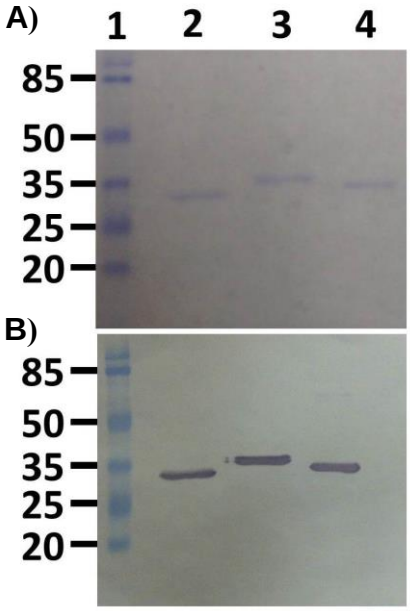
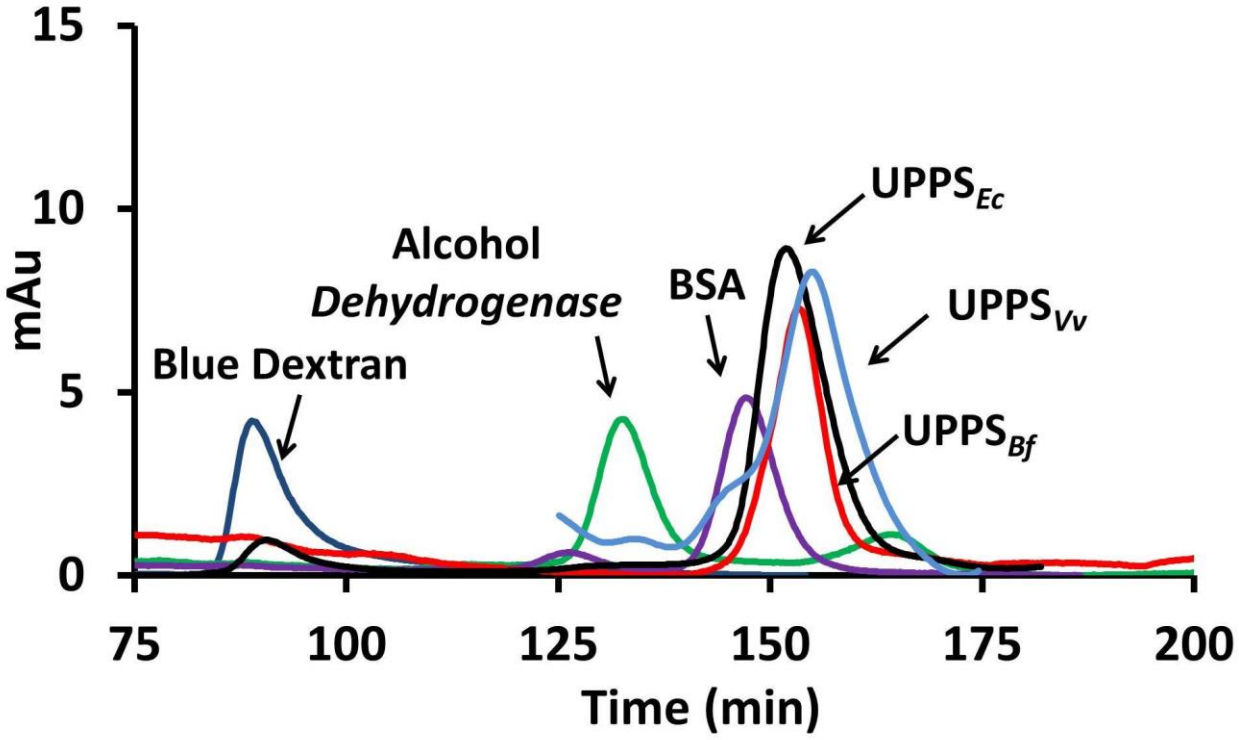
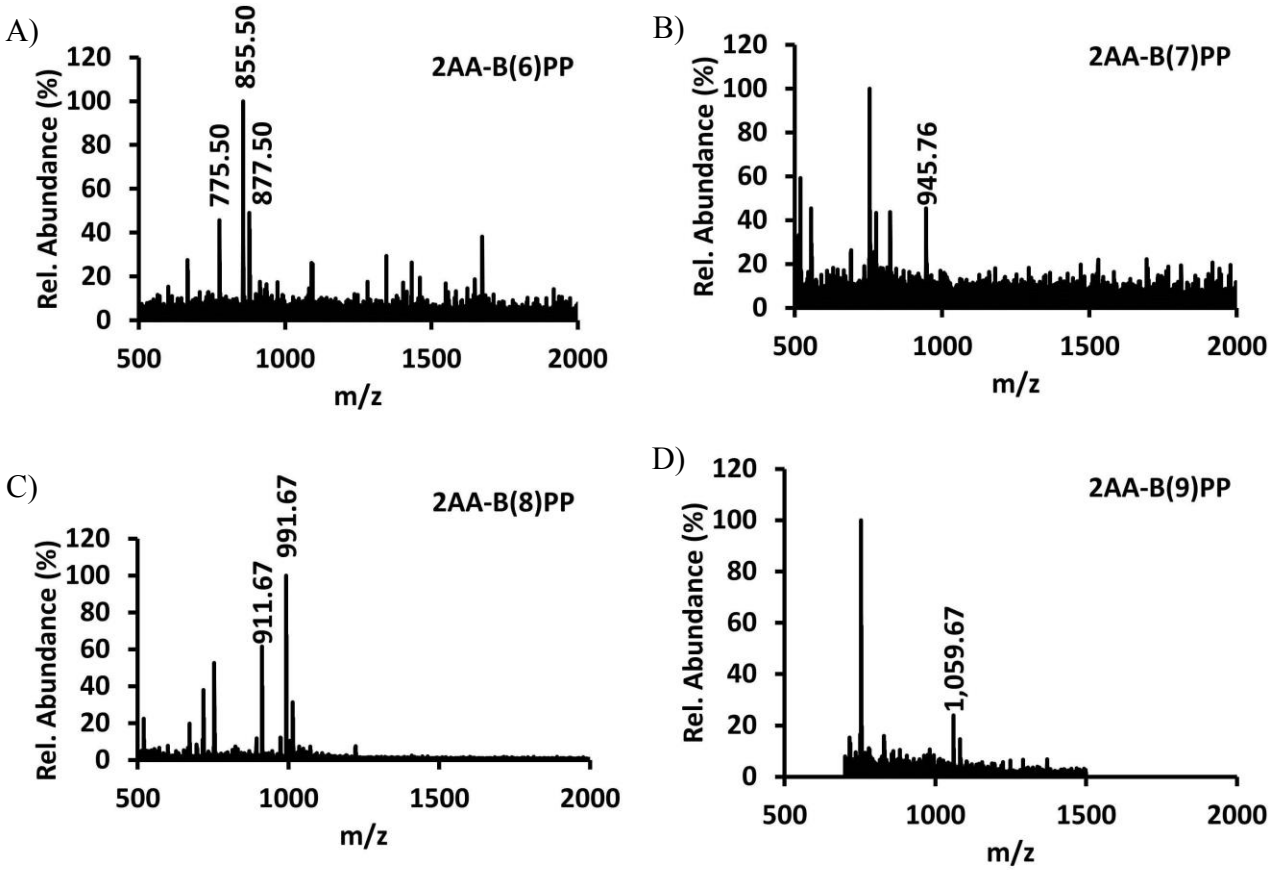
Supporting Figure 1. Expression of UPPS from *B. fragilis, V. vulnificus* and *E. coli.* A) SDS-PAGE and B) anti-T7 Western Blot Analysis of UPPS 1) ladder 2) $UPPS_{Bf}$, 3) $UPPS_{Ec}$ 4) $UPPS_{Vv}$



Supporting Figure 2. SEC of UPPS from *B fragilis*, *E. coli* and *V. vulnificus*. Dark blue: Blue Dextran, Green: Alcohol Dehydrogenase (150 kDa), Purple: Bovine Serum Albumin (66 kDa), Red: *B. fragilis* UPPS, Black: *E. coli* UPPS, Light blue: *V. vulnificus* UPPS. UPPS is 56 kDa in homodimeric state. Protein standards purchased from Sigma-Aldrich.



Supporting Figure 3. Confirmation of isoprenoid length by ESI-MS. Identified peaks are annotated. In 2AA an unidentified peak at 753 is present in 2AA-B(7-9)PP



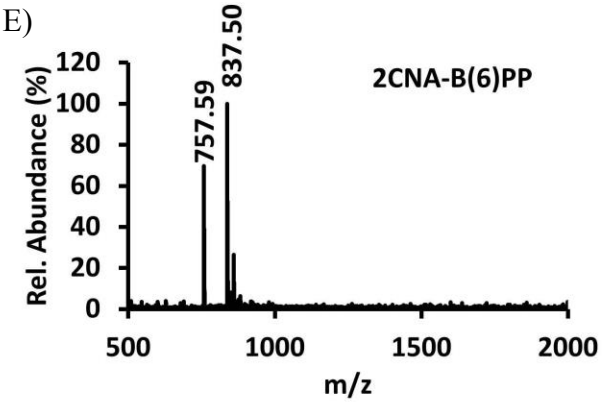
2AA-B(6)PP: HPLC (R_t/k'): 3.7 min/1.5 ESI-MS (M^{-1}) expected: 855.48 actual: 855.50, Na^+: 877.50, 2AA-B(6)P: 775.50

2AA-B(7)PP: HPLC (R_t /k'): 5.6 min/2.8 ESI-MS (M^{-1}) expected: 923.55 actual: Na^+: 945.76

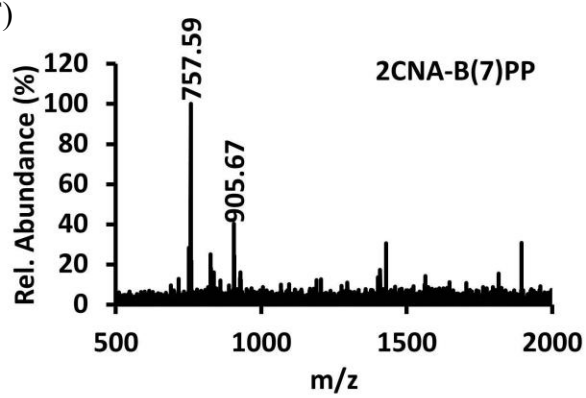
2AA-B(8)PP: HPLC (R_t /k'): 8.7 min/4.9, ESI-MS (M^{-1}) expected: 991.61 actual: 991.67, 2AA-B(8)P: 911.67

2AA-B(9)PP: HPLC (R_t /k'): 13.8 min/8.4, ESI-MS (M^{-1}) expected: 1059.67 actual: 1059.67, Na^+ **:** 1081.67

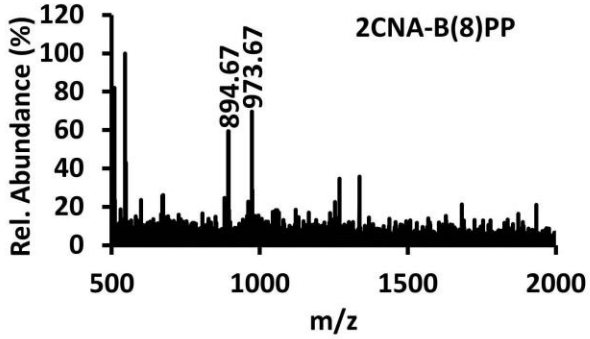
E)



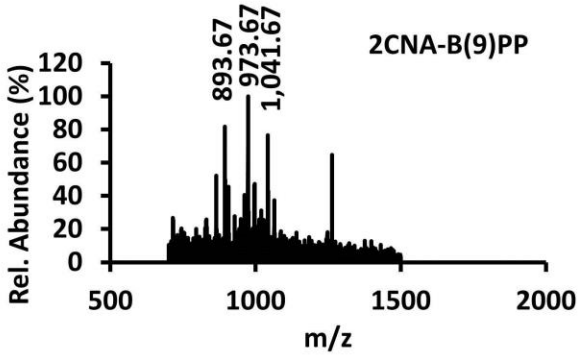
F)



G)



H)



2CNA-B(6)PP: HPLC (R_t /k'): 4.9 min/2.3, ESI-MS (M^{-1}) expected: 837.47 actual:837.50, 2CNA-B(6)P: 757.59

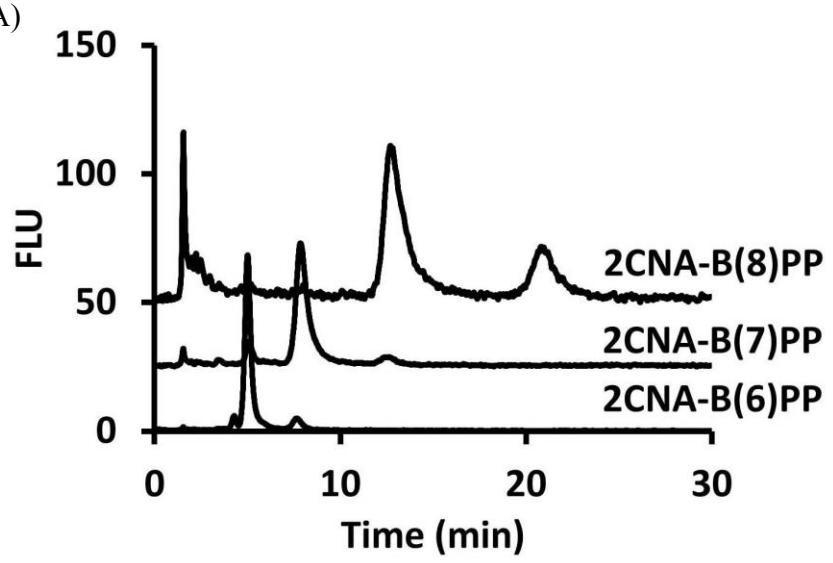
2CNA-B(7)PP: HPLC (R_t /k'): 7.7 min/4.2, ESI-MS (M^{-1}) expected: 905.54 actual: 905.67, contaminated with 2CNA-B(6)P

2CNA-B(8)PP: HPLC (R_t /k'): 12.3 min/7.4, ESI-MS (M^{-1}) expected: 973.60 actual: 973.67, 2CNA-B(8)P: 894.67

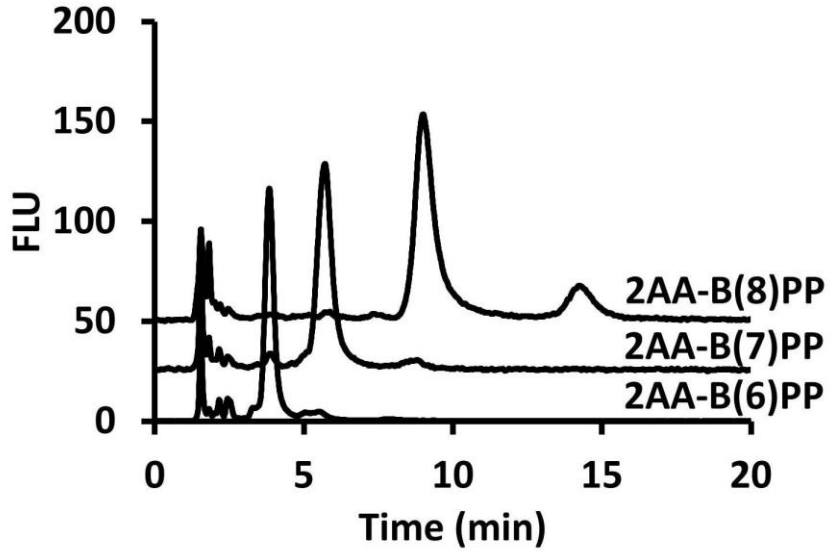
2CNA-B(9)PP: HPLC (R_t /k'): 20 min/12.6, ESI-MS (M^{-1}) expected: 1041.66 actual: 1041.67, contaminated with 2CNA-B(8)PP and B8 monophosphate

Supporting Figure 4. HPLC analysis of purified A) 2AA and B) 2CNA bactoprenyl diphosphates used for MS analysis. Note that the later peak in each trace is monophosphate. Peak heights are normalized to one another.

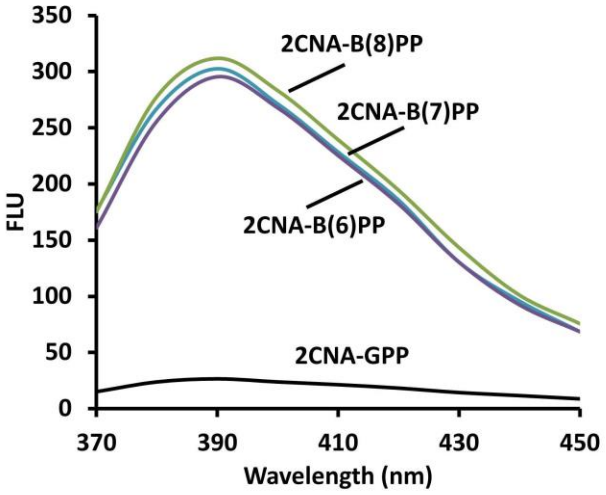
A)



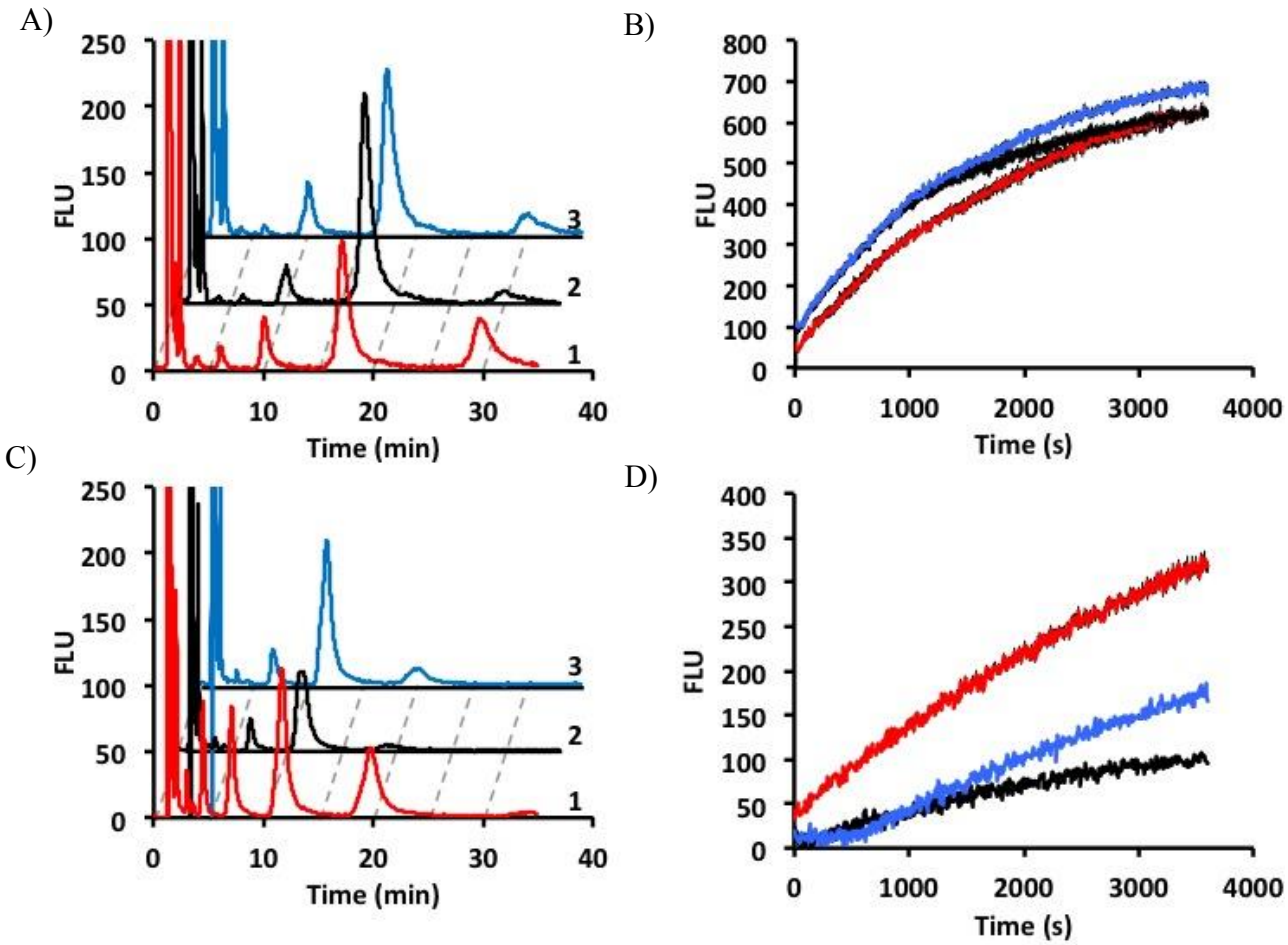
B)



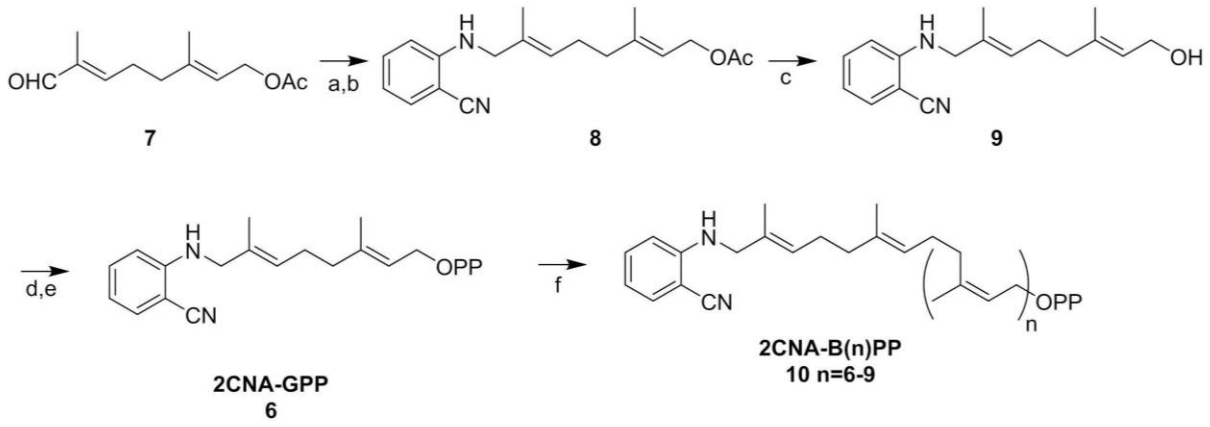
Supporting Figure 5.Fluoresence of 1 μM 2CNA-GPP and 2CNA-B(6-8)PP in aqueous solution



Supporting Figure 6. UPPS assays with matched 2CNA-GPP rates. UPPS concentrations were 6.3, 25 and 100 nM from *B. fragilis*, *E. coli*, and *V. vulnificus* respectively with (A-B) 2CNA-GPP **6** and 22, 88 and 350 nM with 2AA-GPP **5 (**C-D**)**. HPLC was performed in 55%/45% Propanol/100 mM ammonium bicarbonate. A) HPLC analysis of 2CNA-GPP reactions quenched at the end of the plate assay in in B. C) HPLC analysis of 2AA-GPP reactions quenched at the end of the pate assay in D. $UPPS_{Bf}$ (1, red) $UPPS_{Ec}$ (2, black) $UPPS_{Vv}$ (3, blue)



Supporting Scheme 1. Synthesis of 2CNA-GPP. a. $TiCl_4$, 2-aminobenzonitrile, pyridine, CH_2Cl_2; b. acetic acid, $NaBH(OAc)_3$ 57%; c. K_2CO_3/water/MeOH 90%; d. PBr_3/CH_2Cl_2 e. tris(tetra-N-butyl ammonium)diphosphate/CH_2Cl_2 27% f. IPP/UPPS in Bicine buffer with DDM, $MgCl_2$ and KCl.



2CNA-GPP synthesis.

8-(2-nitrile)-Aniline-3, 7-dimethyl-1-acetoxy-2, 6-octadiene (8): Under argon, 150 µL of pyridine was added to 3.0 mL of CH_2Cl_2 and stirred for 15 minutes. To the flask, 26 µL of $TiCl_4$ (94 mg at 4.47g/mL) was allowed to stir for 10 min after which 80 mg (0.067 mmol) of 2-aminobenzonitrile was added along with 100 mg (0.48 mmol) of aldehyde **7**. After 8 hours, 300 µL of acetic acid and 300 mg of $NaHB(OAc)_3$ was added and the reaction was allowed to continue for 24 hours before being quenched with 1.0 mL of water. The reaction was diluted with ether and washed with water, $NaHCO_3$, water and brine then was dried with $MgSO_4$. After the solvent was removed, the mixture was purified using flash column chromatography (5% EtOAc in Hexanes) Yield: 57%. TLC (7:3 Hexanes/ETOAc) R_f=0.51, ^{1}HNMR (500MHz) 7.37 (d, 1H, J=7.9 Hz), 7.33 (t, 1H, J=7.8 Hz), 6.65 (t, 1H, J=7.7 Hz), 6.61 (d, 1H, J=8.7Hz), 5.37 (t, 1H, J=7.2 Hz), 5.31 (t, 1H, J=7.2 Hz), 4.55 (d, 2H, J=7.7 Hz), 3.72 (s, 2H), 2.16 (m, 2H), 2.05 and 2.04 (overlapping t,s, 5H), 1.68 (s, 3H), 1.65 (s, 3H) ^{13}CNMR (125 MHz) 171.2, 150.6, 141.8, 134.3, 132.7, 131.3, 126.3, 118.8, 118.1, 116.4, 111.2, 95.6, 61.4, 50.9, 39.1, 25.9, 21.2, 16.4, 14.6. ESI-MS (M+) expected: 313.19 amu, Actual: 313.05,(M+Na) 334.95, M+K: 350.96.

8-(2-nitrile)-Aniline-3, 7-dimethyl-2, 6-octadien-1-ol (9): To a round bottom flask acetate **8** (1.62 mmol) was added to methanol (15 mL). To this solution, potassium carbonate (485 mmol) dissolved in water (1.5 mL) was added and the reaction was stirred overnight at room temperature. The solvent was removed and the residual oil was extracted with ether and washed with water and brine before being dried with $MgSO_4$. The mixtures were purified using flash column chromatography to yield the corresponding alcohol **9**. Yield: 90% TLC (7:3 Hexanes/ETOAc) R_f=0.22, ^{1}HNMR (500MHz): 7.35 (m, 2H), 6.64 (t, 1H, 7.9 Hz), 6.61 (d, 1H, J=8.4 Hz), 5.37 (m, 2H), 4.12 (d, 2H, J=6.5 Hz), 3.71 (s, 2H), 2.17 (m, 2H), 2.04 (t, 2H, J=8.5

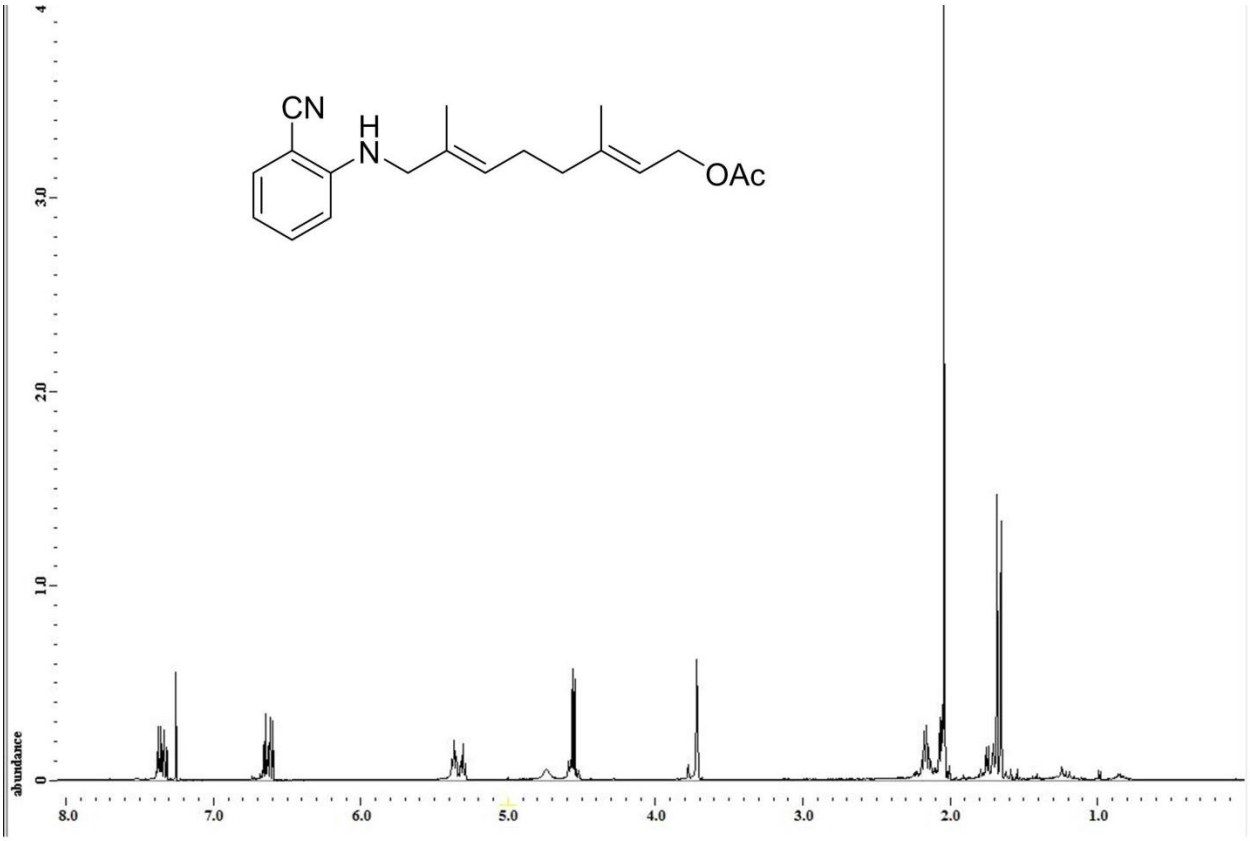
Hz), 1.66, 1.65 (s,s, 6H), ^{13}CNMR (125 MHz): 150.6, 138.8, 134.2, 132.7, 131.2, 126.3, 124.1, 118.2, 116.5, 111.13, 95.6, 59.4, 50.8, 39.1, 25.8, 16.2, 14.7; ESI-MS (M+) expected: 271.18 amu, Actual: 271.30, (M+Na): 293.24

8-(2-nitrile)-Aniline-3, 7-dimethyl-2, 6-octadien-1-yl diphosphate (2CNA-GPP, 6): Alcohol **9** (18.2 μmol) was diluted in 15 mL of dichloromethane and was stirred at room temperature. Phosphorous tribromide (6.16 μmol of a 0.1 M solution) was slowly added to the reaction and left overnight before passing through a short pad of silica in CH_2Cl_2. The solvent was removed by rotary evaporation until approximately 10 mL of CH_2Cl_2 remained. To this suspension, 3-5 equivalents of tris(tetra-N-butyl ammonium) diphosphate was slowly added and he mixture was allowed to stir overnight. The reaction was concentrated and passed through ammonium form AG® 50W-X8 ion-exchange resin. The eluent was collected and lyophilized to yield a white solid, which was dissolved in 25 mM ammonium bicarbonate and purified by reverse phase HPLC monitored at 340 nm. The purification utilized a gradient method with solvent A: 25 mM ammonium bicarbonate, solvent B: acetonitrile 0 min: 20% B, 30 min: 100% B. Preparative scale purifications were performed on a Varian Polaris C18-A 250 x 21.2 mm column at 4 mL/min. Yield: 27% ^{1}HNMR (500MHz): 7.38 (d, 1H, J=7.6 Hz), 7.34 (t, 1H, J=7.9 Hz), 6.67 (d, 1H, J=8.8 Hz), 6.64 (t, 1H, J=7.4 Hz), 5.24 (m, 2H), 4.29 (apparent t, 2H, J=5.9 Hz), 2.05 (m, 2H), 1.94 (t, 2H, J=6.9Hz), 1.54 (s, 3H), 1.50 (s, 3H). ^{13}CNMR (125 MHz): 150.9, 142.6, 134.9, 133.4, 131.7, 125.6, 120.0, 119.0, 117.1, 112.8, 94.9, 62.8, 49.6, 38.6, 25.2, 15.6, 13.5. ^{31}PNMR (202 MHz) -8.0 (bd, 1P, J=22 Hz) -10.1 (d, 1P, J=22 Hz). ESI-MS (M-) expected: 429.10 amu, Actual: 429.18

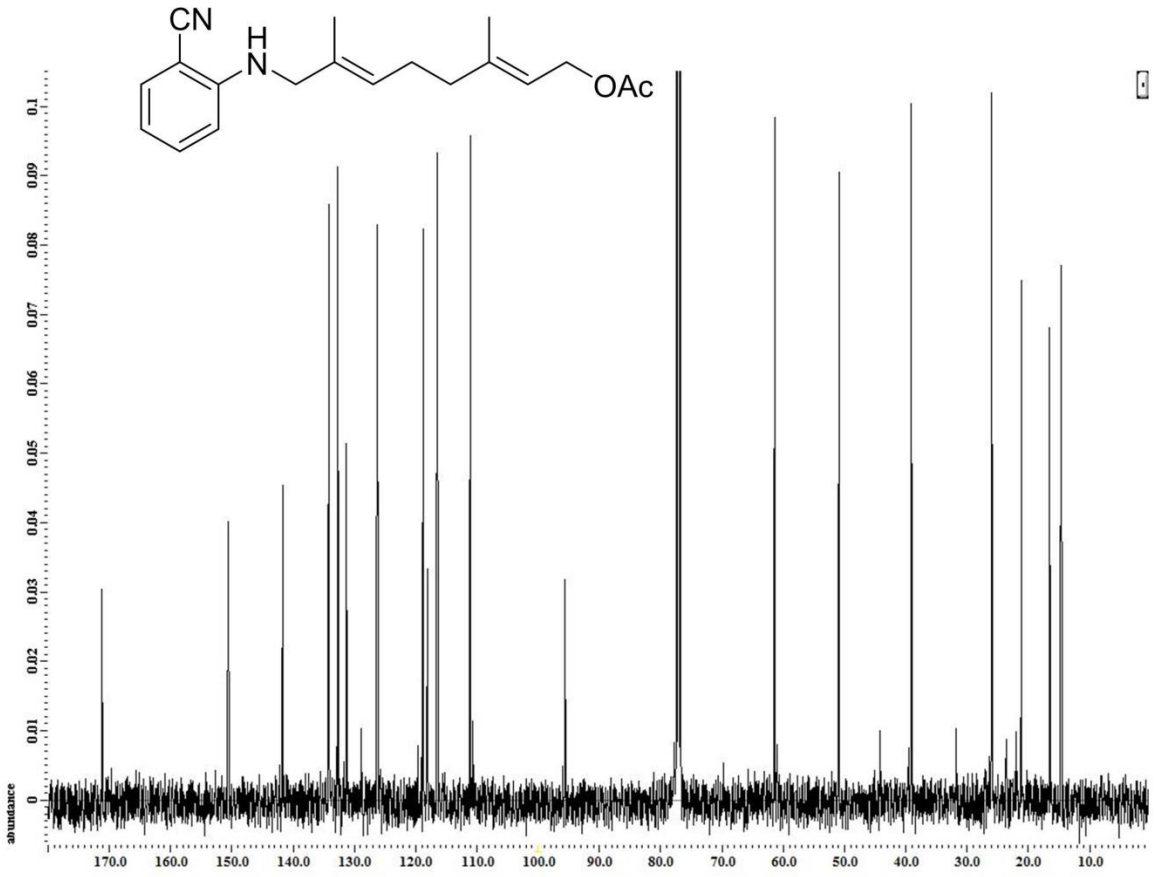
2-ntirileanilinobactoprenyl diphosphate (2CNA-B(n)PP, 10). In a 1.7 mL microcentrifuge tube 190 nmol of **10** was added to a 500 μL (final volume) solution containing 2 mM IPP, 50

mM HEPES (pH=7.4), 2 mM KCl, 0.2 mM $MgCl_2$, 17 nM $UPPS_{Bf}$, 0.1% *n*-dodecyl-β-D-maltoside. The solution was incubated overnight at 37° C then product was purified by reverse phase-HPLC on an Agilent Semi-preparative Eclipse XDB-C18 5 μm 9.4 x 250 mm column in 65% *n*-Propanol/35% 100 mM ammonium bicarbonate monitored at 340 nm at 3 mL/min.

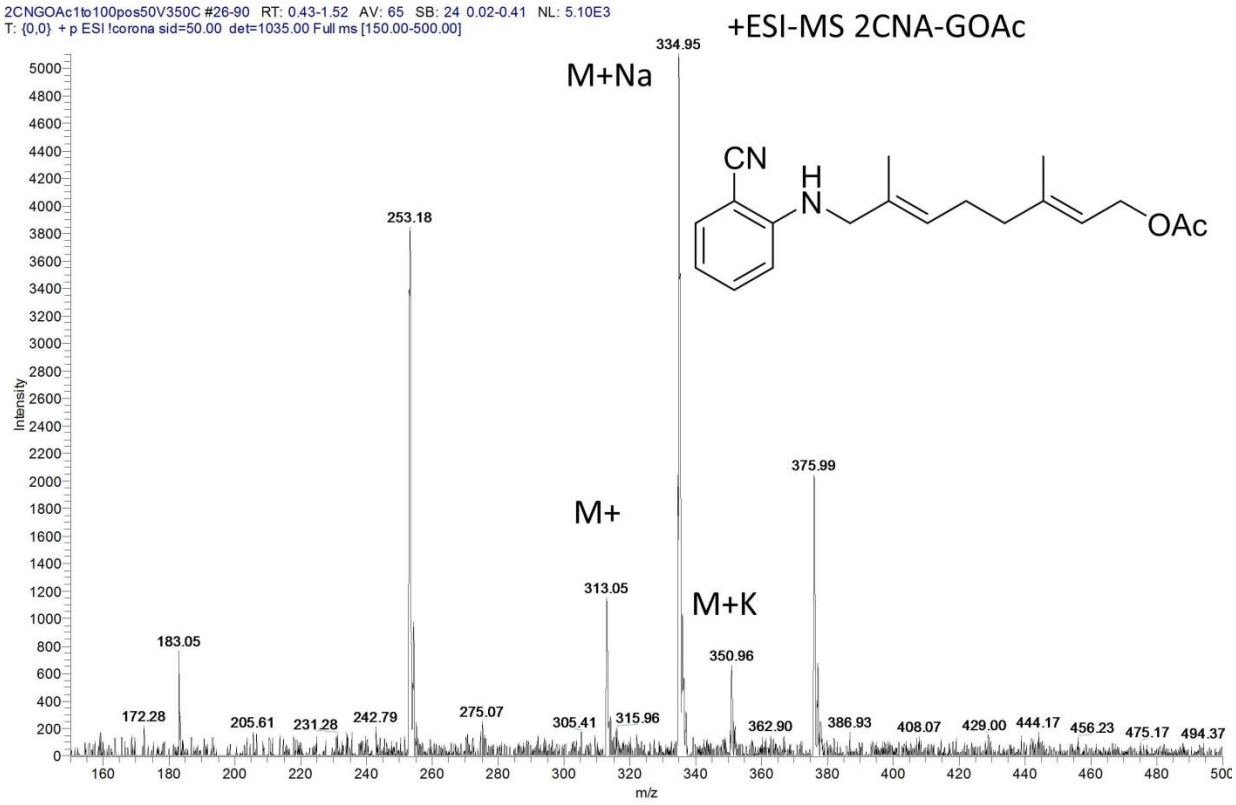
Compound Characterizations
^{1}H-NMR 2CNA-GOAc **8**

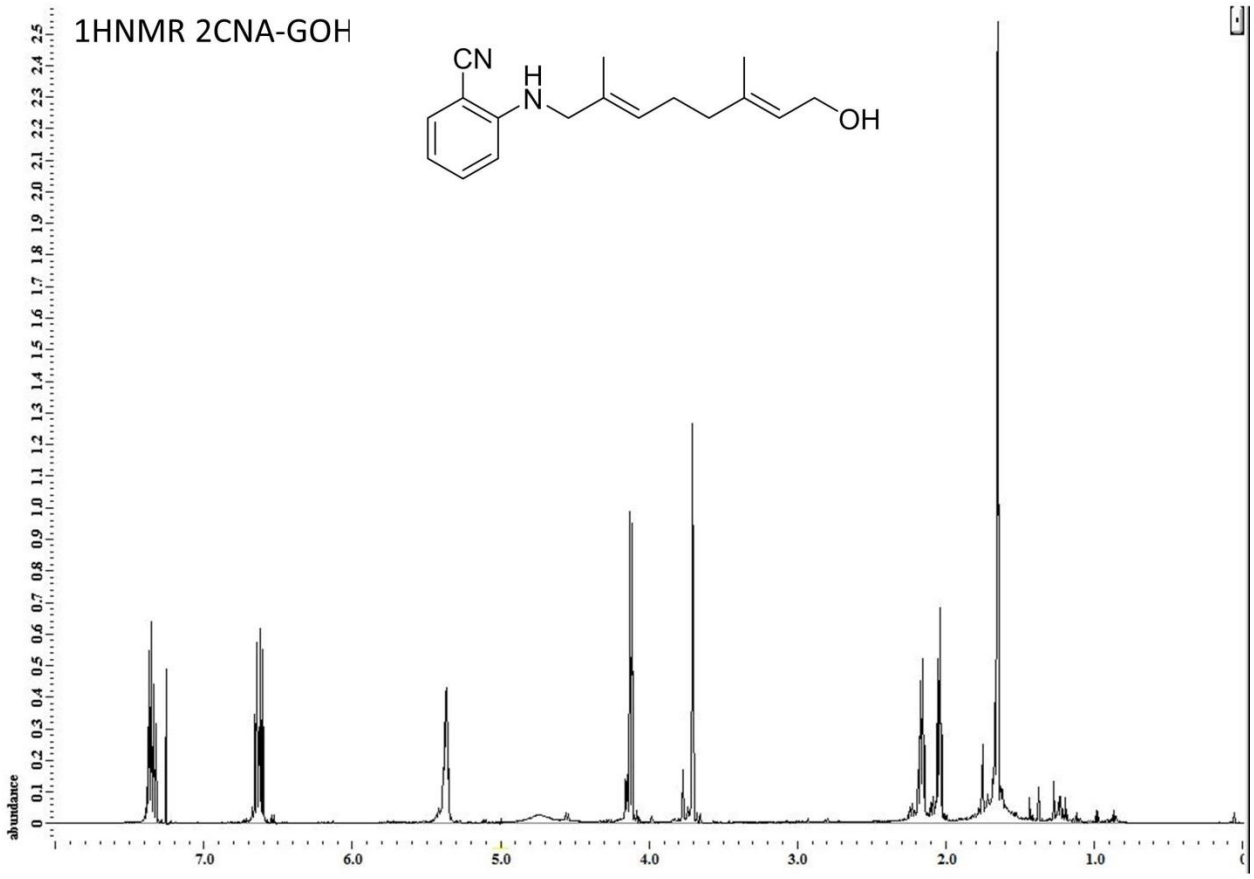


^{13}C-NMR 2CNA-GOAc **8**

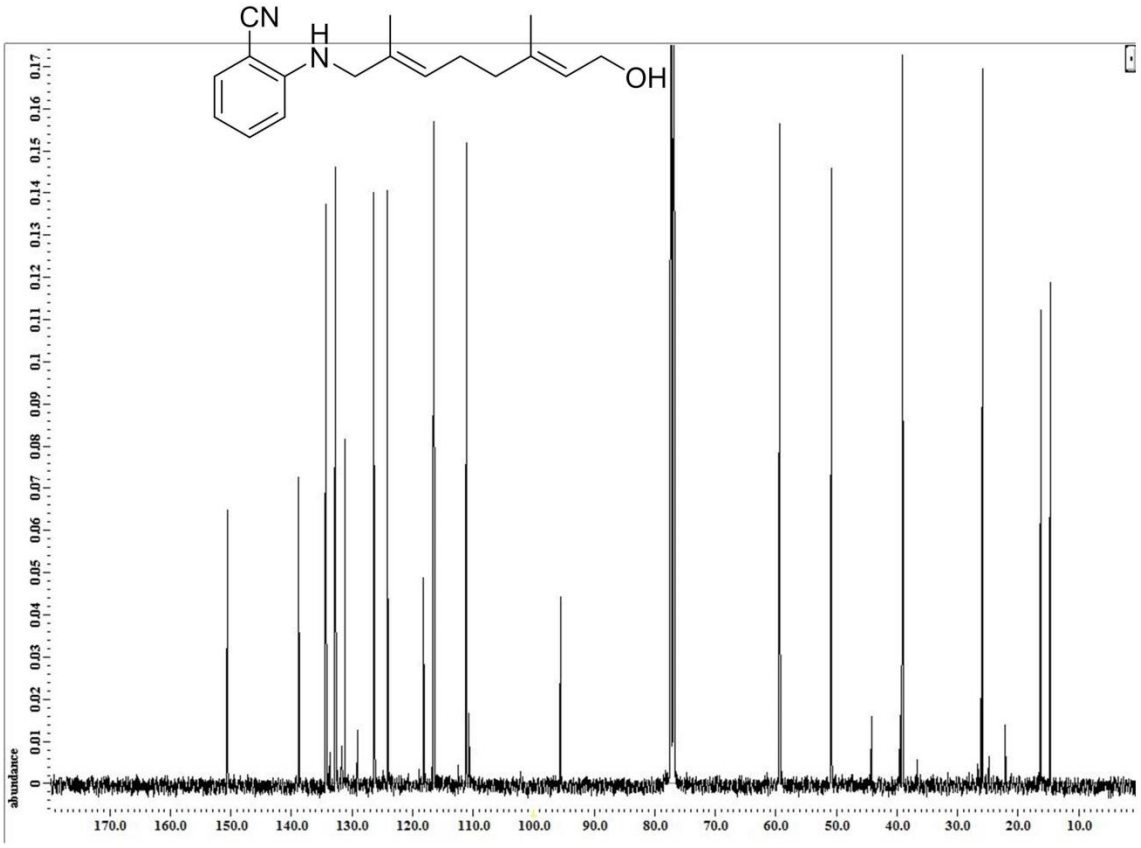


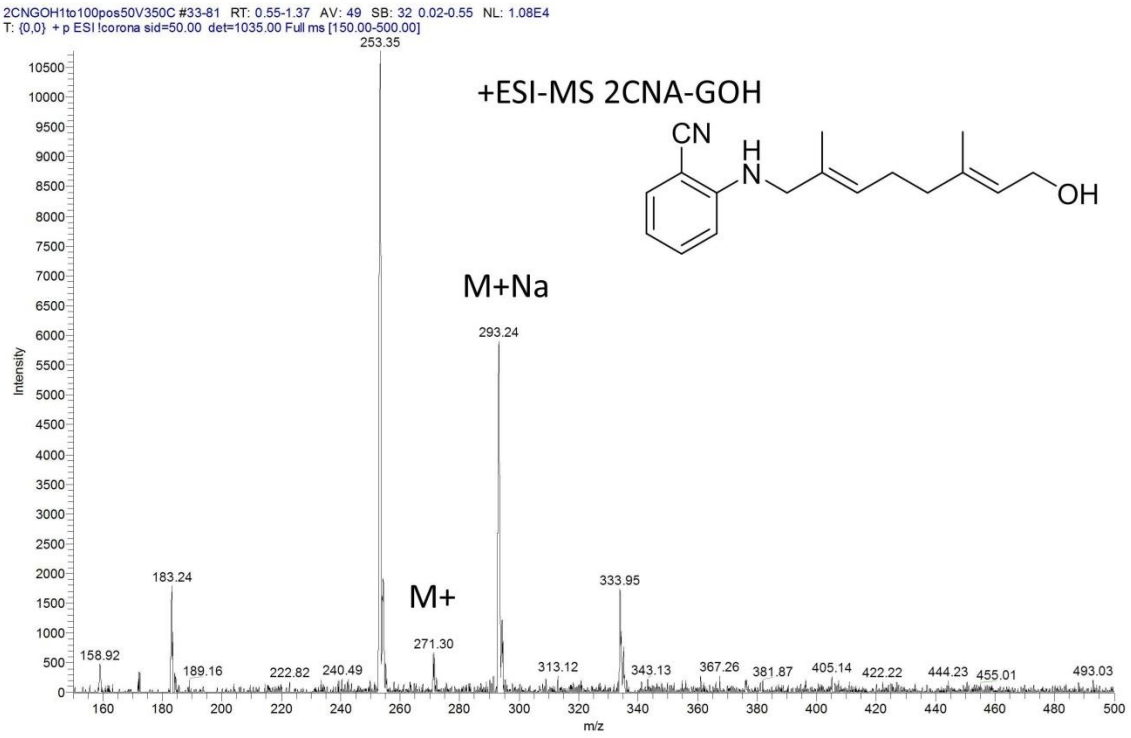
+ESI-MS 2CNA-GOAc **8**




1HNMR 2CNA-GOH
CN
H
N
OH
abundance
7.0
6.0
5.0
4.0
3.0
2.0
1.0

13CNMR 2CNA-GOH

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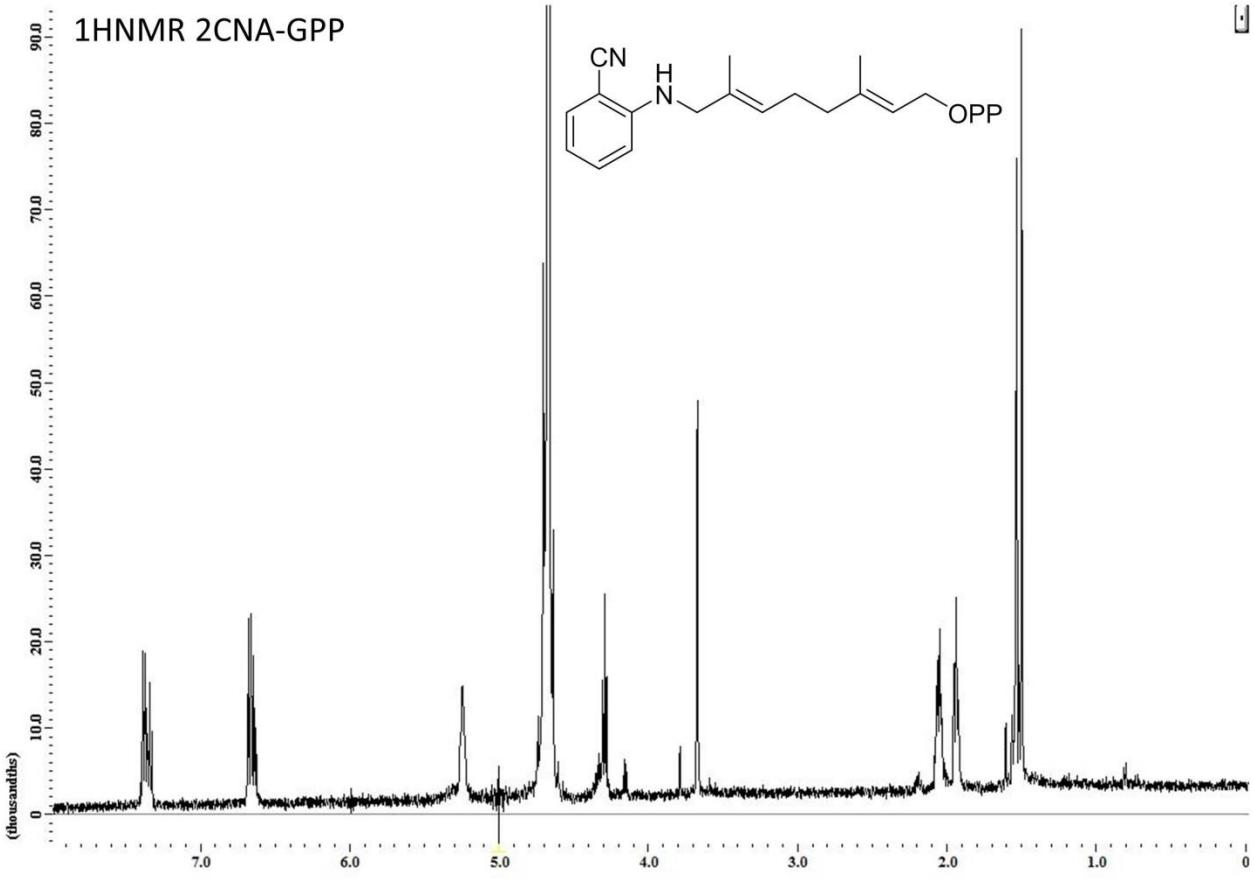
T: {0,0} + p ESI !corona sid=50.00 det=1035.00 Full ms [150.00-500.00]
+ESI-MS 2CNA-GOH
CN
H
N
OH
M+Na
M+
253.35
293.24
183.24
271.30
333.95
158.92
189.16
222.82
240.49
313.12
343.13
367.26
381.87
405.14
422.22
444.23
455.01
493.03
Intensity
m/z


1HNMR 2CNA-GPP
CN
H
N
OPP
(thousands)
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80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
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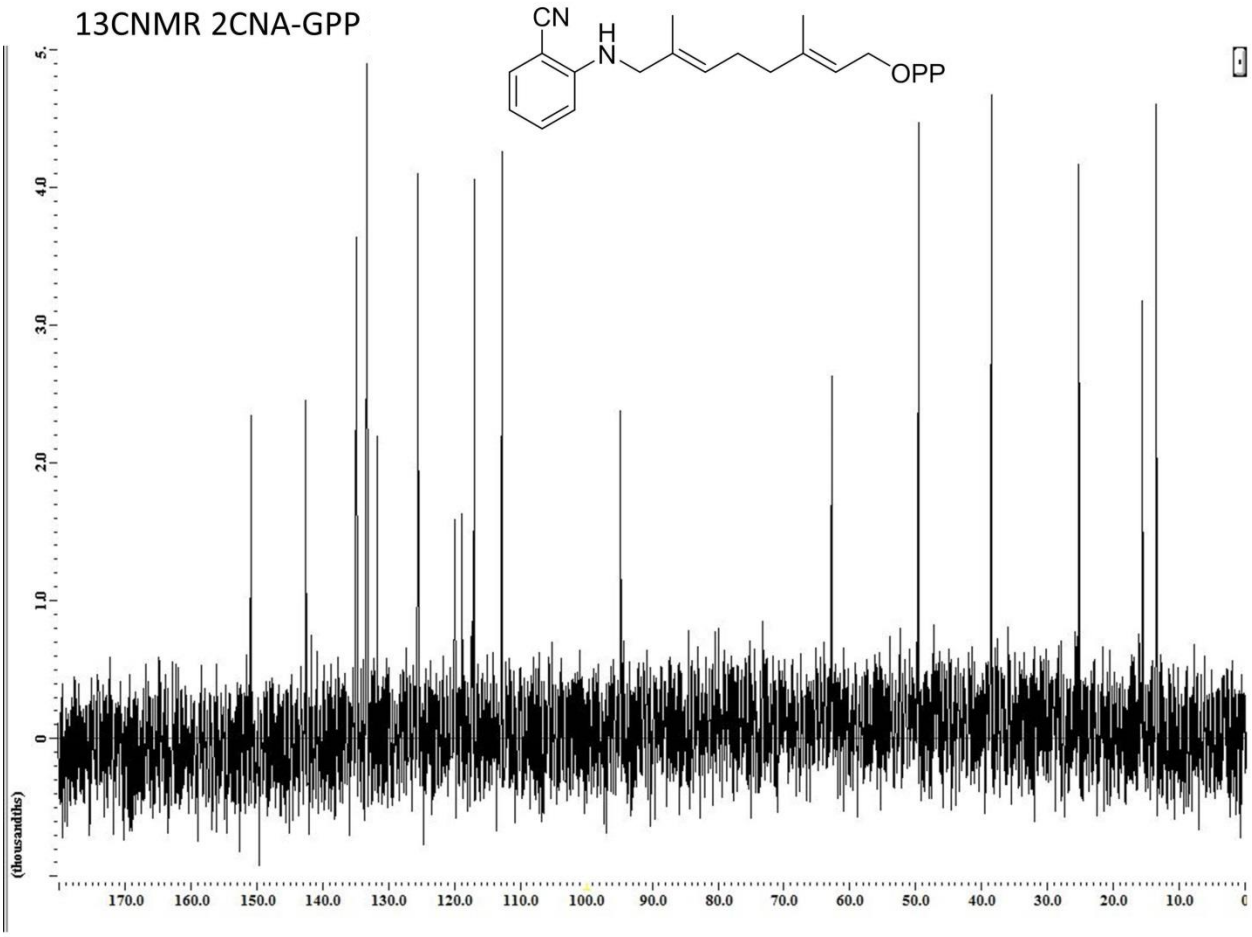
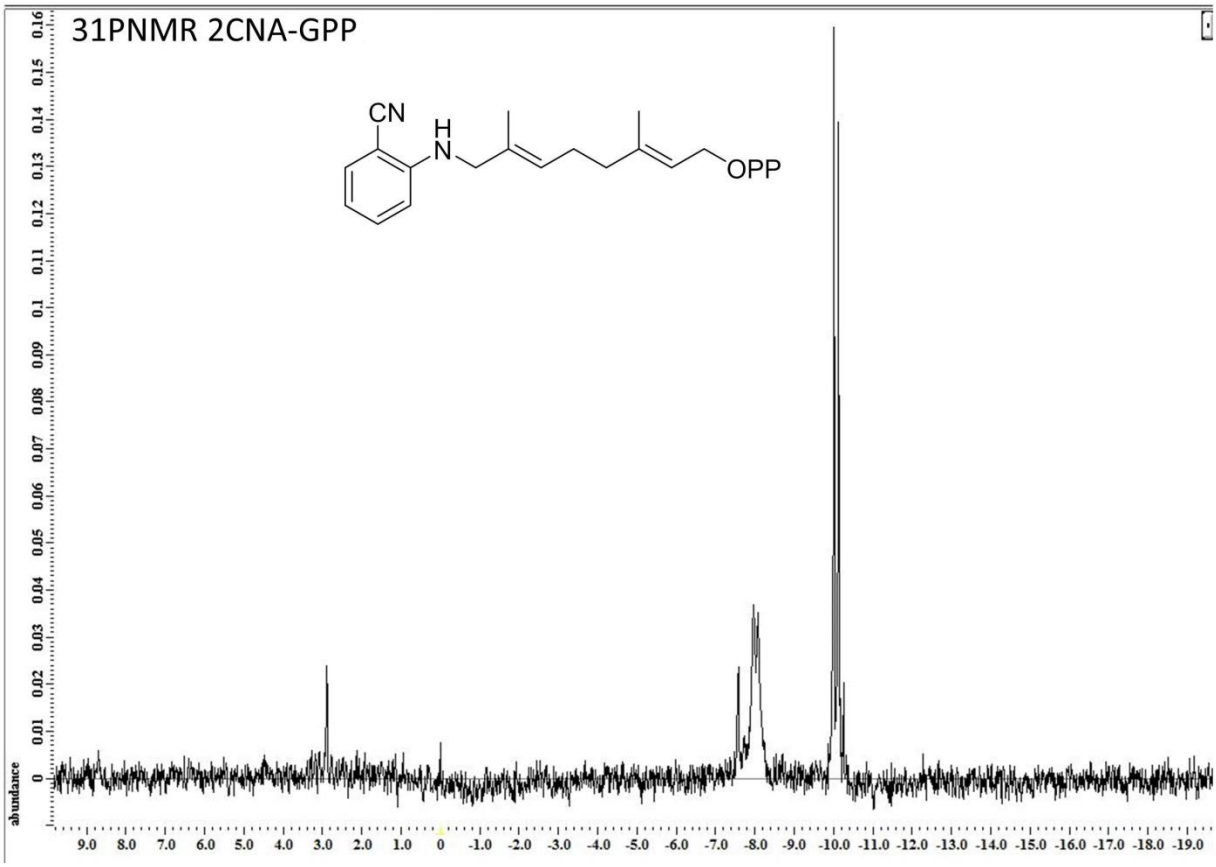
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0

13CNMR 2CNA-GPP




31PNMR 2CNA-GPP
CN
H
N
OPP
abundance
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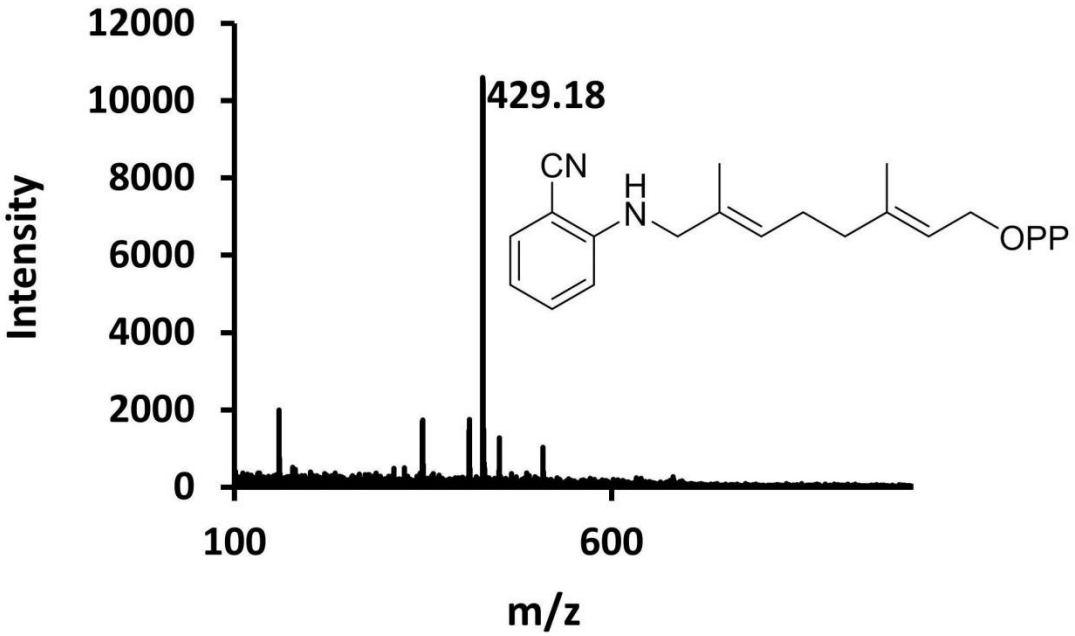
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0 -1.0 -2.0 -3.0 -4.0 -5.0 -6.0 -7.0 -8.0 -9.0 -10.0 -11.0 -12.0 -13.0 -14.0 -15.0 -16.0 -17.0 -18.0 -19.0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4000
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Intensity
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CN
H
N
OPP
100
600
m/z