Electronic Supplementary Information for

Chemoenzymatic preparation of germacrene analogues

Oscar Cascón,[a] Sabrina Touchet,[a] David J. Miller,[a] Verónica Gonzalez,[a] Juan A. Faraldos and Rudolf K. Allemann*[a]

[a]School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT

Contents

1. General Methods

All chemicals were purchased from Sigma-Aldrich unless otherwise stated. All were of analytical quality or better and used as received unless otherwise stated.

^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance III 600 NMR spectrometer, a Bruker Avance 500 NMR spectrometer or a Bruker Avance DPX400 NMR spectrometer and are reported as chemical shifts in parts per million downfield from tetramethylsilane, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (to the nearest 0.5 Hz) and assignment, respectively. Assignments are made to the limitations of COSY, DEPT 90/135, gradient HSQC and gradient HMBC spectra. ^{19}F NMR spectra were recorded on a Jeol Eclipse +300 NMR spectrometer and are reported in chemical shift downfield from $CFCl_3$ followed by multiplicity and coupling constant (to the nearest 0.5 Hz) if appropriate. $CDCl_3$ was filtered through basic alumina prior to use in NMR spectroscopy. EI^+ mass spectra were measured on a Micromass LCT premiere XE mass spectrometer. GCMS was performed on a Hewlett Packard 6890 GC fitted with a J&W scientific DB-5MS column (30 m x 0.25 mm internal diameter) and a Micromass GCT Premiere detecting in the range *m/z* 50-800 in EI^+ mode with scanning once a second with a scan time of 0.9 s. Injections were performed in split mode (split ratio 5:1) at 50 °C. Chromatograms were begun with an oven temperature of 50 °C (unless otherwise stated) rising at 4 °C min^{-1} for 25 min (up to 150 °C) and then at 20 °C min^{-1} for 5 min (250 °C final temperature).

Synthetic genes, codon optimised for expression in *E. coli*, were purchased from Epoch Life Sciences (http://www.epochlifescience.com/Service/Gene_Synthesis.aspx). Genes were bought cloned into pBLUESCRIPT (GAS) or pET28a (GDS) between NcoI and BamHI restriction sites and were followed by two stop codons for GAS and one for GDS.

2. Synthetic methods.

Fluorinated FDPs 2F-FDP,[1] 6F-FDP (**1b**),[2, 3] 10F-FDP (**1c**),[4] 14F-FDP (**1d**)[3] and 15F-FDP (**1e**)[5] were prepared by known literature synthetic methods. FDP analogues with methyl substitutions

at C15 and C14 (**1f**) were also prepared[6, 7] to evaluate the potential for the production of alkyl-modified germacrenes. 15Me-FDP was found to be a poor substrate for both GAS and GDS.

Preparation of 10-fluorofarnesene mixture[8]

An authentic sample of the title compounds as a GC-MS standard was generated as a mixture following the procedure reported by Coates[9] with modifications. A solution of 10-fluorofarnesol[4] (10 mg, 0.042 mmol) and pyridinium *p*-toluensulfonate (4 mg, 0.016 mmol) in 1,2-dichloroethane (1 mL) was stirred and heated (155 °C) in a sealed tube for 10 min. The reaction mixture was directly applied to a preparative TLC plate (silica) previously treated with 0.1% berberine.HCl in EtOH. The loaded plate was allowed to stand at room temperature (1.5 h) to effect evaporation of the solvent (1,2-dichloroethane). The plate was developed once using HPLC grade pentane and the hydrocarbon band was cut and eluted with anhydrous Et_2O to yield 5.3 mg (56%) of fluorinated hydrocarbons. GC/MS measurements indicated a *ca* 3:1:1 mixture of 10F-(*E*)-*β*-farnesene, 10F-Z-α-farnesene, and 10F-*E*-α-farnesene eluting at 25.5, 26.6 and 27.0 min respectively as the major sesquiterpenoids.

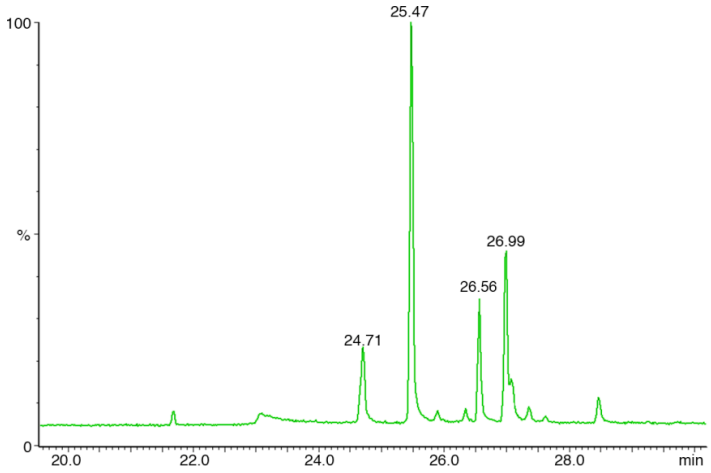


Figure S1. Total ion chromatogram of the 10-fluorofarnesene mixture prepared as described above.

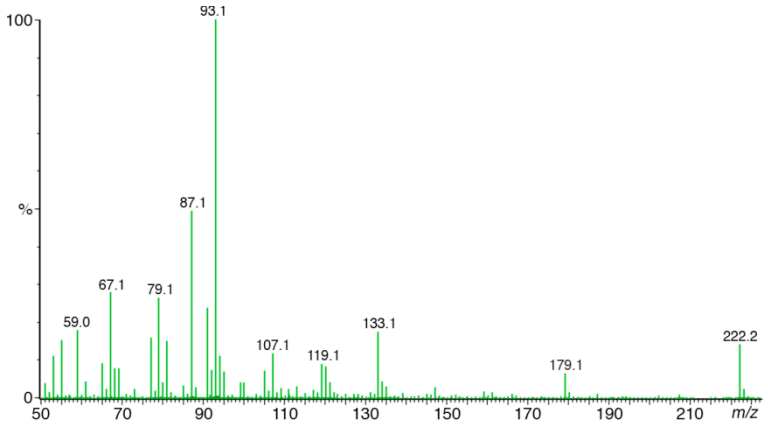


Figure S2. EI^+ mass spectrum of 10F-*E*-β-farnesene (eluting at 25.5 min).

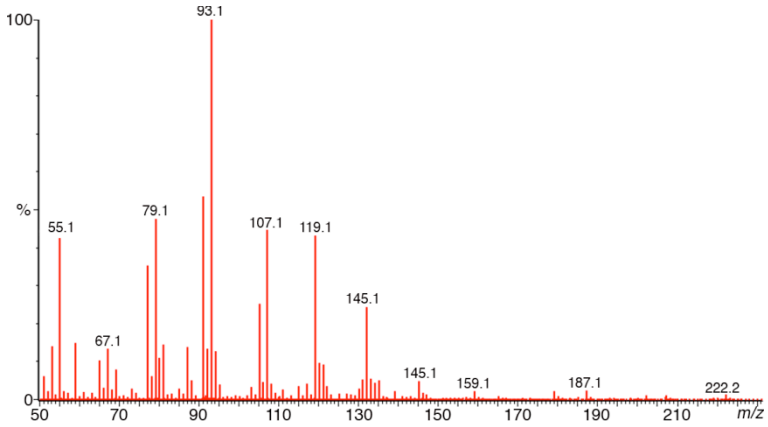


Figure S3. EI^+ mass spectrum of 10F-*Z*-α-farnesene (eluting at 26.65min)

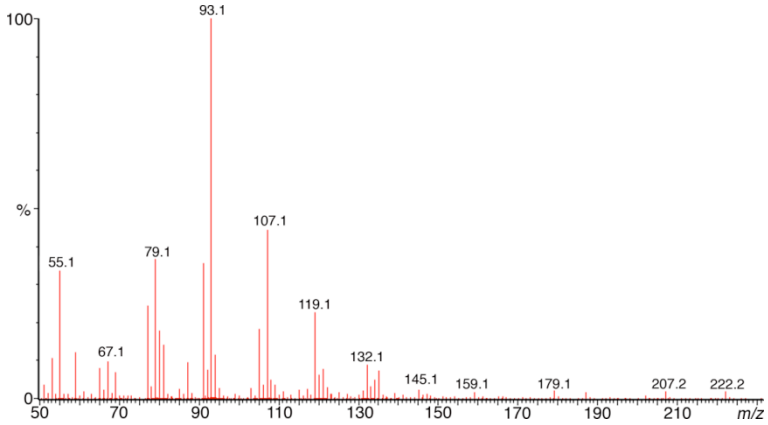


Figure S4. EI^+ mass spectrum of 10F-*E*-α-farnesene (eluting at 26.99 min)

3. Production of Recombinant Sesquiterpene cyclases

Preparation of (*R*)-Germacrene A Synthase (GAS). A gene, codon optimised for expression in *E. coli*, encoding for (*R*)-germacrene A synthase (GAS) from *Solidago canadensis*[10] was purchased from Epoch Biolabs. The synthetic gene was supplied cloned into a pBSK vector between the *Nco*I and *Sac*I restriction sites (pBSK-GAS). pET21d vector and pBSK-GAS were digested with *Nco*I and *Sac*I restriction endonucleases and the fragments corresponding to an open pET21d and GAS gene were ligated using T4 DNA ligase following the manufacturer's protocol. Plasmids were purified from overnight cultures (10 mL LB medium containing ampicillin 50 μg/mL) using a QIAprep Spin Miniprep Kit as described by the manufacturer. Ligation of the GAS gene into the pET21d vector was confirmed by DNA sequence analysis. *E. coli* BL21(DE3) cells were transformed with 1 mL of plasmid solution. One colony of these cells was added to 100 mL of LB medium containing ampillicin (50 μg/mL) and the culture was allowed to grow at 37 °C with shaking (120 rpm) overnight. A portion (5 mL) of the overnight culture was transferred to each of 6 x 500 mL of LB medium containing the same concentration of ampillicin as before. Cells were incubated at 37 °C with shaking at 120 rpm. When the OD_{600} reached 0.6 - 0.8, isopropyl-β-D-thiogalacto-pyranoside (IPTG) was added (500 mM final concentration) and shaking was continued for 4 hours at 37 °C. Cells were harvested by centrifugation at 5 °C (4200 *g*, 10 min). The supernatant solution was discarded and the pellets were stored at -20 °C.

Pellets were defrosted and resuspended in 50 mL of cell lysis buffer (50 mM Tris-Base, 5 mM EDTA, 5 mM β-mercaptoethanol (βME), pH 8). Cells were disrupted by sonication at 5 °C (40 % amplitude for 3 min with 5 s on/10 s off cycles). The resulting suspension was centrifuged at 5 °C (17000 *g* for 30 min) and the supernatant solution was discarded. The protein was recovered from the inclusion bodies by resuspending the pellet in fresh cell lysis buffer (50 mL) and NaOH (0.1 M) was added to the stirred suspension until the solution became clear (pH ~ 12). This solution was stirred at 4 °C for 30 minutes and then the pH was lowered to pH 8.0 by dropwise addition of HCl (1.0 M). βME was added (5 mM final concentration) and the solution was stirred for 30 minutes at 4 °C. This mixture was centrifuged at 5 °C (35000 *g* for 30 minutes). The protein was found in solution and the pellet was discarded. The supernatant solution was loaded

onto a DEAE Fast Flow column (12.5 x 2.5 cm) that had been pre-equilibrated with cell lysis buffer. After loading, the column was washed with 3-5 column volumes (CV) of cell lysis buffer at a flow rate of 10 mL/min. A gradient from 0 to 0.5 M NaCl (4 CV) was followed by a 0.5 to 1 M NaCl gradient (3 CV) and GAS eluted around 0.3 - 0.4 M NaCl. Fractions containing pure protein (SDS-PAGE) were pooled and dialysed overnight against cell lysis buffer (MWCO 30000). The dialysed fractions were concentrated to a final protein volume of ~ 5mL (AMICON system, YM 30). The concentration of protein was estimated using the method of Bradford.[11]

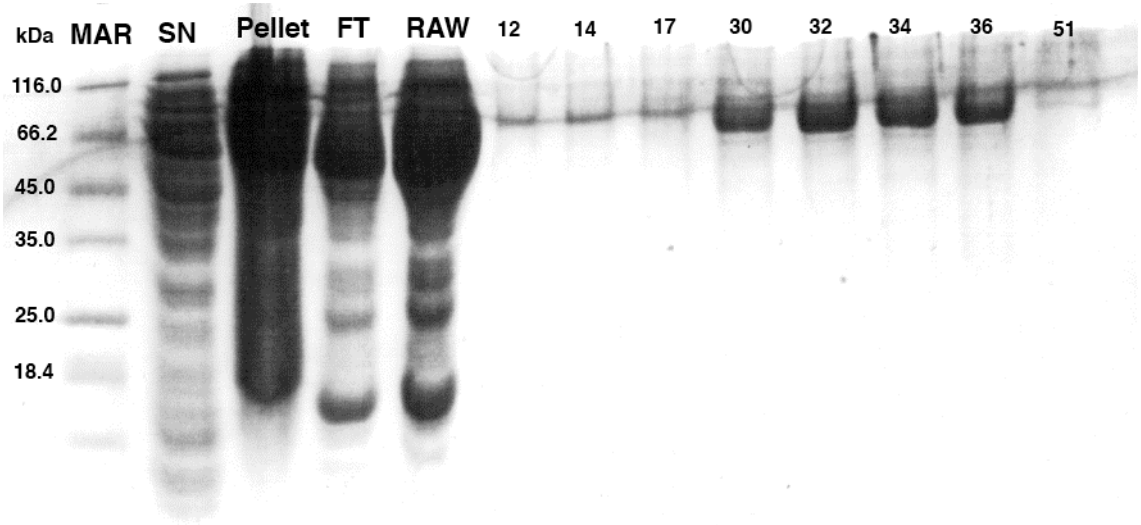


Figure S5. SDS-Polyacrylamide gel for the purification of GAS. MAR = molecular weight marker, SN = supernatant solution after sonication, Pellet = pellet after recovery from inclusion bodies, FT = flow through from DEAE Fast Flow column, Raw = protein solution after base extraction, 12 to 51 = different fractions from the elution of GAS with 0 to 1 M NaCl gradient.

Preparation of (*S*)-Germacrene D Synthase (GDS)

A synthetic gene, codon optimised for expression in *E. coli*, encoding for (*S*)-germacrene D synthase (GDS) from *Solidago canadensis*[12] was purchased from Epoch Biolabs. This gene was supplied cloned into a pET28a vector between the *Nco*I and *BamH*I restriction sites (pET28a-GDS). This plasmid and the pET21d vector were digested with *Nco*I and *Bam*HI restriction

endonucleases. The fragments corresponding to an open pET21d and GDS gene were ligated using T4 DNA ligase following the manufacturer's protocol to give a new plasmid pET21d-GDS. Plasmids were transformed into *E. coli* XLIBlue and then purified from overnight cultures (10 mL LB medium containing ampicillin 100 μg/mL) using a miniprep kit as described by the manufacturer. The gene sequence was confirmed by DNA sequence analysis.

Introduction of C-terminal 6xHis tag into GDS

A single nucleotide deletion was required to bring the 6xHis coding sequence of pET21d in frame with the GDS coding sequence. A Quickchange site-directed mutagenesis kit was used to introduce the desired deletion according to the manufacturer's instructions. The primers used for the deletion were as follows: 5'- CGCCATCAGTGTCAAGGATCCGAATTC- 3' and 5'-GAATTCGGATCCTTGACACTGATGGCG-3'.

Plasmids were transformed into *E. coli* XLIBlue and then purified from overnight cultures (10 mL LB medium containing ampicillin 100 μg/mL) using a miniprep kit as described by the manufacturer. Deletion was confirmed by DNA sequence.

Expression of GDS-His$_6$. GDS was produced in *E. coli* BL21(DE3)Star cells that harboured the cDNA for GDS-His$_6$ under the control of the T7 promoter. *E. coli* BL21(DE3)Star cells were transformed with 1 mL of plasmid. One colony of these cells was added to 100 mL of LB medium containing ampillicin (100 μg/mL) and the culture was allowed to grow at 37 °C with shaking (150 rpm) overnight. 5 mL of the overnight culture was transferred to each of 6 x 500 mL of LB medium containing the same concentration of ampillicin as before. Cells were incubated at 37 °C with shaking, when OD_{600} was between 0.6 - 0.8, IPTG was added (500 mM final concentration) and the cultures were incubated for 4.5 hr at 37 °C. Cells were harvested by centrifugation at 5 °C (4200 *g*, 10 minutes). The supernatant solution was discarded and the pellets were stored at -20 °C.

Purification of GDS-His$_6$

Pellets were defrosted and resuspended in 50 mL of cell lysis buffer (20 mM Tris-Base, 5 mM EDTA, 5 mM βME, pH 8). Cells were disrupted by sonication at 5 °C (40 % amplitude for 3 min

with 5 s on/10 s off cycles) and the resulting suspension was centrifuged at 5 °C (17000 *g*, 30 min) with protein found in the pellet in inclusion bodies. The protein was recovered from the inclusion bodies by adding fresh cell lysis buffer containing 0.2% of Tween® 20 (90 mL) to the pellet and NaOH (1 M) was added until the solution became clear (pH ~ 12). This solution was stirred at 4 °C for 30 min. βME was added (5 mM final concentration) and then the pH was lowered to pH 8 by addition of HCl (1 M). The suspension was stirred for 30 minutes at 4 °C and then centrifuged at 5 °C (35000 *g*, 30 min). The protein was found in solution and the pellet was discarded.

The solution containing the protein was dialysed overnight (50 mM Tris-Base, 50 mM NaCl, 10 mM βME, 0.2% Tween® 20, pH 8 - MWCO 30000). Then the solution was loaded onto a Ni Sepharose™ 6 Fast Flow column (12 mL, the column was eluted under gravity controlled drip flow). After 30 min, the column was washed with 4 CV of binding buffer (50 mM Tris, 500 mM NaCl, 10 mM βME, 5 mM imidazole, pH 8) followed by a gradient from 50 to 500 mM imidazole (20 CV) then a wash with 500 mM imidazole (10 CV). The protein eluted around 50-100 mM imidazole. Fractions were analysed by an SDS-PAGE. The fractions corresponding to a molecular weight of 63800 (GDS) were pooled, dialysed overnight (10 mM Tris-Base, 5 mM βME, 4% glycerol, pH 7.5 - MWCO 30000 Da) and then concentrated to a final volume of ~ 5 mL (AMICON system, YM 30). Glycerol was added to the solution of purified enzyme (10% v/v) and the solution was aliquoted and stored at -20 °C. The concentration of protein was estimated using the method of Bradford.[11]

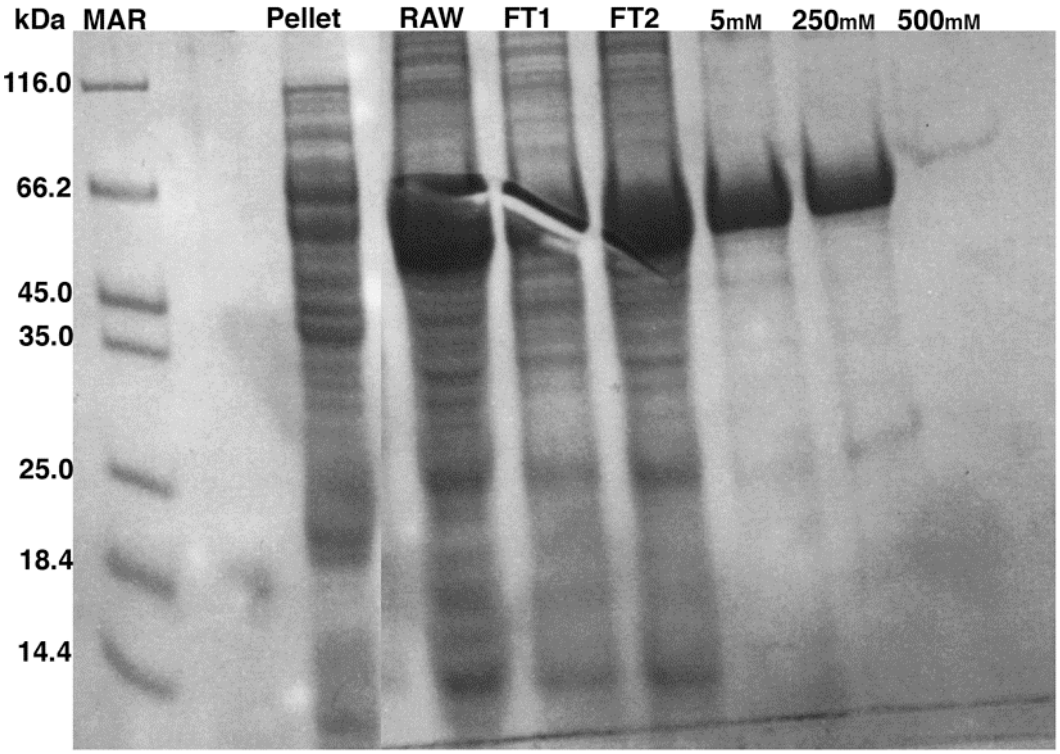


Figure S6. SDS-Polyacrylamide gel of GDS-His_6. MAR = molecular weight marker, Pellet = pellet after recovery from inclusion bodies, Raw = protein solution after base extraction, 5mM, 250mM and 500mM = different fractions of the elution of GDS with this concentration of imidazole. FT1 and FT2 = flow through from Ni SepharoseTM 6 Fast Flow column.

4. Assay of enzyme activity

Assay of GAS and GDS activity was carried out as previously described for aristolochene synthase.[4] Assays (final volume 250 μL) were initiated by addition of enzyme solution (50 μM, 25 μL) to 1-200 μM [1-^{3}H]-farnesyl diphosphate (240000 dpm/nmol) in 20 mM Tris, 10 mM $MgCl_2$ and 5 mM βME (pH 7.5); incubations with GDS also contained 10% glycerol in the buffer). After incubation for 15 min, reactions were stopped by addition of 100 mM EDTA and overlaid with hexane (1 mL). After the samples had been vortexed for 10 s, the hexane layer was removed and the sample extracted with hexane in the same way (2 x 1 mL). The pooled fractions were filtered through approximately 50 mg of silica then emulsified with Ecoscint O (15 mL) and analyzed by scintillation counting.

Kinetic constants were found to be k_{cat} = 0.093 s^{-1} (± 0.01), K_m = 8.49 μM (± 2.00) for GAS and k_{cat} = 0.009 s^{-1} (± 0.0004), K_m = 3.54 μM (± 0.26) for GDS, consistent with previous measurements.[10, 12]

5. Analytical incubation of GAS and GDS with FDP and analogues.

FDP (or FDP analogue) (25 μL, 10 mM) was diluted with 250 μL of incubation buffer (20 mM Tris, 5 mM βME, and 10 mM $MgCl_2$ (5 mM for GAS), pH 7.5 + 10% glycerol for GDS). To this assay solution enzyme (25 μL, 50 μM) was added followed by pentane (1 mL) and the mixture was gently agitated on a shaker. After incubation for 24 h at 25 °C, the olefin products were extracted with pentane (3 x 1 mL). The pooled extracts were passed through a short pad of silica gel (~500 mg) and analyzed by gas chromatography-mass spectrometry (GC-MS) as described above. All incubations were repeated without enzyme as negative controls and an additional experiment was analysed without the filtration step to check for enzyme derived alcohol products.

6. Optimisation of preparative scale enzymatic incubations.

Optimisations were carried out using the analytical scale assays at first using the conditions described above.

Concentrations of sesquiterpenoid and fluorinated sesquiterpenoid hydrocarbons were measured using a GC-flame ionization detection (FID) system and a VF-1ms column (15 m x 0.25 mm (0.25 mm). Carrier gas was Helium (flow rate, 1 ml/min, split ratio 20:1) and chromatograms were begun at 50 °C (injector 200 °C) held for 5 min then rising at 10 °C/min to 250 °C (held for 10 min) with the detector at 250 °C.

Samples of unknown concentration (25 μL) were injected onto the column and the area under the resulting sample peak in the FID-chromatogram used to estimate concentration. For quantification of incubations, the pentane used to overlay the incubations was spiked with α-humulene (35 μM for hydrocarbon products) or the fluorofarnesene mixture described above (35

μM, for fluorinated products) as an internal standard. FID integrations relative to the internal standard were used to calculate percentage conversions from the substrate.

Optimisation of FDP concentration

Farnesyl diphosphate concentration was varied in assay buffer and the area under the peak corresponding to FDP reverse phase high performace liquid chromatogram[1] was determined. Above 0.35 mM FDP, the substrate began to precipitate and this is reflected in the fact that the area under the peak corresponding to FDP did not increase above this point.

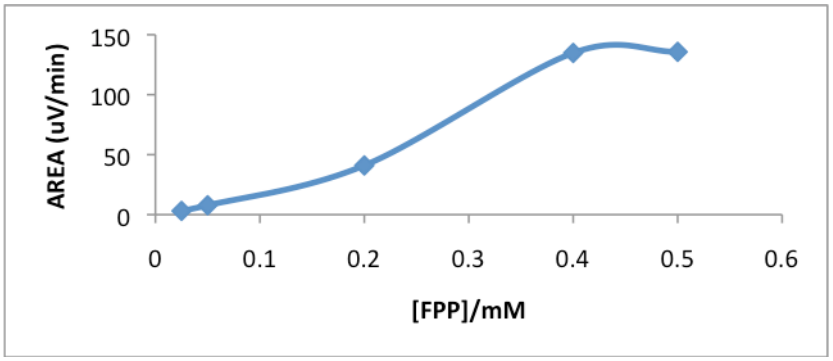


Figure S7. FDP conversion to germacrene D as catalysed by GDS with varying FDP concentrations. The y-axis represents the area under the peak for germacrene D in the FID-chromatogram in μV/min.

Optimisation of Mg^{2+} concentration

Optimisation of [Mg^{2+}] for conversion of FDP by GDS and GAS was carried out using the radiolabelled assay. Production was optimal at a concentration of 10 mM $MgCl_2$ for GDS and 5 mM for GAS.

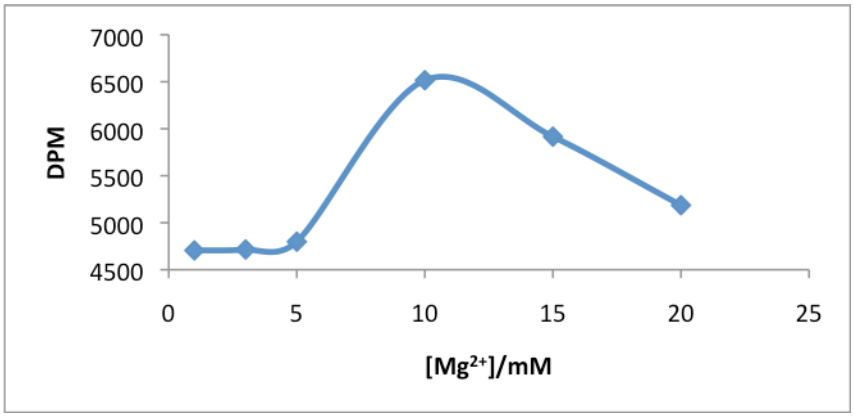


Figure S8. Rate of radiolabelled germacrene D production versus [Mg^{2+}] for GDS.

Optimisation of enzyme concentration

Using [FDP] = 0.35 mM and [Mg^{2+}] = 10 mM, a series of 24 h analytical scale incubations were performed with increasing concentration of GDS measuring the concentration of germacrene D produced using α-humulene (0.35 mM) as an internal standard. As can be seen from Fig. S9 conversions dropped above approx 6 μM enzyme concentration. A similar curve was seen for GAS. However addition of 1% CHAPS to GDS incubations led to an improvement to 76% conversion over 24 h through the ability to raise [GDS] to 12 mM. No such effect was seen for GAS where optimal conversion was remained at ~40%.

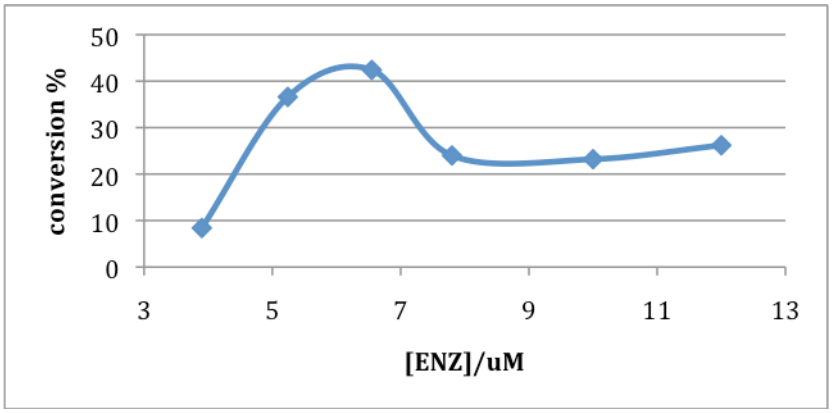


Figure S9. Conversion of FDP to germacrene D by GDS with varying GDS concentration.

7. General procedure for preparative scale incubations of FDP analogues with enzyme

To minimize losses of volatile sesquiterpenoid products during incubation and workup, reactions were performed in sealed ACE tubes containing incubation buffer (15 mL), FDP (or analogue, 0.35 mM), CHAPS (150 mg, 1% - for GDS only - see main text), enzyme (GDS 12 μM or GAS 6 μM) and $CDCl_3$ (2 mL) for direct examination of products by NMR spectroscopy. Each incubation solution was gently agitated for 24 h at room temperature and then rotated using a rotator for 12 h to extract product efficiently into the organic layer. The aqueous layer (a milky suspension) and the organic layer were separated through a phase separator and the resulting $CDCl_3$ solution was dried by passing it through a Pasteur pipette containing anhydrous Na_2SO_4. A further quantity of $CDCl_3$ containing product (~0.5 mL) was isolated by freezing the aqueous suspension remaining in the phase separator in liquid nitrogen and then allowing it to thaw whereupon $CDCl_3$ trapped in the emulsion was freed. The pooled $CDCl_3$ extracts were concentrated to ~ 0.4 mL under a gentle stream of nitrogen for analysis by ^1H NMR spectroscopy and GC-MS.

8. Results from incubation of FDP analogues with GAS and GDS

(*R*)-Germacrene A (3a)

1a was incubated with GAS giving **3a** as the sole pentane extractable product in 40% conversion from FDP. This compound was characterised by GC-MS comparison with authentic standards of (*S*)-germacrene A generated by incubations of FDP (**1a**) with PR-AS.[13]

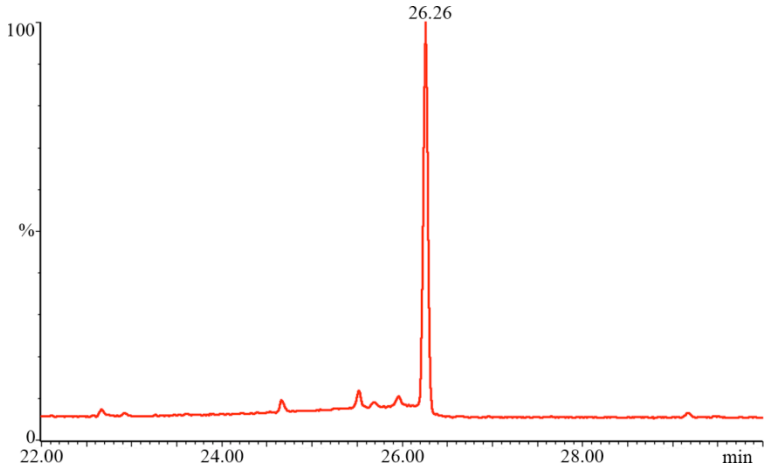


Figure S10. Total ion chromatogram of the pentane extractable products from incubation of FDP (**1a**) with GAS.

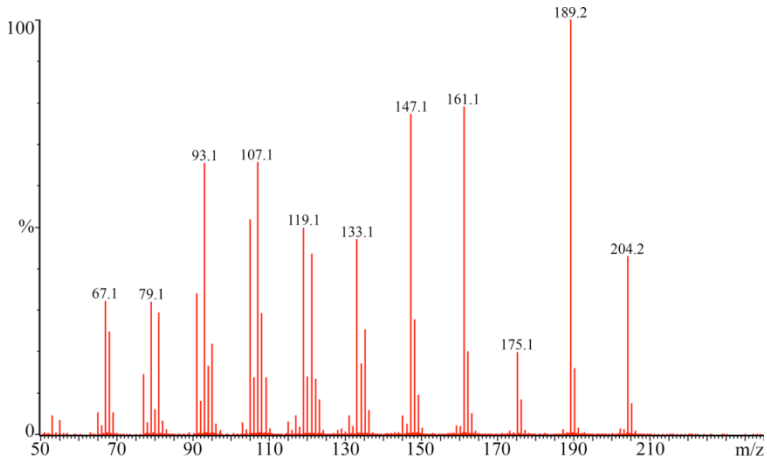


Figure S11. Mass spectrum of germacrene A isolated from incubation of FDP (**1a**) with GAS.

2F-Germacrene A

2F-FDP was incubated with GAS giving **3b** as the sole pentane extractable product but with a very poor (<1%) conversion. The small amount of material generated was characterised by GC-MS comparison with an authentic standard of 2F-germacrene A produced by incubation of **1b** with DCS.[8]

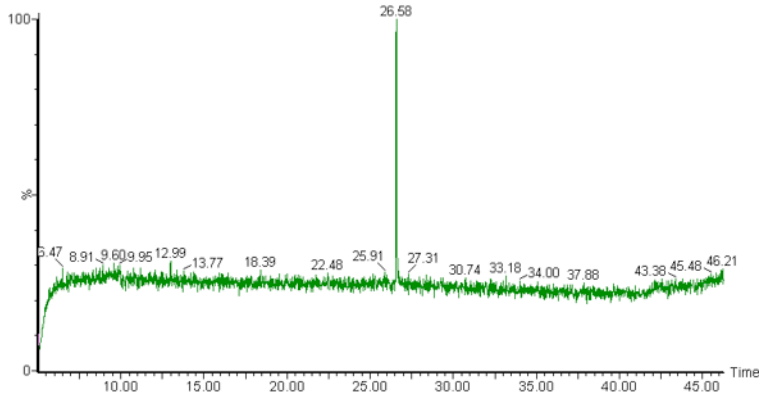


Figure S12. Total ion chromatogram of the pentane extractable products from incubation of GAS and 2F-FDP.

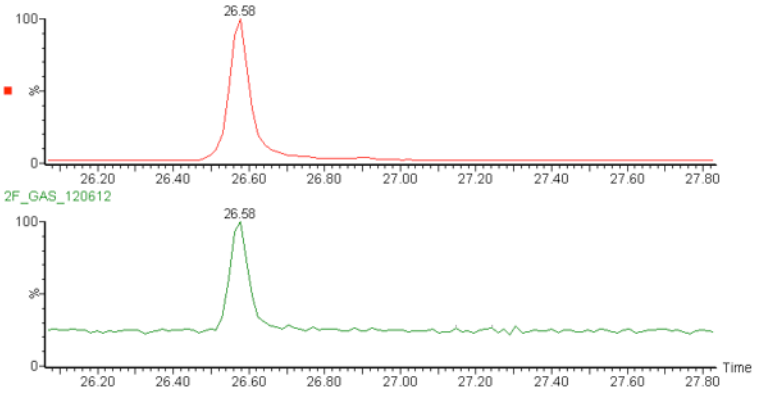


Figure S13. TIC comparison of the pentane extractable products from 2F-FDP with (top) DCS and (bottom) GAS.

6F-Germacrene A (3b)

1b was incubated with GAS giving **3b** as the sole pentane extractable product (29.3% conversion). This compound was characterised by GC-MS comparison with an authentic standard of 6F-germacrene A produced by incubation of **1b** with PR-AS.[3]

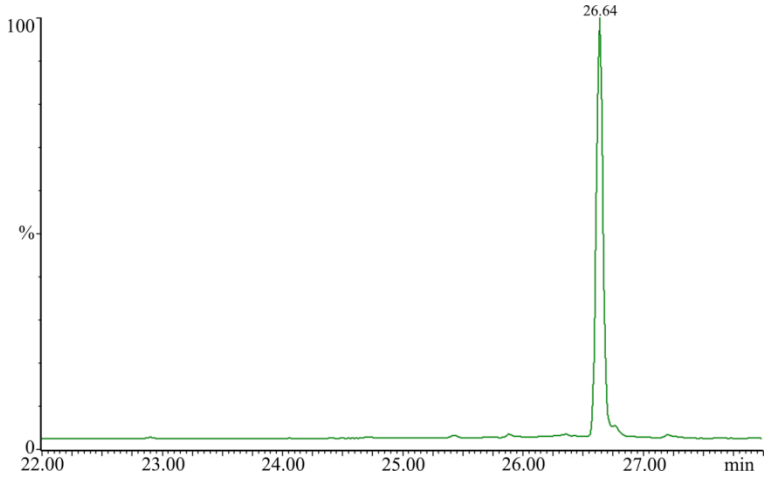


Figure S14. Total ion chromatogram for the pentane extractable products from incubation of 6F-FDP (**1b**) and GAS.

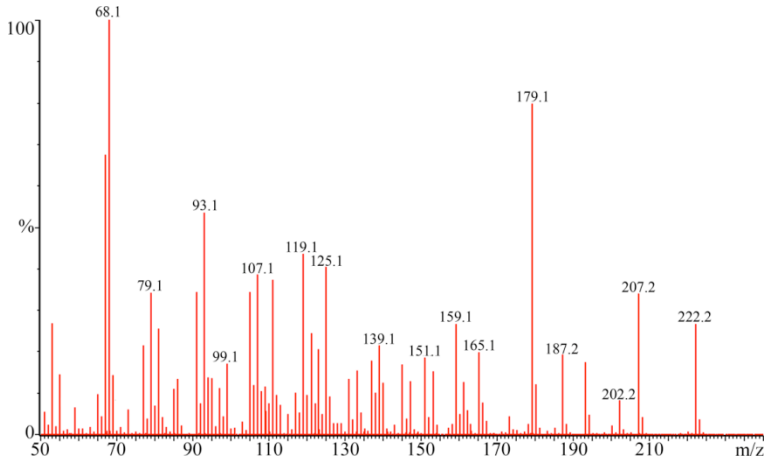


Figure S15. Mass spectrum of 6F-germacrene A isolated from incubation of 6F-FDP (**1b**) and GAS.

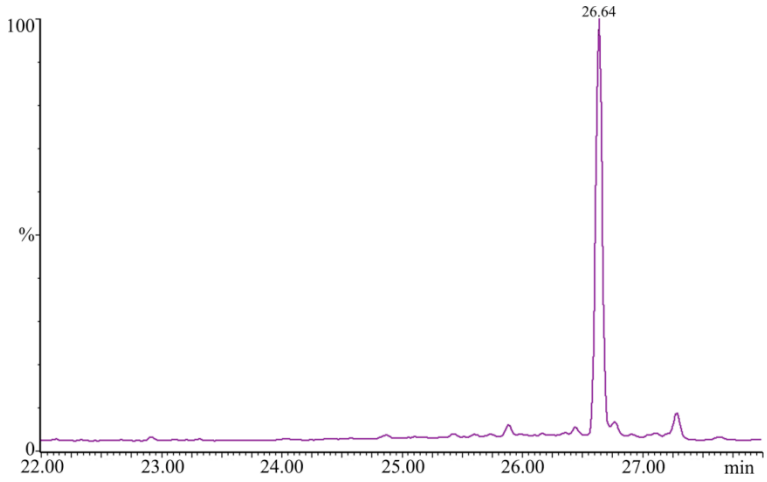


Figure S16. Total ion chromatogram of the mixed pentane extractable products isolated from incubation of **1b** with both GAS and PR-AS.[4]

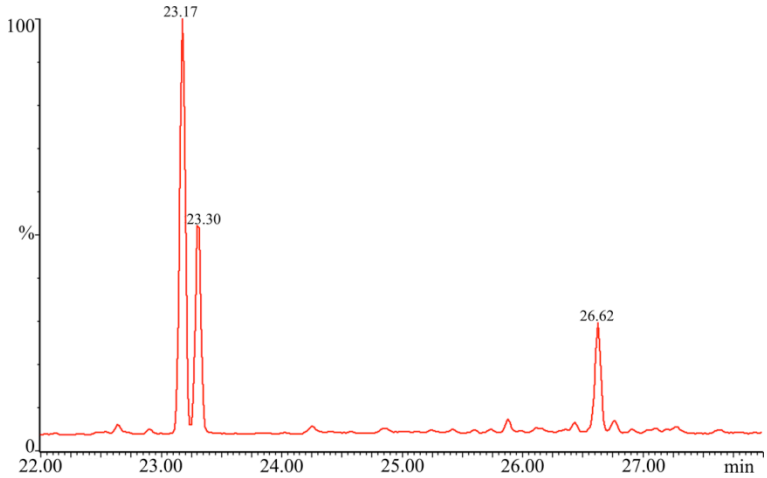


Figure S17. Total ion chromatogram of the mixed pentane extractable products isolated from incubations of 6F-FDP (**1b**) and GAS and PR-AS with an injection port temperature of 250 °C – showing thermally induced Cope rearrangement.

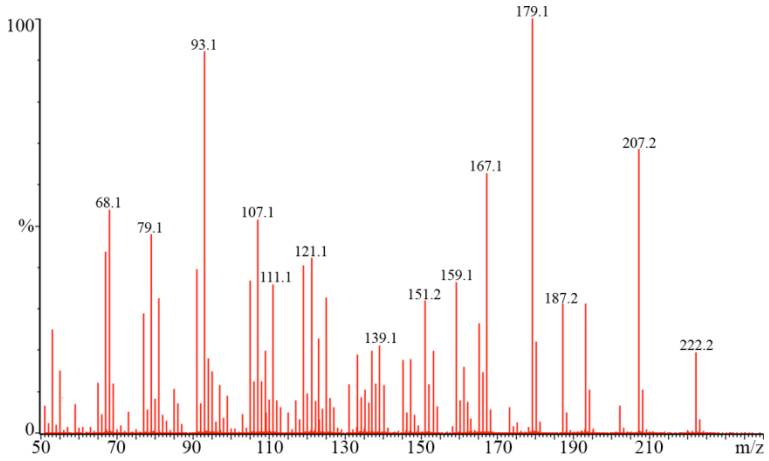
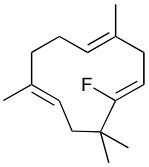


Figure S18. Mass spectrum of Cope rearrangement product eluting at 23.17 min arising from incubations of GAS and PR-AS and 6F-FDP (**1b**).

10F-α-Humulene (6c)



1c was incubated with GAS giving **6c** as the sole pentane extractable product (11.8% conversion). This compound was characterised by GC-MS comparison with authentic material produced by incubation of **1d** with DCS.[8]

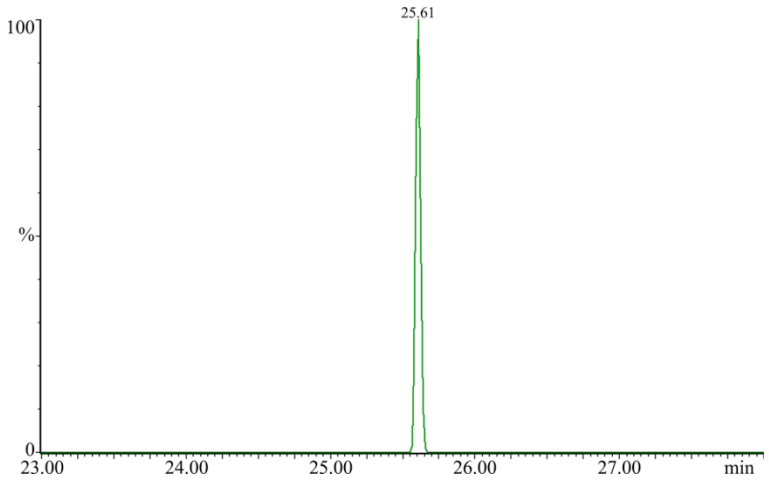


Figure S19. Total ion chromatogram of the pentane extractable products from incubation of 10F-FDP (**1c**) with GAS.

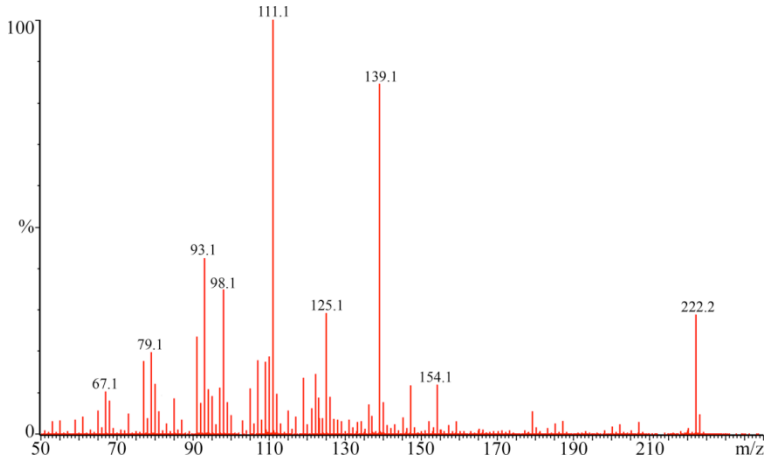


Figure S20. Mass spectrum of product isolated from incubation of 10F-FDP (**1c**) with GAS.

This compound gave identical analytical data to the 10F-α-humulene product previously identified from incubation of **1d** and DCS.[8]

14F-Germacrene A (3d)

1d was incubated with GAS giving **3d** as the sole pentane extractable product (18.9% conversion). This compound was characterised by GC-MS comparison with an authentic standard of 14F-germacrene A produced by incubation of **1d** with PR-AS.[3]

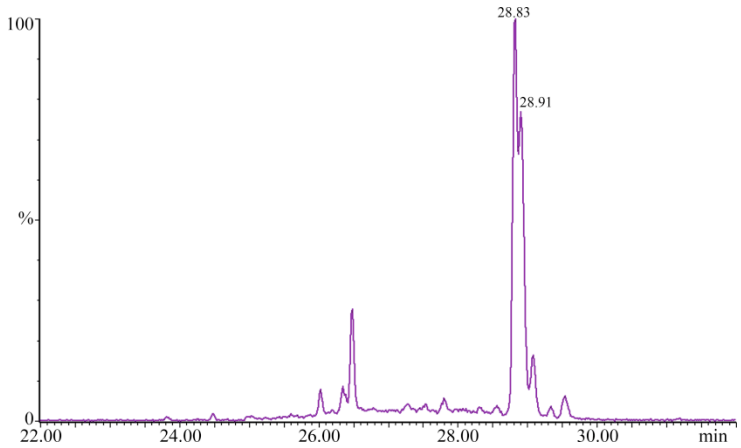


Figure S21. Total ion chromatogram of pentane extractable products isolated from incubation of 14F-FDP (**1d**) with GAS.

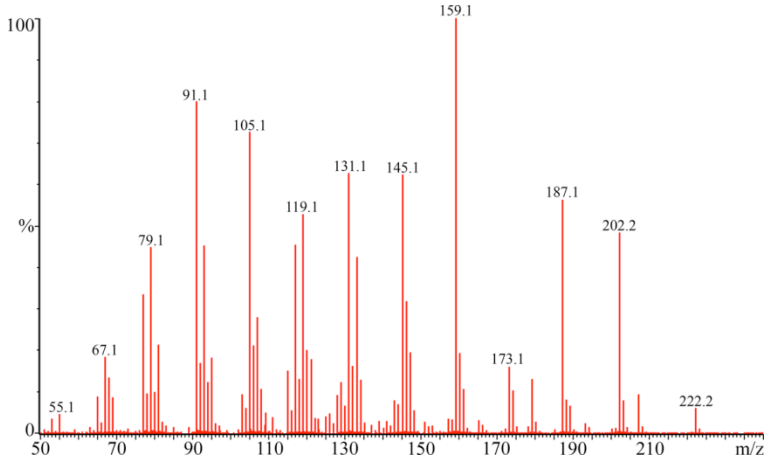


Figure S22. Mass spectrum of the pentane extractable product isolated from incubation of 14F-FDP (**1d**) with GAS.

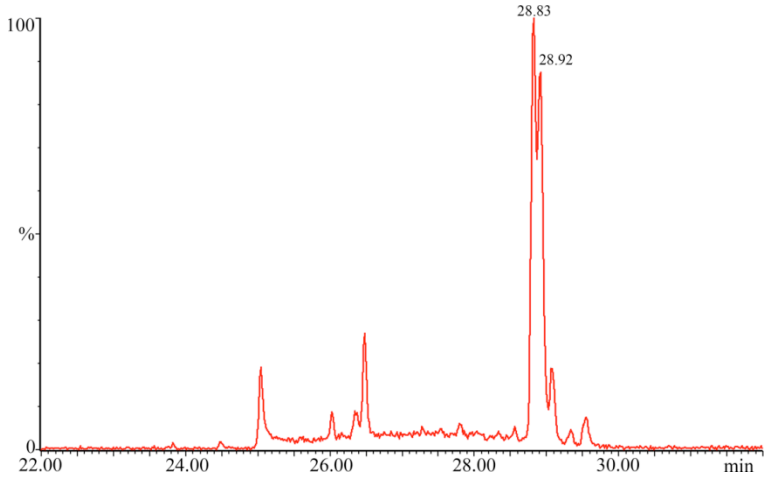


Figure S23. Total ion chromatogram of the mixed pentane extractable products isolated from incubations of 14F-FDP (**1d**) with GAS and PR-AS.

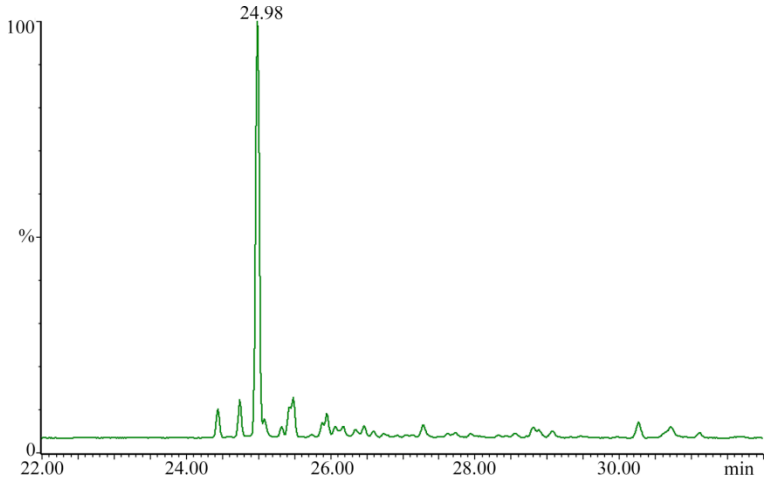


Figure S24. Total ion chromatogram of the mixed pentane extractable products isolated from incubations of 14F-FDP (**1d**) with GAS and PR-AS with an injection port temperature of 250 °C – showing thermally induced Cope rearrangement.

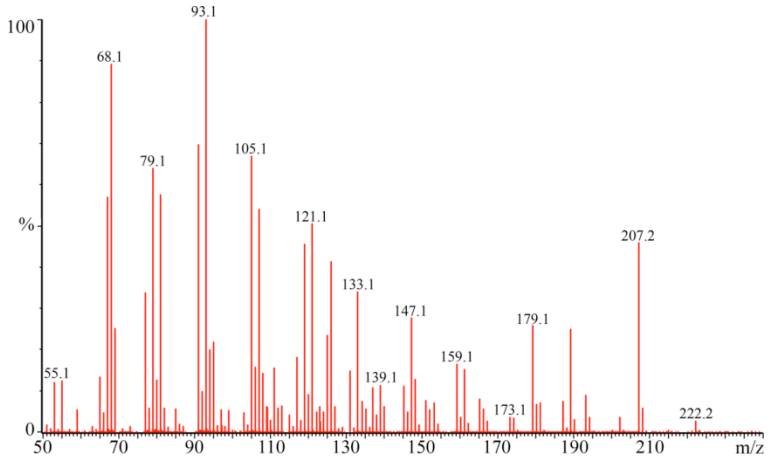


Figure S25. Mass spectrum of Cope rearrangement product eluting at 24.98 min arising from incubations of GAS and PR-AS and 14F-FDP (**1d**).

14Me-Germacrene A (3f)

1f was incubated with GAS giving **3f** as the major compound (50% of hydrocarbon extracts) in a mixture of seven enzyme-derived products (22.7% total conversion). Isolation of the major product by preparative TLC from an identical incubation with PR-AS allowed spectroscopic analysis of pure **3f**.

δ_H (500 MHz, $CDCl_3$, -50 °C) 0.82 (m) and 0.89 (t, *J* 7.5) (3 H, C*H*$_3$CH$_2$), 1.39 (s), 1.40 (s) and 1.42 (s) (6 H, (C*H*$_3$)$_2$CH), 1.47-1.65 (4 H, m, CH_2), 1.70 (1 H, s, CH_2), 1.74-1.84 (1 H, m, CH_2), 1.84-1.96 (3 H, m, CH_2), 2.20-2.35 (1 H, m, CH_2), 2.40-2.55 (1 H, m, (CH$_3$)$_2$C*H*), 4.48 (br s), 4.51 (br s), 4.55 (br s), 4.59 (br s), 4.66 (dd, *J* 4.0 and 8.0), 4.86 (m), 4.96-4.97 and 5.81 (t, *J* = 7.5) (4 H, 2 x C=CH and C=CH_2). δ_C (150 MHz, $CDCl_3$, resonances measured indirectly using HSQC and HMBC spectra at 0 °C) 13.3 (CH_3), 15.6 (CH_3), 19.9, 20.9, 24.5, 25.2, 32.3, 32.9, 34.6, 37.5, 38.3, 38.4, 51.1, 105.9 (C=*C*H$_2$), 120.5, (C=*C*H), 125.2 (C=*C*H), 125.8 (C=*C*H), 127.2 (C=*C*H), 131.4 (C=*C*H), 139.7 (C quaternary), 141.1 (C quaternary), 143.7 (C quaternary), 144.2 (C quaternary), 153.5 (C quaternary); HRMS (EI$^+$-TOF, M^+) Found 218.2028, $C_{16}H_{26}$ requires 218.2035.

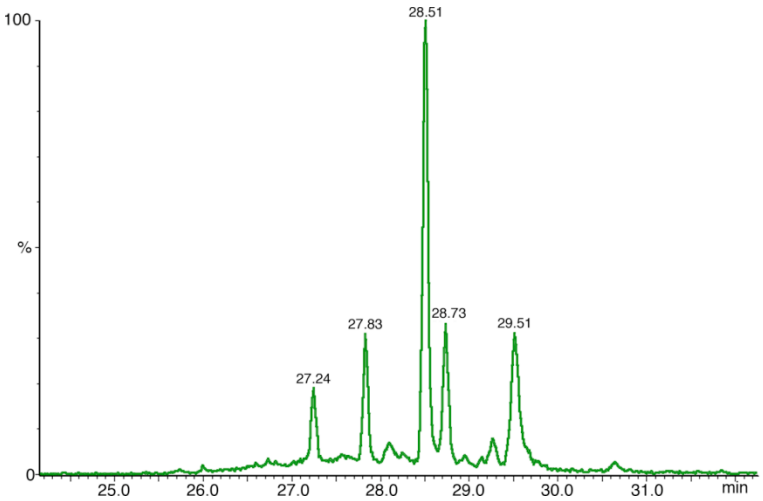


Figure S26. Total ion chromatogram of pentane extractable products isolated from incubation of 14Me-FDP (**1f**) with GAS. Injection temperature 100 °C.

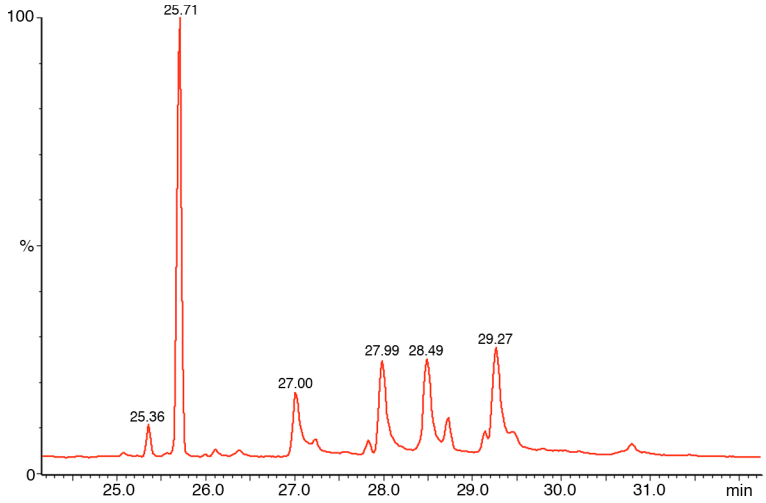


Figure S27. Total ion chromatogram of the pentane extractable products isolated from incubation of 14Me-FDP (**1f**) with GAS, subjected to thermal rearrangement conditions. Injection temperature 250 °C.

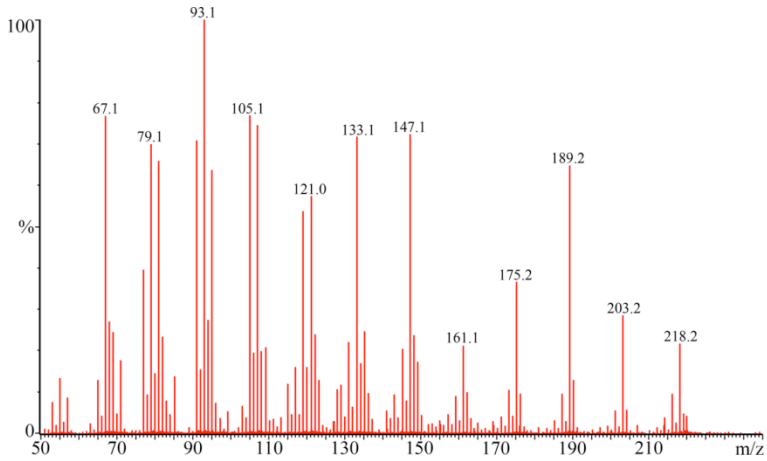


Figure S28. Mass spectrum of the major pentane extractable product (retention time 28.51) isolated from incubation of 14Me-FDP (**1f**) with GAS.

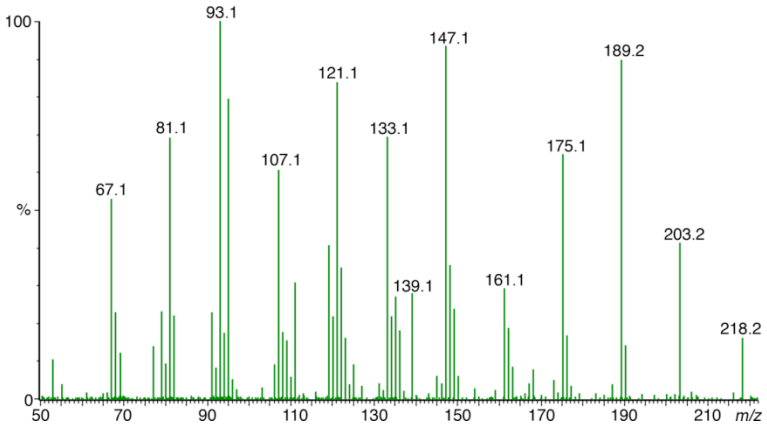


Figure S29. Mass spectrum of the pentane extractable product from incubation of 14Me-FDP (**1f**) and GAS, then subjected to thermal rearrangement (injection temperature 250 °C, retention time 25.71 min).

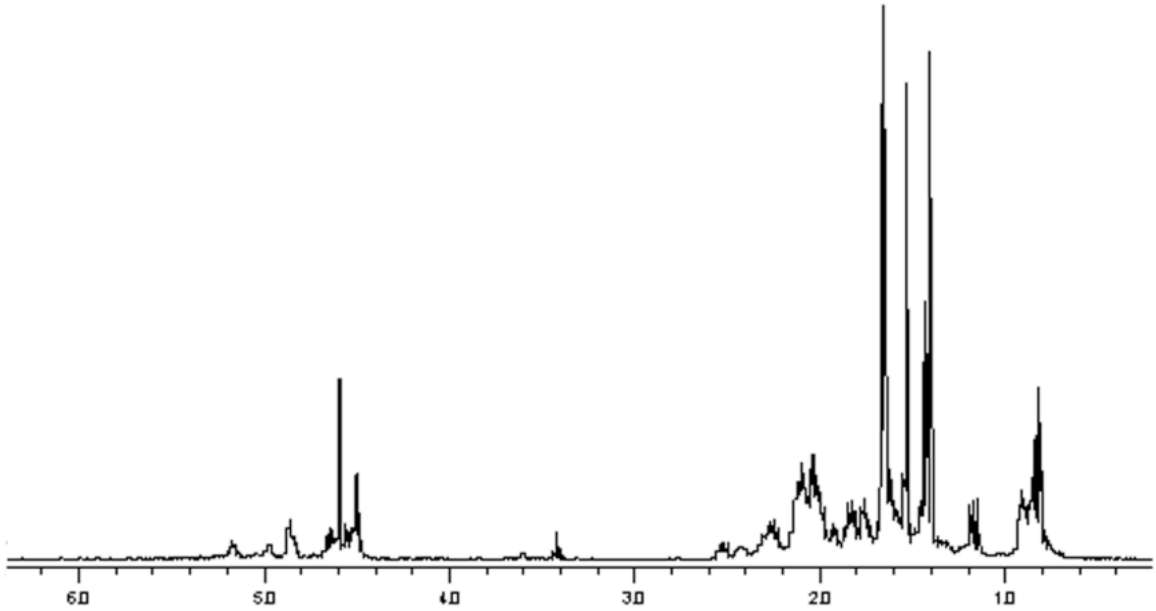


Figure S30. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$, 25 °C) of 14-methyl germacrene A isolated from incubation of **1f** with PR-AS.

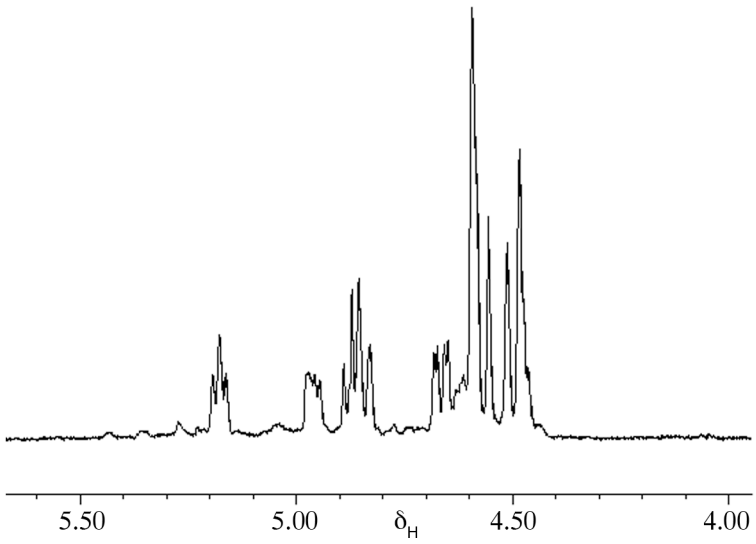


Figure S31. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$, -50 °C) of 14-methyl germacrene A isolated from incubation of **1f** with PR-AS. Image shows the expansion from δ_H 4.0-5.5 ppm showing the alkene protons of the 3 conformers (analogous to those observed for **3a**) that undergo slow exchange at higher temperatures.[14]

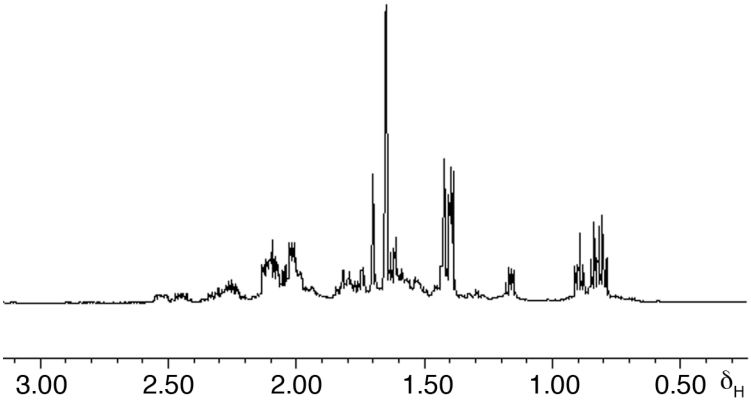


Figure S32. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$, -50 °C) of 14Me-germacrene A isolated from incubation of **1f** with PR-AS. Image shows the expansion from δ_H 0.5-3.0 ppm.

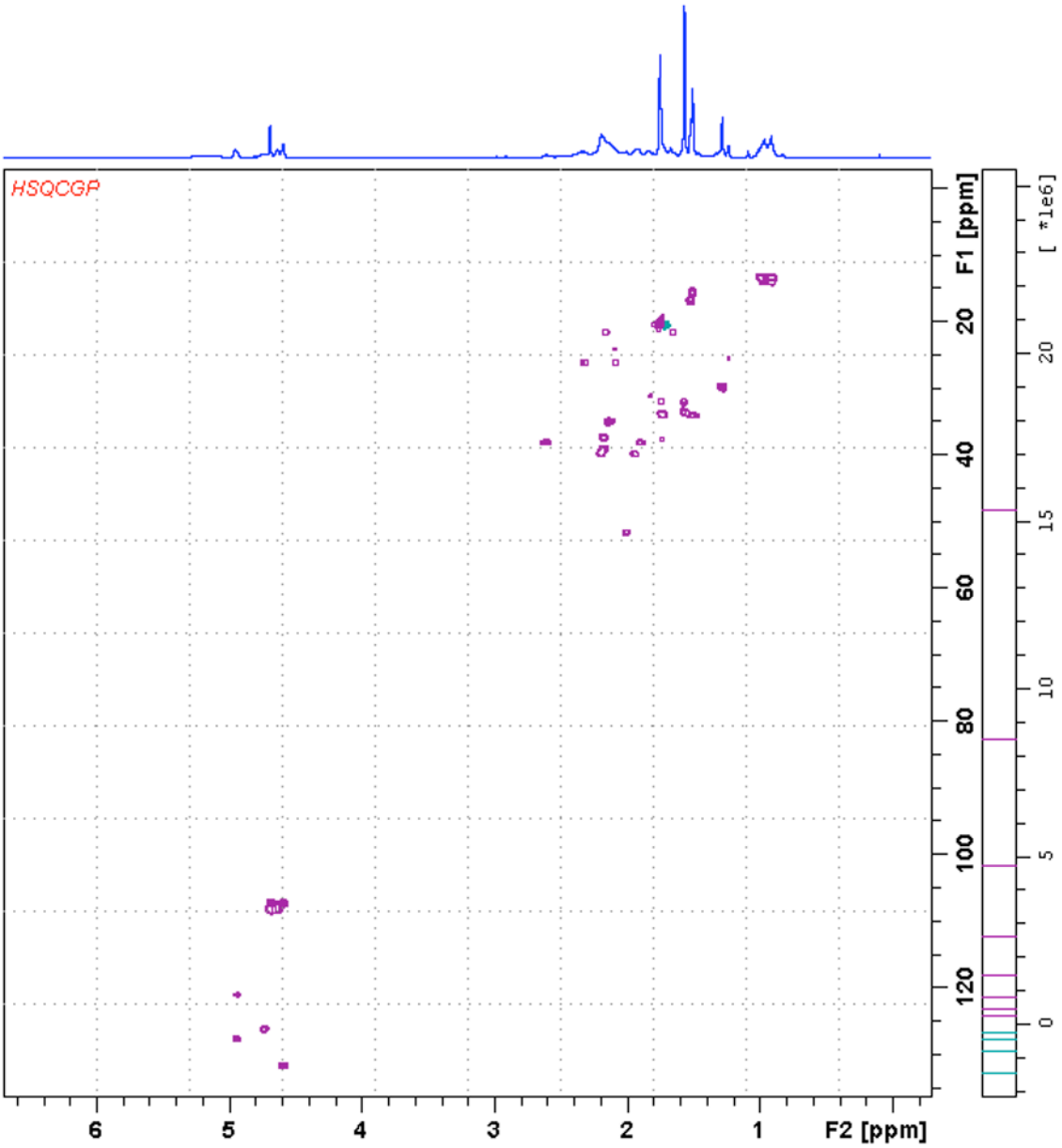


Figure S33. HSQC NMR spectrum (600 MHz, $CDCl_3$, 0 °C) of 14Me-germacrene A (**3f**) produced from incubation of **1f** with GAS.

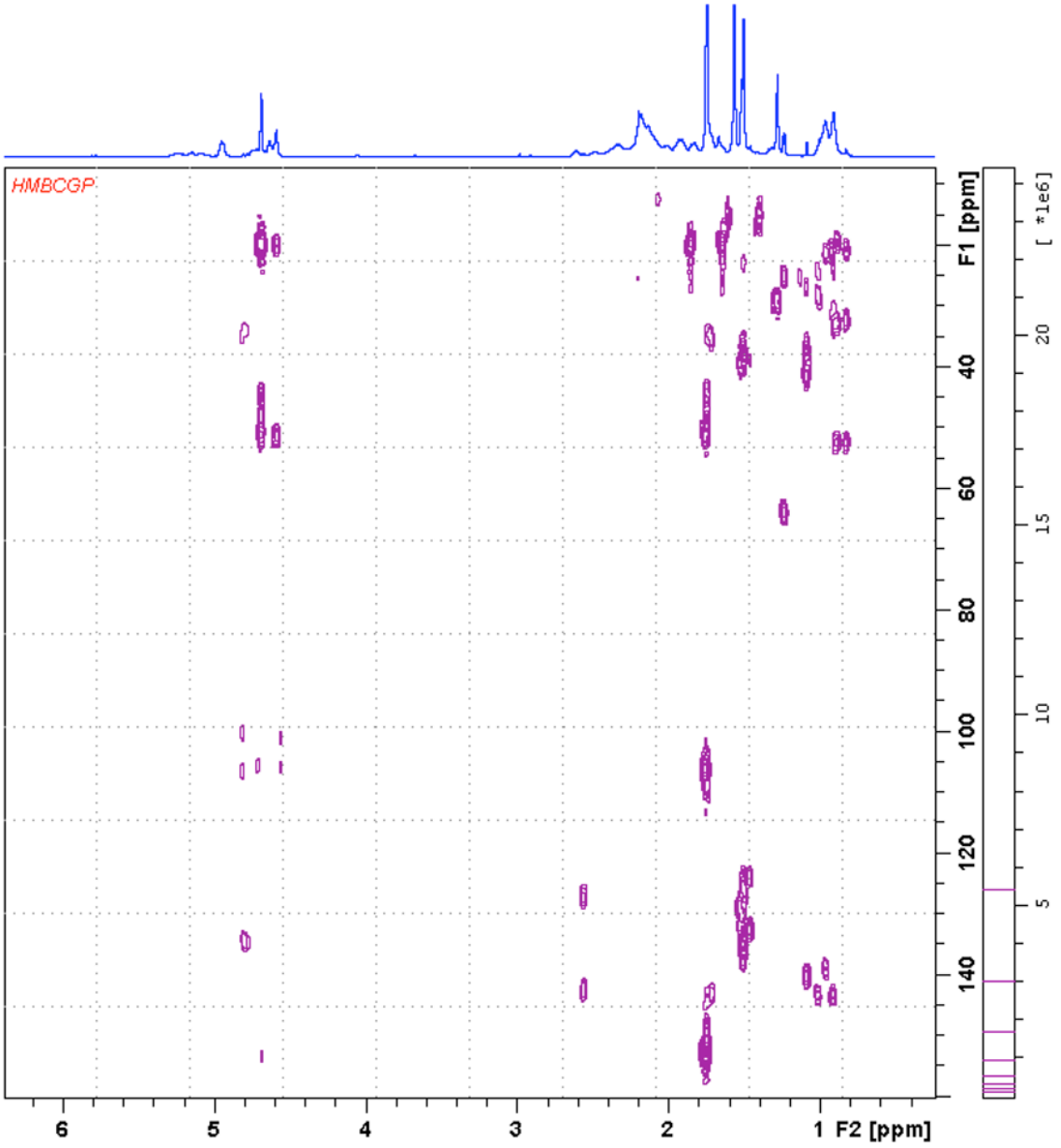


Figure S34. HMBC NMR spectrum (600 MHz, $CDCl_3$, 0 °C) of 14Me-germacrene A (**3f**) produced from incubation of **1f** with GAS.

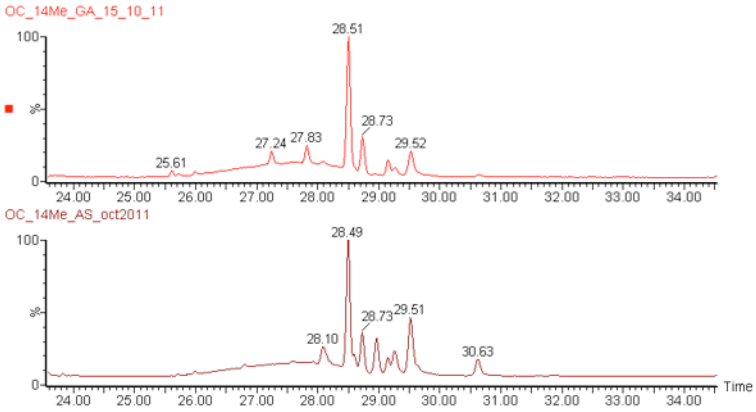


Figure S35. Comparison of the total ion chromatograms of the pentane extractable products from incubation of 14Me-FDP (**1f**) with GAS (top) and PR-AS (bottom).

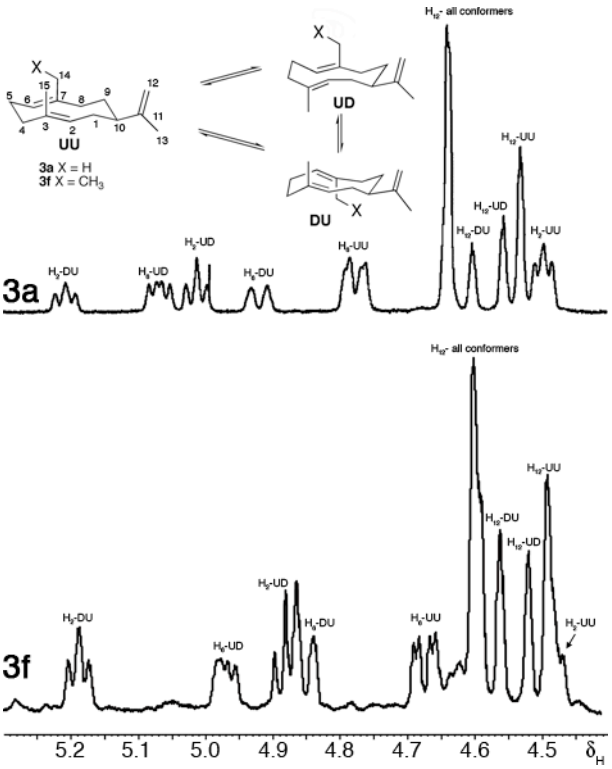


Figure S36. Comparison of the 4.3-5.4 ppm region of the ^{1}H-NMR spectra (500 MHz, $CDCl_3$) of (*R*)-germacrene A (**3a**)[14] and 14-methylgermacrene A (**3h**) recorded at -50 °C.

3f displayed the well documented conformational flexibility exhibited by germacrene A (**3a**),[14] which led to broad lines at ambient temperature in the ^{1}H-NMR spectrum and line sharpening on lowering the temperature with a coalescence temperature of -10 °C. A full conformational analysis of **3h** was not performed, but a comparison with the previously reported ^{1}H-NMR spectra obtained for the conformational analysis of **3a**[14] suggested that at room temperature and below **3h** exists as an interconverting mixture of the same three major conformers as **3a** albeit with different relative populations (Fig. S36).

Assignments of the proton resonances for each conformer of **3f** could be made by comparison with those of the parent hydrocarbon **3a** (Fig. S34). As with **3a**, the most abundant conformation is the 'crossed up-up'[14] form (UU), where two double bonds of the 10-membered ring are approximately perpendicular (crossed) and the alkyl groups at C3 and C7 are both 'up', i.e. cis or syn. The most upfield resonance observed for the olefinic protons at δ_H = 4.48 ppm is a multiplet corresponding to one of the methylene protons on C12 and the methine proton at C2 (UU conformer of **3f**). For **3a** these protons had similar chemical shifts albeit with better resolution.[14] Another noticeable difference between **3a** and **3f** at -50 °C is the overlapping of the signals (δ_H = 4.85 ppm) corresponding to both the methine protons at C2 (UD conformer) and C6 (DU conformer). Well resolved resonances for each of the three conformers at δ_H = 5.18 (H_2-DU, 29%), 4.97 (H_6-UD, 32%) and 4.67 ppm (H_6-UU, 39%) allow estimation of the relative populations of conformers through peak integration. In contrast to **3a**, it is clear that together the 'parallel down-up' DU and 'up-down' UD conformations of **3f** (featuring parallel double bonds and anti sp^2-linked alkyl groups) dominate (61%) over the 'crossed up up' UU conformation (UU) (39%). This conformational distribution is consistent with the increased steric bulk on C14 of **3f** relative to **3a** that likely raises the energy of the UU conformation slightly due to steric clash between the alkyl groups on C3 and C7. This steric interaction is relieved in the UD and DU conformers. Nevertheless, the three observable conformers of **3f** are approximately equally populated at -50 °C in chloroform.

(*S*)-Germacrene D (5a)

1a was incubated with GDS as described above giving germacrene D (**5a**) as the sole pentane extractable product in 76.0% conversion.

δ_H (500 MHz, $CDCl_3$) 0.74 and 0.79 (2 x d, *J* 7.0, $(CH_3)_2CH$), 1.18-1.27 (3 H, m, $(CH_3)_2CH$ and $(CH_3)_2CHCH_2$), 1.50 (3 H, s, $CH_3C{=}CH$), 1.86-1.97, 2.00-2.05, 2.10-2.25, and 2.27-2.39 (7 H, m, $H_2C{=}CCH_2CH_2$, $(CH_3)_2CHCHCH_2$), 4.67 (1 H, d, *J* 2.5, 1 x $C{=}CH_2$) and 4.72 (1 H, dd, *J* 0.5 and 2.5, 1 x $C{=}CH_2$), 5.06 (1 H, dd, *J* = 11.5, and 4.5, $CH_3C{=}CH$), 5.18 (1 H, dd, *J* = 16.0 and 10.0, $H_2C{=}C{-}CH{=}CH$) 5.71 (1 H, d, *J* 16.0, $H_2C{=}C{-}CH{=}CH$); δ_C (125 MHz, $CDCl_3$) 15.90 (CH_3), 19.34 (CH_3), 20.75 (CH_3), 26.54 (CH_2), 29.28 (CH_2), 32.78 (CH_2), 34.54 (CH_2), 40.75 (CH), 52.97 (CH), 109.0 ($C{=}CH_2$), 129.7 ($C{=}CH$), 133.6 ($C{=}CH$), 134.0 ($C{=}CH$), 135.5 (C quaternary), 148.9 (C quaternary); HRMS (EI^+, M^+) Found 204.1881; $C_{15}H_{24}$ requires 204.1878.

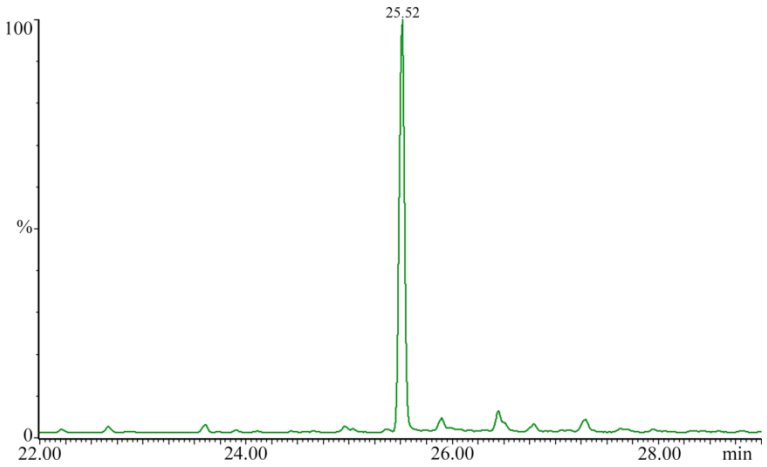


Figure S37. Total ion chromatogram of the pentane extractable products from incubation of FDP (**1a**) with GDS.

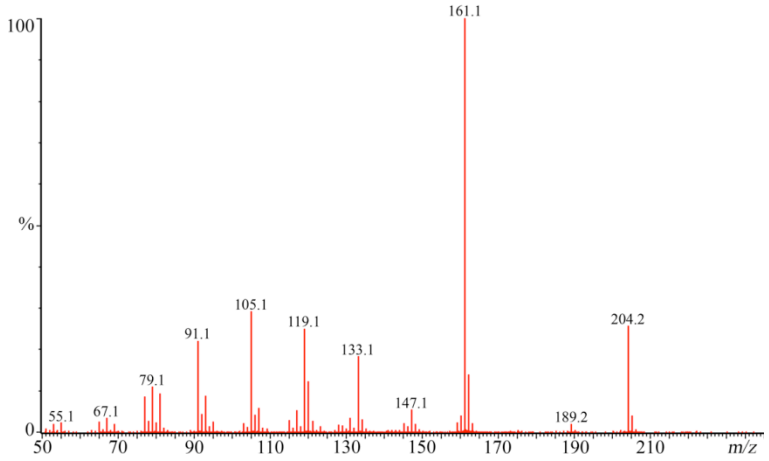


Figure S38. Mass spectrum of germacrene D isolated from incubation of FDP (**1a**) with GDS.

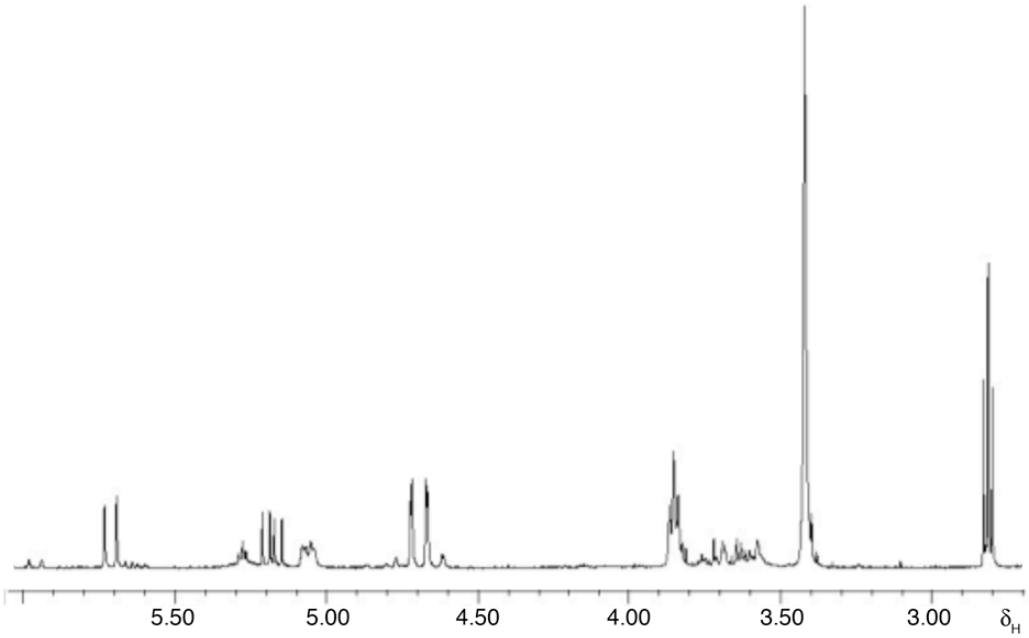


Figure S39. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of germacrene D in the region δ_H = 2.5-6.0 ppm.

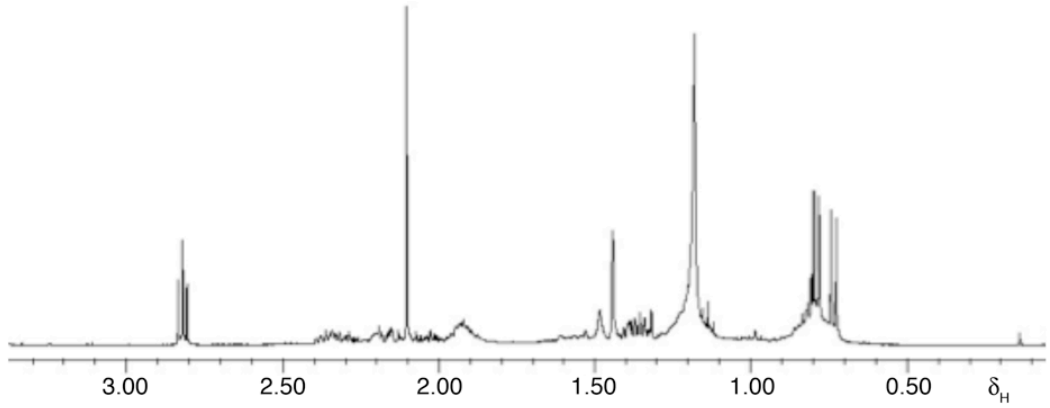


Figure S40. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of germacrene D in the region δ_H = 0.0-3.5 ppm.

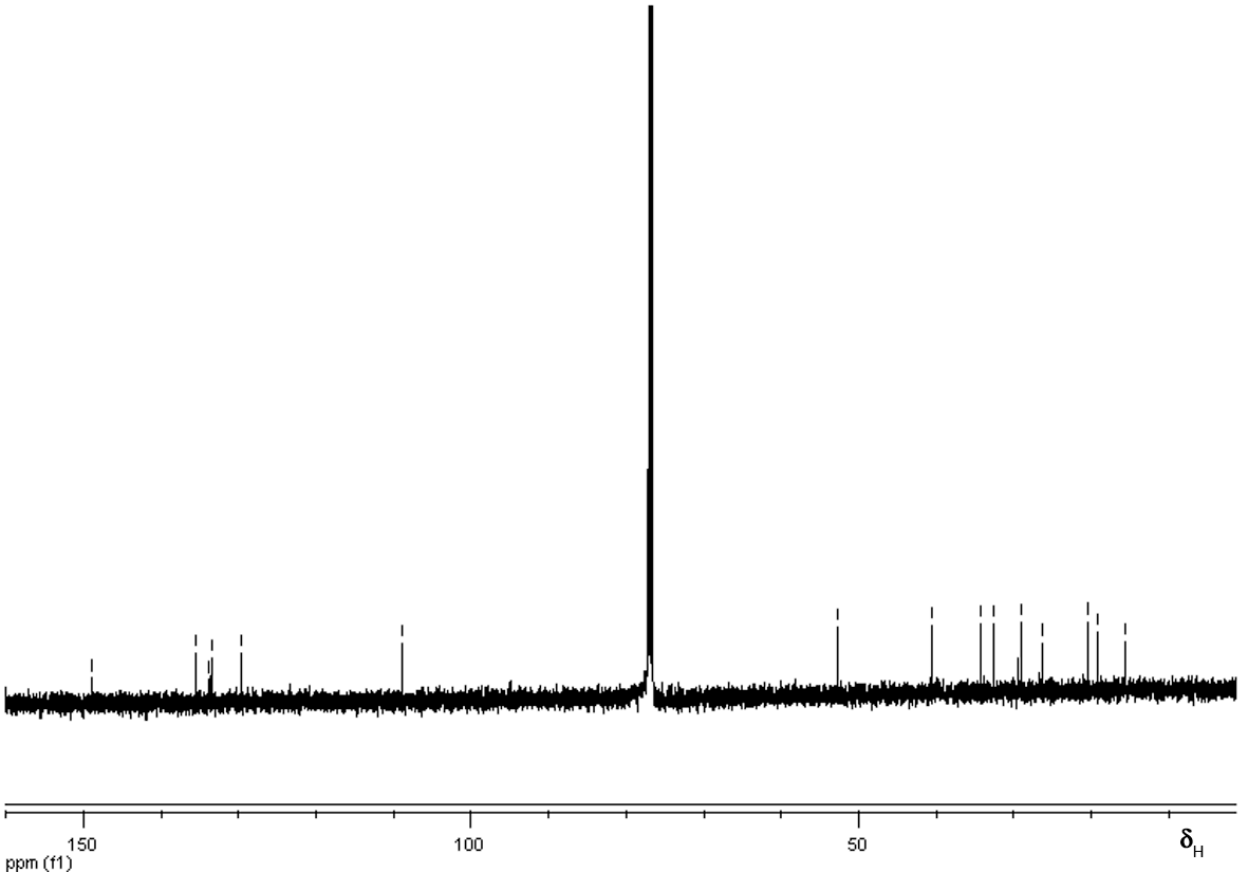


Figure S41. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of germacrene D isolated from incubation of **1a** with GDS.

6F-Germacrene D (5b)

1b was incubated with GDS as described above giving 6F-germacrene D (**5b**) as the sole pentane extractable product in 37.6% conversion.

δ_H (500 MHz, $CDCl_3$) 0.79 and 0.81 (2 x d, *J* 6.5, $(CH_3)_2CH$), 0.84-0.95 (3 H, m, $(CH_3)_2CH$ and $(CH_3)_2CHCH_2$), 1.34 (3 H, d, $J_{H\text{-}F}$ = 2.5, $CH_3C{=}CF$), 1.76-1.87, 1.97-1.99. 2.05-2.10, 2.18-2.35 and 2.60-2.74 (7 H, m, CH_2CH_2CF, $(CH_3)_2CHCHCH_2$), 4.68 and 4.78 (2 H, 2 x s, $C{=}CH_2$), 5.75 (1 H, ddd, *J* = 16.5, 9.5 and 2, $H_2C{=}C\text{-}CH{=}CH$), 6.06 (1 H, d, *J* = 16.5, $H_2C{=}C\text{-}CH{=}CH$); δ_C (150 MHz, $CDCl_3$, resonances measured indirectly by HSQC at 0 °C) 15.4 (CH_3) 17.2 (CH_3) 20.4 (CH_3), 27.1 (CH_2), 28.9 (CH_2), 29.4 (CH), 29.5 (CH_2), 30.7 (CH_2), 31.0 (CH_2) 48.9 (CH), 112.3 ($C{=}CH_2$), 115.2 ($CH_3C{=}CF$), 128.3 ($CH_2{=}CCH{=}CH$), 138.5 ($CH_2{=}CCH{=}CH$), 148.2 ($C{=}CH_2$), 154.7 (d, J_{CF} 248.5, $C{=}CF$); δ_F (282 MHz, C^2HCl_3,) -105.4 (d, *J* = 41.5); HRMS (EI^+-TOF, M^+) Found 222.1785, $C_{15}H_{23}F$ requires 222.1784;.

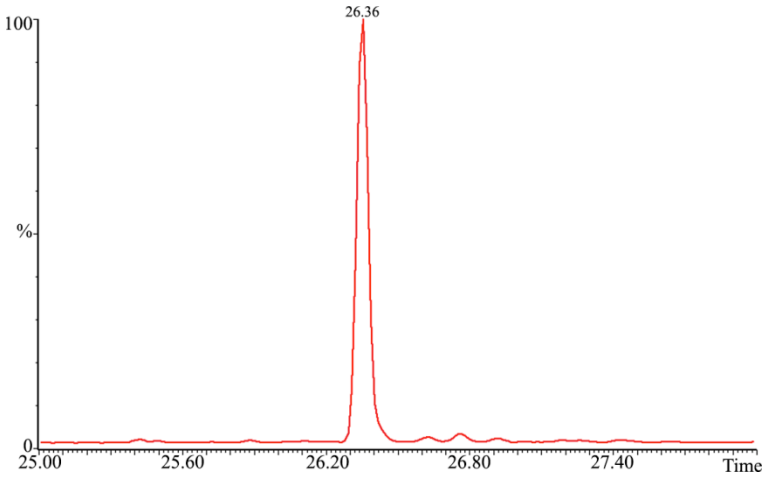


Figure S42. Total ion chromatogram of the pentane extractable products from incubation of 6F-FDP (**1b**) with GDS.

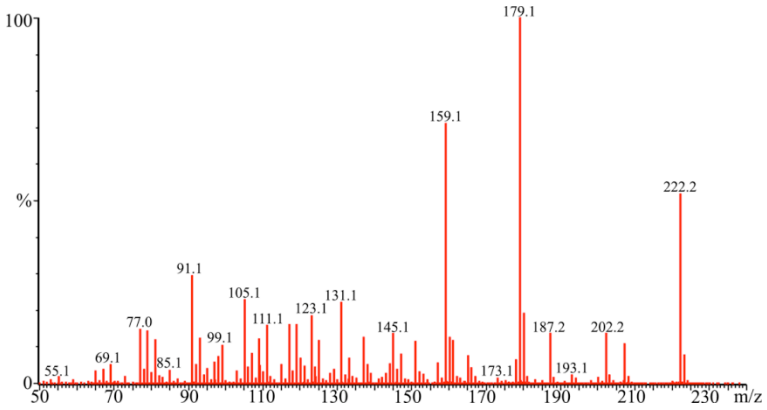


Figure S43. Mass spectrum of 6F-germacrene D (**5b**) isolated from incubation of 6F-FDP (**1b**) with GDS.

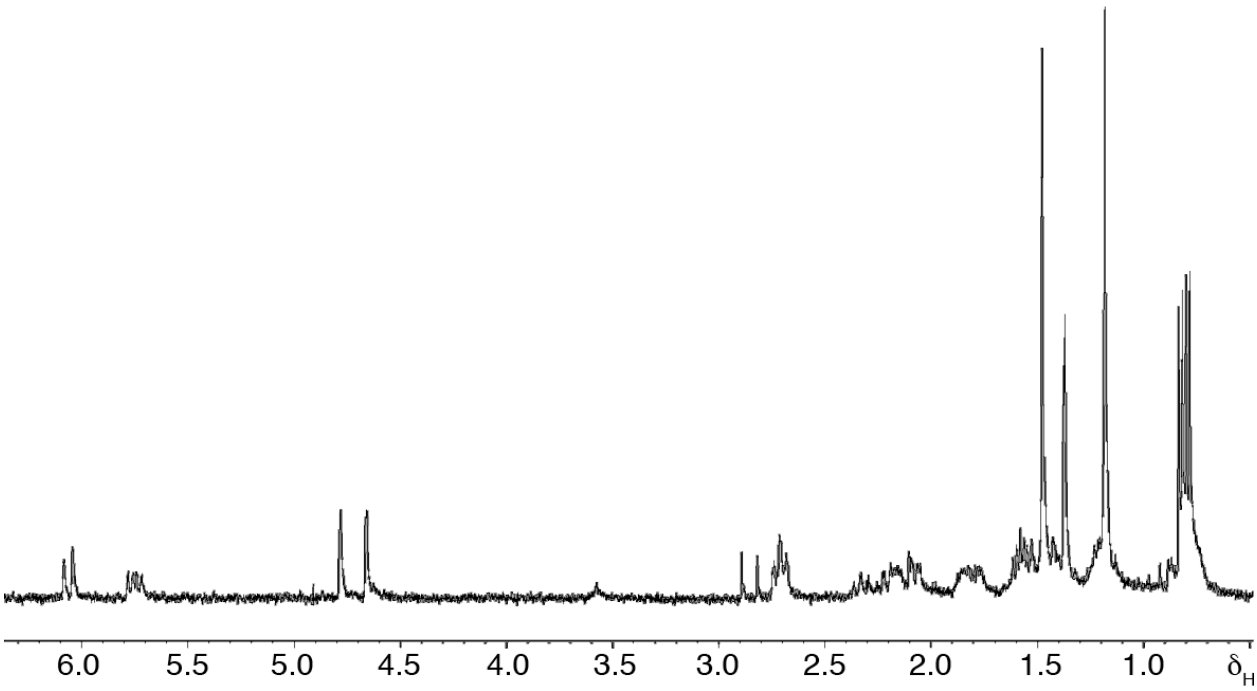


Figure S44. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of 6F-germacrene D isolated from incubation of **1b** with GDS.

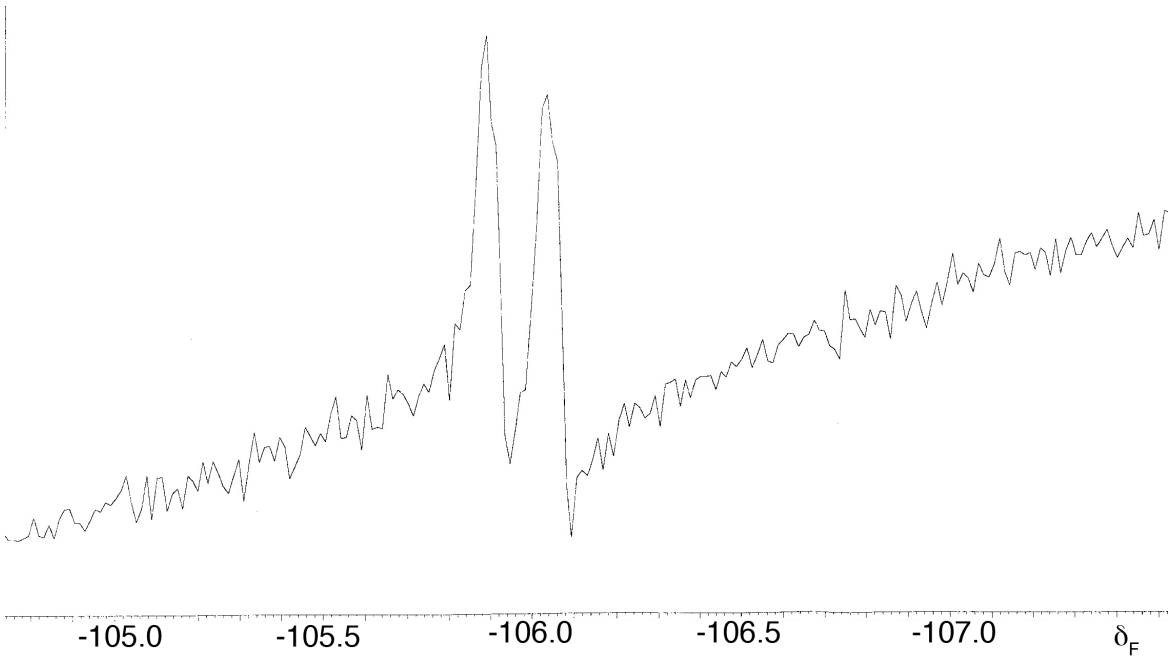


Figure S45. ^{19}F-NMR spectrum (282 MHz, $CDCl_3$) of 6F-germacrene D (**5b**) isolated from incubation of **1b** with GDS.

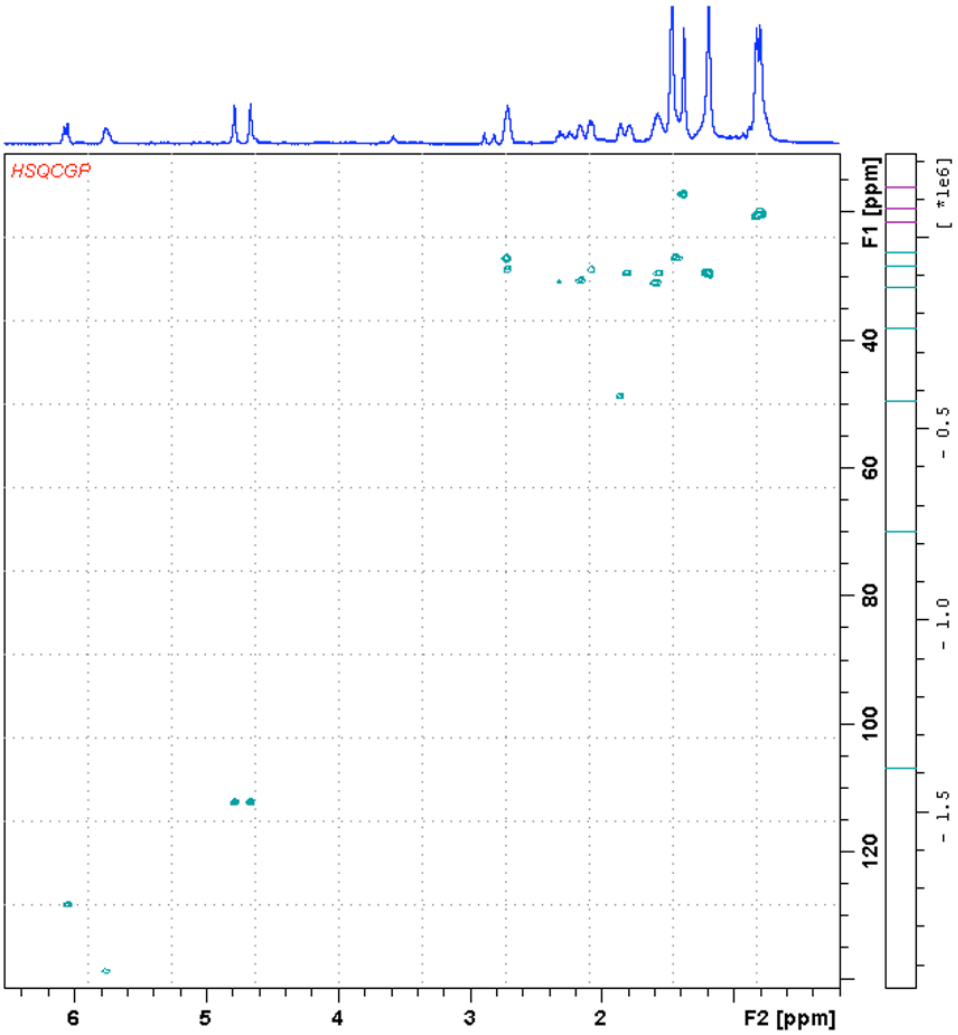


Figure S46. HSQC NMR spectrum (600 MHz, $CDCl_3$) of 6F-germacrene D (**5b**) isolated from incubation of **1b** with GDS.

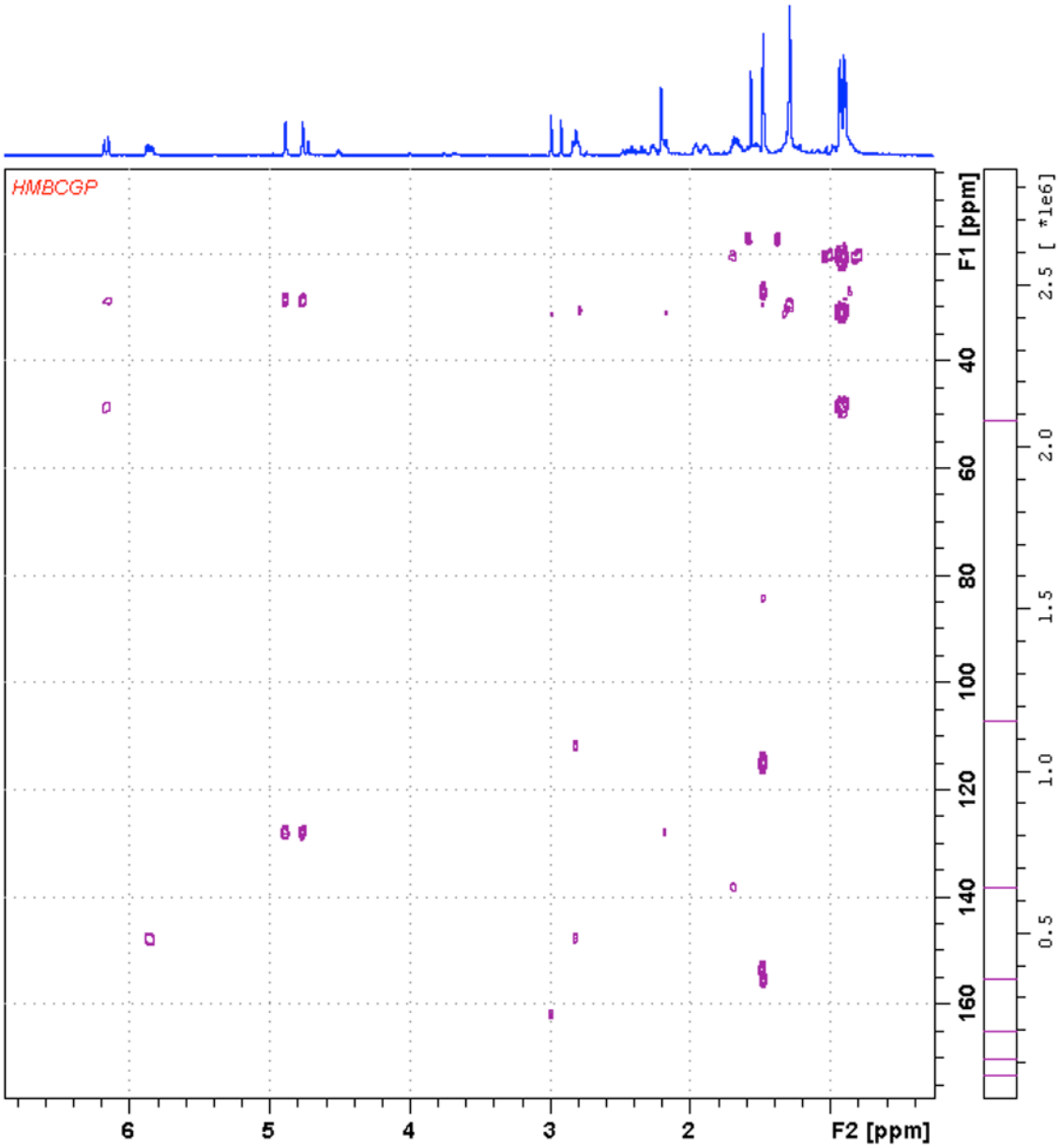


Figure S47. HMBC NMR spectrum (600 MHz, $CDCl_3$) of 6F-germacrene D (**5b**) isolated from incubation of **1b** with GDS.

10F-(*E*)-β-Farnesene (7c)

1c was incubated with GDS giving a mixture of **7c** and **6c** in 3:1 ratio (5.8% total conversion). The two compounds were identified by co-elution with authentic material as described in the main text.

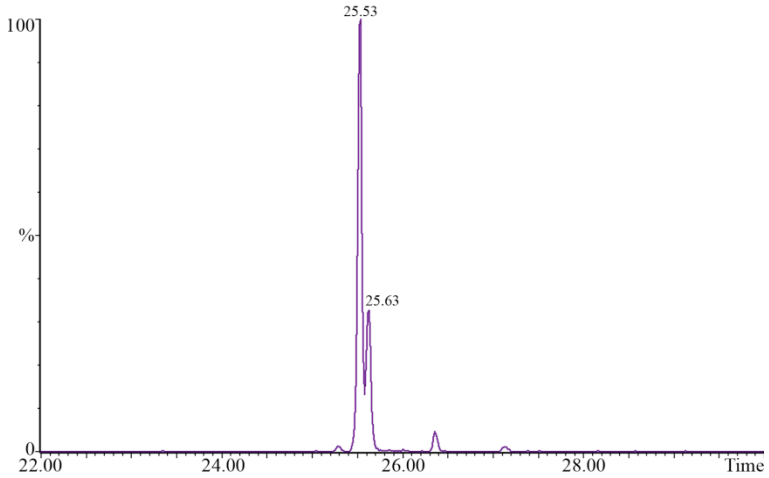


Figure S48. Total ion chromatogram of the pentane extractable products from incubation of 10F-FDP (**1c**) with GDS.

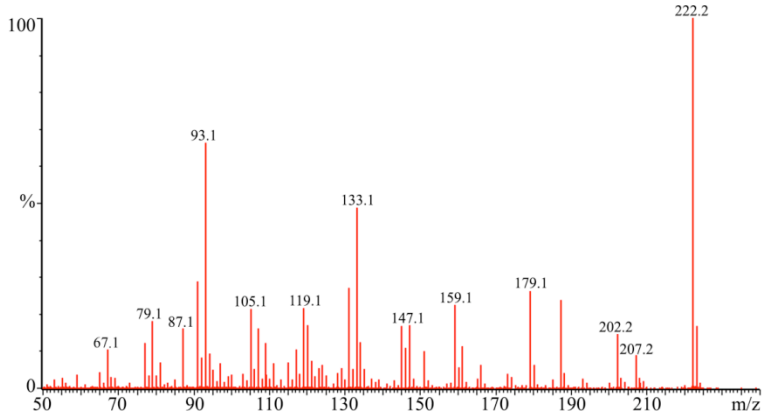


Figure S49. Mass spectrum of major pentane extractable product (retention time 25.53min) from incubation of 10F-FDP (**1c**) with GDS.

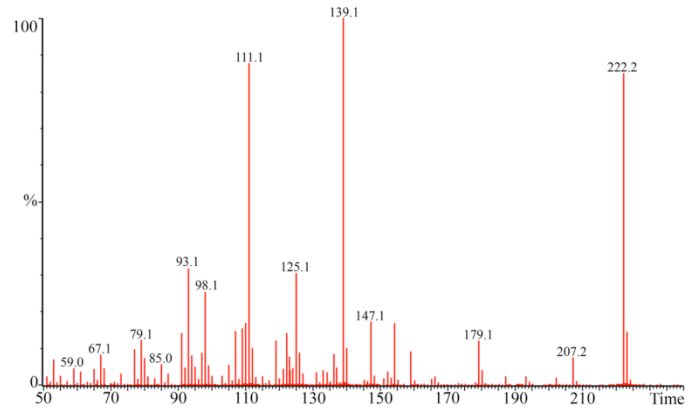


Figure S50. Mass spectrum of minor pentane extractable product (retention time 25.63min) from incubation of 10F-FDP (**1c**) with GDS.

14F-Germacrene D (5d)

1d was incubated with GDS giving a mixture (7.6% conversion) of what was tentatively identified as **5d** and an unknown breakdown product that was tentatively identified as an HF elimination product from ^{1}H and ^{19}F NMR spectroscopic and GC-MS analysis. HRMS (EI^{+}-TOF, M^{+}) Found 222.1789, $C_{15}H_{23}F$ requires 222.1784.

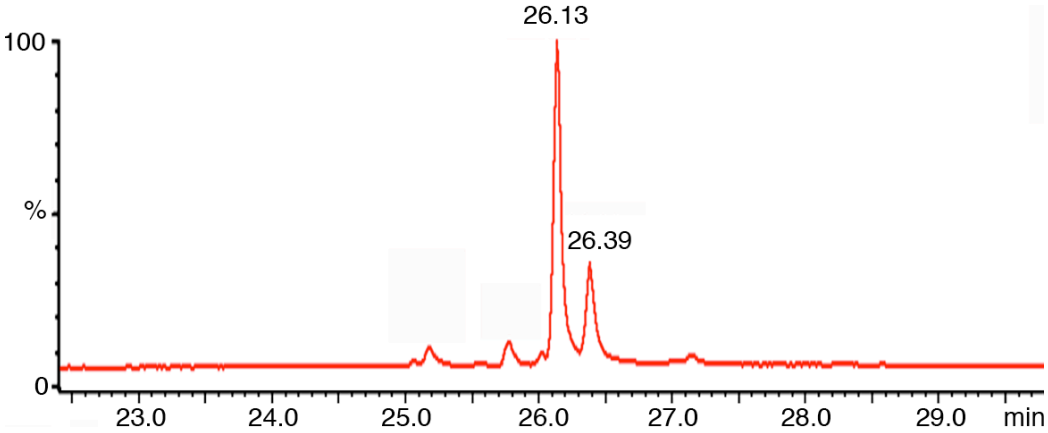


Figure S51. Total ion chromatogram of pentane extractable products isolated from preparative incubation of 14F-FDP (**1d**) with GDS. Note that the product eluting at 26.13 min is the sole product detected in analytical incubations of **1d** with GDS over 16 hr. When time was extended

to several days (as was the case for preparative scale incubations) then the second product eluting at 26.39 min was also observed in analytical incubations.

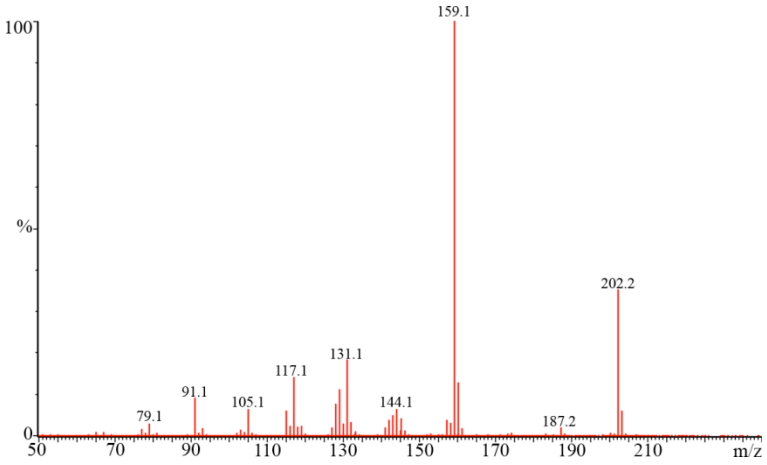


Figure S52. Mass spectrum of major product (retention time 26.13 min) isolated from incubation of 14F-FDP (**1d**) with GDS.

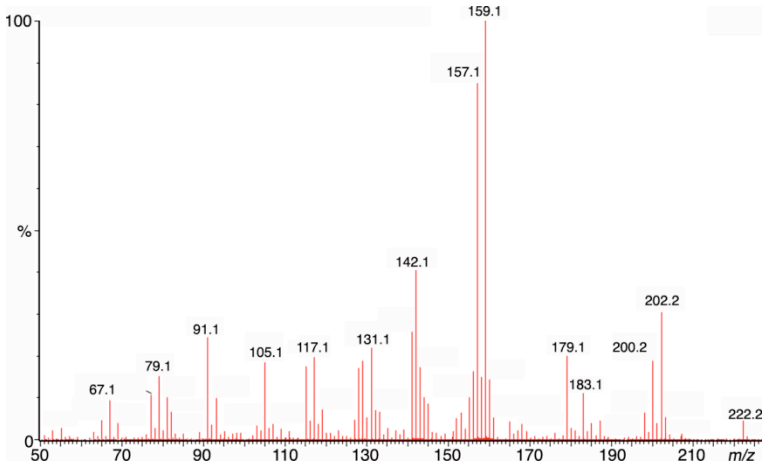


Figure S53. Mass spectrum of minor product (retention time 26.39 min) isolated from incubation of 14F-FDP (**1d**) with GDS.

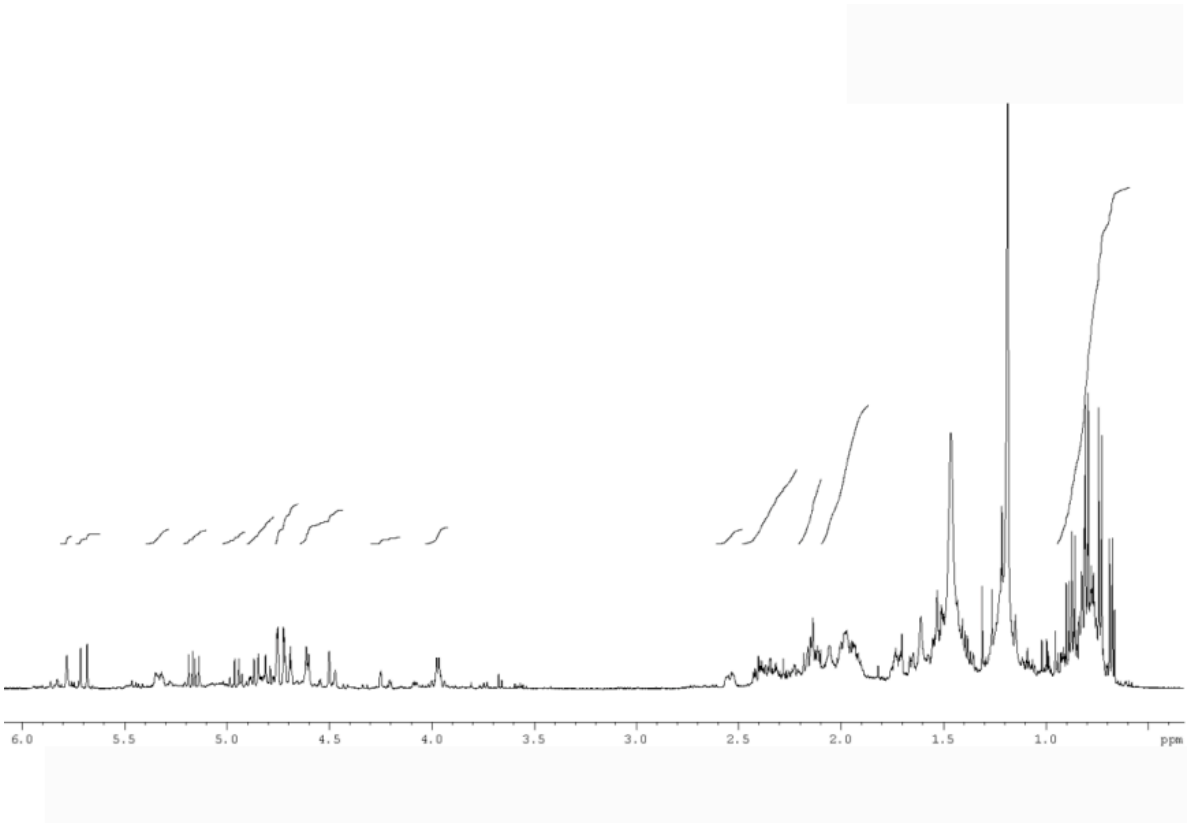


Figure S54. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of product mixture isolated from incubation of 14F-FDP (**1d**) and GDS.

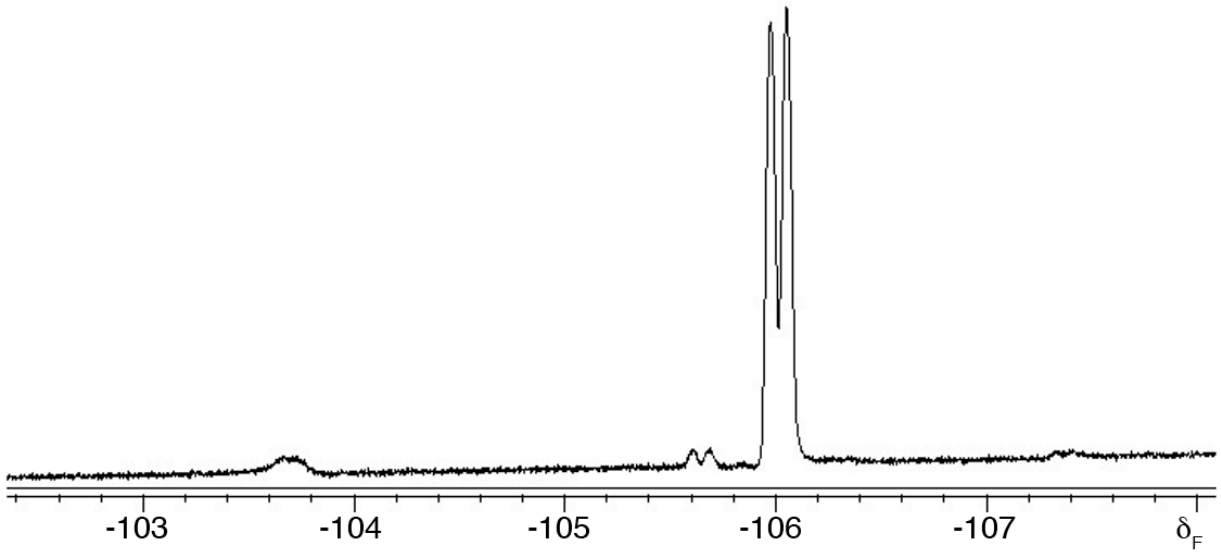


Figure S55. ^{19}F-NMR (565 MHz, $CDCl_3$) of the chloroform extractable products from incubation of 14F-FPP (**1d**) and GDS.

15F-Germacrene D (5e)

No pentane extractable products were isolated from incubation of 15F-FDP (**1e**) with GAS.

1e was incubated with GDS giving a mixture (36.9% total conversion) of what was tentatively identified as **5e** and an unknown breakdown product from ^{1}H and ^{19}F NMR spectroscopic and GC-MS analysis. It was possible to tentatively identify the major product as a germacrene D analogue by comparison of the mass spectrum with germacrene D itself and identify the expected exomethylene proton coupled to fluorine as a wide doublet ($J_{H\text{-}F}$ = 86.0 Hz) δ_H = 6.53 ppm, this was matched by observation of two doublets with the same coupling constant in the ^{19}F NMR spectrum.

δ_F (282 MHz, $CDCl_3$) -138.5 (J_{HF} = 86.0), -136.1 (J_{HF} = 86.0), 184.8 (J_{HF} = 45.0 Hz). HRMS (EI^+-TOF, M^+) Found 222.1789, $C_{15}H_{23}F$ requires 222.1784.

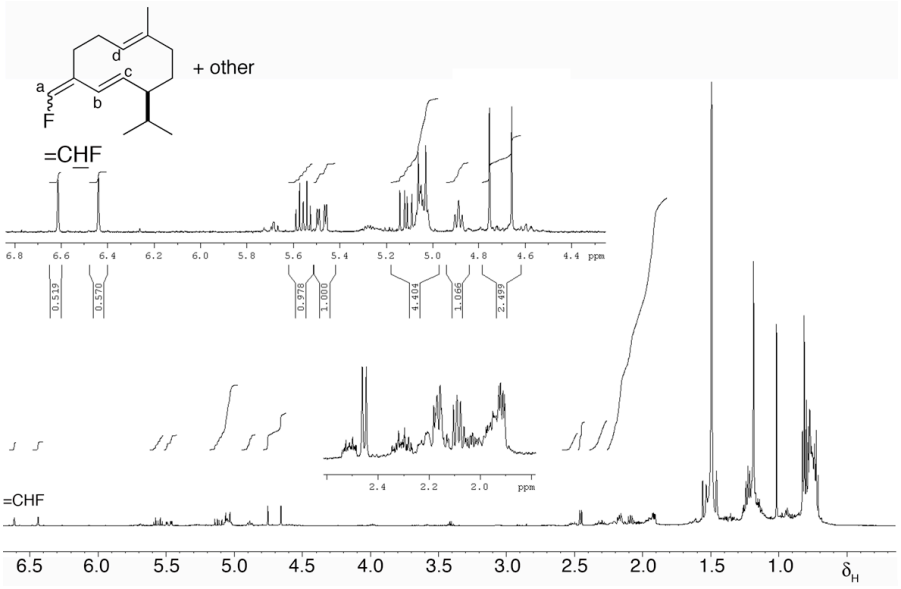


Figure S56. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of the pentane extractable products from incubation of 15F-FDP (**1e**) and GDS.

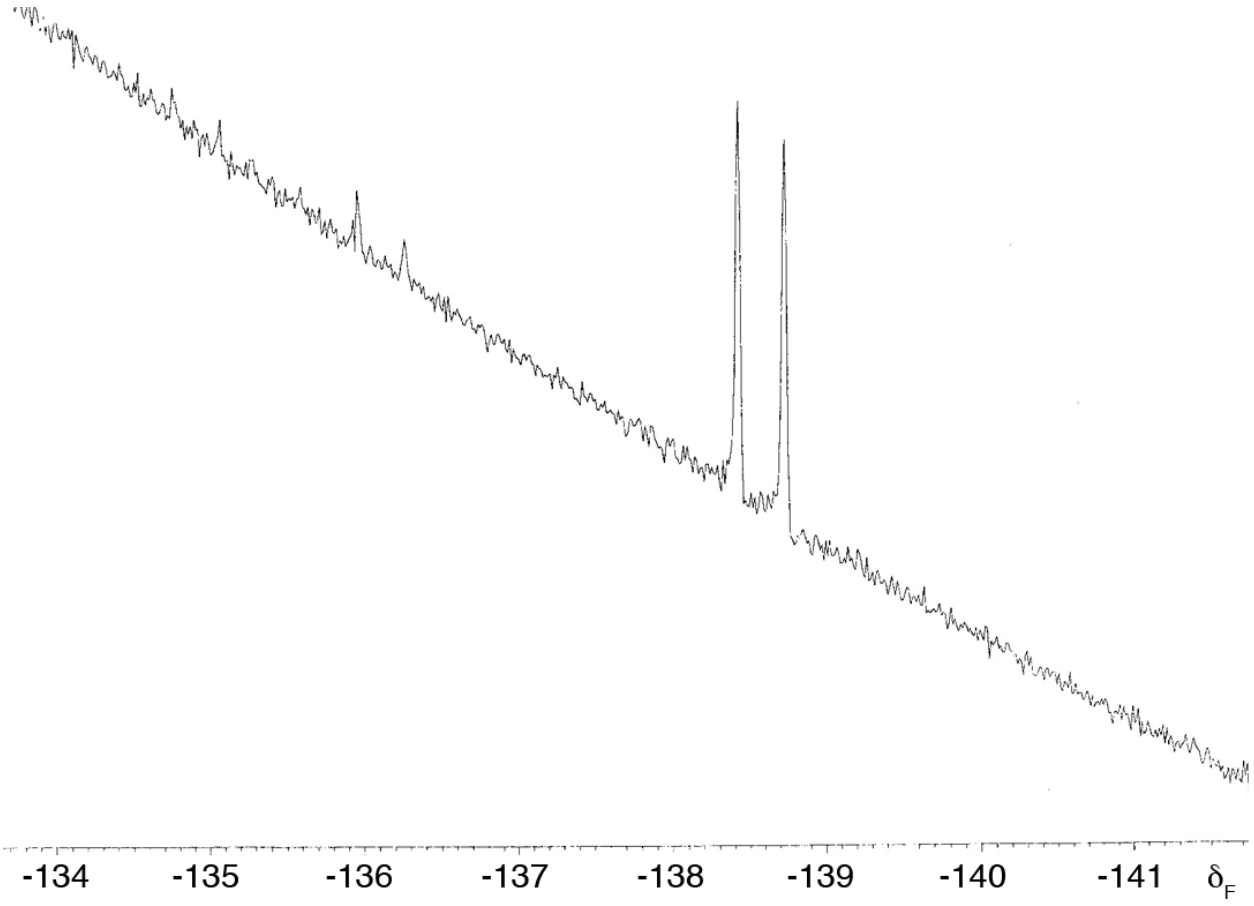


Figure S57. ^{19}F-NMR spectrum (282 MHz, $CDCl_3$) of the major pentane extractable product from incubation of 15F-FDP (**1e**) and GDS.

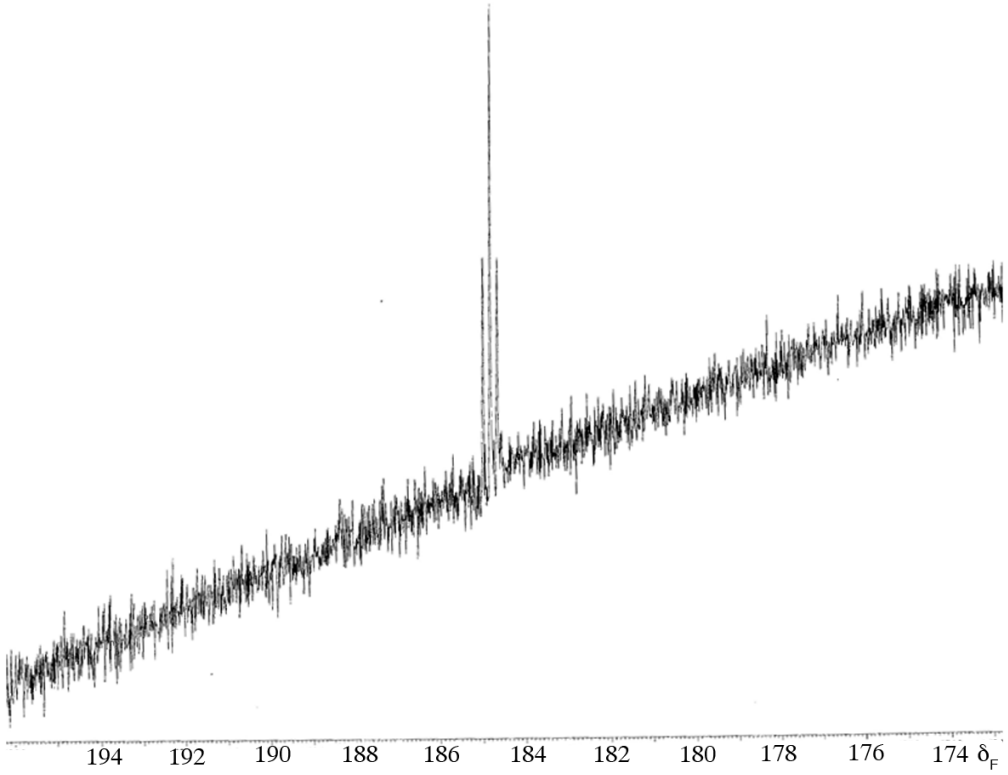


Figure S58. ^{19}F-NMR spectrum (282 MHz, $CDCl_3$) of the minor pentane extractable product from incubation of 15F-FDP (**1e**) and GDS.

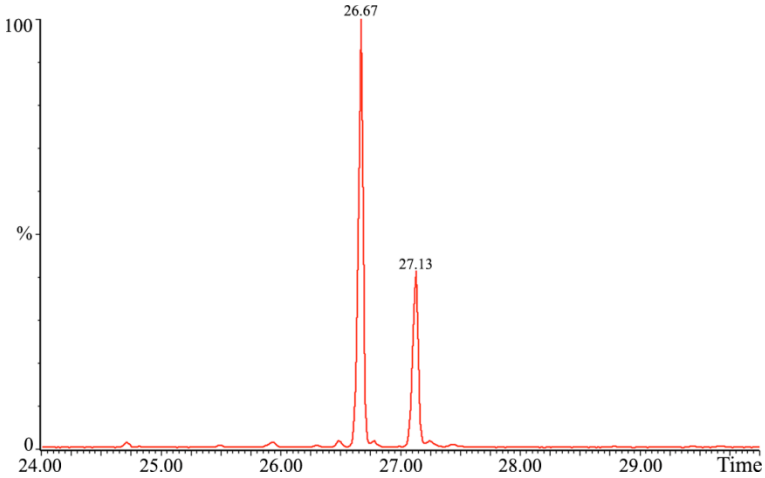


Figure S59. Total ion chromatogram of pentane extractable products isolated from preparative incubation of 15F-FDP (**1e**) and GDS.

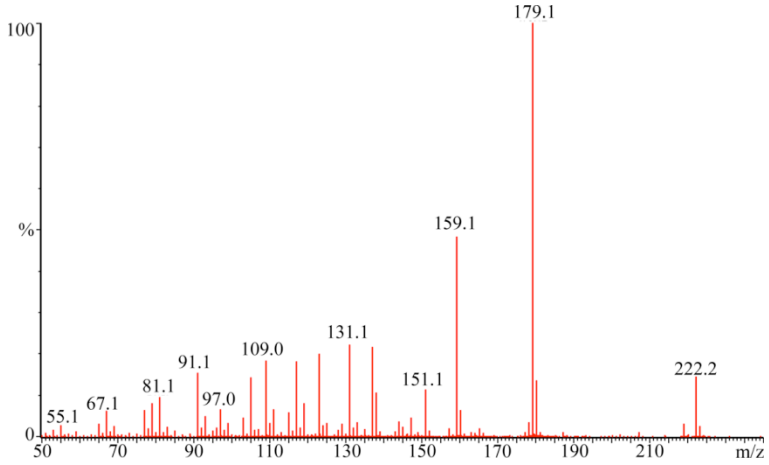


Figure S60. Mass spectrum of major product (retention time 26.67 min) from preparative incubation of 15F-FDP (**1e**) with GDS.

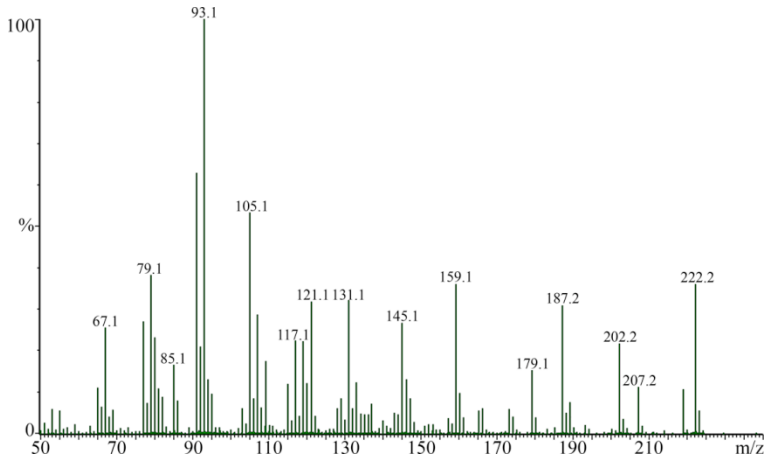


Figure S61. Mass spectrum of minor product (retention time 27.13 min) from preparative incubation of 15F-FDP (**1e**) with GDS.

14Me-Germacrene D (5f)

1f was incubated with GDS giving **5f** as the sole pentane extractable product (44.7% conversion).

δ_H (500 MHz, $CDCl_3$) 0.73 and 0.79 (2 x 3 H, 2 x d, *J* = 6.5, $(CH_3)_2CH$), 0.87 (3 H, t, *J* = 7.5, CH_3CH_2), 0.74-0.82 (2 H, m, $(CH_3)_2CHCHCH_2$), 1.20-1.31, 1.31-1.43, 1.58-1.65, 1.84-2.25 (6 H, m, $CH_3CH_2CCH_2$, $H_2C{=}CCH_2$, $(CH_3)_2CHCH$ and $(CH_3)_2CH$), 4.69 and 4.71 (2 x br s, $H_2C{=}C$), 4.99 (1 H, dd, *J* = 11 and 5, $CH{=}CCH_2CH_3$), 5.12 (1 H, dd, *J* = 16 and 10, $H_2C{=}C\text{-}CH{=}CH$), 5.70 (1 H, d, *J* 16, $H_2C{=}C\text{-}CH{=}CH$); δ_C (600 MHz, $CDCl_3$, peaks measured indirectly via HSQC and HMBC spectra at 0 °C) 12.6 (CH_3CH_2), 19.3 and 20.7 ($(CH_3)_2CH$), 26.8 (CH_2), 28.7 (CH_2), 29.6 (CH_3CH_2), 32.8 (CH), 34.5 (CH_2), 36.8 (CH_2), 52.6 (CH), 109.4 ($C{=}CH_2$), 129.9 ($CH_3CH_2C{=}CH$), 134.8 ($H_2C{=}C\text{-}CH{=}CH$), 135.4 ($H_2C{=}C\text{-}CH{=}CH$), 139.2 and 148.7 (2 x C quaternary); HRMS (EI^+-TOF, M^+) Found 218.2039, $C_{16}H_{26}$ requires 218.2035.

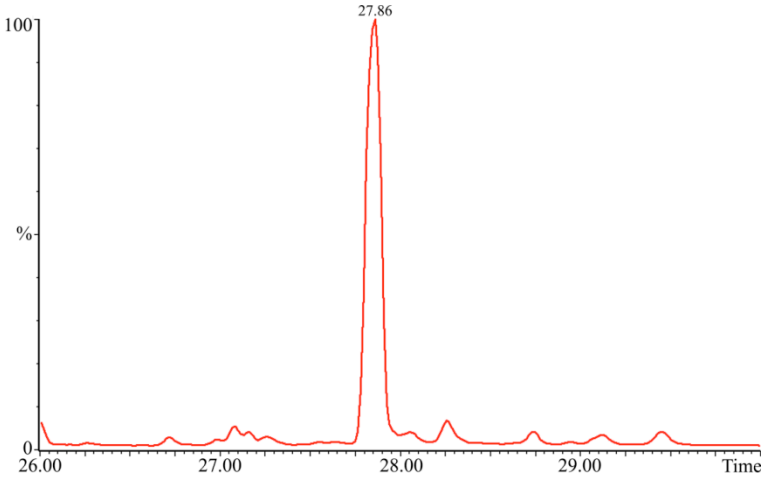


Figure S62. Total ion chromatogram of the pentane extractable products isolated from incubation of 14Me-FDP (**1f**) with GDS.

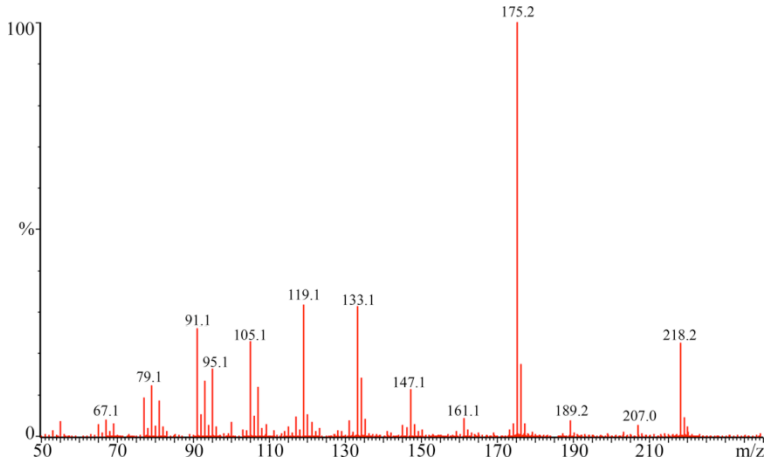


Figure S63. Mass spectrum of the pentane extractable product isolated from incubation of 14Me-FDP (**1f**) with GDS.

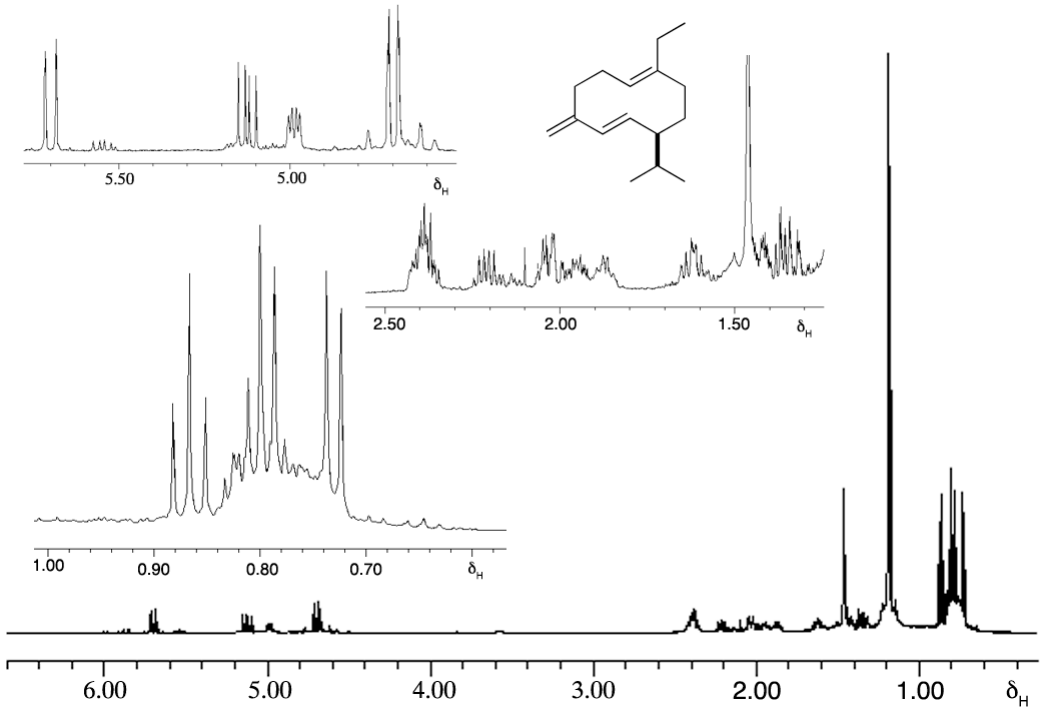


Figure S64. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of 14Me-germacrene D (**5f**).

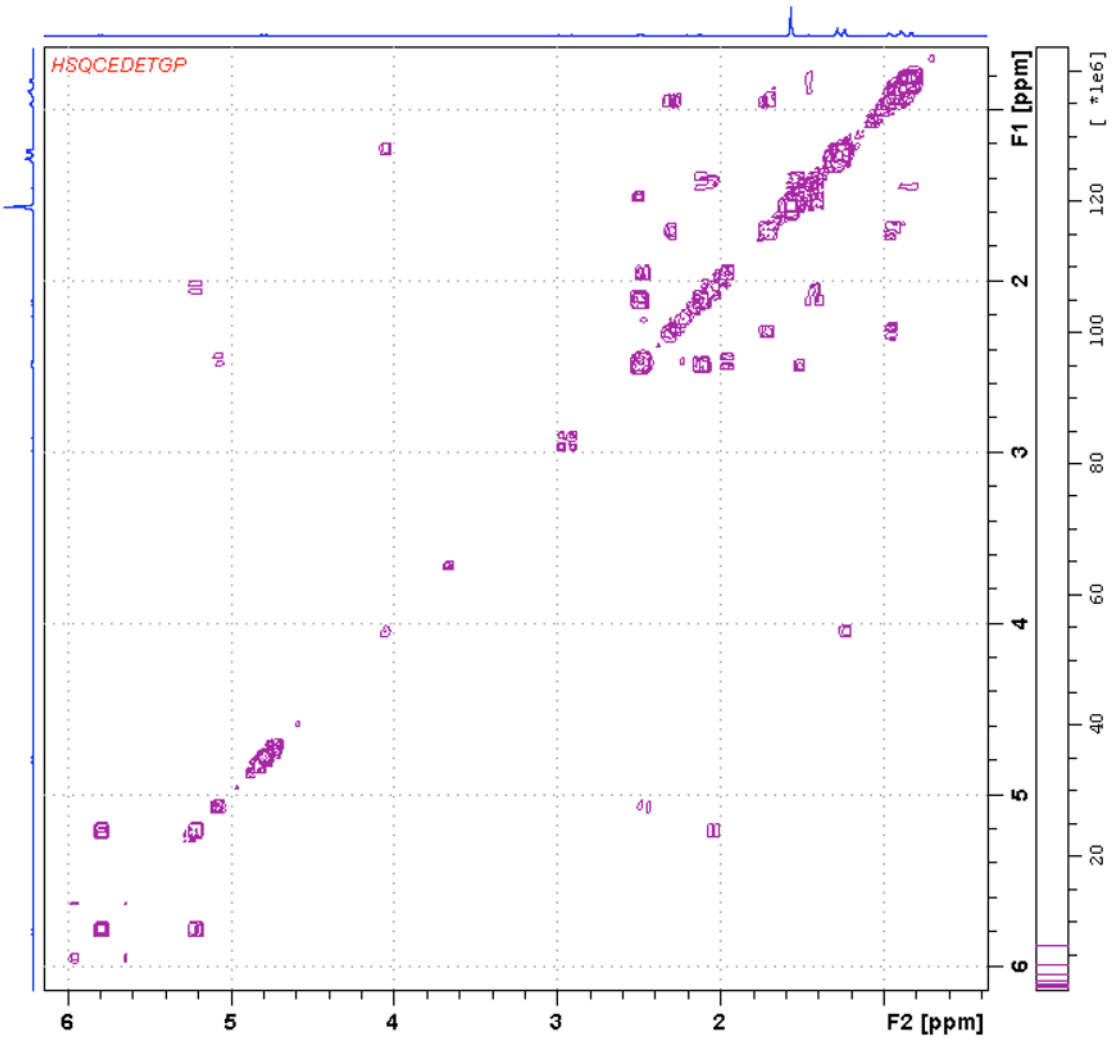


Figure S65. COSY NMR spectrum (600 MHz, $CDCl_3$) of 14Me-germacrene D (**5f**).

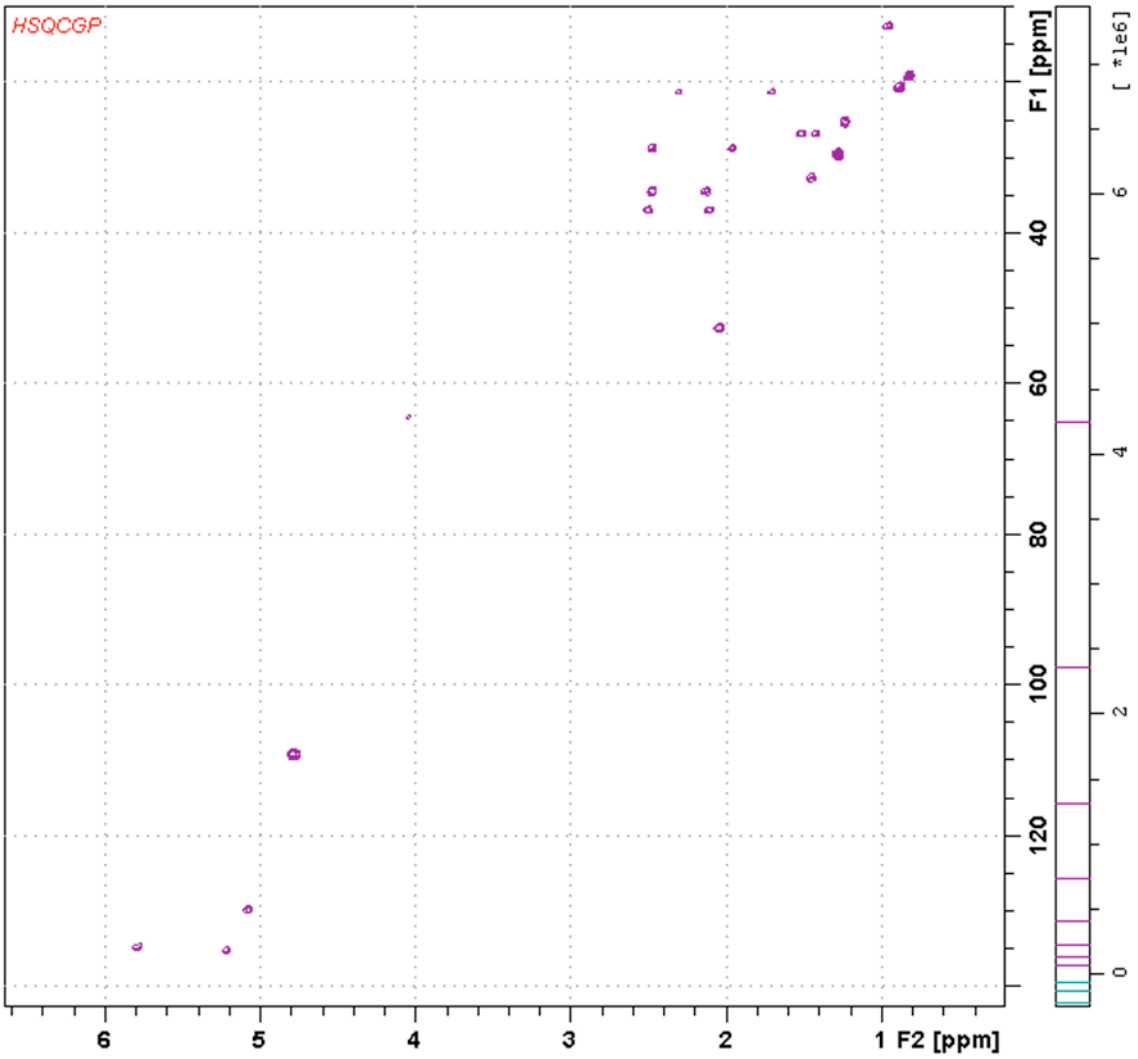


Figure S66. HSQC NMR spectrum (600 MHz, $CDCl_3$ at 0 °C) of 14Me-germacrene D (**5f**).

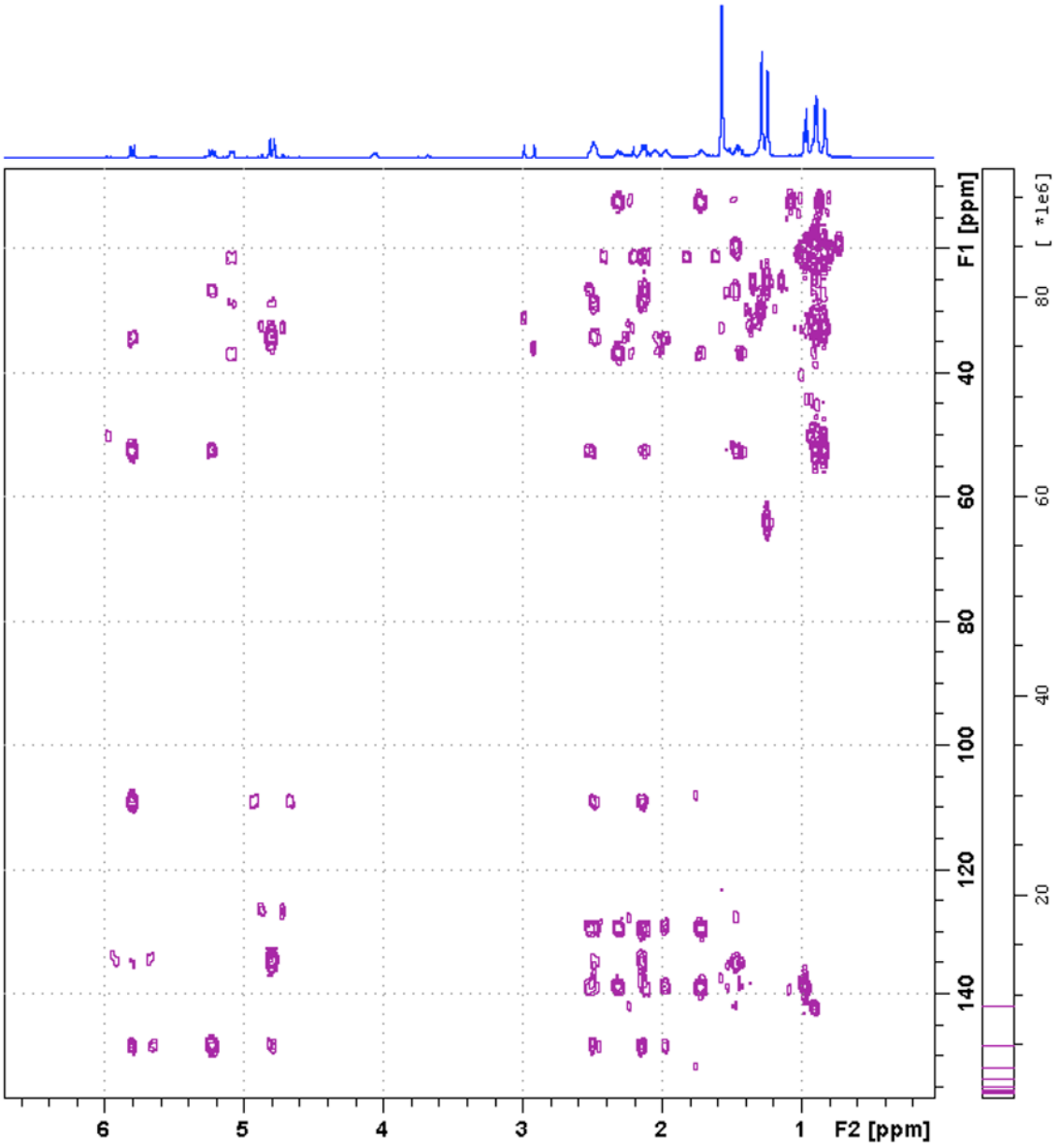


Figure S67. HMBC NMR spectrum (600 MHz, $CDCl_3$ at 0 °C) of 14Me-germacrene D (**5f**).

9. References

1. D. J. Miller, F. L. Yu and R. K. Allemann, *ChemBioChem*, 2007, **8**, 1819-1825.
2. J. A. Faraldos, Y. X. Zhao, P. E. O'Maille, J. P. Noel and R. M. Coates, *ChemBioChem*, 2007, **8**, 1826-1833.
3. D. J. Miller, F. L. Yu, D. W. Knight and R. K. Allemann, *Org. Biomol. Chem.*, 2009, **7**, 962-975.

4. H. A. Gennadios, V. Gonzalez, L. Di Costanzo, A. Li, F. L. Yu, D. J. Miller, R. K. Allemann and D. W. Christianson, *Biochemistry*, 2009, **48**, 6175-6183.
5. J. M. Dolence and C. D. Poulter, *Tetrahedron*, 1996, **52**, 119-130.
6. D. S. Rawat and R. A. Gibbs, *Org. Lett.*, 2002, **4**, 3027-3030.
7. R. A. Gibbs, U. Krishnan, J. M. Dolence and C. D. Poulter, *J. Org. Chem.*, 1995, **60**, 7821-7829.
8. J. A. Faraldos, D. J. Miller, V. Gonzalez, Z. Yoosuf-Aly, O. Cascón, Li Amang and R. K. Allemann, *J. Am. Chem. Soc.*, 2012, **134**, 5900–5908.
9. Y. H. Jin, D. C. Williams, R. Croteau and R. M. Coates, *J. Am. Chem. Soc.*, 2005, **127**, 7834-7842.
10. I. Prosser, A. L. Phillips, S. Gittings, M. J. Lewis, A. M. Hooper, J. A. Pickett and M. H. Beale, *Phytochem.*, 2002, **60**, 691-702.
11. M. M. Bradford, *Anal. Biochem.*, 1976, **72**, 248-254.
12. I. Prosser, I. G. Altug, A. L. Phillips, W. A. Konig, H. J. Bouwmeester and M. H. Beale, *Arch. Biochem. Biophys.*, 2004, **432**, 136-144.
13. M. J. Calvert, P. R. Ashton and R. K. Allemann, *J. Am. Chem. Soc.*, 2002, **124**, 11636-11641.
14. J. A. Faraldos, S. Wu, J. Chappell and R. M. Coates, *Tetrahedron*, 2007, **63**, 7733-7742.