SUPPORTING INFORMATION

Addressing the Chemistry of Germacrene A by Isotope Labeling Experiments
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1. Phylogenetic analysis and sequence of GAS

Figure S1. Location of GAS (shown by a blue arrow) in a phylogenetic tree of bacterial terpene synthases as a member of an uncharacterized branch of TSs in *Micromonospora* (blue). An additional putative germacrene A synthase is also shown, whose host organism *Micromonospora aurantiaca* produces 1 as judged by headspace analysis (Figure S2). Highly conserved residues and motifs are marked in yellow.

Figure S2. Amino acid sequence of GAS (accession number WP_09104378, gene locus tag GA0070215_RS12310). Highly conserved residues and motifs are marked in yellow.
2. General information, gene cloning, expression and incubation experiments

Strains and culture conditions
*Micromonospora marina* DSM 45555 was obtained from the DSMZ (Braunschweig, Germany) and was grown on N-Z-amine medium (20.0 g starch, 10.0 g glucose, 5.0 g yeast extract, 5.0 g N-Z-amine, 1.0 g CaCO₃, 1 L water, pH 7.2) at 28 °C. *Micromonospora aurantiaca* ATCC 27029 was also obtained from the DSMZ and was grown on gym 65 medium (4.0 g glucose, 4.0 g yeast extract, 10.0 g malt extract, 1 L water, pH 7.2) at 37 °C. *Escherichia coli* BL21(DE3) was grown in LB medium (10.0 g tryptone, 5.0 g yeast extract, 5.0 g NaCl, 1 L H₂O, pH 7.2) at 37 °C.

Gene cloning of terpene synthase and IDI
For gene cloning genomic DNA (gDNA) of *M. marina* was isolated from a fresh culture grown in N-Z-amine liquid medium (100 mL) as mentioned above for 7 days. The cultures were centrifuged (8000 x g) and the supernatant was discarded. The cells were suspended in SET buffer (75 mM NaCl, 25 mM EDTA, 20 mM Tris HCl, pH 8.0, 5 mL), lysozyme solution (50 mg mL⁻¹, 100 μL) was added and incubated for 30 min at 37 °C. To this mixture protease K solution (50 mg mL⁻¹, 100 μL) and 10% SDS (600 μL) were added and incubation was carried on for 2 h at 55 °C. Phenol/chloroform/isoamyl alcohol mixture (25:24:1, 5 mL) was added, the phases were mixed and the tube centrifuged at 8000 x g for 45 min. The aqueous layer was transferred to a fresh tube and the DNA was precipitated by addition of ethanol (0.6 vol). The DNA was spun down, washed with 70% ethanol, centrifuged again and dried overnight. The dry DNA was dissolved in water to a final concentration of approximately 1000 ng/μL. The same procedure also yielded gDNA from *E. coli* for cloning of *E. coli* IDI.

Polymerase chain reactions (PCR) were performed according to a standard 3-step PCR protocol provided by the supplier of the used Q5 high-fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA). Using gDNA of *M. marina* and the primers JR132f and JR132r (Table S1) for germacrene A synthase (GAS, WP_091045378), the target gene could be amplified. The same approach yielded the gene for isopentenyl diphosphate isomerase (IDI, ACT44538)² using *E. coli* gDNA and the primers JR077f and JR077r. PCR conditions were: initial denaturation at 98 °C, 30 sec; 3-step cycle: 98 °C, 10 sec; 70 °C, 30 sec; 72 °C, 30 sec; repeated 32 times; final elongation at 72 °C, 5 min). The obtained products were elongated with homology arms by a second PCR using the primers JR133f and JR133r, or JR078f and JR078r, respectively, under the same PCR conditions mentioned before. Homologous recombination in yeast was carried out using the elongated PCR products in combination with the pYE-Express shuttle vector (linearized by digestion with HindIII and EcoRI)³ through a standard protocol using LiOAc, polyethylene glycol and salmon sperm DNA.⁴ Transformed yeast cultures were grown on SM-URA agar plates (425 mg yeast nitrogen base, 1.25 g ammonium sulphate, 5 g glucose, 192.5 mg nutritional supplement minus uracil, 5 g agar, 250 mL water) at 28 °C for 3 days and colonies were collected to obtain the recombined plasmid using the Zymoprep Yeast Plasmid Miniprep II kit (Zymo Research, Irvine, CA, USA). The isolated plasmid was used for electroporation of *E. coli* BL21(DE3) electrocompetent cells, which were grown overnight at 37 °C on LB agar plates supplied with kanamycin (50 μg mL⁻¹). Single colonies were picked and inoculated in 6 mL LB medium with kanamycin and grown for 8 h at 37 °C to isolate single plasmids. The correct insertion of the desired genes was checked by analytical digest with Xhol and Pvull and by sequencing to obtain the plasmids pYE-GAS and pYE-IDI.
Table S1. Primers used for gene cloning.

| Primer | Sequence | Target |
|--------|----------|--------|
| JR132f | ATGC CGC GACT TC GC CCCTC | GAS |
| JR132r | TCAG CGC GTAC CG GG CG | |
| JR133f | GGCAG CCAT ATG GTAC ATG ACT GGT GGA AT GC GG AC TTC GCCCTC | |
| JR133r | TCTC A GTG GTG GTG GTG GTG GTG CTG AG TT CAG GCC TA CCG GCCG | |
| JR077f | ATGCAA AC GGA AC GTC AT TT TG AAT GCA C | IDI |
| JR077r | TTA ATT GGT GCT GC GC GA AA GC Ga CA A AC | |
| JR078f | GGCAG CCAT ATG GTAC GTG GGA AT GCA AA CG GAA ACA GC CAT TTAT TGA AT GC | |
| JR078r | TCTC AGT GTG GTG GTG GTG GTG CTG AG TT AAT TG GC GC GC | |

a Homology arms to pYE-express are underlined.

Gene expression and protein purification

The transformants were inoculated in precultures of LB medium (10 mL) supplied with kanamycin (50 µg/mL final concentration), which were grown with shaking overnight at 37 °C. The preculture (1/500) was then transferred to main cultures in LB medium with kanamycin at the same concentration and the cells were grown with shaking at 37 °C until OD₆₀₀ = 0.4 – 0.6 was reached. The cultures were cooled to 18 °C, before IPTG (0.4 mM final concentration) was added to induce expression. The cultures were shaken at 18 °C overnight and then centrifuged (5000 x g, 35 min, 4 °C). The medium was removed and the cell pellets were resuspended in binding buffer (10 mM/L culture; 20 mM Na₂HPO₄, 500 mM NaCl, 20 mM imidazole, 1 mM MgCl₂, pH = 7.4, 4 °C). Cell lysis was done by ultra sonification on ice (50% power, 5x 1 min). The resulting suspension was centrifuged (5400 x g, 3x 7 min, 4 °C) and the soluble protein fractions were filtrated and loaded on a Ni²⁺-NTA affinity chromatography column (Ni-NTA superfllow, Qiagen, Venlo, Netherlands). The bound target protein was washed with binding buffer (2x 10 mL/L culture) and obtained from the material with elution buffer (2x 6.25 mL/L culture; 20 mM Na₂HPO₄, 500 mM NaCl, 500 mM imidazole, 1 mM MgCl₂, pH = 7.4, 4 °C). Protein containing fractions were analyzed by SDS-PAGE (Figure S3) and used for incubation experiments. Typical protein concentrations in the complete elution fractions using this procedure were 0.2 mg/mL for GAS and 4.1 mg/mL for IDI, as determined by Bradford assay calibrated to bovine serum albumin.
GC/MS analysis
To record GC/MS data, an Agilent (Santa Clara, CA, USA) 7890B GC, which was connected to a 5977A mass detector using a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 µm film) was used. GC parameters were 1) inlet pressure: 77.1 kPa, He at 23.3 mL min⁻¹, 2) injection volume: 2 µL or 1 µL, 3) temperature program: 5 min at 50 °C, then increasing at 5 °C min⁻¹ or 10 °C min⁻¹ to 320 °C, 4) 60 s valve time, and 5) carrier gas: He at 1.2 mL min⁻¹. MS parameters were 1) source: 230 °C, 2) transfer line: 250 °C, 3) quadrupole: 150 °C and 4) electron energy: 70 eV.

Chiral GC analysis
GC with a homochiral stationary phase was carried out on an Agilent 7820A GC system equipped with an FID detector and an Agilent Cyclosil-B capillary column (30 m, 0.25 mm inner diameter, 0.25 µm film). For analysis of β-elemene (2), the GC was programmed as follows: starting from 80 °C, increasing with 1 °C/min to 115 °C, then further increasing with 30 °C/min to 245 °C while holding this temperature for 1 min. Inlet temperature: 250 °C, injection volume: 1 µL, carrier gas: H₂ @ 2.3 mL/min.

NMR spectroscopy
The instruments Bruker (Billerica, MA, USA) Avance I (400 MHz), Avance I (500 MHz), Avance III HD Prodigy (500 MHz) or an Avance III HD Cryo (700 MHz) were used to record NMR data. The obtained spectra were referenced against solvent signals (¹H-NMR, residual proton signals: CDCl₃ δ = 7.26 ppm, C₆D₆ δ = 7.16 ppm, D₂O δ = 4.79 ppm; ¹³C-NMR: CDCl₃ δ = 77.16 ppm, C₆D₆ δ = 128.06 ppm).⁶

Incubation experiments with recombinant GAS and terpenoid precursors
For determination of the GAS terpene cyclase activity, four different experiments were set up by dissolving the diphosphates GPP, FPP, GGPP and GFPP (1 mg each) in substrate buffer (1 mL; 25 mM NH₄HCO₃). After dilution with binding buffer (3.5 mL) and incubation buffer (5 mL: 50 mM Tris/HCl, 10 mM MgCl₂, 20% glycerol, pH = 8.2), GAS elution fraction (0.5 mL) was added. The reactions were incubated at 28 °C with shaking for 3 h and extracted with C₆D₆ (400 µL). The organic layers were dried with MgSO₄, and directly analyzed by GC/MS. Product formation could only be observed in the experiment with FPP (Figure S4).
3. Activity of GAS analysed by GC/MS and chiral GC

Figure S4. A) Total ion chromatogram of the enzymatic conversion of FPP with GAS analyzed by GC/MS with EI-MS spectra of B) 1 and C) 2. The peak labeled with an asterisk represents a non-terpenoid contaminant.
Figure S5. Chromatograms of chiral GC analysis of an incubation experiment with FPP and A) GAS and B) spata-13,17-diene synthase (SpS)\textsuperscript{7} from Streptomyces xinghaiensis, C) W335F variant of intermedeol synthase (STC4)\textsuperscript{8} from Termitomyces and D) coinjection of A) and C). The absolute configuration of 2 was also independently verified by labeling experiments (cf. Figures S22 – S23).
4. $^{13}$C-NMR data and experimental details for $^{13}$C$_1$-labeling of germacrene A

General procedure for incubation experiments with isotopically labeled substrates and recombinant GAS

For $^{13}$C$_1$-labeling, the corresponding $^{13}$C-labeled diphosphates (1.0 mg; (9-$^{13}$C)$_9$ and (10-$^{13}$C)GPP$^{10}$; (1-$^{13}$C), (2-$^{13}$C), (3-$^{13}$C), (4-$^{13}$C), (5-$^{13}$C), (6-$^{13}$C), (7-$^{13}$C), (8-$^{13}$C), (9-$^{13}$C), (10-$^{13}$C), (11-$^{13}$C), (12-$^{13}$C) and (15-$^{13}$C)FPP$^{11}$) were dissolved in substrate buffer (1 mL). A solution of IPP in substrate buffer (1 mL; 2 mg/mL) in case of labeled GPPs or only substrate buffer (1 mL) in case of labeled FPPs was added. To this mixture, incubation buffer (5 mL) was added, FPP synthase$^{12}$ (FPS, S. coelicolor) elution fraction (1 mL, labeled GPPs) or elution buffer (1 mL, labeled FPPs) and GAS elution fraction (2 mL) to give 10 mL of final incubation volume. The mixtures were incubated for 3 h at 28 °C and then directly extracted with C$_6$D$_6$ (650 µL, 350 µL). The combined organic layers were analyzed by GC/MS and NMR. For comparison of the obtained data to unlabeled material, a preparative scale isolation of 1 from spata-13,17-diene synthase (SpS)$^7$ from S. xinghaiensis was used. The same enzyme was also utilized to prepare unlabeled 2 as previously described.$^7$ In case of (7-$^{13}$C)FPP, the labeled product was purified by column chromatography [pentane] to remove peaks from labeled FPP hydrolysis products.

For addressing of the methylene hydrogen positions, (E)- and (Z)-(4-$^{13}$C,4-$^2$H)IPP$^{13}$ (1 mg) were dissolved in substrate buffer (1 mL) and a solution of DMAPP in substrate buffer (1 mL; 0.5 mg/mL) was added, followed by the same setup with FPS and GAS as described above. For PMA analysis, (1R)- and (1S)-(1-$^2$H)FPP$^{14}$ were used as stated above for the (13C$_1$)FPPs and (1R)- and (1S)-(1-$^2$H)GPP$^{15}$ were used as described above for the (13C$_1$)GPPs.
Figure S6. Partial $^{13}$C-NMR spectra of the aliphatic region of the products from singly labeled ($^{13}$C$_1$)FPPs obtained by chemical and enzymatic synthesis. The color code and border of the dots show the site of incorporation into the three conformers of germacrene A (1).
Figure S6. Partial $^{13}$C-NMR spectra of the aliphatic region of the products from singly labeled ($^{13}$C$_1$)FPPs obtained by chemical and enzymatic synthesis. The color code and border of the dots show the site of incorporation into the three conformers of germacrene A (1).
Figure S7. Partial $^{13}$C-NMR spectra of the olefinic region of the products from singly labeled ($^{13}$C$_1$)FPPs obtained by chemical and enzymatic synthesis. The color code and border of the dots show the site of incorporation into the three conformers of germacrene A (1). Signal assignment for C-7 of the minor conformers is tentative, because overlapping signals do not allow for clear integration.
Figure S7. Partial $^{13}$C-NMR spectra of the olefinic region of the products from singly labeled ($^{13}$C$_1$)FPPs obtained by chemical and enzymatic synthesis. The color code and border of the dots show the site of incorporation into the three conformers of germacrene A (1).
5. HSQC spectra of selectively labeled germacrene A samples at C-4 and C-8

Figure S8. Assignment of the $^1$H chemical shifts of germacrene A (1) for C-4. A) Partial HSQC spectrum of (4-13C)-1. The enzymatic conversion of B) (Z)-(4-13C,4-2H)IPP and DMAPP with FPPS and GAS, and C) of (E)-(4-13C,4-2H)IPP and DMAPP with FPPS and GAS results in stereospecifically labeled 1 with known configuration at the deuterated carbon. Red dots indicate 13C-labelings.
Figure S9. Assignment of the $^1$H chemical shifts of germacrene A (1) for C-8. A) Partial HSQC spectrum of (8-$^{13}$C)-1. The enzymatic conversion of B) (Z)-(4-$^{13}$C,4-$^2$H)IPP and DMAPP with FPPS and GAS, and C) of (E)-(4-$^{13}$C,4-$^2$H)IPP and DMAPP with FPPS and GAS results in stereospecifically labeled 1 with known configuration at the deuterated carbon. Red dots indicate $^{13}$C-labelings.
6. Synthesis of (R)- and (S)-(1-13C,1-2H)IPP

**General synthetic methods**
Chemicals were purchased from Thermo Fisher Scientific (Waltham, MA, USA), Sigma Aldrich (St. Louis, MO, USA) or TCI (Tokyo, Japan) and used without further purification. Flash column chromatography was performed on silica Geduran® Si 60 (40 – 63 µm) (Merck, Darmstadt, Germany). Solvents for column chromatography were distilled prior to use. All reactions containing non aqueous solvents or reagents were carried out in flame dried flasks under inert atmosphere (argon). Standard procedures were used to dry the reaction solvents. All reactions and chromatographic steps were monitored, if possible, using TLC (silica, Polygram® SIL G/UV254, Macherey-Nagel, Düren, Germany). A solution of phosphomolybdic acid in EtOH (10 g/100 mL) was used for staining.

**Scheme S1.** Synthesis of (1R)- and (1S)-(1-13C,1-2H)IPP. Red dots represent 13C-labeled carbon atoms.
The synthesis of (1R)-(1-13C,1-2H2)-2-(2,5,5-Trimethyl-1,3-dioxan-2-yl)ethan-1-ol (S2) was performed in analogy to the previously utilized route to (1R)-(1S)-(1-2H)-IPP.

To a stirred suspension of LiAl(2H)4 [0.95 g, 22.7 mmol, 1.0 eq.] in THF (225 mL) was added labeled ester S1 [4.93 g, 22.7 mmol, 1.0 eq.] at 0 °C, which was synthesized from ethyl (1-13C)acetooacetate following a known route. The reaction mixture was stirred for 4 h at 0 °C, before it was quenched carefully with H2O (2 mL). Stirring was continued for 10 min and MgSO4 was added. The mixture was filtered, rinsed with Et2O and the filtrate was concentrated under reduced pressure. Column chromatography on silica gel [cyclohexane/EtOAc (3:1)] yielded the title compound S2 as a colorless liquid (3.48 g, 19.6 mmol, 87%).

1H-NMR (400 MHz, CDCl3): \( \delta = 3.29 \) (d, \( \J_{HH} = 11.3 \) Hz, 2H, 2x 0.5CH2), 3.11 (d, \( \J_{HH} = 11.3 \) Hz, 2H), 2x 0.5CH2), 2.81 (br s, 1H, OH), 1.86 (d, \( \J_{HC} = 4.1 \) Hz, 2H, CH2), 1.17 (s, 3H, CH3), 0.96 (s, 3H, CH3), 0.41 (s, 3H, CH3) ppm. 13C-NMR (101 MHz, CDCl3): \( \delta = 100.1 \) (Cq), 70.2 (2x CH2), 58.1 (quint, \( \J_{CD} = 21.8 \) Hz, *CD2), 42.3 (d, \( \J_{CC} = 36.2 \) Hz, CH2), 29.7 (Ca), 22.8 (CH2), 22.0 (CH3), 19.2 (d, \( \J_{CC} = 1.7 \) Hz, CH3) ppm. GC: \( \mu = 1236 \) (HP-5MS). MS (EI, 70 eV): \( m/z \) (%) = 162 (58), 129 (77), 92 (47), 76 (62), 69 (86), 56 (56), 43 (100). TLC: Rf [cyclohexane/EtOAc (1:1)] = 0.30.

1H-NMR (500 MHz, CDCl3): \( \delta = 3.22 \) (d, \( \J_{HH} = 11.5 \) Hz, 2H, 2x 0.5CH2), 2.45 (d, \( \J_{HC} = 6.6 \) Hz, 2H, CH2), 1.17 (s, 3H, CH3), 0.77 (s, 3H, CH3), 0.51 (s, 3H, CH3) ppm. 13C-NMR (126 MHz, CDCl3): \( \delta = 198.8 \) (t, \( \J_{CD} = 27.0 \) Hz, *CD2), 97.6 (d, \( \J_{CC} = 1.1 \) Hz, Ca), 70.3 (2x CH2), 51.6 (dt, \( \J_{CC} = 40.1 \) Hz, *CD2, \( \J_{CD} = 3.5 \) Hz, CH2), 29.6 (Ca), 22.8 (CH2), 22.2 (CH3), 20.8 (d, \( \J_{CC} = 1.3 \) Hz, CH3) ppm. GC: \( \mu = 1181 \) (HP-5MS). MS (EI, 70 eV): \( m/z \) (%) = 159 (68), 129 (89), 73 (52), 69 (100), 56 (99), 43 (96), 41 (52). TLC: Rf [cyclohexane/EtOAc (1:1)] = 0.72.

According to a literature known procedure, the aldehyde S3 [1.64 g, 9.44 mmol, 1.0 eq.] was added to a (S)-Alpine borane solution [0.5 mL in THF, 22.7 mL, 11.35 mmol, 1.2 eq.]. After stirring for 4 h at room temperature, the reaction mixture was quenched with acetic aldehyde (256 μL) and THF was removed under reduced pressure. The crude product was subjected to high vacuum for 60 min and the crude product was dissolved in Et2O (11.4 mL). The solution was cooled to 0 °C and 2-aminoethanol (618 μL) was added, the resulting precipitate was filtered, rinsed with Et2O (3x 2 mL) and the organic filtrate was washed with H2O (10 mL). The organic phase was dried with MgSO4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography [cyclohexane/EtOAc (3:1)] to give alcohol (1R)-S4 [1.39 g, 7.90 mmol, 84%, 93% ee] as a colorless oil. The reaction was performed with (-)-Alpine borane solution as well under the described conditions, resulting in the alcohol (1S)-S4 [1.32 g, 7.46 mmol, 79%, 84% ee].
(1R)-S4 and (1S)-S4 gave identical analytical data: ¹H-NMR (500 MHz, CDCl₃): δ = 3.92 (dm, 1H), 1.33 (t, 3H), 2.81 (dd, J = 4.8 Hz, 3.5 Hz, CH₂), 1.87 (dd, J = 5.7 Hz, 4.3 Hz, CH₂), 1.17 (s, 3H), 0.96 (s, 3H), 0.40 (t, 3H) ppm. ¹³C-NMR (126 MHz, CDCl₃): δ = 100.1 (C), 70.2 (2x CH₂), 58.4 (t, J = 21.8 Hz, CH), 42.4 (s, J = 36.3 Hz, CH₂), 29.7 (C), 22.8 (CH₃), 22.0 (CH₃), 19.1 (s, J = 1.7 Hz, CH₃) ppm. GC: t = 1231 (HP-5MS). MS (EI, 70 eV): m/z (%) = 161 (72), 129 (91), 91 (46), 75 (62), 73 (36), 69 (88), 56 (62), 42 (100), 41 (49). TLC: Rₖ [cyclohexane/EtOAc (1:1)] = 0.33.

The enantiomeric excess was determined by synthesis of the Mosher esters:¹⁹ Pyridine (1.0 μL) and the stereoselectively deuterated alcohol (0.2 μL) were dissolved in CDCl₃ (100 μL). After addition of (S)-Mosher chloride (1.0 μL), the reaction mixture was stirred for 30 min at room temperature. CDCl₃ (400 μL) was added and the reaction mixture was directly analyzed by ¹H-NMR spectroscopy. The enantiomeric excess of the used alcohol was determined by integration of characteristic peaks for both formed diastereomers (Figure S10).

(1R)- and (1S)-tert-Butyldiphenyl(1-¹³C,1-²H)-2,5,5-trimethyl-1,3-dioxan-2-yl)ethoxy)silane (S5)
The alcohol (1R)-S4 (1.38 g, 7.82 mmol, 1.0 eq.) and imidazole (585 mg, 8.60 mmol, 1.1 eq.) were dissolved in CH₂Cl₂ (39 mL) and the solution was cooled to 0 °C. TBDPSCI (2.36 g, 8.60 mmol, 1.1 eq.) was added dropwise and the reaction mixture was stirred overnight at room temperature. After quenching with H₂O (30 mL), the azeotropic phase was extracted with CH₂Cl₂ (3x 30 mL). The combined organic phases were dried with MgSO₄ and the solvent was removed under reduced pressure. Purification by flash column chromatography on silica [cyclohexane/EtOAc (20:1)] gave the silane (1R)-S5 (2.87 g, 6.93 mmol, 89%) as a colorless oil. The same procedure was used to convert (1S)-S4 (1.30 g, 7.38 mmol) to (1S)-S5 (2.48 g, 5.99 mmol, 81%).

(1R)- and (1S)-S5 gave identical analytical data: ¹H-NMR (500 MHz, CDCl₃): δ = 7.85 – 7.80 (m, 4H), 4.06 (dt, 1H), 3.73 – 3.70 (m, 6H), 3.13 (s, 3H), 1.19 (s, 9H), 0.72 (s, 3H), 0.65 (s, 3H), ppm. ¹³C-NMR (126 MHz, CDCl₃): δ = 136.1 (4x CH), 134.5 (2x C), 129.9 (2x CH), 128.1 (4x CH), 98.2 (C), 70.2 (2x CH₂), 60.2 (t, J = 21.8 Hz, CH), 40.5 (d, J = 39.4 Hz, CH₂), 29.8 (C), 27.2 (3x CH₃), 22.7 (CH₃), 21.8 (CH₃), 19.5 (C) ppm. GC: t = 2555 (HP-5MS). MS (EI, 70 eV): m/z (%) = 399 (2), 283 (2), 271 (100), 253 (3), 239 (36), 221 (3), 193 (77), 175 (77), 167 (11), 161 (11), 139 (18), 135 (12), 129 (40), 77 (9), 69 (18), 43 (20). TLC: Rₖ [cyclohexane/EtOAc (1:1)] = 0.93.

(4R)- and (4S)-(4-¹³C,4-²H)-4-((tert-Butyldiphenylsilyl)oxy)butan-2-one (S6)
The ketal (1R)-S5 (2.86 g, 6.69 mmol, 1.0 eq.) was dissolved in MeOH (41 mL). Then HCl (1 M in H₂O, 4.1 mL, 4.10 mmol, 0.6 eq.) was added dropwise. The reaction mixture was stirred at room temperature for 30 min, quenched with NaHCO₃ (5% in H₂O, 40 mL) and the aqueous phase was extracted with EtO (3x 50 mL). After removing the solvent under reduced pressure, purification via flash column chromatography [cyclohexane/EtOAc (20:1)] yielded the ketone (4R)-S6 (2.06 g, 6.28 mmol, 91%) as a colorless liquid. Using the described procedure, also (1S)-S5 (2.47 g, 5.95 mmol) was converted to (4S)-S6 (1.74 g, 5.30 mmol, 89%).

(4R)- and (4S)-S6 gave identical analytical data: ¹H-NMR (500 MHz, CDCl₃): δ = 7.69 – 7.65 (m, 4H), 7.43 – 7.37 (m, 6H), 3.93 (dt, 1H), 2.64 (dd, 1H), 2.19 (s, 3H), 1.04 (s, CH₃), ppm. ¹³C-NMR (126 MHz, CDCl₃): δ = 213.6 (2x C), 129.9 (2x CH₂), 127.9 (4x CH), 59.5 (t, J = 22.2 Hz, CH), 46.4 (s, J = 38.9 Hz, CH₂), 30.8 (CH₂), 26.9 (3x CH₃), 19.3 (C) ppm. GC: t = 2187 (HP-5MS). MS (EI, 70 eV): m/z (%) = 271 (100), 253 (3), 239 (73), 221 (6), 213 (10), 199 (81), 193 (95), 181 (16), 175 (11), 167 (12), 161 (20), 139 (23), 121 (10), 105 (9), 77 (23), 57 (12), 45 (9), 43 (11). TLC: Rₖ [cyclohexane/EtOAc (5:1)] = 0.39.
(1R)- and (1S)-tert-Butyl-(((1-13C,1-2H)-3-methylbut-3-en-1-yl)oxy)diphenylsilane (S7)
A solution of nBuLi (1.6 mL in THF, 7.79 mL, 12.47 mmol, 2.0 eq.) was added to a solution of PPh3CH2I (5.04 g, 12.47 mmol, 2.0 eq.) in THF (62 mL) at 0 °C. After stirring the reaction mixture for 1 h at 0 °C, it was cooled to -78 °C. Then, ketone (4R)-S6 (2.05 g, 6.23 mmol, 1.0 eq.) was added dropwise and the reaction mixture was stirred overnight without cooling. It was quenched with H2O (60 mL) and the aqueous phase was extracted with Et2O (3x 50 mL). The combined organic layers were dried with MgSO4 and the solvent was removed under reduced pressure. Purification via flash column chromatography on silica [cyclohexane/EtOAc (40:1)] yielded the olefin (1R)-S7 (1.57 g, 4.79 mmol, 77%) as a colorless liquid. Using the same reaction, (4S)-S6 (1.73 g, 5.26 mmol) was converted to (1S)-S7 (1.30 g, 3.97 mmol, 75%).

(1R)-S7 and (1S)-S7 gave identical analytical data: 1H-NMR (500 MHz, CDCl3): δ = 7.71 – 7.63 (m, 4H, 4x CH), 7.45 – 7.36 (m, 6H, 6x CH), 4.77 – 4.74 (m, 1H, CH), 4.70 – 4.68 (m, 1H, CH), 3.75 (dt, 1JH,C = 142.1 Hz, 3JH,H = 6.9, 1H, CHD), 2.28 (dd, 3JH,H = 6.8 Hz, 2JH,C = 5.6 Hz, 2H, CH2), 1.69 (s, 3H, CH3), 1.06 (s, 9H, 3x CH3) ppm. 13C-NMR (126 MHz, CDCl3): δ = 143.1 (d, 2JCC = 1.7 Hz, Cq), 135.7 (4x CH), 134.2 (2x Cq), 129.7 (2x CH), 127.7 (4x CH), 111.8 (d, 2JCC = 3.3 Hz, CH2), 62.6 (t, 1JCD = 21.8 Hz, CHD), 40.9 (d, 1JCC = 38.2 Hz, CH2), 27.0 (3x CH3), 22.9 (d, 3JCC = 1.3 Hz, CH3), 19.4 (3x Cq) ppm. GC: tR = 2067 (HP-5MS). MS (EI, 70 eV): m/z (%) = 269 (55), 237 (15), 227 (23), 213 (6), 199 (12), 191 (100), 183 (16), 181 (14), 173 (5), 159 (31), 135 (17), 121 (13), 113 (6), 105 (18), 77 (14). TLC: Rf [cyclohexane/EtOAc (5:1)] = 0.88.

Trisammonium (1R)- and (1S)(-1-13C,-1-2H)isopentenyl diphosphate (S8)
The silane (1R)-S7 (600 mg, 1.84 mmol, 1.0 eq.) was dissolved in THF (9.2 mL) and the solution was cooled to 0 °C, before a solution of TBAF (1 mL in THF, 2.3 mL, 2.30 mmol, 1.2 eq.) was added dropwise. The reaction mixture was stirred at room temperature for 1 h and quenched with H2O (10 mL). The aqueous phase was extracted with Et2O (3x 10 mL) and the combined organic layers were dried with MgSO4. THF and Et2O were carefully removed under reduced pressure and the crude product was phosphorylated20 with TEAP solution without further purification. TEAP solution was obtained by mixing 1.82 mL of solution A (2.5 mL H2PO4, 9.4 mL acetonitrile) and 3.00 mL of solution B (11 mL NEt3, 10 mL acetonitrile). The crude product of the deprotection reaction was dissolved in trichloroacetonitrile (4.6 mL) and TEAP solution (3x 4.6 mL) was added in intervals of 5 min three times while stirring the reaction mixture at room temperature. The reaction mixture was directly purified by flash column chromatography on silica [PrOH/NH3 (25% in H2O)/H2O (6.0:2.5:0.5)] to yield (1R)-(-1-13C,1-2H)IPP (226 mg, 0.76 mmol, 41%) as an off-white solid. In a repeated procedure, (1S)-S7 (600 mg, 1.84 mmol) was converted to (1S)-(-1-13C,1-2H)IPP (217 mg, 0.72 mmol, 39%).

(1R)-S8 and (1S)-S8 gave identical analytical data: 1H-NMR (500 MHz, D2O): δ = 4.86 – 4.84 (m, 1H, CH), 4.83 – 4.81 (m, 1H, CH), 4.03 (dd, 1JH,C = 146.8 Hz, 3JLP = 6.7, 3JLH = 6.5 Hz, 1H, CHD), 2.37 (dd, 3JL,H = 6.7 Hz, 2JL,C = 5.4 Hz, 2H, CH2), 1.76 (s, 3H, CH3) ppm. 13C-NMR (101 MHz, D2O): δ = 143.7 (d, 2JCC = 1.8 Hz, CH3), 111.5 (d, 3JCC = 3.3 Hz, CH2), 64.0 (td, 1JDD = 22.4 Hz, 2JCC = 5.7 Hz, *CHD), 37.7 (dd, 3JCC = 38.2 Hz, 3JLH = 7.6 Hz, CH2), 21.6 (d, 2JCC = 1.6 Hz, CH3) ppm. 31P-NMR (162 MHz, D2O): δ = -8.69 (d, 2JP,P = 20.9 Hz), -10.62 (dd, 2JP,P = 29.9 Hz, 2JP,C = 5.8 Hz) ppm.
Figure S10. Mosher ester analysis of S4. Partial ¹H-NMR spectra of the reaction product of (S)-MTPA-Cl with A) (1R)-S4 and B) (1S)-S4 showing the signal for H-1. Integration gave an approximated enantiomeric excess of 93% for (1R)-S4 and 84% for (1S)-S4.

7. HSQC spectra of selectively labeled germacrene A samples at C-1, C-5 and C-9

Incubation experiments using (1R)- and (1S)-(1-¹³C,1-²H)IPP, IDI and GAS
The labeled IPPs (1.5 mg each) were dissolved in substrate buffer (1 mL). After dilution with incubation buffer (5 mL), IDI (E. coli) elution fraction (1 mL), FPPS elution fraction (1 mL) and GAS elution fraction (2 mL) were added. The mixtures were incubated, extracted and analyzed as described above for the other labeled substrates.
Figure S11. Assignment of the $^1$H chemical shifts of germacrene A (1) for C-1. A) Partial HSQC spectrum of (1-$^{13}$C)-1. The enzymatic conversion of B) (1$^R$)-(1-$^{13}$C,1-$^2$H)IPP with IDI, FPPS and GAS, and C) of (1$^S$)-(1-$^{13}$C,1-$^2$H)IPP and with IDI, FPPS and GAS results in stereospecifically labeled 1 with known configuration at the deuterated carbon. Red dots indicate $^{13}$C-labelings.
Figure S12. Assignment of the $^1$H chemical shifts of germacrene A (1) for C-5. A) Partial HSQC spectrum of (5-$^{13}$C)-1. The enzymatic conversion of B) $(1R)$-($1^{13}$C,1-$^2$H)IPP with IDI, FPPS and GAS, and C) of $(1S)$-($1^{13}$C,1-$^2$H)IPP and with IDI, FPPS and GAS results in stereospecifically labeled 1 with known configuration at the deuterated carbon. Red dots indicate $^{13}$C-labelings.
Figure S13. Assignment of the \(^1\)H chemical shifts of germacrene A (1) for C-9. A) Partial HSQC spectrum of (9-\(^{13}\)C)-1. The enzymatic conversion of B) (1\(^R\))-\((1-^{13}\)C,1-\(^2\)H)IPP with IDI, FPPS and GAS, and C) of (1\(^S\))-\((1-^{13}\)C,1-\(^2\)H)IPP and with IDI, FPPS and GAS results in stereospecifically labeled 1 with known configuration at the deuterated carbon. Red dots indicate \(^{13}\)C-labelings.
8. HSQC spectra of singly $^{13}$C-labeled germacrene A samples for the remaining positions

**Figure S14.** Assignment of the $^1$H chemical shifts of germacrene A (1) for C-2. Partial HSQC spectrum of (2-$^{13}$C)-1. Red dots indicate $^{13}$C-labelings.

**Figure S15.** Assignment of the $^1$H chemical shifts of germacrene A (1) for C-6. Partial HSQC spectrum of (6-$^{13}$C)-1. Red dots indicate $^{13}$C-labelings.
Figure S16. Assignment of the $^1$H chemical shifts of germacrene A (1) for C-10. Partial HSQC spectrum of (10-$^{13}$C)-1. Red dots indicate $^{13}$C-labelings.

Figure S17. Assignment of the $^1$H chemical shifts of germacrene A (1) for C-12. Partial HSQC spectrum of (12-$^{13}$C)-1. Please note that the labeling is partially scrambled between C-12 and C-13. Because of the much higher signal intensity, the sample originating from (12-$^{13}$C)FPP is used to assign the hydrogen shifts of C-12. Red dots indicate $^{13}$C-labelings.
Figure S18. Assignment of the $^1$H chemical shifts of germacrene A (1) for C-13. Partial HSQC spectrum of (13-13C)-1. Please note that the labeling is partially scrambled between C-13 and C-12. Because of the much higher signal intensity, the sample originating from (13-13C)FPP is used to assign the hydrogen shifts of C-13. Red dots indicate $^{13}$C-labelings.

Figure S19. Assignment of the $^1$H chemical shifts of germacrene A (1) for C-14. Partial HSQC spectrum of (14-13C)-1. Red dots indicate $^{13}$C-labelings.
9. HSQC spectra of selectively labeled β-elemene samples at C-1, C-4, C-5, C-8 and C-9

**Synthesis of labeled β-elemenes (2)**
To prevent any loss of material, the Cope rearrangement reactions were carried out in the NMR solvent C_6D_6. The corresponding labeled germacrene A solutions were transferred to a pressure tube (2 mL; Ace Glass, Vineland, NJ, USA), closed and heated to 115 °C^21 for 2 h. The samples were then carefully concentrated under a stream of argon and analyzed by GC/MS and NMR.
Table S2. NMR data of β-elemene (2) in C₆D₆ recorded at 298 K, cf. literature data.²²

| Cᵃ | proton ¹Hᵇ | ¹³Cᵇ |
|----|------------|------|
| 1  | CH₂        | 1.64 – 1.60 (m, H₆) | 33.3 |
|    |            | 1.58 (dd, ²Jₕₖ = 12.9, ³Jₕₖ = 11.8, H₆) |      |
| 2  | CH         | 1.92 (dd, ³Jₕₖ = 12.5, ⁵Jₕₖ = 3.5) | 52.9 |
| 3  | Cq         | –            | 147.7|
| 4  | CH₂        | 4.92 – 4.91 (m, H₆) | 112.6|
|    |            | 4.69 – 4.68 (m, H₂) |      |
| 5  | CH₂        | 4.945 (dd, ³Jₕₖ = 17.5, ²Jₕₖ = 1.4, H₂) | 110.1|
|    |            | 4.936 (dd, ³Jₕₖ = 10.9, ²Jₕₖ = 1.4, H₆) |      |
| 6  | CH         | 5.80 (dd, ³Jₕₖ = 17.5, ⁵Jₕₖ = 10.9) | 150.5|
| 7  | Cq         | –            | 40.1 |
| 8  | CH₂        | 1.42 – 1.38 (m, H₆) | 40.1 |
|    |            | 1.39 – 1.36 (m, H₆) |      |
| 9  | CH₂        | 1.55 – 1.51 (m, H₆) | 27.2 |
|    |            | 1.40 – 1.36 (m, H₆) |      |
| 10 | CH         | 1.85 – 1.79 (m) | 45.6 |
| 11 | Cq         | –            | 150.2|
| 12 | CH₂        | 4.84 – 4.83 (m, H₂) | 108.8|
|    |            | 4.82 – 4.80 (m, H₆) |      |
| 13 | CH₃        | 1.68 – 1.67 (m, 3H) | 21.1 |
| 14 | CH₃        | 0.98 (s, 3H) | 16.8 |
| 15 | CH₃        | 1.71 – 1.70 (m, 3H) | 25.1 |

ᵃ Carbon numbering as shown in Scheme 1 of main text. ᵇ Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, m = multiplet, coupling constants J are given in Hertz.
Figure S21. Stereochemical course of the Cope rearrangement to β-elemene (2) at C-4. A) Partial HSQC spectrum of 2. The enzymatic conversion of B) (Z)-(4-^{13}C,4-^{2}H)IPP and DMAPP with FPPS and GAS followed by thermal Cope rearrangement, and C) of (E)-(4-^{13}C,4-^{2}H)IPP and DMAPP with FPPS and GAS followed by thermal Cope rearrangement results in stereospecifically labeled 2 with stereoselective incorporation of deuterium into the E' and Z' hydrogen atoms of 2. Please note that the descriptors E and Z refer to the originating positions in IPP, whereas E' and Z' represent the hydrogens in the final product 2. Red dots indicate ^{13}C-labelings.
Figure S22. Stereochemical course of the Cope rearrangement to β-elemene (2) at C-5. A) Partial HSQC spectrum of 2. The enzymatic conversion of B) (1R)-(1-13C,1-2H)IPP with IDI, FPPS and GAS followed by thermal Cope rearrangement, and C) of (1S)-(1-13C,1-2H)IPP with IDI, FPPS and GAS followed by thermal Cope rearrangement results in stereospecifically labeled 2 with stereoselective incorporation of deuterium into the E' and Z' hydrogen atoms of 2. Red dots indicate 13C-labelings.
Figure S23. Determination of the absolute configuration of β-elemene (2) using C-8. A) Partial HSQC spectrum of 2. The enzymatic conversion of B) (Z)-(4-13C,4-2H)IPP and DMAPP with FPPS and GAS followed by thermal Cope rearrangement, and C) of (E)-(4-13C,4-2H)IPP and DMAPP with FPPS and GAS followed by thermal Cope rearrangement results in stereospecifically labeled 2 with known configuration at the deuterated carbon. The absolute configuration of 2 is accessible by solving the relative configuration of the stereocentres with respect to the labeled methylene group. Red dots indicate 13C-labelings.
Figure S24. Determination of the absolute configuration of \( \beta \)-elemene (2) using C-1 and C-9. 
A) Partial HSQC spectrum of 2. The enzymatic conversion of B) (1\(R\))-(1-\(^{13}\)C,1-\(^3\)H)IPP with IDI, FPPS and GAS followed by thermal Cope rearrangement, and C) of (1\(S\))-(1-\(^{13}\)C,1-\(^3\)H)IPP with IDI, FPPS and GAS followed by thermal Cope rearrangement results in stereospecifically labeled 2 with known configuration at the deuterated carbons. The absolute configuration of 2 is accessible by solving the relative configuration of the stereocentres with respect to the labeled methylene group. Red dots indicate \(^{13}\)C-labelings.
10. $^{13}$C-NMR spectra of singly $^{13}$C-labeled $\beta$-elemene samples

Figure S25. $^{13}$C-NMR spectra of the products from singly labeled ($^{13}$C$_{1}$)FPPs in the incubation with GAS after thermal Cope rearrangement to 2. The color code of the dots shows the site of incorporation into $\beta$-elemene (2).
Figure S25. $^{13}$C-NMR spectra of the products from singly labeled ($^{13}$C$_1$)FPPs in the incubation with GAS after thermal Cope rearrangement to 2. The color code of the dots shows the site of incorporation into β-elemene (2).
11. EI-MS spectra of singly $^{13}$C-labeled $\beta$-elemene samples

Figure S26. EI-MS spectra of ($^{13}$C$_1$)-labeled $\beta$-elemene (2) for position-specific mass shift analysis (PMA). Red dots represent $^{13}$C-labeled carbon atoms.
Figure S26. EI-MS spectra of $^{13}$C-labeled β-elemene (2) for position-specific mass shift analysis (PMA). Red dots represent $^{13}$C-labeled carbon atoms.
12. EI-MS spectra of deuterated β-elemene samples

Figure S27. EI-MS spectra of β-elemene (2) arising from incubation experiments of GAS with A) \((1R)-(1^2H)FPP\) and B) \((1S)-(1^2H)FPP\) showing a partially stereo dependent incorporation of deuterium into PMA$_{147}$. 
Figure S28. EI-MS spectra of β-elemene (2) arising from incubation experiments of GAS, FPPS and IPP with A) (1R)-(1-2H)GPP and B) (1S)-(1-2H)GPP showing no stereochemistry dependent incorporation of deuterium into PMA\textsubscript{147}. 
13. Headspace analysis of *M. marina* and *M. aurantiaca*

*M. marina* was grown on a N-Z-amine medium plate for 7 d at 28 °C. *M. aurantiaca* was grown for 3 d on a gym 65 medium plate at 37 °C. The plates were directly connected to a CLSA (closed loop stripping apparatus)\textsuperscript{24} for capturing volatiles emitted by the bacterial culture on a charcoal filter trap. Volatiles were collected for 16 h at room temperature. The charcoal filter was extracted with CH\textsubscript{2}Cl\textsubscript{2} and the extract was directly analysed by GC/MS. Whereas no production of 1 could be observed by *M. marina*, *M. aurantiaca* was shown to produce 1 (Figure S29).

![Figure S29. Total ion chromatogram of a headspace extract from *Micromonospora aurantiaca* ATCC 27029 demonstrating production of 1. The putative corresponding terpene synthase is shown in Figure S1 to be closely related to GAS.](image)

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15. Appendix: NMR spectra of compounds S2-S8 and 2
