

SUPPORTING INFORMATION

A Versatile Photoactivatable Probe Designed to Label the Diphosphate Binding Site of Farnesyl Diphosphate Utilizing Enzymes

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¹H NMR of Compound **6a**

¹H NMR of Compound **7a**

³¹P NMR of Compound **7a**

¹H NMR of Compound **7b**

³¹P NMR of Compound **7b**

¹H NMR of Compound **8a**

³¹P NMR of Compound **8a**

¹H NMR of Compound **8b**

³¹P NMR of Compound **8b**

¹H NMR of Compound **4a**

³¹P NMR of Compound **4a**

HPLC of Compound **4a**

HR ESI MS of Compound **4a**

¹H NMR of Compound **4b**

³¹P NMR of Compound **4b**

HPLC of Compound **4b**

HR ESI MS of Compound **4b**

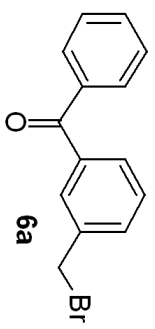
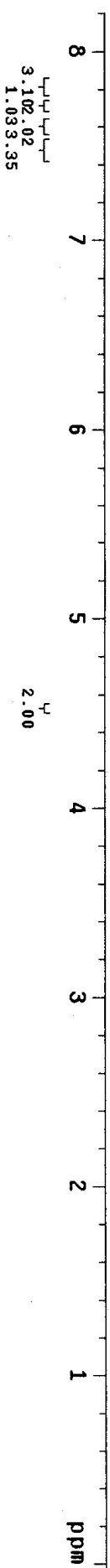
Stereo Pictures of **4b** Bound to FPP-Utilizing Enzymes

Table of Residues within 4 Å of Distal Phosphate (FPP) or Phosphonate (**4b**)

Oh-3-49
 University of Minnesota
 Department of Chemistry
 VAC-300

exp1 std1h
 SAMPLE 7 2008
 date NOV
 solvent CDCl3
 file /O1d/W9/mddon~
 h/081107V3_3202
 ACQUISITION
 strq 299.956
 tn H1
 at 2.000
 mp 23996
 sw 5998.8
 fb 3400
 bs 16
 tpwr 63
 pw 5.3
 dl 1.500
 tof 1318.0
 gain 16
 alock Y
 flags not used
 11 n
 1n n
 dp y
 hs nn
 SP DISPLAY -13.8
 WP 2474.5
 VS 55
 SC 0
 WC 225
 hzmm 11.00
 IS 855.73
 rfi 599.8
 th 0
 ins 6
 al cdc ph 2.000

7.84
 7.838
 7.832
 7.814
 7.810
 7.805
 7.794
 7.787
 7.782
 7.732
 7.727
 7.722
 7.706
 7.702
 7.697
 7.628
 7.624
 7.608
 7.606
 7.599
 7.579
 7.575
 7.511
 7.507
 7.498
 7.490
 7.486
 7.480
 7.462
 7.459
 7.454
 7.429
 5.274
 4.525



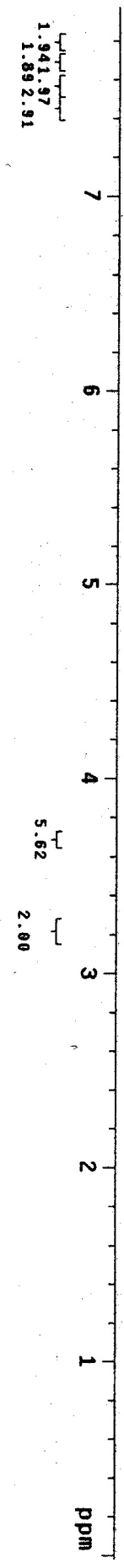
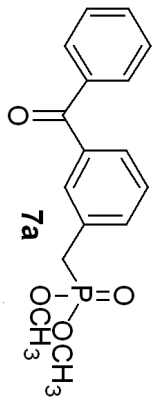
oh-benzo-meta-
University of
Department of
VAC-300

expt std/h

SAMPLE
date Nov 14 20
solvent CDC
file /net/vacc300
xport/home/data/
donh/061114v3_44
ACQUISITION
sfrq 299.9
in 11
at 2.0
mp 239
sw 5998
fb 34
bs 6
tpwr 513
pw 1.500
di 1316.0
tof 16
nt 16
ct 16
atlock Y
gain not used
FLAGS
l1 n
l2 n
l3 n
l4 y
hs nm
SP -5.0
WD 2395.8
VS 46
SC 0
WC 225
hzmm 10.65
ls 79.83
rfl 599.8
th 0
ins 3
ai cdc ph 2.000

DEC 8 VI
299.954
0.10
1.0
200
ft
65536
7
react
procp1ot
werr
wexp
wds
wnt

7.999
7.978
7.974
7.971
7.967
7.965
7.966
7.970
7.964
7.966
7.982
7.975
7.962
7.958
7.953
7.947
7.934
7.928
7.926
7.924
7.922
7.498
7.496
7.491
7.475
7.470
7.465
7.446
7.443
7.431
7.406
3.738
3.709
3.701
3.678
3.673
3.252
3.180

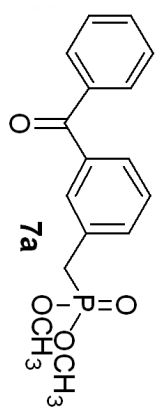


oh-benzo-meta-methyl-ester
 University of Minnesota
 Department of Chemistry
 VAC-300

28.656

exptl s2put1

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 date Nov 14 2008 dfreq H1
 solvent CDC13 dn
 file /net/vac300/e~ dpwr 40
 xport/home/data/md~ dof 0
 donh/081114v3 4404 dm nvy
 ACQUISITION dmw v
 sfrq 121.432 dmw 7233
 tn P31 dsrq
 at 0.880 dres 1.0
 np 68012 hnmw PROCESSING n
 sw 42508.0 lb hnmw 1.50
 fb 23409 1b wfile
 bs 68 pproc
 tpwr 26.2 fn 131072 ft
 pw 1.000 math
 d1 1.908 wefr react
 d2 9017.7 wefr procpilot
 tof .32 wos
 nt 32 wnt
 ct gain not used
 alock n
 flags n
 fl n
 in n
 dp y
 hs yn
 DISPLAY
 sp -6012.7
 wp 12144.8
 vs 22
 sc 0
 wc 225
 hzmm 53.98
 is 1621.74
 rfi 10069.3
 rfp -2076.1
 th 70
 ins 100.000
 ai no ph

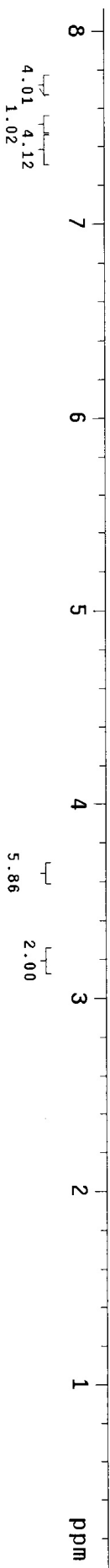
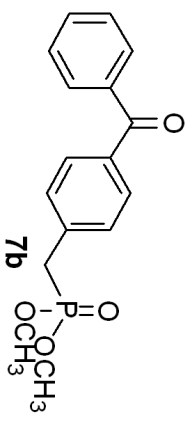


oh-3-37
 University of Minnesota
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 VAC-300

exp1 std1h

SAMPLE
 date Mar 18 2008
 solvent CDC13
 file /01d/k2/mdon~
 h/080318v3_2702
 ACQUISITION
 sfrq 299.956
 tn H1
 at 2.000
 np 23996
 sw 5998.8
 fb 3400
 bs 18
 tpwr 63
 pw 5.3
 dl 1.500
 tof 1318.0
 nt 16
 ct 16
 alock Y
 gain not used
 flags not used
 i1 n
 in n
 dp Y
 hs nm
 DISPLAY
 sp 12.4
 wp 2422.0
 vs 87
 sc 0
 wc 225
 hzmm 10.76
 is 826.17
 rfl 599.8
 rfp 0
 th 4
 ins 4
 ai cdc ph 2.000

Chemical Shift (ppm)	Integration	Phase	Waltz	React	Procplot
7.344	0.10	f			
7.352	0.10	f			
7.359	0.10	f			
7.372	0.10	f			
7.381	0.10	f			
7.384	0.10	f			
7.389	0.10	f			
7.402	0.10	f			
7.408	0.10	f			
7.412	0.10	f			
7.429	0.10	f			
7.434	0.10	f			
7.435	0.10	f			
7.489	0.10	f			
7.493	0.10	f			
7.498	0.10	f			
7.518	0.10	f			
7.542	0.10	f			
7.694	0.10	f			
7.696	0.10	f			
7.704	0.10	f			
7.709	0.10	f			
7.716	0.10	f			
7.722	0.10	f			
7.725	0.10	f			
7.732	0.10	f			
7.736	0.10	f			
3.666	1.0	n			
3.630	1.0	n			
3.623	1.0	n			
3.230	1.0	n			
3.156	1.0	n			



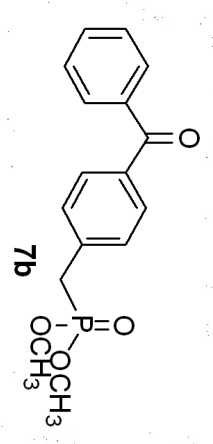
oh-3-37
 University of Minnesota
 Department of Chemistry
 VAC-500

expt 52901

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 date Mar 15 2008
 solvent CDCl3
 file /014/42/...
 h/08033443...
 ACQUISITION
 sfrq 121.492
 tn 431
 nt 4.898
 np 88812
 sv 42505.0
 fb 29480
 be 18
 lgvr 59
 pw 28.2
 d1 1.000
 d2 1.390
 kof 817.7
 nt 32
 ct 32
 atock n
 gain not used
 flags n
 11 n
 1n n
 4p y
 hs y/n
 DISPLAY
 sd -6196.6
 wd 12062.5
 vs 7
 sc 0
 wc 225
 hzmm 53.70
 15 1547.79
 rf1 10064.3
 rfb -2078.1
 ch 48
 line 100.000
 al no ph

PROCCESSING 1.59
 react
 plot

28.285



oh-meta-phosphonate
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expi stdlh

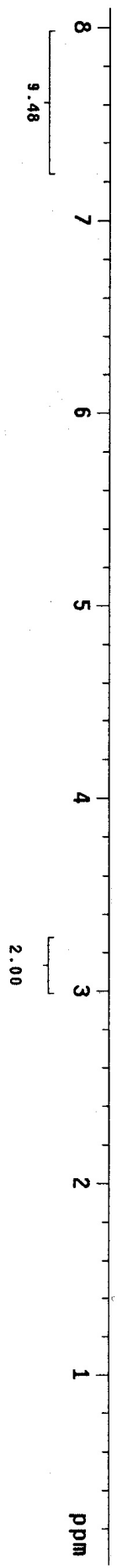
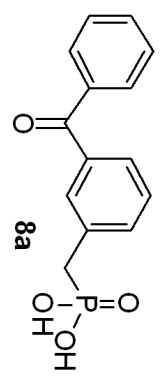
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 solvent GD30D
 file /net/vac300/er
 xport/home/data/md-
 donh/081215V3_1603
 ACQUISITION
 sfreq 299.957
 tn H1
 at 2.000
 np 23396
 sw 5998.8
 fb 3400
 bs 16
 tpwr 63
 pw 5.3
 dl 1.500
 tof 1318.0
 nt 16
 ct Wexp
 atlock Y
 gain not used
 wnt

DEC 8 VT 299.956
 H1
 30
 0
 nmh
 C
 200
 n
 1.0
 hmo
 0.10
 wf file
 proc 65536
 fn f
 math
 react
 procpilot

11 n
 in n
 dp y
 hs nm
 DISPLAY 4.7
 SP 2425.5
 WD 148
 VS 0
 SC 225
 WC 10.78
 hzmm 792.46
 is 599.8
 ffl 0
 rfp 8
 th 8
 ins cdc ph 2.000

5.348

3.212
 3.140



oh-meta-phosphonate
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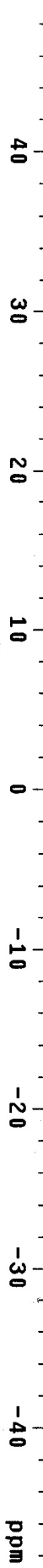
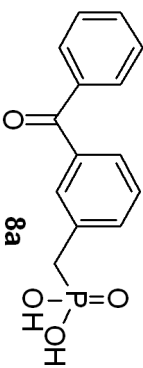
24.423

expt1 s2pu1

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solvent	CD3OD	dn	H1	
file	/net/vac300/er	dpwr	40	
xport	/home/data/md~	dof	0	
donh	/081215V3_1604	dm	nyy	
ACQUISITION		dmf	y	
sfrq	121.433	dmf	7233	
ln	P31	dseq		
at	0.800	dres	1.0	
np	68012	homo	n	
sw	42508.0	PROCESSING		
fb	23400	lb	1.50	
bs	16	wfitle		
lpwr	60	proc		
pw	26.2	fn	131072	
d1	1.000	meth	f	
d2	1.900	werf	react	
tof	9017.7	wexp	procplot	
nt	32	wbs		
ct	32	wnt		
atlock	n			
gain	not used			
flags				
l1	n			
l2	n			
l3	y			
hs	yn			

DISPLAY

SP	-5950.5
WP	11897.0
VS	59
SC	0
WC	225
h2mm	52.88
IS	2227.45
FFI	10069.3
FFP	-2078.1
th	2
ims	100.000
ai	no
ph	



Oh-1-98
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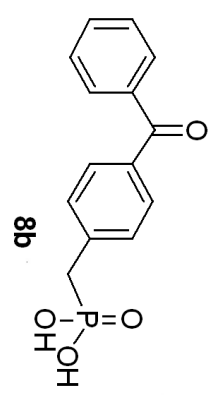
exptl statth

SAMPLE
 date Apr 29 2005
 solvent CDC13
 file /old/j4/madon-
 h/050429V3_0302
 ACQUISITION
 sfrq 299.956
 in H1
 at 2.000
 np 23996
 sw 5998.8
 fb 3400
 bs 16
 tpwr 63
 pw 19.7
 di 1.500
 tof 1318.0
 nt 16
 ct 16
 atlock Y
 gain not used
 flags Y
 11 n
 in n
 dp Y
 hs nm
 DISPLAY
 sp 38.8
 wp 2986.7
 vs 0
 sc 0
 wc 225
 hzmm 10.61
 is 1031.41
 rff1 599.8
 th 0
 ins 10
 ai cdc ph 2.000

4.99
 7.612
 7.529
 7.409
 7.383
 7.314
 7.291
 DEC & VT
 299.954
 H1
 30
 0
 mm
 C
 200
 1.0
 n
 hom
 PROCESSING
 0.10
 1b
 wtfile
 19.7
 proc
 fn
 65536
 math
 f
 react
 procpilot
 wnt

3.681

Dioxane



4.30 2.24
 1.212.17

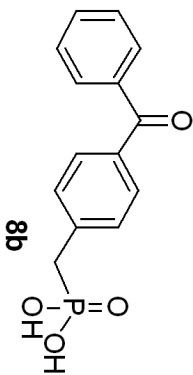
2.00

expt1 s2pu1

```

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solvent    CDCl3          dn         H1
file      /O1d/j4/mddon~  dpwr      40
h/050429v3_0304          dof        0
ACQUISITION              dm         nyy
                          dmm         w
sfrq       121.432        dmf         v
tn         P31           dres      7233
at         0.800         dseq     1.0
np         68012         dres     1.0
sw         42508.0      homo     n
fb         23400        PROCESsing 1.50
bs         16          lb
tpwr       60          wtfile
pw         26.2        proc
d1         1.000       fn         131072
d2         1.900       math
tof        9017.7
nt         32         wefr
ct         32         wexp
alock      not used   wnt
gain       n
          flags
il         n
in         n
dp         y
hs         yn
          DISPLAY
sp         -5950.5
wp         12082.5
vs         7.1
sc         0
wc         225
hzzmm     53.70
is         1584.71
rfl       10069.3
rftp     -2078.1
th         4
ins       100.000
al         no ph

```



STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul

Solvent: D2O

Temp. 25.0 C / 298.1 K

Operator: Ianevich

File: OH_B

INOVA-500 "Inova5001"

Pulse 61.7 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

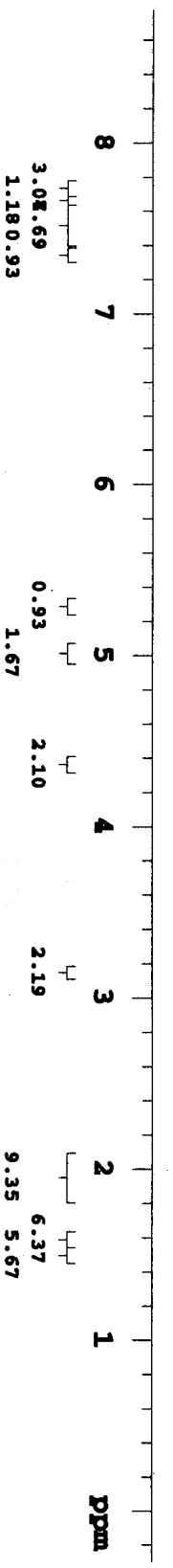
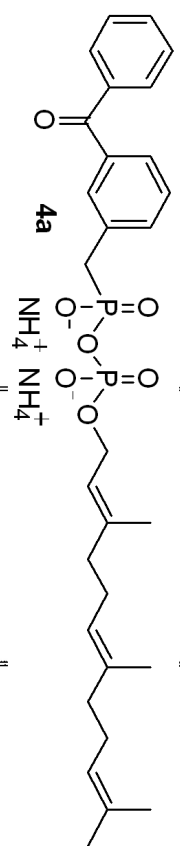
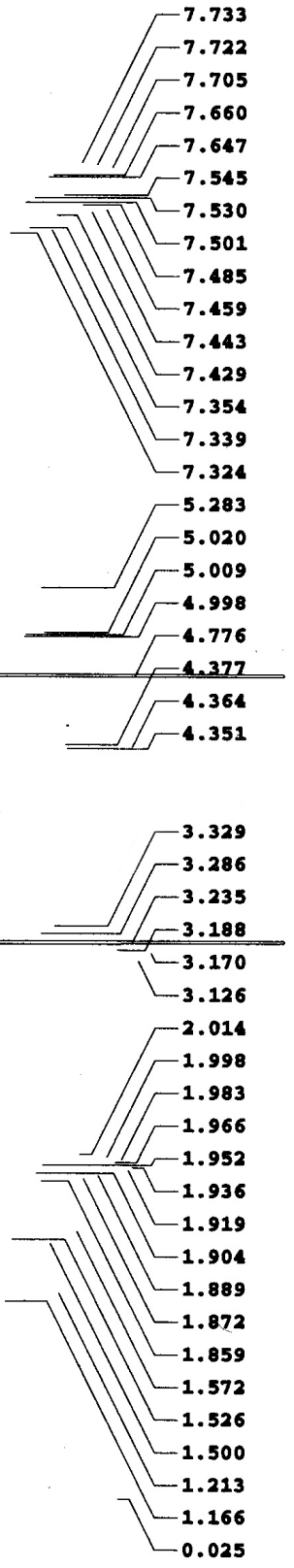
16 repetitions

OBSERVE H1, 499.7831472 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 30 sec



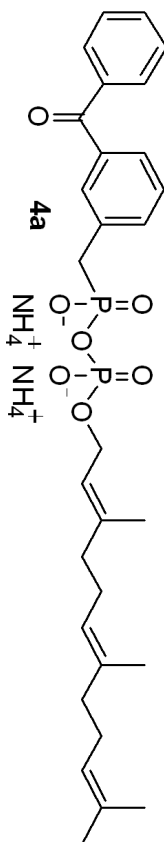
31P SENSITIVITY
0.0485 M TRIPHENYLPHOSPHATE
S/N 255/1

Pulse Sequence: s2pul

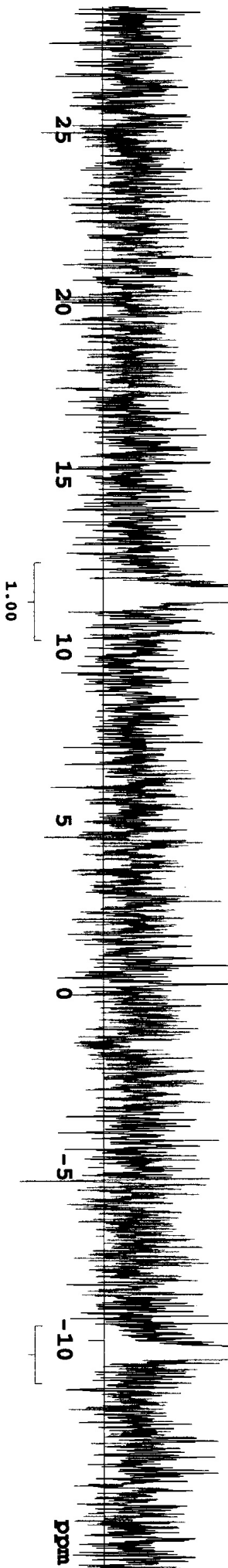
Solvent: CD3OD
Temp. 25.0 C / 298.1 K
Operator: Ienevich
File: OH_B_31P
INOVA-500 "Inova5001"

Relax. delay 2.000 sec
Pulse 90.0 degrees
Acq. time 2.000 sec
Width 20000.0 Hz
256 repetitions
OBSERVE P31, 202.3159351 MHz
DECOUPLE H1, 499.7854255 MHz
Power 44 dB
continuously on
GARP-1 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 131072
Total time 17 min, 6 sec

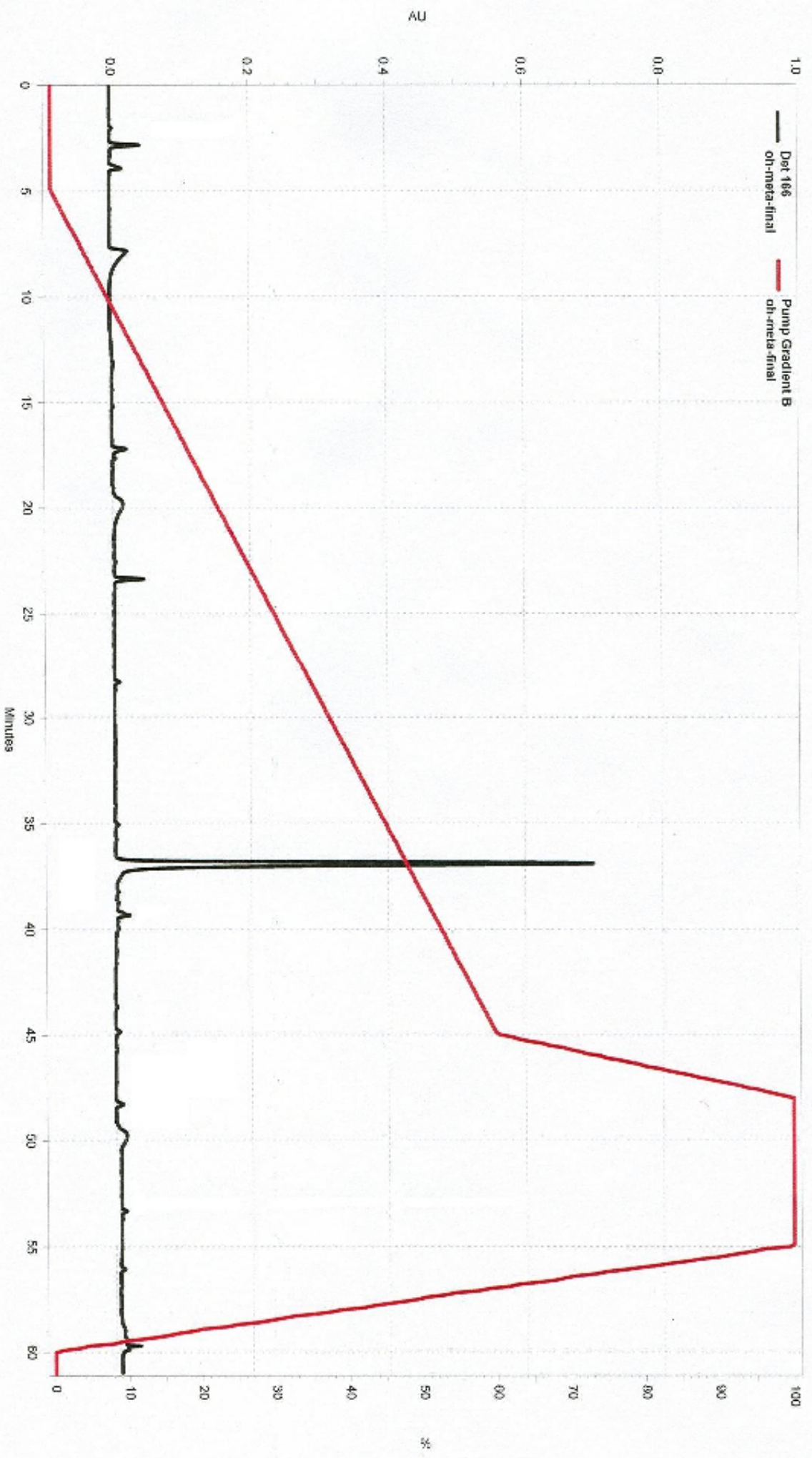
11.558



10.361



1.04



D:\32Karat\Projects\Default\2009\OH\oh-meta-final, Det 166

D:\32Karat\Projects\Default\2009\OH\oh-meta-final, Pump Gradient B

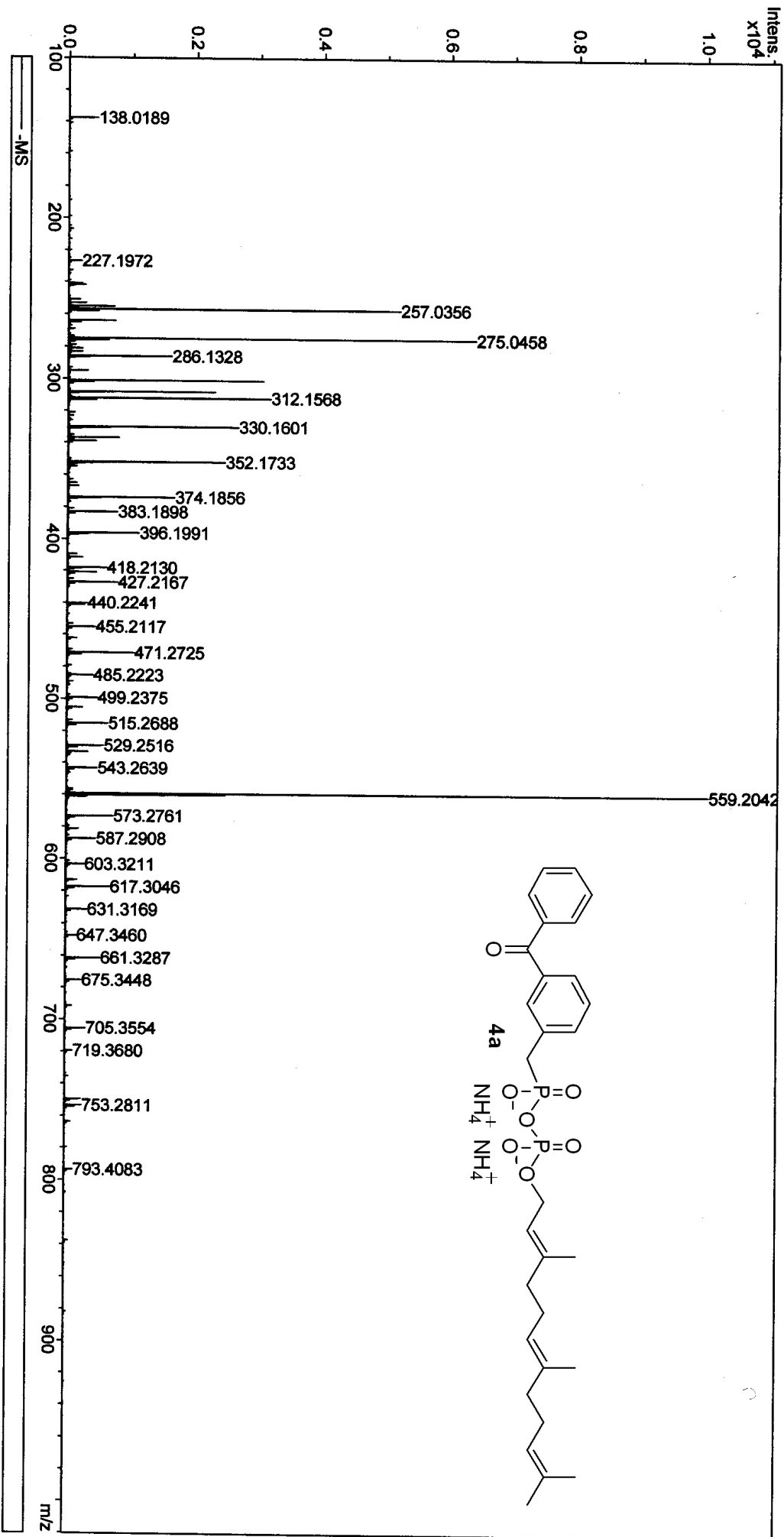
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Sample Name 71020
Comment coupling-metahr-rms

Acquisition Date 3/31/2006 3:02:24 PM
Method negative.tofpar

Operator
Instrument

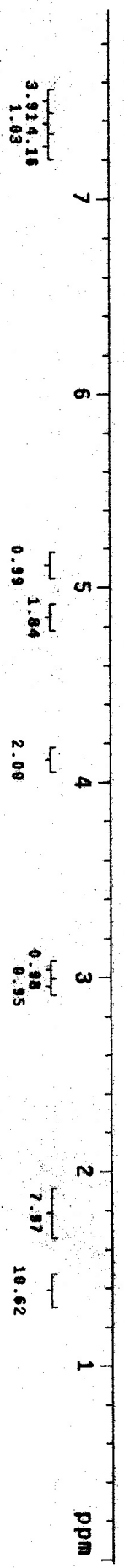
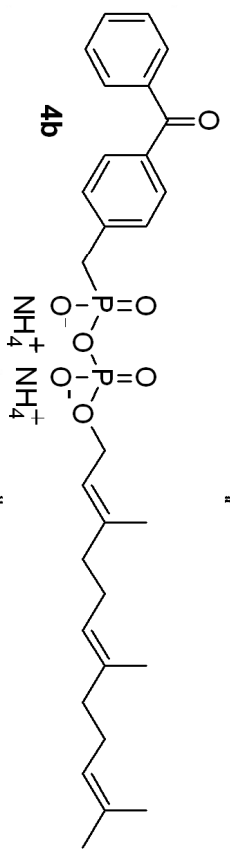
operator name
BiTOF II



Oh-1-87
 University of Minnesota
 Department of Chemistry
 VAC-500

exp1 stain
 SAMPLE
 date May 23 2005
 solvent CDC13
 file /018/14/Modor
 h/050533V3_0202
 ACQUISITION
 sfrq 299.955
 tn H1
 at 2.800
 np 23996
 sv 5388.8
 fb 3480
 bs 16
 ipwr 69
 pw 10.7
 di 1.580
 lof 1518.0
 nt 16
 ct 16
 atlock Y
 gain not used
 flags WRT
 n n
 n n
 y Y
 nm nm
 DISPLAY
 sp -5.0
 wp 2395.8
 vs 662
 sc 0
 wc 225
 hzmm 10.65
 is 1720.85
 rfl 589.8
 rfp 0
 th 2
 ins 2.000
 ai cdc ph
 EC & VT 299.954
 H1 H1
 30 30
 0 0
 nmp C
 200
 1.0
 n
 PROCESSING 0.10
 ft
 65536
 f
 react
 procpilot

- 7.537
- 7.513
- 7.508
- 7.479
- 7.408
- 7.380
- 7.384
- 7.377
- 7.359
- 7.287
- 7.276
- 7.260
- 7.235
- 5.133
- 5.111
- 5.081
- 4.848
- 4.837
- 4.285
- 4.269
- 4.140
- 4.120
- 4.088
- 3.129
- 3.124
- 3.118
- 3.113
- 3.107
- 3.039
- 2.859
- 1.822
- 1.801
- 1.782
- 1.744
- 1.727
- 1.717
- 1.695
- 1.435
- 1.397
- 1.359
- 1.340



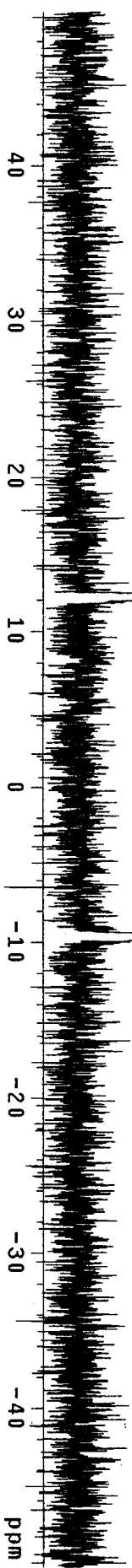
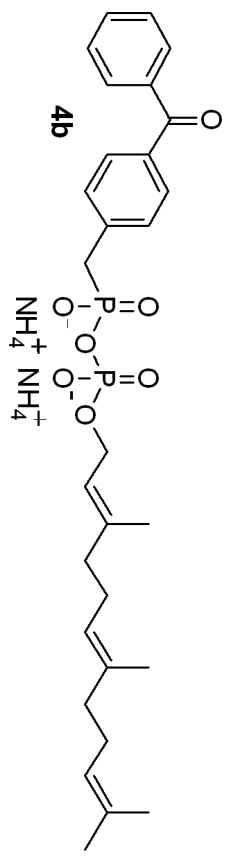
oh-1-97
 University of Minnesota
 Department of Chemistry
 VAC-300

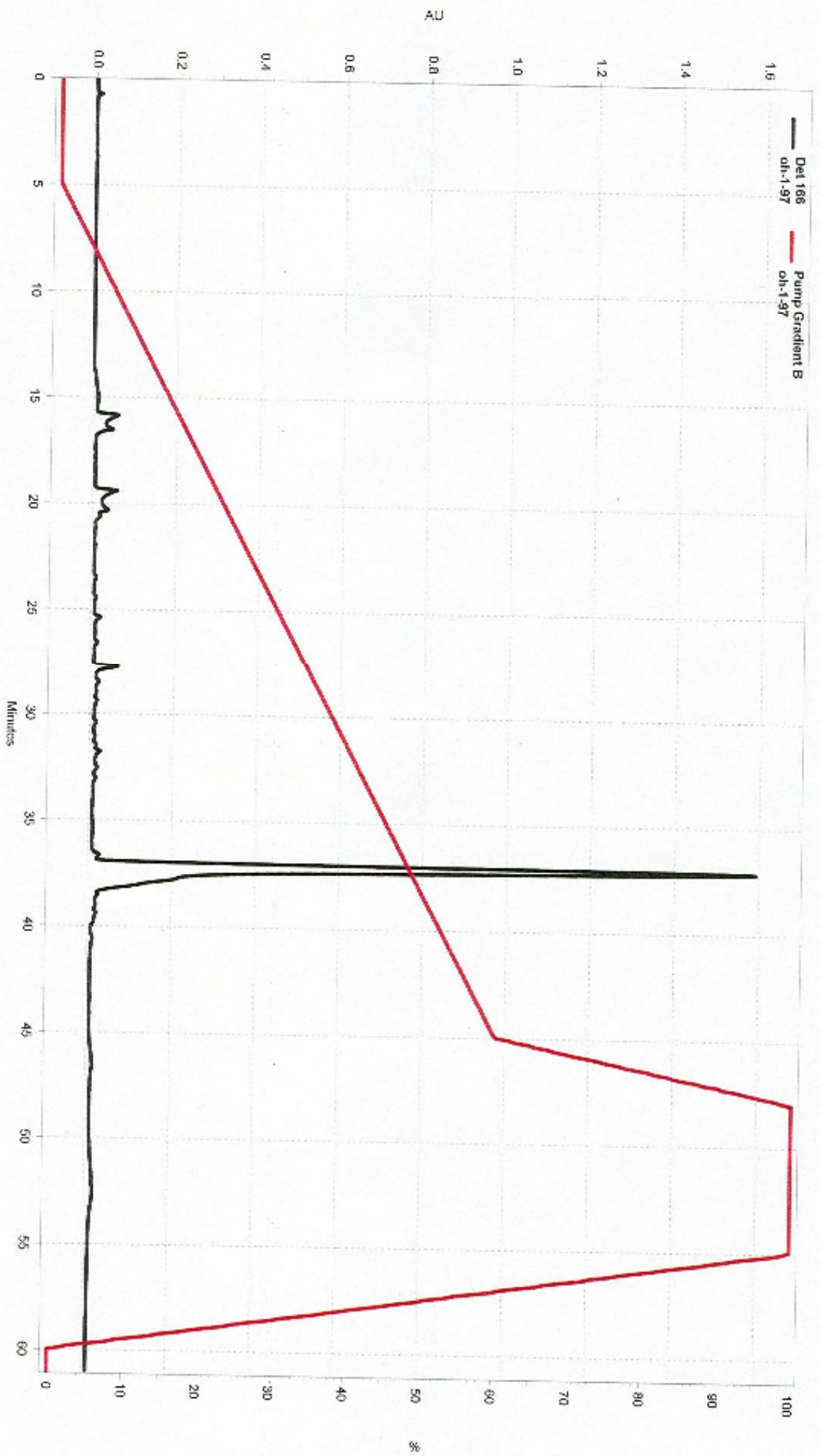
exp1 s2pu1

SAMPLE May 23 2005 dfreq 299.954
 solvent CDC13 dn H1
 file /o1d/14/mddon~ dpwr 40
 h/050523V3_0204 dof 0
 ACQUISITION nyy w
 sfreq 121.432 dmm 7233
 tn P31 dmf
 at 0.800 dseq
 np 68012 dres 1.0
 sw 42508.0 homo
 fb 23400
 bs 16 lb
 tpwr 60 wtfile 1.50
 pw 26.2 proc ft
 dl 1.000 fn 131072
 d2 1.900 math f
 tof 9017.7
 nt 32 wert
 ct 32 wexp
 atock n wbs
 gain not used wrt
 FLAGS
 i1 n
 in n
 dp y
 hs yn
 DISPLAY
 sp -6110.7
 wp 12162.3
 vs 231
 sc 0
 wc 225
 hzmm 54.05
 is 68601.40
 rfi 10069.3
 rfp -2078.1
 th 63
 lns 100.000
 ai no ph

12.275
 12.062

9.546
 9.755





D:\32Karath\Projects\Default\2008\OH\oh-1-97\12-11-2008 5-27-50 PM, Det 166

D:\32Karath\Projects\Default\2008\OH\oh-1-97\12-11-2008 5-27-50 PM, Pump Gradient B

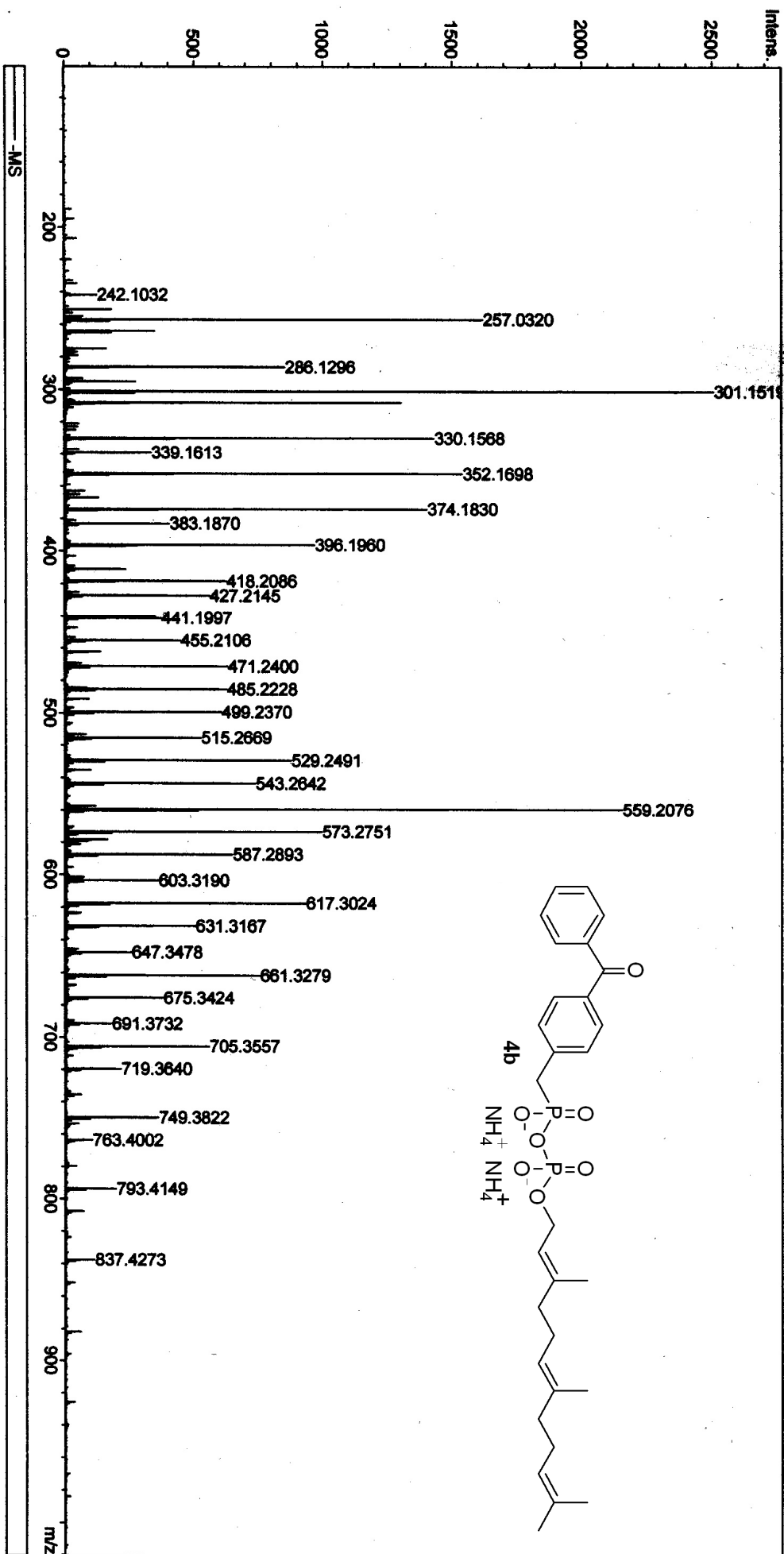
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Comment OH-1-97PEG

Acquisition Date 3/30/2006 3:43:51 PM
Method negative:tofpar

Operator

Operator name
BIO TOF II



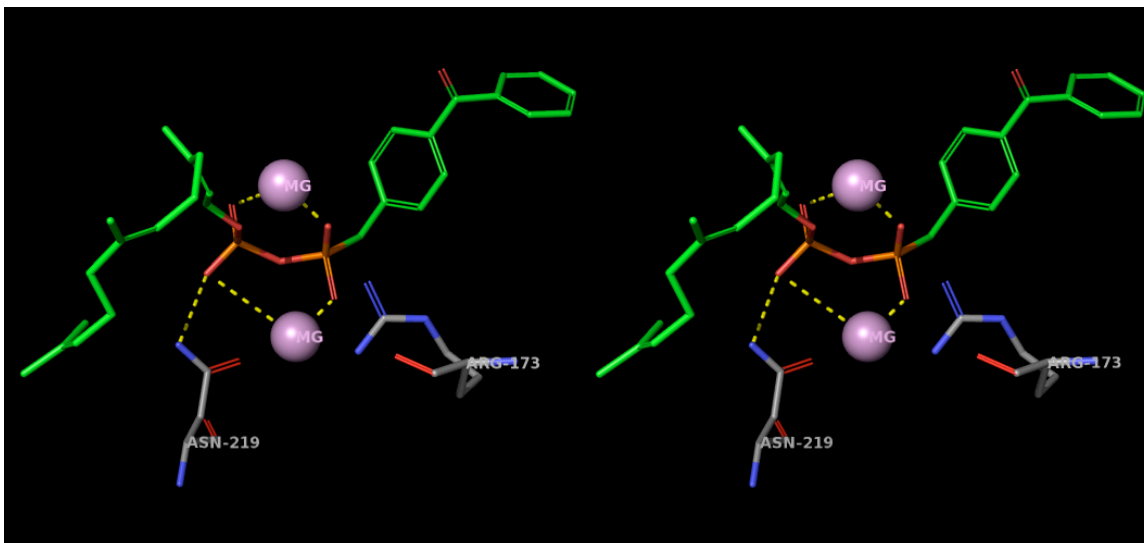
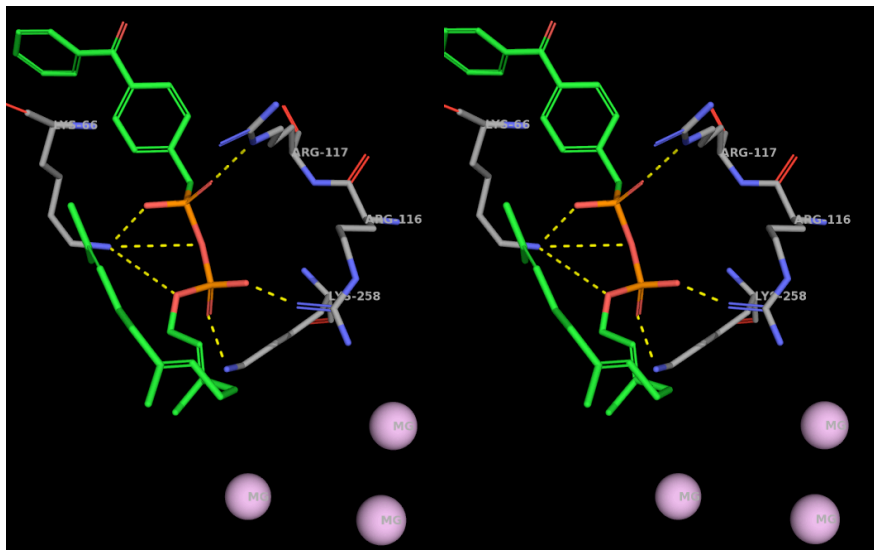
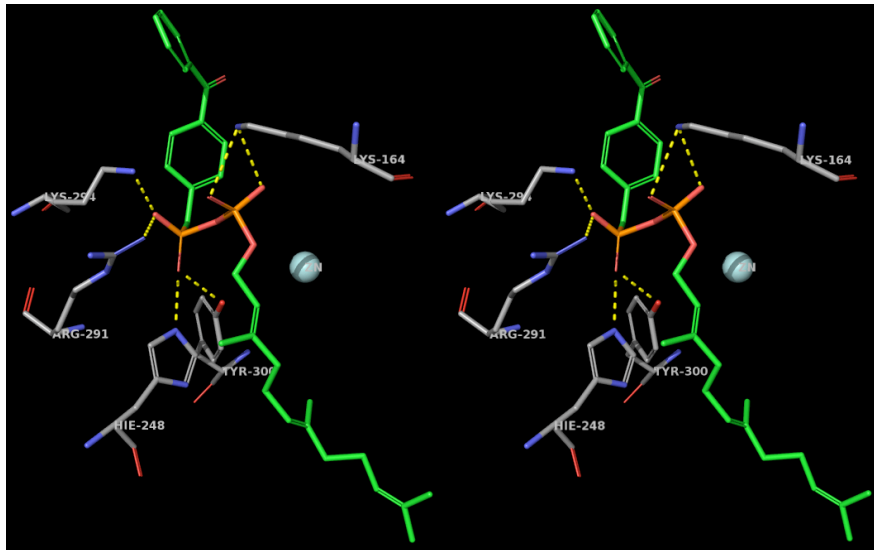


Figure. Modeling of **4b** in the active sites of FPP-utilizing enzymes. This Figure contains stereo views of the pictures shown in Figure 8 in the text. Only the Enzyme•4b complexes are shown. These correspond to the pictures on the right side in Figure 8 (Top Right, Middle Right and Bottom Right). The legend from Figure 8 is reprinted below.

Figure 8. Modeling of **4b** in the active sites of FPP-utilizing enzymes. Top Left: Crystal structure of NrPFTase (1JCR) with bound FPP. Shown, are residues H248 β , R291 β , K294 β , Y300 β , K164 α (colored by element; carbon in grey), FPP (colored by element; carbon in green) and Zn (cyan). The yellow dashes illustrate potential hydrogen bonding or electrostatic interactions (within 4Å) of the distal phosphate. Top Right: Crystal structure of NrPFTase with **4b** built on the bound FPP. The color scheme is the same as that for Top Left. Middle Left: Crystal structure of EcFPPSase (1RQ1) with FPP built by modifying the existing bound IPP. Shown, are residues K66, R116, R117, K258 (colored by element; carbon in grey), FPP (colored by element; carbon in green) and Mg (purple). The yellow dashes illustrate potential hydrogen bonding or electrostatic interactions (within 4Å) of the distal phosphate. Middle Right: Crystal structure of EcFPPSase with **4b** built on the bound IPP. The color scheme is the same as that for Middle Left. Bottom Left: Crystal structure of StSTSase (Pentalenene synthase, 1PS1) with FPP docked in the active site of the enzyme. Shown, are residues R173, N219 (colored by element; carbon in grey), FPP (colored by element; carbon in green) and Mg (purple). The yellow dashes illustrate potential hydrogen bonding or electrostatic interactions (within 4Å) of the distal phosphate. Bottom Right: Crystal structure of StSTSase with **4b** docked in the active site of the enzyme. The color scheme is the same as that for Bottom Left.

Residues within 4 Å of the distal phosphate (FPP) or phosphonate (**4b**)**RnPFTase**

Ligand	Residue	Dist in Å	Ligand	Residue	Dist Å
FPP			Benzophenone		
O2A	NZ (K164, α)	2.9	O2A	NZ (K164, α)	2.8
O1A	NZ (K164, α)	3.3	O1A	NZ (K164, α)	3.0
O1A	NZ (K294, β)	3.3			
O1B	NZ (K294, β)	2.8	O1B	NE2 (H248, β)	2.7
O2B	NZ (K294, β)	3.8	O1B	OH (Y300, β)	2.7
O2B	NH2 (R291, β)	3.2			
O2B	NE2 (H248, β)	3.0			
O3B	NE2 (H248, β)	4.0	O3B	NZ (K294, β)	2.5
O3B	OH (Y300, β)	2.7	O3B	NH2 (R291, β)	3.7
O3A	OH (Y300, β)	3.8			
O1A	NH2 (R291, β)	2.9			
O2B	NE (R291, β)	2.8			

EcFPPSase

Ligand	Residue	Dist in Å	Ligand	Residue	Dist Å
FPP			Benzophenone		
O8	NZ (K258)	2.6	O9	NH2 (R116)	2.1
O6	NZ (K258)	3.1	O8	NZ (K258)	2.6
O9	NZ (K66)	2.6	O6	NZ (K66)	3.9
O10	NZ (K66)	3.5	O10	NZ (K66)	3.6
OUKN1	NZ (K66)	2.6	O12	NZ (K66)	2.4
OUKN1	N (K66)	3.5	O12	N (K66)	3.6
O12	NH2 (R117)	3.1	O14	NE (R117)	2.6
O12	NE (R117)	2.6			
O14	NE (R117)	4.0			

StSTSase

Ligand	Residue	Dist in Å	Ligand	Residue	Dist Å
FPP			Benzophenone		
O3	NH2 (R173)	3.4	O2	ND2 (N219)	3.0
O1	NH2 (R173)	3.5	O4	MG701 (Mg1)	1.8
O1	NH1 (R173)	3.0	O	MG701 (Mg1)	2.6
O1	ND2 (N219)	3.9	O2	MG701 (Mg1)	3.7
O	ND2 (N219))	3.5	O5	MG702 (Mg2)	2.0
O2	MG702 (Mg2)	2.0	O	MG702 (Mg2)	3.3
O	MG702 (Mg2)	2.8	O3	MG702 (Mg2)	2.0
O6	MG702 (Mg2)	1.8	O1	MG702 (Mg2)	3.1
O4	MG702 (Mg2)	3.8			
O5	MG701 (Mg1)	1.8			
O	MG701 (Mg1)	3.0			
O1	MG701 (Mg1)	3.6			