transfer line: 250 °C, 3) quadrupole: 150 °C and 4) electron energy: 70 eV. Retention indices (I) were determined from retention times in comparison to the retention times of of *n*-alkanes (C₇-C₄₀).
Figure S1. Total ion chromatograms of A) a CLSA headspace extract from an agar plate culture of *S. mobaraensis* NBRC 13819, and B) an extract of an incubation of GFPP with SmTS1. The asterisks indicate non-enzymatic degradation products from GFPP.
Figure S2. El mass spectra of A) sestermobaraene A (6), B) sestermobaraene B (7), C) sestermobaraene C (8), D) sestermobaraene D (9), sestermobaraene E (10), F) sestermobaraene F (11), G) sestermobaraol (12), and further unknown compounds (a, b, c). The mass spectra on the left are from compounds in CLSA headspace extracts and the mass spectra on the right are from enzyme products.
Figure S2 (continued). EI mass spectra of A) sestermobaraene A (6), B) sestermobaraene B (7), C) sestermobaraene C (8), D) sestermobaraene D (9), sestermobaraene E (10), F) sestermobaraene F (11), G) sestermobaraol (12), and further unknown compounds (a, b, c). The mass spectra on the left are from compounds in CLSA headspace extracts and the mass spectra on the right are from enzyme products.
Table S1. Type I terpene synthase homologs from *S. mobaraensis*.

| accession number | TS no.      | (predicted) function                        |
|------------------|-------------|---------------------------------------------|
| WP_004941320     | SmTS1       | sestermobaraene synthase                    |
| WP_004945508     | SmTS2       | 2-MIB synthase                              |
| EME96605         | SmTS3       | geosmin synthase                            |
| WP_004939181     | SmTS4       | (insoluble)                                 |
| WP_004942276     | SmTS5       | (insoluble)                                 |
| WP_004954463     | SmTS6       | sesquiterpene/diterpene synthase            |
| WP_004952180     | SmTS7       | no activity                                 |
| WP_004952004     | SmTS8       | sesquiterpene/diterpene synthase (low activity) |
| WP_004954462     | SmTS9       | sesquiterpene/diterpene synthase (low activity) |
| WP_004954459     | SmTS10      | sesquiterpene synthase (low activity)       |
Figure S3. A) Phylogenetic tree constructed from 3267 amino acid sequences of bacterial terpene synthase homologs using the tree builder function of Geneious (alignment type: global alignment with free end gaps, cost matrix: Blosum45, genetic distance model: Jukes-Cantor, tree build method: neighbor-joining, gap open penalty: 8, gap extension penalty: 2). The largest branches representing functionally characterized enzymes and their closest relatives with likely the same function are shown in green and blue. B) Expansion for the branch containing SmTS1 and its closest relatives. The red arrows indicate the terpene synthases from S. mobaraensis. The scale bar indicates the number of substitutions per site.
**Figure S4.** Amino acid sequences of SmTS1–SmTS10. Highly conserved motifs are marked by yellow background.
Figure S4 (continued). Amino acid sequences of SmTS1 – SmTS10. Highly conserved motifs are marked by yellow background.
Gene cloning and expression

*S. mobaraensis* was grown in medium 65 (0.4% glucose, 0.4% yeast extract, 1% malt extract, pH 7.2) at 28°C for 1 week. The cells were collected by centrifugation and the genomic DNA was extracted by using a standard phenol/chloroform extraction protocol. The obtained gDNA, the primers according to Table S2, and Q5®-polymerase (NEB, Ipswich, MA, USA) were used for PCR. The standard protocol for PCR was: initial denaturation at 98 °C for 40 sec, followed by 30 cycles of a 3 steps program (denaturation at 98 °C for 10 sec, annealing at 60 – 67 °C for 30 sec, elongation at 72 °C for 45 sec) and final elongation at 72 °C for 2 min. The primers indicate the target sequence (accession number) in their names. Short primers were used to amplify the gene from the gDNA, then the longer primers were used in a second PCR with the first PCR product as a template to attach the homology arms (underlined) for homologous recombination in yeast with the linearized pYE-Express vector (EcoRI and HindIII digestion). All PCR products were analyzed by gel electrophoresis and purified by using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Homologous recombination was performed by the PEG/LiOAc method. After culturing the yeast for three days, the plasmid containing the integrated gene was isolated from the yeast by using the Zymoprep™ Yeast Plasmid Miniprep II kit (Zymoresearch, Irvine, CA, USA), followed by introduction to *E. coli* BL21 (DE3) through electroporation. The transformants were cultured on LB agar plates (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1.5% agar) with kanamycin (50 μg/mL) at 37 °C overnight. Single colonies were selected and grown in liquid LB medium overnight for plasmid DNA isolation by using PureYield™ Plasmid Miniprep System (Promega, Madison, WI, USA). The sequences of the cloned genes were verified by DNA sequencing. The *E. coli* transformants harbouring a correct plasmid were pre-cultured in liquid LB medium and grown at 37 °C overnight. The preculture was used to inoculate an expression culture (0.1%) and incubated at 37 °C until an OD₆₀₀ of 0.4 – 0.6 was reached. The expression culture was then cooled to 18 °C and IPTG (0.4 mM) was added to induce protein expression, followed by incubation at 18 °C for 16 – 18 h. The grown cells were collected by centrifugation and used for protein purification immediately or stored at –80 °C.
| Primer name | Target       | Nucleotide sequence (5’ → 3’, homology arms are underlined)          |
|-------------|--------------|---------------------------------------------------------------------|
| AH005r_WP004941320 (SmTS1) | GTGACGCTCAACCCGGTGC       | CTATCCCAAGTCCTGCTGCG                                                |
| AH005r_WP004941320 (SmTS1) | GTGACGCTCAACCCGGTGC       | CTATCCCAAGTCCTGCTGCG                                                |
| AH006r_WP004941320 (SmTS1) | GTGACGCTCAACCCGGTGC       | CTATCCCAAGTCCTGCTGCG                                                |
| AH012f_WP004941318 GFPPS  | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH012r_WP004941318 GFPPS  | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH013f_WP004941318 GFPPS  | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH013r_WP004941318 GFPPS  | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH001f_WP004939181 (SmTS4) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH001r_WP004939181 (SmTS4) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH003f_WP004939181 (SmTS4) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH003r_WP004939181 (SmTS4) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR028f_WP004942276 (SmTS5) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR028r_WP004942276 (SmTS5) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR027f_WP004942276 (SmTS5) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR027r_WP004942276 (SmTS5) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH004f_WP004954463 (SmTS6) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| JR124r_WP004954463 (SmTS6) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH002f_WP004954463 (SmTS6) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| JR125r_WP004954463 (SmTS6) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH007f_WP004952180 (SmTS7) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH007r_WP004952180 (SmTS7) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH008f_WP004952180 (SmTS7) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| AH008r_WP004952180 (SmTS7) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR030f_WP004952004 (SmTS8) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR030r_WP004952004 (SmTS8) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR029f_WP004952004 (SmTS8) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR029r_WP004952004 (SmTS8) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| JR122f_WP004954462 (SmTS9) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| JR122r_WP004954462 (SmTS9) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| JR123f_WP004954462 (SmTS9) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| JR123r_WP004954462 (SmTS9) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR032f_WP004954459 (SmTS10) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR032r_WP004954459 (SmTS10) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR031f_WP004954459 (SmTS10) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
| PR031r_WP004954459 (SmTS10) | ATGAGGAGGCACCTGC          | TGCAAGCTTCTCGACCGACAG                                               |
Enzyme purification
The collected cell pellets (from 200 mL culture) were suspended in binding buffer (5 mL; 20 mM Na₂HPO₄, 500 mM NaCl, 20 mM imidazole, 1 mM MgCl₂, pH 7.4, 4 °C) and lysed by ultrasonification (5 x 1 min). The lysate was centrifuged (14000 x g, 7 min) to remove the cell debris. The supernatant was loaded onto a Ni²⁺-NTA affinity chromatography column (Super Ni-NTA, Generon, Slough, UK), followed by washing with binding buffer (2 x 2 mL) and elution of the target protein with elution buffer (2 mL; 20 mM Na₂HPO₄, 500 mM NaCl, 500 mM imidazole, 1 mM MgCl₂, pH 7.4, 4 °C). The protein purity and concentration in eluted fractions were analyzed by SDS-PAGE and Bradford assay. Typical protein concentrations were 0.5 mg/mL for SmTS1 and 1.2 mg/mL for GFPPS.

Figure S5. SDS-PAGE of purified His-tagged SmTS1 and GFPPS.
Incubation experiments

For incubations with GFPP, purified SmTS1 (0.2 mL; ca. 0.5 mg/mL), a solution of GFPP (0.2 mL; 1.0 mg/mL in 25 mM NH₄HCO₃) and incubation buffer (0.5 mL; 20 mM Na₂HPO₄, 4 mM MgCl₂, 10% glycerol, pH 7.4) were mixed and incubated at 28 °C overnight. The enzyme products were extracted with hexane (0.2 mL), and the obtained extract was dried with MgSO₄ and analyzed by GC/MS.

For incubations with GGPP, FPP, GPP or DMAPP, a solution of each substrate (1 mL; 1.5 mg/mL in 25 mM NH₄HCO₃), purified GFPPS (2 mL; ca. 1.2 mg/mL), SmTS1 (2 mL; ca. 0.5 mg/mL) and a solution of IPP (1 mL; 2.0 mg/mL in 25 mM NH₄HCO₃) were mixed with incubation buffer (5 mL) and incubated at 28 °C overnight. The products were extracted with hexane (0.6 mL), and the obtained extract was dried with MgSO₄ and analyzed by GC/MS.

For preparative scale incubation, solutions of GFPPS (45 mL) and SmTS1 (45 mL), each obtained from 4 L E. coli culture, were used to set up 30 small scale reactions. The small scale reaction system was: GFPPS (1.5 mL), SmTS1 (1.5 mL), FPP (0.8 mL; 1.5 mg/mL in 25 mM NH₄HCO₃), IPP (0.8 mL; 2.0 mg/mL in 25 mM NH₄HCO₃) and incubation buffer (5 mL). After incubation at 28 °C overnight, the reaction mixtures of all small scale reactions were combined. The obtained products were extracted with hexane (3 x 50 mL) and concentrated under reduced pressure.
Figure S6. Incubation experiments with SmTS1. Products obtained from A) GFPP, B) GGPP with IPP and GFPPS, C) FPP with IPP and GFPPS, D) GPP with IPP and GFPPS, and E) DMAPP with IPP and GFPPS. The asterisks indicate non-enzymatic degradation products from GFPP. For comparison, the Y-axis of all five chromatograms was set to the same absolute scaling.
Compound purification

The crude products obtained from the preparative scale enzyme incubation were purified via silica gel chromatography to afford pure 10 (0.4 mg) and 12 (0.7 mg). Compound 6 (0.9 mg) was obtained via HPLC purification. HPLC purifications were performed on a Smartline series HPLC system (Knauer, Berlin, Germany), equipped with a UV/Vis-Detector S-2550 (190–1000 nm) and a Knauer Eurosep II 100-5 C18P column (5 μm; 8 × 250 mm). Elution was performed with acetonitrile at 6 mL/min (86 bar). The UV/Vis absorption was monitored at 205 nm.

The other compounds were purified via repeated preparative TLC using AgNO₃ coated TLC plates. The AgNO₃ coated TLC plates were obtained by treatment of commercial TLC plates (TLC Silica gel F₂₅₄, 20 × 20 cm, Merck, Darmstadt, Germany) with a solution of AgNO₃ in methanol (5 g/100 mL) for 10 min, followed by drying at 65 °C for 40 min. After TLC separation with a mixture solvent of cyclohexane and ethyl acetate, a small stripe of the TLC plate was cut off and stained with molybodophosphoric acid in EtOH (10 g/100 mL). The silica of regions containing the target compounds was scratched off and extracted with diethyl ether. After evaporation of the solvent, the pure compounds 7 (1.5 mg), 8 (1.5 mg), 9 (0.5 mg), 11 (0.8 mg) were obtained.

NMR spectroscopy

NMR spectra were recorded on a Bruker (Billerica, MA, USA) Avance I (300 MHz), Avance I (400 MHz), Avance I (500 MHz), Avance III HD Prodigy (500 MHz) or an Avance III HD Cryo (700 MHz) NMR spectrometer. Spectra were measured in C₆D₆ and referenced against solvent signals (¹H-NMR, residual proton signal: δ = 7.16; ¹³C-NMR: δ = 128.06).[¹⁹]

GC/MS-QTOF

GC/MS-QTOF analyses were performed on a 7890B GC connected to a 7200 accurate-mass Q-TOF detector (Agilent) equipped with a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 μm film). MS parameters were 1) inlet pressure: 83.2 kPa, He at 24.6 mL min⁻¹, 2) transfer line: 250 °C, 3) electron energy 70 eV. GC parameters were 1) temperature program: 5 min at 50 °C increasing at 5 °C min⁻¹ to 320 °C, 2) injection volume: 1 μL, 3) split ratio: 50:1, 60 s valve time, and 4) carrier gas: He at 1 mL min⁻¹.

IR spectroscopy and optical rotations

IR spectra were recorded on an ALPHA II FTIR Spectrometer (Bruker Optics, MA, USA), and the scan range was set to 500 to 4000 cm⁻¹. Optical rotations were recorded on a MCP 150 Modular Circular Polarimeter (Anton Paar GmbH, Graz, Austria).

(−)-Sestermobiaraene A ((3S,3aR,5aR,6R,7R,11aR,12S,2)-3-isopropyl-5a,9a,11a,12-tetramethyl-2,3,3a,5a,6,7,8,9,9a,10,11,11a-dodecahydro-1H-5,7,6-(epipropane[1,1,3]triyl)-benzo[a]cyclopenta[e][8]annulene, 6). TLC (pentane): Rᵣ = 0.95. Optical rotation: [α]D²⁰ = −4.4 (c 0.09, CH₂Cl₂). HRMS (ToF): m/z = 340.3122 (calc. for [C₂₅H₄₀]⁺ 340.3124). GC (HP-5MS): l = 2358. MS (El, 70 eV): Figure S2. IR (diamond ATR): ν / cm⁻¹ = 2591 (s), 2924 (s), 2857 (m), 1736 (m), 1718 (m), 1458 (m), 1376 (m), 1366 (m), 1228 (w), 1206(w), 1094 (w), 1028 (w), 800 (w), 543 (w). NMR data are given in Table S3 and Figures S7–S14.

(−)-Sestermobiaraene B ((3R,3aR,6S,6aR,7aR,10S,10aS,11aS,11bS)-10-isopropyl-6,7a,11b-trimethyl-12-methylenehexadecahydro-3,11a-methanoindeno[5,6-e]azulene, 7). TLC (AgNO₃ coated, cyclohexane): Rᵣ = 0.45. Optical rotation: [α]D²⁰ = −21.3 (c 0.15, DCM). HRMS (ToF): m/z = 340.3130 (calc. for [C₂₅H₄₀]⁺ 340.3124). GC (HP-5MS): l = 2350. MS (El, 70 eV): Figure S2. IR (diamond ATR): ν / cm⁻¹ = 2948 (s), 2924 (s), 2895 (m), 2869 (m), 1645 (w), 1460 (m), 717 (m), 665 (m), 583 (m). NMR data are given in Table S3 and Figures S7–S14.
1375 (m), 1260 (m), 1086 (m), 1016 (m), 876 (m), 800 (m). NMR data are given in Table S4 and Figures S15–S22.

(−)-Sestermobaraene C ((3S,3aR,6aR,7aR,10S,10aS,11aR,11bS)-10-isopropyl-7a,11b,12-trimethyl-6-methylenehexadecahydro-3,11a-methanoidenono[5,6]-azulene, 8). TLC [AgNO₃ coated, cyclohexane/ethyl acetate (40/1)]; Rᵣ = 0.38. Optical rotation: [α]D⁰ = −14.7 (c 0.15, CH₂Cl₂). HRMS (ToF): m/z = 340.3129 (calc. for [C₃S₄H₄O]⁺ 340.3124). GC (HP-5MS): l = 2432. MS (EI, 70 eV); Figure S2. IR (diamond ATR): ν / cm⁻¹ = 2946 (s), 2925 (s), 2924 (s), 2872 (m), 2855 (m), 1738 (w), 1658 (w), 1465 (m), 1376 (m), 1260 (m), 1086 (m), 1019 (m), 888 (m), 801 (m). NMR data are given in Table S5 and Figures S23–S30.

(−)-Sestermobaraene D ((3S,3aR,3a′S,6aR,7aR,10S,10aS,11aS)-10-isopropyl-3a,7a,12-trimethyl-6-methylenehexadecahydro-1H,4H-3,11a-methanophtho[1,8-f]azulene, 9). TLC (AgNO₃ coated, cyclohexane): Rᵣ = 0.27. Optical rotation: [α]D⁰ = −37.5 (c 0.04, CH₂Cl₂). HRMS (ToF): m/z = 340.3124 (calc. for [C₃S₄H₄O]⁺ 340.3124). GC (HP-5MS): l = 2470. MS (EI, 70 eV); Figure S2. IR (diamond ATR): ν / cm⁻¹ = 2953 (s), 2925 (s), 2872 (m), 2855 (m), 1738 (w), 1644 (w), 1438 (m), 1377 (m), 1260 (w), 1164 (m), 1045 (m), 977 (m), 884 (m), 800 (m). NMR data are given in Table S6 and Figures S31–S38.

(−)-Sestermobaraene E ((1S,3aR,9E,13E,15E,16aS)-1-isopropyl-3a,10,14-trimethyl-6-methylene-12,2,3a,4,5,6,7,8,11,12,16a-dodecahydrocyclopenta[15]annulene 10). TLC (pentane): Rᵣ = 0.42. Optical rotation: [α]D⁰ = +0.05 (c 0.08, CH₂Cl₂). HRMS (ToF): m/z = 340.3124 (calc. for [C₃S₄H₄O]⁺ 340.3124). GC (HP-5MS): l = 2362. MS (EI, 70 eV); Figure S2. IR (diamond ATR): ν / cm⁻¹ = 2948 (s), 2925 (s), 2870 (m), 1738 (w), 1658 (w), 1465 (m), 1376 (m), 1260 (w), 1229 (w), 1217 (w), 1093 (m), 1019 (m), 888 (m), 803 (m). NMR data are given in Table S7 and Figures S39–S46.

(+)-Sestermobaraene F ((3S,3aS,6aS,7aR,10S,10aS,11aS,11bS,11cS)-10-isopropyl-3,7a,11b-trimethyl-6-methylenehexadecahydro-1H-cyclopenta[2′,3′]cyclopropa[1′,2′;3,4]cyclohepta[1,2-f]indene 11). TLC [AgNO₃ coated, cyclohexane/ethyl acetate (40/1)]; Rᵣ = 0.42. Optical rotation: [α]D⁰ = +15 (c 0.08, CH₂Cl₂). HRMS (ToF): m/z = 340.3123 (calc. for [C₃S₄H₄O]⁺ 340.3124). GC (HP-5MS): l = 2362. MS (EI, 70 eV); Figure S2. IR (diamond ATR): ν / cm⁻¹ = 2948 (s), 2925 (s), 2870 (m), 1738 (w), 1658 (w), 1465 (m), 1376 (m), 1260 (w), 1229 (w), 1217 (w), 1093 (m), 1019 (m), 888 (m), 803 (m). NMR data are given in Table S8 and Figures S47–S54.

(−)-Sestermobaraol ([(3S,3aS,5S,5aS,6R,7R,9aS,10S,11aR,12S)-3-isopropyl-5a,10,11a,12-tetramethyltetradecahydro-9aH-5,7,6-(epipropene[1,1,3]triy]benz[a]cyclopenta[e][8]-annulen-9a-ol 12). TLC [cyclohexane/ethyl acetate (5/1)]; Rᵣ = 0.55. Optical rotation: [α]D⁰ = −35.7 (c 0.07, CH₂Cl₂). HRMS (ToF): m/z = 358.3230 (calc. for [C₃S₄H₄O]⁺ 358.3230). GC (HP-5MS): l = 2608. MS (EI, 70 eV); Figure S2. IR (diamond ATR): ν / cm⁻¹ = 3274 (w), 2953 (s), 2924 (s), 2875 (m), 1735 (w), 1672 (w), 1652 (w), 1463 (m), 1375 (m), 1345 (m), 1261 (m), 1092 (m), 1016 (m), 798 (m), 544 (m). NMR data are given in Table S9 and Figures S55–S62.
Figure S7. Structure elucidation of 6. Bold: $^1$H,$^1$H-COSY correlations, single-headed arrows: key HMBC correlations, and double-headed arrows: key NOESY correlations.
Table S3. NMR data of sestermobaraene A (6) in C₆D₆ recorded at 298 K.

|   | C[a] | ¹³C[b] | ¹H[b]            |
|---|------|--------|------------------|
| 1 | CH₂  | 35.39  | 1.47 (m, 2H)     |
| 2 | CH₂  | 34.69  | 1.47 (m, 1H, H₉) |
|   |      |        | 1.39 (m, 1H, H₆) |
| 3 | C_q  | 43.28  |                  |
| 4 | CH₂  | 34.17  | 1.53 (m, 1H, H₁) |
|   |      |        | 1.02 (m, 1H, H₈) |
| 5 | CH₂  | 20.47  | 1.64 (m, 1H, H₆) |
|   |      |        | 1.47 (m, 1H, H₅) |
| 6 | CH   | 49.68  | 1.41 (m, 1H)     |
| 7 | C_q  | 52.31  |                  |
| 8 | CH₂  | 39.91  | 1.47 (m, 1H, H₁)[c] |
|   |      |        | 1.37 (m, 1H, H₈)[c] |
| 9 | CH₂  | 23.19  | 1.59 (m, 1H, H₉)[c] |
|   |      |        | 1.38 (m, 1H, H₆)[c] |
|10 | CH   | 49.03  | 1.90 (d, J = 4.0, 1H) |
|11 | C_q  | 50.63  |                  |
|12 | C_q  | 156.19 |                  |
|13 | CH   | 120.18 | 5.15 (d, J = 9.3, 1H) |
|14 | CH   | 48.38  | 2.28 (dd, J = 10.9, 9.6, 1H) |
|15 | C_q  | 49.09  |                  |
|16 | CH₂  | 39.65  | 1.43 (m, 1H, H₈)  |
|   |      |        | 1.30 (m, 1H, H₆)  |
|17 | CH₂  | 25.88  | 1.80 (m, 1H, H₆)  |
|   |      |        | 1.35 (m, 1H, H₅)  |
|18 | CH   | 50.30  | 1.66 (m, 1H)      |
|19 | CH   | 33.09  | 1.56 (m, 1H)      |
|20 | CH₃  | 22.24  | 0.98 (d, J = 6.9, 3H) |
|21 | CH₃  | 20.65  | 0.92 (d, J = 6.6, 3H) |
|22 | CH₃  | 21.33  | 0.87 (s, 3H)      |
|23 | CH₃  | 20.12  | 1.30 (s, 3H)      |
|24 | CH₃  | 17.04  | 1.14 (s, 3H)      |
|25 | CH₃  | 25.47  | 0.94 (s, 3H)      |

[a] Carbon numbering as shown in Figure S7. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, coupling constants J are given in Hertz. [c] For assignment of H₁ and H₈ cf. Figure S101.
Figure S8. $^1$H-NMR spectrum (700 MHz, C$_6$D$_6$) of 6.
Figure S9. $^{13}$C-NMR spectrum (176 MHz, C$_6$D$_6$) of 6.
Figure S10. $^{13}$C-DEPT135 spectrum (176 MHz, C$_6$D$_6$) of 6.
Figure S11. $^1$H-$^1$H-COSY spectrum (C$_6$D$_6$) of 6.
Figure S12. HSQC spectrum (C$_6$D$_6$) of 6.
Figure S13. HMBC spectrum (C₆D₆) of 6.
Figure S14. NOESY spectrum (C₆D₆) of 6.
Figure S15. Structure elucidation of 7. Bold: \(^1\)H,\(^1\)H-COSY correlations, single-headed arrows: key HMBC correlations, and double-headed arrows: key NOESY correlations.
Table S4. NMR data of sestermobaraene B (7) in C$_6$D$_6$ recorded at 298 K.

|       | $^{13}$C$^a$ | $^1$H$^b$     |                  |
|-------|--------------|---------------|------------------|
| 1 CH$_2$ | 45.93        | 1.84 (m, 1H, H$_a$) |                  |
| 2 CH    | 42.54        | 1.51 (m, 1H)    |                  |
| 3 CH    | 38.37        | 1.49 (m, 1H)    |                  |
| 4 CH$_2$ | 32.54        | 1.87 (m, 1H, H$_b$) | 1.45 (m, 1H, H$_a$) |
| 5 CH$_2$ | 25.42        | 1.84 (m, 1H, H$_a$) | 1.53 (m, 1H, H$_b$) |
| 6 CH    | 51.91        | 1.53 (m, 1H)    |                  |
| 7 C$_q$  | 54.43        | –              |                  |
| 8 CH$_2$ | 36.73        | 1.93 (ddd, J = 12.5, 9.1, 3.0, 1H, H$_b$)$^c$ | 1.14 (m, 1H, H$_a$)$^c$ |
| 9 CH$_2$ | 29.80        | 1.69 (m, 1H, H$_a$)$^c$ | 1.32 (m, 1H, H$_b$)$^c$ |
| 10 CH   | 56.26        | 2.33 (dd, J = 5.0, 1.1, 1H) |                  |
| 11 C$_q$ | 165.27       | –              |                  |
| 12 C$_q$ | 49.46        | –              |                  |
| 13 CH$_2$ | 35.13        | 1.54 (m, 1H, H$_b$) | 1.23 (t, J = 13.0, 1H, H$_a$) |
| 14 CH   | 46.08        | 1.59 (m, 1H)    |                  |
| 15 C$_q$ | 41.38        | –              |                  |
| 16 CH$_2$ | 39.91        | 1.49 (m, 1H, H$_a$) | 1.18 (m, 1H, H$_b$) |
| 17 CH$_2$ | 23.85        | 1.72 (m, 1H, H$_a$) | 1.42 (m, 1H, H$_b$) |
| 18 CH   | 46.83        | 1.50 (m, 1H)    |                  |
| 19 CH   | 29.64        | 1.73 (m, 1H)    |                  |
| 20 CH$_3$ | 22.21        | 0.97 (d, J = 6.9, 3H) |                  |
| 21 CH$_2$ | 17.99        | 0.89 (d, J = 6.8, 3H) |                  |
| 22 CH$_3$ | 17.79        | 0.75 (s, 3H)    |                  |
| 23 CH$_2$ | 103.51       | 4.98 (d, J = 1.1, 1H, H$_b$)$^d$ | 4.90 (d, J = 1.0, 1H, H$_e$)$^d$ |
| 24 CH$_3$ | 16.98        | 1.08 (s, 3H)    |                  |
| 25 CH$_3$ | 23.32        | 1.00 (d, J = 6.4, 3H) |                  |

$^a$ Carbon numbering as shown in Figure S15. $^b$ Chemical shifts $\delta$ in ppm, multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, coupling constants $J$ are given in Hertz. $^c$ For assignment of H$_a$ and H$_b$ cf. Figure S102. $^d$ Assignment according to CIP priority rules.
Figure S16. $^1$H-NMR spectrum (700 MHz, C$_6$D$_6$) of 7.
Figure S17. $^{13}$C-NMR spectrum (176 MHz, C$_6$D$_6$) of 7.
**Figure S18.** $^{13}$C-DEPT135 spectrum (176 MHz, C$_6$D$_6$) of 7.
Figure S19. $^1$H,$^1$H-COSY spectrum (C$_6$D$_6$) of 7.
Figure S20. HSQC spectrum (C₆D₆) of 7.
Figure S21. HMBC spectrum (C$_6$D$_6$) of 7.
Figure S22. NOESY spectrum ($C_6D_6$) of 7.
Figure S23. Structure elucidation of 8. Bold: $^1$H-$^1$H-COSY correlations, single-headed arrows: key HMBC correlations, and double-headed arrows: key NOESY correlations.
### Table S5. NMR data of sestermobaraene C (8) in C$_6$D$_6$ recorded at 298 K.

|   | $^1$C$^{[a]}$ | $^{13}$C$^{[b]}$ | $^1$H$^{[b]}$ |
|---|-------------|-----------------|-------------|
| 1 | CH$_2$      | 44.34           | 1.80 (dd, $J = 12.4, 3.3, 1H, H_\alpha$) |
|   |             |                 | 1.38 (t, $J = 12.6, 1H, H_\beta$) |
| 2 | CH          | 44.45           | 2.52 (dm, $J = 12.6, 1H$) |
| 3 | C$_q$       | 156.75          | –           |
| 4 | CH$_2$      | 36.89           | 2.35 (m, 1H, H$_\alpha$) |
|   |             |                 | 2.28 (m, 1H, H$_\beta$) |
| 5 | CH$_2$      | 29.65           | 1.93 (dddd, $J = 14.4, 10.1, 5.7, 4.6, 1H, H_\alpha$) |
|   |             |                 | 1.46 (m, 1H, H$_\beta$) |
| 6 | CH          | 51.42           | 1.62 (dt, $J = 9.9, 2.0, 1H$) |
| 7 | C$_q$       | 53.78           | –           |
| 8 | CH$_2$      | 37.39           | 1.84 (ddd, $J = 12.6, 9.2, 3.2, 1H, H_\beta$)$^{[c]}$ |
|   |             |                 | 1.08 (m, 1H, H$_\alpha$)$^{[c]}$ |
| 9 | CH$_2$      | 20.80           | 1.47 (m, 1H, H$_\beta$)$^{[c]}$ |
|   |             |                 | 1.27 (m, 1H, H$_\alpha$)$^{[c]}$ |
| 10| CH          | 52.33           | 1.50 (m, 1H) |
| 11| CH          | 36.32           | 2.32 (m, 1H) |
| 12| C$_q$       | 45.49           | –           |
| 13| CH$_2$      | 29.30           | 1.68 (m, 1H, H$_\beta$) |
|   |             |                 | 1.05 (m, 1H, H$_\alpha$) |
| 14| CH          | 47.00           | 1.19 (m, 1H) |
| 15| C$_q$       | 41.32           | –           |
| 16| CH$_2$      | 39.72           | 1.46 (m, 1H, H$_\alpha$) |
|   |             |                 | 1.10 (m, 1H, H$_\beta$) |
| 17| CH$_2$      | 22.94           | 1.66 (m, 1H, H$_\alpha$) |
|   |             |                 | 1.40 (m, 1H, H$_\beta$) |
| 18| CH          | 47.52           | 1.49 (m, 1H) |
| 19| CH          | 28.71           | 1.76 (m, 1H) |
| 20| CH$_3$      | 22.65           | 0.98 (d, $J = 6.8, 3H$) |
| 21| CH$_3$      | 17.38           | 0.88 (d, $J = 6.8, 3H$) |
| 22| CH$_3$      | 17.57           | 1.17 (s, 3H) |
| 23| CH$_3$      | 15.62           | 1.09 (d, $J = 7.2, 3H$) |
| 24| CH$_3$      | 17.61           | 0.76 (d, $J = 0.9, 3H$) |
| 25| CH$_2$      | 112.33          | 4.97 (m, 1H, H$_2$)$^{[d]}$ |
|   |             |                 | 4.85 (t, $J = 1.8, 1H, H_2$)$^{[d]}$ |

$^{[a]}$ Carbon numbering as shown in Figure S23. $^{[b]}$ Chemical shifts $\delta$ in ppm, multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, coupling constants $J$ are given in Hertz. $^{[c]}$ For assignment of H$_\alpha$ and H$_\beta$ cf. Figure S103. $^{[d]}$ Assignment according to CIP priority rules.
Figure S24. ^1^H-NMR spectrum (700 MHz, C₆D₆) of 8.
Figure S25. $^{13}$C-NMR spectrum (176 MHz, $C_6D_6$) of 8.
Figure S26. $^{13}$C-DEPT135 spectrum (176 MHz, C$_6$D$_6$) of 8.
Figure S27. $^1$H,$^1$H-COSY spectrum (C$_6$D$_6$) of 8.
Figure S28. HSQC spectrum (C$_6$D$_6$) of 8.
Figure S29. HMBC spectrum (C₆D₆) of 8.
Figure S30. NOESY spectrum (C$_6$D$_6$) of 8.
Figure S31. Structure elucidation of 9. Bold: $^1$H,$^1$H-COSY correlations, single-headed arrows: key HMBC correlations, and double-headed arrows: key NOESY correlations.
Table S6. NMR data of sestermobaraene D (9) in C₆D₆ recorded at 298 K.

| C  | ¹³C [b] | ¹H [b] |
|----|--------|--------|
| 1  | CH₂    | 48.45  | 1.80 (m, 1H, H₆) |
|    |        |        | 1.55 (m, 1H, H₅) |
| 2  | CH     | 49.93  | 2.25 (d, J = 12.7, 1H) |
| 3  | Cq     | 153.80 | – |
| 4  | CH₂    | 27.31  | 2.48 (m, 1H, H₆) |
|    |        |        | 2.16 (m, 1H, H₅) |
| 5  | CH₂    | 26.41  | 1.46 (m, 1H, H₆) |
|    |        |        | 1.34 (m, 1H, H₅) |
| 6  | CH     | 52.86  | 1.00 (dd, J = 13.6, 5.1, 1H) |
| 7  | Cq     | 46.08  | – |
| 8  | CH₂    | 32.76  | 1.76 (m, 1H, H₆) |
|    |        |        | 1.19 (m, 1H, H₅) |
| 9  | CH₂    | 28.26  | 1.78 (m, 1H, H₆) |
|    |        |        | 1.20 (m, 1H, H₅) |
| 10 |        | 47.17  | 1.50 (br d, J = 4.9, 1H) |
| 11 | CH     | 43.12  | 2.28 (q, J = 6.8, 1H) |
| 12 | Cq     | 55.36  | – |
| 13 | CH₂    | 27.24  | 1.29 (m, 1H, H₆) |
|    |        |        | 1.20 (m, 1H, H₅) |
| 14 | CH     | 49.88  | 1.30 (m, 1H) |
| 15 | Cq     | 44.91  | – |
| 16 | CH₂    | 40.92  | 1.32 (m, 1H, H₆) |
|    |        |        | 1.13 (m, 1H, H₅) |
| 17 | CH₂    | 21.43  | 1.61 (m, 1H, H₆) |
|    |        |        | 1.45 (m, 1H, H₅) |
| 18 | CH     | 47.79  | 1.52 (m, 1H) |
| 19 | CH     | 27.31  | 1.78 (m, 1H) |
| 20 | CH₃    | 16.03  | 0.89 (d, J = 6.7, 3H) |
| 21 | CH₃    | 23.60  | 0.95 (d, J = 6.9, 3H) |
| 22 | CH₃    | 18.41  | 0.83 (s, 3H) |
| 23 | CH₃    | 10.87  | 0.86 (d, J = 6.6, 3H) |
| 24 | CH₃    | 29.20  | 1.07 (s, 3H) |
| 25 | CH₂    | 109.88 | 4.88 (q, J = 2.3, 1H, H₂) |
|    |        |        | 4.83 (q, J = 2.2, 1H, H₂) |

[a] Carbon numbering as shown in Figure S31. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, q = quartet, br = broad, m = multiplet, coupling constants J are given in Hertz. [c] For assignment of H₅ and H₆ cf. Figure S104. [d] Assignment according to CIP priority rules.
Figure S32. $^1$H-NMR spectrum (700 MHz, C$_6$D$_6$) of 9.
Figure S33. $^{13}$C-NMR spectrum (176 MHz, $^{13}$C$_6$D$_6$) of 9.
Figure S34. $^{13}$C-DEPT135 spectrum (176 MHz, C₆D₆) of 9.
Figure S35. $^1$H, $^1$H-COSY spectrum (C$_6$D$_6$) of 9.
Figure S36. HSQC spectrum (C₆D₆) of 9.
Figure S37. HMBC spectrum (C$_6$D$_6$) of 9.
Figure S38. NOESY spectrum (C₆D₆) of 9.
Figure S39. Structure elucidation of 10. Bold: $^1$H-$^1$H-COSY correlations, single-headed arrows: key HMBC correlations, and double-headed arrows: key NOESY correlations.
Table S7. NMR data of sestermobaraene E (10) in C$_6$D$_6$ recorded at 298 K.

| $^1$H[a] | $^{13}$C[b] | $^1$H[b] |
|---------|------------|---------|
| 1 CH$_2$ | 39.27      | 1.59 (m, 1H, H$_b$) |
|         |            | 1.52 (m, 1H, H$_a$) |
| 2 CH$_2$ | 30.66      | 2.17 (m, 1H) |
|         |            | 1.80 (m, 1H) |
| 3 C$_q$  | 150.12     | –       |
| 4 CH$_2$ | 37.64      | 2.10 (m, 1H, H$_b$) |
|         |            | 2.07 (m, 1H, H$_a$) |
| 5 CH$_2$ | 25.41      | 2.10 (m, 2H) |
| 6 CH     | 126.31     | 5.06 (m, 1H) |
| 7 C$_q$  | 132.97     | –       |
| 8 CH$_2$ | 39.48      | 2.07 (m, 1H, H$_a$) |
|         |            | 1.95 (m, 1H, H$_b$) |
| 9 CH$_2$ | 25.31      | 2.30 (m, 1H, H$_a$) |
|         |            | 1.96 (m, 1H, H$_b$) |
| 10 CH    | 131.57     | 5.09 (dd, $J$ = 10.9, 3.2, 1H) |
| 11 C$_q$ | 133.76     | –       |
| 12 CH    | 138.38     | 6.13 (d, $J$ = 15.7, 1H) |
| 13 CH    | 127.91     | 5.38 (dd, $J$ = 15.4, 9.8, 1H) |
| 14 CH    | 58.54      | 1.98 (m, 1H) |
| 15 C$_q$ | 46.25      | –       |
| 16 CH$_2$| 40.00      | 1.38 (m, 1H, H$_b$) |
|         |            | 1.34 (m, 1H, H$_a$) |
| 17 CH$_2$| 23.96      | 1.71 (m, 1H, H$_a$) |
|         |            | 1.38 (m, 1H, H$_b$) |
| 18 CH    | 49.06      | 1.85 (m, 1H) |
| 19 CH    | 30.59      | 1.70 (m, 1H) |
| 20 CH$_3$| 22.32      | 0.95 (d, $J$ = 6.9, 3H) |
| 21 CH$_3$| 18.44      | 0.89 (d, $J$ = 6.8, 3H) |
| 22 CH$_3$| 19.65      | 0.83 (s, 3H) |
| 23 CH$_3$| 12.45      | 1.62 (s, 3H) |
| 24 CH$_3$| 15.30      | 1.41 (s, 3H) |
| 25 CH$_2$| 108.09     | 4.93 (br s, 1H, H$_2$)[c] |
|         |            | 4.92 (br s, 1H, H$_E$)[c] |

[a] Carbon numbering as shown in Figure S39. [b] Chemical shifts $\delta$ in ppm, multiplicity: s = singlet, d = doublet, br = broad, m = multiplet, coupling constants $J$ are given in Hertz. [c] Assignment according to CIP priority rules.
Figure S40. $^1$H-NMR spectrum (700 MHz, C$_6$D$_6$) of 10.
Figure S41. $^{13}$C-NMR spectrum (176 MHz, C$_6$D$_6$) of 10.
Figure S42. $^{13}$C-DEPT135 spectrum (176 MHz, C$_6$D$_6$) of 10.
Figure S43. $^1$H-$^1$H-COSY spectrum ($\text{C}_6\text{D}_6$) of 10.
Figure S44. HSQC spectrum (C$_6$D$_6$) of 10.
Figure S45. HMBC spectrum (C₆D₆) of 10.
Figure S46. NOESY spectrum (C₆D₆) of 10.
Figure S47. Structure elucidation of 11. Bold: $^1$H,$^1$H-COSY correlations, single-headed arrows: key HMBC correlations, and double-headed arrows: key NOESY correlations.
Table S8. NMR data of sestermobaraene F (11) in C₆D₆ recorded at 298 K.

|   | C₁   | ¹³C[b] | ¹¹H[b]                      |
|---|------|--------|-----------------------------|
| 1 | CH₂  | 47.20  | 1.67 (m, 1H, Hₐ)            |
|   |      |        | 1.30 (m, 1H, Hₙ)            |
| 2 | CH   | 45.99  | 2.71 (td, J = 12.2, 3.7, 1H) |
| 3 | Cq   | 153.51 | –                           |
| 4 | CH₂  | 31.30  | 2.38 (m, 2H)                |
| 5 | CH₂  | 33.74  | 1.99 (m, 1H, Hₐ)            |
|   |      |        | 1.92 (m, 1H, Hₙ)            |
| 6 | Cq   | 39.05  | –                           |
| 7 | CH   | 45.42  | 2.20 (m, 1H)                |
| 8 | CH₂  | 35.51  | 1.86 (m, 1H, Hₐ)            |
|   |      |        | 1.14 (m, 1H, Hₙ)            |
| 9 | CH₂  | 25.92  | 1.96 (m, 1H, Hₐ)            |
|   |      |        | 1.72 (m, 1H, Hₙ)            |
|10 | CH   | 37.64  | 0.62 (d, J = 5.7, 1H)       |
|11 | Cq   | 30.64  | –                           |
|12 | CH   | 50.82  | 0.98 (m, 1H)                |
|13 | CH₂  | 27.21  | 1.61 (m, 1H, Hₐ)            |
|   |      |        | 1.25 (m, 1H, Hₙ)            |
|14 | CH   | 51.90  | 1.04 (m, 1H)                |
|15 | Cq   | 42.62  | –                           |
|16 | CH₂  | 39.43  | 1.43 (m, 1H, Hₐ)            |
|   |      |        | 1.10 (m, 1H, Hₙ)            |
|17 | CH₂  | 23.68  | 1.68 (m, 1H, Hₐ)            |
|   |      |        | 1.36 (m, 1H, Hₙ)            |
|18 | CH   | 46.29  | 1.55 (m, 1H)                |
|19 | CH   | 29.52  | 1.69 (m, 1H)                |
|20 | CH₃  | 22.43  | 0.95 (d, J = 6.9, 3H)       |
|21 | CH₃  | 17.97  | 0.83 (d, J = 6.8, 3H)       |
|22 | CH₃  | 19.25  | 0.85 (s, 3H)                |
|23 | CH₃  | 11.55  | 1.11 (s, 3H)                |
|24 | CH₃  | 16.53  | 1.14 (d, J = 7.1, 3H)       |
|25 | CH₂  | 112.68 | 4.91 (d, J = 2.6, 1H, H₂)   |
|   |      |        | 4.82 (m, 1H, H₂)            |

[a] Carbon numbering as shown in Figure S47. [b] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, coupling constants J are given in Hertz. [c] Assignment according to CIP priority rules.
Figure S48. $^1$H-NMR spectrum (700 MHz, C$_6$D$_6$) of 11.
Figure S49. $^{13}$C-NMR spectrum (176 MHz, $C_6D_6$) of 11.
Figure S50. $^{13}$C-DEPT135 spectrum (176 MHz, C$_6$D$_6$) of 11.
Figure S51. $^1\text{H},^1\text{H}$-COSY spectrum (C$_6$D$_6$) of 11.
Figure S52. HSQC spectrum (C6D6) of 11.
Figure S53. HMBC spectrum (C\textsubscript{6}D\textsubscript{6}) of 11.
Figure S54. NOESY spectrum (C$_6$D$_6$) of 11.
Figure S55 Structure elucidation of 12. Bold: $^1$H,$^1$H-COSY correlations, single-headed arrows: key HMBC correlations, and double-headed arrows: key NOESY correlations.
### Table S9. NMR data of sestermobaraol (12) in C₆D₆ recorded at 298 K.

|   | \( ^{13}\text{C} \) [a] | \( ^{1}\text{H} \) [b] |
|---|---|---|
| 1 | CH₂ | 45.68 | 1.71 (dd, \( J = 15.7, 9.9, 1\text{H}, H_\beta \)) |
|   |   |   | 1.21 (d, \( J = 15.6, 1\text{H}, H_\alpha \)) |
| 2 | CH | 34.58 | 2.02 (dq, \( J = 10.0, 6.8, 1\text{H} \)) |
| 3 | C_q | 79.59 | – |
| 4 | CH₂ | 35.57 | 1.77 (m, 1H, \( H_\alpha \)) |
|   |   |   | 1.40 (m, 1H, \( H_\beta \)) |
| 5 | CH₂ | 17.42 | 1.43 (m, 1H, \( H_\alpha \)) |
|   |   |   | 1.09 (m, 1H, \( H_\beta \)) |
| 6 | CH | 49.05 | 1.51 (m, 1H) |
| 7 | C_q | 49.33 | – |
| 8 | CH₂ | 29.89 | 1.62 (m, 1H, \( H_\alpha \)) [c] |
|   |   |   | 1.06 (m, 1H, \( H_\alpha \)) [c] |
| 9 | CH₂ | 22.28 | 1.54 (m, 1H, \( H_\alpha \)) [c] |
|   |   |   | 1.33 (m, 1H, \( H_\alpha \)) [c] |
| 10 | CH | 46.94 | 1.95 (dd, \( J = 4.8, 1.8, 1\text{H} \)) |
| 11 | C_q | 48.56 | – |
| 12 | CH | 41.24 | 1.51 (m, 1H) |
| 13 | CH₂ | 26.13 | 1.64 (m, 1H, \( H_\alpha \)) |
|   |   |   | 0.94 (m, 1H, \( H_\beta \)) |
| 14 | CH | 46.28 | 1.63 (m, 1H) |
| 15 | C_q | 45.35 | – |
| 16 | CH₂ | 45.81 | 1.30 (m, 2H) |
| 17 | CH₂ | 21.29 | 1.55 (m, 1H, \( H_\alpha \)) |
|   |   |   | 1.30 (m, 1H, \( H_\beta \)) |
| 18 | CH | 53.62 | 1.65 (m, 1H) |
| 19 | CH | 28.93 | 1.76 (m, 1H) |
| 20 | CH₃ | 22.55 | 0.93 (d, \( J = 6.9, 3\text{H} \)) |
| 21 | CH₃ | 16.41 | 0.83 (d, \( J = 6.8, 3\text{H} \)) |
| 22 | CH₃ | 17.84 | 0.87 (s, 3H) |
| 23 | CH₃ | 17.84 | 0.89 (s, 3H) |
| 24 | CH₃ | 17.92 | 1.12 (s, 3H) |
| 25 | CH₃ | 21.87 | 0.97 (d, \( J = 6.7, 3\text{H} \)) |

[a] Carbon numbering as shown in Figure S55. [b] Chemical shifts \( \delta \) in ppm, multiplicity: s = singlet, d = doublet, q = quartet, m = multiplet, coupling constants \( J \) are given in Hertz. [c] For assignment of \( H_\alpha \) and \( H_\beta \) cf. Figure S107.
Figure S56. $^1$H-NMR spectrum (700 MHz, C$_6$D$_6$) of 12.
Figure S57. $^{13}$C-NMR spectrum (176 MHz, $C_6D_6$) of 12 (asterisk indicates an impurity from commercial $C_6D_6$).
Figure S58. $^{13}$C-DEPT135 spectrum (176 MHz, C$_6$D$_6$) of 12.
Figure S59. $^{1}H,^{1}H$-COSY spectrum ($C_{6}D_{6}$) of 12.
Figure S60. HSQC spectrum (C₆D₆) of 12.
Figure S61. HMBC spectrum (C₆D₆) of 12.
Figure S62. NOESY spectrum (C\textsubscript{6}D\textsubscript{6}) of 12.
Incubation experiments with labeled substrates
Isotopic labeling experiments were performed with the precursors of GFPP (ca. 1.5 mg, in 1 mL 25 mM NH₄HCO₃), incubation buffer (5 mL), enzyme elution fractions, and the substrates and enzyme preparations as listed in Table S10. After incubation at 28 °C overnight, the products were extracted twice with C₆D₆ (600 μL and 300 μL), the extracts were dried with MgSO₄ and analyzed by NMR and/or GC/MS.

Table S10. Labeling experiments with SmTS1.

| entry | substrates | enzymes | results shown in |
|-------|------------|---------|-----------------|
| 1     | GGPP + (1-13)CIPP[10] | GFPPS, SmTS1 | Figure S63 |
| 2     | GGPP + (2-13)CIPP[20] | GFPPS, SmTS1 | Figure S64 |
| 3     | GGPP + (3-13)CIPP[10] | GFPPS, SmTS1 | Figure S65 |
| 4     | GGPP + (4-13)CIPP[10] | GFPPS, SmTS1 | Figure S66 |
| 5     | (1-13)CGGPP + IPP     | GFPPS, SmTS1 | Figure S67 |
| 6     | (2-13)CGGPP[10] + IPP | GFPPS, SmTS1 | Figure S68 |
| 7     | (3-13)CGGPP + IPP     | GFPPS, SmTS1 | Figure S69 |
| 8     | (4-13)CGGPP + IPP     | GFPPS, SmTS1 | Figure S70 |
| 9     | (1-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S71 |
| 10    | (2-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S72 |
| 11    | (3-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S73 |
| 12    | (4-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S74 |
| 13    | (5-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S75 |
| 14    | (6-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S76 |
| 15    | (7-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S77 |
| 16    | (8-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S78 |
| 17    | (9-13)CIPP[21] + IPP   | GFPPS, SmTS1 | Figure S79 |
| 18    | (10-13)CIPP[21] + IPP  | GFPPS, SmTS1 | Figure S80 |
| 19    | (11-13)CIPP[21] + IPP  | GFPPS, SmTS1 | Figure S81 |
| 20    | (12-13)CIPP[21] + IPP  | GFPPS, SmTS1 | Figure S82 |
| 21    | (13-13)GPP[22] + IPP   | GFPPS, SmTS1 | Figure S83 |
| 22    | (14-13)CIPP[21] + IPP  | GFPPS, SmTS1 | Figure S84 |
| 23    | (15-13)CIPP[21] + IPP  | GFPPS, SmTS1 | Figure S85 |
| 24    | (20-13)CIPP[21] + IPP  | GFPPS, SmTS1 | Figure S86 |
| 25    | GGPP + (5-13)CIPP (Scheme S6) | GFPPS, SmTS1 | Figure S87 |
| 26    | (7-13)CIPP[23] + (E)-(4-13)C,4-2H)IPP[24] | GFPPS, SmTS1 | Figures S88 – S94 |
| 27    | (7-13)CIPP[23] + (Z)-(4-13)C,4-2H)IPP[24] | GFPPS, SmTS1 | Figures S88 – S94 |
| 28    | (R)-(1-13)C,1-2H)GPP[10] + (2-13)CIPP[20] | GFPPS, SmTS1 | Figures S95, S96 |
| 29    | (S)-(1-13)C,1-2H)GPP[10] + (2-13)CIPP[20] | GFPPS, SmTS1 | Figures S95, S96 |
| 30    | (3-13)C,2-2H)GPP[10] + IPP | GFPPS, SmTS1 | Figure S97 |
| 31    | GPP + (Z)-(4-2H)IPP[25] + (2-13)CIPP[20] | FPPS,[26] GFPPS, SmTS1 | Figure S98 |
| 32    | GPP + (3-13)C,4-2H)IPP (Scheme S7) | GFPPS, SmTS1 | Figures S99, S100 |
| 33    | GPP + (R)-(1-13)C,1-2H)IPP[2] | GFPPS, SmTS1 | Figures S101 – S107 |
| 34    | GPP + (S)-(1-13)C,1-2H)IPP[2] | GFPPS, SmTS1 | Figures S101 – S107 |
| 35    | GPP + (E)-(4-13)C,4-2H)IPP[24] | GFPPS, SmTS1 | Figures S101 – S107 |
| 36    | GPP + (Z)-(4-13)C,4-2H)IPP[24] | GFPPS, SmTS1 | Figures S101 – S107 |
Figure S63. Enzymatic conversion of (1-\(^{13}\)C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \(^{13}\)C-NMR spectra. Figures A) – G) show the \(^{13}\)C-NMR spectra of unlabeled 6 – 12, H) shows the \(^{13}\)C-NMR spectrum of the enzyme products from (1-\(^{13}\)C)GFPP.
Figure S64. Enzymatic conversion of (2-\(^{13}\)C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \(^{13}\)C-NMR spectra. Figures A) – G) show the \(^{13}\)C-NMR spectra of unlabeled 6 – 12, H) shows the \(^{13}\)C-NMR spectrum of the enzyme products from (2-\(^{13}\)C)GFPP.
Figure S65. Enzymatic conversion of (3-\textsuperscript{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \textsuperscript{13}C-NMR spectra. Figures A) – G) show the \textsuperscript{13}C-NMR spectra of unlabeled 6 – 12, H) shows the \textsuperscript{13}C-NMR spectrum of the enzyme products from (3-\textsuperscript{13}C)GFPP.
Figure S66. Enzymatic conversion of (4-^{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the $^{13}$C-NMR spectra. Figures A) – G) show the $^{13}$C-NMR spectra of unlabeled 6 – 12, H) shows the $^{13}$C-NMR spectrum of the enzyme products from (4-^{13}C)GFPP.
Figure S67. Enzymatic conversion of (5-13C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the 13C-NMR spectra. Figures A) – G) show the 13C-NMR spectra of unlabeled 6 – 12, H) shows the 13C-NMR spectrum of the enzyme products from (5-13C)GFPP.
Figure S68. Enzymatic conversion of (6-$^{13}$C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the $^{13}$C-NMR spectra. Figures A) – G) show the $^{13}$C-NMR spectra of unlabeled 6 – 12, H) shows the $^{13}$C-NMR spectrum of the enzyme products from (6-$^{13}$C)GFPP.
Figure S69. Enzymatic conversion of (7-13C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the 13C-NMR spectra. Figures A) – G) show the 13C-NMR spectra of unlabeled 6 – 12, H) shows the 13C-NMR spectrum of the enzyme products from (7-13C)GFPP.
Figure S70. Enzymatic conversion of (8-^{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the $^{13}$C-NMR spectra. Figures A) – G) show the $^{13}$C-NMR spectra of unlabeled 6 – 12, H) shows the $^{13}$C-NMR spectrum of the enzyme products from (8-^{13}C)GFPP.
Figure S71. Enzymatic conversion of (9-\textsuperscript{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \textsuperscript{13}C-NMR spectra. Figures A) – G) show the \textsuperscript{13}C-NMR spectra of unlabeled 6 – 12, H) shows the \textsuperscript{13}C-NMR spectrum of the enzyme products from (9-\textsuperscript{13}C)GFPP.
Figure S72. Enzymatic conversion of (10-^{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the $^{13}$C-NMR spectra. Figures A) – G) show the $^{13}$C-NMR spectra of unlabeled 6 – 12, H) shows the $^{13}$C-NMR spectrum of the enzyme products from (10-^{13}C)GFPP.
Figure S73. Enzymatic conversion of (11-13C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the 13C-NMR spectra. Figures A) – G) show the 13C-NMR spectra of unlabeled 6 – 12, H) shows the 13C-NMR spectrum of the enzyme products from (11-13C)GFPP.
Figure S74. Enzymatic conversion of (12-\textsuperscript{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \textsuperscript{13}C-NMR spectra. Figures A) – G) show the \textsuperscript{13}C-NMR spectra of unlabelled 6 – 12, H) shows the \textsuperscript{13}C-NMR spectrum of the enzyme products from (12-\textsuperscript{13}C)GFPP.
Figure S75. Enzymatic conversion of (13-13C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the 13C-NMR spectra. Figures A) – G) show the 13C-NMR spectra of unlabeled 6 – 12, H) shows the 13C-NMR spectrum of the enzyme products from (13-13C)GFPP.
Figure S76. Enzymatic conversion of (14-13C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the 13C-NMR spectra. Figures A) – G) show the 13C-NMR spectra of unlabeled 6 – 12, H) shows the 13C-NMR spectrum of the enzyme products from (14-13C)GFPP.
Figure S77. Enzymatic conversion of (15-13C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the $^{13}$C-NMR spectra. Figures A) – G) show the $^{13}$C-NMR spectra of unlabeled 6 – 12, H) shows the $^{13}$C-NMR spectrum of the enzyme products from (15-13C)GFPP.
Figure S78. Enzymatic conversion of (16-$^{13}$C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the $^{13}$C-NMR spectra. Figures A) – G) show the $^{13}$C-NMR spectra of unlabeled 6 – 12, H) shows the $^{13}$C-NMR spectrum of the enzyme products from (16-$^{13}$C)GFPP.
Figure S79. Enzymatic conversion of (17-\textsuperscript{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \textsuperscript{13}C-NMR spectra. Figures A) – G) show the \textsuperscript{13}C-NMR spectra of unlabeled 6 – 12, H) shows the \textsuperscript{13}C-NMR spectrum of the enzyme products from (17-\textsuperscript{13}C)GFPP.
Figure S80. Enzymatic conversion of (18-\textsuperscript{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \textsuperscript{13}C-NMR spectra. Figures A) – G) show the \textsuperscript{13}C-NMR spectra of unlabeled 6 – 12, H) shows the \textsuperscript{13}C-NMR spectrum of the enzyme products from (18-\textsuperscript{13}C)GFPP.
Figure S81. Enzymatic conversion of (19-\(^{13}\)C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \(^{13}\)C-NMR spectra. Figures A) – G) show the \(^{13}\)C-NMR spectra of unlabeled 6 – 12, H) shows the \(^{13}\)C-NMR spectrum of the enzyme products from (19-\(^{13}\)C)GFPP.
Figure S82. Enzymatic conversion of (20-13C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the 13C-NMR spectra. Figures A) – G) show the 13C-NMR spectra of unlabeled 6 – 12, H) shows the 13C-NMR spectrum of the enzyme products from (20-13C)GFPP.
**Figure S83.** Enzymatic conversion of (21-\(^{13}\)C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \(^{13}\)C-NMR spectra. Figures A) – G) show the \(^{13}\)C-NMR spectra of unlabeled 6 – 12, H) shows the \(^{13}\)C-NMR spectrum of the enzyme products from (21-\(^{13}\)C)GFPP.
Figure S84. Enzymatic conversion of (22-^{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the ^{13}C-NMR spectra. Figures A) – G) show the ^{13}C-NMR spectra of unlabeled 6 – 12, H) shows the ^{13}C-NMR spectrum of the enzyme products from (22-^{13}C)GFPP.
Figure S85. Enzymatic conversion of (23-\textsuperscript{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \textsuperscript{13}C-NMR spectra. Figures A) – G) show the \textsuperscript{13}C-NMR spectra of unlabeled 6 – 12, H) shows the \textsuperscript{13}C-NMR spectrum of the enzyme products from (23-\textsuperscript{13}C)GFPP.
Figure S86. Enzymatic conversion of (24-\textsuperscript{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the \textsuperscript{13}C-NMR spectra. Figures A) – G) show the \textsuperscript{13}C-NMR spectra of unlabeled 6 – 12, H) shows the \textsuperscript{13}C-NMR spectrum of the enzyme products from (24-\textsuperscript{13}C)GFPP.
Figure S87. Enzymatic conversion of (25-^{13}C)GFPP with SmTS1. Coloured dots indicate labeled carbons and the corresponding peaks in the $^{13}$C-NMR spectra. Figures A) – G) show the $^{13}$C-NMR spectra of unlabeled 6 – 12, H) shows the $^{13}$C-NMR spectrum of the enzyme products from (25-^{13}C)GFPP.
Scheme S1. Biosynthesis of labeled 6 – 12 from (7-\(^{13}\)C)GPP and (E)- or (Z)-(4-\(^{13}\)C,4-\(^{2}\)H)IPP with GFPPS and SmTS1.
Figure S88. 1,5-Hydride shift from A to B in the formation of compound 6. A) $^{13}$C-NMR signal for C19 of unlabeled 6. B) $^{13}$C-NMR signal for deuterated C19 of labeled 6 obtained from (7-$^{13}$C)GPP with (E)-(4-$^{13}$C,4-$^2$H)IPP. C) $^{13}$C-NMR signal for non-deuterated C19 of labeled 6 obtained from (7-$^{13}$C)GPP with (Z)-(4-$^{13}$C,4-$^2$H)IPP. The slightly upfield shifted triplet in B) is indicative for a direct $^{13}$C-$^2$H bond and supports the proposed 1,5-hydride shift.
Figure S89. 1,5-Hydride shift from A to B in the formation of compound 7. A) $^{13}$C-NMR signal for C19 of unlabeled 7, B) $^{13}$C-NMR signal for deuterated C19 of labeled 7 obtained from (7-$^{13}$C)GPP with (E)-(4-$^{13}$C,4-$^{2}$H)IPP, C) $^{13}$C-NMR signal for non-deuterated C19 of labeled 7 obtained from (7-$^{13}$C)GPP with (Z)-(4-$^{13}$C,4-$^{2}$H)IPP. The slightly upfield shifted triplet in B) is indicative for a direct $^{13}$C-$^{2}$H bond and supports the proposed 1,5-hydride shift.
Figure S90. 1,5-Hydride shifts from A to B in the formation of compound 8, A) $^{13}$C-NMR signal for C19 of unlabeled 8, B) $^{13}$C-NMR signal for deuterated C19 of labeled 8 obtained from $(7-^{13}$C)GPP with $(E)$-$(4-^{13}$C,4-2$^2$H)IPP, C) $^{13}$C-NMR signal for non-deuterated C19 of labeled 8 obtained from $(7-^{13}$C)GPP with $(Z)$-$(4-^{13}$C,4-2$^2$H)IPP. The slightly upfield shifted triplet in B) is indicative for a direct $^{13}$C-2$^2$H bond and supports the proposed 1,5-hydride shift.
Figure S91. 1,5-Hydride shift from A to B in the formation of compound 9, A) $^{13}$C-NMR signal for C19 of unlabeled 9, B) $^{13}$C-NMR spectrum for deuterated C19 of labeled 9 obtained from (7-$^{13}$C)GPP with (E)-(4-$^{13}$C,4-2H)IPP, C) $^{13}$C-NMR signal for non-deuterated C19 of labeled 9 obtained from (7-$^{13}$C)GPP with (Z)-(4-$^{13}$C,4-2H)IPP. As compound 9 was a minor product, the expected triplet signal in B) could not be observed. However, the presence of a signal for C19 in C) together with the absence in B) indicates deuteration of C19 for H$_E$ = $^2$H and thus supports the 1,5-hydride shift.
Figure S92. 1,5-Hydride shifts from A to B in the formation of compound 10. A) $^{13}$C-NMR signal for C19 of unlabeled 10, B) $^{13}$C-NMR spectrum for deuterated C19 of labeled 10 obtained from (7-$^{13}$C)GPP with ($E$)-(4-$^{13}$C,4-$^2$H)IPP, C) $^{13}$C-NMR signal for non-deuterated C19 of labeled 10 obtained from (7-$^{13}$C)GPP with ($Z$)-(4-$^{13}$C,4-$^2$H)IPP. As compound 10 was a minor product, the expected triplet signal in B) could not be observed. However, the presence of a signal for C19 in C) together with the absence in B) indicates deuteration of C19 for $H_E = ^2$H and thus supports the 1,5-hydride shift.
Figure S93. 1,5-Hydride shift from A to B in the formation of compound 11, A) $^{13}$C-NMR signal for C19 of unlabeled 11, B) $^{13}$C-NMR spectrum for deuterated C19 of labeled 11 obtained from (7-$^{13}$C)GPP with (E)-(4-$^{13}$C,4-$^{2}$H)IPP, C) $^{13}$C-NMR signal for non-deuterated C19 of labeled 11 obtained from (7-$^{13}$C)GPP with (Z)-(4-$^{13}$C,4-$^{2}$H)IPP. As compound 11 was a minor product, the expected triplet signal in B) could not be observed (the triplet that is visible at 29.1 ppm has a smaller $\Delta\delta = -0.40$ than expected (ca. $\Delta\delta = -0.5$) and originates from another compound in the sample). However, the presence of a signal for C19 in C) together with the absence in B) indicates deuteration of C19 for $H_E = ^2$H and thus supports the 1,5-hydride shift.
Figure S94. 1,5-Hydride shift from A to B in the formation of compound 12, A) $^{13}$C-NMR signal for C19 of unlabeled 12, B) $^{13}$C-NMR spectrum for deuterated C19 of labeled 12 obtained from (7-$^{13}$C)GPP with (E)-(4-$^{13}$C,4-$^2$H)IPP, C) $^{13}$C-NMR signal for non-deuterated C19 of labeled 12 obtained from (7-$^{13}$C)GPP with (Z)-(4-$^{13}$C,4-$^2$H)IPP. As compound 12 was a minor product, the expected triplet signal in B) could not be observed. However, the presence of a signal for C19 in C) together with the absence in B) indicates deuteration of C19 for $H_E=^2$H and thus supports the 1,5-hydride shift.
Scheme S2. Biosynthesis of labeled 6 – 12 from (R)- or (S)-(1-\(^{13}\)C,1-\(^2\)H)GPP and (2-\(^{13}\)C)IPP with GFPPS and SmTS1.
Figure S95. 1,5-Hydride shift from B to C in the formation of compound 10. A) $^{13}$C NMR signal for C2 of unlabeled 10, B) $^{13}$C NMR signal for non-deuterated C2 of labeled 10 obtained from (S)-(1-$^{13}$C,1-$^2$H)GPP with (2-$^{13}$C)IPP, C) the $^{13}$C NMR signal for deuterated C2 of labeled 10 obtained from (R)-(1-$^{13}$C,1-$^2$H)GPP with (2-$^{13}$C)IPP could not be observed, D) total ion chromatogram of the products obtained from (S)-(1-$^{13}$C,1-$^2$H)GPP and (2-$^{13}$C)IPP, E) from (R)-(1-$^{13}$C,1-$^2$H)GPP and (2-$^{13}$C)IPP (peak for 10 is marked by arrow), F) HSQC signals for C2 of unlabeled 10, labeled 10 ($H_S$=2H) and labeled 10 ($H_R$=2H, one crosspeak missing, indicating deuteration at C2), G) HSQC signals for C13 of unlabeled 10, labeled 10 ($H_S$=2H, crosspeak missing, indicating deuteration at C13) and labeled 10 ($H_R$=2H).
Figure S96. The specific loss of the 1-pro-S proton of GPP in the final deprotonation step to compound 6. A) Mass spectrum of compound 6 obtained from \((R)-(1^{13}\text{C},1^{2}\text{H})\text{GPP and (2}^{13}\text{C})\text{IPP}, B) mass spectrum of compound 6 obtained from \((S)-(1^{13}\text{C},1^{2}\text{H})\text{GPP and (2}^{13}\text{C})\text{IPP, C) }^{13}\text{C NMR spectrum for C13 of unlabeled 6, D) }^{13}\text{C} \text{NMR spectrum for deuterated C13 of labeled 6 obtained from (R)-(1}^{13}\text{C},1^{2}\text{H})\text{GPP and (2}^{13}\text{C})\text{IPP.}
Scheme S3. Biosynthesis of 6 – 12 from (3-$^{13}$C,2-$^2$H)GGPP and IPP with GFPPS and SmTS1.
Figure S97. The 1,2-hydride shift from D to E in the formation of compound 11. A) $^{13}$C NMR spectrum for C7 of unlabeled 11, B) $^{13}$C NMR spectrum for deuterated C7 of 11 obtained from (3-$^{13}$C,2-$^{2}$H)GGPP and IPP.
Scheme S4a. Biosynthesis of 6 – 12 from GPP, \((Z)-(4,2H)IPP\) and \((2,13C)IPP\) with FPPS, GFPPS and SmTS1, showing the formation of the target isotopomer of 6.
Scheme S4b. Biosynthesis of 6 – 12 from GPP, (Z)-(4-²H)IPP and (2-¹³C)IPP with FPPS, GFPPS and SmTS1. First, the incubation of GPP and (Z)-(4-²H)IPP with FPPS was performed, followed by addition of (2-¹³C)IPP, GFPPS and SmTS1. In side reactions, after the first step unreacted GPP can be elongated with the later added (2-¹³C)IPP to form other (e. g. as shown here non-deuterated) isotopomers of 6. Further isotopomers are possible by statistical incorporation of (Z)-(4-²H)IPP and (2-¹³C)IPP.
Figure S98. The 1,4-hydride shift from H to J in the formation of compound 6. A) $^{13}$C NMR spectrum for C2 of unlabeled 6, B) $^{13}$C NMR spectrum of labeled 6 obtained from GPP, (Z)-(4-$^2$H)IPP and (2-$^{13}$C)IPP. The upfield shifted triplet for C2 of 6a supports the 1,4-hydride shift. Additional signals for C2 of other isotopomers such as 6b are also observed.
Scheme S5. Biosynthesis of 6 – 12 from GPP and (3-13C,4-2H2)IPP with GFPPS and SmTS1.
Figure S99. The 1,2-hydride shift from G to L in the formation of compound 8. \(^{13}\text{C}\) NMR spectra showing the signals for C11 of A) unlabeled 8 and B) labeled 8, for C3 of C) unlabeled 8 and D) labeled 8, and for C7 of E) unlabeled 8 and F) labeled 8. Labeled 8 was obtained from GPP and \((3-{^{13}\text{C}},4-{^2\text{H}}_2)\)IPP. The upfield shifted triplet in B) supports the 1,2-hydride shift. The upfield shifts in D) and F) are a result of double deuteration at the neighboring carbon.
Figure S100. The 1,2-hydride shift from G to L in the formation of compound 7. $^{13}$C NMR spectra showing the signal for C3 of A) unlabeled 7 and B) labeled 7, for C7 of C) unlabeled 7 and D) labeled 7, and for C11 of E) unlabeled 7 and F) labeled 7. Labeled 7 was obtained from GPP and (3-$^{13}$C,4-$^{2}$H$_{2}$)IPP. The upfield shifted triplet in B) supports the 1,2-hydride shift. The $\Delta\delta = -0.68$ is a result of one directly bound deuterium at C3 and two deuterium atoms at the neighboring carbon C4.
Figure S101. Determination of the absolute configuration of 6. A) Overlaid HSQC spectra from two labeling experiments using GPP with (S)- or (R)-(1-13C, 1-2H)IPP. The experiment with (S)-(1-13C, 1-2H)IPP resulted in vanished crosspeaks for H1_S, H5_S and H9_S, but crosspeaks for H1_R, H5_R and H9_R were detected (blue), while in the experiment with (R)-(1-13C, 1-2H)IPP the red crosspeaks were observed. B) Overlaid HSQC spectra from two labeling experiments using GPP with (Z)- or (E)-(4-13C, 4-2H)IPP. The experiment with (Z)-(4-13C, 4-2H)IPP resulted in vanished crosspeaks for H4_Z and H8_Z, but crosspeaks for H4_E and H8_E were detected (blue), while in the experiment with (E)-(4-13C, 4-2H)IPP the red crosspeaks were observed. Figures C) and D) show the GFPP isotopomers with the stereogenic anchors at the labeled carbons of known absolute configuration and their conversion into labeled 6, which together with the NOESY based assignments of diastereotopic hydrogens at C1, C4, C5, C8 and C9 indicates the absolute configuration of 6 as shown.
Figure S102. Determination of the absolute configuration of 7. A) Overlaid HSQC spectra from two labeling experiments using GPP with (S)- or (R)-(1-13C,1-2H)IPP. The experiment with (S)-(1-13C,1-2H)IPP resulted in vanished crosspeaks for H1_S, H5_S and H9_S, but crosspeaks for H1_R, H5_R and H9_R were detected (blue), while in the experiment with (R)-(1-13C,1-2H)IPP the red crosspeaks were observed. B) Overlaid HSQC spectra from two labeling experiments using GPP with (Z)- or (E)-(4-13C,4-2H)IPP. The experiment with (Z)-(4-13C,4-2H)IPP resulted in vanished crosspeaks for H4_Z and H8_Z, but crosspeaks for H4_E and H8_E were detected (blue), while in the experiment with (E)-(4-13C,4-2H)IPP the red crosspeaks were observed. Figures C) and D) show the GFPP isotopomers with the stereogenic anchors at the labeled carbons of known absolute configuration and their conversion into labeled 7, which together with the NOESY based assignments of diastereotopic hydrogens at C1, C4, C5, C8 and C9 indicates the absolute configuration of 7 as shown.
Figure S103. Determination of the absolute configuration of 8. A) Overlaid HSQC spectra from two labeling experiments using GPP with (S)- or (R)-\((1^{13}C,1^{2}H)\)IPP. The experiment with (S)-\((1^{13}C,1^{2}H)\)IPP resulted in vanished crosspeaks for H1_S, H5_S and H9_S, but crosspeaks for H1_R, H5_R and H9_R were detected (blue), while in the experiment with (R)-\((1^{13}C,1^{2}H)\)IPP the red crosspeaks were observed. B) Overlaid HSQC spectra from two labeling experiments using GPP with (Z)- or (E)-\((4^{13}C,4^{2}H)\)IPP. The experiment with (Z)-\((4^{13}C,4^{2}H)\)IPP resulted in vanished crosspeaks for H4_Z and H8_Z, but crosspeaks for H4_E and H8_E were detected (blue), while in the experiment with (E)-\((4^{13}C,4^{2}H)\)IPP the red crosspeaks were observed. Figures C) and D) show the GFPP isotopomers with the stereogenic anchors at the labeled carbons of known absolute configuration and their conversion into labeled 8, which together with the NOESY based assignments of diastereotopic hydrogens at C1, C4, C5, C8 and C9 indicates the absolute configuration of 8 as shown.
Figure S104. Determination of the absolute configuration of 9. A) Overlaid HSQC spectra from two labeling experiments using GPP with (S)- or (R)-{(1,13C,1,2H)}IPP. The experiment with (S)-{(1,13C,1,2H)}IPP resulted in vanished crosspeaks for H1S, H5S and H9S, but crosspeaks for H1R and H5R were detected (blue, signal for H9R was covered), while in the experiment with (R)-{(1,13C,1,2H)}IPP the red crosspeaks were observed. B) Overlaid HSQC spectra from two labeling experiments using GPP with (Z)- or (E){(4,13C,4,2H)}IPP. The experiment with (Z){(4,13C,4,2H)}IPP resulted in vanished crosspeaks for H4Z and H8Z, but the crosspeaks for H4E was detected (blue, H8E was weak), while in the experiment with (E){(4,13C,4,2H)}IPP the red crosspeaks were observed. Figures C) and D) show the GFPP isotopomers with the stereogenic anchors at the labeled carbons of known absolute configuration and their conversion into labeled 8, which together with the NOESY based assignments of diastereotopic hydrogens at C1, C4, C5, C8 and C9 indicates the absolute configuration of 8 as shown.
Figure S105. Determination of the absolute configuration of 10. A) Overlaid HSQC spectra from two labeling experiments using GPP with (S)- or (R)-(1-13C,1-2H)IPP. The experiment with (S)-(1-13C,1-2H)IPP resulted in vanished crosspeaks for H1S, H5S and H9S, but crosspeaks for H1R, H5R and H9R were detected (blue), while in the experiment with (R)-(1-13C,1-2H)IPP the red crosspeaks were observed. B) Overlaid HSQC spectra from two labeling experiments using GPP with (Z)- or (E)-(4-13C,4-2H)IPP. The experiment with (Z)-(4-13C,4-2H)IPP resulted in vanished crosspeaks for H4Z and H8Z, but crosspeaks for H4E and H8E were detected (blue), while in the experiment with (E)-(4-13C,4-2H)IPP the red crosspeaks were observed. Figures C) and D) show the GFPP isotopomers with the stereogenic anchors at the labeled carbons of known absolute configuration and their conversion into labeled 10, which together with the NOESY based assignments of diastereotopic hydrogens at C1, C4, C5, C8 and C9 indicates the absolute configuration of 10 as shown.
Figure S106. Determination of the absolute configuration of 11. A) Overlaid HSQC spectra from two labeling experiments using GPP with (S)- or (R)-(1-13C,1-2H)IPP. The experiment with (S)-(1-13C,1-2H)IPP resulted in vanished crosspeaks for H1, H5 and H9, but crosspeaks for H1, H5 and H9 were detected (blue), while in the experiment with (R)-(1-13C,1-2H)IPP the red crosspeaks were observed. B) Overlaid HSQC spectra from two labeling experiments using GPP with (Z)- or (E)-(4-13C,4-2H)IPP. The experiment with (Z)-(4-13C,4-2H)IPP resulted in vanished crosspeaks for H4 and H8, but crosspeaks for H4 and H8 were detected (blue), while in the experiment with (E)-(4-13C,4-2H)IPP the red crosspeaks were observed. Figures C) and D) show the GFPP isotopomers with the stereogenic anchors at the labeled carbons of known absolute configuration and their conversion into labeled 11, which together with the NOESY based assignments of diastereotopic hydrogens at C1, C4, C5, C8 and C9 indicates the absolute configuration of 11 as shown.
Figure S107. Determination of the absolute configuration of 12. A) Overlaid HSQC spectra from two labeling experiments using GPP with (S)- or (R)-(1-13C,1-2H)IPP. The experiment with (S)-(1-13C,1-2H)IPP resulted in vanished crosspeaks for H1_S, H5_S and H9_S, but crosspeaks for H1_R, H5_R and H9_R were detected (blue), while in the experiment with (R)-(1-13C,1-2H)IPP the red crosspeaks were observed. B) Overlaid HSQC spectra from two labeling experiments using GPP with (Z)- or (E)-(4-13C,4-2H)IPP. The experiment with (Z)-(4-13C,4-2H)IPP resulted in vanished crosspeaks for H4_Z and H8_Z, but crosspeaks for H4_E and H8_E were detected (blue), while in the experiment with (E)-(4-13C,4-2H)IPP the red crosspeaks were observed. Figures C) and D) show the GFPP isotopomers with the stereogenic anchors at the labeled carbons of known absolute configuration and their conversion into labeled 12, which together with the NOESY based assignments of diastereotopic hydrogens at C1, C4, C5, C8 and C9 indicates the absolute configuration of 12 as shown.
The synthetic route towards (5-\textsuperscript{13}C)IPP.

**Scheme S6.** The synthetic route towards (5-\textsuperscript{13}C)IPP.

### Synthesis of (5-\textsuperscript{13}C)IPP

**3-((\textit{t}ert-\textit{B}utyldiphenylsilyl)\textit{o}xy)propanol (S1)**

To a suspension of NaH (60% in mineral oil, 1.0 g, 25.0 mmol, 1.0 eq) in THF (25 mL) was slowly added 1,3-propanediol (1.9 g, 25.0 mmol, in 12.5 mL THF) at 0 °C. The reaction mixture was stirred for 45 min at room temperature. Then TBDPSCI (6.87 g, 25 mmol, 1.0 eq, in 12.5 mL THF) was added dropwise, and the reaction mixture was stirred for 1.5 h at room temperature. The reaction was quenched by adding sat. NaHCO\textsubscript{3} (75 ml) at 0 °C, followed by extraction with Et\textsubscript{2}O (3 x 100 mL). The combined organic layers were dried with MgSO\textsubscript{4} and concentrated under reduced pressure. The product S1 (6.4 g, 20.4 mmol, 82%) was obtained via silica gel chromatography [cyclohexane/EA (4/1), \textit{Rf} = 0.40] as a colorless oil.

**3-((\textit{t}ert-\textit{B}utyldiphenylsilyl)\textit{o}xy)propanol S1.** \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta = 7.72 – 7.66\) (m, 4H, 4 x CH), \(7.47 – 7.38\) (m, 6H, 6 x CH), \(3.89 – 3.82\) (m, 4H, 2 x CH\textsubscript{2}), \(2.33\) (s, 1H, OH), \(1.82\) (p, \(\textit{J}_{H,H} = 5.7\) Hz, 2H, CH\textsubscript{2}), \(1.07\) (s, 9H, 3 x CH\textsubscript{3}). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}): \(\delta = 135.67\) (4 x CH), 133.38 (2 x C\textsubscript{q}), 129.90 (2 x CH), 127.88 (4 x CH), 63.37 (CH\textsubscript{2}), 62.04 (CH\textsubscript{2}), 34.41 (CH\textsubscript{2}), 26.97 (3 x CH\textsubscript{3}), 19.22 (C\textsubscript{q}). GC (HP-5MS): \(I = 2200\). MS (EI, 70 eV): \(m/z\) (%) = 257 (28), 239 (1), 229 (10), 211 (5), 199 (100), 179 (96), 167 (2), 149 (6), 139 (10), 121 (7), 117 (7), 105 (7), 91 (11), 77 (14), 57 (14), 45 (4).

**3-((\textit{t}ert-\textit{B}utyldiphenylsilyl)\textit{o}xy)propanal (S2)**

To a solution of IBX (6.72 g, 24.4 mmol, 1.2 eq) in DMSO (62.5 mL) was added S1 (6.4 g, 20.4 mmol, in 12.5 mL DMSO) dropwise at room temperature. The reaction mixture was stirred overnight. Et\textsubscript{2}O (100 mL) was added, followed by cooling to 0 °C and addition of sat. NaHCO\textsubscript{3} (200 mL). The organic layer was separated and the aqueous layer was extracted with Et\textsubscript{2}O (2 x 100 mL). The combined organic layers were dried with MgSO\textsubscript{4} and concentrated under reduced pressure. Purification via silica gel chromatography [cyclohexane/EA (10/1), \textit{Rf} = 0.46] afforded S2 (5.63 g, 18.0 mmol, 88%) as a colorless oil.

**3-((\textit{t}ert-\textit{B}utyldiphenylsilyl)\textit{o}xy)propanal S2.** \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta = 9.82\) (t, \(\textit{J}_{H,H} = 2.1\) Hz, 1H, CH), \(7.69 – 7.64\) (m, 4H, 4 x CH), \(7.45 – 7.38\) (m, 6H, 6 x CH), \(4.03\) (t, \(\textit{J}_{H,H} = 6.0\) Hz, 2H, CH\textsubscript{2}), \(2.61\) (td, \(\textit{J}_{H,H} = 6.0, 2.2\) Hz, 2H, CH\textsubscript{2}), \(1.05\) (s, 9H, 3 x CH\textsubscript{3}). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}): \(\delta = 202.03\) (CH), 135.68 (4 x CH), 133.38 (2 x C\textsubscript{q}), 129.95 (2 x CH), 127.90 (4 x CH), 58.44 (CH\textsubscript{2}), 46.52 (CH\textsubscript{2}), 26.89 (3 x CH\textsubscript{3}), 19.29 (C\textsubscript{q}). GC (HP-5MS): \(I = 2135\). MS (EI, 70 eV): \(m/z\) (%) = 255 (75), 225 (79), 211 (22), 199 (25), 183 (100), 177 (62), 147 (10), 135 (8), 117 (72), 105 (22), 100 (5), 91 (12), 77 (20), 57 (4), 45 (9).

**3-((\textit{t}ert-\textit{B}utyldiphenylsilyl)\textit{o}xy)butan-2-ol (S3)**

Magnesium turnings (0.38 g, 15.84 mmol, 1.1 eq) were covered with Et\textsubscript{2}O (5 mL) in an oven-dried flask and a small piece of iodine was added. \textsuperscript{13}CH\textsubscript{3}I (2.06 g, 14.42 mmol, 1.0 eq) was added dropwise at a rate to maintain the reaction. The reaction mixture was stirred for 1.5 h at...
room temperature. After cooling to 0 °C, S2 (4.5 g, 14.40 mmol, in 20 mL Et2O) was added, followed by stirring at room temperature for 4 h. The reaction was quenched by the addition of sat. NH4Cl (100 mL) and extracted with Et2O (3 x 100 mL). The combined organic layers were dried with MgSO4 and concentrated under reduced pressure. Through silica gel chromatography [cyclohexane/EA (4/1), Rf = 0.44], S3 (3.5 g, 10.65 mmol, 74%) was obtained as a colorless oil.

(1-13C)-4-((tert-Butyldiphenylsilyl)oxy)butan-2-ol S3. 1H NMR (400 MHz, CD2Cl2): δ = 7.78 – 7.73 (m, 4H, 4 x CH), 7.25 – 7.19 (m, 6H, 6 x CH), 4.02 – 3.88 (m, 1H, CH), 3.83 – 3.66 (m, 2H, CH2), 2.42 (t, Jw,H = 3.8 Hz, 3Jc,H = 3.8 Hz, 1H, OH), 1.67 – 1.35 (m, 2H, CH2), 1.12 (s, 9H, 3 x CH3), 1.08 (dd, Jw,H = 125.1 Hz, 4Jw,H = 6.2 Hz, 3H, CH3). 13C NMR (101 MHz, CD2Cl2): δ = 136.00 (4 x CH), 133.85 (2 x Cδ), 130.09 (2 x CH), 128.16 (4 x CH), 66.72 (d, 3Jc,C = 39.1 Hz, CH), 63.07 (d, 3Jc,C = 3.9 Hz, CH2), 41.22 (CH2), 27.07 (3 x CH3), 23.97 (13CH3), 19.35 (Cδ).

GC (HP-5MS): tR = 2199. MS (EI, 70 eV): m/z (%) = 272 (4), 254 (4), 229 (40), 211 (7), 199 (100), 181 (7), 167 (3), 151 (4), 139 (14), 121 (3), 105 (3), 91 (5), 77 (7), 56 (3), 45 (2).

(1-13C)-4-((tert-Butyldiphenylsilyl)oxy)butan-2-one (S4). Following the same procedure as for the preparation of S2, S3 (3.5 g, 10.65 mmol) was converted into S4 (2.8 g, 8.55 mmol, 80 %) as a colorless oil.

(1-13C)-4-((tert-Butyldiphenylsilyl)oxy)butan-2-one S4. TLC [cyclohexane/EA (10/1)]: Rf = 0.34. 1H NMR (400 MHz, CDCl3): δ = 7.68 – 7.64 (m, 4H, 4 x CH), 7.44 – 7.36 (m, 6H, 6 x CH), 3.94 (t, Jw,H = 6.3 Hz, 2H, CH2), 2.64 (t, 3Jc,H = 6.2 Hz, 2H, CH2), 2.19 (d, Jw,H = 127.2 Hz, 3H, CH3), 1.04 (s, 9H, 3 x CH3). 13C NMR (101 MHz, CDCl3): δ = 207.98 (d, 3Jc,C = 40.4 Hz, Cδ), 135.68 (4 x CH), 133.56 (2 x Cδ), 129.85 (2 x CH), 127.84 (4 x CH), 59.86 (CH2), 46.49 (d, 3Jc,C = 13.5 Hz, CH2), 30.85 (13CH3), 26.92 (3 x CH3), 19.29 (Cδ). GC (HP-5MS): tR = 2209. MS (EI, 70 eV): m/z (%) = 270 (90), 240 (73), 222 (6), 211 (10), 199 (80), 192 (100), 181 (20), 174 (16), 165 (20), 162 (23), 152 (5), 139 (25), 121 (13), 114 (11), 105 (13), 91 (8), 77 (27), 57 (3), 45 (11).

tert-Butyl((3-((13C)methyl)but-3-en-1-yl)oxy)diphenylsilane (S5). To a suspension of CH3PPh3 (6.93 g, 17.14 mmol, 2.0 eq) in THF (80 mL) was added n-BuLi (1.6 m in hexane, 10.71 mL, 17.14 mmol, 2.0 eq) dropwise at 0 °C. The reaction mixture was stirred at the same temperature for 1 h. After cooling the mixture to –78 °C, S4 (2.8 g, 8.55 mmol) was added dropwise, followed by stirring overnight without further cooling. The reaction was quenched by the addition of water and extracted with Et2O (3 x 100 mL). The combined organic layers were dried with MgSO4 and the solvent was removed under reduced pressure. The product S5 (1.78 g, 5.46 mmol, 64%) was obtained via silica gel chromatography [cyclohexane/EA (100/1); Rf = 0.37].

tert-Butyl((3-((13C)methyl)but-3-en-1-yl)oxy)diphenylsilane S5. 1H NMR (500 MHz, CDCl3): δ = 7.71 – 7.68 (m, 4H, 4 x CH), 7.45 – 7.35 (m, 6H, 6 x CH), 4.80 – 4.65 (m, 2H, CH2), 3.78 (t, Jw,H = 6.9 Hz, 2H, CH2), 2.34 – 2.25 (m, 2H, CH2), 1.70 (d, 3Jc,H = 125.7 Hz, 3H, CH3), 1.07 (s, 9H, 3 x CH3). 13C NMR (126 MHz, CDCl3): δ = 143.12 (d, 3Jc,C = 41.7 Hz, Cδ), 135.74 (4 x CH), 134.15 (2 x Cδ), 129.68 (2 x CH), 127.74 (4 x CH), 111.84 (d, 3Jc,C = 2.8 Hz, CH2), 62.90 (d, 3Jc,C = 1.5 Hz, CH2), 41.02 (d, 3Jc,C = 3.6 Hz, CH2), 27.00 (3 x CH3), 22.90 (13CH3), 19.36 (Cδ). GC (HP-5MS): tR = 2083. MS (EI, 70 eV): m/z (%) = 268 (68), 250 (1), 238 (17), 225 (37), 211 (6), 199 (14), 190 (100), 183 (21), 160 (32), 135 (18), 122 (8), 112 (7), 105 (19), 91 (5), 77 (15), 68 (1), 53 (2), 45 (6).

3-((13C)Methyl)but-3-en-1-yl 4-methylbenzenesulfonate (S6). To a solution of S5 (1.78 g, 5.46 mmol) in THF (26 mL) was added TBAF (1 M in THF, 6.60 mL, 6.60 mmol, 1.2 eq) dropwise at 0 °C. The reaction mixture was stirred for 2 h at room temperature and then quenched by the addition of water and extracted with Et2O (3 x 50 mL). The combined organic layers were dried with MgSO4. The solvent was removed carefully under reduced pressure (600 mbar, 40 °C, 40 min). The residue containing the product alcohol was dissolved in CH2Cl2 (48 mL) and the solution was cooled to 0 °C, followed by addition of DMAP (2.17 g, 17.79 mmol, 3.3 eq). TsCl (2.61 g, 13.69 mmol, 2.5 eq, suspended in 10 mL CH2Cl2)
was added dropwise. After stirring the mixture overnight at room temperature, the reaction was quenched by the addition of sat. NH₄Cl (100 mL), followed by extraction with Et₂O (3 x 100 mL). The organic layers were combined and dried with MgSO₄, and the solvent was removed by vacuum evaporation. Via silica gel chromatography [cyclohexane/EA (5/1): Rᵣ = 0.44], S6 (0.60 g, 2.48 mmol, 45%) was obtained as a colorless oil.

3-((^13C)Methyl)but-3-en-1-yl 4-methylbenzenesulfonate S6. \(^1^H\) NMR (499 MHz, CDCl₃): \(\delta = 7.81 - 7.77 \text{ (m, 2H, 2 x CH)}, 7.36 - 7.32 \text{ (m, 2H, 2 x CH)}, 4.81 - 4.72 \text{ (m, 1H, 1/2 x CH₂)}, 4.72 - 4.61 \text{ (m, 1H, 1/2 x CH₂)}, 4.13 \text{ (t, } ^3J_{\text{H,H}} = 6.9 \text{ Hz, 2H, CH₂)}, 2.45 \text{ (s, 3H, CH₃)}, 2.37 - 2.32 \text{ (m, 2H, CH₂)}, 1.66 \text{ (d, } ^1J_{\text{C,H}} = 126.1 \text{ Hz, 3H, CH₃}). \(^{13}\text{C}\) NMR (126 MHz, CDCl₃): \(\delta = 144.85 \text{ (C₉)}, 140.27 \text{ (d, } ^1J_{\text{C,C}} = 42.2 \text{ Hz, C₉)}, 133.33 \text{ (C₉)}, 129.94 \text{ (2 x CH)}, 128.05 \text{ (2 x CH)}, 113.23 \text{ (d, } ^2J_{\text{C,C}} = 2.7 \text{ Hz, CH₂)}, 68.67 \text{ (d, } ^2J_{\text{C,C}} = 1.7 \text{ Hz, CH₂)}, 36.90 \text{ (d, } ^2J_{\text{C,C}} = 4.0 \text{ Hz, CH₂)}, 22.48 \text{ (}^{13}\text{CH₃}), 21.79 \text{ (CH₃)}. GC (HP-5MS): \(I = 1832\). MS (EI, 70 eV): \(m/z\) (%) = 173 (4), 155 (26), 139 (1), 107 (1), 91 (54), 77 (2), 69 (100), 56 (10), 41 (5).

Trismamonium (5-^{13}\text{C})isopentenyl diphosphate

( Nob₄)₃HP₂O₇ (1.13 g, 1.25 mmol, 3.0 eq) was added to acetonitrile (1 mL), followed by the dropwise addition of S6 (0.10 g, 0.42 mmol, in 1 mL acetonitrile). The reaction mixture was stirred overnight, and the solvent was removed under reduced pressure. The residue was loaded onto an ion exchange resin column (DOWEX® 50W-X8, 100-200 mesh, NH₄⁺ form), followed by elution with two column volumes of elution buffer (25 mM NH₄HCO₃ in 2% iPrOH/H₂O). Lyophilization gave the crude product which was dissolved in aqueous NH₄HCO₃ (0.1 M, 3 mL), followed by extraction with acetonitrile/iPrOH (1/1, 3 x 7 mL).[^27] The extracts were combined and evaporated under reduced pressure. The residue was freeze-dried to afford (5-^{13}\text{C})IPP (107 mg, 0.36 mmol, 86%) as a colorless powder.

Trisammonium (5-^{13}\text{C})isopentenyl diphosphate (5-^{13}\text{C})IPP. \(^1^H\) NMR (500 MHz, D₂O): \(\delta = 4.89 - 4.80 \text{ (m, 2H, =CH₂)}, 4.05 \text{ (q, } ^3J_{\text{H,H}} = 6.7 \text{ Hz, CH₂)}, 2.41 - 2.35 \text{ (m, 2H, CH₂)}, 1.76 \text{ (d, } ^1J_{\text{C,H}} = 126.1 \text{ Hz, 3H, CH₃}). \(^{13}\text{C}\) NMR (126 MHz, D₂O): \(\delta = 143.89 \text{ (d, } ^1J_{\text{C,C}} = 41.4 \text{ Hz, C₉)}, 111.56 \text{ (d, } ^2J_{\text{C,C}} = 2.7 \text{ Hz, CH₂)}, 64.26 \text{ (d, } ^2J_{\text{P,C}} = 5.3 \text{ Hz, CH₂)}, 37.91 \text{ (dd, } ^2J_{\text{P,C}} = 7.6, ^3J_{\text{P,C}} = 3.8 \text{ Hz, CH₂)}, 21.73 \text{ (}^{13}\text{CH₃}). ^{31}\text{P}\) NMR (202 MHz, D₂O): \(\delta = -7.78 \text{ (d, } ^2J_{\text{P,P}} = 15.9 \text{ Hz, 1P)}, -10.47 \text{ (d, } ^2J_{\text{P,P}} = 16.0 \text{ Hz, 1P)}.

[^27]: Reference or citation
**Scheme S7.** The synthetic route towards (3-13C,4-2H2)IPP.

**Synthesis of (3-13C,4-2H2)IPP**

Ethyl 2-((2-13C)-2,5,5-trimethyl-1,3-dioxan-2-yl)acetate (S7)
Following a published procedure,[28] (3-13C)acetoacetate (1.00 g, 7.63 mmol) and neopentyl glycol (1.74 g, 16.72 mmol, 2.2 eq) were dissolved in CH2Cl2 (40 mL) at room temperature. TMSCI (3.63 g, 33.44 mmol, 4.4 eq) was added dropwise, followed by stirring the reaction mixture under reflux. The reaction mixture was cooled to room temperature and then quenched by the addition of a mixture of sat. NaHCO3 (60 mL) and ice-water (100 mL). The product was extracted with Et2O (3 x 80 mL), the combined organic layers were dried with MgSO4, and the solvent was removed under reduced pressure. Via silica gel chromatography [cyclohexane/EA (5/1), Rf=0.36], S7 (1.44 g, 6.63 mmol, 87%) was obtained as a colourless oil.

**Ethyl 2-((2-13C)-2,5,5-trimethyl-1,3-dioxan-2-yl)acetate S7.** 1H NMR (300 MHz, CDCl3): δ = 3.98 (q, 3JCH = 7.1 Hz, 2H, CH2), 3.43 – 3.21 (m, 4H, 2 x CH2), 2.78 (d, 3JCH = 5.9 Hz, 2H, CH2), 1.68 (d, 2JCH = 4.8 Hz, 3H, CH3), 0.96 (t, 3JCH = 7.1 Hz, 3H, CH3), 0.67 (s, 3H, CH3). 13C NMR (75 MHz, CDCl3): δ = 169.13 (Cq), 97.79 (13Cq), 70.55 (d, 3JC,C = 2.1 Hz, 2 x CH2), 60.26 (CH2), 41.25 (d, 3JC,C = 44.1 Hz, CH2), 29.76 (d, 3JC,C = 2.4 Hz, CH3), 23.32 (d, 1JC,C = 48.0 Hz, CH3), 22.60 (CH3), 22.43 (CH3), 14.23 (CH3). GC (HP-5MS): l = 1340. MS (EI, 70 eV): m/z (%) = 202 (32), 130 (100), 116 (16), 104 (12), 86 (28), 69 (52), 56 (32), 44 (81).

**2-((2-13C)-2,5,5-Trimethyl-1,3-dioxan-2-yl)ethan-1-ol (S8)**
LiAlH4 (0.25 g, 6.63 mmol, 1.0 eq) was suspended in THF (50 mL) and the suspension was cooled to 0 ºC. Then S7 (1.44 g, 6.63 mmol, in 5 mL THF) was added dropwise, and the mixture was stirred overnight without further cooling. After cooling the reaction mixture to 0 ºC, water (2.5 mL) was carefully added to quench the reaction, followed by the addition of MgSO4 for drying the solvent. The solids were removed by filtration, and the filtrate was concentrated under reduced pressure to afford S8 (1.15 g, 6.56 mmol, 99%) without further purification.

**2-((2-13C)-2,5,5-Trimethyl-1,3-dioxan-2-yl)ethan-1-ol S8.** 1H NMR (300 MHz, CDCl3): δ = 4.00 – 3.87 (m, 2H, CH2), 3.34 – 3.02 (m, 4H, 2 x CH2), 2.81 (t, 3JCH = 5.7 Hz, 1H, OH), 1.94 – 1.78 (m, 2H, CH2), 1.17 (d, 2JCH = 4.4 Hz, 3H, CH3), 0.96 (s, 3H, CH3), 0.40 (s, 3H, CH3). 13C NMR (75 MHz, CDCl3): δ = 100.10 (13Cq), 70.23 (d, 3JC,C = 2.1 Hz, 2 x CH2), 58.79 (d, 2JC,C = 2.0 Hz,
CH₂), 42.48 (d, Jₑ₋C = 46.9 Hz, CH₂), 29.75 (d, Jₑ₋C = 2.4 Hz, C₆), 22.81 (CH₃), 22.03 (CH₃), 19.08 (d, Jₑ₋C = 45.9 Hz, CH₃), GC (HP-5MS): l = 1236. MS (EI, 70 eV): m/z (%): 160 (42), 130 (56), 90 (24), 74 (39), 69 (45), 56 (46), 44 (100), 41 (47), 31 (23).

tert-Butyldiphenyl(2-((2-13C)-2,5,5-trimethyl-1,3-dioxan-2-yl)ethoxy)silane (S9)
Compound S8 (1.15 g, 6.56 mmol) and imidazole (0.63 g, 9.24 mmol) were added into DCM (40 mL). After the solids were dissolved, TBDPSCI (2.20 g, 7.92 mmol, 1.2 eq in 2 mL DCM) was added dropwise, and the reaction mixture was stirred overnight. The reaction was quenched by adding water (80 mL) and extracted with Et₂O (3 x 80 mL). The combined organic layers were washed with brine and dried with MgSO₄. The solvent was removed under reduced pressure and the product S9 (2.25 g, 82.4%) was obtained via silica gel chromatography [cyclohexane/EA (20/1), Rᵣ=0.25] as a colorless oil.

tert-Butyldiphenyl(2-((2-13C)-2,5,5-trimethyl-1,3-dioxan-2-yl)ethoxy)silane S9. ¹H NMR (300 MHz, CDCl₃): δ = 7.86 – 7.77 (m, 4H, 4 x CH₂), 7.27-7.16 (m, 6H, 6 x CH), 4.07 (td, Jₑ₋H = 7.3 Hz, 3Jₑ₋C = 2.5 Hz, 2H, CH₂), 3.21 (m, 4H, 2 x CH₂), 2.24 (tt, Jₑ₋H, H = 7.4 Hz, 2Jₑ₋H = 4.9 Hz, 2H, CH₂), 1.34 (d, 2Jₑ₋H = 4.5 Hz, 3H, CH₃), 1.19 (s, 9H, 3 x CH₃), 0.72 (s, 3H, CH₃), 0.65 (s, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃): δ = 136.07 (4 x CH), 134.46 (2 x C₆), 129.92 (2 x CH), 128.09 (4 x CH), 98.24 (¹³C₄), 70.20 (d, 2Jₑ₋C = 2.1 Hz, 2 x CH₂), 60.57 (CH₂), 29.79 (d, Jₑ₋H = 2.5 Hz, CH₂), 27.18 (3 x CH₂), 22.69 (CH₂), 22.54 (CH₂), 19.47 (C₆). GC (HP-5MS): l = 2585. MS (EI, 70 eV): m/z (%) = 398 (4), 356 (1), 283 (3), 270 (100), 252 (2), 240 (42), 225 (6), 211 (6), 199 (61), 192 (48), 181 (13), 174 (5), 165 (9), 162 (8), 148 (5), 139 (14), 135 (12), 130 (41), 121 (6), 105 (9), 91 (6), 77 (11), 69 (24), 56 (28), 44 (28).

(2-13C)-4-((tert-Butyldiphenylylsilyl)oxy)butan-2-one (S10)
Compound S9 (2.25 g, 5.44 mmol) was dissolved in MeOH (34 mL), followed by the dropwise addition of HCl (1 M, 2.6 mL, 2.6 mmol, 0.48 eq). After stirring the reaction mixture at room temperature for 30 min, the reaction was quenched by the addition of NaHCO₃ (5%, 56 mL). The product was extracted with Et₂O (3 x 60 mL). The combined organic layers were dried with MgSO₄ and concentrated under reduced pressure. Purification by silica gel chromatography [cyclohexane/EA (20/1), Rᵣ=0.20] afforded S10 (1.37 g, 4.18 mmol, 77%) as a colorless oil.

(2-13C)-4-((tert-Butyldiphenylylsilyl)oxy)butan-2-one S10. ¹H NMR (300 MHz, CDCl₃): δ = 7.79 – 7.73 (m, 4H, 4 x CH₂), 7.25 – 7.20 (m, 6H, 6 x CH), 3.85 (td, Jₑ₋H = 6.1 Hz, 3Jₑ₋H = 4.4 Hz, 2H, CH₂), 2.19 (q, 2Jₑ₋H = 5.9 Hz, 2Jₑ₋C = 5.9 Hz, 2H, CH₂), 1.70 (d, 2Jₑ₋H = 5.9 Hz, 3H, CH₃), 1.14 (s, 9H, 3 x CH₃). ¹³C NMR (75 MHz, CDCl₃): δ = 205.06 (¹³C₄), 136.01 (4 x CH), 133.98 (2 x C₆), 130.07 (2 x C₆), 128.13 (4 x C₆), 59.98 (d, 2Jₑ₋C = 2.0 Hz, CH₂), 46.03 (d, 2Jₑ₋H = 39.7 Hz, CH₂), 30.08 (d, 1Jₑ₋C = 40.6 Hz, CH₃), 27.05 (3 x CH₃), 19.42 (C₆). GC (HP-5MS): l = 2204. MS (EI, 70 eV): m/z (%) = 270 (60), 240 (44), 199 (100), 192 (36), 181 (17), 174 (6), 162 (8), 152 (7), 139 (9), 121 (8), 114 (4), 105 (10), 91 (6), 77 (22), 71 (4), 57 (21), 44 (25), 41 (18).

tert-Butyl(((3-13C,4-2H₂)-3-methylbut-3-en-1-yl)oxy)diphenylsilane (S11)
CD₃PPh₃I (3.07 g, 7.53 mmol, 1.8 eq) was added to THF (40 mL) and the mixture was cooled to 0 °C. Then n-BuLi (1.6 M in hexane, 4.7 mL, 7.53 mmol, 1.8 eq) was added dropwise, followed by stirring the mixture at 0 °C for 1 h. The reaction mixture was cooled to –78 °C, S10 (1.37 g, 4.18 mmol) was added dropwise, and stirring was continued overnight without further cooling. The reaction was poured onto ice-water (150 mL), and the product was extracted with Et₂O (3 x 70 mL). The combined organic layers were dried with MgSO₄ and concentrated under reduced pressure. The product S11 (1.16 g, 3.54 mmol, 85%) was obtained as a colorless oil via silica gel chromatography [cyclohexane/EA (40/1), Rᵣ=0.40].

tert-Butyl(((3-13C,4-2H₂)-3-methylbut-3-en-1-yl)oxy)diphenylsilane S11. ¹H NMR (700 MHz, CDCl₃): δ = 7.80 – 7.77 (m, 4H, 4 x CH₂), 7.24 – 7.20 (m, 6H, 6 x CH), 3.77 (td, 3Jₑ₋H = 6.7 Hz, 2Jₑ₋H = 3.6 Hz, 2H, CH₂), 2.24 (td, 3Jₑ₋H = 6.7 Hz, 2Jₑ₋H = 5.9 Hz, 2H, CH₂), 1.58 (d, 2Jₑ₋H = 6.3 Hz, 3H, CH₃), 1.18 (s, 9H, 3 x CH₃). ¹³C NMR (176 MHz, CDCl₃): δ = 142.77 (¹³C₄), 136.04 (4 x CH), 134.39 (2 x C₆), 129.96 (2 x CH₂), 128.07 (4 x CH), 63.04 (d, 2Jₑ₋C = 1.8 Hz, CH₂), 41.16 (d, 1Jₑ₋C = 41.1 Hz, CH₂), 27.13 (3 x CH₃), 22.65 (d, 1Jₑ₋C = 42.3 Hz, CH₂), 19.51 (C₆). GC (HP-5MS): l = 2078. MS (EI, 70 eV): m/z (%) = 270 (100), 240 (25), 225 (45), 211 (11), 207 (11), 193 (11), 189 (11), 173 (11), 159 (11), 145 (11), 131 (11), 117 (11), 103 (11), 91 (11), 79 (11), 67 (11), 55 (11), 43 (11), 31 (11).
199 (22), 192 (86), 181 (41), 162 (27), 155 (4), 147 (5), 135 (25), 121 (9), 114 (5), 105 (28), 91 (9), 77 (16), 57 (42), 41 (30).

(3-13C,4-2H2)-3-Methylbut-3-en-1-yl-4-methylbenzenesulfonate (S12)

Compound S11 (1.16 g, 3.54 mmol) was dissolved in THF (15 mL) and the solution was cooled to 0 °C. TBAF (1 M in THF, 4.25 mL, 4.25 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured onto ice-water, followed by extraction with Et2O (3 x 60 mL). The combined organic layers were then with MgSO4 and concentrated carefully under reduced pressure (600 mbar, 40 °C, 40 min) to afford the crude alcohol which was used for next step directly.

CH2Cl2 (40 mL) and DMAP (1.43 g, 11.68 mmol, 3.3 eq) were added to the residue and the solution was cooled to 0 °C. TsCl (1.68 g, 8.85 mmol, 2.5 eq, suspended in 8 mL DCM) was added dropwise and the reaction mixture was stirred overnight without further cooling. The reaction was quenched by pouring onto a mixture of sat. NH4HCO3 (60 mL) and ice-water (100 mL). The product was extracted with Et2O (3 x 60 mL) and the combined extracts were dried with MgSO4. The solvent was removed under reduced pressure. The product S12 (0.68 g, 2.79 mmol, 79%) was obtained as colorless oil via silica gel chromatography [pentane/Et2O(5/1), Rf = 0.44].

(3-13C,4-2H2)-3-Methylbut-3-en-1-yl-4-methylbenzenesulfonate S12. 1H NMR (700 MHz, CDCl3): δ = 7.76 – 7.73 (m, 2H, 2 x CH), 6.69 – 6.65 (m, 2H, 2 x CH), 3.93 (td, 3J_H,H = 6.7 Hz, 3J_C,H = 3.8 Hz, 2H, CH2), 1.98 (q, 3J_C,H = 6.5 Hz, 2H, CH2), 1.82 (s, 3H, CH3), 1.36 (d, 1H_C,H = 6.3 Hz, 3H, CH3). 13C NMR (176 MHz, CDCl3): δ = 144.15 (Cq), 140.31 (13Cq), 134.56 (Cq), 129.78 (2 x CH), 128.18 (2 x Cq), 68.23 (d, 2J_C,C = 1.8 Hz, CH2), 36.83 (d, 1J_C,C = 41.1 Hz, CH2), 22.02 (d, 1J_C,C = 41.2 Hz, CH2), 21.13 (CH3). GC (HP-5MS): /=1824. MS (EI, 70 eV): m/z (%) = 173 (5), 155 (43), 134 (3), 107 (5), 91 (94), 71 (100), 65 (40), 58 (13), 41 (13).

Trisammonium (3-13C,4-2H2)isopentenyl diphosphate (3-13C, 4-2H2)IPP

Following the same procedure as described above for the preparation of (5-13C)IPP, S12 (0.28 g, 1.15 mmol) was converted into (3-13C, 4-2H2)IPP (310 mg, 1.03 mmol, 90%) as a colorless powder.

(3-13C, 4-2H2)IPP. 1H NMR (499 MHz, D2O): δ = 4.02 – 3.92 (m, 2H, CH2), 2.31 (q, 3J_H,H = 6.5 Hz, 2H, CH2), 1.69 (d, 2J_C,H = 6.3 Hz, 3H, CH3). 13C NMR (126 MHz, D2O): δ = 143.72 (13Cq), 64.07 (dd, 2J_P,C = 5.7 Hz, 2J_C,C = 1.9 Hz, CH2), 37.77 (dd, 1J_C,C = 41.8 Hz, 3J_P,C = 7.6 Hz, CH2), 21.57 (d, 1J_C,C = 41.3 Hz, CH3). 31P NMR (202 MHz, D2O): δ = −7.12 (d, 2J_P,P = 21.7 Hz, 1P), −10.54 (d, 2J_P,P = 21.8 Hz, 1P).
References

[1] K. Grob, F. Zürcher, *J. Chromatogr.* 1976, 117, 285.
[2] J. Rinkel, J. S. Dickschat, *Org. Lett.* 2019, 21, 2426.
[3] S.-Y. Kim, P. Zhao, M. Igarashi, R. Sawa, T. Tomita, M. Nishiyama, T. Kuzuyama, *Chem. Biol.* 2009, 16, 736.
[4] C. Nakano, T. Tezuka, S. Horinouchi, Y. Ohnishi, *J. Antibiost.* 2012, 65, 551.
[5] P. Rabe, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2013, 52, 1810.
[6] W. K. W. Chou, I. Fanizza, T. Uchiyama, M. Komatsu, H. Ikeda, D. E. Cane, *J. Am. Chem. Soc.* 2010, 132, 8850.
[7] D. E. Cane, J. K. Sohng, C. R. Lamberson, S. M. Rudnicki, Z. Wu, M. D. Lloyd, J. S. Oliver, B. R. Hubbard, *Biochemistry* 1994, 33, 5846.
[8] P. Rabe, M. Samborskyy, P. F. Leadlay, J. S. Dickschat, *Org. Biomol. Chem.* 2017, 15, 2353.
[9] P. Rabe, J. Rinkel, T. A. Klapschinski, L. Barra, J. S. Dickschat, *Org. Biomol. Chem.* 2016, 14, 158.
[10] P. Rabe, J. Rinkel, E. Dolja, T. Schmitz, B. Nubbemeyer, T. H. Luu, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2017, 56, 2776.
[11] P. Baer, P. Rabe, K. Fischer, C. A. Citron, T. A. Klapschinski, M. Groll, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2014, 53, 7652.
[12] C. Nakano, S. Horinouchi, Y. Ohnishi, *J. Biol. Chem.* 2011, 286, 27980.
[13] J. Rinkel, J. S. Dickschat, *Org. Lett.* 2019, 21, 9442.
[14] X. Lin, R. Hopson, D. E. Cane, *J. Am. Chem. Soc.* 2006, 128, 6022.
[15] B. Neumann, A. Pospiech, H. U. Schaire, *Trends Genet.* 1992, 8, 332.
[16] J. S. Dickschat, K. A. K. Pahirulzaman, P. Rabe, T. A. Klapschinski, *ChemBioChem* 2014, 15, 810.
[17] R. D. Giets, R. H. Schiestl, *Nat. Protoc.* 2007, 2, 31.
[18] M. M. Bradford, *Anal. Biochem.* 1976, 72, 248.
[19] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* 2010, 29, 2176.
[20] J. Rinkel, L. Lauterbach, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2019, 58, 452.
[21] P. Rabe, L. Barra, J. Rinkel, R. Riclea, C. A. Citron, T. A. Klapschinski, A. Janusko, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2015, 54, 13448.
[22] G. Bian, J. Rinkel, Z. Wang, L. Lauterbach, A. Hou, Y. Yuan, Z. Deng, T. Liu, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2018, 57, 15887.
[23] T. Mitsuhashi, J. Rinkel, M. Okada, I. Abe, J. S. Dickschat, *Chem. Eur. J.* 2017, 23, 10053.
[24] L. Lauterbach, J. Rinkel, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2018, 57, 8280.
[25] J. Rinkel, L. Lauterbach, P. Rabe, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2018, 57, 3238.
[26] P. Rabe, J. Rinkel, B. Nubbemeyer, T. G. Köllner, F. Chen, J. S. Dickschat, *Angew. Chem. Int. Ed.* 2016, 55, 15420.
[27] V. J. Davisson, A. B. Woodside, T. R. Neal, K. E. Stremler, M. Muehlbacher, C. D. Poulter, *J. Org. Chem.* 1986, 51, 4768.
[28] L. Barra, B. Schulz, J. S. Dickschat, *ChemBioChem* 2014, 15, 2379.