Supporting Information

A Fluorogenic, Small Molecule Reporter for Mammalian Phospholipase C Isozymes

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This document contains supporting figures (3 pages), experimental procedures and data (5 pages), and NMR spectra of key compounds (17 pages)

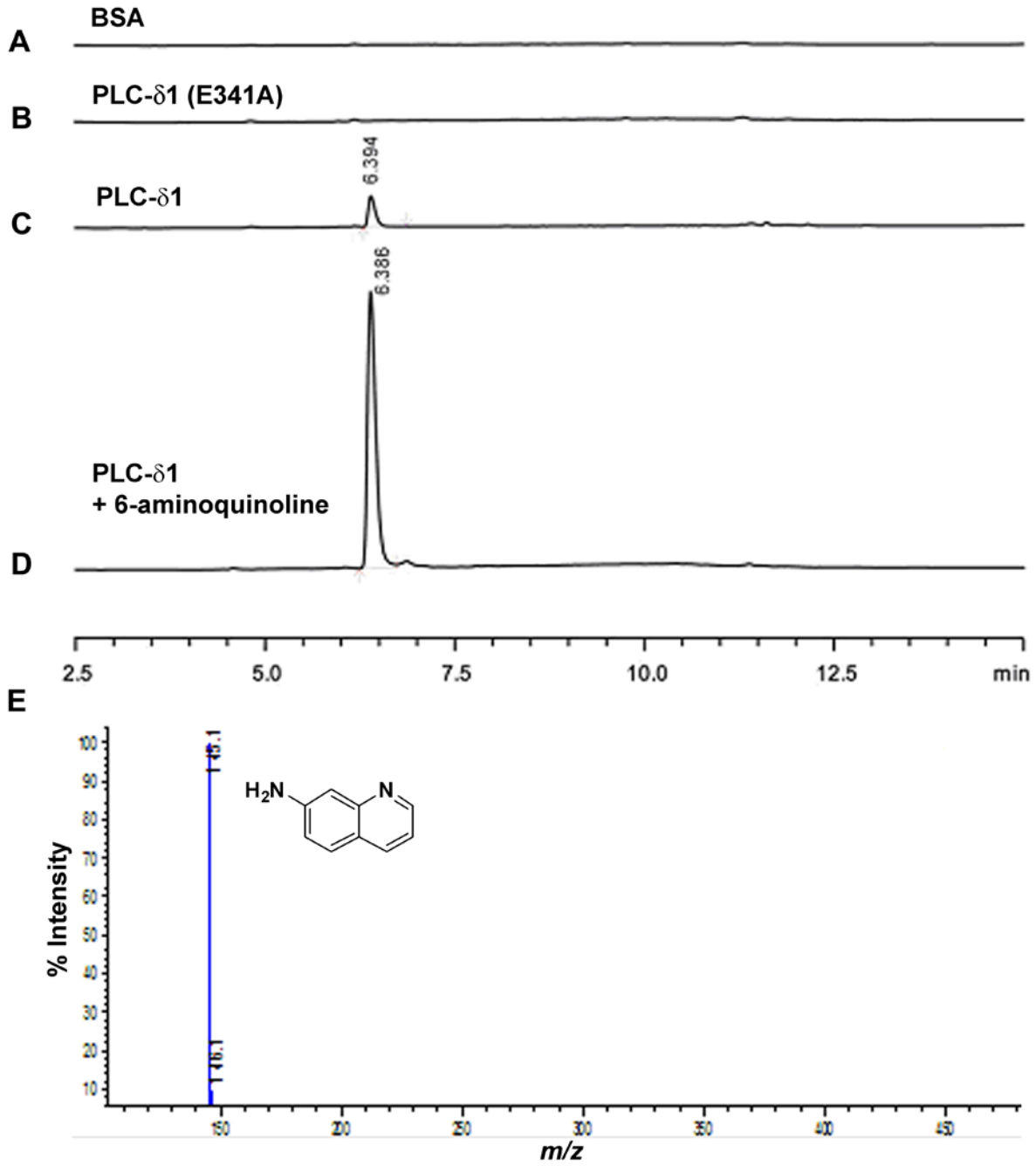


Figure S1. HPLC and LC-MS analyses confirm PLC-δ1 cleaves WH-15 to generate free 6-aminoquinoline. WH-15 (58 μM, final concentration) was used in the PLC assay buffer as described in the Experimental with the presence of BSA, PLC-δ1(E341A), or PLC-δ1. The reaction was stopped by adding MeOH and the mixture was analyzed by HPLC. The column was eluted in a gradient that starts with 10% MeOH in H_2O and ends with 100% MeOH in 10 min. The HPLC chromatograms for (A) BSA; (B) PLC-δ1(E341A); (C) PLC-δ1; and (D) co-injection of PLC-δ1 sample (20 μL) with 6-aminoquinoline (10 μL of 1.0 mM in H_2O) are shown. LC-MS (ESI-Pos) (E) analysis of the PLC-δ1 sample after incubation with **WH-15** demonstrates the formation of a compound with the same molecular ion as 6-aminoquinoline.

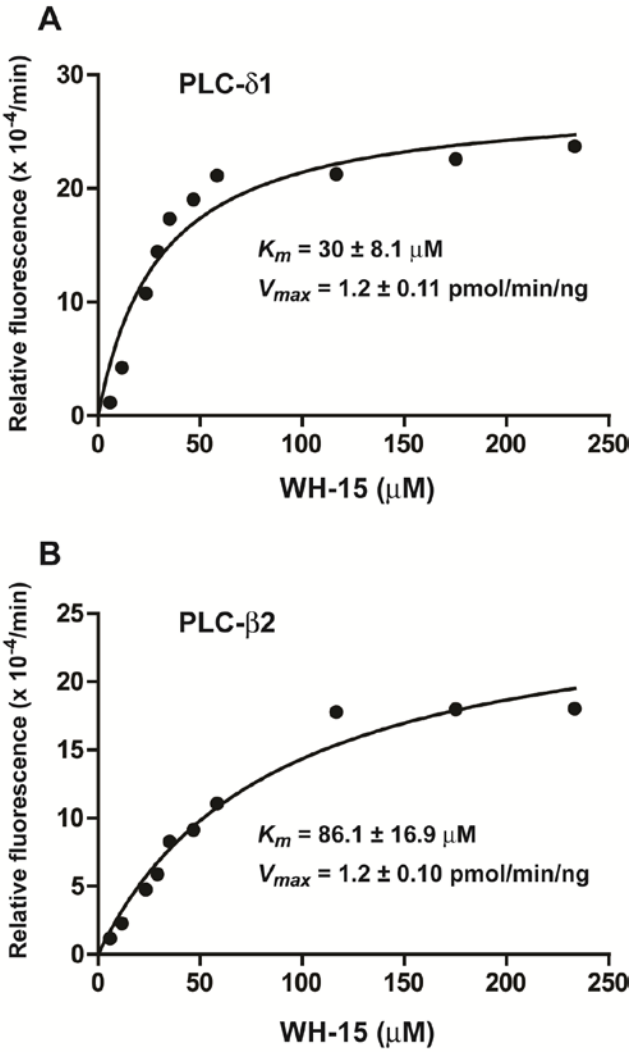


Figure S2. Kinetic studies of WH-15 with PLC-δ1 (A) and PLC-β2 (B).

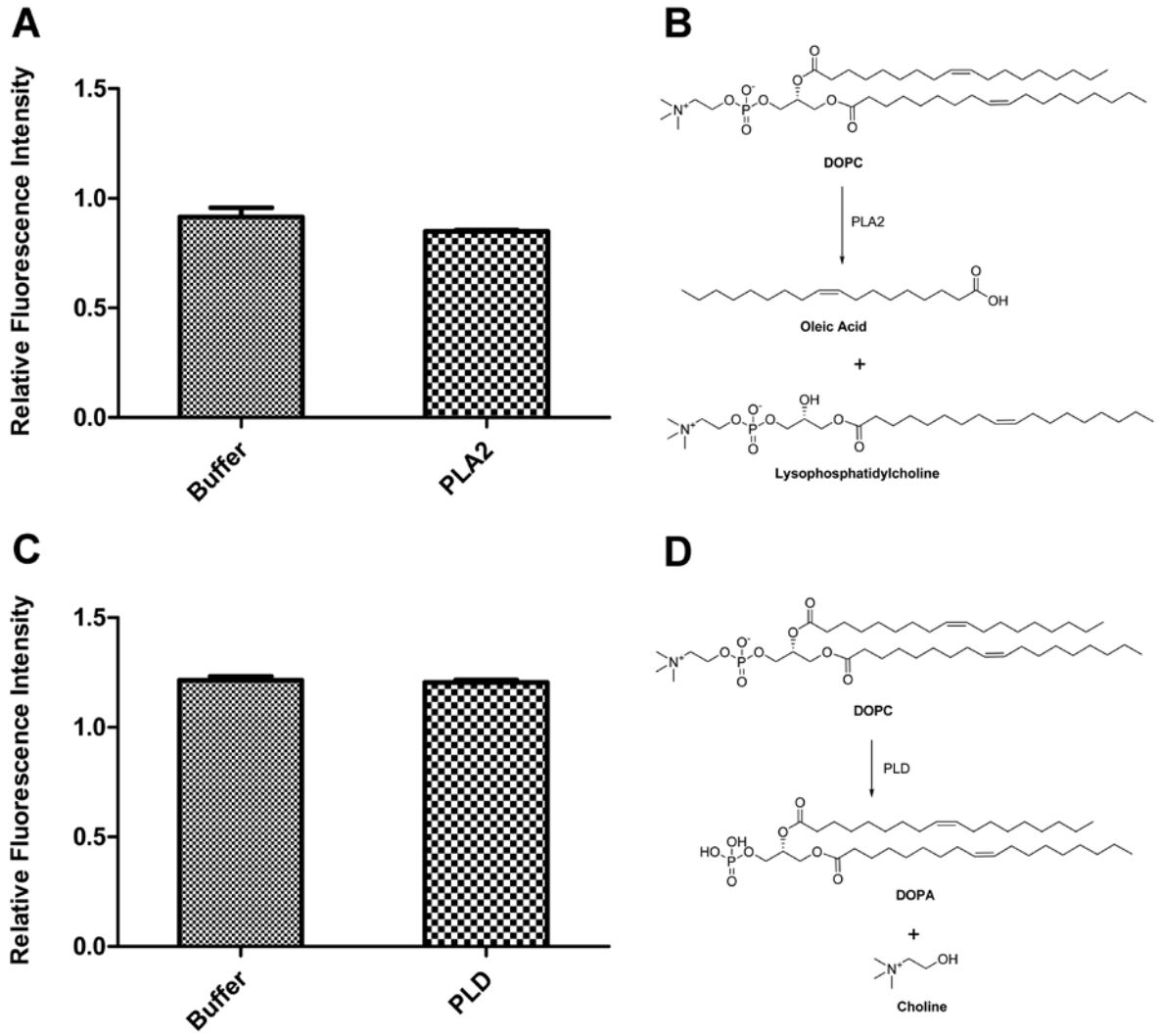


Figure S3. PLA2 and PLD did not generate fluorescence increase from WH-15. (A) **WH-15** was incubated with PLA2 under conditions that were described in the Experimental and fluorescence was recorded. The relative fluorescence intensity is defined as the ratio of fluorescence reading to the initial reading in reaction mixture without PLA2 at t = 0 min. (B) The same batch of PLA2 catalyzed the hydrolysis of dioleoylphosphatidylcholine (DOPC) to olelic acid and lysophosphatidylcholine under the same reaction conditions. Lysophosphatidylcholine was detected by ^{31}P-NMR and mass spectrometry (MS). The specific activity of PLA2 was measured as 5.9 nmol/min/unit. (C) **WH-15** was incubated with PLD under conditions that were described in the Experimental and fluorescence was recorded. The relative fluorescence intensity is defined as the ratio of fluorescence reading to the initial reading in the reaction mixture without PLD at t = 0 min. (B) The same batch of PLD catalyzed the hydrolysis of DOPC to dioleoylphosphatidic acid (DOPA) and choline under the same reaction conditions. DOPA was detected by 31P-NMR and MS. The specific activity of PLD was measured as 14.4 nmol/min/unit.

Experimental

General. Chemicals were purchased from Aldrich and Acros Chemical Corporation and used without further purification. Solvents were purchased from suppliers as anhydrous grade. NMR spectra were recorded at room temperature on Gemini-300 MHz, Inova-400 MHz or Inova-500 MHz spectrometer. Chemical shifts are reported in ppm with TMS as the internal standard for ^{1}H NMR and 85% H_3PO_4 as the external standard for ^{31}P NMR spectra. High-resolution mass spectra were obtained on a Bruker Daltonics (Billerica, Massachusetts) BioToF (ESI-TOF; Electrospray Time of Flight Mass Spectrometer) mass spectrometer or LTQ Orbitrap (Thermo Fisher Scientific, Bremen, Germany). HPLC analyses were performed on a Thermo Betasil C18 reverse phased column (150 x 4.6 mm, 5 μm) with the SHIMADZU LC-6AD system. Preparative HPLC was performed on a Thermo Betasil C18 reverse phase column (150 x 10 mm, 5 μm). Phospholipase A_2 from honey bee venom and Phospholipase D from *Streptomyces chromofuscus* were purchased from Sigma.

4-Hydroxy-3-(octyloxy)benzaldehyde (3)

To the solution of 4-(benzyloxy)-3-hydroxybenzaldehyde (190 mg, 0.83 mmol) in anhydrous DMF (2.0 mL) was added 60% NaH (40 mg, 1.00 mmol) at 0 ^{o}C followed by the addition of 1-iodooctane (300 mg, 1.25 mmol). The reaction mixture was stirred at room temperature (r.t.) overnight and NH_4Cl solution was then added. The mixture was extracted with ethyl acetate 3 times and the combined organic layers were dried and concentrated under vacuum. The resulting residue was purified through flash column chromatography (Hexane: Ethyl Acetate = 10:1) to yield 4-(benzyloxy)-3-(octyloxy)benzaldehyde (235 mg, 83%) as light yellow oil, which was subsequently subjected to hydrogenolysis in ethyl acetate in the presence of 10% Pd/C. After filtration and concentration, the crude residue was purified by column chromatography (Hexane: Ethyl Acetate = 5:1) to give **3** (125 mg, 72%) as light yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.80 (s, 1H), 7.36−7.42 (m, 2H), 7.03 (d, *J* = 8.6 Hz, 1H), 6.44 (s, 1H), 4.09 (t, *J* = 6.9 Hz, 2H), 1.82 (hexatet, *J* = 6.7 Hz, 2H), 1.20−1.50 (m, 10H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 191.09, 151.96, 146.70, 129.90, 127.42, 114.44, 109.68, 69.30, 31.86, 29.37, 29.28, 29.09, 26.03, 22.73, 14.17; ESI-HRMS for $[M + H]^+$

$C_{15}H_{23}O_3$: calcd 251.1647, found 5251.1638.

Benzyl 4-formyl-2-(octyloxy)phenyl diisopropylphosphoramidite (4)

A solution of **3** (66.0 mg, 0.26 mmol) in anhydrous CH_2Cl_2 was added to the mixture of 1*H*-tetrazole (9.0 mg, 0.13 mmol) and 1-(benzyloxy)-N,N,N',N'-tetraisopropylphosphinediamine (1.0 M, in CH_2Cl_2) (0.53 mL, 0.53 mmol) at r.t. under argon. After stirring at r.t. for 3 h, the reaction mixture was concentrated and purified by column chromatography (Hexane: Acetone: TEA= 100: 5: 3) to give phosphoramidite **4** (127 mg, 100%) as colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.85 (s, 1H), 7.40 (d, *J* = 2.1 Hz, 1H), 7.24–7.37 (m, 6H), 7.20 (dd, *J* = 8.1 Hz, 1.8 Hz, 1H), 4.88 (dd, *J* = 12.4, 8.5 Hz, 1H), 4.82 (dd, *J* = 12.4, 8.5 Hz, 1H), 4.01 (t, *J* = 6.5 Hz, 2H), 3.74–3.88 (m, 2H), 1.72–1.85 (m, 2H), 1.41–1.52 (m, 2H), 1.20–1.38 (m, 20H), 0.88 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 191.33, 151.57 (d, J_{CP} = 2.3 Hz), 150.26 (d, J_{CP} = 4.4 Hz), 139.48 (d, J_{CP} = 7.4 Hz), 131.58, 128.39, 127.54, 127.09, 125.64, 120.10 (d, J_{CP} = 12.5 Hz), 111.17, 68.86, 66.09 (d, J_{CP} = 17.1 Hz), 43.85 (d, J_{CP} = 13.1 Hz), 31.92, 29.50, 29.37, 26.22, 24.71, 24.62, 24.50, 22.76, 14.20; ^{31}P NMR ($CDCl_3$, 162 MHz) δ149.01 (s,1P); ESI-HRMS for $[M + Na]^+$ $C_{28}H_{42}NO_4PNa$: calcd 510.2749, found 510.2770.

Compound 6

A solution of phosphoramidite **4** (127 mg, 0.26 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added to compound **5** (65 mg, 78 μmol) and 1*H*-tetrazole (29 mg, 0.39 mmol) in anhydrous CH_2Cl_2 (1.0 mL) at r.t. in one portion under argon. The mixture was stirred at r.t. for 12 h, and a solution of *t*-BuOOH (5.0~6.0 M, 0.24 mL) in decane was added at -40 ^{o}C. The resulting reaction mixture was warmed to room temperature gradually, concentrated and purified by column chromatography (Hexane: Acetone= 2:1) to provide the product **6** (39 mg, 40%) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.90 (s, 1H), 7.53 (dd, *J* = 8.5, 8.2 Hz, 1H), 7.20–7.45 (m, 27H), 5.26 (conformation 1) and 5.24 (conformation 2) (2H), 4.85–5.15 (m, 9H), 4.57–4.80 (m, 4H), 4.51 (d, *J* = 6.6 Hz, 1H), 4.32–4.45 (m, 4H), 4.18 (dd, *J* = 16.0, 8.0 Hz, 1H), 3.94–4.05 (m, 2H), 3.55 (dd, *J* = 9.5, 7.4 Hz, 1H), 3.37 (conformation 1) and 3.32 (conformation 2) (s, 3H), 3.33 (conformation 2) and 3.27 (conformation 1) (s, 3H), 3.19

(s, 3H), 1.76 (hexatet, J = 6.9 Hz, 2H), 1.20–1.44 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 191.07, 151.07, 150.99, 144.49, 144.46, 144.38, 136.06, 136.03, 135.96, 135.93, 135.87, 135.77, 135.35, 135.33, 135.22, 134.45, 134.42, 134.41, 128.93, 128.88, 128.85, 128.74, 128.65, 128.59, 128.57, 128.55, 128.50, 128.16, 128.05, 128.00, 127.92, 124.82, 124.80, 124.66, 121.84, 121.79, 111.86, 98.89, 97.74, 96.75, 77.71, 77.40, 77.36, 77.01, 75.75, 74.41, 70.82, 70.74, 70.03, 69.94, 69.84, 69.79, 69.77, 69.72, 69.57, 69.50, 69.27, 56.83, 55.99, 55.93, 55.90, 31.92, 29.41, 29.39, 29.34, 29.06, 25.94, 25.91, 22.75, 14.22; ^{31}P NMR ($CDCl_3$, 162 MHz) δ conformation 1: −0.07 (2P), −6.05 (1P); conformation 2: −0.10 (2P), −6.25 (1P); ESI (Pos)-HRMS for $[M + Na]^+$ $C_{62}H_{77}O_{20}P_3Na$: calcd 1257.4119, found 1257.4169.

Compound 7

Aldehyde **6** (30 mg, 24 μmol) in anhydrous THF (2.0 mL) was treated with $NaBH_4$ (5.0 mg, 0.132 mmol) at r.t. under argon for 4 h. The reaction mixture was concentrated and purified by column chromatography (hexane: acetone= 2:1) to give the product **7** (28 mg, 95%) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25–7.38 (m, 26 H), 6.95 (s, 1H), 6.83 (d, J = 8.2 Hz, 1H), 5.23–5.27 (m, 2H), 4.85–5.17 (m, 9H), 4.58–4.76 (m, 6H), 4.50 (dd, J = 6.9, 6.3 Hz, 1H), 4.08–4.44 (m, 5H), 3.81–3.98 (m, 2H), 3.50 (dd, J = 11.3, 10.2 Hz, 1H), 3.36 (conformation 1) and 3.32 (conformation 2) (s, 3H), 3.33 (conformation 2) and 3.27 (conformation 1) (s, 3H), 3.19 (s, 3H), 1.76 (tt, J = 7.4, 7.0 Hz, 2H), 1.20–1.44 (m, 10H), 0.87 (t, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.45, 150.40, 139.32, 139.27, 138.98, 138.91, 136.26, 136.16, 136.15, 136.07, 135.79, 135.71, 128.67, 128.60, 128.52, 128.44, 128.35, 128.20, 128.15, 128.04, 127.84, 121.75, 121.72, 121.47, 121.45, 118.97, 118.91, 112.46, 98.84, 97.72, 96.60, 96.36, 78.87, 77.36, 75.69, 75.60, 74.50, 74.41, 74.17, 70.42, 70.12, 69.86, 69.80, 69.70, 69.64, 69.60, 69.54, 69.35, 69.30, 69.09, 69.05, 65.10, 56.87, 56.85, 55.94, 55.88, 31.96, 29.49, 29.46, 29.38, 29.31, 26.04, 26.00, 22.78, 14.24; ^{31}P NMR ($CDCl_3$, 162 MHz): δ conformation 1: 0.07 (2P), −5.34 (1P); conformation 2: 0.04 (2P), −5.44 (1P); ESI-HRMS for $[M + Na]^+$ $C_{62}H_{79}O_{20}P_3Na$: calcd 1259.4275, found 1259.4267. The ^{31}P NMR signals of the two conformers collapsed when the temperature of measurement increases to 50 ^{o}C.

Compound WH-15

The mixture of compound **7** (20 mg, 16 μmol), 4-Dimethylaminopyridine (DMAP) (12 mg, 100 μM) and N-(quinolin-6-yl)-1*H*-imidazole-1-carboxamide **8** (12 mg, 50 μmol) was stirred in anhydrous acetonitrile (3.0 mL) under argon at 60 ^{o}C. The reaction was monitored by TLC (Hexane: acetone=1:1) on silica gel. After 4 h, the reaction mixture was concentrated and subjected to a flash column purification (Hexane:acetone = 1.5:1) to remove most of the starting material **6** and DMAP. The purified compound **9** was dried and re-dissolved anhydrous CH_2Cl_2 (2.0 mL). Bromotrimethylsilane (2.0 mL) was then added at -10 ^{o}C under argon. The reaction mixture was slowly warmed to r.t. and stirred for another 2 h. The solvents and volatile compounds were removed by evaporation, and the residue was dried under vacuum for 1 h. Methanol (4.0 mL) was subsequently added and stirred at r.t. for 2 h. After removal of the solvent, the residue was dried under vacuum for 2 h. Then the crude product was re-dissolved in 30% MeOH and purified by HPLC on a Thermo Betasil C18 reverse Phase column (150 x 10 mm, 5 μm). The desired fractions were combined to give the product (5 mg, 36%) as white solid. The compound was treated with 1.0 M TEAB buffer to form the triethyl amine salt, which was stable at -20 ^{o}C for 2 months. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.6 (d, *J* = 3.6 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 8.06 (s, 1H), 7.84 (d, *J* = 9.0 Hz, 1H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.39 (dd, *J* = 8.4, 4.3 Hz, 1H), 6.96 (s, 1H), 6.84 (d, *J* = 7.7 Hz, 1H), 5.06 (s, 2H), 4.30 (dd, *J* = 17.9, 8.8 Hz, 1H), 4.22 (br. s, 1H), 4.07 (dd, *J* = 7.7, 8.4 Hz, 1H), 3.85–3.96 (m, 4H), 3.51 (d, *J* = 9.3 Hz, 1H), 1.71 (tt, *J* = 7.8, 6.9 Hz, 2H), 1.30–1.41 (m, 2H), 1.10–1.30 (m, 8H), 0.77 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD, 126 MHz) δ 155.89, 151.52 (d, J_{CP} = 6.0 Hz), 149.63 (d, J_{CP} = 9.9 Hz), 145.55, 144.06 (d, J_{CP} = 6.0 Hz), 139.17, 137.86, 133.54, 130.70, 129.78, 124.59, 123.05, 122.42, 121.74, 115.46, 115.41, 115.29, 80.22, 78.36, 72.94 (d, J_{CP} = 9.9 Hz), 72.39, 70.57, 67.82, 60.28, 33.18, 30.70, 30.62, 30.53, 27.23, 23.87, 14.61; ^{31}P NMR (CD_3OD, 162 MHz) δ 3.88 (1P), 3.11 (1P), −3.39 (1P); ESI (Pos)-HRMS for $[M + H]^+$ $C_{31}H_{44}N_2O_{18}P_3$: calcd 825.1799, found 825.1793.

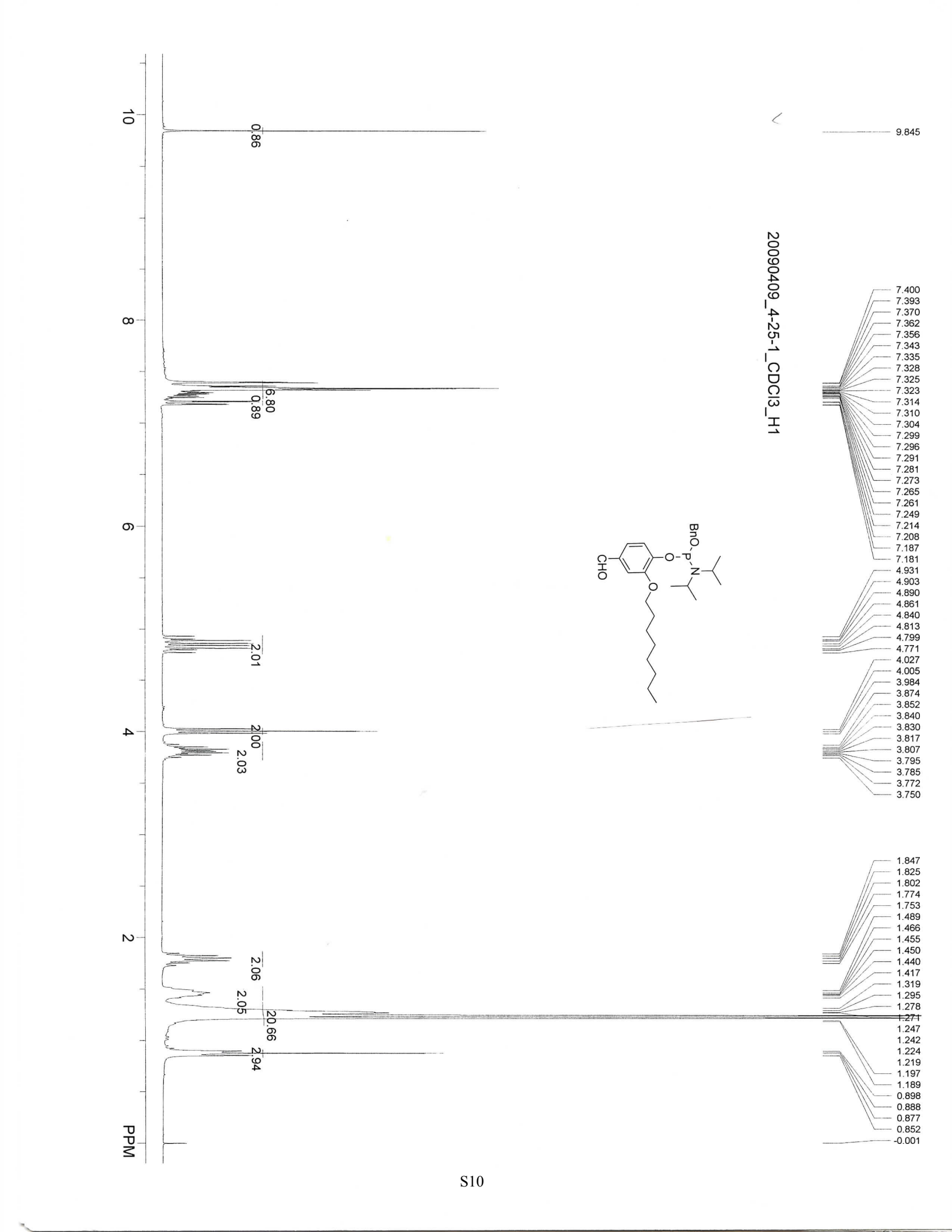
Phospholipase A2 (PLA2) Assay

The reporter **WH-15** (50 μM) was dissolved in the assay buffer (10 μL) that contains

150 mM of NaCl and 5 mM of $CaCl_2$ at 37 °C. The assay was initiated by the addition of 2 μL of PLA2 (10 unit/mL, Sigma). The fluorescence was measured as described above. For the control reaction to demonstrate PLA2 is functional, dioleoylphosphatidylcholine (DOPC) was used instead of **WH-15** under otherwise identical conditions. The enzymatic product lysophosphatidylcholine was detected by ^{31}P-NMR and mass spectrometry (MS). The specific activity of PLA2 was measured as 5.9 nmol/min/unit.

Phospholipase D (PLD) Assay

The reporter **WH-15** (50 μM) was dissolved in the assay buffer (10 μL) that contains 12.4 mM of Tris (pH 8.4), 3.1 mM of SDS, and 50 mM of $CaCl_2$. The assay was initiated by the addition of 2 μL of PLD (10 unit/mL, Sigma). The fluorescence was measured as described above. For the control reaction to demonstrate PLD is functional, DOPC was used instead of **WH-15** under otherwise identical conditions. The enzymatic product dioleoylphosphatidic acid (DOPA) was detected by mass spectrometry (MS) and ^{31}P-NMR. The specific activity of PLD was measured as 14.4 nmol/min/unit.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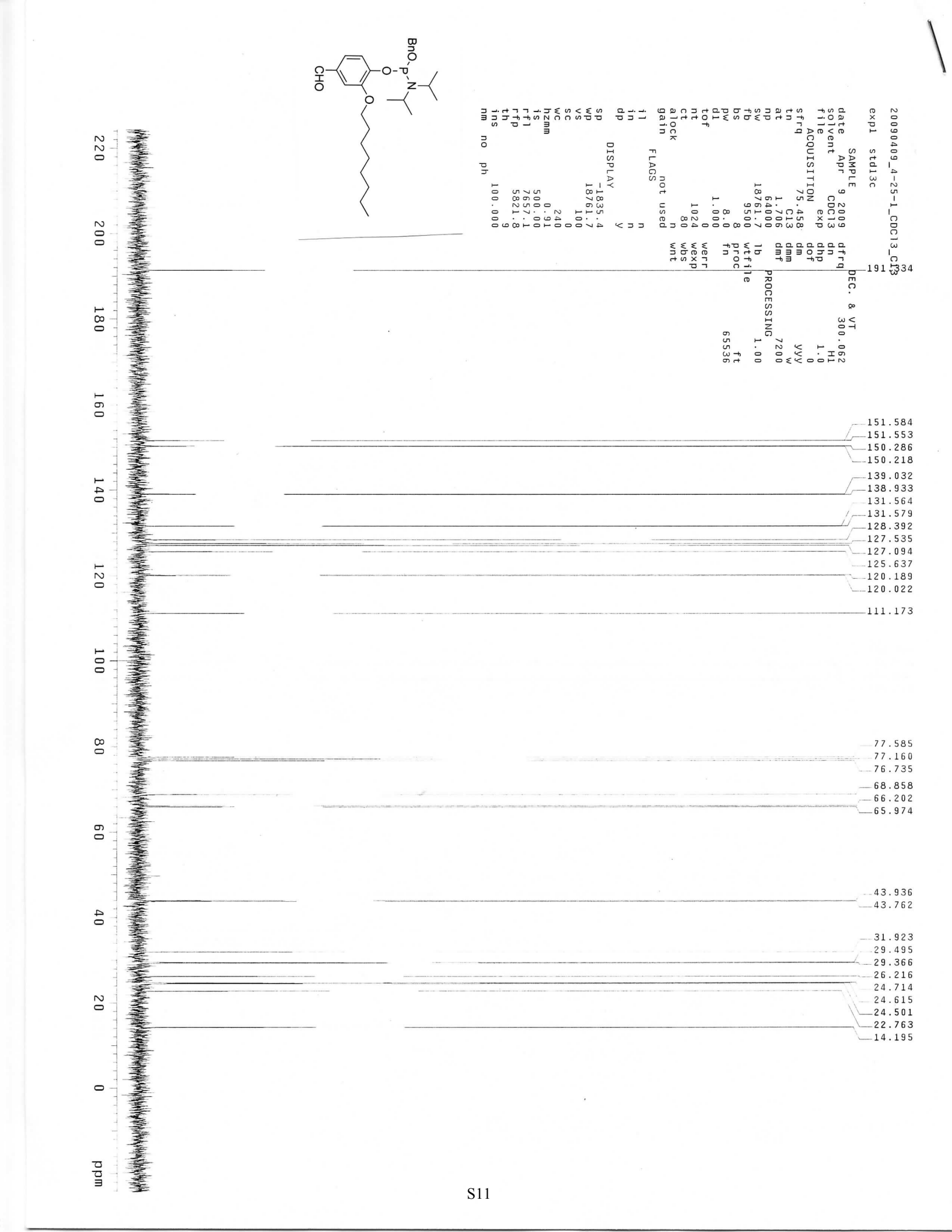
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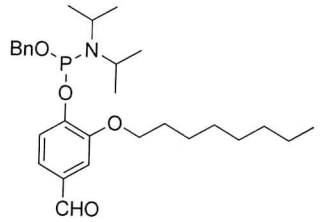

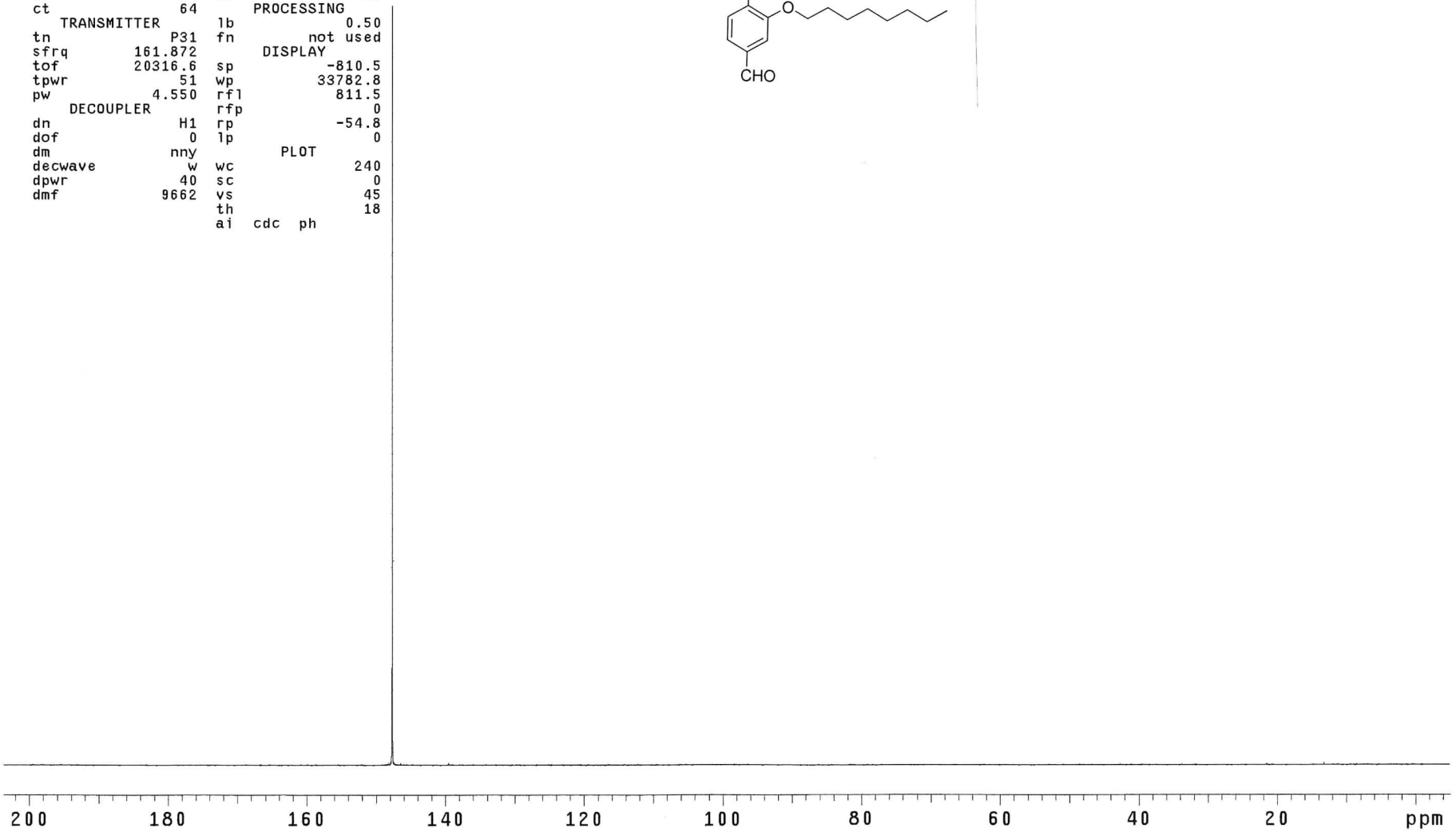

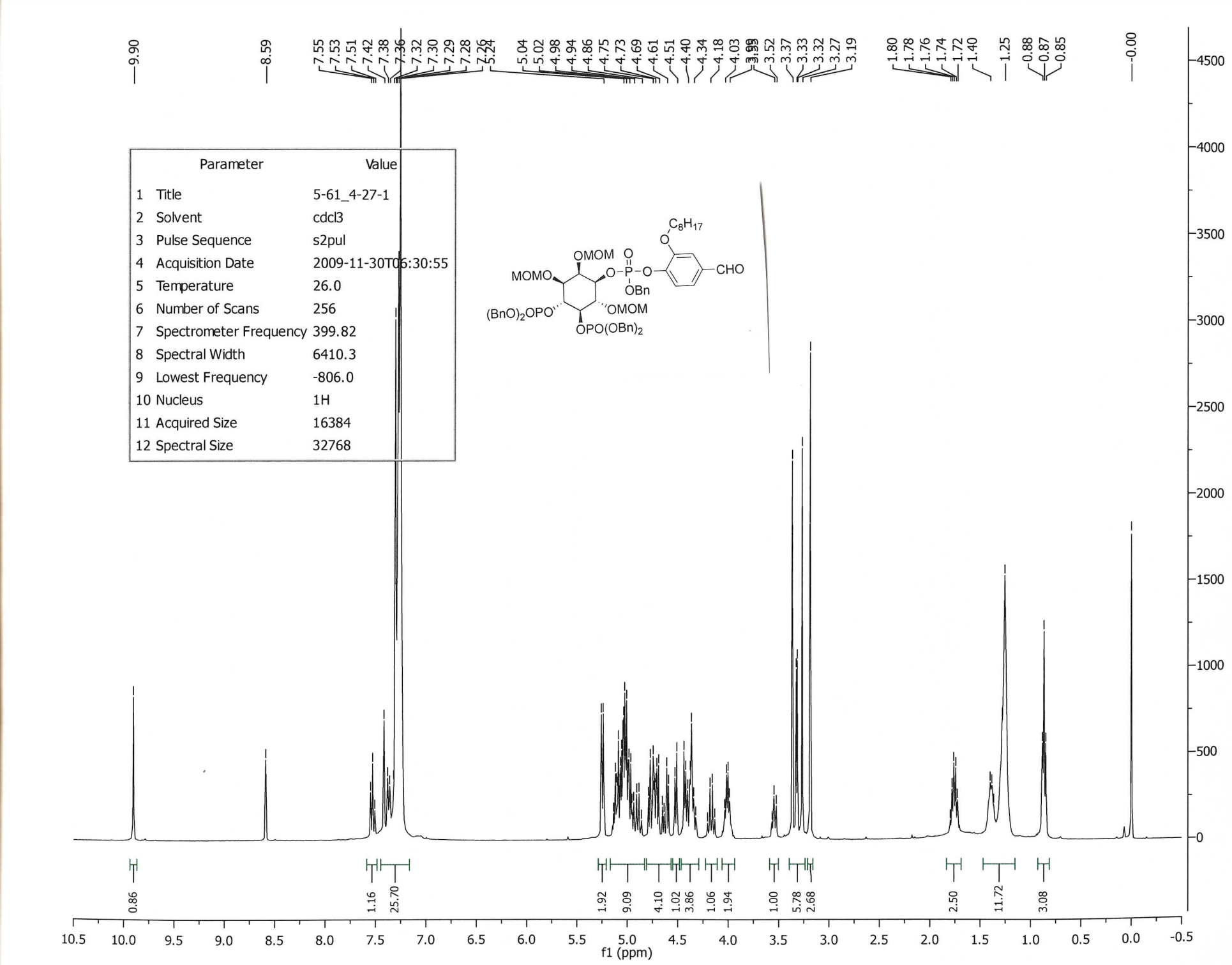
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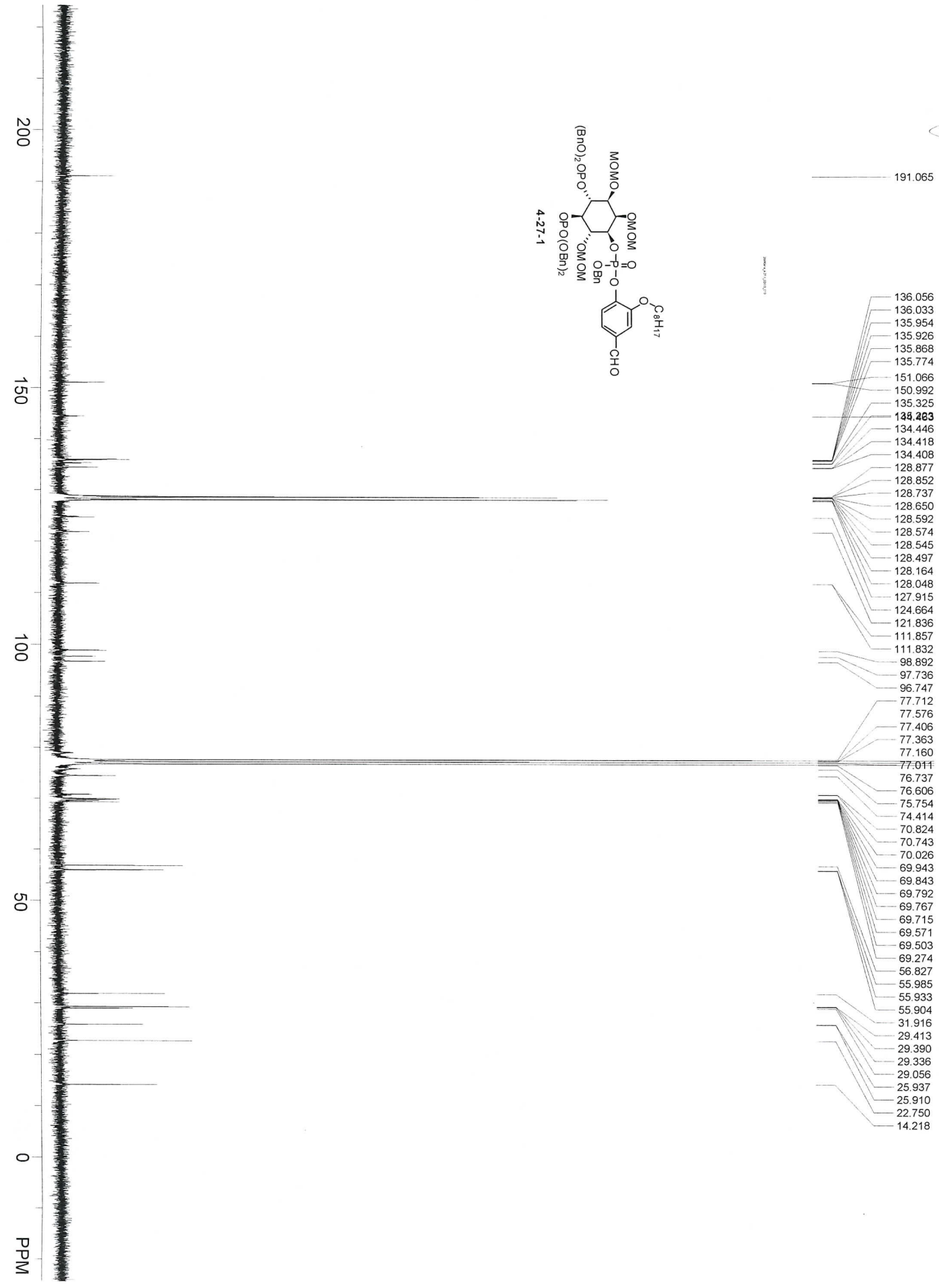
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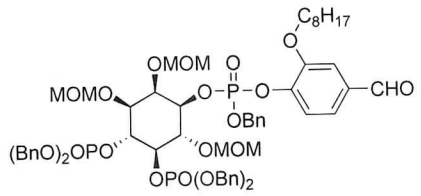
151.066
150.992
135.325
134.446
134.418
134.408
128.877
128.852
128.737
128.650
128.592
128.574
128.545
128.497
128.164
128.048
127.915
124.664
121.836
111.857
111.832
98.892
97.736
96.747
77.712
77.576
77.406
77.363
77.160
77.011
76.737
76.606
75.754
74.414
70.824
70.743
70.026
69.943
69.843
69.792
69.767
69.715
69.571
69.503
69.274
56.827
55.985
55.933
55.904
31.916
29.413
29.390
29.336
29.056
25.937
25.910
22.750
14.218
200
150
100
50
0
PPM

	Parameter	Value
1	Title	5-61_4-27-1
2	Solvent	cdcl3
3	Pulse Sequence	s2pul
4	Acquisition Date	2009-11-30T07:25:50
5	Temperature	26.0
6	Number of Scans	512
7	Spectrometer Frequency	161.86
8	Spectral Width	41666.7
9	Lowest Frequency	-8694.7
10	Nucleus	31P
11	Acquired Size	32768
12	Spectral Size	65536

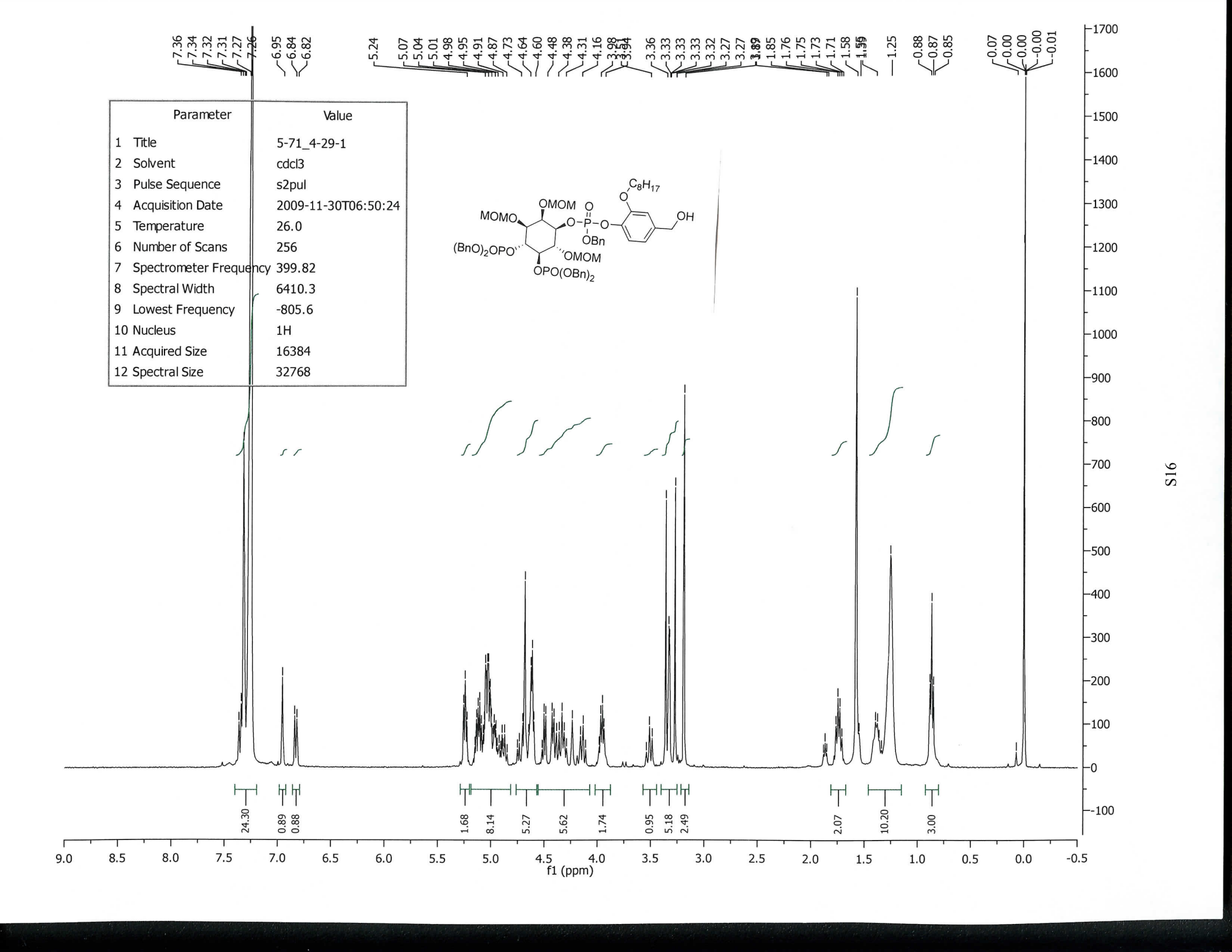


-1.42
-1.45
-7.40
-7.60

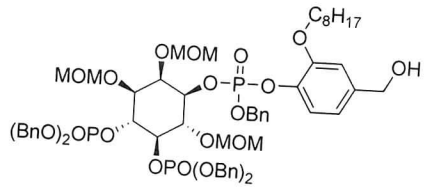
25000
20000
15000
10000
5000
0

1.99
1.00

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50
f1 (ppm)


Parameter Value
1 Title 5-71_4-29-1
2 Solvent cdcl3
3 Pulse Sequence s2pul
4 Acquisition Date 2009-11-30T06:50:24
5 Temperature 26.0
6 Number of Scans 256
7 Spectrometer Frequency 399.82
8 Spectral Width 6410.3
9 Lowest Frequency -805.6
10 Nucleus 1H
11 Acquired Size 16384
12 Spectral Size 32768
OMOM
MOMO
O
O-P-O
OBn
OH
C_8H_{17}
$(BnO)_2OPO$
OMOM
$OPO(OBn)_2$
f1 (ppm)

	Parameter	Value
1	Title	5-95_4-29-1
2	Solvent	cdcl3
3	Pulse Sequence	s2pul
4	Acquisition Date	2009-12-02T13:04:41
5	Temperature	26.0
6	Number of Scans	20000
7	Spectrometer Frequency	100.54
8	Spectral Width	25510.2
9	Lowest Frequency	-1679.7
10	Nucleus	13C
11	Acquired Size	32768
12	Spectral Size	65536

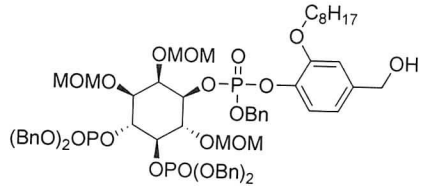


150.45
150.40
139.32
136.16
135.71
127.84
118.91
112.46
98.84
97.72
96.60
96.36
77.16
75.69
74.50
74.17
69.05
65.10
56.87
55.94
55.88
31.96
29.46
29.38
29.31
26.04
26.00
22.78
14.24
0.14

26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

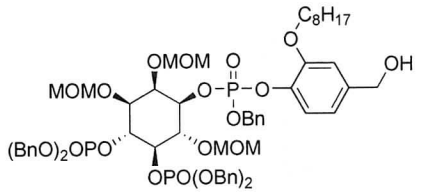
	Parameter	Value
1	Title	5-71_4-29-1
2	Solvent	cdcl3
3	Pulse Sequence	s2pul
4	Acquisition Date	2009-11-30T07:06:52
5	Temperature	26.0
6	Number of Scans	512
7	Spectrometer Frequency	161.86
8	Spectral Width	41666.7
9	Lowest Frequency	-8694.7
10	Nucleus	31P
11	Acquired Size	32768
12	Spectral Size	65536



4-29-1 CDCl3 P31 at -17

exp9 s2pul

SAMPLE		DEC. & VT	
date	Aug 3 2010	dfrq	499.749
solvent	CDCl3	dn	H1
file	/export/home/~	dpwr	35
	qszhang/vnmrsys/da~	dof	49.2
	ta/WGHUANG/20100803~	dm	yyy
	3_4-29-1_P31_minus1~	dmm	w
	17.fid	dmf	8742
ACQUISITION		dseq	
sfrq	202.311	dres	1.0
tn	P31	homo	n
at	1.600	temp	-17.0
np	194236	DEC2	
sw	60698.0	dfrq2	0
fb	33000	dn2	
bs	16	dpwr2	1
tpwr	63	dof2	0
pw	15.0	dm2	n
d1	0	dmm2	c
tof	15148.5	dmf2	9900
nt	256	dseq2	
ct	256	dres2	1.0
alock	n	homo2	n
gain	60	DEC3	
FLAGS		dfrq3	0
il	n	dn3	
in	n	dpwr3	1
dp	y	dof3	0
hs	ny	dm3	n
DISPLAY		dmm3	c
sp	-1260.5	dmf3	9900
wp	1474.5	dseq3	
vs	40	dres3	1.0
sc	0	homo3	n
wc	250	PROCESSING	
hzmm	5.90	lb	5.00
is	28.57	wtfile	
rfl	20233.8	proc	ft
rfp	0	fn	not used
th	5	math	f
ins	1.000		
nm cdc ph		werr	
		wexp	
		wbs	
		wnt	



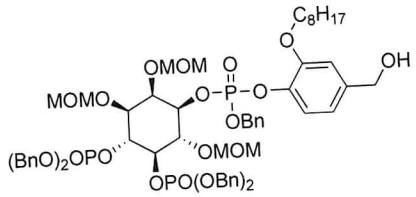
7

^{31}P NMR at -17 °C

4-29-1_CDCl3_P31 at 25

exp9 s2pul

SAMPLE		DEC. & VT	
date	Aug 3 2010	dfrq	499.749
solvent	CDCl3	dn	H1
file	/export/home/~	dpwr	35
	qszhang/vnmrsys/da~	dof	49.2
	ta/WGHUANG/2010080~	dm	yyy
	3_4-29-1_P31_25.fi~	dmm	w
	d	dmf	8742
ACQUISITION		dseq	
sfrq	202.311	dres	1.0
tn	P31	homo	n
at	1.600	temp	25.0
np	194236	DEC2	
sw	60698.0	dfrq2	0
fb	33000	dn2	
bs	16	dpwr2	1
tpwr	63	dof2	0
pw	15.0	dm2	n
d1	0	dmm2	c
tof	15148.5	dmf2	9900
nt	128	dseq2	
ct	128	dres2	1.0
alock	n	homo2	n
gain	60	DEC3	
FLAGS		dfrq3	0
il	n	dn3	
in	n	dpwr3	1
dp	y	dof3	0
hs	ny	dm3	n
DISPLAY		dmm3	c
sp	-1649.5	dmf3	9900
wp	2153.4	dseq3	
vs	33	dres3	1.0
sc	0	homo3	n
wc	250	PROCESSING	
hzmm	8.61	lb	5.00
is	28.57	wtfile	
rfl	20233.8	proc	ft
rfp	0	fn	not used
th	5	math	f
ins	1.000		
nm cdc ph		werr	
		wexp	
		wbs	
		wnt	



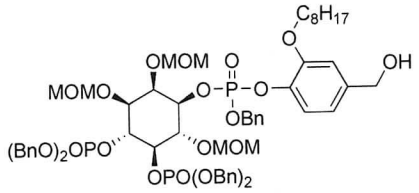
7

^{31}P NMR at 25 °C

4-29-1 CDCl3 P31 at 50

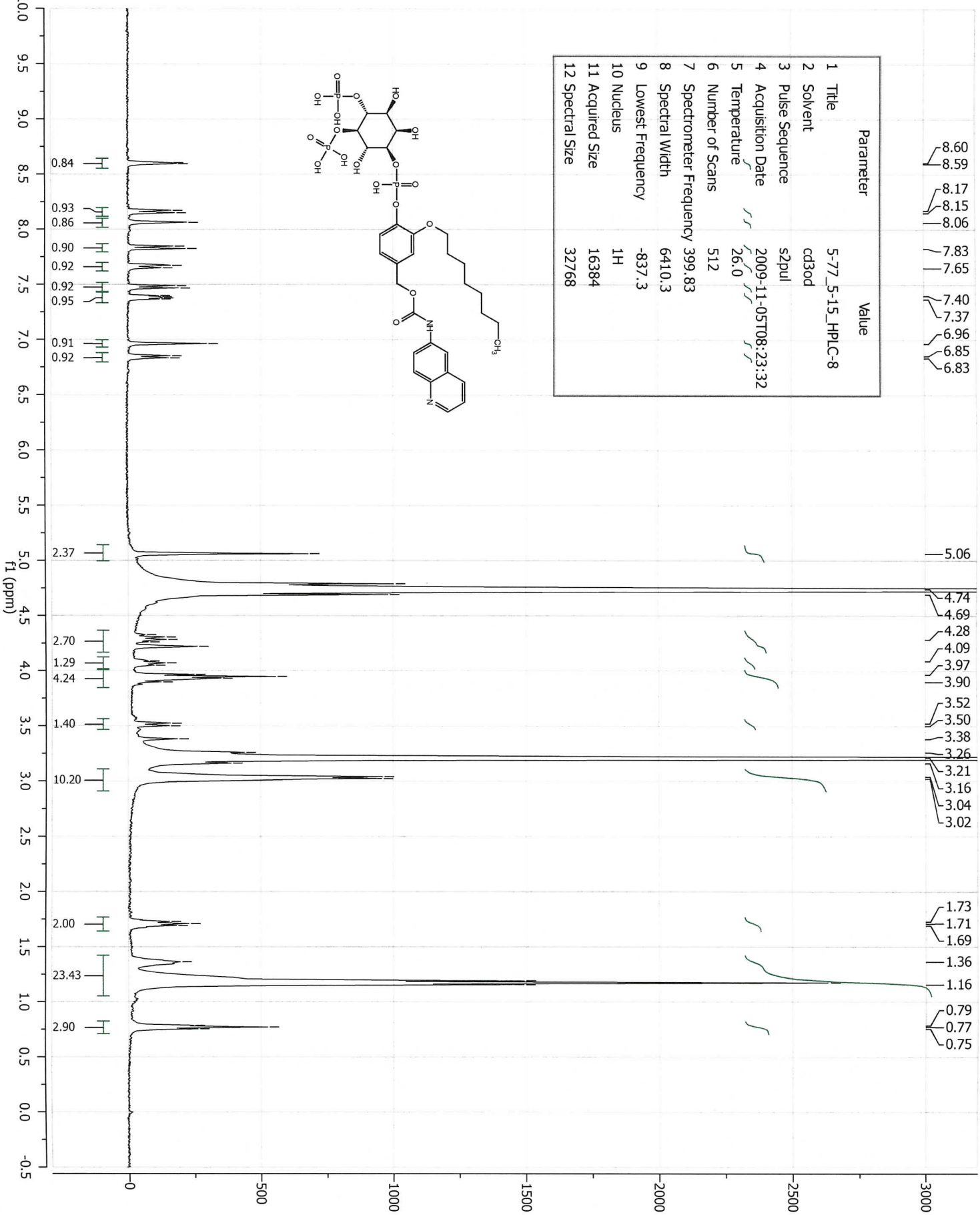
exp9 s2pul

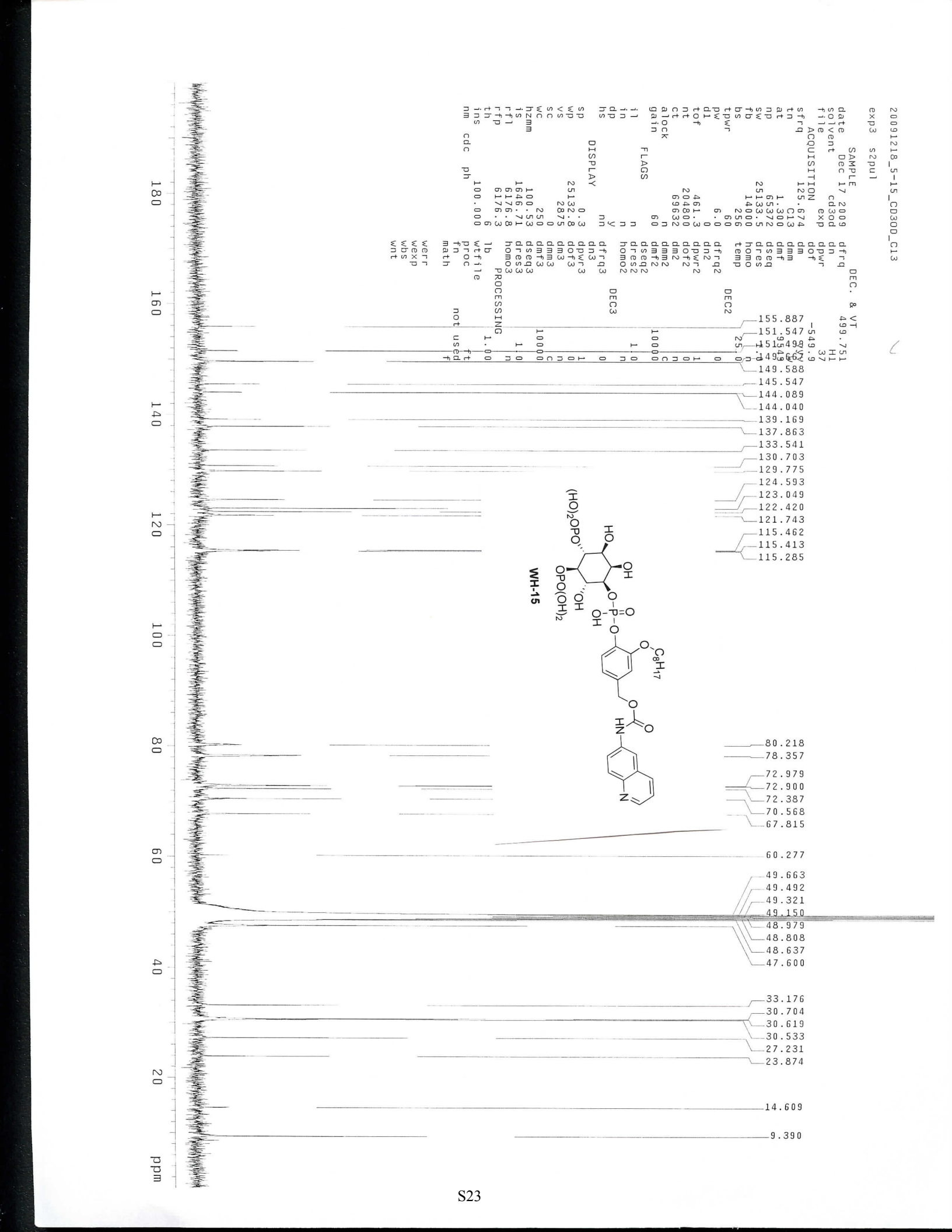
SAMPLE		DEC. & VT	
date	Aug 3 2010	dfrq	499.749
solvent	CDCl3	dn	H1
file	/export/home/~	dpwr	35
	qszhang/vnmrsys/da~	dof	49.2
	ta/WGHUANG/2010080~	dm	yyy
	3_4-29-1_P31_50.fi~	dmm	w
	d	dmf	8742
ACQUISITION		dseq	
sfrq	202.311	dres	1.0
tn	P31	homo	n
at	1.600	temp	50.0
np	194236	DEC2	
sw	60698.0	dfrq2	0
fb	33000	dn2	
bs	16	dpwr2	1
tpwr	63	dof2	0
pw	15.0	dm2	n
d1	0	dmm2	c
tof	15148.5	dmf2	9900
nt	256	dseq2	
ct	256	dres2	1.0
alock	n	homo2	n
gain	60	DEC3	
FLAGS		dfrq3	0
il	n	dn3	
in	n	dpwr3	1
dp	y	dof3	0
hs	ny	dm3	n
DISPLAY		dmm3	c
sp	-1729.2	dmf3	9900
wp	2312.7	dseq3	
vs	34	dres3	1.0
sc	0	homo3	n
wc	250	PROCESSING	
hzmm	9.25	lb	5.00
is	28.57	wtfile	
rfl	20233.8	proc	ft
rfp	0	fn	not used
th	6	math	f
ins	1.000		
nm cdc ph		werr	
		wexp	
		wbs	
		wnt	



7

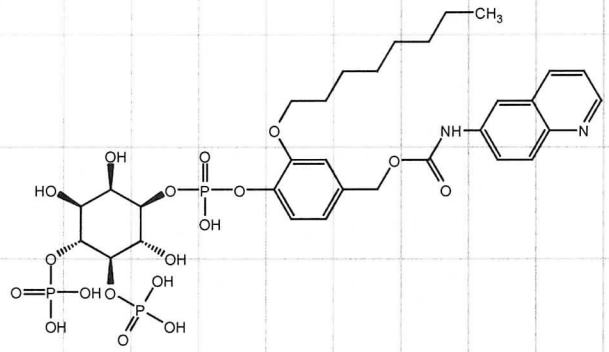
^{31}P NMR at 50 °C


Parameter Value
1 Title 5-77_5-15_HPLC-8
2 Solvent cd3od
3 Pulse Sequence s2pul
4 Acquisition Date 2009-11-05T08:23:32
5 Temperature 26.0
6 Number of Scans 512
7 Spectrometer Frequency 399.83
8 Spectral Width 6410.3
9 Lowest Frequency -837.3
10 Nucleus 1H
11 Acquired Size 16384
12 Spectral Size 32768
f1 (ppm)
0.84
0.93
0.86
0.90
0.92
0.92
0.95
0.91
0.92
2.37
2.70
1.29
4.24
1.40
10.20
2.00
23.43
2.90
8.60
8.59
8.17
8.15
8.06
7.83
7.65
7.40
7.37
6.96
6.85
6.83
5.06
4.74
4.69
4.28
4.09
3.97
3.90
3.52
3.50
3.38
3.26
3.21
3.16
3.04
3.02
1.73
1.71
1.69
1.36
1.16
0.79
0.77
0.75


20091218_5-15_CD3OD_C13
exp3 s2pul
SAMPLE
date Dec 17 2009
solvent cd3od
file exp
ACQUISITION
sfrq 125.674
tn C13
at 1.300
np 65372
sw 25133.5
fb 14000
bs 256
tpwr 60
pw 6.0
d1 0
tof 461.3
nt 204800
ct 69632
alock n
gain 60
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp 0.3
wp 25132.8
vs 2875
sc 0
wc 250
hzmm 100.53
is 1646.71
rfl 6176.8
rfp 6176.3
th 6
ins 100.000
nm cdc ph
DEC. & VT
dfrq 499.751
dn H1
dpwr 37
dof -549.9
dm nny
dmm c
dmf 25.0
dfrq2 0
dn2
dpwr2 1
dof2 0
dm2 n
dmm2 c
dmf2 10000
dseq2
dres2 1.0
homo2 n
DEC3
dfrq3 0
dn3
dpwr3 1
dof3 0
dm3 n
dmm3 c
dmf3 10000
dseq3
dres3 1.0
homo3 n
PROCESSING
lb 1.00
wtfile
proc ft
fn
math not used
werr f
wexp
wbs
wnt
155.887
151.547
151.495
149.588
145.547
144.089
144.040
139.169
137.863
133.541
130.703
129.775
124.593
123.049
122.420
121.743
115.462
115.413
115.285
80.218
78.357
72.979
72.900
72.387
70.568
67.815
60.277
49.663
49.492
49.321
49.150
48.979
48.808
48.637
47.600
33.176
30.704
30.619
30.533
27.231
23.874
14.609
9.390
WH-15
ppm

	Parameter	Value
1	Title	5-77_5-15_HPLC-8
2	Solvent	cd3od
3	Pulse Sequence	s2pul
4	Acquisition Date	2009-11-05T09:49:24
5	Temperature	26.0
6	Number of Scans	1024
7	Spectrometer Frequency	161.86
8	Spectral Width	41666.7
9	Lowest Frequency	-8694.4
10	Nucleus	31P
11	Acquired Size	32768
12	Spectral Size	65536

^{31}P NMR (162 MHz, cd_3od) δ 2.53, 1.76, -4.74.



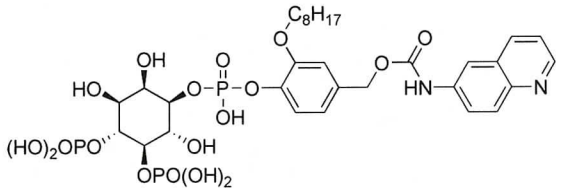
5-15_Cosy

Sample Name:
5-15_Cosy
Data Collected on:
GMB400-vnmrs400
Archive directory:
/home/UNC/vnmrsys/data/wghuang
Sample directory:
05_986
FidFile: 05_986_gCOSY_001

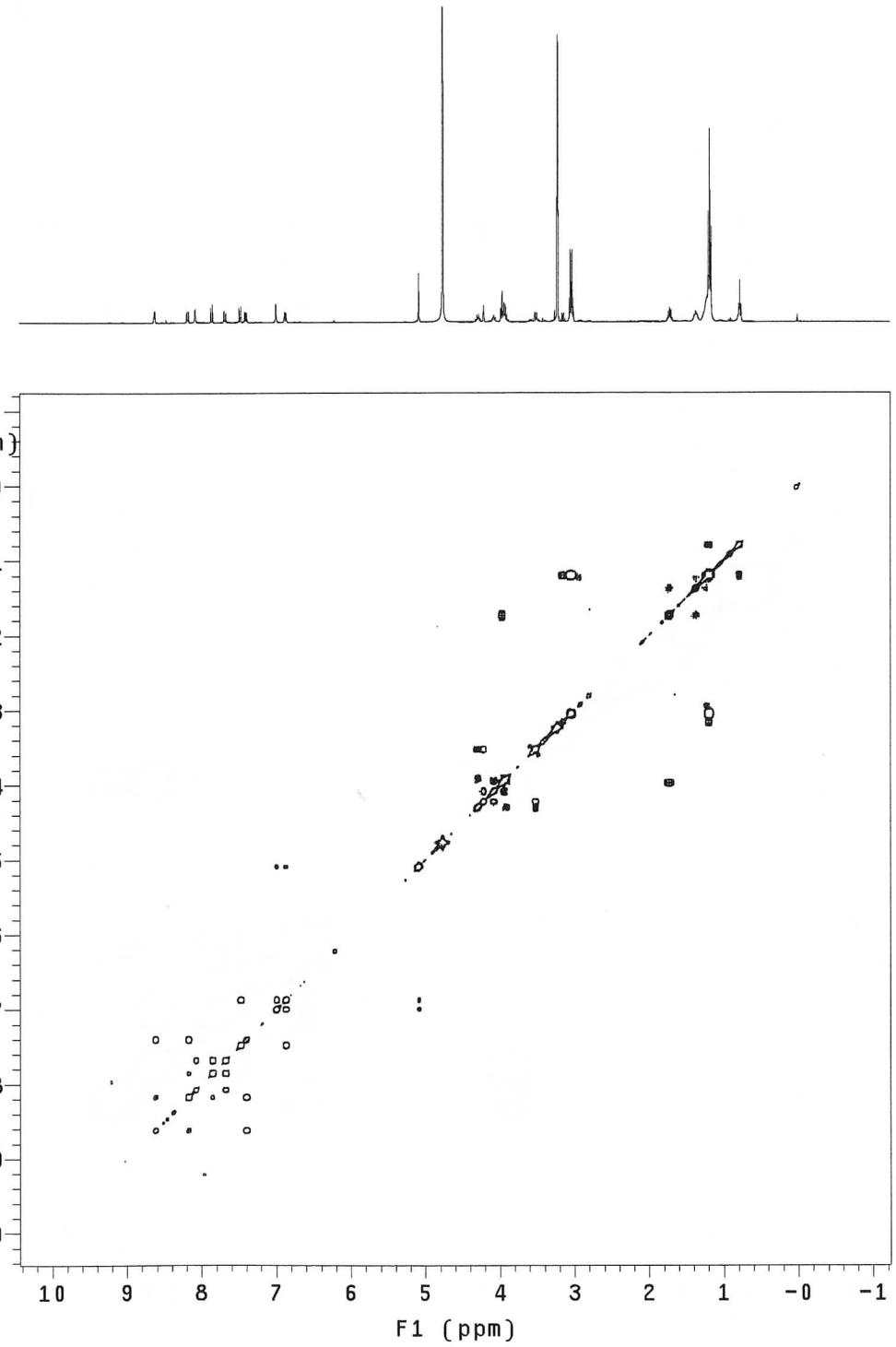
Pulse Sequence: gCOSY
Solvent: cd3od
Data collected on: Dec 20 2009

Temp. 26.0 C / 299.1 K
Sample #13, Operator: wghuang

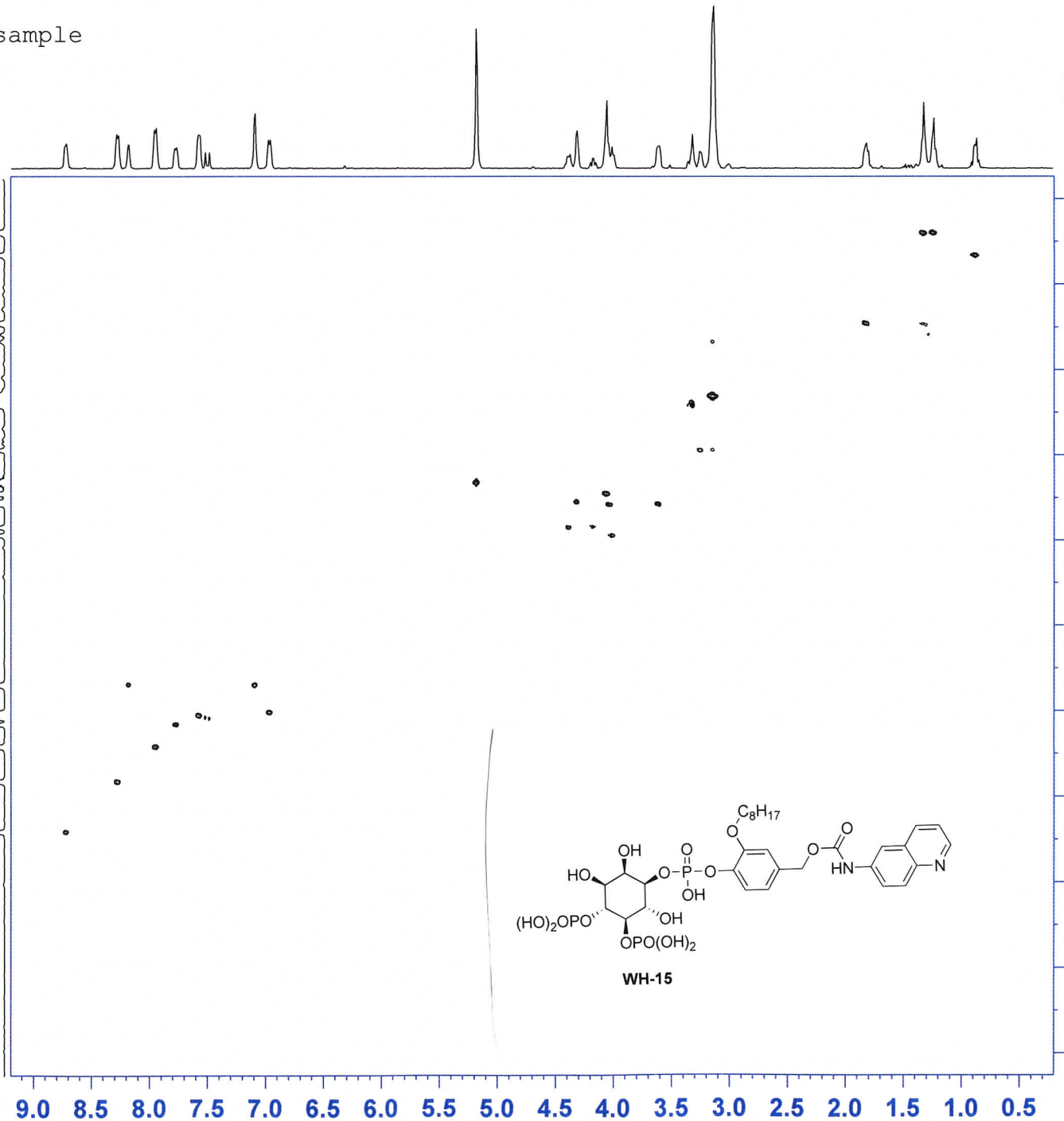
Relax. delay 1.000 sec
Acq. time 0.150 sec
Width 4664.2 Hz
2D Width 4664.2 Hz
Single scan
512 increments
OBSERVE H1, 399.8173940 MHz
DATA PROCESSING
Sq. sine bell 0.075 sec
F1 DATA PROCESSING
Sq. sine bell 0.080 sec
FT size 4096 x 4096
Total time 11 min



WH-15



weigang sample
gHMQC



WH-15

```
Current Data Parameters
NAME                  weigang-bbi
EXPNO                           8
PROCNO                          1
                                rs
Date_                    20100126
Time_                        21.51
INSTRUM                      spect
PROBHD    5 mm PABBI 1H/
PULPROG                  hmqcgpqf
TD                           2048
SOLVENT                      MeOD
NS                             40
DS                             16
SWH                    4496.403 Hz
FIDRES                 2.195509 Hz
AQ                     0.2278988 sec
RG                         13004
DW                      111.200 usec
DE                        6.00 usec
TE                       293.5 K
CNST2                145.0000000
d0                     0.00000300 sec
D1                     1.50000000 sec
d2                     0.00344828 sec
d12                    0.00002000 sec
d13                    0.00000400 sec
D16                    0.00010000 sec
DELTA1                 0.00232428 sec
IN0                    0.00001890 sec

======== CHANNEL f1 ========
NUC1                          1H
P1                         7.80 usec
p2                        15.60 usec
PL1                        0.00 dB
SFO1                500.1323506 MHz

======== CHANNEL f2 ========
CPDPRG2                    garp
NUC2                        13C
P3                        14.00 usec
PCPD2                     70.00 usec
PL2                        0.00 dB
PL12                      13.98 dB
SFO2                125.7703604 MHz

====== GRADIENT CHANNEL =====
GPNAM1                SINE.100
GPNAM2                SINE.100
GPNAM3                SINE.100
GPZ1                      50.00 %
GPZ2                      30.00 %
GPZ3                      40.10 %
P16                     1000.00 usec

F1 - Acquisition parameters
ND0                            2
TD                           256
SFO1                125.7704 MHz
FIDRES              103.339951 Hz
SW                     210.344 ppm
FnMODE                       QF

F2 - Processing parameters
SI                           1024
SF                  500.1300000 MHz
WDW                        SINE
SSB                           0
LB                         0.00 Hz
GB                            0
PC                         1.40

F1 - Processing parameters
SI                           1024
MC2                          QF
SF                  125.7577900 MHz
WDW                        SINE
SSB                           0
LB                         0.00 Hz
GB                            0
```