Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Terpenoid cyclopropylmethyl diphosphates can serve as a substrate for the sesquiterpene synthase BcBot2

Supporting Information

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1 Materials and Methods

1.1 General Information

All commercially obtained solvents and reagents were supplied by ABCR, ACROS ORGANICS, ALFA-AESAR, CARBOLUTION, CARL ROTH, FLUOROCHEM, HONEYWELL, MERCK, NEW ENGLAND BIOLABS, SIGMA-ALDRICH, and TCI and were used as provided unless stated otherwise. Dry solvents were purchased or freshly distilled. Petroleum ether (bp.: 40–60 °C) and CH_2Cl_2 were always distilled before use. All deuterated solvents for NMR measurements were purchased from DEUTERO. Amine bases were dried over KOH or CaH_2 and freshly distilled before use. All reactions with reagents sensitive to air or humidity were carried out in flame-dried glassware and under argon atmosphere using Schlenk techniques.

High-resolution mass spectra (HRMS) were measured with a MICROMASS LCT spectrometer which uses a Lockspray dual ion source and a WATERS Alliance 2695 system. In addition, a WATERS QTOF Premier spectrometer was used in combination with a WATERS Acquity UPLC system. An electron spray ionizer (ESI) was used for ionization. The calculated and actual ion mass signals (m/z) are reported in atomic mass units.

Silica gel-coated aluminium plates (type 60 F254) by MERCK, were used for qualitative thin-layer-chromatography (TLC) analysis. The spots were visualized with UV light (λ = 248 nm) and/or with staining solutions ($KMnO_4$, vanillin, anisaldehyde).

For manual column chromatography, silica gel (type: 60 M, grain size: 40–63 µm) from MACHEREY-NAGEL was used. The elution was performed under slight overpressure. For automated flash column chromatography, the flash purification system Sepacore by BÜCHI and prepacked cartridges (Chromabond by MACHEREY-NAGEL and FlashPure by BÜCHI) were used.

Bruker instrument	frequency [MHz]		
	^{1}H	^{13}C	^{31}P
Ultrashield-400	400	100	-
Ascend-400	400	100	162
Ultrashield-500	500	125	-
Avance-600 Ascend-600	600	151	-

Table 1: Employed BRUKER NMR devices and the respective frequencies for ^{1}H, ^{13}C, and ^{31}P-NMR experiments.

All reported NMR experiments were performed at room temperature using various BRUKER instruments (Table 1). Chemical shifts (δ) are reported in parts per million (ppm) and were calibrated with the residual proton signals of the solvents used [$\delta(CDCl_3)$ = 7.26 ppm (^{1}H), 77.16 ppm (^{13}C); $\delta(H_2O)$ = 4.79 ppm (^{1}H)]. Coupling constants (J) are reported in Herz (Hz) and signal multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, qi = quintet, sex = sextet, m = multiplet, br = broad signal.

GC-MS measurements were carried out on a GC-MS device with the following components. Manufacturer: AGILENT; instrument: GC = 7890B, MS = 5977B; columns (length, inner diameter, film thickness): Optima 5HT (30 m, 0.25 mm, 0.25 µm); mass spectrometer: Quadrupol (mass range = 30–650 amu); detector: flame ionization detector, MS; ion source: electron ionization, 70 eV; injector: KAS 4 (60 °C–300 °C, 12 °C/s); temperature gradient: 50 °C (1 min)–300 °C (6.5 min), 20 °C/s; carrier gas: He (rate = 15 mL/min). Samples were dissolved in GC-grade *n*-hexane and injected splitless. The total measuring time (20 min) and injection volume (1 µL) were kept constant.

For chiral gas chromatography, samples were manually injected and a GC unit with the following components was used. Manufacturer: AGILENT; instrument: HP 6890 Series; columns (length, inner

diameter, film thickness): Hydrodex-β-6-tbdm (25 m, 0.25 mm, 0.25 µm), Lipodex G (25 m, 0.25 mm, 0.25 µm); detector: flame ionization detector; injector: hot injection at 300 °C; split: 1:10. The injection volume was kept constant (5 µL) but the temperature gradient, as well as the run time, were optimized for each sample.

Preparative GC was performed to purify biotransformation products. The substances were dissolved in *n*-pentane. The injection volume was kept constant (1 µL) but the temperature gradient, as well as the measuring time, were optimized for each sample. A GC device with the following components was used. Manufacturer: AGILENT; instrument: HP 6890 Series; columns (length, inner diameter, film thickness): Zebron ZB-1 (30 m, 0.53 mm, 3 µm); detector: flame ionization detector; injector: hot injection; split: splitless; carrier gas: hydrogen.

In addition to retention times (t_R), retention indices (RI) are also reported. For calibration, a mixture of *n*-alkanes with distinct retention values was measured. To calculate the retention indices of the substances the following equation (1) is used:

(1): $$RI = 100\left[n + \frac{\log t_R(sample) - \log t_R(s)}{\log t_R(B) - \log t_R(s)}\right]$$

RI retention index
t_R(s) retention time of the alkane eluting right before the sample
t_R(B) retention time of the alkane eluting right after the sample
n number of carbons of the alkane eluting right before the sample

Specific optical rotations $\left([\alpha]_D^T\right)$ were measured using a glass cuvette (volume = 1 mL, length = 1 dm) and the PERKIN-ELMER 341/343 series spectrometer and are reported in degrees $\left(^\circ = \frac{^\circ \cdot mL}{g \cdot dm}\right)$. Concentrations (c) are reported in $10\frac{mg}{ml}$ and measurements were performed at varying temperatures (T) with a sodium lamp (sodium-D-line, λ = 589 nm).

2 General Microbiological Procedures

2.1 Heterologous Expression

Overexpression of the sesquiterpene cyclase presilphiperfolan-8-β-ol synthase (BcBot2) was performed in *E. coli* BL21 (DE3). The respective genes are encoded in a pET28a(+) vector containing a kanamycin resistance, T7 polymerase, a lac-operator, and polyhistidine (His)-tags. The initial cloning and transformation of the synthetic genes to produce the first stock cultures was performed in the past by our group.[1]
First, a preculture was prepared by mixing LB-medium (4 mL), kanamycin (50 µg/mL, 4 µL), and a cryogenic stock culture (50 µL). The preculture mixture was incubated for 4 h at 37 °C and 180 rpm. At this stage, a portion of the preculture can be diluted with an aq. glycerol (30 %) solution and stored at -80 °C to be used as stock culture. To synthesize the main culture, the preculture (1 mL) was mixed with 2TY-medium (50 mL) and kanamycin (50 µg/mL, 50 µL) and incubation at 37 °C and 180 rpm was continued until the required optical density value (≥ 0.4, λ = 600 nm) was achieved (2–5 h). To induce protein expression, the cloudy suspension was charged with *iso*propyl-β-thiogalactopyranoside (IPTG, 1 M, 50 µL) and incubation was continued for 20–24 h at 16 °C and 180 rpm. After centrifugation

(4500 rpm, 10 min, 4 °C), the supernatant was decanted and the obtained cell pellet was stored at -20 °C.

2.2 Protein Purification

The cell pellet obtained after heterologous expression was resuspended in lysis buffer (1 mL/100 mg pellet) and the cells ultrasonically lysed (sonotrode: KE76, amplitude: 45 %, 10 min, pulse/pause: 4 s/6 s). To remove all solid cell components, the resulting cloudy suspension was centrifuged (10 000 rpm, 4 °C) and the supernatant cell lysate collected. Immobilized metal chelate affinity chromatography (IMAC) was used to purify the proteins obtained. For this purpose, a nickel-nitrilotriacetic acid-agarose (Ni-NTA-agarose) column (Protino by MACHEREY-NAGEL) was utilized. The cell lysate was loaded onto the Ni-NTA-column and aq. imidazole solutions with increasing concentrations were used for elution (fractions: 25/50/100/250/500 mM, 5 mL each). Fractions containing the desired enzymes (determined via SDS PAGE or Bradford assay) were combined in a concentration/membrane tube (Amicon Ultra-15 by MERCK, 30 kDa MWCO) and concentrated via centrifugation (4500 rpm, 30 min, 4 °C). To remove excess salts, a desalting column (Cytiva PD-10 by FISHER SCIENTIFIC) containing cross-linked dextran gel (Sephadex G-25 Medium) was used. Finally, the proteins were collected in a centrifugal filter unit and after centrifugation (4500 rpm, 30 min, 4 °C), the concentrated and purified enzymes were fractionated into aliquots. Protein concentrations were determined via UV/VIS spectroscopy.

2.3 Analytical *in vitro* Enzyme Tests

Analytical enzyme *in vitro* assays (qualitative biotransformations) with the natural substrate FPP were conducted to determine the activity of BcBot2 (positive controls). Additionally, negative controls were performed when a new unnatural substrate was examined (incubation either without enzyme or substrate).

Table 2: Deployed conditions for *in vitro* analytical enzyme assays.

conditions per vial	
total volume	0.5 mL
HEPES	50 mM
DTT	5 mM
$MgCl_2$	5 mM
enzyme	0.1 g/L
substrate	0.15 mM
pH	7.5

First, reactions vials with the reported conditions (Table 2) were prepared by mixing all necessary reagents and solutions except for the active enzyme. With the addition of BcBot2, the biotransformation reaction was initiated. The reaction solutions were incubated for 20–30 min at 34 °C, cooled to 0 °C, and *n*-hexane (100 µL) was added. After extraction with a vortex mixer (30 s), the resulting suspensions were centrifuged (6000 rpm, 4 °C, 30 s) to achieve phase separation. The organic phases (60 µL) were transferred to vials with micro glass inserts and analyzed by GC-MS.

2.4 SemiPreparative Enzyme Tests

The conditions and procedure for semi-preparative biotransformation were slightly changed compared to the analytical tests (Table 3). The main difference is the supplemental addition of polysorbate 20 (Tween 20) as a stabilizer and emulsifier as well as pyrophosphatase (PPase). Moreover, the work-up procedure and the order and method of reagent addition were changed.

conditions per batch	
total volume	50 mL
HEPES	50 mM
DTT	5 mM
$MgCl_2$	15 mM
enzyme	0.2 g/L
substrate	1.5 mM
pH	7.5
Tween 20	0.01 %
PPase	1 µL

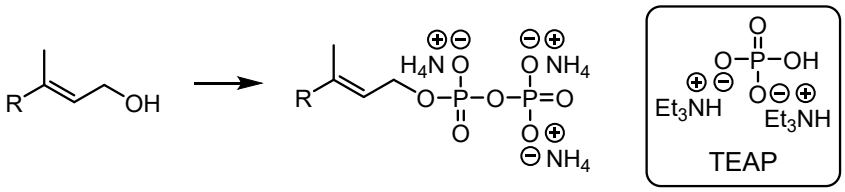
Table 3: Deployed conditions for semi-preparative biotransformation.

First, all necessary reagents and solutions except for the enzyme and substrate were added to a round bottom flask (Table 3). After adding the sesquiterpene cyclase (0.1 g/L), the substrate was added continuously using a syringe pump (1 mL/h) at room temperature. The resulting colorless suspension was then incubated for 6 h at 34 °C and 150 rpm. Afterwards, another portion of BcBot2 (0.05 g/L) was added and incubation was continued for 16 h. After this time, a final portion of enzyme solution (0.05 g/L) was added and incubation was continued for 4 h. The reaction mixture was extracted with Et_2O and the phases separated. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure (≥800 mbar, 40 °C). The crude mixture was purified by column chromatography (silica, *n*-pentane:Et_2O) to yield the desired biotransformation products.

3 Chemical Syntheses

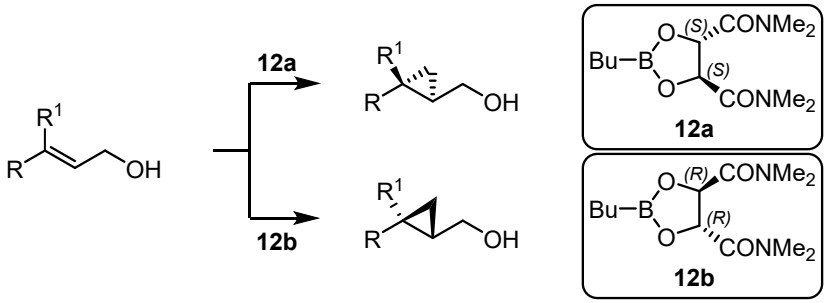
3.1 General ChemicalSynthetic Procedures

One-Step Synthesis of Diphosphates Starting from Allyl Alcohols



For the phosphorylation of allyl alcohols, a bis-triethylammonium phosphate (TEAP) solution was utilized. For this purpose, two solutions (A and B) were prepared in advance. For solution A, phosphoric acid (conc., 1.5 mL) was mixed with acetonitrile (9.4 mL) resulting in a diluted phosphoric acid/acetonitrile (3 M) solution. Similarly, freshly distilled triethylamine (11 mL) was diluted with acetonitrile (10 mL) to obtain solution B (3.8 M). To prepare the desired TEAP (3.5 M) solution, solutions A (0.91 mL) and B (1.5 mL) were mixed and stirred for 5–10 min at 36 °C. In a second flask, trichloroacetonitrile (25 eq.) was slowly added to the respective allyl alcohol (1 eq.) at room temperature. To this reaction mixture, freshly prepared TEAP (3.5 M, 27 eq.) was added in three portions at 5 min intervals. After the addition of the last portion, stirring was continued for 5 min and the crude mixture was purified by column chromatography (silica, *i*PrOH:NH_3(conc.):H_2O = 6:3:1) to yield the desired diphosphates.[2]

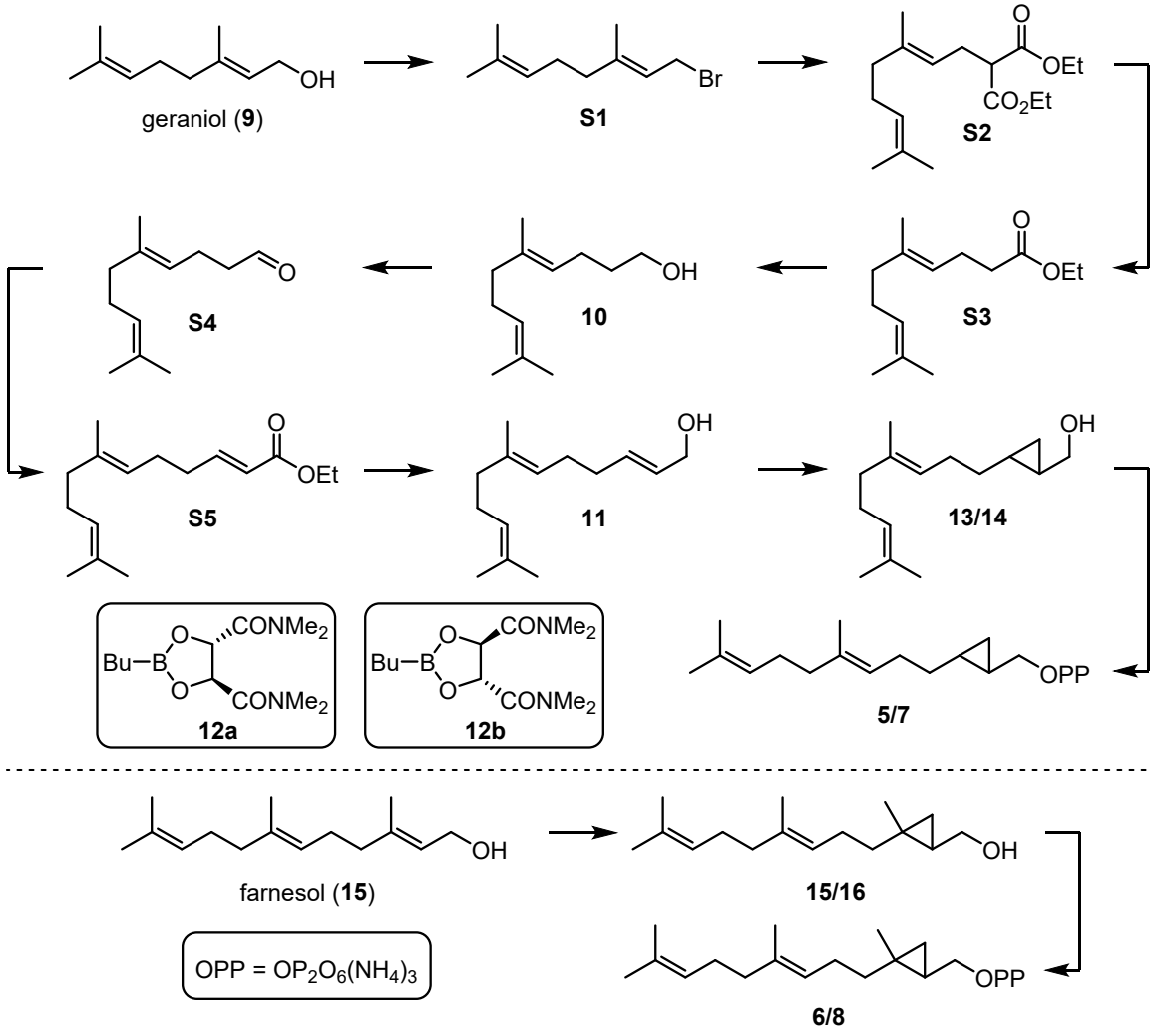
Asymmetric Cyclopropanation of Allyl Alcohols



Diethylzinc (2 M, 2 eq.), DME (2.2 eq.), and CH_2Cl_2 were added to a two-neck flask and the mixture was cooled to 0 °C. (A two-neck flask with an inlet adapter was used for this reaction because

diethylzinc vapors clog cannulas very quickly, which can lead to dangerous pressure increases during the reaction). A solution of diiodomethane (1 M, 2.2 eq.) in CH_2Cl_2 was added dropwise and stirring was continued for 30 min at 0 °C. In a second flask, the respective allyl alcohol (1 M, 1 eq.) and ligand (1 M, 1.5 eq.) were mixed in CH_2Cl_2 at 0 °C. The resulting solution was added quickly to the reaction flask and the mixture was allowed to warm to room temperature. Stirring was continued at room temperature until no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were subsequently washed with aq. HCl (1 M), a sat. aq. $NaHCO_3$ and NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:Et_2O = 10:1 → 4:1) to yield the desired cyclopropyl alcohol.[3,4]

3.2 Syntheses of Cyclopropyl Diphosphates 5-8



S1: (*E*)-1-Bromo-3,7-dimethylocta-2,6-diene

Phosphorus tribromide (4.93 mL, 0.05 mol, 0.4 eq.) was added dropwise to a stirring solution of geraniol (**9**) (20 g, 0.13 mol, 1 eq.) in dry THF (60 mL) at 0 °C. After stirring for 1 h at 0 °C, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and

concentrated under reduced pressure. The obtained colorless oil was used in the next reaction without further purification. The analytical data are consistent with those reported in the literature.[5]
R_f = 0.8 (PE:EtOAc = 4:1).

S2: Diethyl (*E*)-2-(3,7-dimethylocta-2,6-dien-1-yl)malonate
To a solution of K_2CO_3 in DMF (0.5 M, 25 mL) was added diethyl malonate (2.07 g, 12.89 mmol, 1 eq.) and geranyl bromide (**S1**) (2.8 g, 12.89 mmol, 1 eq.) at room temperature. After stirring for 18 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of aq. HCl (1 M). The phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with H_2O and a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained crude colorless oil was used in the next reaction without further purification.[5]
R_f = 0.6 (PE:EtOAc = 4:1).

S3: Ethyl (*E*)-5,9-dimethyldeca-4,8-dienoate
a): To a stirring solution of crude diester **S2** (1.59 g, 5.36 mmol, 1 eq.) in DMSO (25 mL) was added H_2O (125.5 mg, 6.97 mmol, 1.3 eq.) and lithium chloride (0.58 g, 13.68 mmol, 2.55 eq.) at room temperature. After heating to 160 °C and stirring the reaction mixture for 18 h under refluxing conditions, no further conversion of the starting material was observed. The crude mixture was cooled to room temperature and extracted with Et_2O. The combined organic phases were washed with H_2O and a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:EtOAc = 20:1) to yield the desired ester **S3** (314 mg, 1.4 mmol, 26 % o3s) as a yellow oil.[6]
b): *n*-Butyllithium (1.6 M in hexane, 4.26 g, 66.45 mmol, 3.05 eq.) was added to freshly-distilled di*iso*propylamine (6.67 g, 66.45 mmol, 2.05 eq.) in dry THF (80 mL) at 0 °C. The mixture was stirred at the same temperature for 1 h to yield lithium di*iso*propylamide. In a second flask, EtOAc (5.85 g, 66.45 mmol, 2.05 eq.) was added to a suspension of copper(I)-iodide (24.69 g, 129.7 mmol, 4 eq.) in THF (240 mL) at -110 °C. The freshly prepared lithium di*iso*propylamide mixture was added to the second flask at -100 °C and stirring was continued for 1.5 h at -50 °C. Then, freshly prepared geranyl bromide (**S1**) (7.04 g, 32.41 mmol, 1 eq.) in THF (60 mL) was added and stirring was continued for 2 h at -30 °C, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. NH_4Cl solution. The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained crude yellow oil was used in the next reaction without further purification. The analytical data are consistent with those reported in the literature.[7]
R_f = 0.45 (PE:EtOAc = 20:1); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.13–5.05 (m, 2 H, H-3, H-7), 4.12 (q, J = 7.1 Hz, 2 H, CO_2-CH_2), 2.32–2.30 (m, 4 H, H-8, H-9), 2.08–1.94 (m, 4 H, H-4, H-5), 1.67 (m, 3 H, H-1), 1.62-1.57 (m, 6 H, 2×CH_3), 1.25 (t, J = 7.1 Hz, 3 H, CO_2CH_2-CH_3) ppm.

10: (*E*)-5,9-Dimethyldeca-4,8-dien-1-ol
Di*iso*butylaluminium hydride (1 M in hexane, 10.41 g, 71.30 mmol, 2.2 eq.) was slowly added to a solution of ester **S3** (7.27 g, 31.41 mmol, 1 eq., 1 M) in dry Et_2O at -78 °C. After stirring for 18 h at room temperature, no further conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na, K-tartrate solution. Stirring was continued for 16 h at room temperature, after which the phases were separated and the aqueous phase extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica,

PE:EtOAc = 5:1) to yield the desired alcohol **10** (2.43 g, 13.33 mmol, 41 % o3s starting from **9**) as a colorless oil. The analytical data are consistent with those reported in the literature.[6,7]
R_f = 0.3 (PE:EtOAc = 4:1); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.17–5.13 (m, 1 H, H-3/7), 5.10–5.06 (m, 1 H, H-3/7), 3.65 (t, J = 6.3 Hz, 2 H, H-10), 2.11–2.05 (m, 4 H, H-4, H-5), 2.07–1.96 (m, 2 H, H-8), 1.68 (m, 3 H, H-1), 1.64–1.59 (m, 8 H, H-9, 2×CH_3), 1.41 (bs, 1 H, OH) ppm.

S4: (*E*)-5,9-Dimethyldeca-4,8-dienal
To a stirring solution of oxalyl chloride (0.34 mL, 3.92 mmol, 1.5 eq.) in CH_2Cl_2 (3.9 mL) was slowly added DMSO (0.56 mL, 7.83 mmol, 3 eq.) at -78 °C and stirring was continued for 1.5 h. Then, alcohol **10** (476 mg, 2.61 mmol, 1 eq.) in CH_2Cl_2 (1.3 mL) was slowly added. After stirring the mixture for 2 h at -78 °C, freshly-distilled triethylamine (1.81 mL, 13.05 mmol, 5 eq.) was slowly added. The reaction mixture was allowed to warm to room temperature and stirred for 14 h, after which no further conversion of the starting material was observed. The reaction was terminated by the addition of H_2O, the phases were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat aq. NH_4Cl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:Et_2O = 20:1 → 4:1) to yield the desired aldehyde **S4** (375 mg, 2.09 mmol, 80 %) as a colorless oil.[8] The analytical data are consistent with those reported in the literature.[7]
R_f = 0.5 (PE:EtOAc = 10:1); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 9.76 (t, J = 1.8 Hz, 1 H, H-10), 5.12–5.05 (m, 2 H, H-3, H-7), 2.48–2.43 (m, 2 H, H-9), 2.36–2.30 (m, 2 H, H-8), 2.07–1.96 (m, 4 H, H-4, H-5), 1.68 (m, 3 H, H-1), 1.62–1.59 (m, 6 H, 2×CH_3) ppm.

S5: **Ethyl (2*E*,6*E*)-7,11-dimethyldodeca-2,6,10-trienoate**
To a stirring solution of aldehyde **S4** (187 mg, 1.04 mmol, 1 eq.) in toluene (2 mL) was added ethyl (triphenylphosphoranylidene)acetate (542 mg, 1.56 mmol, 1.5 eq.) at room temperature. After stirring for 18 h, no further conversion of the starting material was observed. The solvent was removed under reduced pressure and the crude mixture was dry loaded onto a column. After purification via column chromatography (silica, PE:Et_2O = 20:1), the desired ester **S5** (174 mg, 0.69 mmol, 67 %) was obtained as a colorless oil. The analytical data are consistent with those reported in the literature.[6]
R_f = 0.55 (PE:EtOAc = 10:1); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 6.96 (dt, J = 15.6 Hz, 6.7 Hz, 1 H, H-10), 5.82 (d, J = 15.6 Hz, 1 H, H-11), 5.13–5.07 (m, 2 H, H-3, H-7), 4.18 (q, J = 7.1 Hz, 2 H, CO_2-CH_2), 2.26–2.14 (m, 2 H, H-8, H-9), 2.09–1.96 (m, 4 H, H-4, H-5), 1.68 (s, 3 H, H-1), 1.60 (s, 6 H, 2×CH_3), 1.28 (t, J = 7.1 Hz, 3 H, CO_2CH_2-CH_3) ppm.

11: **(2*E*,6*E*)-7,11-Dimethyldodeca-2,6,10-trien-1-ol**
To a stirring solution of ester **S5** (350 mg, 1.4 mmol, 1 eq.) in Et_2O (3.5 mL) was dropwise added di*iso*butylaluminium hydride (1 M in hexane, 3.08 mL, 3.08 mmol, 2.2 eq.) at -78 °C. Stirring was continued for 18 h at room temperature, after which full conversion of the starting material was observed. The reaction was terminated by the addition of a sat. aq. Na, K-tartrate solution. After stirring the mixture for 16 h at room temperature, the phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with a sat. aq. NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, PE:Et_2O = 5:1) to yield the desired alcohol **11** (207 mg, 0.99 mmol, 71 %) as a colorless oil. The analytical data are in agreement with the literature data.[6]
R_f = 0.25 (PE:EtOAc = 10:1); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.73–5.62 (m, 2 H, H-10, H-11), 5.12–5.07 (m, 2 H, H-3, H-7), 4.09 (d, J = 5.1 Hz, 2 H, H-12), 2.09–1.96 (m, 8 H, H-4, H-5, H-8, H-9), 1.68 (s, 3 H, H-1), 1.60 (s, 6 H, 2×CH_3) ppm.

13: ((1*R*,2*R*)-2-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)cyclopropyl)methanol

Starting from allyl alcohol **11** (100 mg, 0.48 mmol, 1 eq.), the desired cyclopropyl alcohol **13** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 5) using (4*S*,5*S*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**12a**, 194.5 mg, 1.06 mmol, 1.5 eq.). Purification via column chromatography (silica, PE:EtOAc = 10:1 → 5:1) delivered alcohol **13** (61 mg, 0.27 mmol, 73 %) as a colorless oil with minor impurities.

$\mathbf{R_f}$ = 0.3 (PE:EtOAc = 4:1); $\mathbf{[\alpha]^{24}_D}$ = -8.4° (c = 1.0, $CHCl_3$); **HR-GC-MS**: calculated: $C_{15}H_{27}O$ $[M+H]^+$: 223.2062, found: 223.2065 m/z; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.15 (t, *J* = 13.7 Hz, 1 H, H-6), 5.09 (t, *J* = 6.8 Hz, 1 H, H-10), 3.43 (d, *J* = 7.1 Hz, 2 H, H-1), 2.11–2.04 (m, 4 H, H-5, H-9), 2.00–1.95 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.61 (s, 3 H, H-14), 1.60 (s, 3 H, H-15), 1.29–1.20 (m, 2 H, H-4), 0.91–0.81 (m, 1 H, H-2), 0.65–0.57 (m, 1 H, H-3), 0.39–0.30 (m, 2 H, H-13) ppm; **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 135.4 (C-7), 131.5 (C-11), 124.5 (C-6), 124.4 (C-10), 67.4 (C-1), 39.9 (C-8), 33.9 (C-4), 28.1 (C-5/9), 26.8 (C-5/9), 25.8 (C-12), 21.5 (C-2), 17.8 (C-15), 17.1 (C-3), 16.1 (C-14), 10.1(C-13) ppm.

14: ((1*S*,2*S*)-2-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)cyclopropyl)methanol

Starting from allyl alcohol **11** (100 mg, 0.48 mmol, 1 eq.), the desired cyclopropyl alcohol **14** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 5) using (4*R*,5*R*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**12b**, 194.5 mg, 1.06 mmol, 1.5 eq.). Purification via column chromatography (silica, PE:EtOAc = 10:1 → 7:1) delivered alcohol **14** (92 mg, 0.41 mmol, 89 %) as a colorless oil with minor impurities.

$\mathbf{R_f}$ = 0.3 (PE:EtOAc = 4:1); $\mathbf{[\alpha]^{24}_D}$ = 7.2° (c = 1.1, $CHCl_3$); **HR-GC-MS**: calculated: $C_{15}H_{27}O$ $[M+H]^+$: 223.2062, found: 223.2060 m/z; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.15 (t, *J* = 13.4 Hz, 1 H, H-6), 5.09 (t, *J* = 6.8 Hz, 1 H, H-10), 3.45 (d, *J* = 7.1 Hz, 2 H, H-1), 2.11–2.04 (m, 4 H, H-5, H-9), 2.00–1.95 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.61 (s, 3 H, H-14), 1.60 (s, 3 H, H-15), 1.29–1.20 (m, 2 H, H-4), 0.89–0.83 (m, 1 H, H-2), 0.65–0.57 (m, 1 H, H-3), 0.39–0.30 (m, 2 H, H-13) ppm; **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 135.4 (C-7), 131.5 (C-11), 124.5 (C-6), 124.4 (C-10), 67.4 (C-1), 39.9 (C-8), 33.9 (C-4), 28.1 (C-5/9), 26.8 (C-5/9), 25.8 (C-12), 21.5 (C-2), 17.8 (C-15), 17.1 (C-3), 16.1 (C-14), 10.1(C-13) ppm.

15: ((1*R*,2*R*)-2-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)-2-methylcyclopropyl)methanol

Starting from farnesol (**15**) (300 mg, 1.35 mmol, 1 eq.), the desired cyclopropyl methyl alcohol **15** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 5) using (4*S*,5*S*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**12a**, 546.6 mg, 2.02 mmol, 1.5 eq.) and CH_2I_2 (4 eq.). Purification via column chromatography (silica, PE:EtOAc = 10:1 → 4:1) delivered alcohol **15** (299 mg, 1.27 mmol, 94 %, 94 % *ee*) as a colorless oil.

$\mathbf{R_f}$ = 0.3 (PE:EtOAc = 4:1); $\mathbf{[\alpha]^{24}_D}$ = -6.6° (c = 1.0, $CHCl_3$); **HR-GC-MS**: calculated: $C_{16}H_{26}$ $[M-H_2O]$: 218.2035, found: 218.2032 m/z; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.14–5.07 (m, 2 H, H-6, H-10), 3.71 (dd, *J* = 11.4 Hz, 6.6 Hz, 1 H, H-1), 3.50 (dd, *J* = 11.3 Hz, 8.6 Hz, 1 H, H-1), 2.10–2.03 (m, 4 H, H-5, H-9), 1.99–1.95 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.60 (s, 3 H, H-15), 1.60 (s, 3 H, H-16), 1.40–1.32 (m, 1 H, H-4), 1.20–1.15 (m, 1 H, H-4), 1.10 (s, 3 H, H-14), 0.95–0.88 (m, 1 H, H-2), 0.51 (dd, *J* = 8.6 Hz, 4.5 Hz, 1 H, H-13), 0.13 (t, *J* = 4.9 Hz, 1 H, H-13) ppm; **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 135.1 (C-7), 131.5 (C-11), 124.5 (d, C-6, C-10), 64.1 (C-1), 41.3 (C-4), 39.9 (C-8), 26.8 (C-5/9), 26.4 (C-5/9), 25.8 (C-12), 25.5 (C-2), 20.1 (C-3), 17.9 (C-13), 17.8 (C-16), 17.2 (C-14), 16.1 (C-15) ppm.

16: ((1*S*,2*S*)-2-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)-2-methylcyclopropyl)methanol

Starting from farnesol (**15**) (300 mg, 1.35 mmol, 1 eq.), the desired cyclopropyl methyl alcohol **16** was prepared according to the general procedure for the asymmetric cyclopropanation of allyl alcohols (p. 5) using (4*R*,5*R*)-2-butyl-N^4,N^4,N^5,N^5-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide (**12b**, 546.6 mg, 2.02 mmol, 1.5 eq.) and CH_2I_2 (4 eq.). Purification via column chromatography (silica, PE:EtOAc = 10:1 → 4:1) delivered alcohol **16** (318.9 mg, 1.35 mmol, quant.) as a colorless oil.
The analytical data are consistent with those reported in the literature.[4]
$\mathbf{R_f}$ = 0.3 (PE:EtOAc = 4:1); $\mathbf{[\alpha]^{24}_D}$ = 6.7° (c = 1.0, $CHCl_3$; Lit.: 5.5°, c = 3.5, $CHCl_3$); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.14–5.07 (m, 2 H, H-6, H-10), 3.71 (dd, *J* = 11.4 Hz, 6.6 Hz, 1 H, H-1), 3.50 (dd, *J* = 11.4 Hz, 8.6 Hz, 1 H, H-1), 2.10–2.03 (m, 4 H, H-5, H-9), 1.99–1.95 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.60 (s, 3 H, H-15), 1.60 (s, 3 H, H-16), 1.40–1.32 (m, 1 H, H-4), 1.27 (bs, 1 H, OH), 1.20–1.12 (m, 1 H, H-4), 1.10 (s, 3 H, H-14), 0.95–0.88 (m, 1 H, H-2), 0.51 (dd, *J* = 8.6 Hz, 4.8 Hz, 1 H, H-13), 0.13 (t, *J* = 4.8 Hz, 1 H, H-13) ppm; **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 135.1 (C-7), 131.5 (C-11), 124.5 (d, C-6, C-10), 64.1 (C-1), 41.2 (C-4), 39.8 (C-8), 26.8 (C-5/9), 26.3 (C-5/9), 25.8 (C-12), 25.5 (C-2), 20.1 (C-3), 17.9 (C-13), 17.8 (C-16), 17.2 (C-14), 16.1 (C-15) ppm.

5: Ammonium ((1*R*,2*R*)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)cyclopropyl)methyl diphosphate
Cyclopropyl diphosphate **5** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 5) starting from alcohol **13** (30 mg, 135 µmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **5** (20 mg, 46.1 µmol, 34 %) as a colorless solid.
$\mathbf{R_f}$ = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1); **^{1}H-NMR** (400 MHz, D_2O): δ = 5.29 (t, *J* = 7.3 Hz, 1 H, H-6), 5.20 (tt, *J* = 6.9 Hz, 1.3 Hz, 1 H, H-10), 4.00–3.89 (m, 1 H, H-1), 3.68–3.61 (m, 1 H, H-1), 2.16–2.09 (m, 4 H, H-5, H-9), 2.09–2.02 (m, 2 H, H-8), 1.69 (s, 3 H, H-12), 1.64 (s, 3 H, H-14), 1.63 (s, 3 H, H-15), 1.37–1.30 (m, 2 H, H-4), 0.99–0.93 (m, 1 H, H-2), 0.77–0.71 (m, 1 H, H-3), 0.51–0.46 (m, 1 H, H-13), 0.42–0.36 (m, 1 H, H-13) ppm; **^{13}C-NMR** (100 MHz, D_2O): δ = 136.3 (C-7), 133.5 (C-11), 124.9 (C-6), 124.5 (C-10), 71.0 (d, C-1), 38.8 (C-8), 33.0 (C-4), 27.2 (C-5/9), 25.7 (C-5/9), 24.8 (C-12), 18.2 (d, C-2), 16.9 (C-15), 16.7 (C-3), 15.2 (C-14), 9.8 (C-13) ppm. **^{31}P-NMR** (160 MHz, D_2O): δ = -10.38 (d, *J* = 20.4 Hz, 1 P, terminal P), -10.71 (dt, *J* = 20.5 Hz, 6.3 Hz, 1 P, RO-*P*O_3-) ppm.

7: Ammonium ((1*S*,2*S*)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)cyclopropyl)methyl diphosphate
Cyclopropyl diphosphate **7** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 5) starting from alcohol **14** (30 mg, 135 µmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **7** (14 mg, 32.3 µmol, 24 %) as a colorless solid.
$\mathbf{R_f}$ = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1); **^{1}H-NMR** (400 MHz, D_2O): δ = 5.31–5.26 (m, 1 H, H-6), 5.23–5.16 (m, 1 H, H-10), 3.99–3.90 (m, 1 H, H-1), 3.72–3.60 (m, 1 H, H-1), 2.16–2.09 (m, 4 H, H-5, H-9), 2.09–2.02 (m, 2 H, H-8), 1.69 (s, 3 H, H-12), 1.64 (s, 3 H, H-14), 1.62 (s, 3 H, H-15), 1.39–1.33 (m, 2 H, H-4), 0.99–0.92 (m, 1 H, H-2), 0.79–0.71 (m, 1 H, H-3), 0.52–0.47 (m, 1 H, H-13), 0.42–0.36 (m, 1 H, H-13) ppm; **^{13}C-NMR** (100 MHz, D_2O): δ = 136.2 (C-7), 133.3 (C-11), 124.9 (C-6), 124.5 (C-10), 70.9 (d, C-1), 38.8 (C-8), 33.1 (C-4), 27.3 (C-5/9), 25.8 (C-5/9), 24.8 (C-12), 18.1 (d, C-2), 17.0 (C-15), 16.7 (C-3), 15.2 (C-14), 9.8 (C-13) ppm. **^{31}P-NMR** (160 MHz, D_2O): δ = -10.48 (d, *J* = 20.9 Hz, 1 P, terminal P), -10.73 (d, *J* = 20.9 Hz, 6.3 Hz, 1 P, RO-*P*O_3-) ppm.

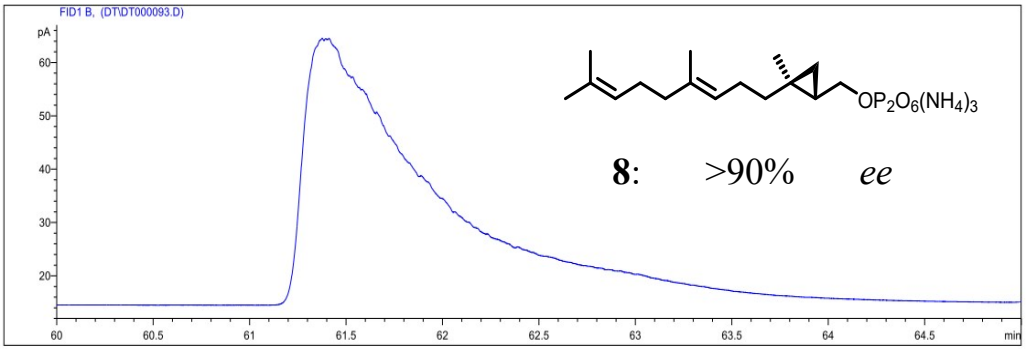
6: Ammonium ((1*R*,2*R*)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)-2-methylcyclopropyl)methyl diphosphate
Cyclopropylmethyl diphosphate **6** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 5) starting from alcohol **15** (95 mg, 0.40 mmol, 1 eq.). Purification of the

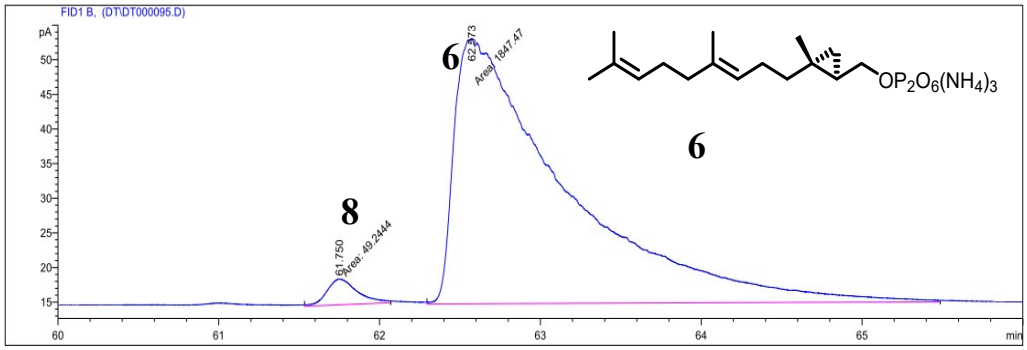
crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **6** (88 mg, 0.20 mmol, 49 %) as a colorless solid.
$\mathbf{R_f}$ = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1); **^{1}H-NMR** (400 MHz, D_2O): δ = 5.23 (t, *J* = 6.9 Hz, 1 H, H-6), 5.17 (t, *J* = 6.6 Hz, 1 H, H-10), 4.05–3.97 (m, 1 H, H-1), 3.90–3.84 (m, 1 H, H-1), 2.16–2.06 (m, 4 H, H-5, H-9), 2.03–1.98 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.62 (s, 3 H, H-15), 1.61 (s, 3 H, H-16), 1.28–1.25 (m, 2 H, H-4), 1.09 (s, 3 H, H-14), 1.01–0.94 (m, 1 H, H-2), 0.56 (dd, *J* = 8.4 Hz, 4.2 Hz, 1 H, H-13), 0.23 (t, *J* = 4.8 Hz, 1 H, H-13) ppm; **^{13}C-NMR** (100 MHz, D_2O): δ = 136.0 (C-7), 133.4 (C-11), 125.2 (C-6), 124.5 (C-10), 64.2 (C-1), 40.4 (C-4), 38.8 (C-8), 25.7 (C-5/9), 24.8 (C-5/9, C-12), 23.1 (d, C-2), 20.0 (C-3), 17.1 (C-13), 16.9 (C-16), 16.4 (C-14), 15.1 (C-15) ppm. **^{31}P-NMR** (160 MHz, D_2O): δ = -7.55 (d, *J* = 21.4 Hz, 1 P, terminal P), -10.44 (dt, *J* = 21.5 Hz, 5.5 Hz, 1 P, RO-*P*O_3-) ppm.

8: Ammonium ((1*S*,2*S*)-2-((*E*)-4,8-dimethylnona-3,7-dien-1-yl)-2-methylcyclopropyl)methyl diphosphate

Cyclopropylmethyl diphosphate **8** was prepared according to the general one-step procedure for the synthesis of diphosphates (p. 5) starting from alcohol **16** (10 mg, 42 µmol, 1 eq.). Purification of the crude mixture via column chromatography (silica, *i*PrOH:NH_3:H_2O = 6:3:1) yielded the desired diphosphate **8** (8 mg, 17.9 µmol, 43 %) as a colorless solid.
$\mathbf{R_f}$ = 0.27 (*i*PrOH:NH_3:H_2O = 6:3:1); **^{1}H-NMR** (400 MHz, D_2O): δ = 5.24 (t, *J* = 6.6 Hz, 1 H, H-6), 5.18 (t, *J* = 6.4 Hz, 1 H, H-10), 4.06–4.00 (m, 1 H, H-1), 3.91–3.84 (m, 1 H, H-1), 2.16–2.06 (m, 4 H, H-5, H-9), 2.03–2.00 (m, 2 H, H-8), 1.67 (s, 3 H, H-12), 1.62 (s, 3 H, H-15), 1.61 (s, 3 H, H-16), 1.28–1.24 (m, 2 H, H-4), 1.10 (s, 3 H, H-14), 1.01–0.99 (m, 1 H, H-2), 0.57 (dd, *J* = 8.4 Hz, 4.0 Hz, 1 H, H-13), 0.23 (t, *J* = 4.6 Hz, 1 H, H-13) ppm; **^{13}C-NMR** (100 MHz, D_2O): δ = 136.0 (C-7), 133.4 (C-11), 125.2 (C-6), 124.5 (C-10), 64.2 (C-1), 40.4 (C-4), 38.8 (C-8), 25.7 (C-5/9), 24.8 (C-5/9, C-12), 23.1 (d, C-2), 20.0 (C-3), 17.1 (C-13), 16.9 (C-16), 16.4 (C-14), 15.1 (C-15) ppm. **^{31}P-NMR** (160 MHz, D_2O): δ = -9.54 (d, *J* = 20.7 Hz, 1 P, terminal P), -10.61 (dt, *J* = 20.7 Hz, 5.8 Hz, 1 P, RO-*P*O_3-) ppm.

GC analysis





Signal 1: FID1 B,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
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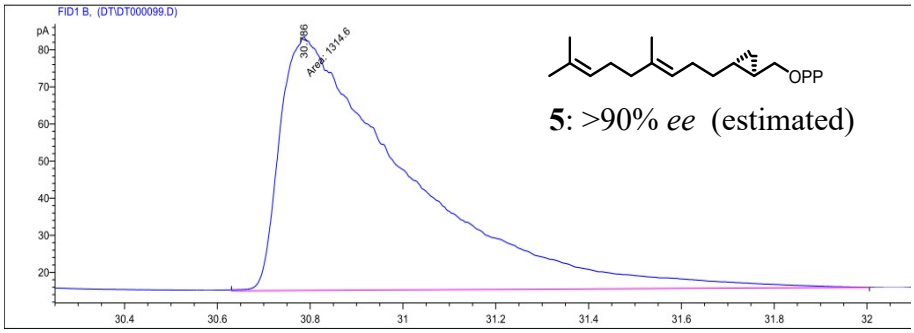
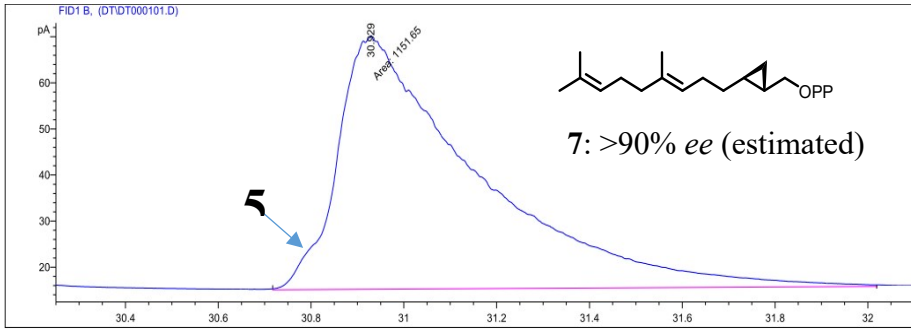
2	62.573	MM	0.8037	1847.47021	38.31194	97.40370

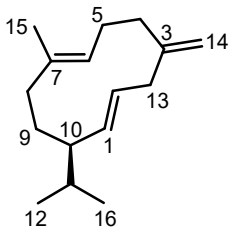




4 SemiPreparative Biotransformation of 6 with BcBot2

Following the general procedure for semi-preparative biotransformation, three new cyclic terpenes were obtained using the sesquiterpene cyclase BcBot2 and diphosphate **6** (33.5 mg, 75 µmol). Purification of the crude mixture via column chromatography (silica, *n*-pentane:Et_2O = 10:1 ⟶ 4:1 ⟶ 2:1) allowed separation of the triene **17** from the more polar alcohols **18** and **19**, which were obtained as a mixture (**18**:**19** = 2:3). Separation attempts via preparative GC were unsuccessful.

Biotransformation product 17: (*R*,1*E*,6*E*)-5-Isopropyl-2-methyl-9-methylenecycloundeca-1,6-diene

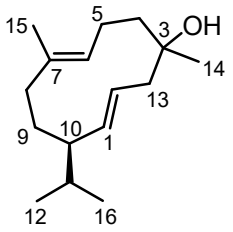


17

(*R*,1*E*,6*E*)-5-Isopropyl-2-methyl-9-methylenecycloundeca-1,6-diene (**17**) (4 mg, 18 µmol, 24 %) was obtained as a highly volatile colorless oil with impurities. $\mathbf{R_f}$ = 0.90 (PE:EtOAc = 4:1); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.12–5.15 (m, 1 H, H-6), 5.11 (ddd, *J* = 15.1 Hz, 9.4 Hz, 5.5 Hz, 1 H, H-2), 5.02 (dd, *J* = 15.3 Hz, 9.5 Hz, 1 H, H-1), 4.81 (d, *J* = 8.2 Hz, 2 H, H-14), 2.73 (dd, *J* = 12.5 Hz, 5.4 Hz, 1 H, H-13), 2.48 (dd, *J* = 12.7 Hz, 9.4 Hz, 1 H, H-13), 2.28–2.32 (m, 1 H, H-5), 2.22–2.25 (m, 1 H, H-8), 2.12–2.15 (m, 1 H, H-4), 2.06–2.10 (m, 1 H, H-8), 1.94–1.96 (m, 1 H, H-4), 1.90–1.92 (m, 1 H, H-5), 1.84–1.87 (m, 1 H, H-10), 1.40–1.43 (m, 2 H, H-9), 1.49 (m, 4 H, H-11, H-15), 0.83 (d, *J* = 6.8 Hz, 3 H, H-12/16), 0.80 (d, *J* = 6.9 Hz, 3 H, H-12/16) ppm; **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 150.1 (C-3) 135.2 (C-7), 133.5 (C-1), 128.9 (C-2), 127.3 (C-6), 111.2 (C-14), 53.1 (C-10), 43.4 (C-13), 41.6 (C-8), 33.6 (C-11), 34.2 (C-4), 29.6 (C-5), 20.8 (C-12), 28.2 (C-9), 15.9 (C-15), 19.0 (C-16) ppm.

Biotransformation product 18: (3*E*,5*R*,8*E*)-5-Isopropyl-1,8-dimethylcycloundeca-3,8-dien-1-ol

(3*E*,5*R*,8*E*)-5-Isopropyl-1,8-dimethylcycloundeca-3,8-dien-1-ol (**18**) (2 mg, 8.5 µmol, 11 %) was obtained as a volatile colorless oil.

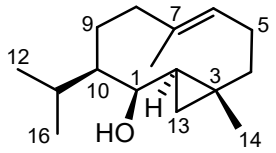


18

R_f = 0.20 (PE:EtOAc = 4:1); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.28 (ddd, *J* = 15.4 Hz, 10.0 Hz, 5.2 Hz, 1 H, H-2) 5.13 (dd, *J* = 15.4 Hz, 9.8 Hz, 1 H, H-1), 5.06–5.09 (m, 1 H, H-6), 2.26–2.33 (m, 1 H, H-5), 2.23–2.25 (m, 1 H, H-8), 2.18–2.19 (m, 1 H, H-13), 2.15–2.17 (m, 1 H, H-8), 2.01 (dd, *J* = 13.0 Hz, 10.1 Hz, 1 H, H-13), 1.89–1.95 (m, 1 H, H-10), 1.82 (m, 1 H, H-5), 1.80 (m, 1 H, H-4), 1.67–1.69 (m, 1 H, H-4), 1.57 (s, 3 H, H-15), 1.55 (br, 1 H, OH), 1.49–1.52 (m, 1 H, H-11), 1.36–1.40 (m, 2 H, H-9), 1.24 (m, 3 H, H-14), 0.83 (d, *J* = 6.8 Hz, 3 H, H-12/16), 0.80 (d, *J* = 6.9 Hz, 3 H, H-12/16) ppm; **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 135.9 (C-1) 133.2 (C-7), 128.8 (C-2), 130.1 (C-6), 72.8 (C-3), 53.5 (C-10), 48.0 (C-13), 33.8 (C-11), 42.2 (C-8), 30.1 (C-14), 40.0 (C-4), 27.2 (C-9), 23.9 (C-5), 16.0 (C-15), 18.8 (C-12/16), 20.7 (C-12/16) ppm.

Biotransformation product 19: (1*R*,2*S*,3*R*,10*R*,*E*)-3-Isopropyl-6,10-dimethyl-bicyclo[8.1.0]undec-6-en-2-ol

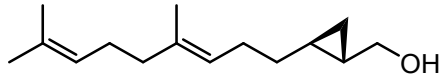
(1*R*,2*S*,3*R*,10*R*,*E*)-3-Isopropyl-6,10-dimethyl-bicyclo[8.1.0]undec-6-en-2-ol (**19**) (3 mg, 13 µmol, 17 %) was obtained as a volatile colorless oil.



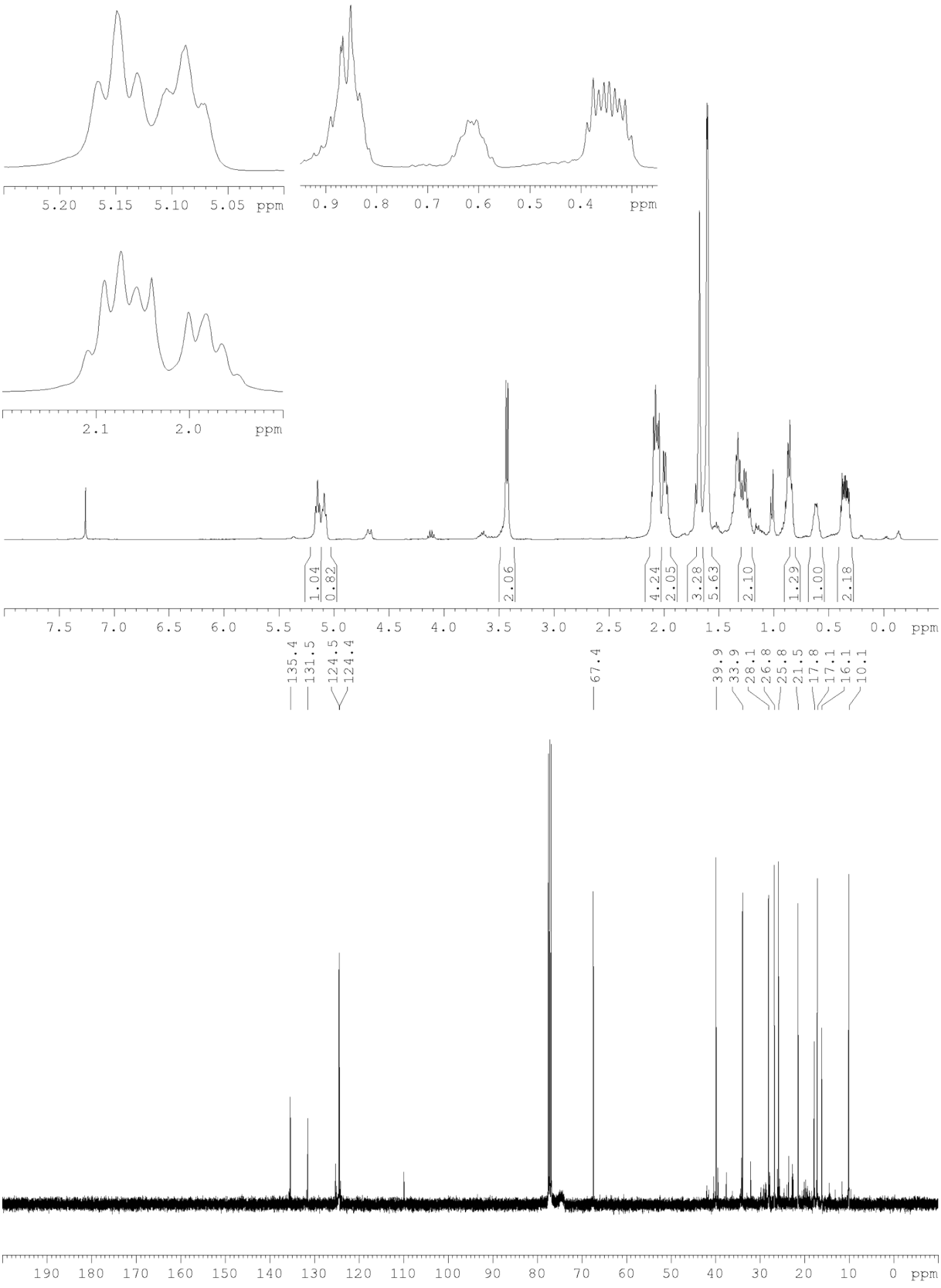
19

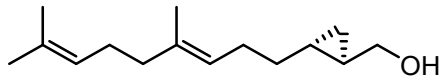
R_f = 0.20 (PE:EtOAc = 4:1); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 5.32 (t, *J* = 7.6 Hz, 1 H, H-6) 3.36 (dd, *J* = 9.9 Hz, 1.7 Hz, 1 H, H-1), 2.20–2.25 (m, 2 H, H-5, H-8), 2.13–2.17 (m, 1 H, H-5), 1.84–1.88 (m, 1 H, H-4), 1.80 (m, 1 H, H-8), 1.73–1.76 (m, 1 H, H-9), 1.67–1.69 (m, 1 H, H-11), 1.64 (s, 3 H, H-15), 1.55 (br, 1 H, OH), 1.32–1.35 (m, 1 H, H-9), 1.05–1.08 (m, 1 H, H-10), 1.00 (d, *J* = 6.7 Hz, 3 H, H-12/16), 0.98 (s, 3 H, H-14), 0.93 (d, *J* = 6.6 Hz, 3 H, H-12/16), 0.85–0.87 (m, 1 H, H-4), 0.62 (ddd, *J* = 9.7 Hz, 8.3 Hz, 5.6 Hz, 1 H, H-2), 0.40 (dd, *J* = 8.3 Hz, 4.3 Hz, 1 H, H-13), 0.12 (dd, *J* = 5.5 Hz, 4.3 Hz, 1 H, H-13) ppm; **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 133.2 (C-7) 124.0 (C-6), 74.7 (C-1), 48.4 (C-10), 39.2 (C-4), 38.8 (C-8), 32.2 (C-11), 30.0 (C-2), 25.6 (C-9), 24.2 (C-5), 21.7 (C-12/16), 21.3 (C-12/16), 18.8 (C-15), 18.5 (C-3), 17.3 (C-14), 17.2 (C-13) ppm.

5 Spectra and Chromatograms

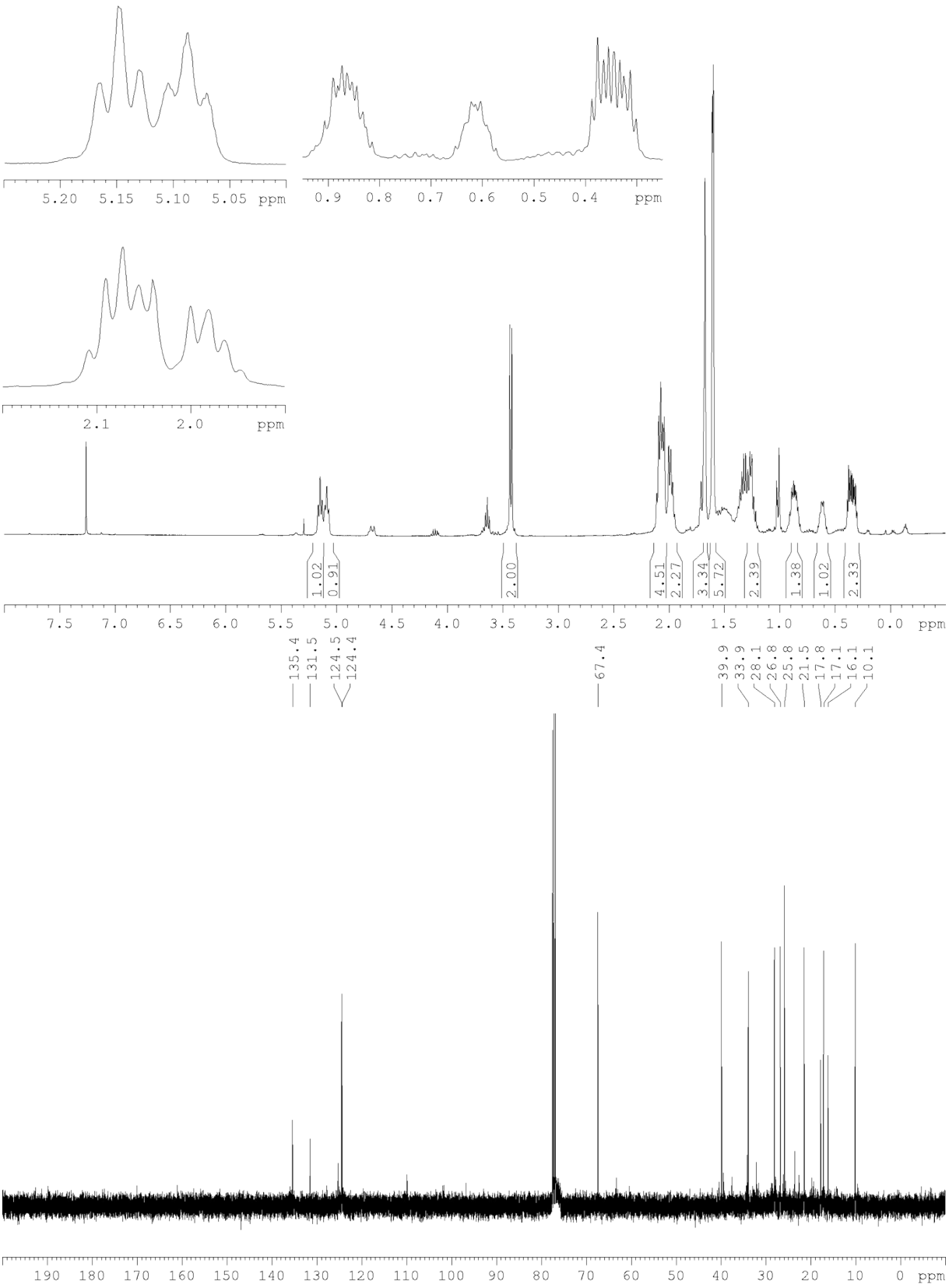


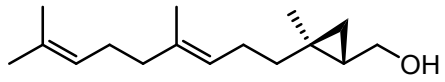
14



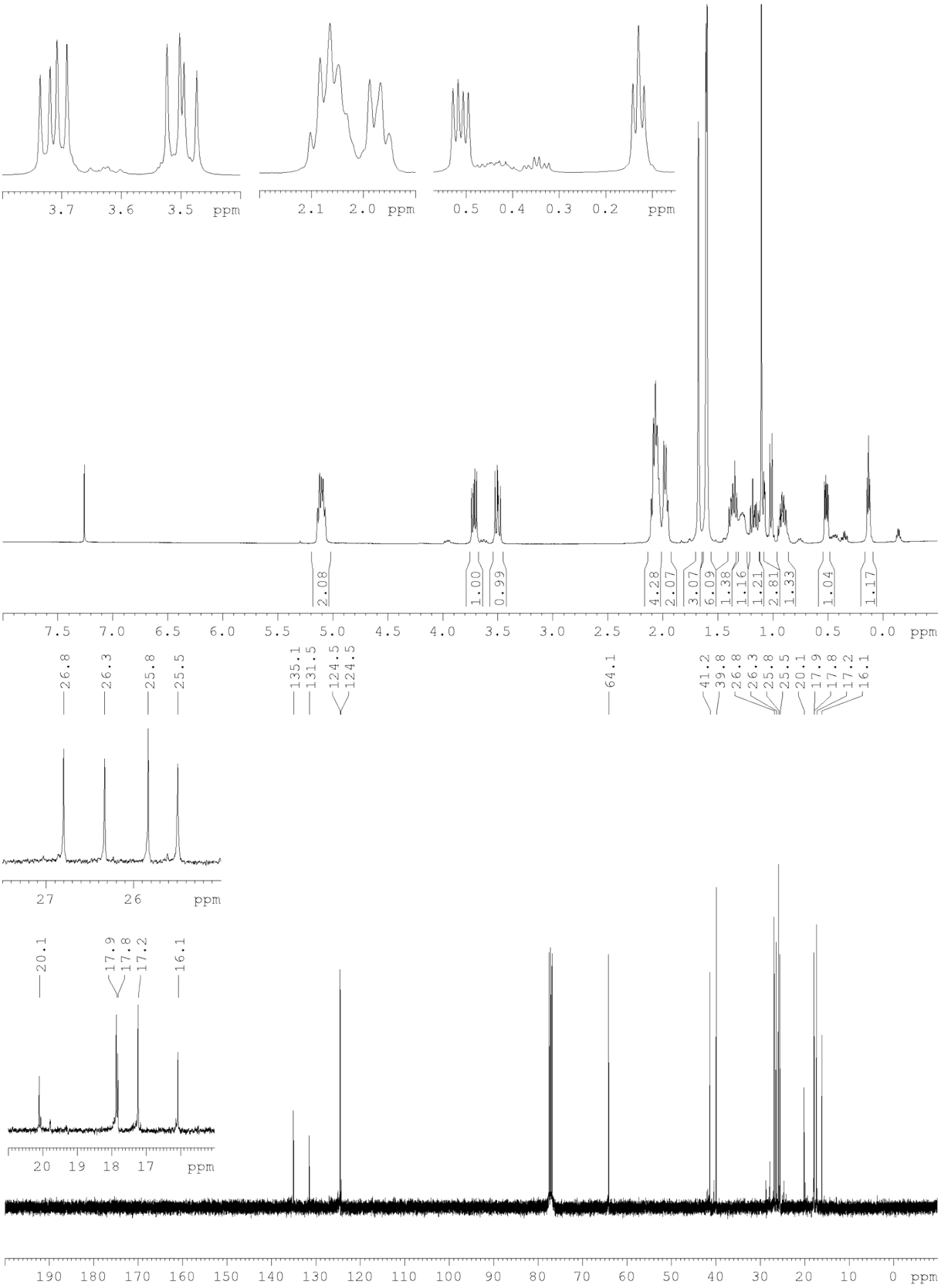

OH

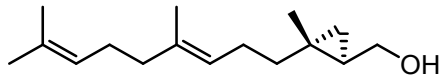
13


5.20 5.15 5.10 5.05 ppm
0.9 0.8 0.7 0.6 0.5 0.4 ppm
2.1 2.0 ppm
1.02 0.91 2.00 4.51 2.27 3.34 5.72 2.39 1.38 1.02 2.33
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
135.4 131.5 124.5 124.4 67.4 39.9 33.9 28.1 26.8 25.8 21.5 17.8 17.1 16.1 10.1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

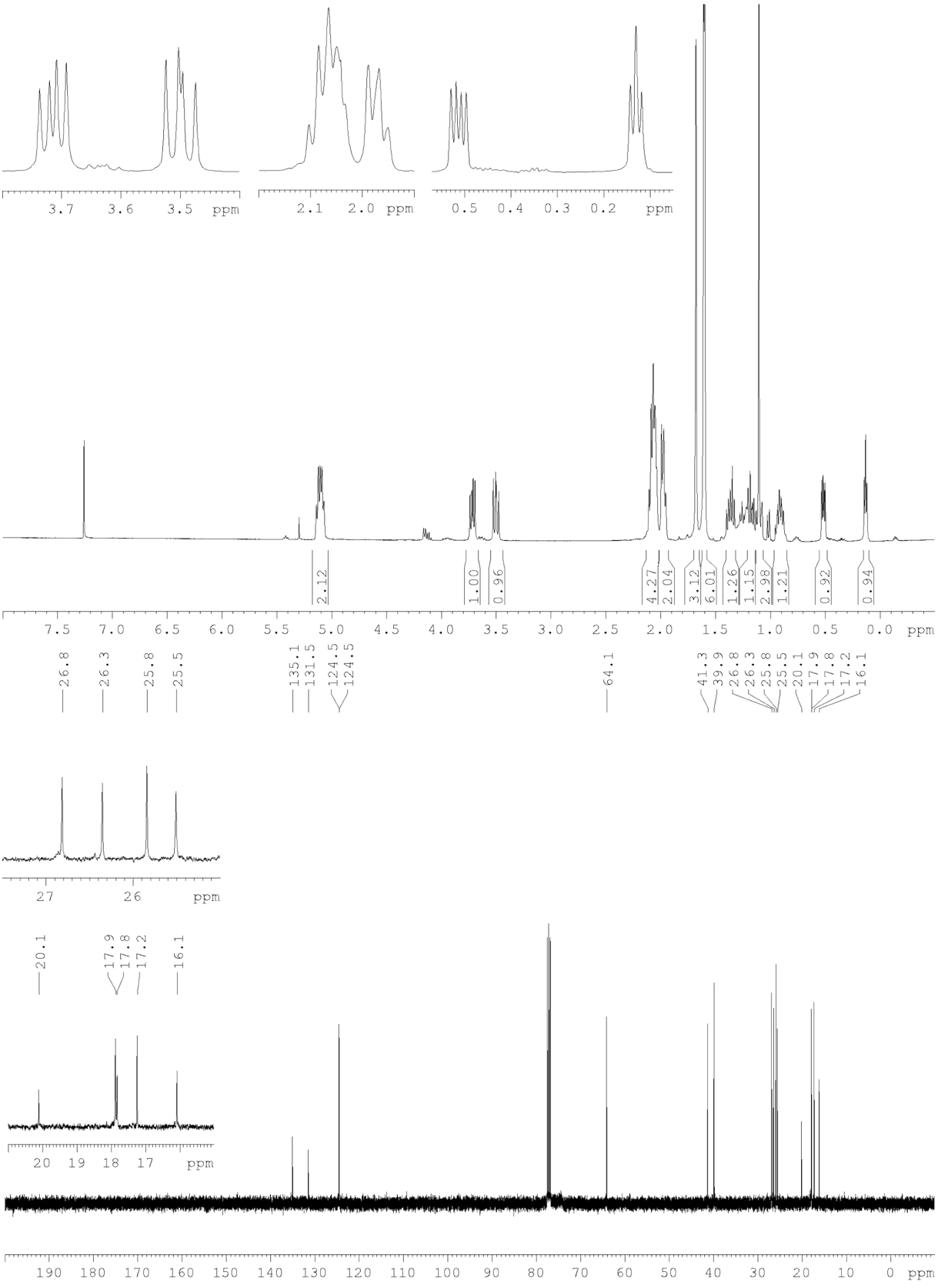

OH

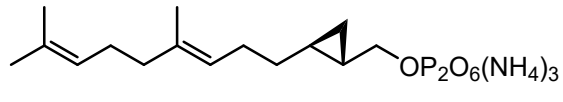
16


3.7 3.6 3.5 ppm
2.1 2.0 ppm
0.5 0.4 0.3 0.2 ppm
2.08
1.00
0.99
4.28
2.07
3.07
6.09
1.38
1.16
1.21
2.81
1.33
1.04
1.17
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
26.8
26.3
25.8
25.5
135.1
131.5
124.5
124.5
64.1
41.2
39.8
26.8
26.3
25.8
25.5
20.1
17.9
17.8
17.2
16.1
27 26 ppm
20.1
17.9
17.8
17.2
16.1
20 19 18 17 ppm
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

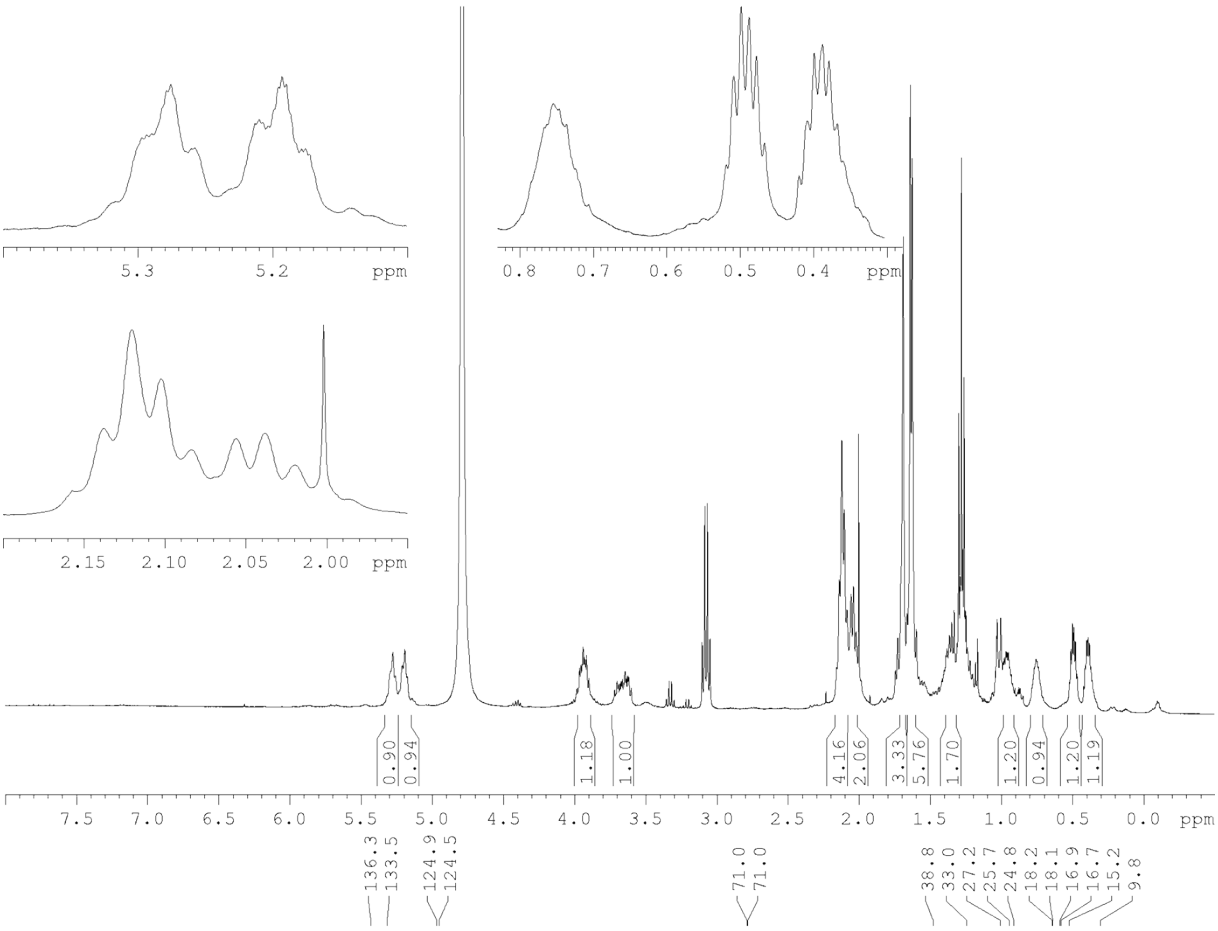

OH

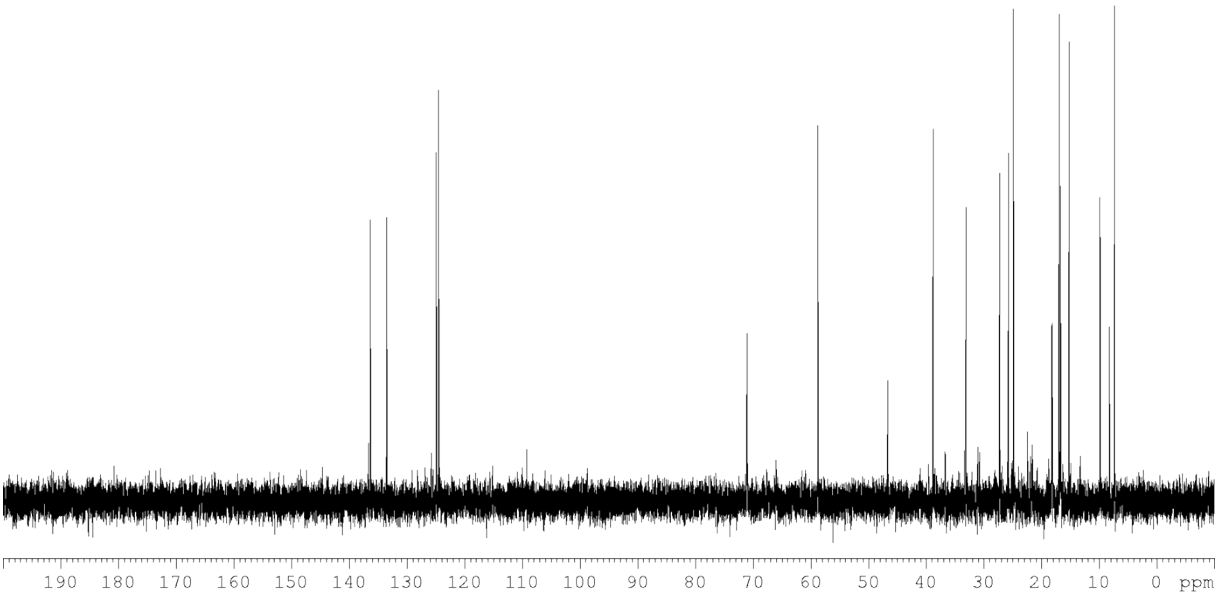
15

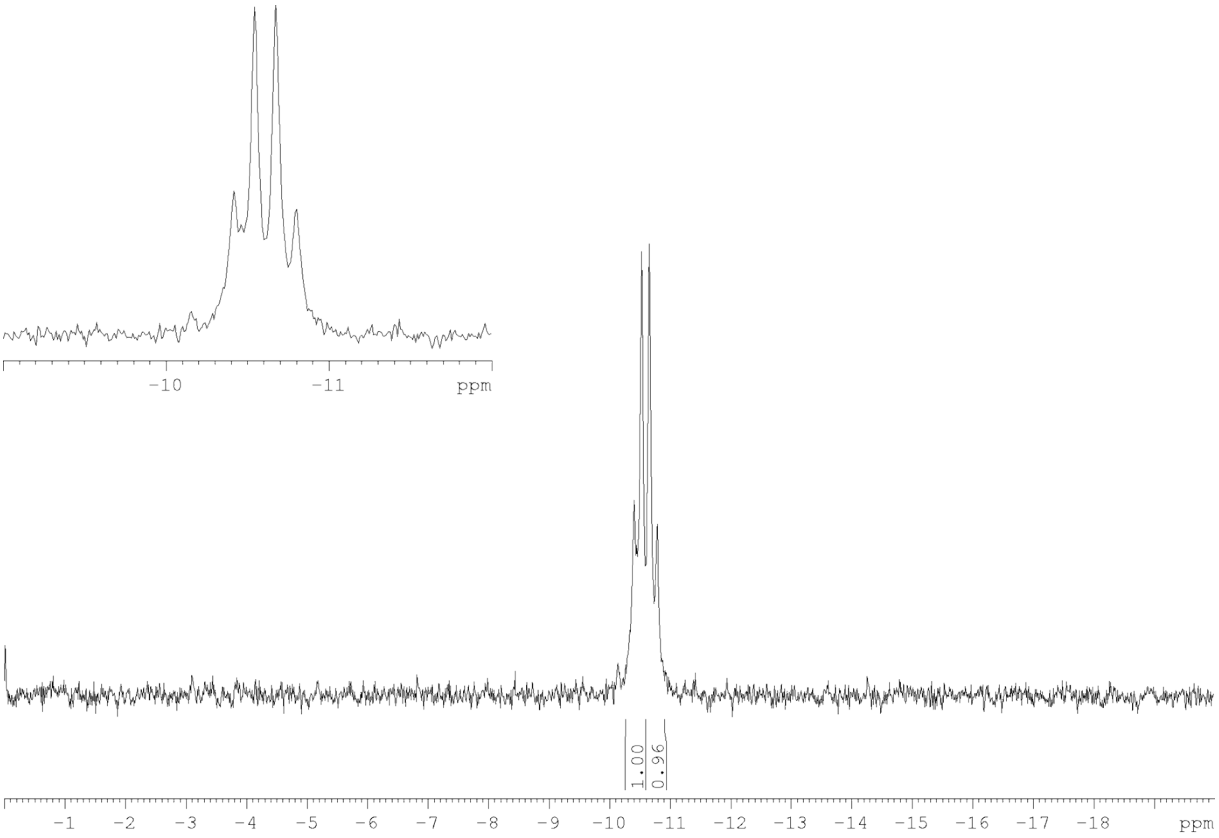

3.7 3.6 3.5 ppm
2.1 2.0 ppm
0.5 0.4 0.3 0.2 ppm
2.12
1.00
0.96
4.27
2.04
3.12
6.01
1.26
1.15
2.98
1.21
0.92
0.94
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
26.8
26.3
25.8
25.5
135.1
131.5
124.5
124.5
64.1
41.3
39.9
26.8
26.3
25.8
25.5
20.1
17.9
17.8
17.2
16.1
27 26 ppm
20.1
17.9
17.8
17.2
16.1
20 19 18 17 ppm
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

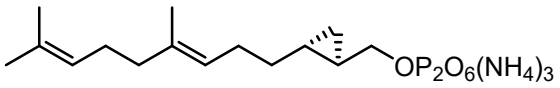

OP2O6(NH4)3

7

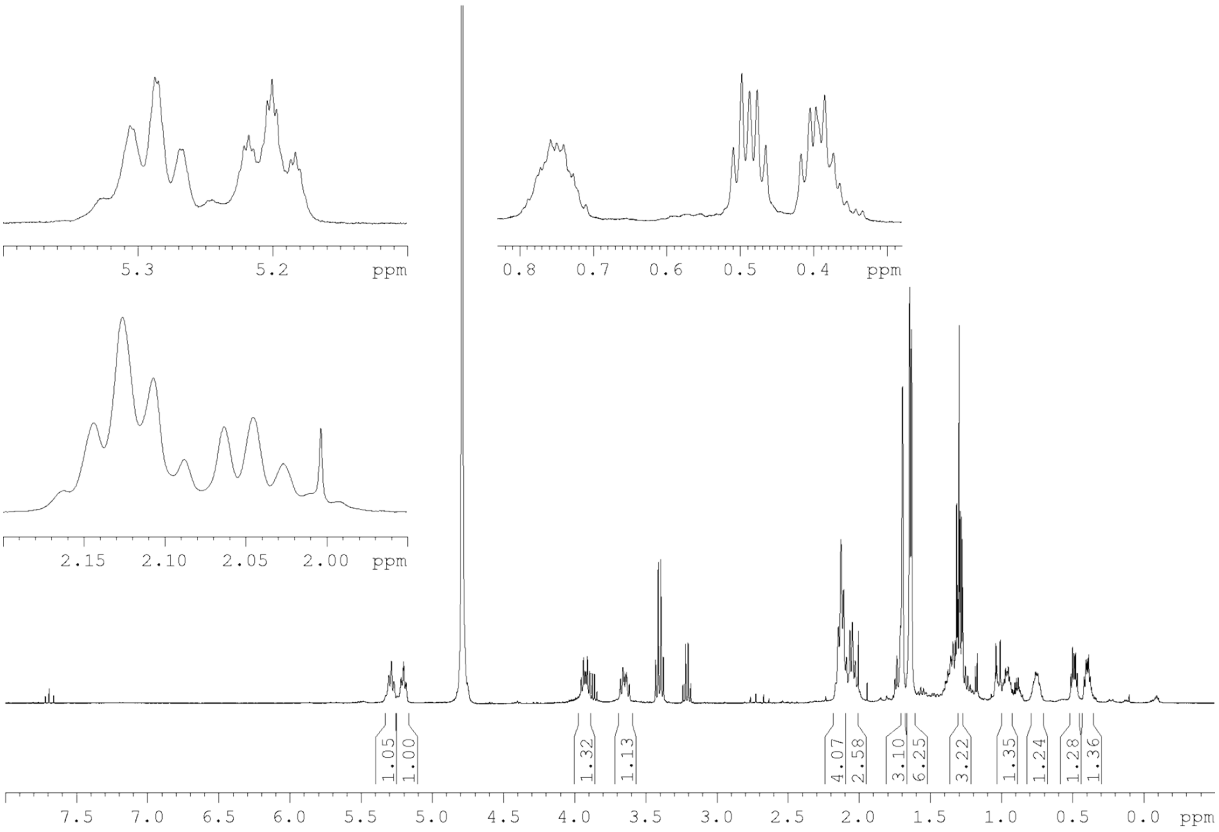

5.3
5.2
ppm
0.8
0.7
0.6
0.5
0.4
ppm
2.15
2.10
2.05
2.00
ppm
0.90
0.94
1.18
1.00
4.16
2.06
3.33
5.76
1.70
1.20
0.94
1.20
1.19
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
136.3
133.5
124.9
124.5
71.0
71.0
38.8
33.0
27.2
25.7
24.8
18.2
18.1
16.9
16.7
15.2
9.8

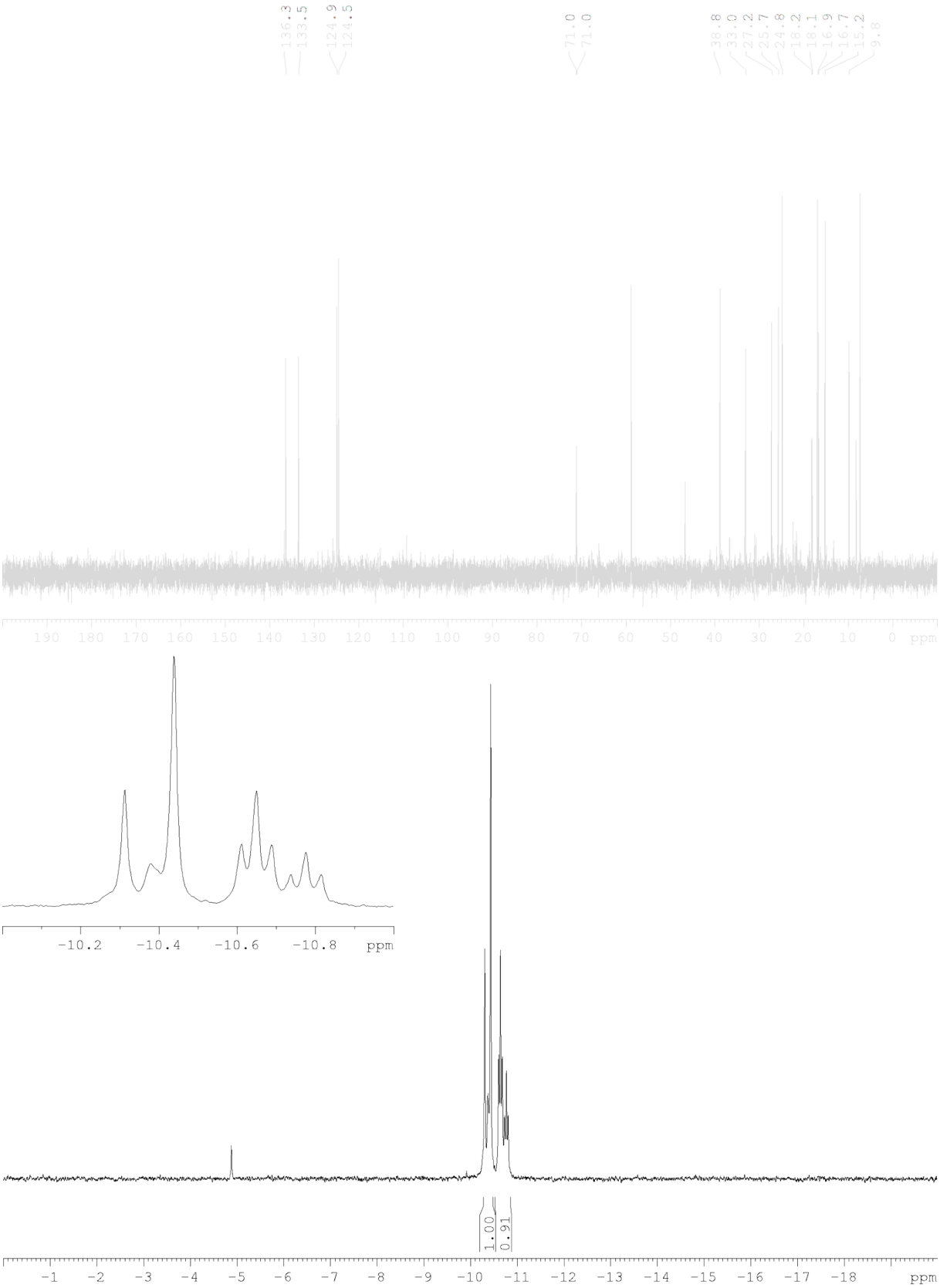

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

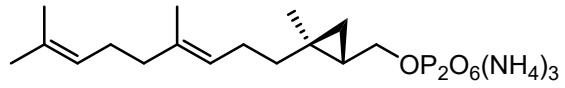

-10
-11
ppm
1.00
0.96
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18
ppm


OP2O6(NH4)3

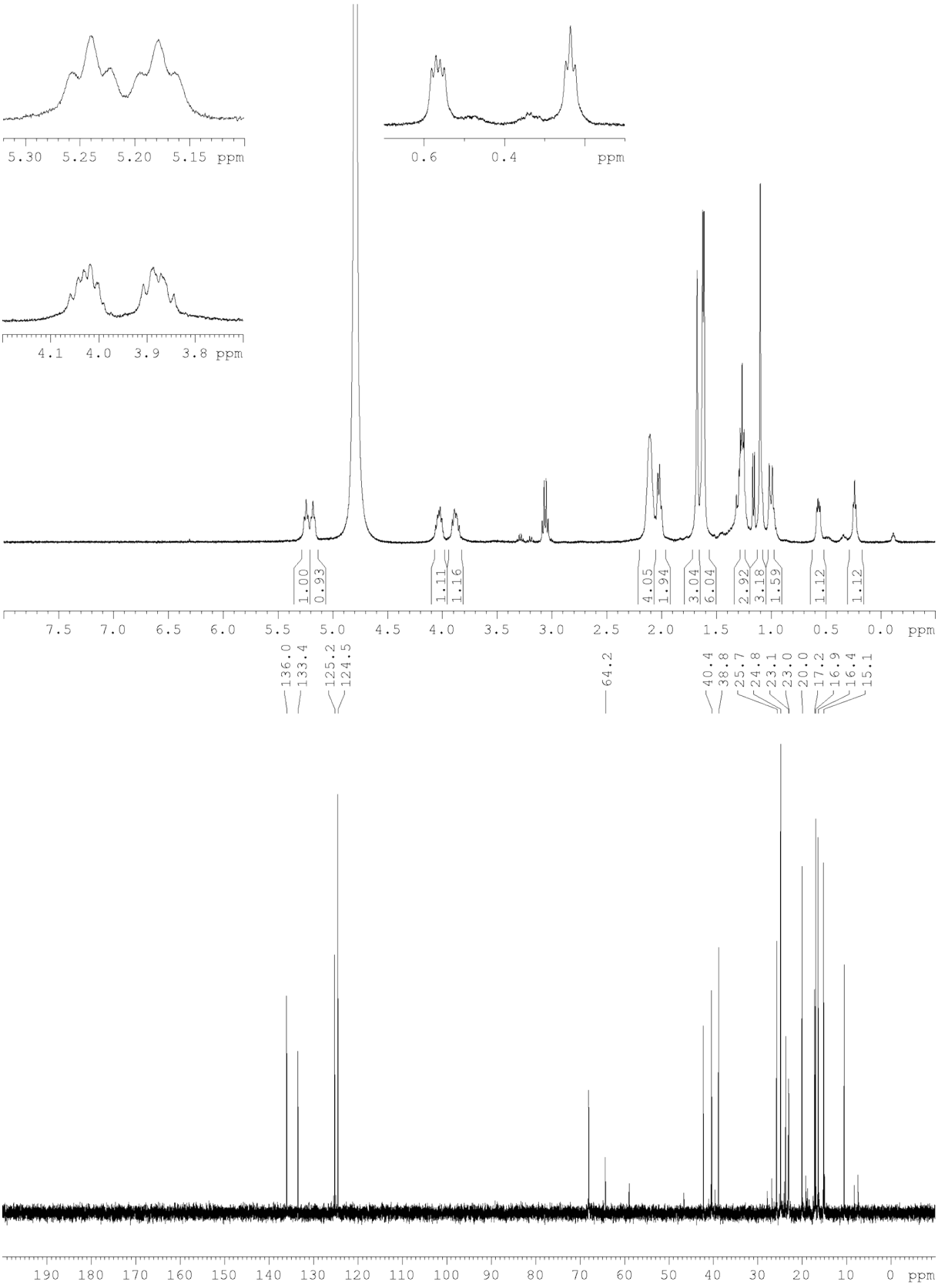
5

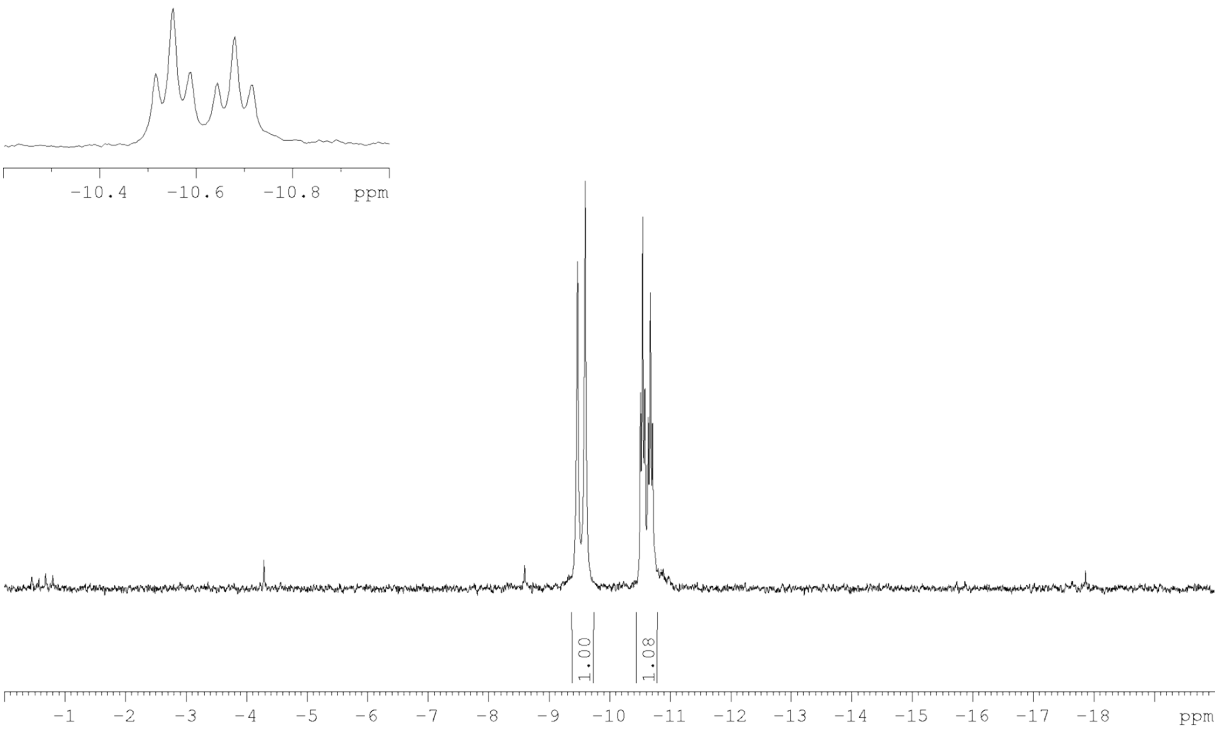

5.3
5.2
ppm
0.8 0.7 0.6 0.5 0.4
ppm
2.15 2.10 2.05 2.00
ppm
1.05
1.00
1.32
1.13
4.07
2.58
3.10
6.25
3.22
1.35
1.24
1.28
1.36
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm

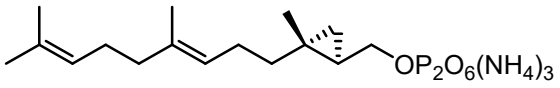

136.3
133.5
124.9
124.5
71.0
71.0
38.8
33.0
27.2
25.7
24.8
18.2
18.1
16.9
16.7
15.2
9.8
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
-10.2 -10.4 -10.6 -10.8 ppm
1.00
0.91
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 ppm


OP2O6(NH4)3

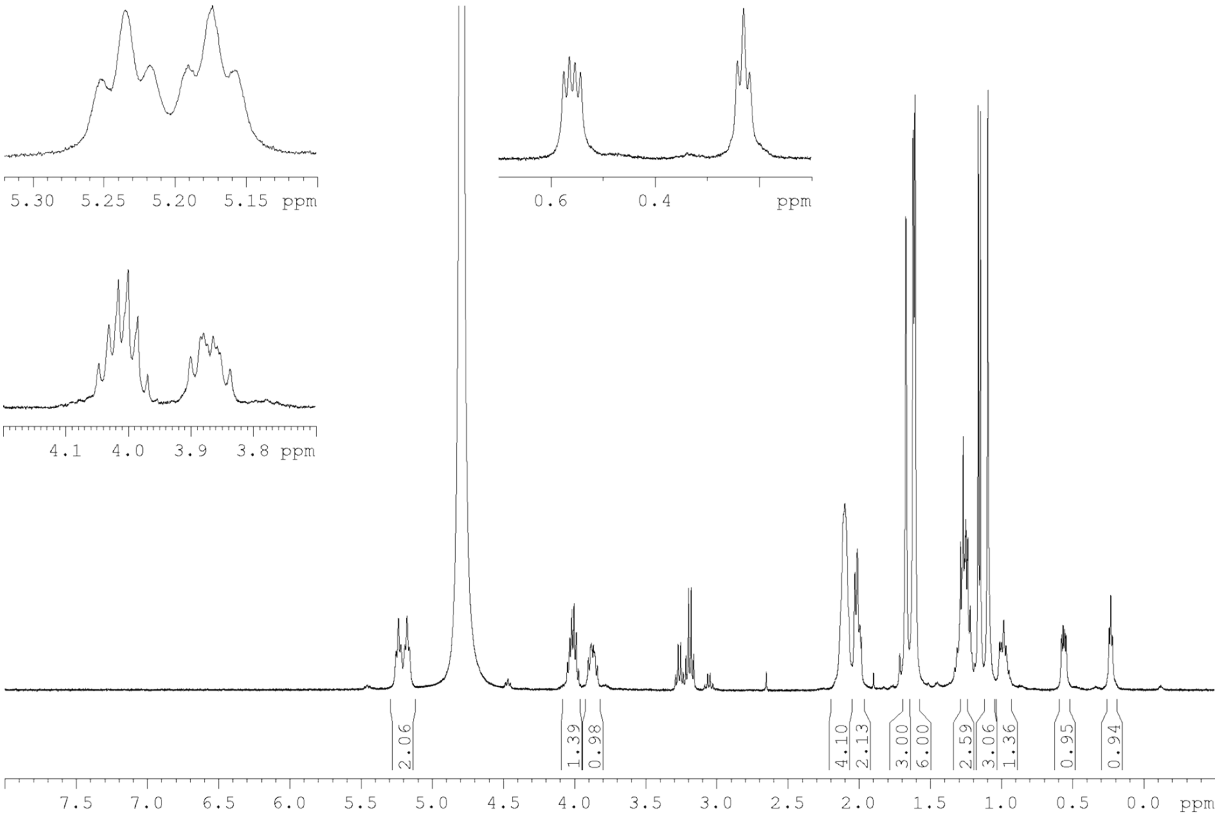
8

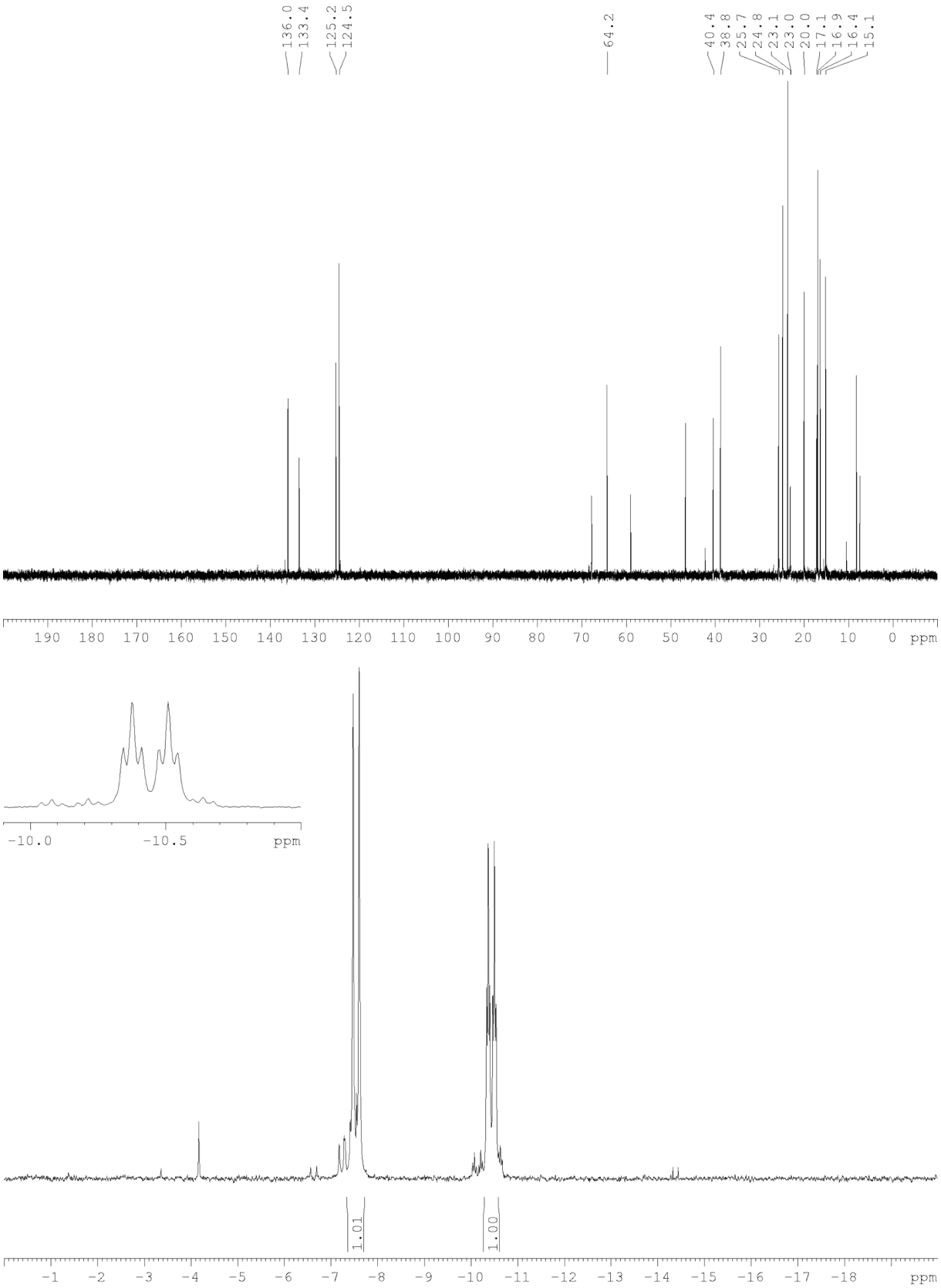

5.30 5.25 5.20 5.15 ppm
0.6 0.4 ppm
4.1 4.0 3.9 3.8 ppm
1.00 0.93 1.11 1.16 4.05 1.94 3.04 6.04 2.92 3.18 1.59 1.12 1.12
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
136.0 133.4 125.2 124.5 64.2 40.4 38.8 25.7 24.8 23.1 23.0 20.0 17.2 16.9 16.4 15.1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

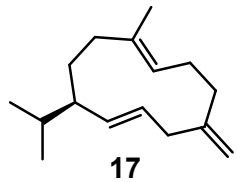

-10.4
-10.6
-10.8
ppm
1.00
1.08
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
ppm


OP2O6(NH4)3

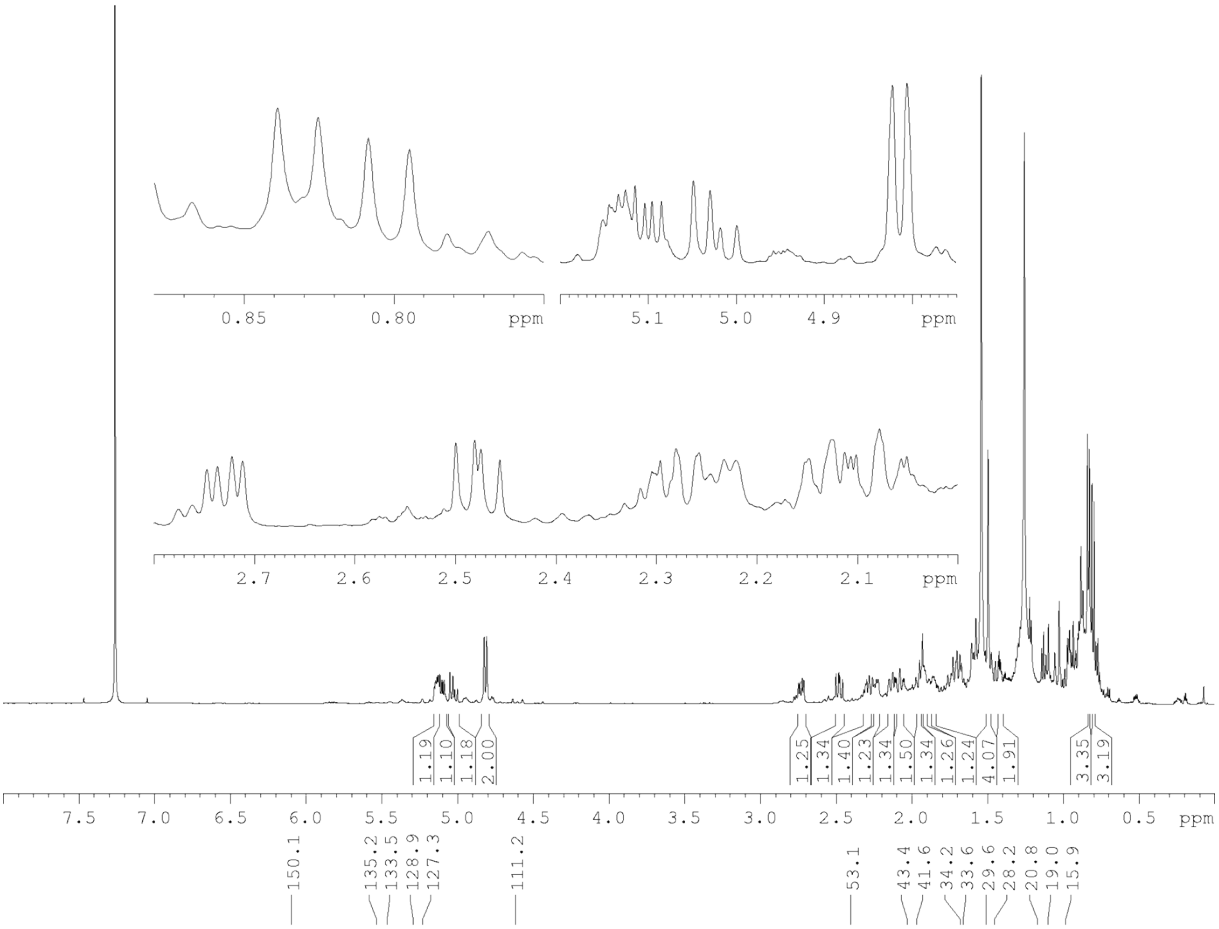
6


5.30
5.25
5.20
5.15
ppm
0.6
0.4
ppm
4.1
4.0
3.9
3.8
ppm
2.06
1.39
0.98
4.10
2.13
3.00
6.00
2.59
3.06
1.36
0.95
0.94
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm


136.0
133.4
125.2
124.5
64.2
40.4
38.8
25.7
24.8
23.1
23.0
20.0
17.1
16.9
16.4
15.1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
-10.0
-10.5
ppm
1.01
1.00
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 ppm



17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0.80
ppm
5.1
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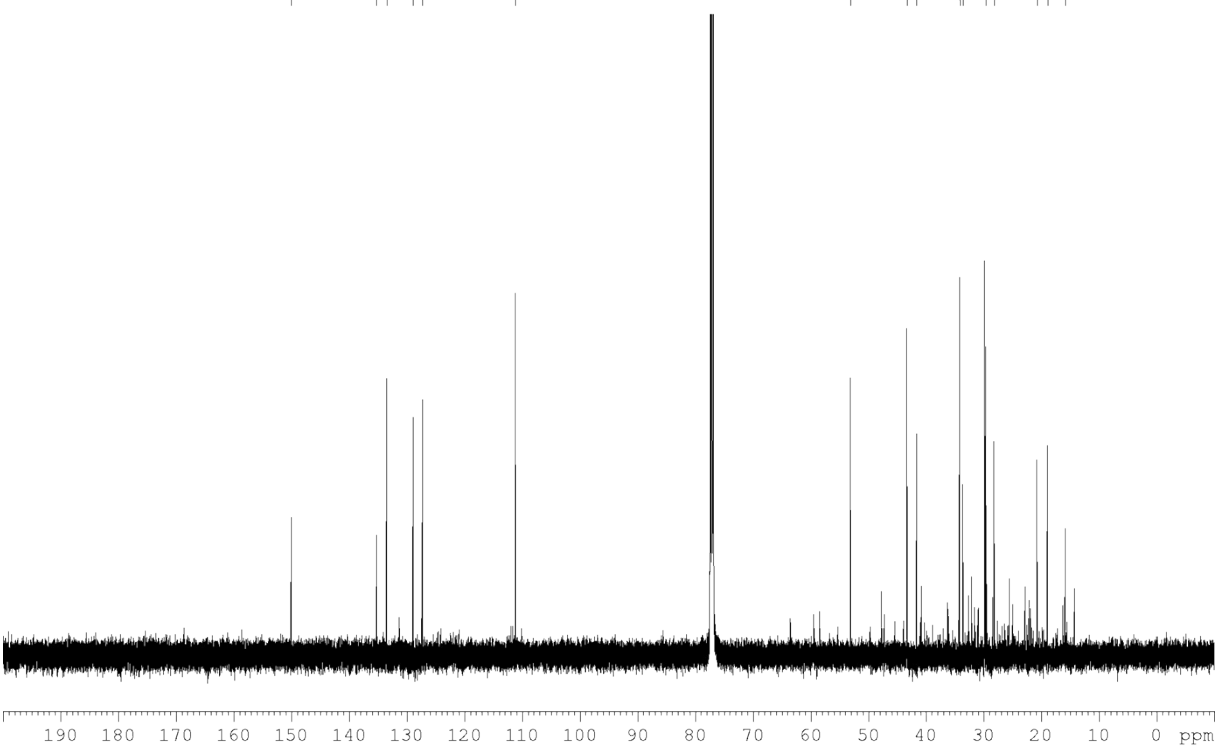
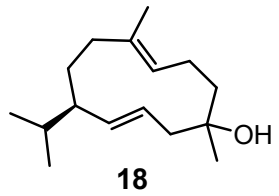
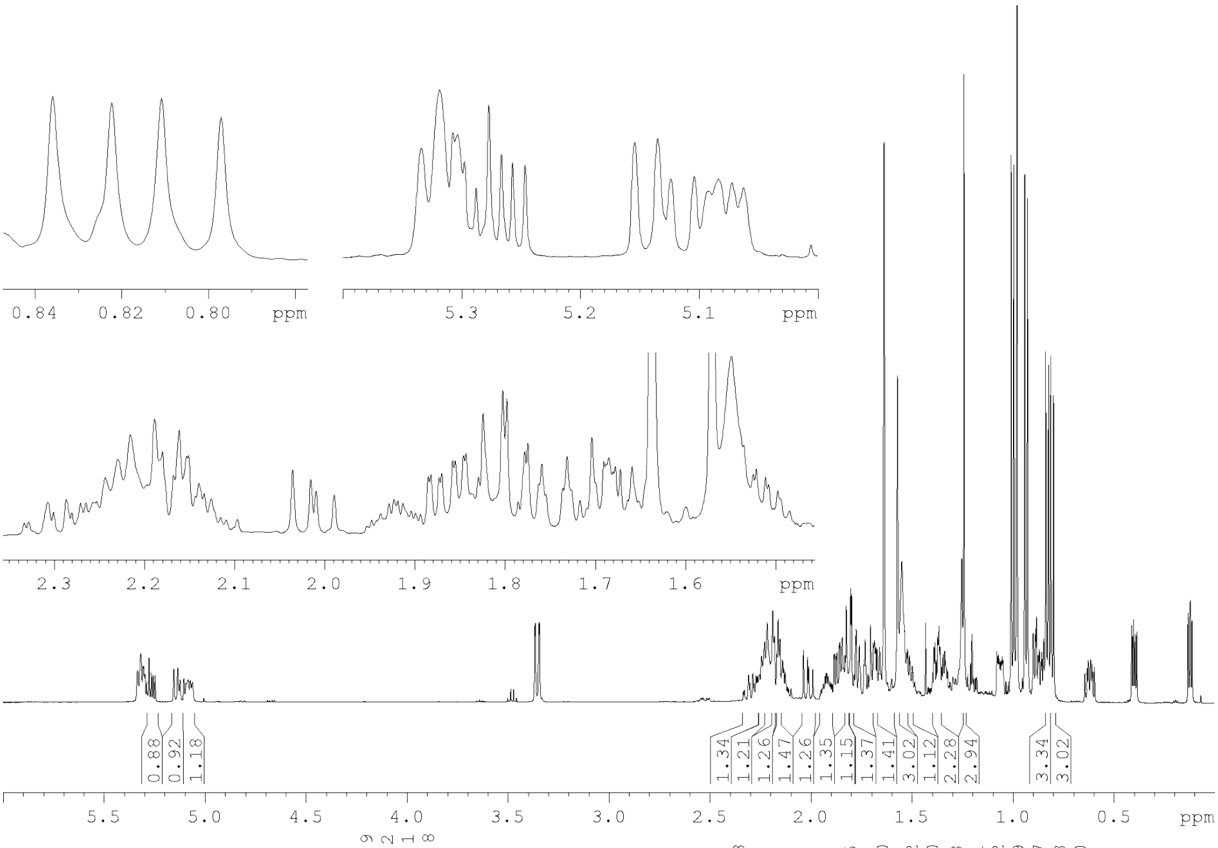
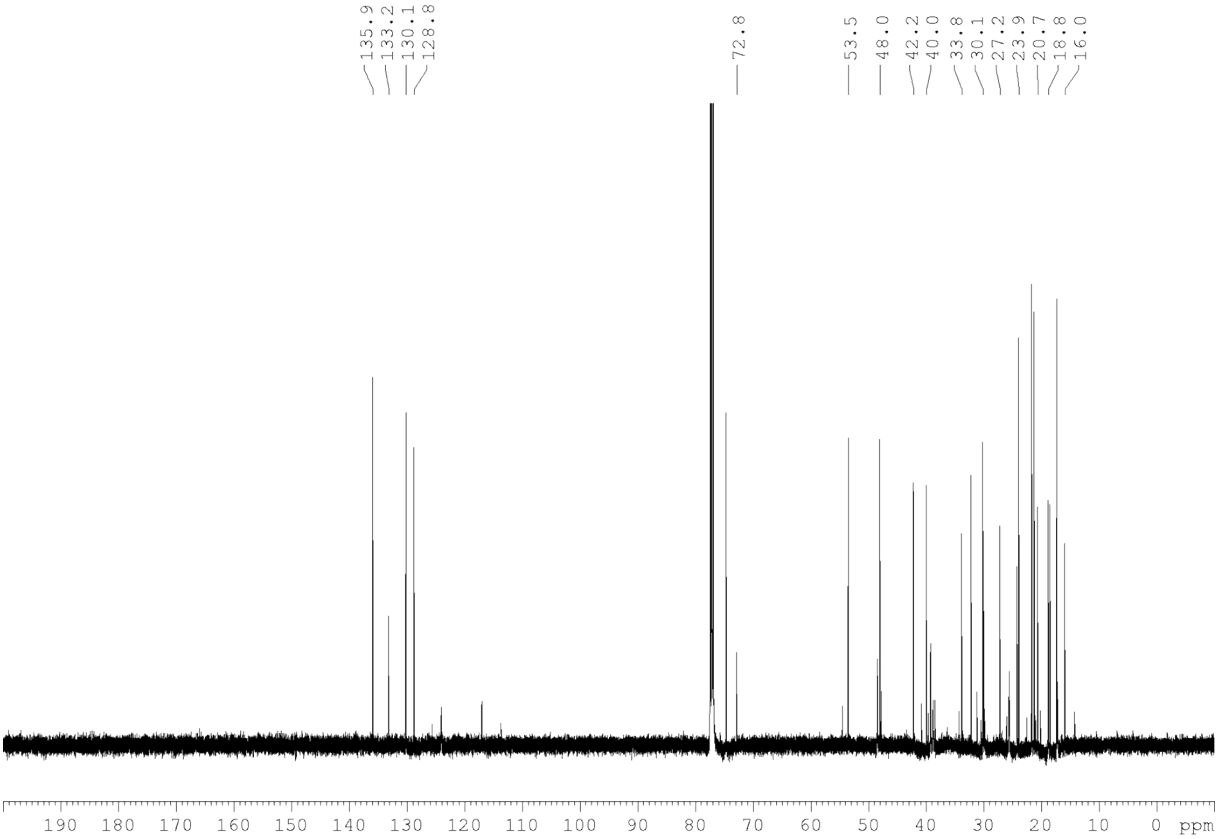
4.9
ppm
2.7
2.6
2.5
2.4
2.3
2.2
2.1
ppm
1.19
1.10
1.18
2.00
1.25
1.34
1.40
1.23
1.34
1.50
1.34
1.26
1.24
4.07
1.91
3.35
3.19
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

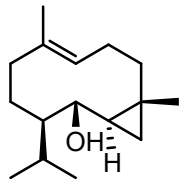

150.1
135.2
133.5
128.9
127.3
111.2
53.1
43.4
41.6
34.2
33.6
29.6
28.2
20.8
19.0
15.9
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


OH

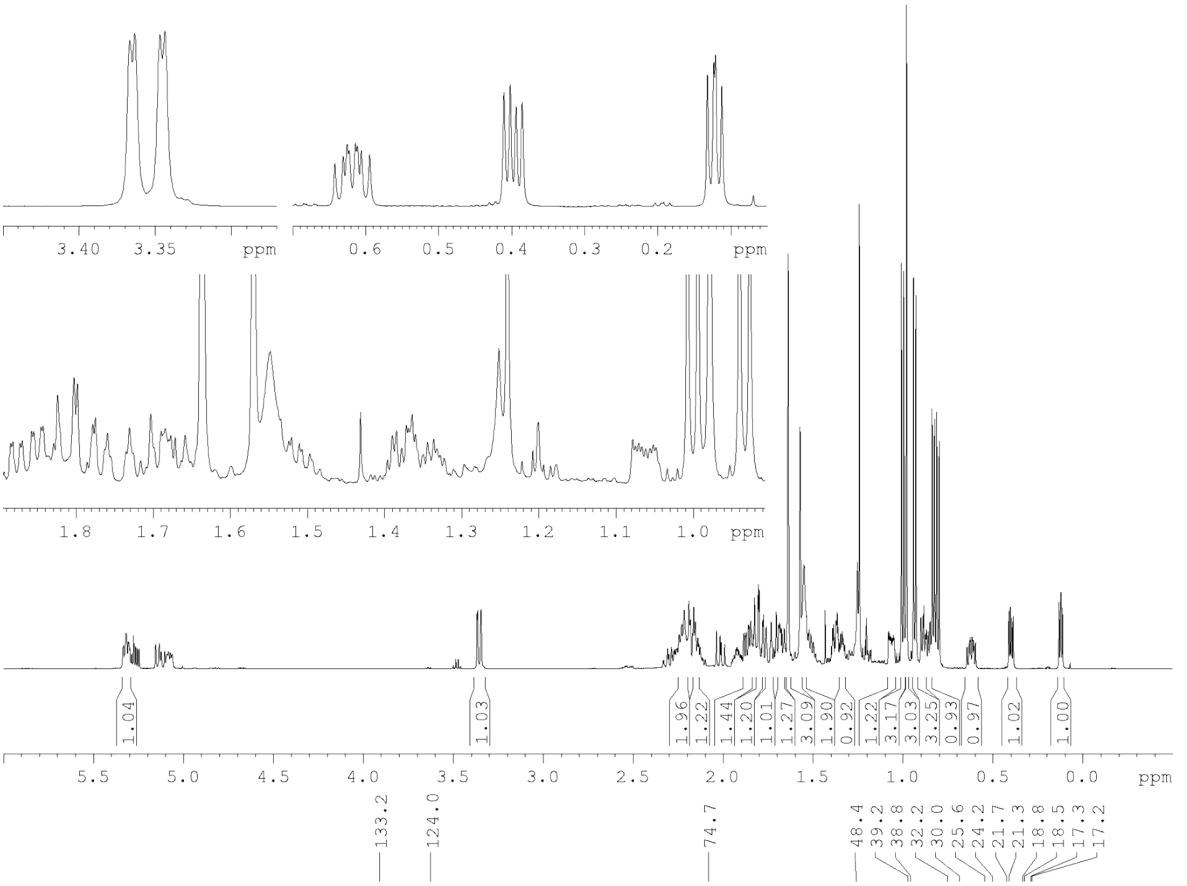
18

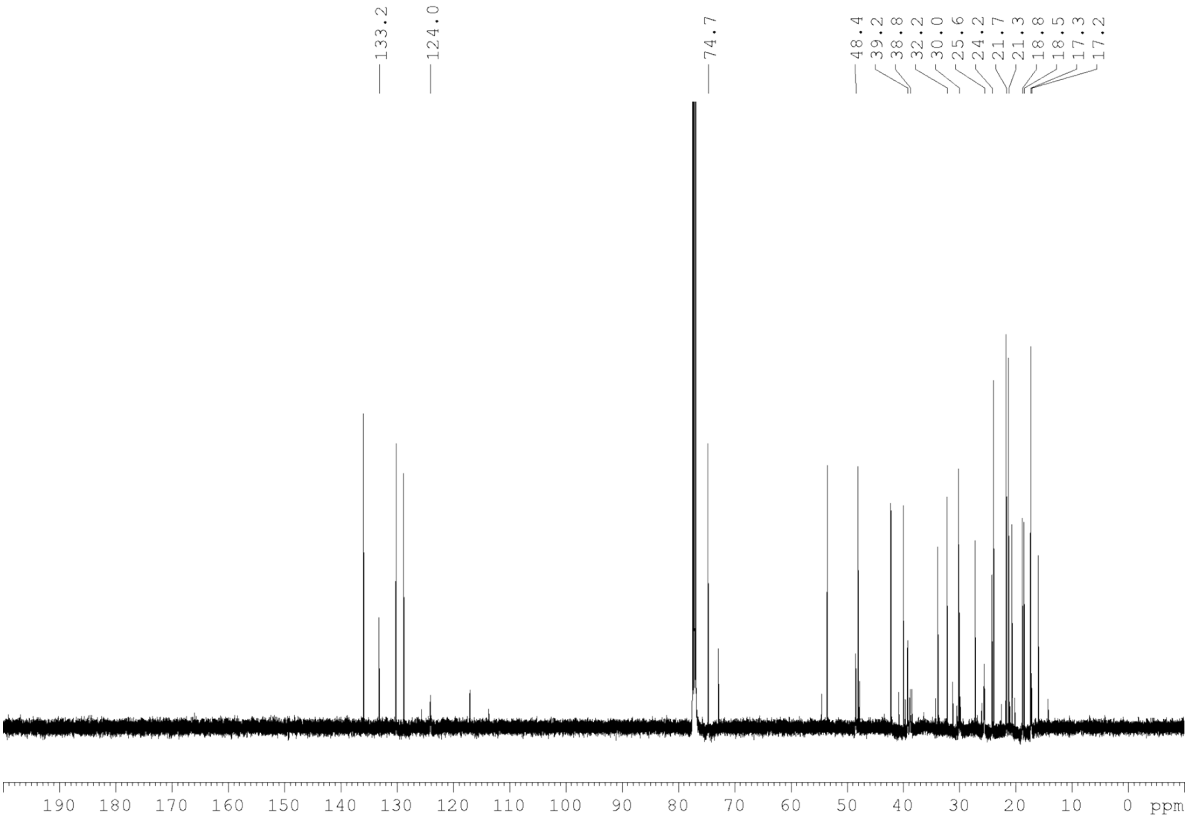

0.84
0.82
0.80
ppm
5.3
5.2
5.1
ppm
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
ppm
0.88
0.92
1.18
1.34
1.21
1.26
1.47
1.26
1.35
1.15
1.37
1.41
3.02
1.12
2.28
2.94
3.34
3.02
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


135.9
133.2
130.1
128.8
72.8
53.5
48.0
42.2
40.0
33.8
30.1
27.2
23.9
20.7
18.8
16.0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

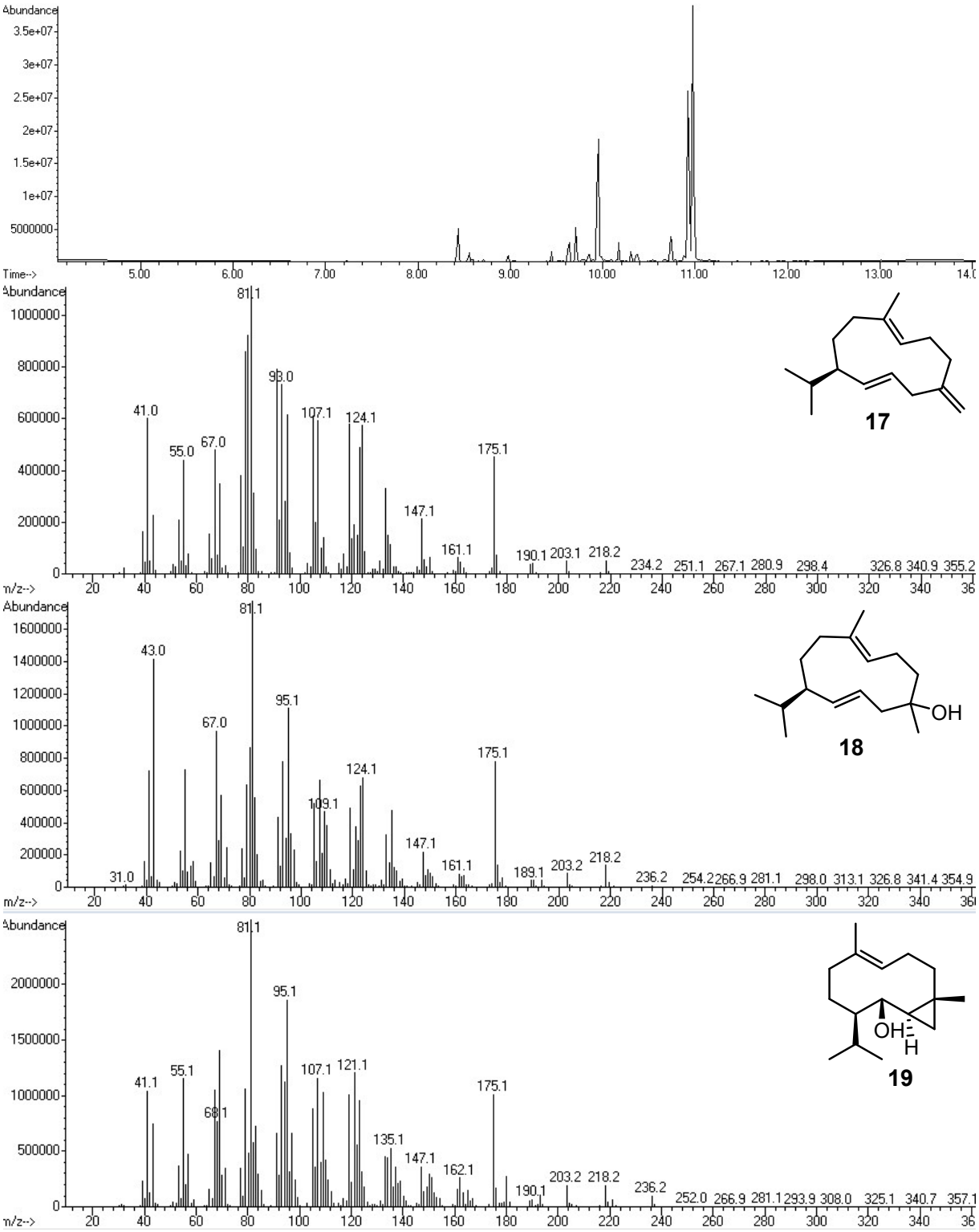

OH
H

19


3.40 3.35 ppm
0.6 0.5 0.4 0.3 0.2 ppm
1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 ppm
1.04 1.03 1.96 1.22 1.44 1.20 1.01 1.27 3.09 1.90 0.92 1.22 3.17 3.03 3.25 0.93 0.97 1.02 1.00
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm


133.2
124.0
74.7
48.4
39.2
38.8
32.2
30.0
25.6
24.2
21.7
21.3
18.8
18.5
17.3
17.2
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

6+BcBot2 – m/z at 9.95 (17), 10.93 (18/19), and 10.98 min (18/19); (Sought: m/z = 218, 236)



6 Computations

6.1 General Information

All calculations of the quantum mechanical raw data, static and dynamic, were carried out with the Gaussian 16 set of programs [9] applying a long-range corrected hybrid density functional with damped atom-atom dispersion corrections wb97xd [10] and a standard Pople basis set, 6-31G(d). Implicit water solvation was accounted for in terms of a Polarizable Continuum Model (PCM). Ab initio MD trajectories were computed applying the Atom Centered Density Matrix Propagation (ADMP). [11] The simulations were carried out to 2000 femtoseconds (fs) with a time interval 0.1 fs and is sampled with the initial internal energy 1.0 eV. A total of 20000 steps is run for each trajectory.

All stationary points of the static calculations were characterized as minima or transition structures in terms of their energy second derivative matrices. A modified Moore–Penrose algorithm developed by Kai Brandhorst at the TU Braunschweig was used in order to obtain the relaxed force constants (compliance constants) as measures for the kinetic bond strength. [121-14]

Our COMPLIANCE algorithm avoids the dependencies of the calculated force constants on the coordinate system. Using a (pseudo) inversion of the Hesse matrix, the construction and the nontrivial inversion of the complete B matrix (Wilson) is bypassed. If a Cartesian Hessian matrix is available as input, all internal force constants for arbitrary atom–atom pairs, as well as their couplings, can be selected and calculated within seconds. It does not matter whether or not the atom pair is bound in a Lewis sense. The transformation of the Cartesian force constants into the relaxed internal force constants (compliance constants) was carried out with our freely available graphical interface, which allows the import the Cartesian Hesse matrix in its compact lower triangle.

6.2 Model Structures

Based on the crystallographic data (pdb: 4OKZ) of selina-4(15),7(11)-diene synthase (SdS) with a non-reactive substrate surrogate 2,3-dihydrofarnesyl diphosphate (DHFPP), five diphosphate model structures A-E were created, each containing three Mg^{2+} ions and two significant water molecules.

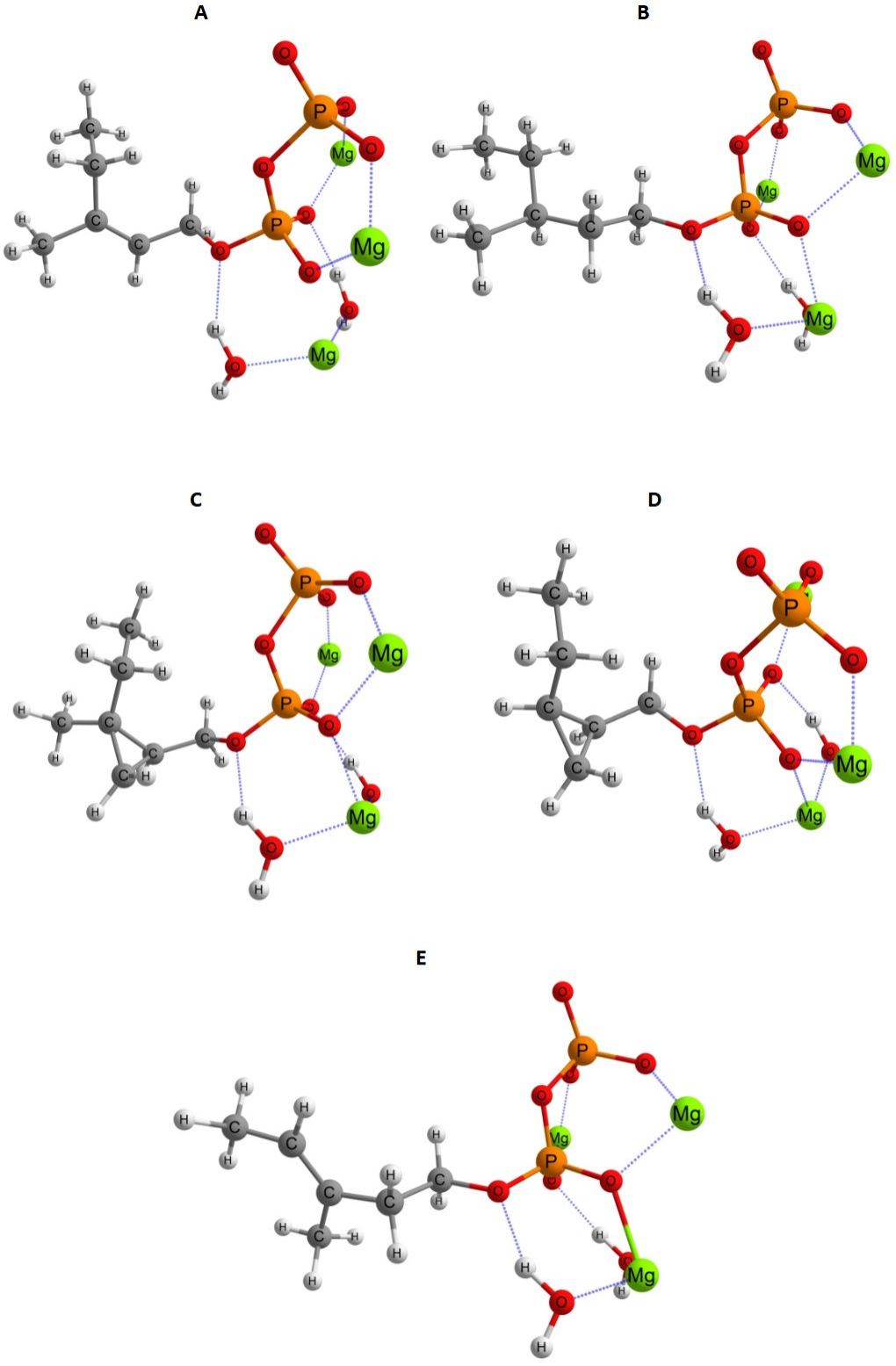


Fig. SI 1: Five diphosphate model structures A-E each containing three Mg^{2+} ions and two significant water molecules used for our calculations of the relaxed force constants describing the activated c-o bonds. See also table 3 in the main text.

6.3 Description of additional files

B.gif - E.gif: Animated gif files showing possible rearrangements of the nascent carbocations after S_N1 dissociation of the diphosphate.

B.gif: Due to 10 independent ADMP (Atom Centered Density Matrix Propagation) trajectories, again at the wb97xd/6-31G(d), the hydrogen shift was found to occur rapidly (~ 1.4 ps) and barrier-free. Even assuming a S_N1 type dissociation as the very first step leading to a nascent, primary cation, a tertiary carbocation is formed instantly via H migration.

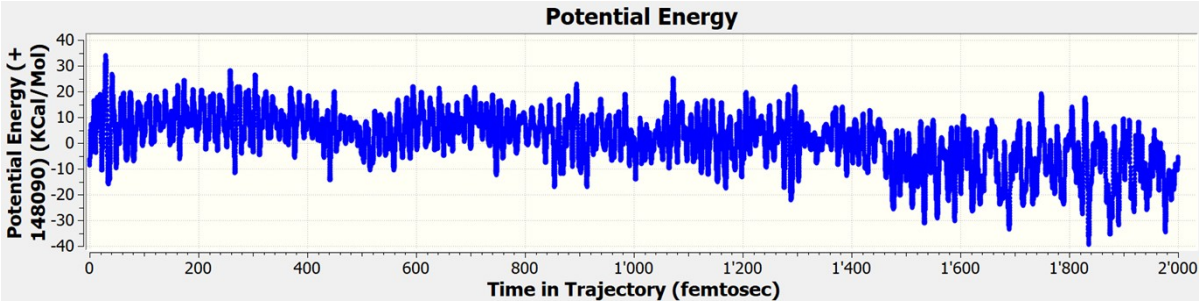


Fig. SI 2: A typical wb97xd/6-31G(d) trajectory for the carbocation **B**. After ~ 1.4 ps, the H^+ migration occurs visible as a drop of the potential energy by more than 10 kcal/mol.

C.gif: A stable tertiary carbocation with strong hyperconjugation is the result after diphosphate dissociation. The cyclopropane moiety opens up. **D.gif**: The resulting secondary carbocation is stabilized by a newly formed three membered ring. Such cyclopropylcarbinyl cations are rare but were discussed in the context of pentalenene. **E.gif**: A secondary homoallyl carbocation is formed.

TS.gif

Animated gif file showing the ring closure for **24** at the wb97xd/6-31G(d) level of theory.

6.4 Optimized Cartesian coordinates of the Models A-E

Terpene diphosphate model A

12 2.579943 0.261842 -2.151169
12 1.635192 3.247026 -0.464177
12 2.045843 -1.259124 2.469994
6 -1.991475 0.508443 1.069700
8 -0.918910 0.992399 0.180339
6 -3.296077 0.947231 0.498679
6 -4.130348 0.164628 -0.200436
6 -5.440757 0.701444 -0.711811
6 -3.866855 -1.295553 -0.5013250
6 -4.507735 -2.226556 0.537919
15 0.521072 0.325592 0.225280
8 1.093395 0.316568 1.641169
8 1.366454 1.194937 -0.742240
8 0.335875 -1.120748 -0.347092
15 1.624807 -2.288447 -0.513106
8 2.127740 -2.436533 0.932705
8 0.971924 -3.468808 -1.131773
8 2.632913 -1.499711 -1.372166
8 -0.364637 3.498931 -0.942199
8 2.202261 2.859131 1.476632
1 -4.349337 -3.275019 0.266622
1 -5.587267 -2.056719 0.611413
1 -2.792596 -1.498582 -0.564399
1 -4.281476 -1.525351 -1.490304
1 -5.482256 0.624717 -1.805297
1 -6.279333 0.109770 -0.323898
1 -5.596949 1.746358 -0.428789
1 -1.903275 -0.577376 1.145622
1 -1.804583 0.952043 2.050161
1 -0.895153 2.736423 -0.638219
1 -0.841164 4.299040 -0.679862

1 1.895387 1.988611 1.811679

1 2.057437 3.507064 2.180119

1 -4.073394 -2.060010 1.529602

1 -3.562583 1.987793 0.677799

Terpene diphosphate model B

12 2.964870 0.323762 -1.983901
12 1.808574 3.259615 -0.267353
12 2.080455 -1.310179 2.499746
6 -1.967955 0.209574 0.476718
8 -0.775957 0.900336 -0.005688
6 -3.152741 0.791599 -0.269091
6 -4.509455 0.216650 0.171960
6 -5.631450 1.010954 -0.505782
6 -4.615973 -1.289471 -0.124325
6 -5.928025 -1.927612 0.334997
15 0.676711 0.278657 0.170405
8 1.117415 0.250475 1.632597
8 1.582712 1.207181 -0.683393
8 0.585665 -1.154619 -0.452033
15 1.906531 -2.293977 -0.511068
8 2.240055 -2.490048 0.978000
8 1.353195 -3.462268 -1.240007
8 2.986551 -1.452633 -1.221494
8 -0.152113 3.492059 -0.855256
8 2.367424 2.739340 1.650887
1 -5.902743 -3.011930 0.180796
1 -6.790786 -1.535515 -0.215799
1 -3.788362 -1.818663 0.365585
1 -4.486224 -1.446137 -1.205447
1 -5.615369 0.851137 -1.591747
1 -6.617211 0.714929 -0.135034
1 -5.516175 2.084896 -0.321282
1 -4.601714 0.357031 1.259237
1 -3.011165 0.621797 -1.344232
1 -3.156971 1.876718 -0.108197
1 -1.838155 -0.856251 0.280153
1 -2.039592 0.377372 1.554770
1 -0.682144 2.691339 -0.677061
1 -0.674960 4.250748 -0.560107
1 1.989885 1.874244 1.921433
1 2.229747 3.353839 2.385482
1 -6.099451 -1.742881 1.403723

Terpene diphosphate model C

12 2.556952 0.248357 -2.189665
12 1.564796 3.268564 -0.625301
12 2.201656 -1.112436 2.511218
6 -1.836815 0.622826 1.306373
8 -0.880212 1.004933 0.273049
6 -3.256692 0.763709 0.808906
6 -3.896860 -0.175094 -0.189838
6 -5.343104 -0.554456 0.064192
6 -3.044832 -1.260525 -0.831075
6 -2.957302 -2.552193 -0.014928
15 0.575556 0.365330 0.270631
8 1.209112 0.430755 1.657487
8 1.350209 1.206359 -0.776741
8 0.391877 -1.105688 -0.228527
15 1.691370 -2.256472 -0.422675
8 2.310294 -2.297044 0.984576
8 1.018525 -3.486134 -0.909963
8 2.610849 -1.510951 -1.409757
8 -0.455779 3.449918 -1.079917
8 2.149737 3.021852 1.340975
1 -2.236944 -3.242454 -0.464642
1 -3.924308 -3.062803 0.038542
1 -2.034322 -0.878194 -1.004158
1 -3.461656 -1.484738 -1.821910
1 -5.821919 -0.895015 -0.861593
1 -5.419779 -1.361695 0.800953
1 -5.914368 0.298624 0.445124
1 -1.618812 -0.404494 1.610770
1 -1.659104 1.285685 2.154737
1 -0.958200 2.728795 -0.654661
1 -0.935106 4.271370 -0.902140
1 1.916631 2.150668 1.728235
1 1.960866 3.694616 2.009535
1 -2.631940 -2.355096 1.012867
6 -3.604995 1.250984 -0.579832
1 -2.793959 1.414668 -1.283809
1 -4.428478 1.953256 -0.672550
1 -3.916631 1.116608 1.598084

Terpene diphosphate model D

12 2.450038 0.613315 -2.302144
12 0.916496 3.312833 -0.493495
12 2.491628 -0.886058 2.529857
6 -1.891084 -0.497493 0.767923
8 -0.931731 0.448848 0.198113
6 -3.260363 0.031298 0.487530

6 -4.349333 -0.924665 0.068309
15 0.637313 0.211319 0.250834
8 1.198003 0.405565 1.656729
8 1.199558 1.261133 -0.743885
8 0.862023 -1.244666 -0.279063
15 2.433823 -1.982249 -0.469840
8 3.041004 -1.840455 0.935490
8 2.139192 -3.354459 -0.952711
8 3.096725 -1.005712 -1.459573
8 -1.106839 3.053223 -0.748386
8 1.753696 3.113110 1.389595
1 -1.722389 -1.462978 0.284465
1 -1.688993 -0.581161 1.838543
1 -1.408359 2.159925 -0.490327
1 -1.747365 3.685636 -0.394547
1 1.645994 2.207756 1.754183
1 1.536913 3.735906 2.097120
6 -3.760169 0.019136 -0.939687
1 -3.097547 -0.380005 -1.702563
1 -4.365678 0.861988 -1.260993
1 -3.562796 0.873009 1.106286
6 -5.768859 -0.731593 0.554563
1 -5.883994 -1.201983 1.538860
6 -6.791063 -1.312557 -0.422811
1 -6.628754 -2.386712 -0.567136
1 -7.812884 -1.172382 -0.056099
1 -6.709901 -0.827342 -1.401750
1 -4.046942 -1.966196 -0.031796
1 -5.960113 0.339990 0.694796

Terpene diphosphate model E

12 3.156359 0.311399 -1.749368
12 1.721307 3.240745 -0.411737
12 1.726580 -1.208967 2.609804
6 -2.049234 0.145569 0.232059
8 -0.854126 0.810062 -0.272177
6 -3.233299 0.677429 -0.559420
6 -4.534774 0.072445 -0.070373
6 -5.109069 0.707132 1.170282
6 -5.074759 -0.955512 -0.735395
6 -6.331095 -1.712290 -0.420488
15 0.598097 0.252566 0.055670
8 0.906513 0.303157 1.549984
8 1.539683 1.180778 -0.754259
8 0.624117 -1.205685 -0.513434
15 1.982621 -2.292273 -0.344665
8 2.070814 -2.469989 1.180634
8 1.602947 -3.483051 -1.144795

8 3.133231 -1.410135 -0.872220
8 -0.243564 3.452760 -0.993627
8 2.255854 2.758206 1.522094
1 -6.109558 -2.775896 -0.270476
1 -7.035118 -1.653490 -1.259403
1 -5.342343 1.762529 0.981691
1 -6.020282 0.217854 1.518696
1 -4.387023 0.688904 1.995770
1 -3.070583 0.447046 -1.616940
1 -3.262518 1.769788 -0.456387
1 -1.934739 -0.930476 0.081534
1 -2.126723 0.361850 1.300170
1 -0.764190 2.637893 -0.857662
1 -0.783098 4.191932 -0.680034
1 1.839177 1.917187 1.808676
1 2.137437 3.394660 2.241102
1 -6.842524 -1.347105 0.472838
1 -4.550013 -1.302647 -1.626621

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