Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information for

Probing the reaction mechanism of aristolochene synthase with 12,13-difluorofarnesyl diphosphate

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Experimental details

All chemicals were purchased from Sigma-Aldrich unless otherwise stated. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium/benzophenone ketyl under nitrogen. Acetonitrile, dichloromethane, toluene and triethylamine were distilled from calcium hydride under nitrogen. 9-BBN was used as the commercially available crystalline form, solutions were not found to work adequately in the hydroboration reactions employed in this study. Ecoscint scintillation fluid was purchased from National Diagnostics. All other chemicals were of analar quality or better and used as received unless otherwise stated. Reactions were stirred at room temperature in air unless otherwise stated. All glassware was clean and dry before use.

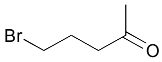
^{1}H NMR spectra were measured on a Bruker Avance 500 NMR spectrometer or a Bruker Avance DPX400 NMR spectrometer and are reported as chemical shifts in parts per million downfield from tetramethylsilane, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (to the nearest 0.5 Hz) and assignment respectively. ^{13}C NMR spectra were measured on a Bruker Avance 500 NMR spectrometer and are reported as chemical shift downfield from tetramethylsilane, coupling constant where appropriate and assignment. Assignments are made to the limitations of COSY, DEPT 90/135, gradient HSQC and gradient HMBC spectra. ^{19}F and ^{31}P NMR spectra were recorded on a Jeol Eclipse +300 NMR spectrometer and are reported in chemical shift downfield from $CFCl_3$ and 85% H_3PO_4 respectively followed by multiplicity and coupling constant (to the nearest 0.5 Hz) if appropriate. IR spectra were recorded on a Perkin ELMER 1600 series FTIR spectrometer and samples were prepared as thin films of neat liquid on sodium chloride discs for oils and as KBr disks for solids. EI^+ mass spectra were measured on a Micromass LCT premiere XE mass spectrometer ES^- mass spectra were provided by the UK EPSRC mass spectrometry service, Swansea UK.

Reverse phase HPLC was performed on a system comprising of a Dionex P680 pump and a Dionex UVD170U detector unit.

Expression and Purification of AS and AS-F112A in E. coli. Enzymes were produced in *E. coli* BL21(DE3) cells harbouring a cDNA for AS or AS-F112A under the control of the T7 promoter as previously described.[1] Cells were grown at 37 °C in LB medium with 0.3 mM ampicillin until they reached an A_{600} of 0.5. They were induced with 0.5 mM isopropyl-β-D-1-thiogalactopypyranoside, incubated for a further 3 h and harvested by centrifugation at 8000*g* for 10 min. Proteins were then extracted from the inclusion bodies and purified following our established protocols.[1, 2] Each enzyme was pure as judged by SDS-gel electrophoresis.

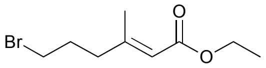
Details of synthetic work

5-Bromopentan-2-one (8).[3]



A solution of α-acetylbutyrolactone (6.46 cm^3, 60.0 mmol) in toluene (40 cm^3) was stirred as hydrobromic acid (48% in H_2O, 10.2 cm^3, 90.0 mmol) was added. The whole reaction mixture was heated at 80 °C for 14 h under N_2. Water (30 cm^3) and diethyl ether (30 cm^3) were added, and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 20 cm^3). The combined ethereal extracts were washed with water (2 × 20 cm^3) and brine (20cm^3), dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (4 : 1) as eluent gave **8** as a pale yellow oil (8.7 g, 89%); R$_f$ 0.25 (Hexane : EtOAc = 4 : 1); HRMS (EI$^+$, [M(^{79}Br)]$^+$) found 163.9829, $C_5H_9O^{79}Br$ requires 163.9831; υ_{max} (thin film)/cm^{-1} 2963.8, 1714.7, 1434.6, 1367.2, 1301.3, 1247.1, 1179.0, 908.6, 735.0; δ_H (500 MHz, C^2HCl_3) 2.12 (2 H, quintet, *J* 7.0, $CH_2CH_2CH_2$), 2.17 (3 H, s, CH_3), 2.65 (2 H, t, *J* 7.0, $CH_2C{=}O$), 3.45 (2 H, t, *J* 6.5, $BrCH_2$); δ_C (125 MHz, C^2HCl_3) 26.37 ($CH_2CH_2CH_2$), 30.07 (CH_3), 33.28 ($BrCH_2$), 41.43 ($CH_2C{=}O$), 207.30 (C=O); *m/z* (EI$^+$) 165.9 (40%, [M(^{81}Br)]$^+$), 164.0 (40, [M(^{79}Br)]$^+$), 92.8 (100).

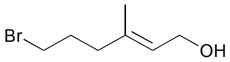
(*E*)-Ethyl 6-bromo-3-methyl-hex-2-enoate (9).[4]



To a suspension of sodium hydride (0.12 g, 4.80 mmol) in anhydrous THF (10 cm^3) at 0 °C, triethyl phosphonoacetate (0.95 cm^3, 4.80 mmol) and a solution of **8** (0.66 g, 4.00 mmol) in anhydrous THF (5 cm^3) were sequentially added dropwise and with stirring. The reaction mixture was stirred for 14 h under N_2. Water (20 cm^3) and diethyl ether (20 cm^3) were added, and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 15 cm^3). The combined ethereal extracts were washed with water (2 × 10 cm^3) and brine (10 cm^3), dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (9 : 1) as eluent gave the Z isomer of **9** as a minor by-product (56 mg, 6 %) followed by **9** (*E* isomer) as a pale yellow oil (640 mg, 69%); R$_f$ 0.27 (Hexane : EtOAc = 9 : 1); HRMS (ES$^+$, [M+H]$^+$) found 235.0327, $C_9H_{16}O_2{}^{79}Br$ requires 235.0328; υ_{max} (thin film)/cm^{-1} 2977.6, 1713.6, 1648.9, 1438.4, 1368.1, 1272.6, 1217.4, 1147.1, 1041.7, 951.3, 865.9, 810.7; δ_H (500 MHz, C^2HCl_3) 1.30 (3 H, t, *J* 7.0, CH_2CH_3), 2.06 (2 H, m, $BrCH_2CH_2$), 2.18 (3 H, d, *J* 1.0, $CH_3C{=}CH$), 2.32 (2 H, t, *J* 8.0, $BrCH_2CH_2CH_2$), 3.42 (2 H, t, *J* 7.5, $BrCH_2$), 4.16 (2 H, q, *J* 7.0, CH_2CH_3), 5.72 (1 H, m, C=C*H*); δ_C (125 MHz, C^2HCl_3) 14.30 (CH_2CH_3), 18.67 ($CH_3C{=}CH$), 30.25 ($BrCH_2CH_2$), 32.61 ($BrCH_2$), 38.99 ($BrCH_2CH_2CH_2$), 59.61 (CH_2CH_3), 116.65 (C=*C*H), 157.51(*C*=CH), 166.57 (*C*=O); *m/z* (CI$^+$) 252.0 (100%, [M(^{79}Br)+NH$_4$]$^+$), 254.0 (100%, [M(^{81}Br)+NH$_4$]$^+$).

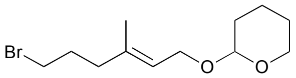
Z-isomer; R$_f$ 0.35 (Hexane : EtOAc = 9 : 1); HRMS (ES$^+$, [M+H]$^+$) found 235.0328, $C_9H_{16}O_2{}^{79}Br$ requires 235.0328; υ_{max} (thin film)/cm^{-1} 2978.7, 1713.4, 1649.9, 1439.6, 1366.7, 1349.0, 1222.7, 1149.6, 1044.4, 868.0 cm^{-1}; δ_H (500 MHz, C^2HCl_3) 1.28 (3 H, t, *J* 7.0, $CH_2C\mathit{H}_3$), 1.91 (3 H, d, *J* 1.5, $C\mathit{H}_3C{=}CH$), 2.05 (2 H, m, $BrCH_2C\mathit{H}_2$), 2.74 (2 H, t, *J* 8.0, $BrCH_2CH_2C\mathit{H}_2$), 3.44 (2 H, t, *J* 7.0, $BrC\mathit{H}_2$), 4.15 (2 H, q, *J* 7.0, $C\mathit{H}_2CH_3$), 5.71 (1 H, d, *J* 1.5, $C{=}C\mathit{H}$); δ_C (125 MHz, C^2HCl_3) 14.30 ($CH_2\mathit{C}H_3$), 18.65 ($\mathit{C}H_3C{=}CH$), 31.39 ($BrCH_2\mathit{C}H_2$), 32.24 ($BrCH_2CH_2\mathit{C}H_2$), 33.24 ($Br\mathit{C}H_2$), 59.58 ($\mathit{C}H_2CH_3$), 117.19 ($C{=}\mathit{C}H$), 158.22 ($\mathit{C}{=}CH$), 166.16 ($\mathit{C}{=}O$); *m/z* (CI+) 252.0 (60%, $[M(^{79}Br)+NH_4]^+$), 254.0 (60%, $[M(^{81}Br)+NH_4]^+$), 174.0 (100%).

(*E*)-6-Bromo-3-methyl-hex-2-en-1-ol (10a).



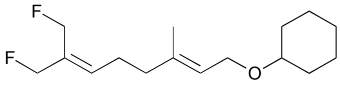
A solution of **9** (1.50 g, 6.40 mmol) in anhydrous THF (60 cm^3) was stirred under N_2 and cooled to –78 °C then diisobutylaluminium hydride (1 M solution in hexane, 15.4 cm^3, 15.4 mmol) was added dropwise. The resulting mixture was stirred at –78 °C for 2 h and then allowed to warm to 0 °C at which time the reaction was judged complete by TLC analysis. Saturated potassium sodium tartrate solution (50 cm^3) and diethyl ether (50 cm^3) were added. The mixture was stirred at room temperature for another 30 min, and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 30 cm^3). The combined ethereal extracts were washed with brine (30 cm^3), dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (2 : 1) as eluent gave **10a** as a light yellow oil (1.20 g, 98%); R_f 0.29 (Hexane : EtOAc = 2 : 1); υ_{max} (thin film)/cm^{-1} 3327.9, 2936.0, 1668.1, 1437.5, 1382.1, 1284.6, 1244.0, 1202.3, 1092.2, 1000.9, 865.9 cm^{-1}; δ_H (500 MHz, C^2HCl_3) δ 1.40 (1 H, b, $O\mathit{H}$), 1.61 (3H, s, $C\mathit{H}_3C{=}CH$), 1.92 (2 H, quintet, *J* 7.0, $BrCH_2C\mathit{H}_2$), 2.11 (2 H, t, *J* 7.0, $BrCH_2CH_2C\mathit{H}_2$), 3.33 (2 H, t, *J* 7.0, $BrC\mathit{H}_2$), 4.10 (2 H, d, *J* 7.0, $C\mathit{H}_2OH$), 5.39 (1 H, m, $C{=}C\mathit{H}$); δ_C (125 MHz, C^2HCl_3) 16.19 ($\mathit{C}H_3C{=}CH$), 30.63 ($BrCH_2\mathit{C}H_2$), 33.18 ($Br\mathit{C}H_2$), 37.71 ($BrCH_2CH_2\mathit{C}H_2$), 59.29 ($\mathit{C}H_2OH$), 124.61 ($C{=}\mathit{C}H$), 137.76 ($\mathit{C}{=}CH$); *m/z* (EI^+) 192.0 (100%, $[M(^{79}Br)]^{+}$,), 194.0 (100% , $[M(^{81}Br)]^+$).

2-((*E*)-6-bromo-3-methylhex-2-enyloxy)-tetrahydro-2*H*-pyran (10b).



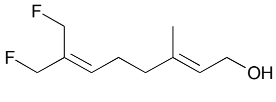
To a stirred solution of **10a** (2.82 g, 14.6 mmol) and 3,4-dihydro-*2H*-pyran (2.68 cm^3, 29.2 mmol) in CH_2Cl_2 (80 cm^3) under N_2 at 0 °C was added p-toluenesulfonic acid (0.14 g, 0.74 mmol), and the mixture stirred for 16 h whilst warming to room temperature. The solution then was diluted with diethyl ether (80 cm^3) and washed with a saturated $NaHCO_3$ solution (50 cm^3). The aqueous layer was extracted with diethyl ether (2 × 50 cm^3) and the combined ethereal extracts were washed with water (50 cm^3) and brine (50 cm^3), dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (9 : 1) as eluent to gave **10b** as a yellow oil (3.72 g, 93%); R_f 0.23 (Hexane : EtOAc = 9 : 1); HRMS (ES^+, $[M+NH_4]^+$) found 294.1065, $C_{12}H_{25}O_2NBr$ requires 294.1063; υ_{max} (thin film)/cm^{-1} 2940.1, 2865.2, 1669.3, 1439.8, 1383.6, 1353.0, 1245.5, 1199.8, 1116.8, 1076.2, 1023.2, 905.6, 868.8, 813.8 cm^{-1}; δ_H (500 MHz, C^2HCl_3) 1.60-1.85 (6 H, m, $CH(CH_2)_3$), 1.69 (3 H, s, CH_3C=CH), 2.00 (2 H, quintet, *J* 7.0, $BrCH_2CH_2$), 2.19 (2 H, t, *J* 7.0, $BrCH_2CH_2CH_2$), 3.40 (2 H, t, *J* 7.0, $BrCH_2$), 3.51 and 3.88 (2 H, m, $(CH_2)_2CH_2O$), 4.01 and 4.23 (2 H, m, $C=CHCH_2O$), 4.62 (1 H, m, OCHO), 5.42 (1 H, m, $C=CHCH_2O$); δ_C (125 MHz, C^2HCl_3) 16.34 (CH_3C=CH), 19.61, 25.49, and 30.66 ($(CH_2)_3CH_2O$), 30.71 ($BrCH_2CH_2$), 33.25 ($BrCH_2$), 37.80 ($BrCH_2CH_2CH_2$), 62.32 ($(CH_2)_3CH_2O$), 63.58 ($CHCH_2O$), 97.95 (OCHO), 121.98 ($CHCH_2O$), 138.13 (CH_3C=CH); *m/z* (EI^+) 294.2 (50%, $[M(^{79}Br)+NH_4]^+$), 296.2 (50%, $[M(^{81}Br)+NH_4]^+$), 102.1 (100%).

(*E*)-2-(8-Fluoro-7-fluoromethyl-3-methyl-octa-2,6-dienyloxy)-tetrahydro-*2H*-pyran (12a).[5]



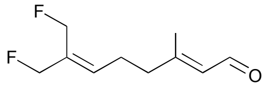
A mixture of **10b** (1.10 g, 3.97 mmol) and triphenylphosphine (1.57 g, 5.99 mmol) in anhydrous acetonitrile (20 cm^3), under a nitrogen atmosphere, was heated under reflux for 14 h. After cooling, the solvent was evaporated to afford an oily mixture. Excess triphenylphosphine was removed by triturating the mixture with anhydrous diethyl ether, and the sticky residue was dried *in vacuo* for 5 h to afford the intermediate phosphonium salt **11** as a viscous oil. To a stirred solution of crude **11** (1.96 g, 3.63 mmol) in anhydrous THF (40 cm^3) under N_2 at –78 ˚C, was added lithium hexamethyldisilazide (1 M solution in THF, 4.00 cm^3, 4.00 mmol), the mixture was stirred for 30 min at –78 ˚C, and then the 1,3-difluoro acetone (0.30 cm^3, 3.64 mmol) was added dropwise. The whole reaction mixture was allowed to warm to –20 ˚C over 1 h. and maintained at –20 ˚C for 2 h. The reaction was then quenched by addition of diethyl ether (40 cm^3) and water (40 cm^3). The aqueous layer was extracted with diethyl ether (3 × 30 cm^3) and the combined ethereal extracts were washed brine (30 cm^3), dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (6 : 1) as eluent gave **12a** as a yellow oil (0.66 g, 66%); R_f 0.27 (Hexane : EtOAc = 6 : 1); HRMS (CI^+, $[M+NH_4]^+$) found 292.2081, $C_{15}H_{28}O_2NF_2$ requires 292.2083; υ_{max} (thin film)/cm^{-1} 2942.1, 2364.1, 1672.0, 1441.6, 1317.6, 1261.0, 1200.1, 1117.4, 1076.4, 1203.8, 970.8, 904.9, 868.8, 813.9 cm^{-1}; δ_H (500 MHz, C^2HCl_3) 1.39-1.68 (6 H, m, $(C\mathit{H}_2)_3CH_2O$), 1.53 (3 H, s, $C\mathit{H}_3C{=}CH$), 1.98 (2 H, t, *J* 7.5, $FCH_2C{=}CHCH_2C\mathit{H}_2$), 2.14 (2 H, m, $FCH_2C{=}CHC\mathit{H}_2$), 3.35 and 3.71 (2 H, m, $(CH_2)_3C\mathit{H}_2O$), 3.85 and 4.07 (2 H, m, $C{=}CHC\mathit{H}_2O$), 4.47 (1 H, m, OCHO), 4.67 (2 H, d, J_{HF} 47.5, $C\mathit{H}_2F$), 4.80 (2 H, d, J_{HF} 47.5, $C\mathit{H}_2F$), 5.23 (1 H, dt, *J* 7.5, *J* 1.0, $C{=}C\mathit{H}CH_2O$), 5.70 (1 H, m, $CH_2FC{=}C\mathit{H}$); δ_C (125 MHz, C^2HCl_3) 16.35 (CH_3), 19.61, 25.48 and 30.70 ($(\mathit{C}H_2)_3CH_2O$), 25.76 ($FCH_2C{=}CH\mathit{C}H_2$), 38.77 (t, J_{CF} 2.5, $CH_2FCCHCH_2\mathit{C}H_2$), 62.32 ($(CH_2)_3\mathit{C}H_2O$), 63.54 ($CH\mathit{C}H_2O$), 76.86 (d, J_{CF} = 161, $\mathit{C}H_2F$), 83.92 (d, J_{CF} = 164, $\mathit{C}H_2F$), 97.95 (OCHO), 121.81 ($\mathit{C}HCH_2O$), 130.89 (t, J_{CF} = 14.0, $\mathit{C}CH_2F$), 137.12 (t, J_{CF} = 9, $CH_2FC\mathit{C}H$), 138.49 ($CH_2\mathit{C}CH_3$); δ_F (283 MHz, C^2HCl_3) –210.13 (t, J_{HF} 47.0), –214.88 (t, J_{HF} 48.5); *m/z* (CI^+) 292.3 (100%, $[M+NH_4]^+$), 102.2 (95%).

(*E*)-8-Fluoro-7-fluoromethyl-3-methylocta-2,6-dien-1-ol (12b).



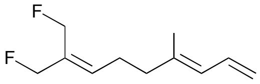
A solution of **12a** (0.48 g, 1.75 mmol) and pyridinium p-toluenesulphonate (0.04 g, 0.17mmol) in ethanol (20 cm^3) was stirred at 55 °C for 3 h. Concentration of the solvent under reduced pressure gave a yellow oil, then water (20 cm^3) and diethyl ether (20 cm^3) were added and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 10 cm^3). The combined organic layers were washed with brine (10 cm^3), dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (2 : 1) as eluent gave **12b** as a pale yellow oil (0.30 g, 91%); R_f 0.24 (hexane : EtOAc = 2 : 1); HRMS (CI^+, $[M+NH_4]^+$) found 208.1508, $C_{10}H_{20}ONF_2$ requires 208.1507; υ_{max} (thin film)/cm^{-1} 3364.5, 2932.0, 2361.2, 1670.5, 1443.4, 1373.6, 1236.9, 987.1 cm^{-1}; δ_H (500 MHz, C^2HCl_3) 1.65 (1 H, s, $O\mathit{H}$), 1.69 (3 H, s, CH_3), 2.14 (2 H, t, J 7.5, $C{=}CHCH_2C\mathit{H}_2$), 2.32 (2 H, m, $C{=}CHC\mathit{H}_2CH_2$), 4.16 (2 H, d, J 7.0, $C\mathit{H}_2OH$), 4.84 (2 H, d, $J_{H\text{-}F}$ 47.5, $C\mathit{H}_2F$), 4.96 (2 H, d, $J_{H\text{-}F}$ 47.5, $C\mathit{H}_2F$), 5.43 (1 H, dt, J 7.0, J 1.0, $C\mathit{H}CH_2OH$), 5.86 (1 H, m, $C{=}C\mathit{H}CH_2CH_2$); δ_C (125 MHz, C^2HCl_3) 16.19 (CH_3), 25.76 ($C{=}CHCH_2\mathit{C}H_2$), 38.63 ($C{=}CH\mathit{C}H_2CH_2$), 59.22 (CH_2OH), 76.88 (d, J_{CF} = 161, $\mathit{C}H_2F$), 83.94 (d, J_{CF} = 164, $\mathit{C}H_2F$), 124.54 ($\mathit{C}HCH_2OH$), 131.08 (t, J_{CF} = 15.0, $\mathit{C}CH_2F$), 137.09 (t, $J_{C\text{-}F}$ = 9, $CH_2FC{=}\mathit{C}H$), 137.92 ($CH_2\mathit{C}CH_3$); δ_F (283 MHz, C^2HCl_3) –209.94 (t, J_{HF} 47.5), –214.59 (t, J_{HF} 47.5); *m/z* (CI^+) 190.2 (90%, $[M]^+$), 208.2 (100%, $[M+NH_4]^+$).

(*E*)-8-Fluoro-7-fluoromethyl-3-methylocta-2,6-dienal (13a).



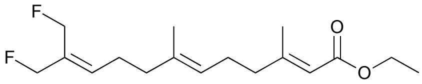
A mixture of **12b** (1.60 g, 8.42 mmol), *N*-methylmorpholine-*N*-oxide (1.38 g, 11.8 mmol) and freshly activated powdered 4 Å molecular sieves (0.70 g) in anhydrous acetonitrile (70 cm^3) was stirred for 10 min whereupon tetra-*n*-propyl perruthenate (0.15 g, 0.42 mmol) was added. The reaction became warm and was then stirred at room temperature for 16 h. The mixture was filtered through Celite® and the solvent was concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (2 : 1) as eluent gave **13a** as a pale yellow oil (1.40 g, 90%); R_f 0.29 (Hexane : EtOAc = 2 : 1); HRMS (CI^+, $[M+NH_4]^+$) found 206.1354, $C_{10}H_{18}ONF_2$ requires 206.1351; υ_{max} (thin film)/cm^{-1} 2952.3, 2359.2, 1672.8, 1438.4, 1381.1, 1194.9, 1123.2, 967.7, 865.9 cm^{-1}; δ_H (500 MHz, d_6-benzene) 1.57 (3 H, s, $C\mathit{H}_3$), 1.67 (2 H, m, $C{=}CHCH_2C\mathit{H}_2$), 1.83 (2 H, m, $C{=}CHC\mathit{H}_2CH_2$), 4.62 (2 H, d, J_{HF} 47.5, $C\mathit{H}_2F$), 4.68 (2 H, d, J_{HF} 47.5, $C\mathit{H}_2F$), 5.35 (1 H, b, $C{=}C\mathit{H}CH_2CH_2$), 5.78 (1 H, d, J 7.5, $C{=}C\mathit{H}CHO$), 9.92 (1 H, d, J = 7.5, $CH{=}O$); δ_C (125 MHz, C^2HCl_3) 16.54 ($\mathit{C}H_3$), 24.69 ($C{=}CH\mathit{C}H_2CH_2$), 39.03 (C=CHCH$_2$*C*H$_2$), 76.29 (d, *J*$_{CF}$ = 162.5, *C*H$_2$F), 83.14 (d, *J*$_{CF}$ = 166.5, *C*H$_2$F), 127.53 (C=*C*HCHO), 131.96 (t, *J*$_{CF}$ = 15.0, FCH$_2$*C*=CH), 134.59 (t, *J*$_{CF}$ = 7.5, FCH$_2$C=*C*H), 159.61 (CH$_3$*C*=CH), 189.50 (C=O); δ$_F$ (283 MHz, C^{2}HCl$_3$) –212.04 (t, *J*$_{HF}$ 47.5), –216.79 (t, *J*$_{H\text{-}F}$ 47.5); *m/z* (CI$^+$) 206.2 (100%, [M+NH$_4$]$^+$).

(*E*)-9-Fluoro-8-fluoromethyl-4-methylnona-1,3,7-triene (13b).



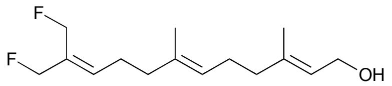
A stirred suspension of methyltriphenylphosphonium bromide (4.5 g, 12.60 mmol) in anhydrous THF (50 cm^3) was cooled to –78 °C then *n*-BuLi (2.5 M, 5.04 cm^3, 12.6 mmol) was added dropwise under argon. The reaction mixture was allowed to warm to 0 °C giving a clear deep yellow solution. After stirring at 0 °C for 30 min, the aldehyde **13a** (1.40 g, 7.45 mmol) was added dropwise and the complete reaction mixture was stirred for 16 h. whilst slowly warming to room temperature. Water (20 cm^3) and diethyl ether (20 cm^3) were added and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 15 cm^3). The combined ethereal extracts were washed with water (2 × 20 cm^3) and brine (20 cm^3), dried over MgSO$_4$, filtered and then concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (9 : 1) as eluent gave **13b** as a light yellow oil (1.27 g, 92%); R$_f$ 0.34 (Hexane : EtOAc = 9 : 1); HRMS (CI$^+$, [M+H]$^+$) found 187.1290, C$_{11}$H$_{17}$F$_2$ requires 187.1293; υ$_{max}$ (thin film)/cm^{-1} 2925.6, 2359.8, 1674.4, 1651.5, 1448.4, 1372.9, 1217.4, 986.8, 901.0 cm^{-1}; δ$_H$ (500 MHz, C^{2}HCl$_3$) 1.56 (3 H, s, CH$_3$), 1.95 (2 H, t, *J* 7.5, C=CHCH$_2$C*H*$_2$), 1.83 (2 H, m, C=CHC*H*$_2$CH$_2$), 4.62 (2 H, d, *J*$_{HF}$ 47.5, CH$_2$F), 4.75 (2 H, d, *J*$_{HF}$ 47.5, CH$_2$F), 4.80 (1 H, dd, *J* 10.0, *J* 1.5, CHCH=CH$_{trans}$*H*$_{cis}$), 4.90 (1 H, dd, *J* 17.0, *J* 1.5, CHCH=C*H*$_{trans}$H$_{cis}$), 5.64 (2 H, m, C=C*H*CH$_2$CH$_2$ and C*H*=CHCH$_2$), 6.35 (1 H, dt, *J* 17.0, *J* 10.0, CHC*H*=CH$_2$); δ$_C$ (125 MHz, C^{2}HCl$_3$) 16.56 (CH$_3$), 25.91 (C=CH*C*H$_2$CH$_2$), 39.01 (C=CCH$_2$*C*H$_2$), 76.86 (d, *J*$_{CF}$ 161.0, CH$_2$F), 83.92 (d, *J*$_{C\text{-}F}$ 165.0, CH$_2$F), 115.44 (CHCH=*C*H$_2$), 126.34 (*C*HCH=CH$_2$), 131.07 (t, *J*$_{C\text{-}F}$ 15.0, FCH$_2$*C*=CH), 133.04 (CH*C*H=CH$_2$), 136.98 (dd, *J*$_{C\text{-}F}$ 10.0, *J*$_{C\text{-}F}$ 7.5, FCH$_2$C=*C*H), 137.62 (CH$_3$*C*=CH); δ$_F$ (300 MHz, C^{2}HCl$_3$) –211.97 (t, *J*$_{H\text{-}F}$ 47.5), –216.70 (t, *J*$_{H\text{-}F}$ 47.5); *m/z* (CI$^+$) 204.2 (100%, [M+NH$_4$]$^+$), 187.1 (45%, [M+H]$^+$).

(2*E*,6*E*)-Ethyl 12-fluoro-11-fluoromethyl-3,7-dimethyldodeca-2,6,10-trienoate (15).



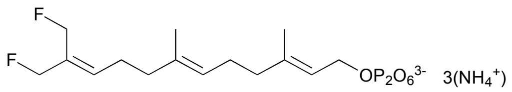
A mixture of the **13b** (1.25 g, 6.72 mmol) and 9-BBN (2.46 g, 10.1 mmol) solution in anhydrous THF (50 cm^3) was stirred at room temperature under nitrogen atmosphere until all the starting material had been consumed as judged by TLC (approx 2 h.). The iodide **14**^{6} (1.62 g, 6.72 mmol), triphenylarsine (0.21 g, 0.68 mmol), $PdCl_2$dppf (0.28 g, 0.34 mmol) and 6 M NaOH (4.55 cm^3, 27.3 mmol) were added in quick succession, the complete solution was then stirred at 50 °C for 15 h. After cooling to room temperature aqueous hydrogen peroxide solution (1M, 15 cm^3) was added and the solution was stirred for a further 30 min. Water (30 cm^3) and diethyl ether (30 cm^3) were added, and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 25 cm^3). The combined ethereal extracts were washed with water (2 × 20 cm^3) and brine (20 cm^3), dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Purification by flash column chromatography on silica gel with hexane and ethyl acetate (9 : 1) as eluent gave **15** as a light yellow oil (0.55 g, 29%); R_f 0.32 (Hexane : EtOAc = 9 : 1); HRMS (ES$^+$, $[M+H]^+$) found 318.2239, $C_{17}H_{30}O_2NF_2$ requires 318.2239; υ_{max} (thin film)/cm^{-1} 2932.0, 1713.8, 1648.7, 1446.7, 1370.8, 1350.3, 1274.9, 1222.6, 1145.3, 1096.0, 1040.0, 970.6, 865.3, 735.4 cm^{-1}; δ_H (500 MHz, C^2HCl_3) 1.28 (3 H, t, *J* 7.0, CH_2CH_3), 1.62 and 2.17 (6 H, s, 2 × CH_3), 2.07-2.30 (8 H, m, 2 × CH_2CH_2), 4.14 (2 H, q, *J* 7.0, CH_2CH_3), 4.83 (2 H, d, J_{HF} 47.5, CH_2F), 4.96 (2 H, d, J_{HF} 47.5, CH_2F), 5.12 and 5.85 (2 H, m, 2 × $C{=}CHCH_2CH_2$), 5.67 (1 H, s, $C{=}CHCO_2$); δ_C (125 MHz, C^2HCl_3) 14.31 (CH_2CH_3), 15.91 and 18.74 (2 × CH_3), 25.85 (CH_2), 25.99 (CH_2), 38.82 (t, J_{CF} 2.5, $FCH_2C{=}CHCH_2$), 40.75 (CH_2), 59.48 (CH_2CH_3), 76.87 (d, J_{CF} 161.5, CH_2F), 83.95 (d, J_{CF} 164.0, CH_2F), 115.77 ($C{=}CHCO_2$), 124.11 ($CH_3C{=}CHCH_2CH_2$), 130.84 (t, $J_{C\text{-}F}$ 15.0, $FCH_2C{=}CH$), 137.37 (dd, J_{CF} 10.0, J_{CF} 7.5, $FCH_2C{=}CH$), 134.65 and 159.41 (quaternary *C*), 166.81 (C=O); δ_F (283 MHz, C^2HCl_3) –211.76 (t, J_{HF} 54.0), –216.68 (t, J_{HF} 54.0); *m/z* (CI$^+$) 318.3 (100%, $[M+NH_4]^+$), 301.3 (20%, $[M+H]^+$), 281.2 (40%, $[M–F]^+$).

(2*E*,6*E*)-12-Fluoro-11-(fluoromethyl)-3,7-dimethyldodeca-2,6,10-trien-1-ol (16a).



This compound was prepared in a manner identical to that for the alcohol **10a**; the crude alcohol was purified by silica column chromatography using hexane and ethyl acetate (2: 1) as eluent to give **16a** as a light yellow oil (0.12 g, 68%); R_f 0.26 (Hexane : EtOAc = 2 : 1); HRMS (CI^+, $[M+NH_4]^+$) found 276.2132, $C_{15}H_{28}ONF_2$ requires 276.2133; υ_{max} (thin film)/cm^{-1} 3349.3, 2922.0, 2361.2, 1668.2, 1445.4, 1376.8, 1235.0, 987.5, 569.1 cm^{-1}; δ_H (500 MHz, C^2HCl_3) 1.37 (1 H, s, O*H*), 1.53 and 1.61 (6 H, s, 2 × CH_3), 1.95-2.22 (8 H, m, 2 × CH_2CH_2), 4.08 (2 H, d, *J* 7.0, $C\mathit{H}_2OH$), 4.75 (2 H, d, J_{HF} 47.5, CH_2F), 4.88 (2 H, d, J_{HF} 47.5, CH_2F), 5.04 (1 H, dt, *J* 7.0, *J* 1.0, $CH_3C{=}C\mathit{H}CH_2CH_2$), 5.34 (1 H, dt, *J* 7.0, *J* 1.0, $C{=}C\mathit{H}CH_2OH$), 5.78 (1 H, m, $FCH_2C{=}C\mathit{H}$); δ_C (125 MHz, C^2HCl_3) 15.91 and 16.25 (2 × CH_3), 26.03 (CH_2), 26.21 (CH_2), 38.84 (t, J_{CF} 2.5, $FCH_2C{=}CH\mathit{CH}_2$), 39.38 (CH_2), 59.36 (CH_2OH), 76.93 (d, J_{CF} 161.5, CH_2F), 84.00 (d, J_{CF} 165.0, CH_2F), 123.52 ($C{=}\mathit{C}HCH_2OH$), 125.00 ($CH_3C{=}\mathit{C}HCH_2CH_2$), 130.74 (t, J_{CF} 15.0, $FCH_2\mathit{C}{=}CH$), 133.86 and 139.44 (2 x $CH_3\mathit{C}{=}CH$), 137.61 (dd, J_{CF} 10.0, J_{CF} 9.0, $FCH_2C{=}\mathit{C}H$); δ_F (283 MHz, C^2HCl_3) –211.62 (t, $J_{H\text{-}F}$ 47.5), –216.60 (t, $J_{H\text{-}F}$ 47.5); *m/z* (CI^+) 241.1 (100%, $[M–OH]^+$), 276.2 (60%, $[M+NH_4]^+$), 258.2 (20%, M^+).

(2*E*,6*E*)-12-Fluoro-11-fluoromethyl-3,7-dimethyldodeca-2,6,10-trien-1-yl diphosphate tris-ammonium salt (1b)[7, 8]



A stirred solution of **16a** (0.12 g, 0.45 mmol) and triethylamine (0.13 cm^3, 0.90 mmol) in anhydrous THF (5 cm^3) was cooled to –45 °C then methansulfonyl chloride (45 mm^3, 0.59 mmol) was added. The resulting milky mixture was stirred at – 45 °C for 45 min then a solution of lithium bromide (0.16 g, 1.80 mmol) in THF (5 cm^3) was added via a cannula. The resulting suspension was allowed to warm to 0 °C and stirred for additional 1 h. Cold water (10 cm^3) and hexane (10 cm^3) were added and the two layers were separated. The aqueous layer was extracted with hexane (2 × 10 cm^3) and the combined organic layers were washed with saturated $NaHCO_3$ solution (10 cm^3) and brine (10 cm^3) then dried over Na_2SO_4 and filtered. Evaporation of the solvent gave **16b** as a light yellow oil which was used without further purification. To a stirred solution of **16b** in anhydrous acetonitrile (10 cm^3) under N_2 was added freshly recrystallized tris(tetra-n-butylammonium) hydrogenpyrophosphate[8] (0.81 g, 0.90 mmol). The complete reaction mixture was stirred for 2 h. Solvent was then removed under reduced pressure and the resulting opaque residue was dissolved in 2 cm^3 of 1:49 (v/v) isopropyl alcohol and 25 mM ammonium bicarbonate (ion-exchange buffer). The pale yellow solution was slowly passed through a column containing 30 equiv. of DOWEX 50W-X8 (100-200 mesh) cation-exchange resin that had been equilibrated with two column volumes of ion-exchange buffer. The column was eluted with two column volumes of same buffer at a flow rate of one column volume per 15 min. The clear light yellow eluent was lyophilized to dryness to give a solid, which was purified by reverse phase HPLC (150 × 21.2 mm Phenomenex Luna column, eluting with 10% B for 20 min, then a linear gradient to 60% B over 25 min and finally a linear gradient to 100% B over 5 min.; solvent B: CH_3CN, solvent A: 25 mM NH_4HCO_3 in water, flow rate 5.0 cm^3/min, detecting at 220 nm) to give **1b** as a white solid (21.2 mg, 10% for two steps); HPLC t_R 36.58 min; HRMS (ES$^-$, $[M–H]^-$) found 417.1044, $C_{15}H_{25}O_7F_2P_2$ requires 447.1049; υ_{max}(KBr disc)/cm^{-1} 2924.0, 2362.7, 1704.8, 1494.4, 1457.1, 1412.6, 1201.7, 1123.5, 1091.4, 1036.1, 911.4, 826.1, 721.4; δ_H (500 MHz, ^2H_2O at pH 8.5 buffered with $N^2H_4O^2H$)[8] 1.48 (3 H, s, CH_3), 1.57 (3 H, s, CH_3), 1.93-2.20 (8 H, m, 2 × CH_2CH_2), 4.33 (2 H, t, *J* 6.5, CH_2O), 4.76 (2 H, d, J_{HF} 47.5, CH_2F), 4.91 (2 H, d, J_{HF} 47.5, CH_2F), 5.07 (1 H, t, *J* 7.0, $CH_3C{=}C\mathit{H}CH_2CH_2$), 5.31 (1 H, t, *J* 7.0, $C{=}C\mathit{H}CH_2O$), 5.87 (1 H, m, $FCH_2C{=}C\mathit{H}$); δ_C (125 MHz, ^2H_2O at pH 8.5 buffered with $N^2H_4O^2H$) 15.15 and 15.65 (2 × CH_3), 25.58 (CH_2), 25.63 (CH_2), 38.08 (CH_2), 38.80 (CH_2), 62.71 (d, J_{CP} 5.0, CH_2O), 78.58 (d, J_{CF} 154.0, CH_2F), 85.67 (d, J_{CF} 156.5, CH_2F), 119.72 ($C{=}\mathit{C}HCH_2O$), 125.11 ($CH_3C{=}\mathit{C}HCH_2CH_2$), 129.97 (t, $J_{C\text{-}F}$ 14.0, $FCH_2\mathit{C}{=}CH$), 135.38 and142.87 (2 x $CH_3\mathit{C}{=}CH$), 140.61 (t, J_{CF} 9.5, $FCH_2C{=}\mathit{C}H$); δ_F (283 MHz, ^2H_2O at pH 8.5 buffered with $N^2H_4O^2H$) –207.21 (d, J_{HF} 47.5), –216.83 (d, $J_{F\text{-}F}$ 47.5); δ_P (122 MHz, ^2H_2O at pH 8.5 buffered with $N^2H_4O^2H$) –6.61 (d, J_{PP} 21.0), –10.47 (d, J_{PP} 21.0); *m/z* (ES$^-$) 417.1 (100%, $[M–H]^-$).

Characterisation of products from incubation of 12,13-difluorofarnesyl diphosphate (1b) with AS and AS-F112A.

Purified enzyme (50 μM) was incubated with **1b** (200 μM) in 20 mM Tris, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol and 15% glycerol (pH 7.5) in a final volume of 500 mm^3 overlayed with hexane (200 mm^3) at 30 °C for 1-7 days. Reactions were terminated by addition of EDTA (100 mM, 100 mm^3) and the products were extracted by vortexing against hexane (3 x 3 cm^3). The pooled extracts were vigorously mixed with 1.5 g of silica then decanted and concentrated under reduced pressure on ice.

The hexane extractable products were analysed by GC-MS. This was performed on a system comprising of 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 mass-spectrometer 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 and used helium as the carrier gas. The oven temperature was raised from 50 °C with a gradient of 4 °C min^{-1} for 25 minutes followed by a gradient of 20 °C min^{-1} for 5 min (250 °C final temperature). For AS no terpenoid products were detected in this experiment leading to the conclusion that compound **1b** is not a substrate of AS.

For AS-F112A, three products with *m/z* 240 were detected (Figures S1-S4).

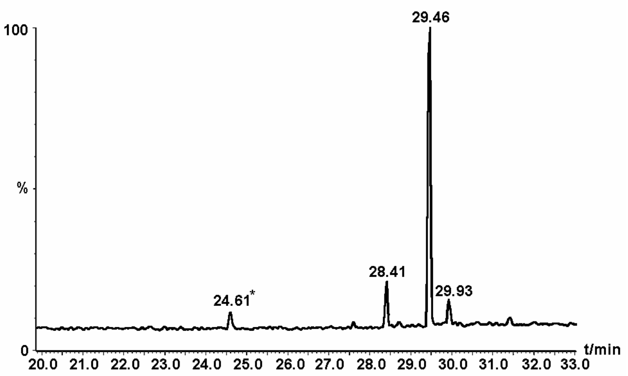


Figure S1. Total ion chromatogram of the hexane extractable products from the incubation of 12,13-difluoro-FPP (**1b**) with AS-F112A. Three peaks were observed that contained a molecular ion peak at *m/z* 240 (corresponding to $C_{15}H_{22}F_2$). The compound eluting at 24.6 min (marked *) was not a terpenoid.

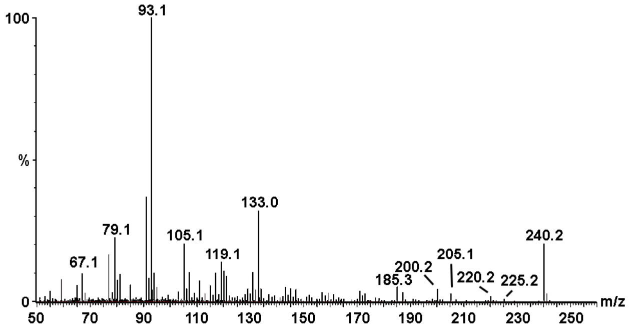


Figure S2. EI^+-mass spectrum of the compound eluting at 28.41 min in the gas chromatogram of the hexane extractable products resulting from the incubation of **1b** with AS-F112A.

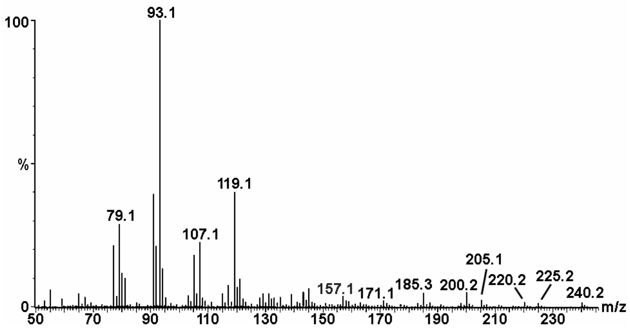


Figure S3. EI^+-mass spectrum of the compound eluting at 29.46 min in the gas chromatogram of the hexane extractable products resulting from the incubation of **1b** with AS-F112A.

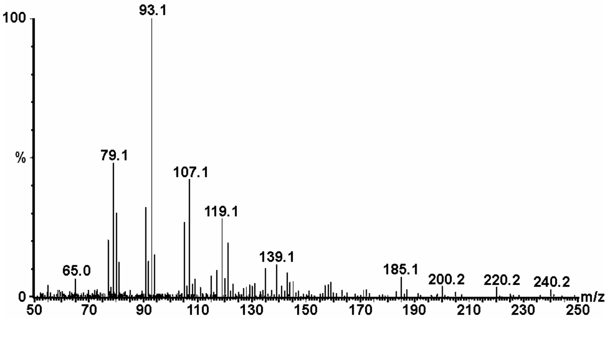


Figure S4. EI^+-mass spectrum of the compound eluting at 29.93 min in the gas chromatogram of the hexane extractable products resulting from the incubation of **1b** with AS-F112A.

Kinetic Characterisation of 12,13-difluorofarnesyl diphosphate (1b) as an inhibitor of AS

Assays (250 mm^3 final volume) were initiated by addition of purified AS solution. Assays contained 0.1-5 μM [1-^{3}H]-farnesyl diphosphate (240000 dpm/nmol), 0-1 μM **1b**, 10 mM Tris, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol and 15% glycerol and were prewarmed to 30 °C prior to addition of enzyme solution. After incubation for 4 min. each assay was stopped by addition of 100 mM EDTA and overlayed with hexane (500 mm^3). After vortexing for 10 s. the hexane was removed and the sample extracted with hexane in the same way (2 x 500 mm^3). The pooled hexane extracts were vortexed with silica (50 mg) the sample was centrifuged at 13000 rpm for 5 min and then the hexane was decanted into a scintillation vial containing 15 cm^3 of Ecoscint and analysed for radioactivity. K_M and $K_{M(app)}$ values were determined by a non-linear fit of the data to the equation $V = V_{max}[S]/(K_M + [S])$. Mode of action of the inhibitor was determined by examination of double reciprocal plots of 1/v versus 1/[S]. K_i was determined using a plot of [I] versus $K_{M(app)}$ once the inhibitor was observed to be competitive.

ESI-References

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