

## **Supporting Information**

### **Synthesis of Chemically Stabilized Phosmidosine Analogs and the Structure-Activity Relationship of Phosmidosine**

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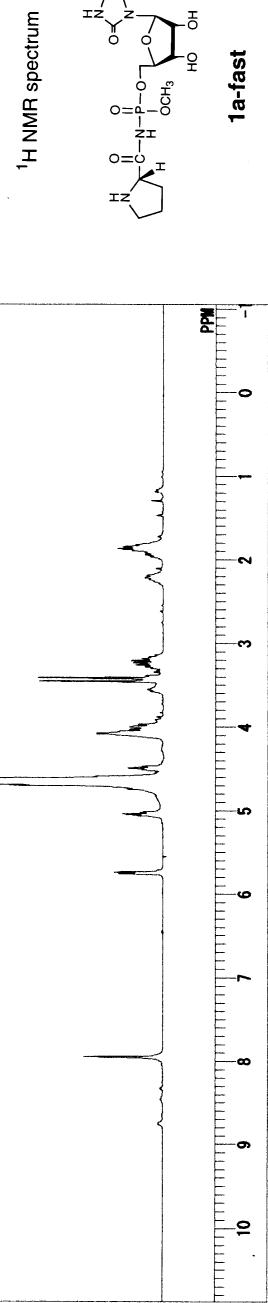
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**General Methods.**  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{31}\text{P}$  NMR spectra were obtained on a GX-270 apparatus at 270, 68 and 109 MHz, respectively. The chemical shifts were measured from tetramethylsilane (0 ppm) or DMSO- $d_6$  (2.49 ppm) for  $^1\text{H}$  NMR,  $\text{CDCl}_3$  (77.0 ppm), DMSO- $d_6$  (39.7 ppm) or DMF- $d_7$  (2.74 ppm) for  $^{13}\text{C}$  NMR, and 85% phosphoric acid (0 ppm) for  $^{31}\text{P}$  NMR. Column chromatography was performed with Wako silica gel C-200. Reverse-phase column chromatography was performed by use of  $\mu$ Bondasphere 37-55  $\mu\text{m}$  C-18 (125A) particles which was set up in a glass column of a medium pressure preparative HPLC system. Elution was performed with the following solvent systems I-III for 50 min at a flow rate of 2.0 mL/min. Solvent system I: water-acetonitrile (100:0 to 70:70, v/v); solvent system II: water-MeOH-trifluoroacetic acid (93:7:0.1, v/v/v); solvent system III: 0.1 M ammonium acetate (pH 7.0)-acetonitrile (100:0 to 50:50, v/v). Reverse-phase HPLC was performed using  $\mu$ Bondasphere and  $\mu$ Bondapak C-18 columns (Waters Co., Ltd., 3.9 x 150 mm and 7.8 x 300 mm, respectively) with a linear gradient of 0 – 15%  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  containing 0.1 M  $\text{NH}_4\text{OAc}$  (pH 7.0) at 50 °C at a flow rate of 1.0 mL/min and 3.0 mL/min, respectively, for 30 min. ESI mass spectra were measured on Mariner<sup>TM</sup>. MALDI-TOF mass spectra were measured on Voyager RP. UV spectra were measured by a U-2000 spectrophotometer. TLC was performed with Merck silica gel 60 ( $F_{254}$ ) plates. 8-Bromoadenosine was purchased from Sigma-Aldrich Co., Ltd. Triethylamine was distilled from  $\text{CaH}_2$  and stored over Molecular Sieves 4A. In vitro analysis of the antitumor activity in various cancer cell lines was carried out by the literature method reported by Carmichael.<sup>1</sup> The morphological reversion activity test was conducted according to the literature method.<sup>2</sup> Compounds **5**<sup>3</sup> and **25a-d**<sup>4,5</sup> were synthesized according to the previous method reported.<sup>6</sup> Compounds **6a-d** were synthesized according to the literature methods.<sup>7</sup>

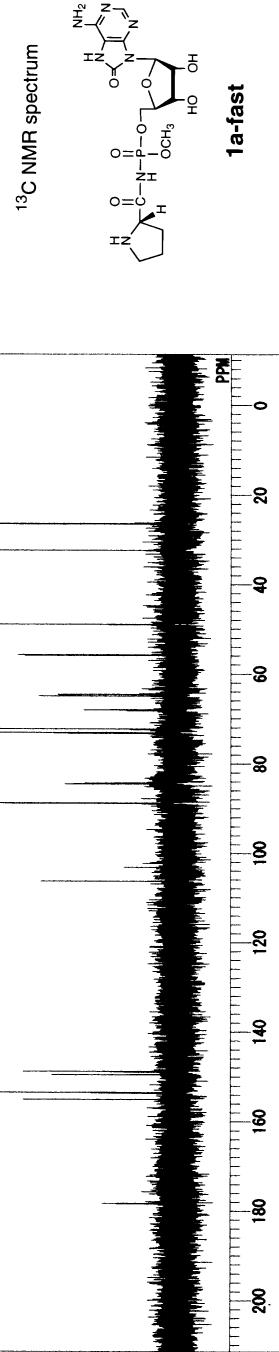
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2. Matsuura, N.; Onose, R.; Osada, H. *J. Antibiot.* **1996**, *49*, 361-3654.
3. Lannoye, G. S.; Moerlein, Stephen M.; Parkinson, D.; Welch, M. J. *J. Med. Chem.* **1990**, *33*, 2430-7.
4. Mamos, P.; Dalatsis, E.; Athanassopoulos, C.; Balayiannis, G.; Papaioannou, D.;

- Francis, G. W. *Acta Chem. Scand.* **1998**, *52*, 227-231.
5. Barlos, K.; Papaioannou, D.; Voliotis, S. *Liebigs Ann. Chem.* **1988**, *12*, 1127-1133.
  6. Moriguchi, T.; Yanagi, T.; Kunimori, M.; Wada, T.; Sekine, M. *J. Org. Chem.* **2000**, *24*, 8229-8238.
  7. (a) Hamamoto, S.; Takaku, H. *Chemistry Lett.* **1986**, 1401-4. (b) Hayakawa, Y.; Hirose, M.; Hayakawa, M.; Noyori, R. *J. Org. Chem.* **1995**, *60*, 925-30. (c) Uhlmann, E.; Peyman, A.; O'Malley, G.; Helsberg, M.; Winkler, I. Eur. Pat. EP 552767 (1993). (d) Broeders, N. L. H. L.; Koole, L. H.; Buck, H. M. *J. Am. Chem. Soc.* **1990**, *112*, 7475-82.

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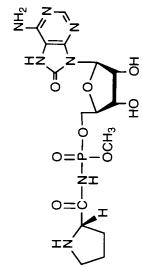
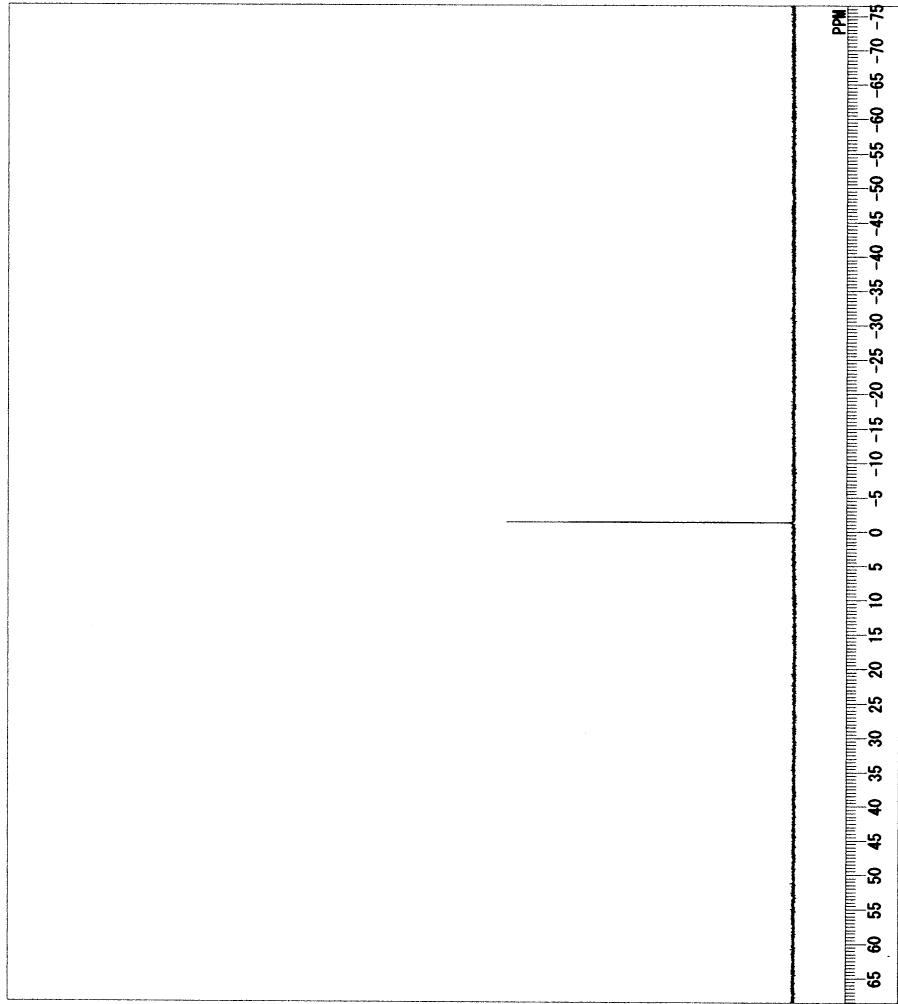
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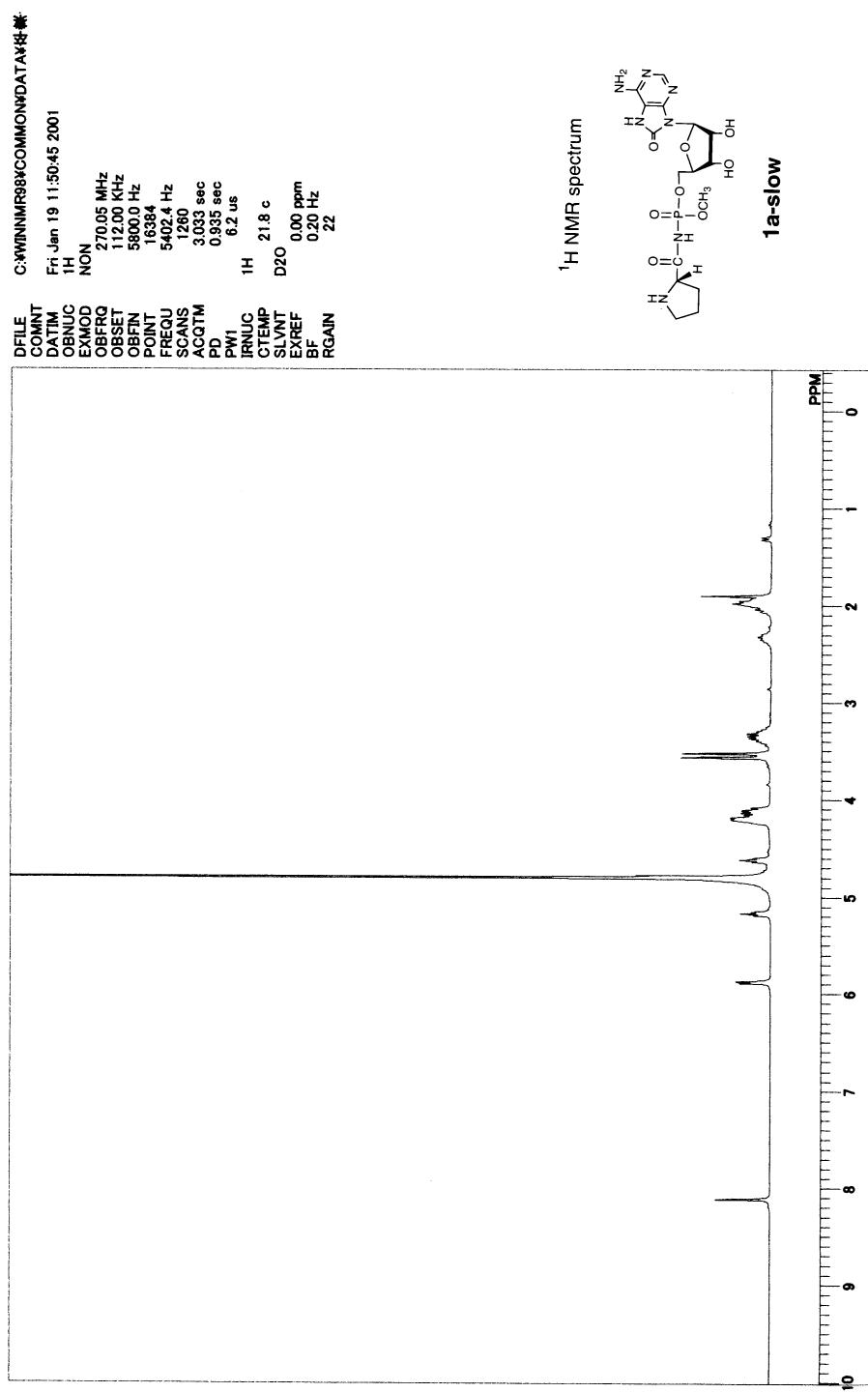
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$^{31}\text{P}$  NMR spectrum

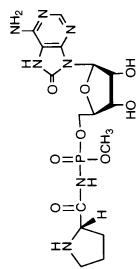
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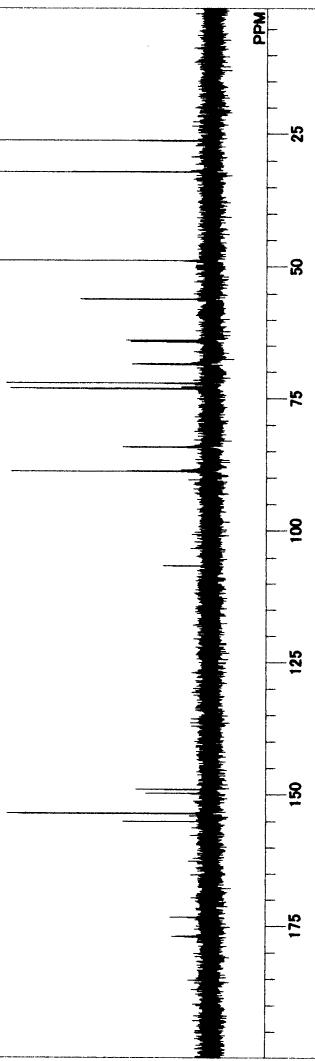
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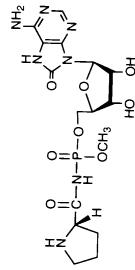


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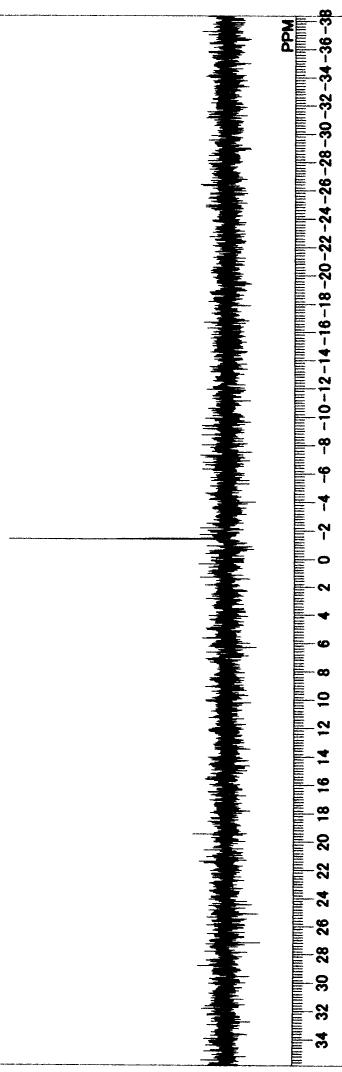
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<sup>31</sup>P NMR spectrum



1a-slow



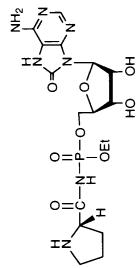
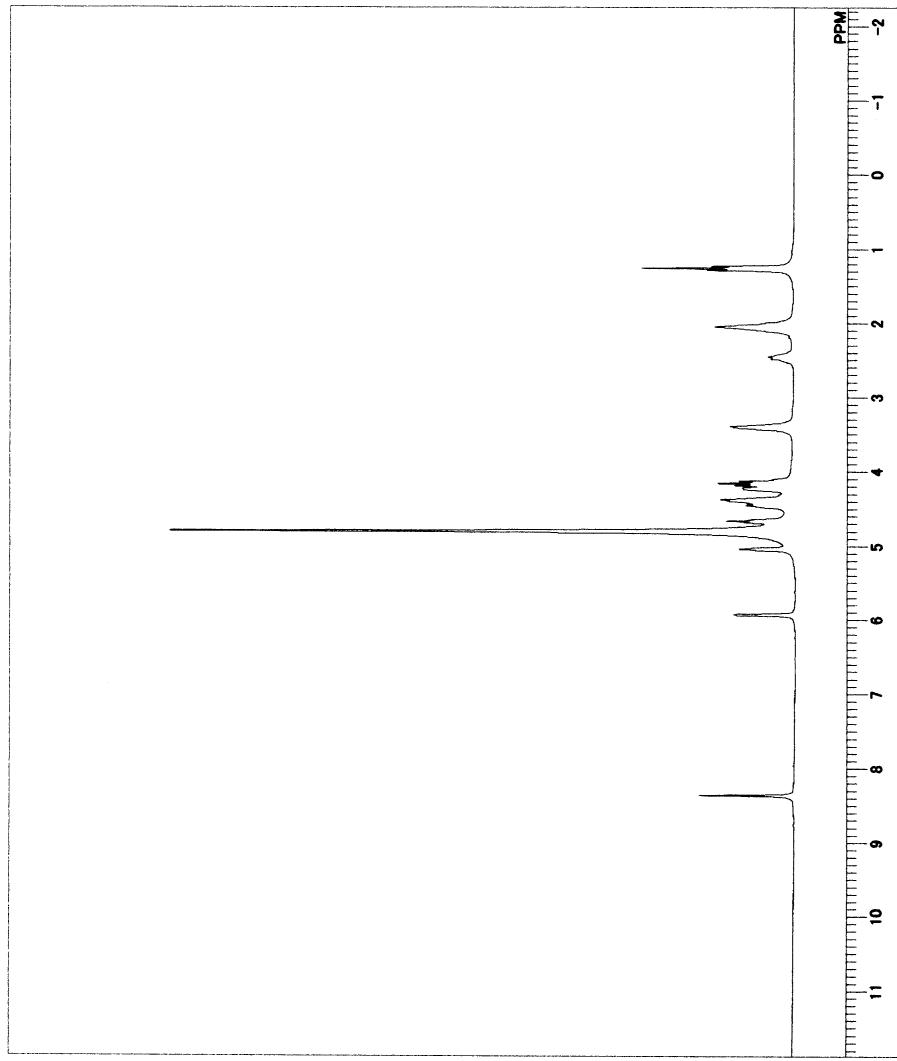
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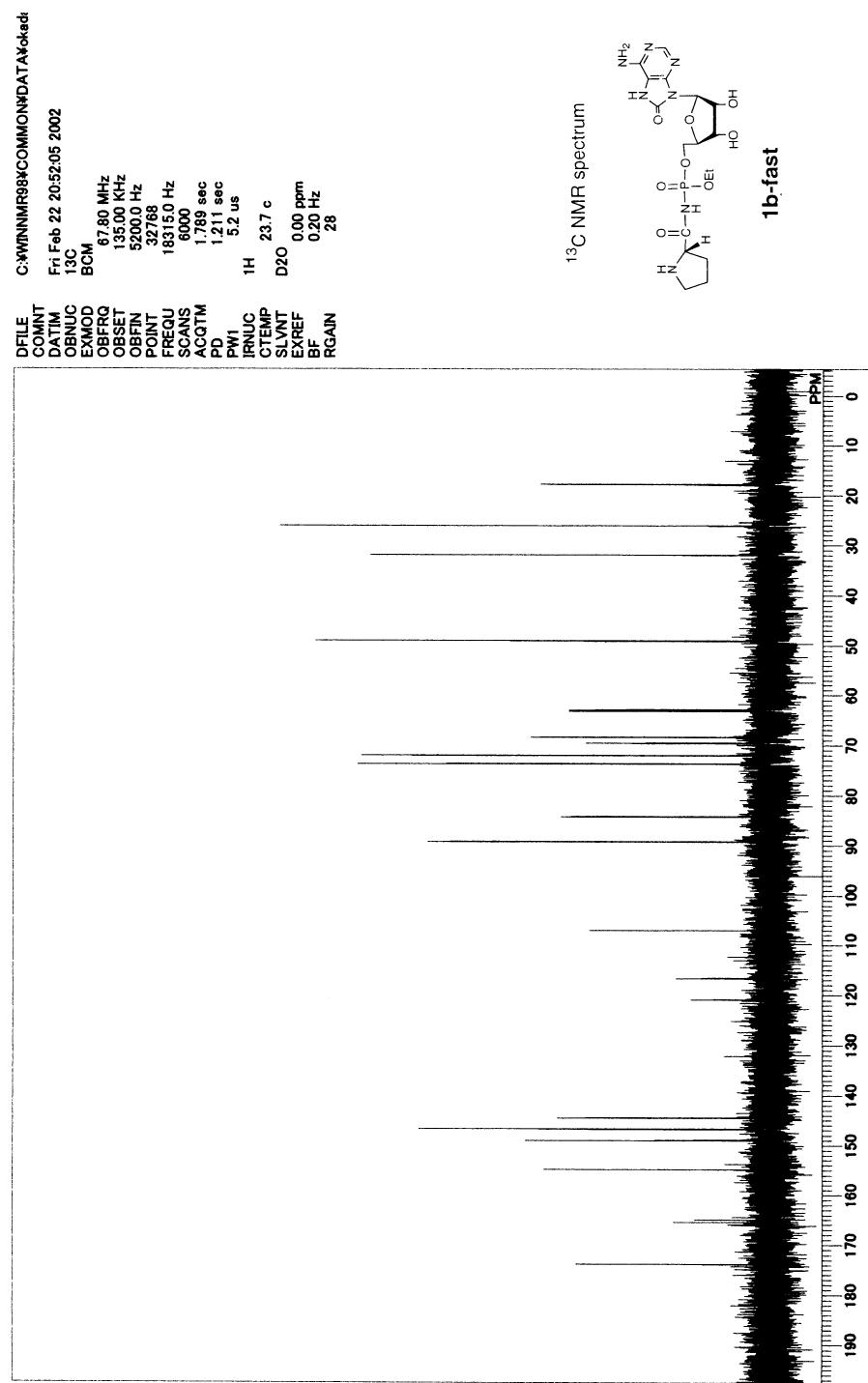
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<sup>1</sup>H NMR spectrum

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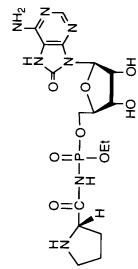


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<sup>31</sup>P NMR spectrum



1b-fast

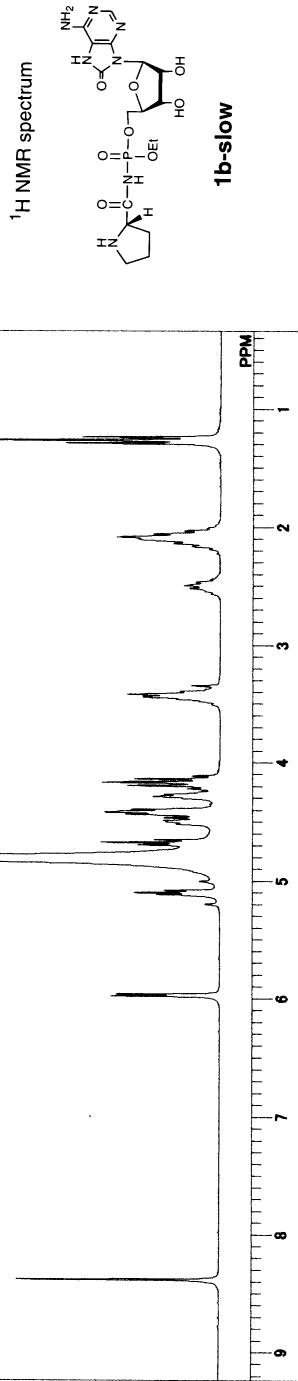


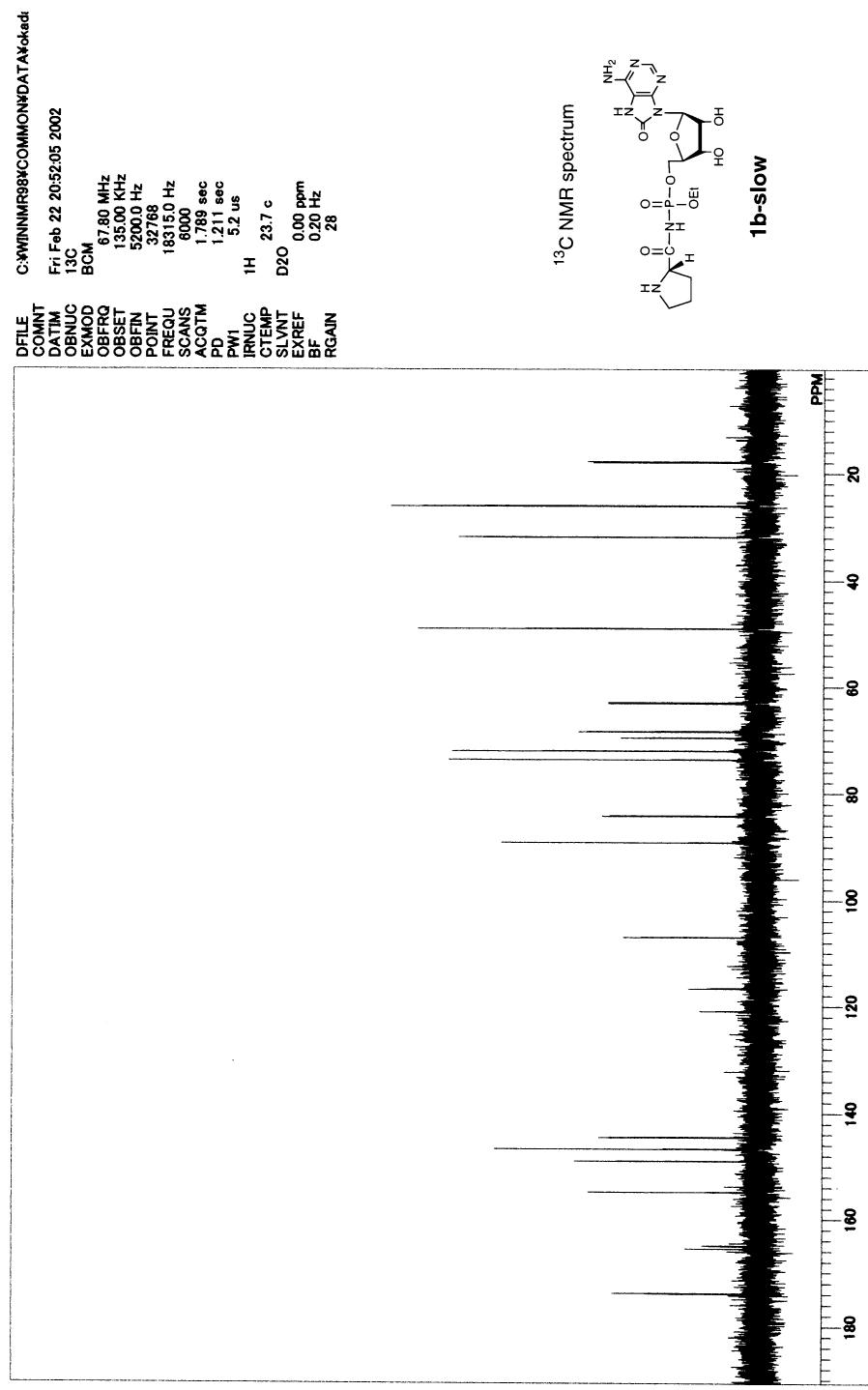
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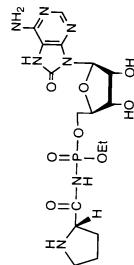
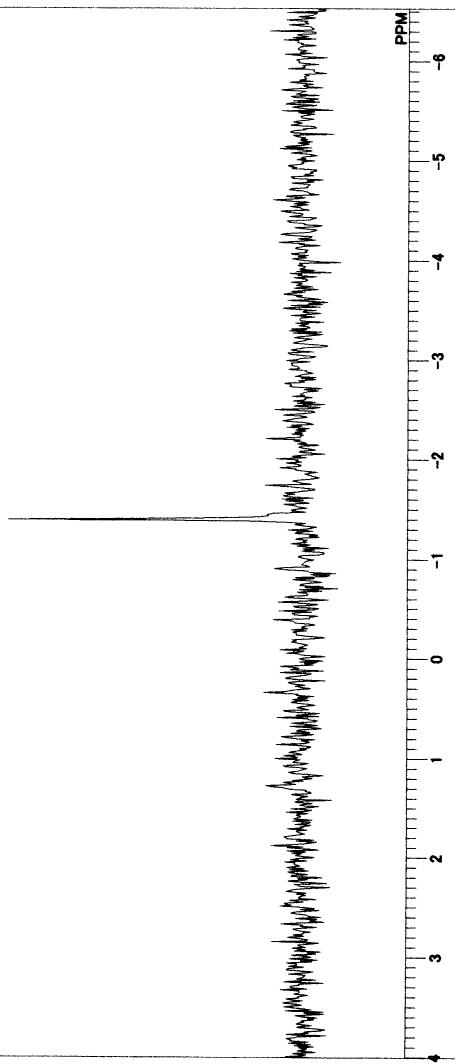


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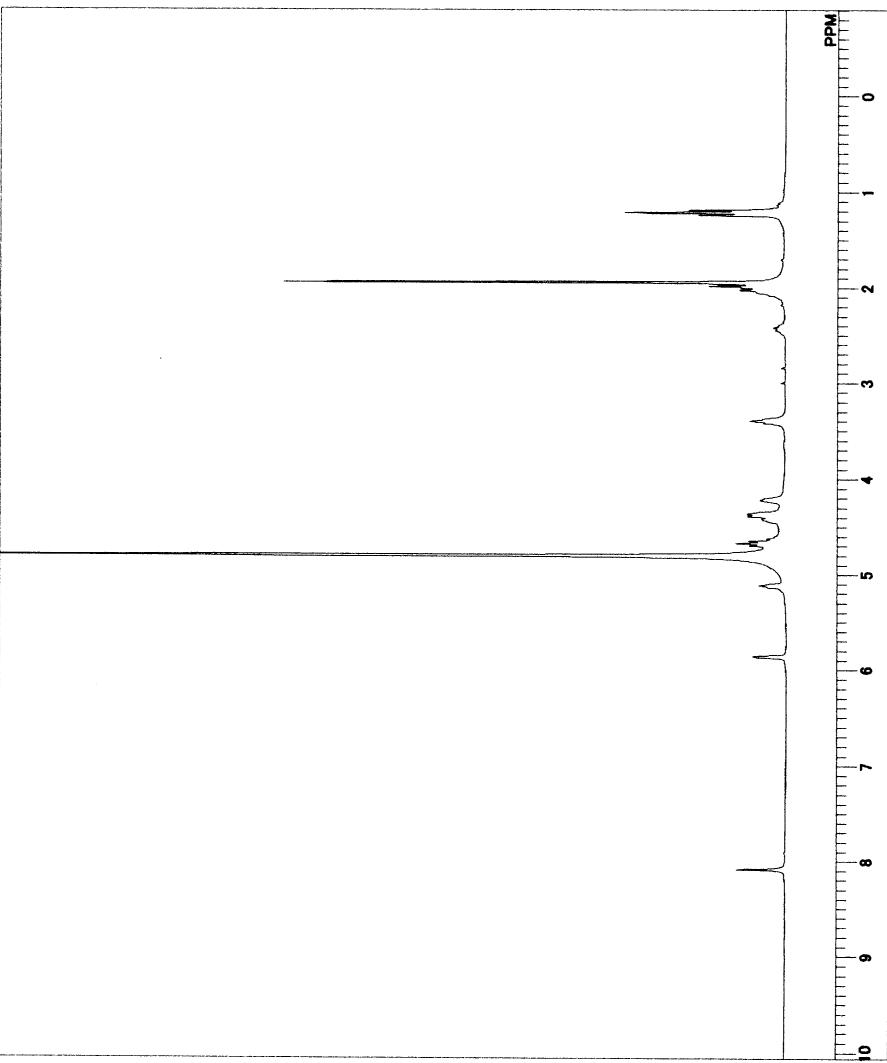


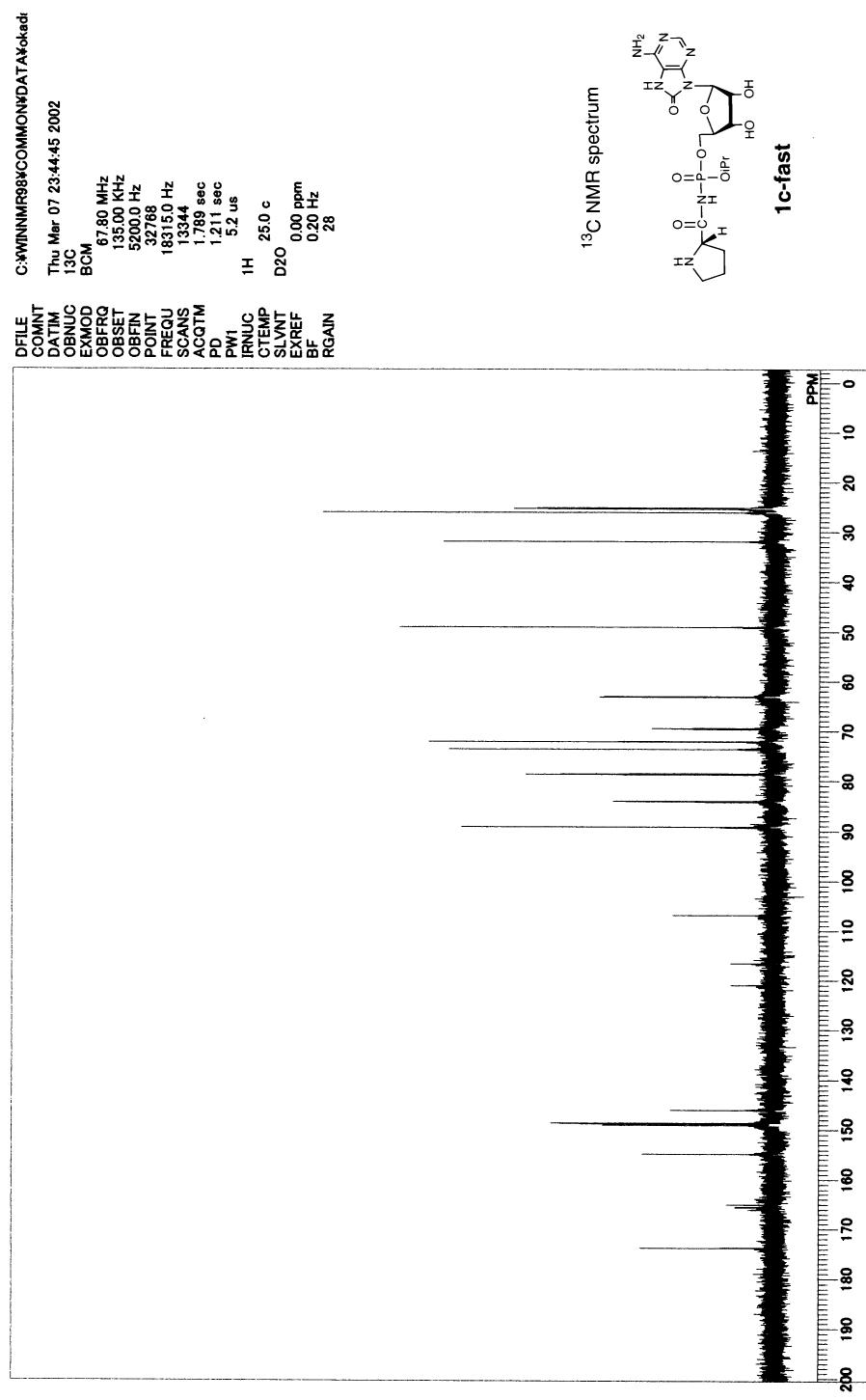
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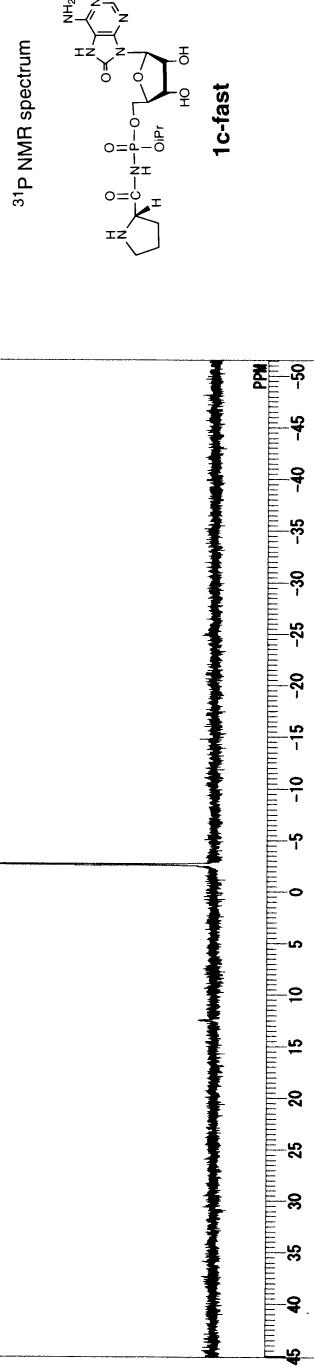
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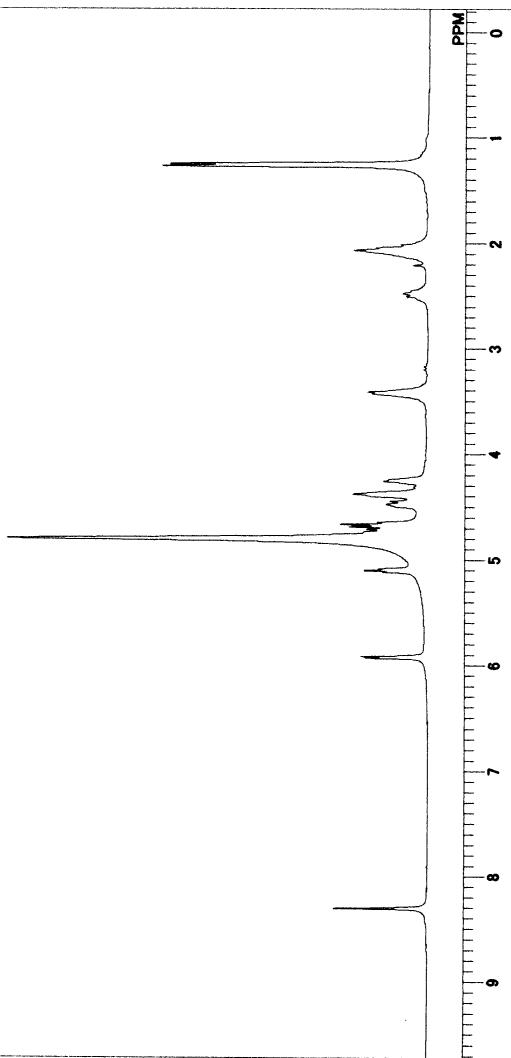


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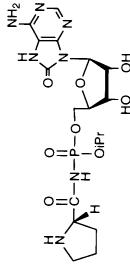


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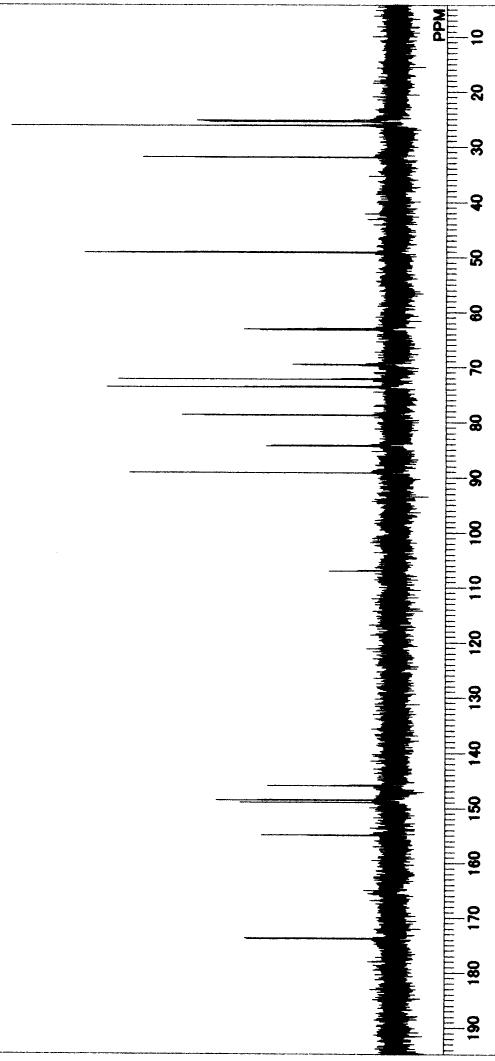
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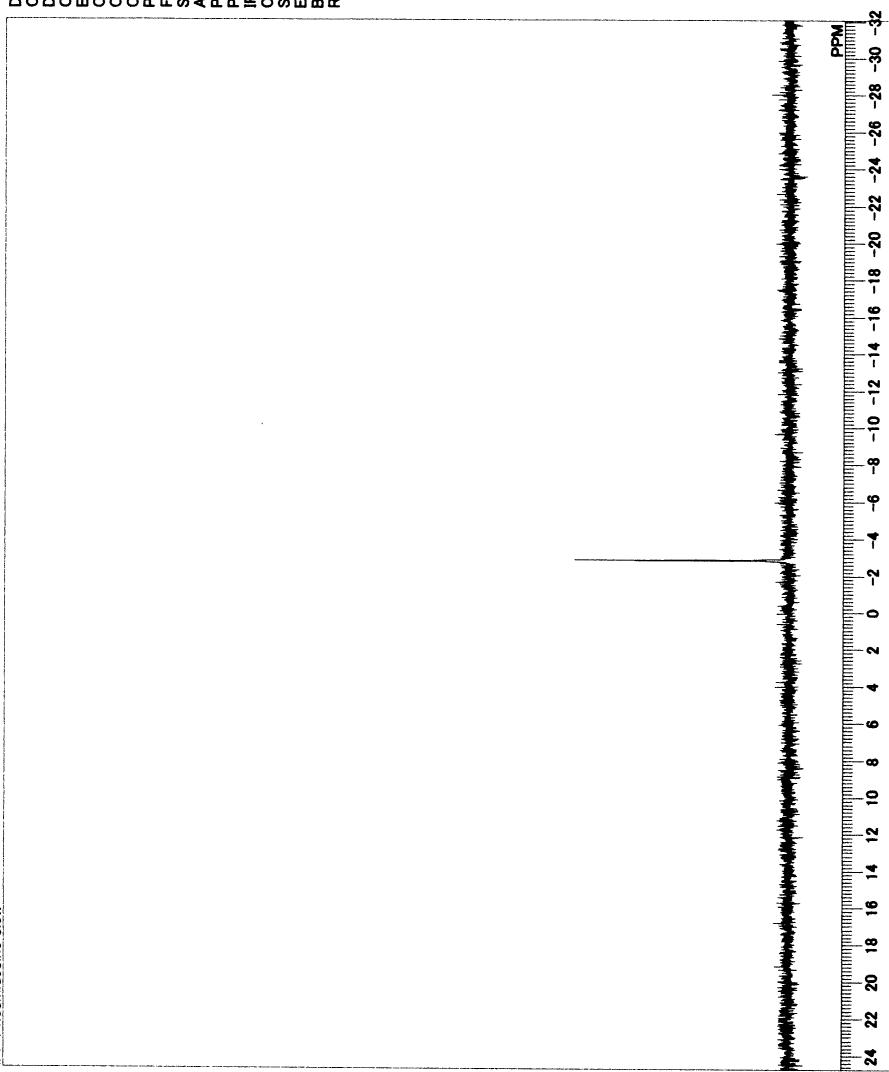
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<sup>13</sup>C NMR spectrum

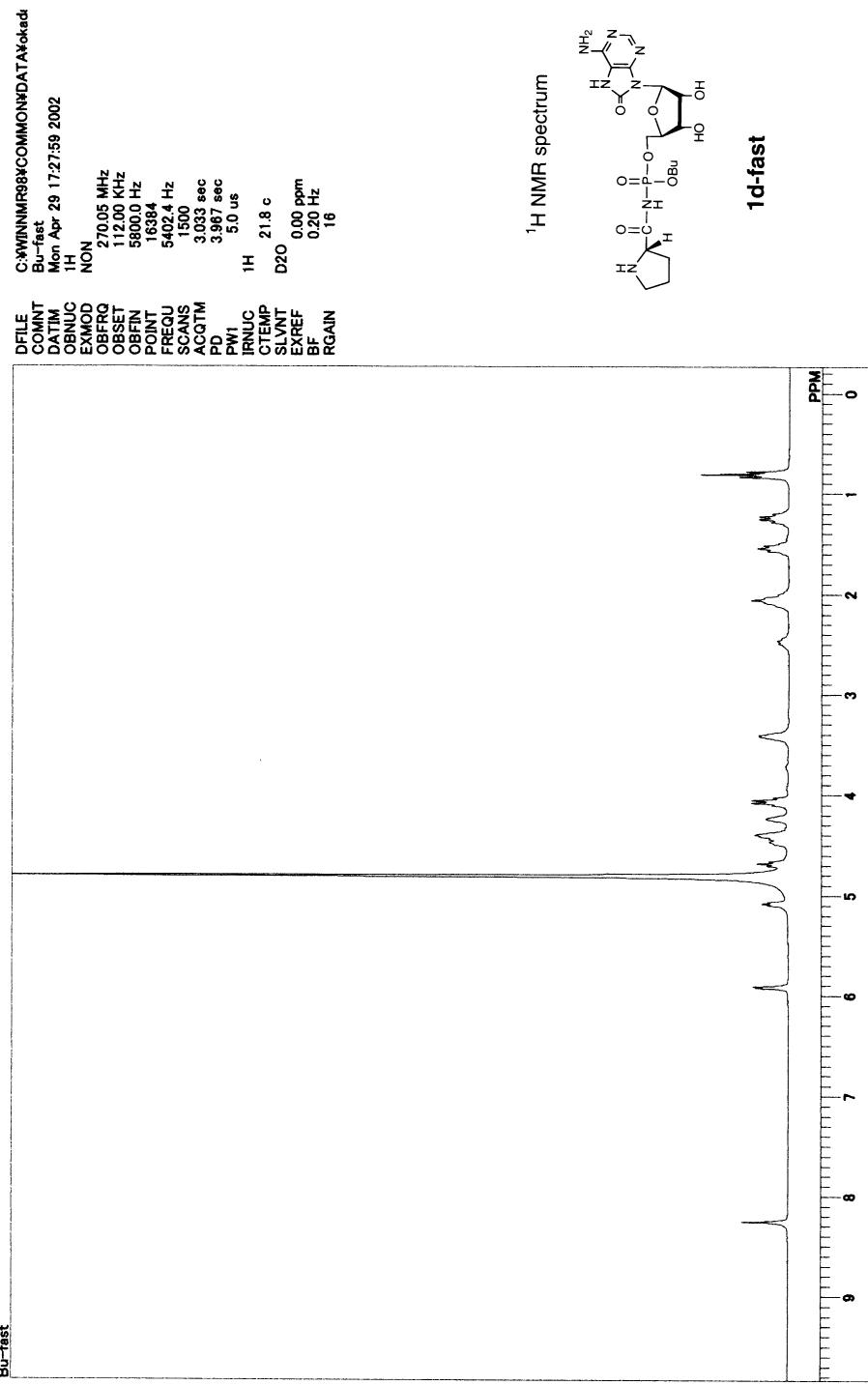


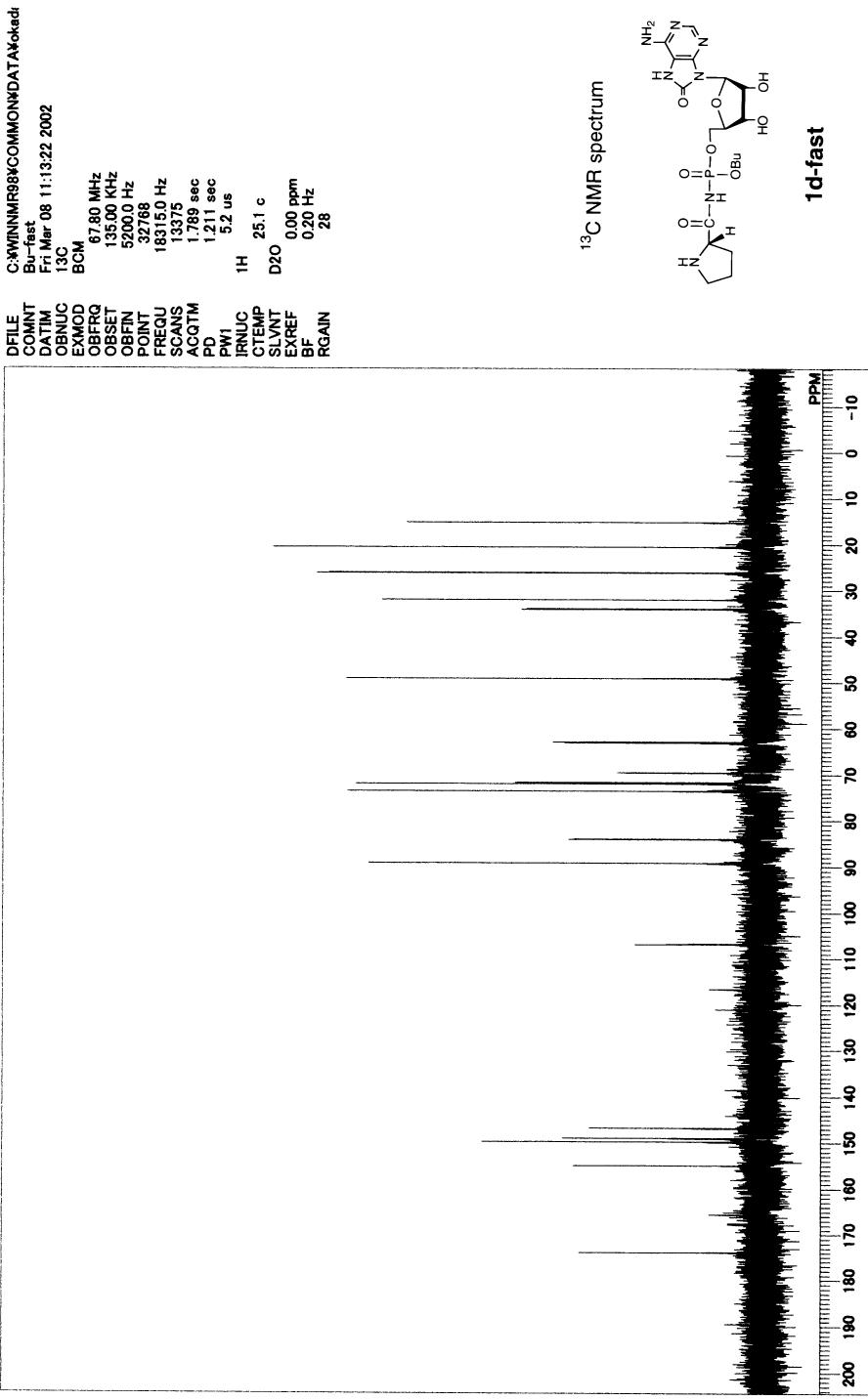
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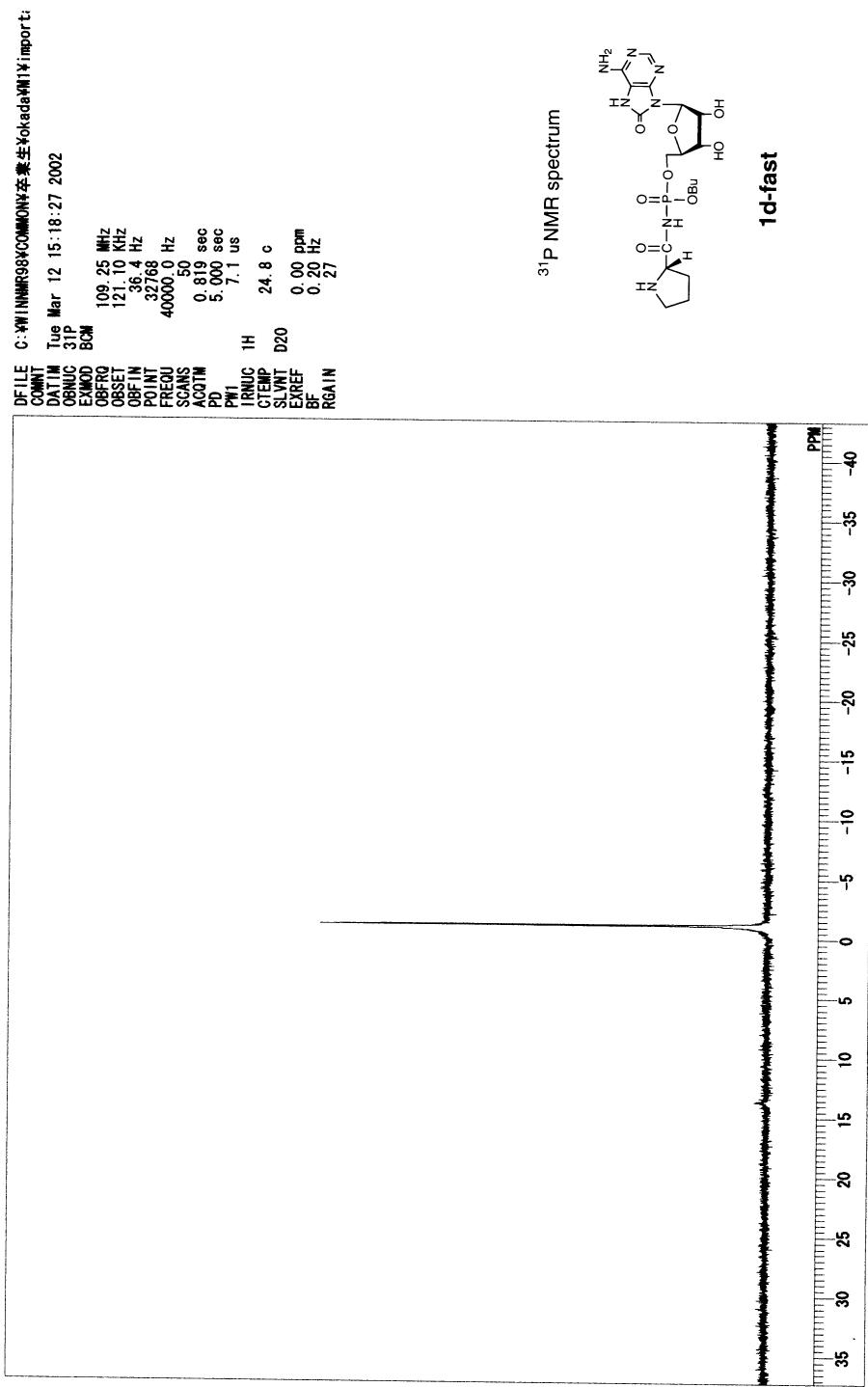




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RGAIN  
25





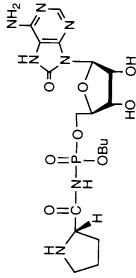


C:\WINNMR\84\COMMON\DATA\Yokada\Important\Important\BuSlow\1Hals  
Bu-Slow  
Wed Mar 13 2021 13:36 2002

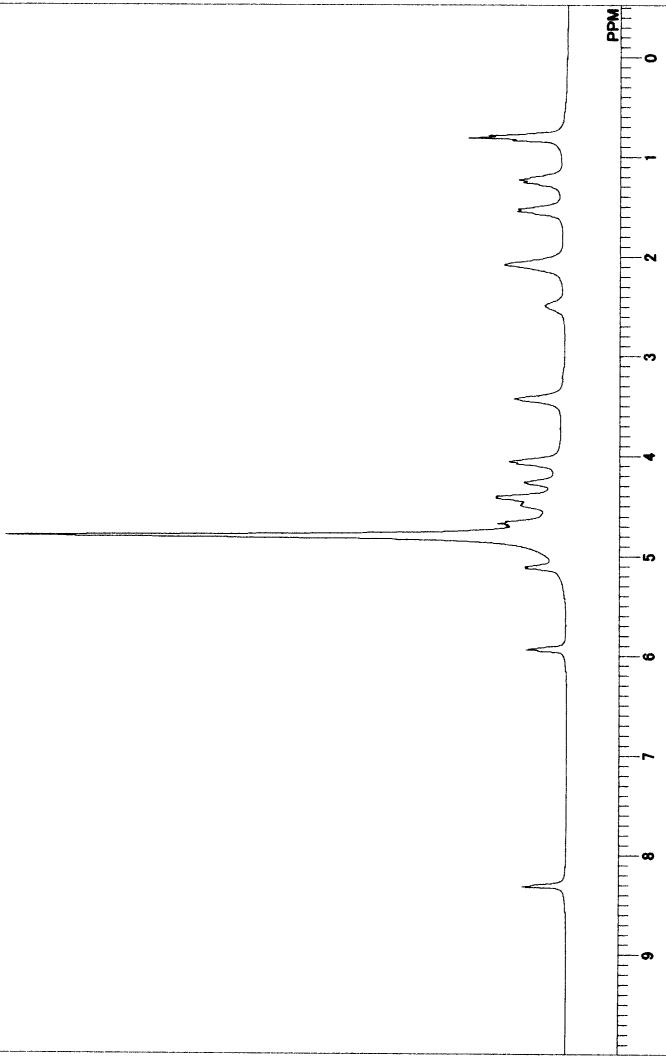
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EXMOD  
OBFRQ  
OBET  
OBIN  
POINT  
FREQU  
SCANS  
ACQTM  
PD  
PWN  
IRNUC  
CTEMP  
SLVNT  
EXREF  
BF  
RGAIN

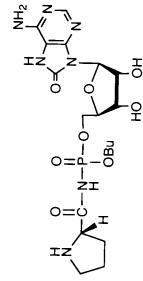
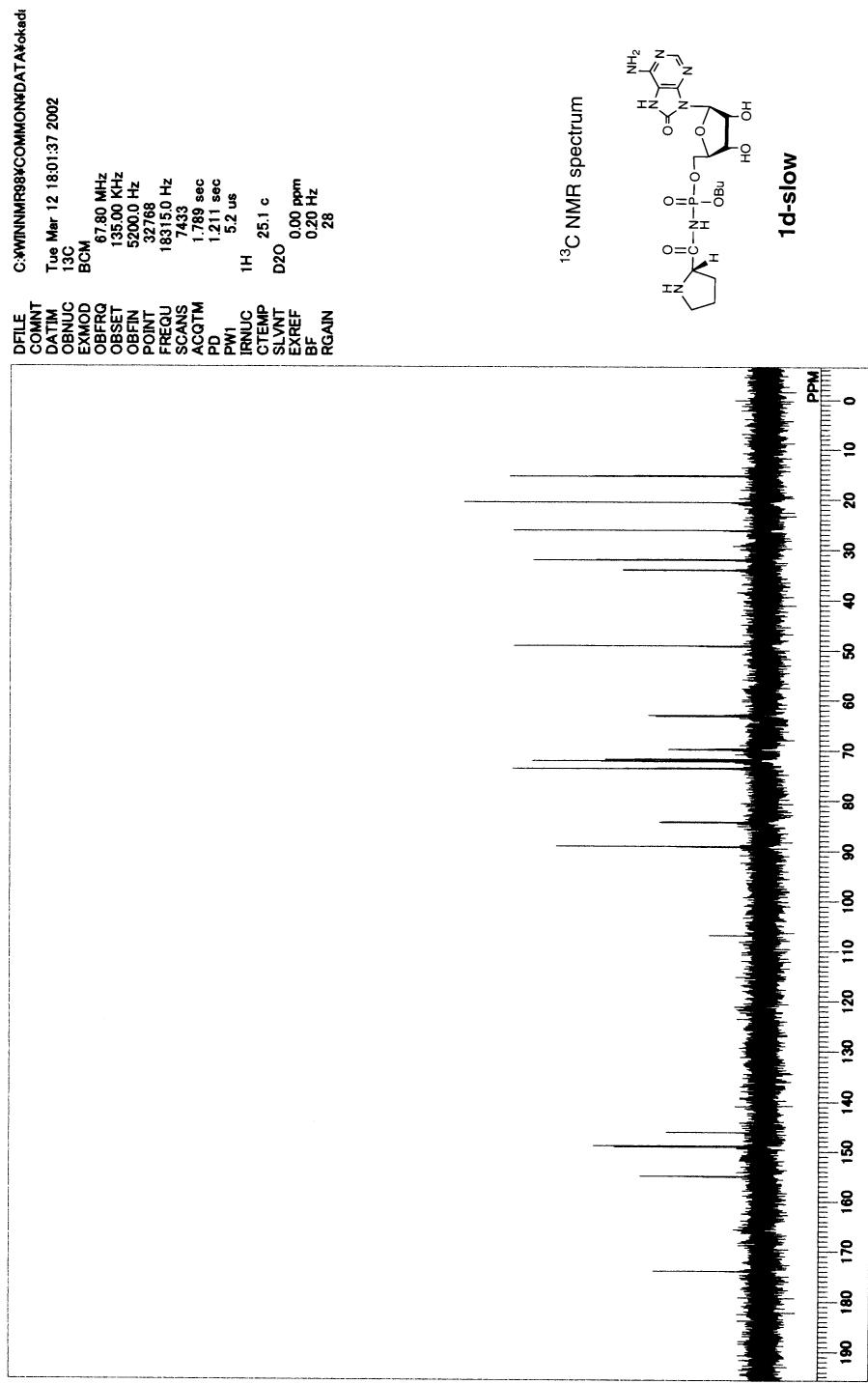
1H  
NON  
270.05 MHz  
112.00 kHz  
5800.0 Hz  
163.84  
5402.4 Hz  
226  
3.033 sec  
3.987 sec  
5.0 us

1H NMR spectrum



1d-slow



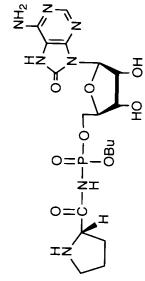


C:\WINNMR\8\COMMON\DATA\Yokada\Important\Important\Bu\slow\31P.xls

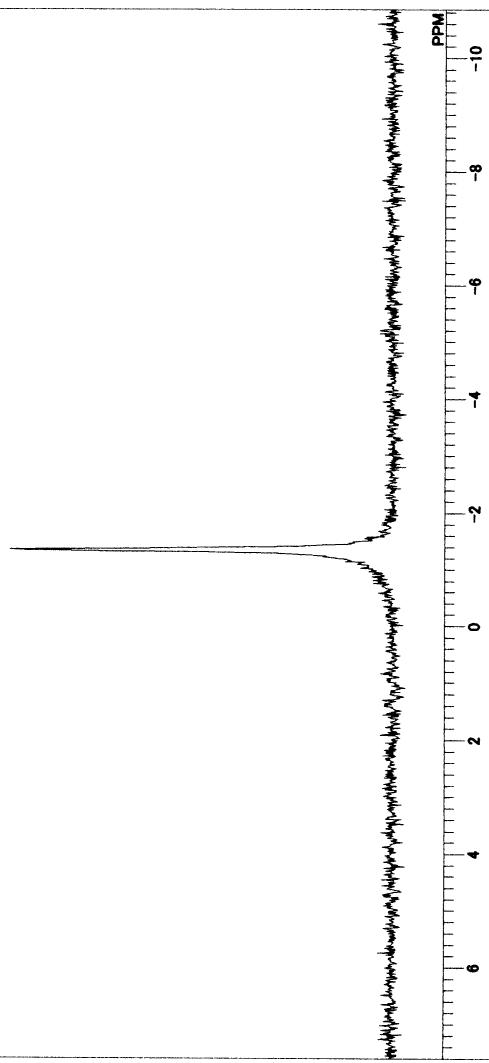
Bu->slow  
Wed Mar 13 2025 31 2002

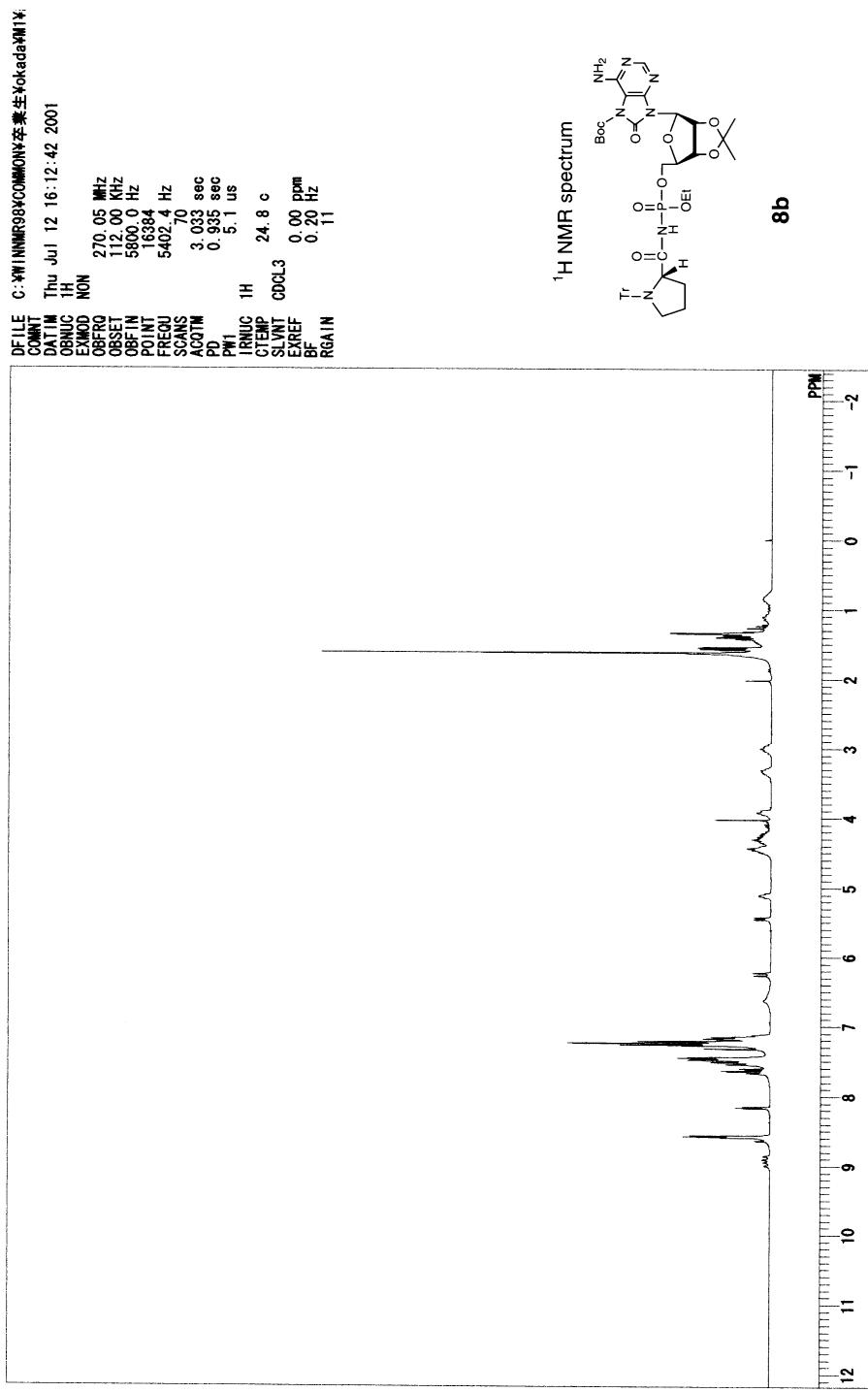
DFILE  
CONINT  
DATIM  
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EXMOD  
BCM  
OBFRQ 109.25 MHz  
OBSET 121.10 kHz  
OBFIN 36.4 Hz  
POINT 32768  
FREQU 400000 Hz  
SCANS 16  
ACQTM 0.819 sec  
PD 5.000 sec  
PW1 7.1 us  
TRNUC 1H  
CTEMP 25.2 c  
SLVNT D2O  
EXREF 0.00 ppm  
BF 0.20 Hz  
RGAIN 25

<sup>31</sup>P NMR spectrum



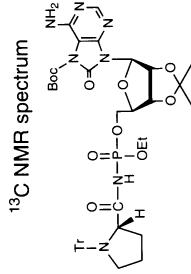
1d-slow





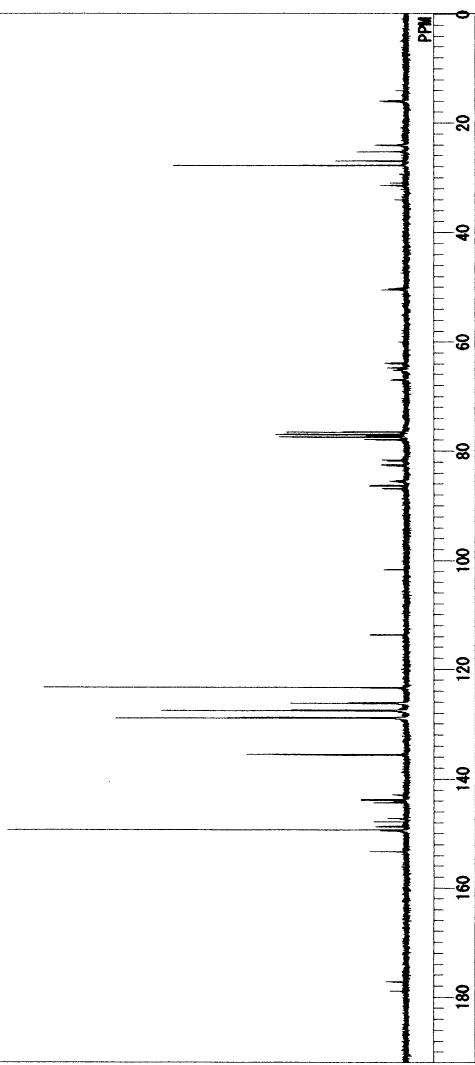
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DATM 130  
CBNUC 130  
EAD0D BCM  
OBFRQ 67.80 MHz  
OFSSET 135.00 kHz  
OBFTW 5200.0 Hz  
POINT 32768  
FREQU 18315.0 Hz  
SCANS 4/4  
ACQTM 1.789 sec  
PD 1.211 sec  
PW 4.4 us  
IRNUC 1H  
CTEMP 26.5 c  
SLVNT CDCl<sub>3</sub>  
EXREF 77.00 ppm  
BF 0.20 Hz  
RGAIN 32



<sup>13</sup>C NMR spectrum

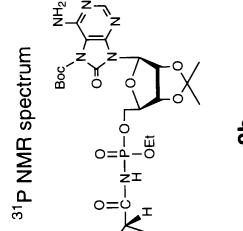
8b



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DFILE C:\WINNMR\98\COMMON\卒業生\Yokada\XWY結合関係\ET\編合、カラム後-31P.als
COUNT
DATUM Thu Jul 12 16:16:34 2001
GBIN 31P
EXND B64
FREQ 109.25 MHz
OBST 121.10 kHz
OBFIN 36.4 Hz
POINT 32768
FREQU 40000.0 Hz
SCANS 25
ACQTM 0.819 sec
PD 5.000 sec
PW1 7.1 us
IRNUC 1H
CTEMP 25.6 c
SLWIT CDCL3
EXREF 0.00 ppm
BF 0.20 Hz
RGAIN 26

```



DFILE C:\WINNMR\98\COMMON\卒業生\Yokada\MM2\

COUNT

DATIM Tue Apr 16 15:23:39 2002

ORINC 1H

EXND N0W

OBRQ 270.05 MHz

OBSET 112.00 kHz

OBFIN 5800.0 Hz

POINT 16384

FREQU 5402.4 Hz

SCANS 32

ACQW 3.033 sec

PD 3.967 sec

PW1 5.1 us

IRUC 1H

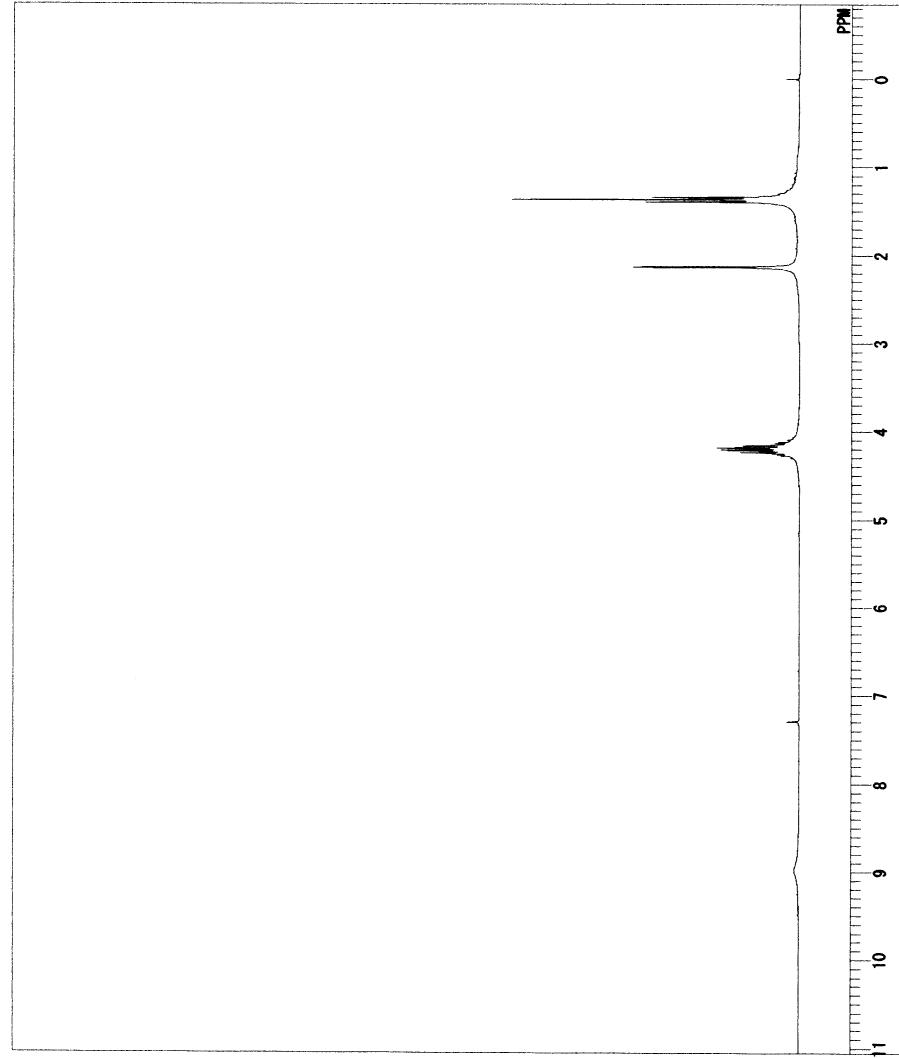
CTEMP 23.5 c

SLWT CDCL3

EXREF 0.00 ppm

BF 0.20 Hz

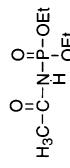
RGAIN 16



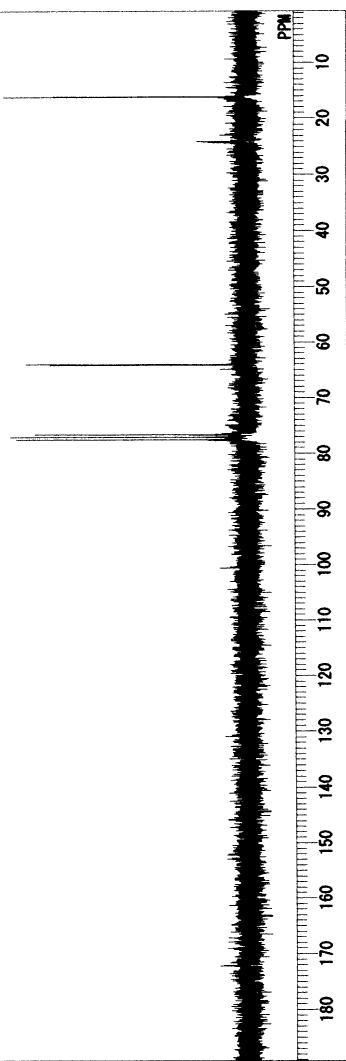
FILE C:\WINNMR90\COMMON\卒業生\Yokada\MM2\W-acetyl phosphoramidateカラム2 13C

DATE Tue Apr 16 15:49:18 2002

COINT  
 DATW  
 DNUC 13C  
 EXMOD B6M  
 F2FREQ 67.80 MHz  
 OFFSET 135.00 kHz  
 OBSV.FIN 5290.0 Hz  
 POINTS 32768  
 FREQU 18315.0 Hz  
 SCANS 300  
 ACQTM 1.769 sec  
 PD 1.211 sec  
 PW1 4.4 us  
 IRNUC 1H  
 CTMP 24.5 c  
 SVNT CDCL3  
 EAREF 77.00 ppm  
 BF 0.20 Hz  
 RGAIN 32

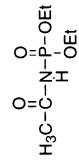
<sup>13</sup>C NMR spectrum

11

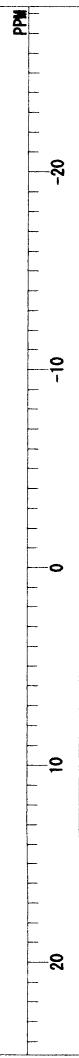


DFILE C:\WINNMR98\COMMON\卒業生\Yokada\NN2NN-acetyl phosphoramideカラム後2.als  
COMMENT  
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ORIGIN 31P  
EXMD B0M  
OBPRQ 109.25 Hz  
OBSET 121.10 kHz  
OBFIN 36.4 Hz  
POINT 32768  
FREQU 40000.0 Hz  
SCANS 16  
ACQIM 0.819 sec  
PD 5.000 sec  
PW1 7.1 us  
IRUC 1H  
CTEMP 24.6 c  
SLWIT CDCl3  
EXREF 0.00 ppm  
BF 0.20 Hz  
RGAIN 24

<sup>31</sup>P NMR spectrum



11



C:\WINNMR\88\COMMON\DATA\Yokada\MM2\Pro-Et-Prolinamide-Di-Et-Phosphoramidate 1H.als

C:\WINNMR\88\COMMON\DATA\Yokada\MM2\Pro-Et-Prolinamide-Di-Et-Phosphoramidate

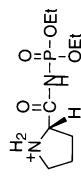
Prolinamide-Di-Et-Phosphoramidate  
Thu Jun 13 20:35:17 2002

FILE  
CONT  
DATIM  
OBNUC  
EXMOD  
OBFRQ  
OBSET  
OBPN  
POINT  
FREQU  
SCANS  
ACQTM  
PD  
PW1  
TRNUC  
CTEMP  
SLVNT  
EXREF  
BF  
RGAIN

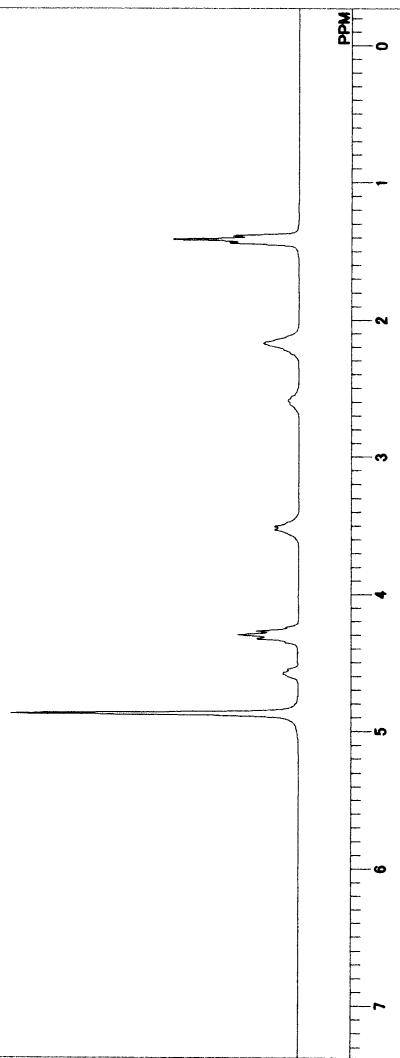
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163B4  
54.024 Hz  
25  
3.033 sec  
3.987 sec  
5.0 us

1H  
24.6 c  
D2O  
0.00 ppm  
0.20 Hz  
12

<sup>1</sup>H NMR spectrum



13



C:\WINNMR\98\COMMON\DATA\Yokada\MM2\Prolinamide-Di-Et-Phosphoramidate 13C.als

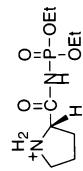
C:\WINNMR\98\COMMON\DATA\Yokada\MM2\Prolinamide-Di-Et-Phosphoramidate

FILE  
COMMENT  
DATE  
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EXMOD  
OBFRQ  
OBSET  
OBPN  
POINT  
FREQU  
SCANS  
ACQTM  
PD  
PW1  
IRNUC  
CTEMP  
SLVNT  
EXREF  
BF  
RGAIN

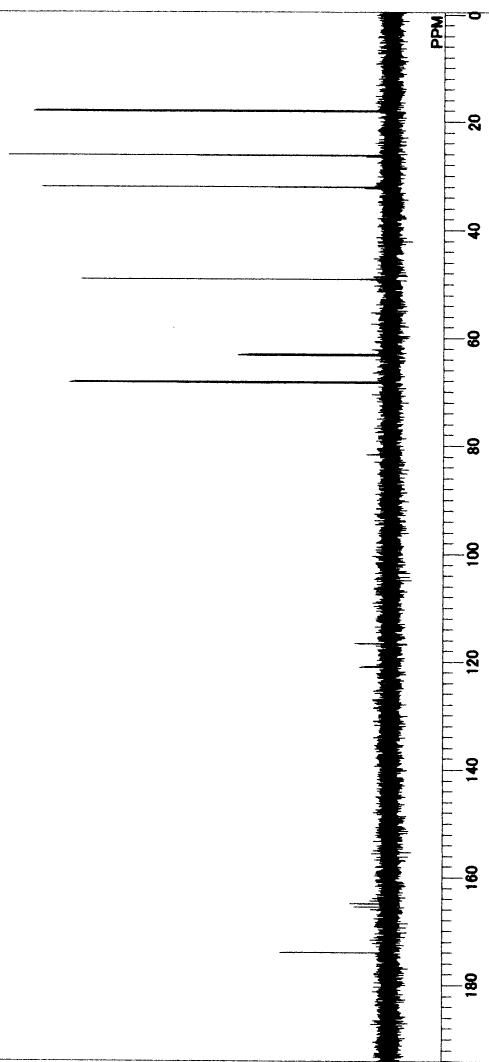
Thu Jun 13 21:24:33 2002  
13C  
BGM  
13C  
67.80 MHz  
135.00 kHz  
5200.0 Hz  
32768  
18315.0 Hz  
888  
1.789 sec  
1.211 sec  
5.2 us

13C  
BGM  
13C  
67.80 MHz  
135.00 kHz  
5200.0 Hz  
32768  
18315.0 Hz  
888  
1.789 sec  
1.211 sec  
5.2 us

<sup>13</sup>C NMR spectrum



13

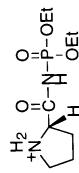


C:\WINNMR\98\COMMON\DATA\Yokade\WIN2K\Pro-Et2P-Prolinamide-Di-Et-Phosphoramidate  
Prolinamide-Di-Et-Phosphoramidate  
Thu Jun 13 20:29:41 2002

DFILE  
COMNT  
DATIM  
OBNUC  
EXMOD  
BCM  
OBFRQ  
OBSET  
OBFIN  
POINT  
FREQJ  
SCANS  
ACQTM  
PD  
PW1  
IRNUC  
CTEMP  
SLVNT  
EXREF  
BF  
RGAIN

31P  
108.25 MHz  
121.10 kHz  
38.4 Hz  
32768  
40000.0 Hz  
16  
0.819 sec  
5.000 sec  
7.1 us  
1H  
25.6 c  
D2O  
0.00 ppm  
0.20 Hz  
22

<sup>31</sup>P NMR spectrum



13



C:\WINNMR\8\COMMON\DATA\Acetamide-Phosmididine

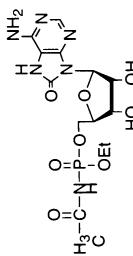
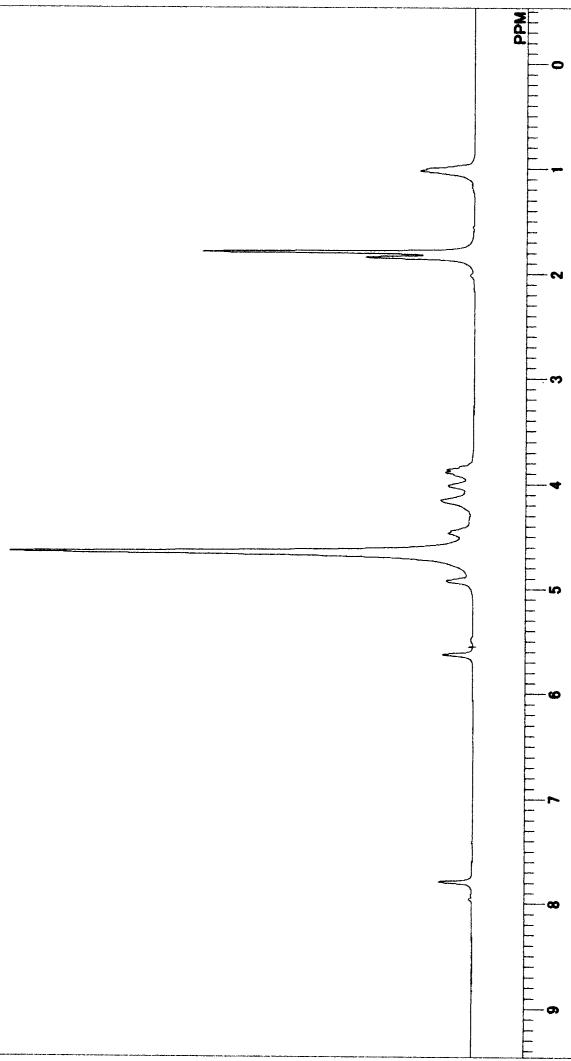
C:\WINNMR\8\COMMON\DATA\Acetamide-Phosmididine

Acetamide-Phosmididine

```

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EXMOD          270.05 MHz
OBFRQ          112.00 kHz
OBSET          5800.0 Hz
OBPN          16384
POINT          54024.4 Hz
FREQU          22
SCANS          3.033 sec
ACQTM          3.867 sec
PD              5.0 us
PW1            1H
TRNUC          22.5 c
CTEMP          D2O
SLVNT          4.65 ppm
EXREF          0.20 Hz
BF              11
RGAIN

```

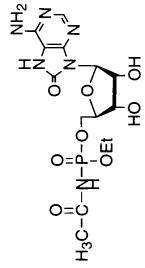
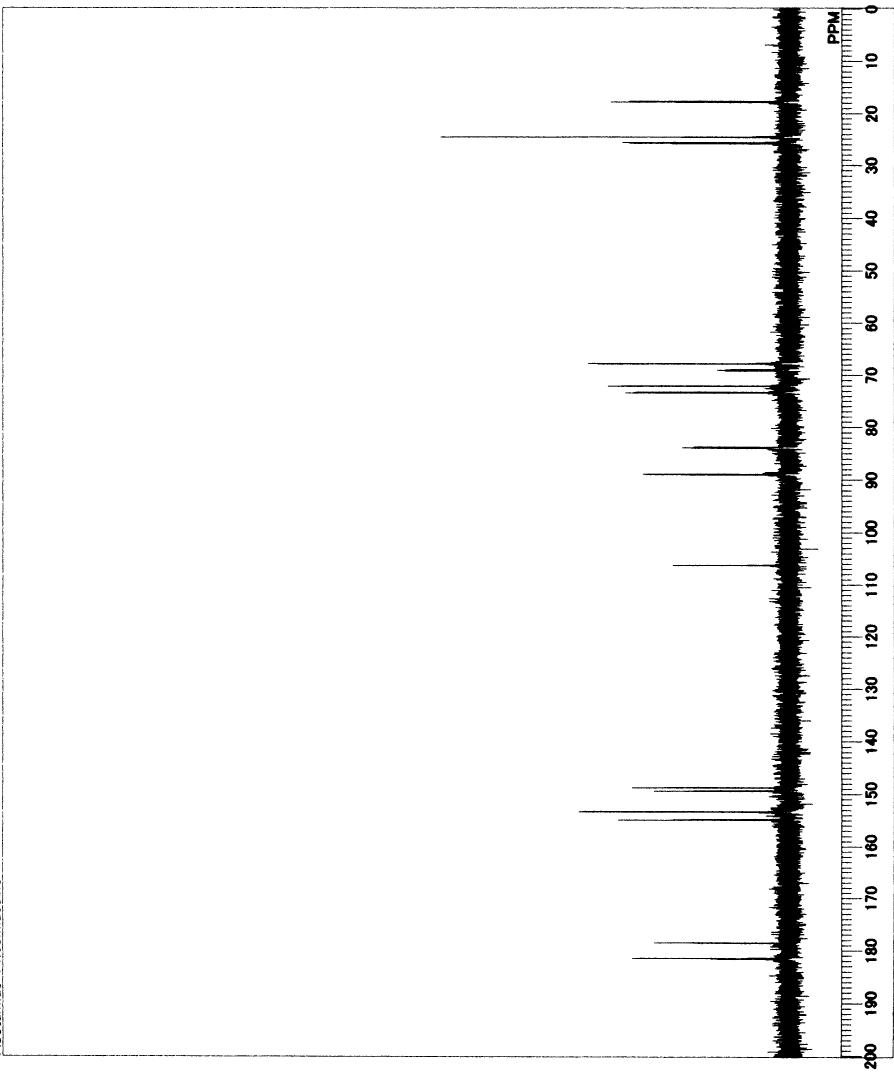


C:\WININNMR98\COMMON\DATA\okada\Acetamide-Phosmidosine 13Cals

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C:\WININNMR98\COMMON\DATA\okada\Acetamide-Phosmidosine
Acetamide-Phosmidosine
13C
Fri Jun 21 14:47:50 2002
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COMNT
DATIM
OBNUC
EXMOD
OBFRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
TRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN
13C
BCM
67.80 MHz
133.00 kHz
5200.0 Hz
32768
18315.0 Hz
4000
1.789 sec
1.211 sec
5.2 us
1H
24.2 c
D2O
0.00 ppm
0.20 Hz
28

```



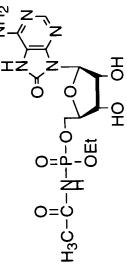
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Fri Jun 21 11:24:13 2002
Acetamide-Phosmidosine

DFILE
CONINN
DTIM
ORNUC
EXMOD
BCM
OBFRQ
109.25 MHz
121.10 kHz
OBSET
36.4 Hz
OBPN
327.88
POINT
40000.0 Hz
FREQU
SCANS
40
ACQTM
0.819 sec
PD
5.000 sec
PW1
7.1 us
TRNUC
1H
CTEMP
24.8 c
SLVNT
D2O
0.00 ppm
EXREF
BF
0.20 Hz
RGAIN
23

```

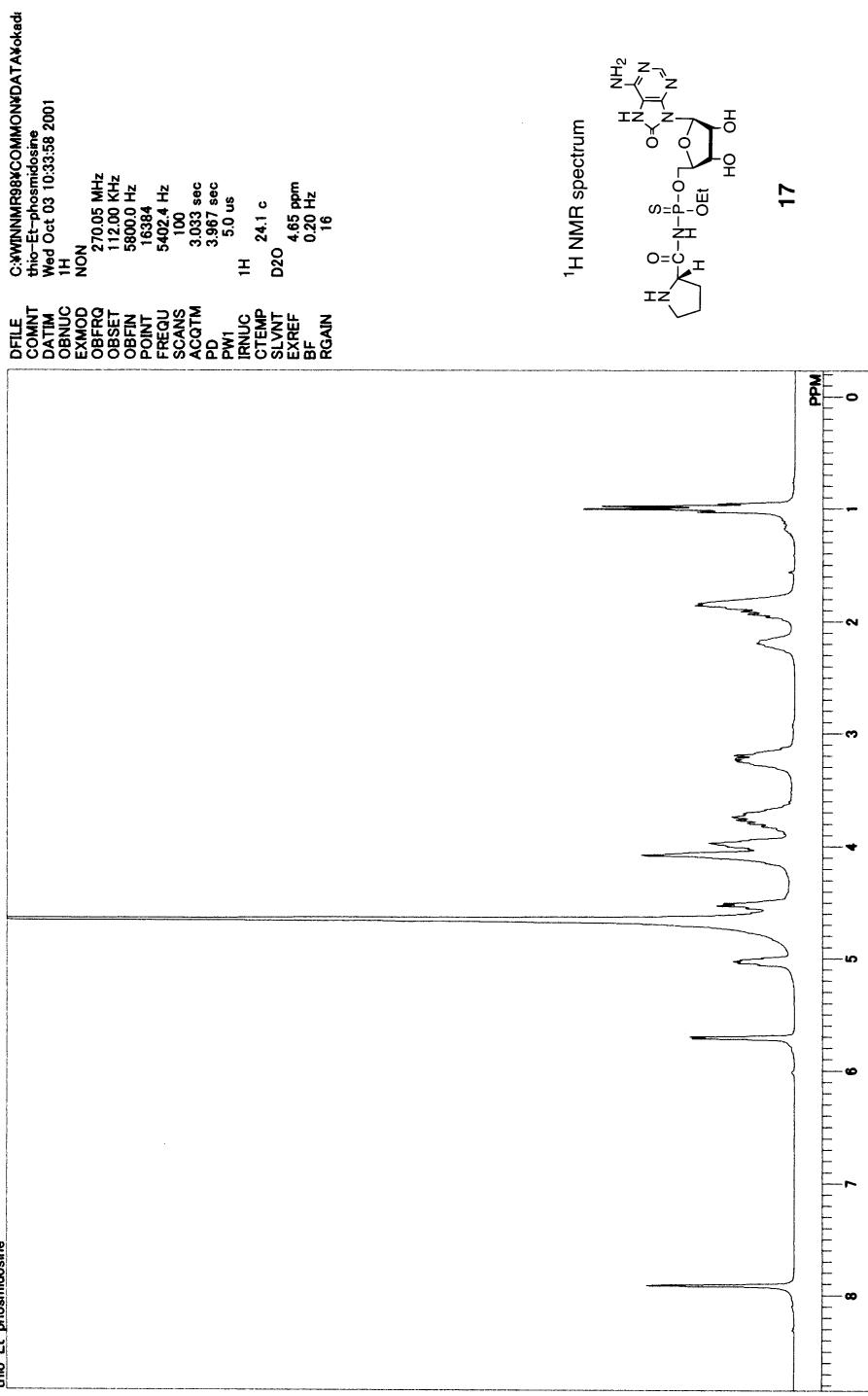
$^{31}\text{P}$  NMR spectrum



15



C:\WINNMR\98\COMMON\DATA\okada\thio-Et-phosmidosine\1Hals



C:\WININNMR98\COMMON\DATA\okada\Y13C\als  
thio-Et-phosmidosine

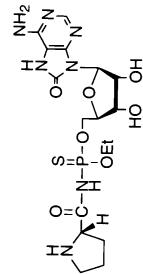
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thio-Et-phosmidosine  
Wed Oct 03 13:58:33 2001

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EXMOD  
OBFRQ  
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OBFIN  
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PW1  
TRNUC  
CTEMP  
SLVNT  
EXREF  
BF  
RGAIN

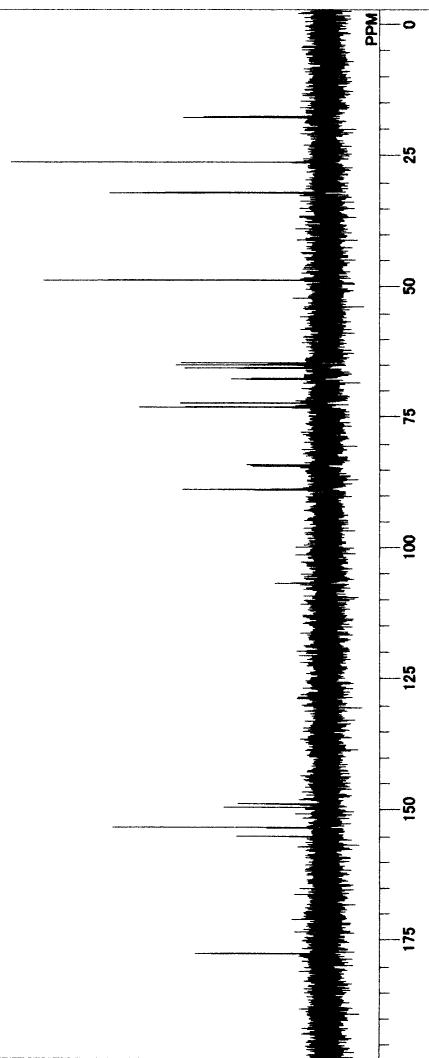
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BCM  
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135.00 kHz  
5200.0 Hz  
32768  
18315.0 Hz  
2536  
1.789 sec  
1.211 sec  
5.2 us

1H  
D2O  
24.7 c  
0.00 ppm  
0.20 Hz  
28

<sup>13</sup>C NMR spectrum



17



C:\WINNMR\98\COMMON\DATA\Yokada\MM1\thio-Et-phosmidosine31P.xls

thio-Et-phosmidosine

Wed Oct 03 10:18:37 2001

DFILE COMNT  
DATM thio-Et-phosmidosine  
OBNUC 31P  
EXMOD BCM  
OBFRQ 109.25 MHz  
OBSET 121.10 kHz  
OBFIN 36.1 Hz  
POINT 327.98

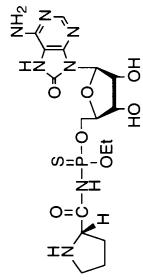
FREQU 40000.0 Hz  
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PD 5.000 sec  
PWI 7.1 us

JNUC 1H  
CTEMP 25.3 c

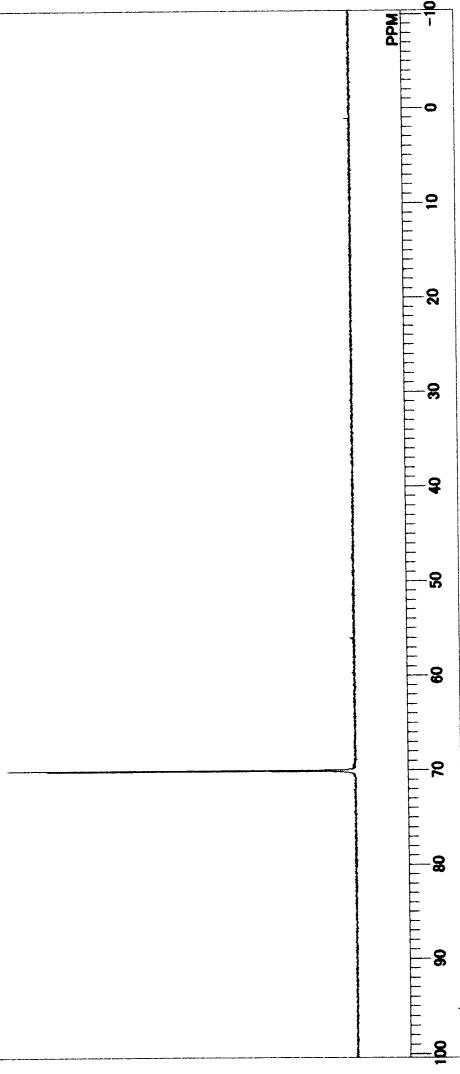
SLVNT D2O  
EXREF 0.00 ppm

BF 0.20 Hz  
RGAIN 25

<sup>31</sup>P NMR spectrum



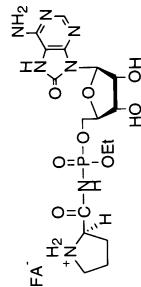
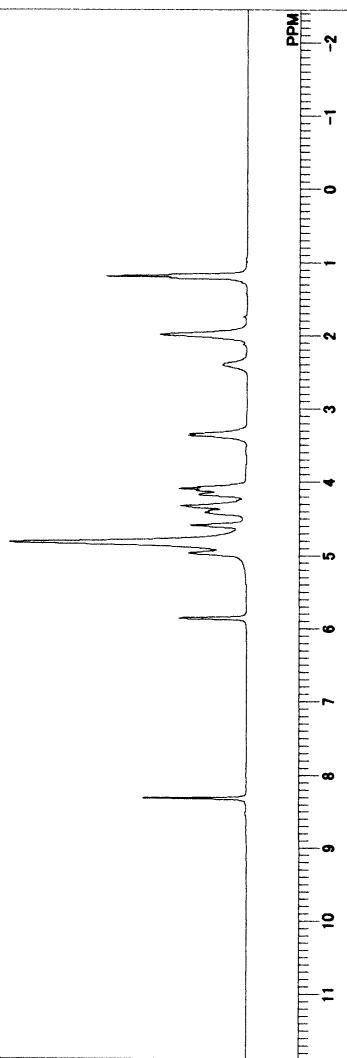
17



C:WINNMR98\COMMON\DATA\kada\W\amino acid substitution\D-proline\Phosmidosine 1H final.xls  
D-Proline Phosmidosine

C:\WINNNMB98\COMMONDATA\TAX\dat

DFILE	COMNT
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OBNUC	EXMOD
OBFRQ	OBSET
OBFIN	POINT
FREQU	SCANS
ACQTM	PD
PW1	IRNUC
CTEMP	SILVNT
EXREF	BF
RGAIN	



<sup>1</sup>H NMR spectrum

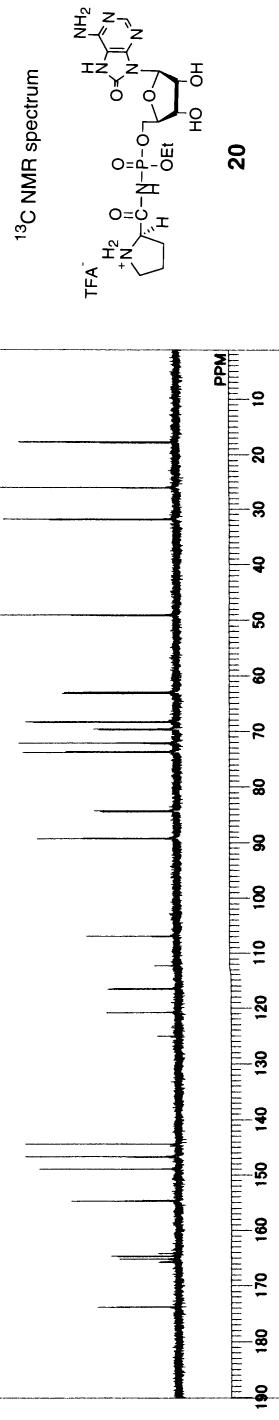
20

C:\WINNMR\98\COMMON\DATA\Wokada\W2\amino acid substitution\MD-Proline Phosmidosine 13C final.sis  
D-Proline Phosmidosine

```

DFILE
COMNT
DATM
OBNUC
EXMOD
OBFRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
IRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN
1H
24.0.c
D2O
0.00 ppm
0.20 Hz
28
13C
Wed May 29 14:13:37 2002
BCM
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135.00 kHz
5200.0 Hz
32788
18315.0 Hz
2950
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5.2 us

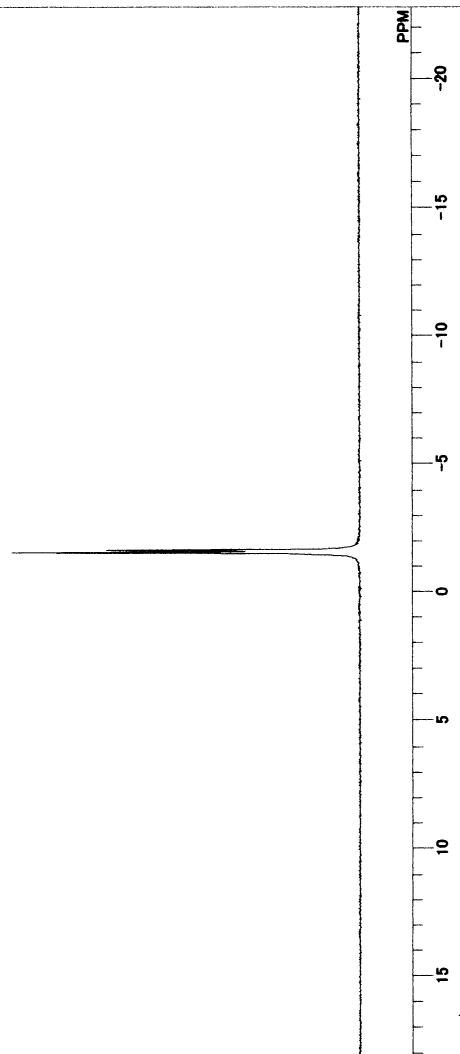
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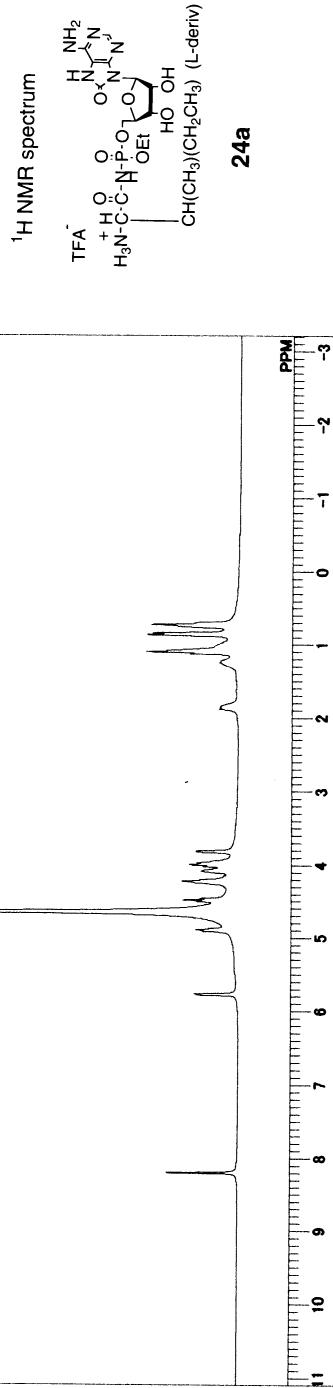
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C:\WINNMR\88\COMMON\DATA\Yokada\W2\amino acid substitution\Ile-Phosmidosine.siw slow peak 1H.als

DFILE  
CONNT  
DTIMC  
OBNUC  
EXMOD  
OBFRQ  
OBSET  
OBPN  
POINT  
FREQU  
SCANS  
ACQTM  
PD  
PW1  
TRNUC  
CTEMP  
SLVNT  
EXREF  
BF  
RGAIN

Thu May 30 20:33:45 2002  
Ile-Phosmidosine slow peak  
1H  
NON  
270.05 MHz  
112.00 kHz  
5800.0 Hz  
163B4  
5402.4 Hz  
64  
3.033 sec  
3.987 sec  
5.00 us



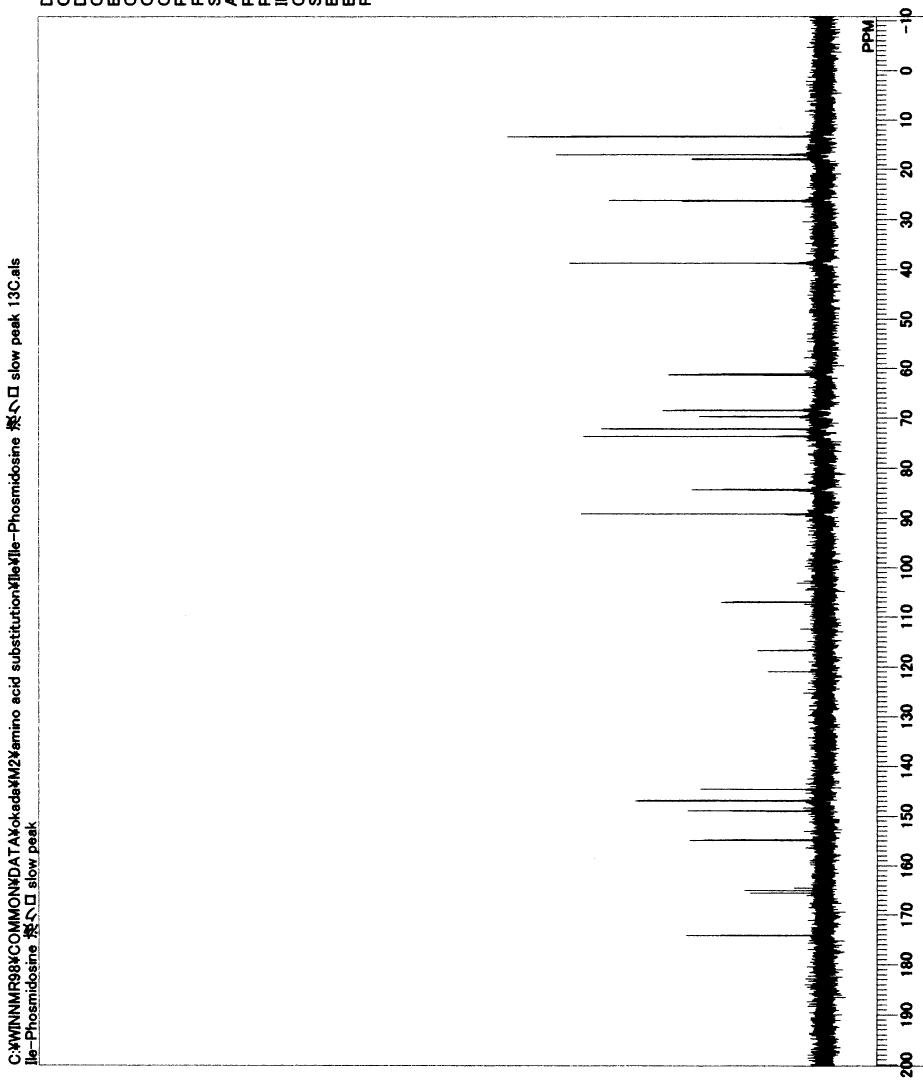
C:\WINNMR\COMMON\DATA\Amokade\W2\fermino acid substitution\Ile-Phosmidosine \ slow peak

```

C:\WINNMR\COMMON\DATA\Amokade\W2\fermino acid substitution\Ile-Phosmidosine \ slow peak
Thu May 30 19:10:49 2002

DFILE
COMNT
DATIM
OBNUC
EXMOD
OBFRQ
OBSET
OBFIN
POINT
32768
FREQU
18315.0 Hz
SCANS
8985
ACQTM
1.785 sec
PD
1.211 sec
PW1
5.2 us
IRNUC
1H
CTEMP
24.7 c
SLVNT
D2O
0.00 ppm
EXREF
BF
0.20 Hz
RGAIN
28

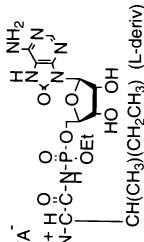
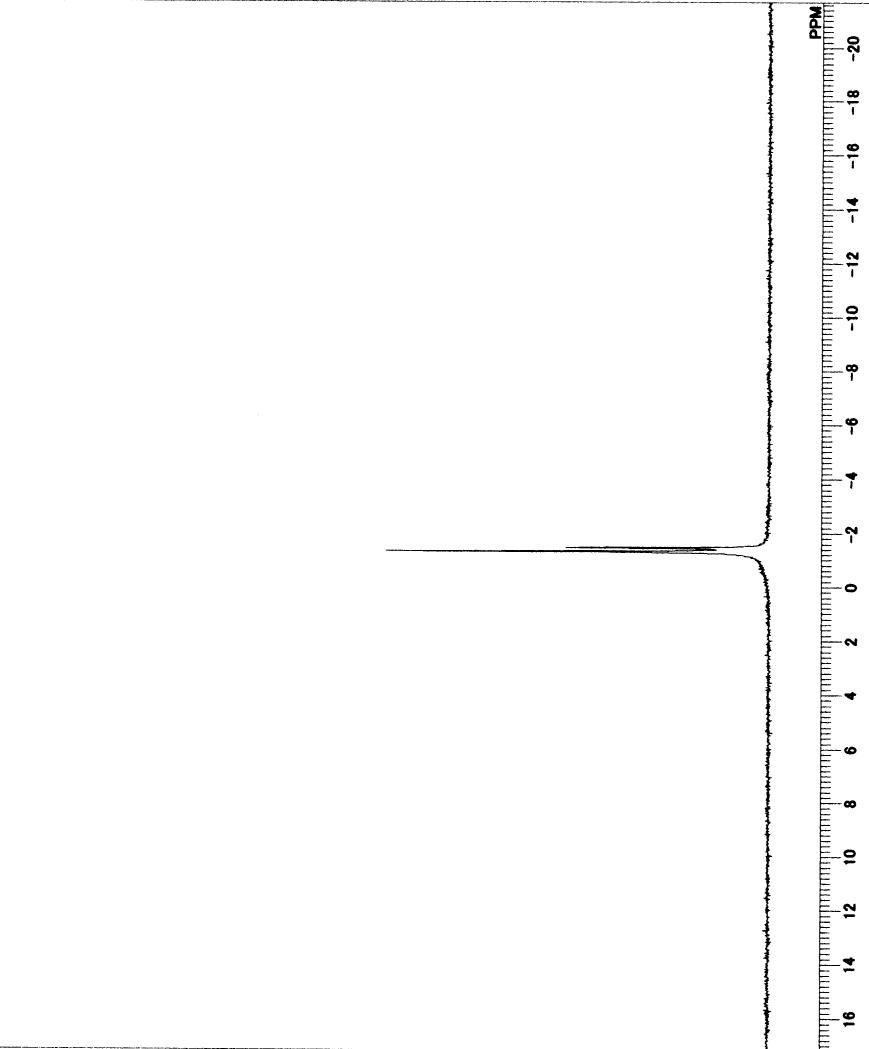
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C:\WINMM\98\COMMONDATA\kodata\W2Y amino acid substitution\Ile-Phosmidosine 慢い slow peak.als  
Ile-Phosmidosine 慢い slow peak

C:\WINNMR98\COMMON\DATA\Xokadi

DFILE

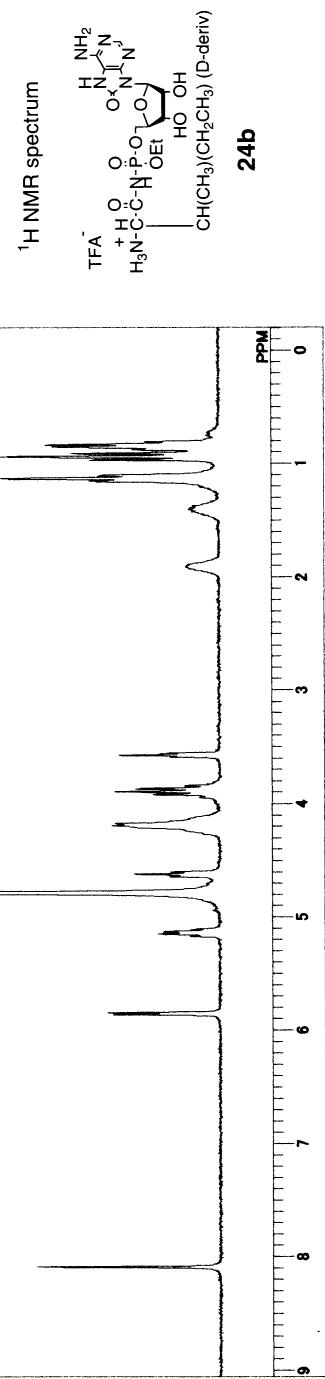


24a

C:\WINNMR8\COMMON\DATA\Yokada\\*.\* D-Ile-D-Ile-Phos 1H.ais  
D-Ile-Phosmidosine

C:\WINNMR\98\COMMON\DATA\Yokada  
D-ile-Phosmidosine  
Thu Jan 16 10:26:05 2003  
1H  
NON

DFILE COMNT DATIM OBNUC EXMOD OBFRQ OBSET OBFN POINT FREQU SCANS ACQTM PD PW1 IJNUC CTEMP SLVNT EXREF BF RGAIN



C:\WINNMR\98\COMMON\DATA\okada\NM2\amino acid substitution\Phosmididine.spc slow peak 13Cals

D-ile-Phosmididine.spc slow peak

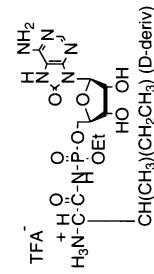
DFILE  
COMNT  
DATIM  
OBNUC  
EXMOD  
OBFRQ  
OBSET  
OBFIN  
POINT  
FREQU  
SCANS  
ACQTM  
PD  
PW1  
IRNUC  
OTEMP  
SLVNT  
EXREF  
BF  
RGAIN

Thu May 30 19:10:49 2002

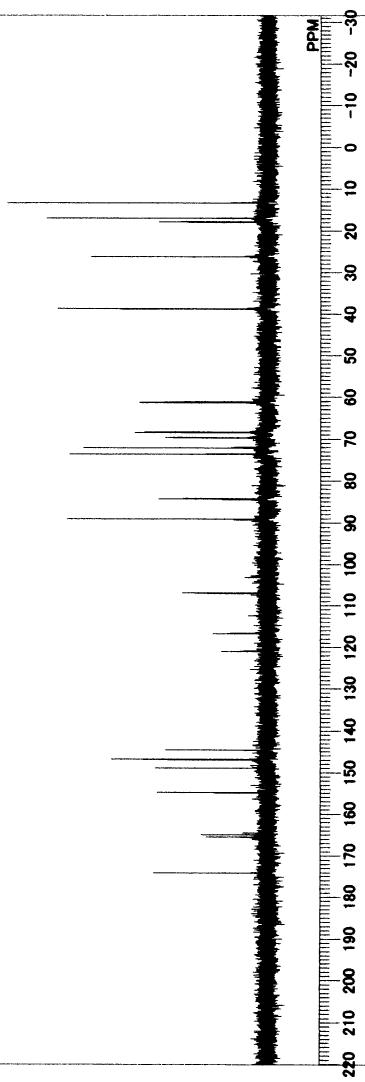
13C  
BCM  
67.80 MHz  
135.00 kHz  
5200.0 Hz  
32768  
18315.0 Hz  
8095  
1.789 sec  
1.211 sec  
5.2 us

1H  
D2O  
24.1 c  
0.00 ppm  
0.20 Hz  
28

<sup>13</sup>C NMR spectrum



24b



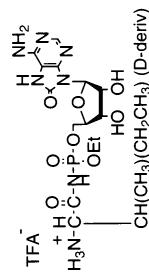
C:\WINNMR\98\COMMON\DATA\Nokada\W2\Amino acid substitution\Ile\Phosmidosine \&\ slow peak  
 D-Ile-Phosmidosine \&\ slow peak

```

C:\WINNMR\98\COMMON\DATA\Nokada\W2\Amino acid substitution\Ile\Phosmidosine \&\ slow peak
D-Ile-Phosmidosine \&\ slow peak
Thu May 30 11:50:42 2002
31P
BCM
EXMOD
OBNUC
OBFRQ
108.25 MHz
121.10 kHz
OBSET
36.4 Hz
OBFIN
32768
POINT
400000 Hz
FREQU
SCANS
77
ACQTM
0.819 sec
PD
5.000 sec
PW1
7.1 us
IRNUC
1H
CTEMP
25.1 c
SLANT
D2O
0.00 ppm
EXREF
BF
0.20 Hz
RGAIN
25

```

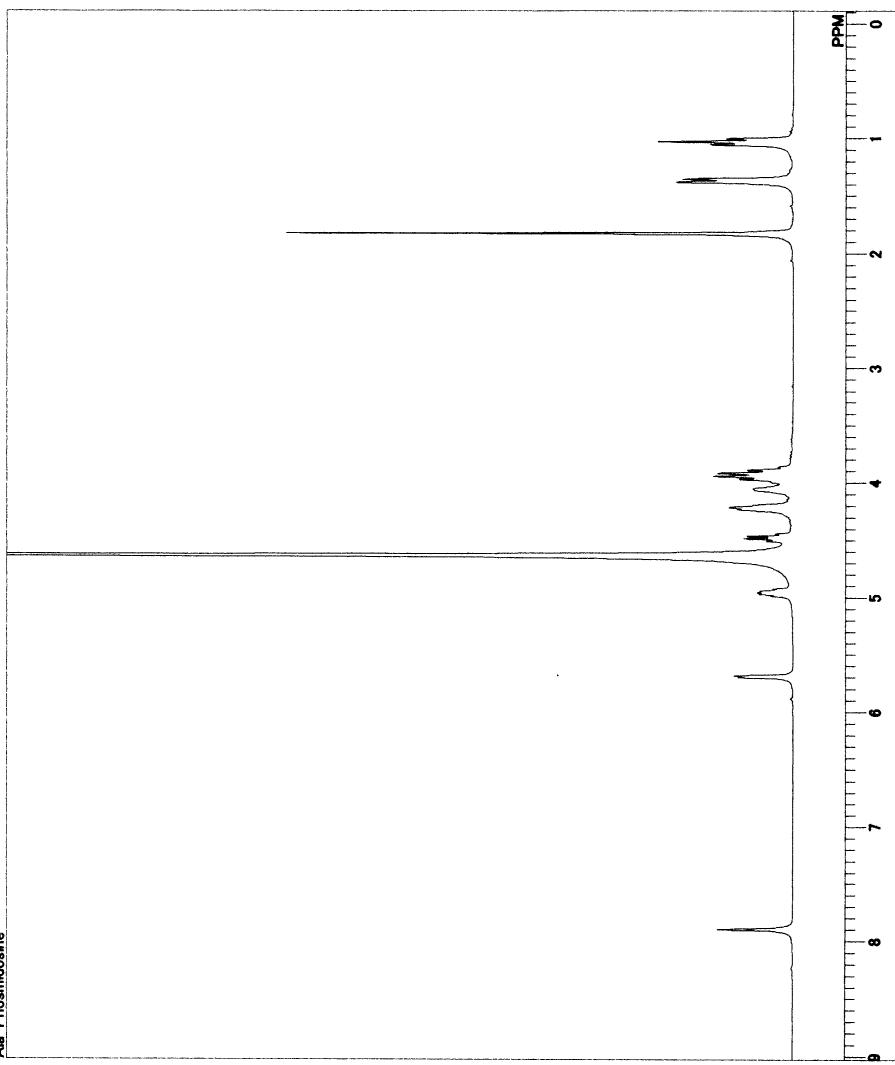
<sup>31</sup>P NMR spectrum



C:\WINNMR\99\COMMON\NDATA\Yokokawa\2\Xmino acid substitution\X\Ala\14C\Ala-Phosmididine 1H-als

C:\WINNMR\98\COMMON\NDATA\Yokokawa\1\Ala-Phosmididine

Ala-Phosmididine  
1H  
Fri Aug 09 15:30:01 2002

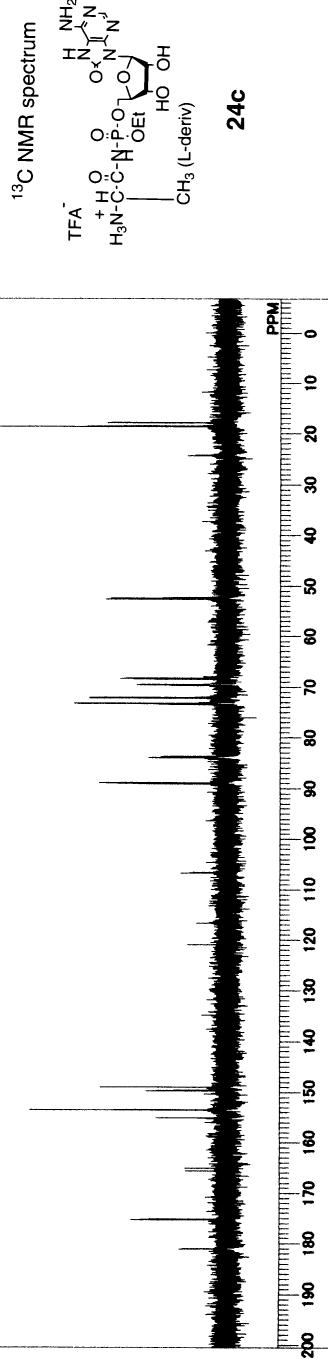


```

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C:\WINNMR98\COMMON\DATA\Yokada\W2\Amino acid substitution\Ala\Ala-Phosmidosine 13C.als

DFILE
COMINT
OBNUC
EXMOD
OBFRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
IRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN
13C
Tue Aug 06 22:14:53 2002
BCM
67.80 MHz
135.00 KHz
5200.0 Hz
32788
18315.0 Hz
12005
1.789 sec
1.211 sec
5.2 us
1H
24.4 C
D2O
0.00 ppm
0.20 Hz
28

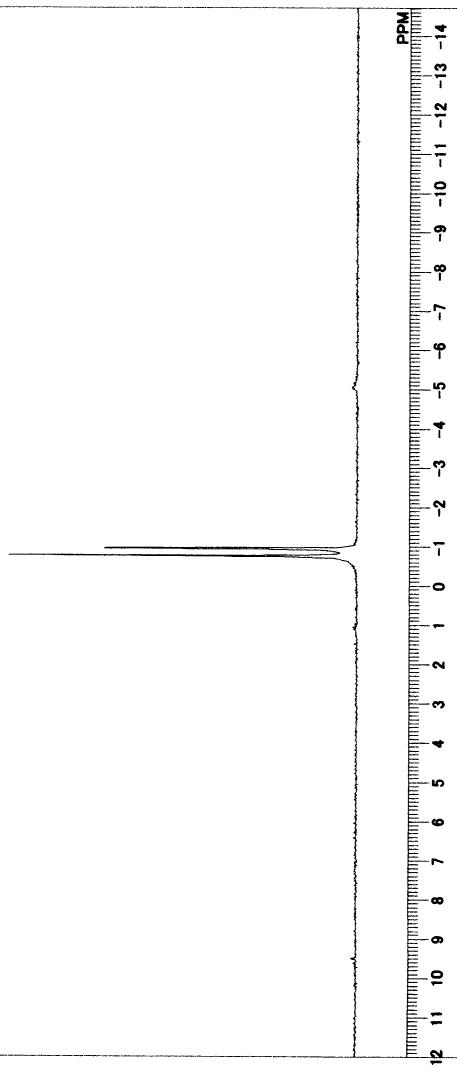
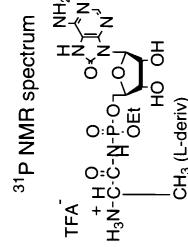
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```

C:\WINNNMR98\COMMON\NDAT\A\okada\Y\Ala-Y\Ala-Phosmidosine.xls
Ala-Phosmidosine
Tue Aug 06 11:28:45 2002
31P
B6M
B6M
109.25 MHz
121.10 kHz
36.4 Hz
32768
40000.0 Hz
121
0.819 sec
5.000 sec
7.1 us
1H
25.7 c
D2O
25.7 c
SLW111
0.00 ppm
0.20 Hz
25
ACQTM
PD
PW1
IRNUC
CTEMP
SLW111
EXRF
BF
RGAIN

```



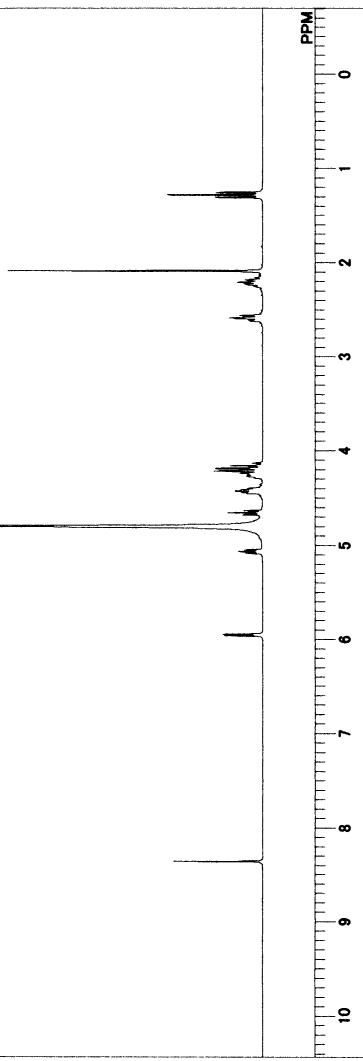
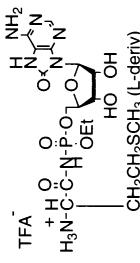
C:\WINNMR\COMMON\DATA\okada\N2\amino acid substitution\Met\Met-Phosmidosine fast 1Hals  
Met-Phosmidosine fast

C:\WINNMR\COMMON\DATA\okada\N2\amino acid substitution\Met\Met-Phosmidosine fast 1Hals

```
DFILE
COMNT
DATIM
OBNUC
EXMOD
OBFRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
TRNUC
OTEMP
SLVNT
EXREF
BF
RGAIN
```

```
1H
Tue Sep 03 11:17:43 2002
Met-Phosmidosine fast
1H
NON
270.05 MHz
112.00 kHz
5800.0 Hz
16384
5402.4 Hz
484
3.033 sec
3.967 sec
5.0 us
1H
23.3 c
D2O
0.00 ppm
0.20 Hz
19
```

<sup>1</sup>H NMR spectrum

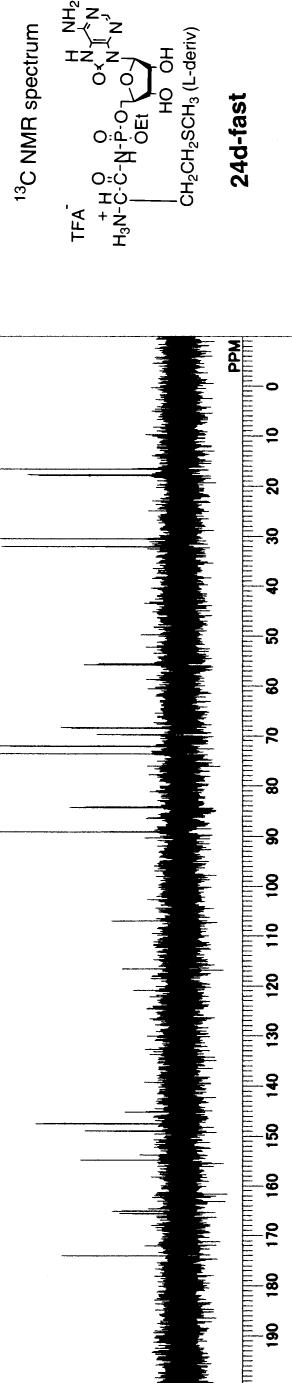


**24d-fast**

C:\WINNMR\98\COMMON\DATA\okada\

```
DFILE          COMNT
              DATIM
              OBNUC   13C
              EXMOD
              OBFRQ  67.80 MHz
              OBSET  135.00 kHz
              OBFIN  5200.0 Hz
              POINT  32768
              POINT
              FREQU  18315.0 Hz
              SCANS  12706
              ACQTM
              PD
              PW1    1.211 sec
              PW1    5.2 us
              IRNUC
              CTEMP
              SLVNT
              EXREF
              BF
              RGAIN
```

```
C:\WINNMR\98\COMMON\DATA\okada\fast
Met-Phosmididine fast
```



C<sub>2</sub>H<sub>11</sub>NNR<sub>2</sub>COMMON DAT Atokada W2 amino acid substitution Met<math>\rightarrow</math>Met-Phosmidosine fast 31P als  
Met-Phosmidosine fast

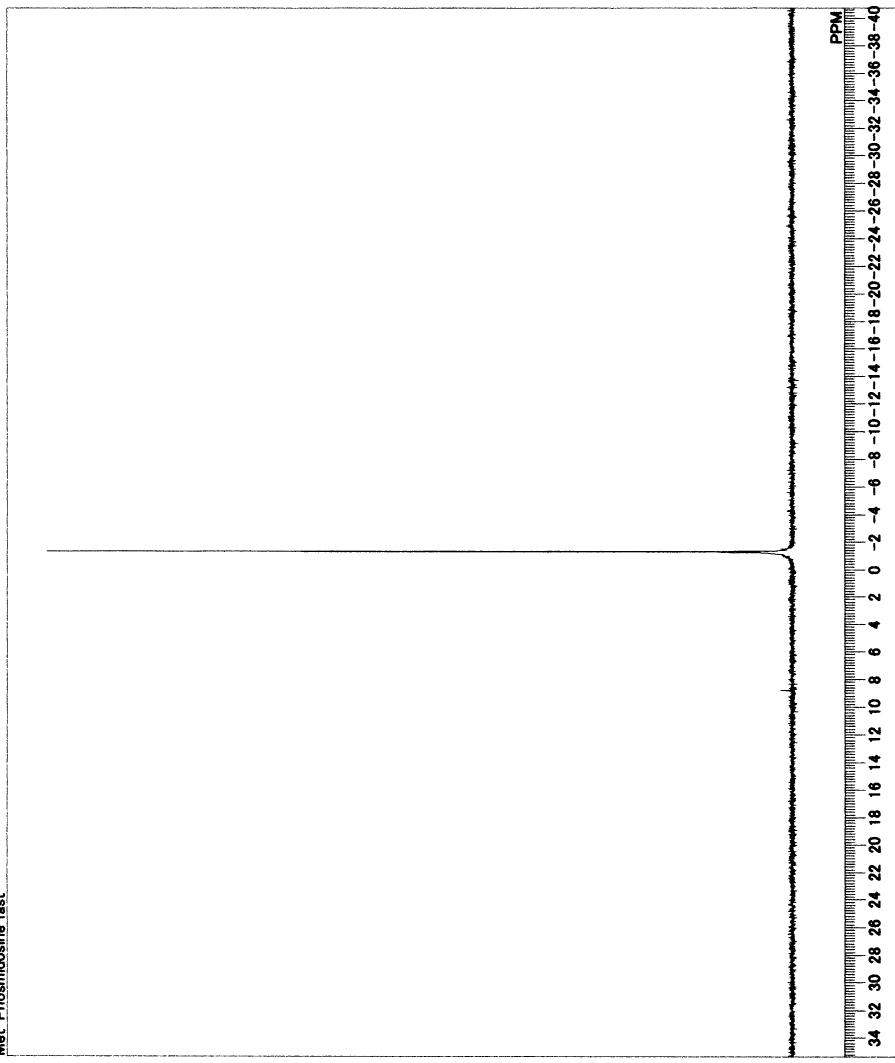
C:\WINNMR98\COMMON\DATA\skach

```

C:\win32\pysim\simulations\metpharm\metpharm.psf

Met-Pharmodine Fast
Mon Sep 02 10:54:52 2002
31P
BCM
109.25 MHz
121.10 kHz
36.4 Hz
32/68
40000.0 Hz
196
0.819 sec
ACQ7M
5.000 sec
PD
PMW
IRNUC
CTEMP
SLWNT
EXPERF
BF
RGAIN

```

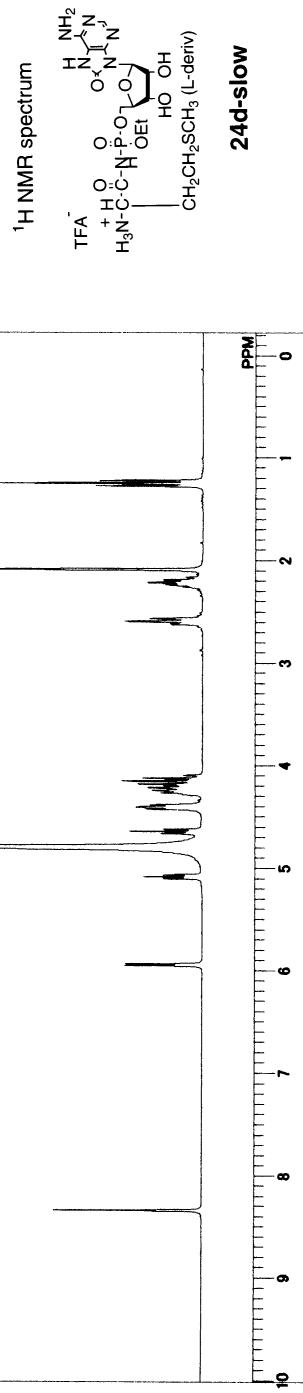


C:\WINNMR\98\COMMON\DATA\Yokada\W2\Amino acid substitution\Met\Met-Phosmididine slow\1Hals  
Met-Phosmididine slow

```

DFILE: C:\WINNMR\98\COMMON\DATA\Yokada\W2\Amino acid substitution\Met\Met-Phosmididine slow\1Hals
COMNT: Met-Phosmididine slow
DTIM: Tue Sep 03 10:11:37 2002
OBNUC: 1H
EXMOD: NON
OBFRQ: 270.05 MHz
OBSET: 112.00 kHz
OBFIN: 5800.0 Hz
POINT: 16384
FREQU: 5402.4 Hz
SCANS: 174
ACQTM: 3.033 sec
PD: 3.96 sec
PW1: 5.0 us
IRNUC: 1H
CTEMP: 22.8 c
SLVLT: D2O
EXREF: 0.00 ppm
BF: 0.20 Hz
RGAIN: 19

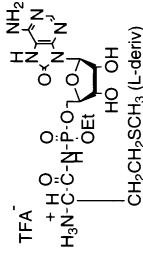
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C:\WINNMR\98X\COMMON\DATA\Akada\13C\aln  
Met-Phosmididine slow

DFILE COMINT  
COMMENT  
OBNUC DATIM  
EXMOD Tue Sep 03 09:06:44 2002  
OBFRQ 13C  
BCM 67.80 MHz  
OBSET 135.00 kHz  
OBFIN 5200.0 Hz  
POINT 32768  
FREQU 183150 Hz  
SCANS 1350  
ACQTM 1.780 sec  
PD 1.211 sec  
PW1 5.2 us  
IRNUC 1H  
CTEMP 23.8 c  
SLVNT D2O  
EXREF 0.00 ppm  
BF 0.20 Hz  
RGAIN 28

<sup>13</sup>C NMR spectrum



C:\WINNMR\98\COMMON\DATA\Nakada\N2\Amino acid substitution\Met\2\Met-Phosmidosine slow\Met-Phosmidosine slow

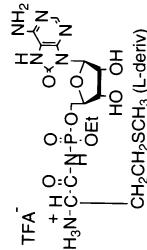
C:\WINNMR\98\COMMON\DATA\Nakada\

```

DFILE      COMINT
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OBNUC     Met-Phosmidosine slow
31P
EXMOD    BCM
OBFRQ    109.25 MHz
OBSET    121.10 kHz
OBFIN    38.4 Hz
POINT    32768
FREQUJ   40000.0 Hz
SCAIS    457
ACQTM    0.819 sec
PD       5.000 sec
PW1      7.1 us
IRNUC    1H
CTEMP    D2O 23.9 c
SLVNT    0.00 ppm
EXREF    BF 0.20 Hz
RGAIN    27

```

<sup>31</sup>P NMR spectrum



24d-slow

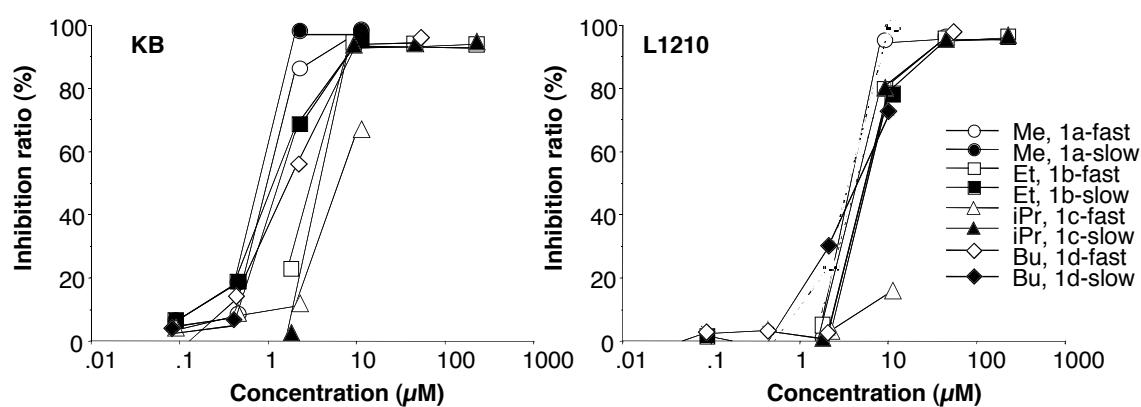


## **Assay of *In Vitro* Antitumor Activity**

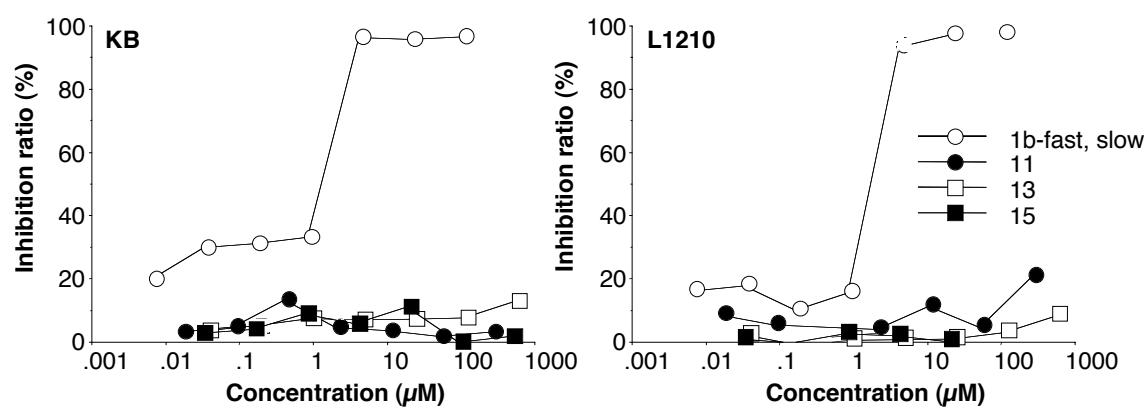
The tetrazolium-based semi-automated colorimetric assay (MTT assay) developed by Carmichael *et al.*\* was modified and used to determine the *in vitro* antitumor activity of phosphomodine analogs. The activity was determined by using mouse leukemia L1210 cells and human epidermoid carcinoma KB cells. Roswell Park Memorial Institute Medium 1610 supplemented with 10% heat-inactivated fetal bovine serum and 50 µg/ml of kanamycin was used as the cell culture medium. Tumor cells ( $2 \times 10^3$  cells/well) plated into flat-bottomed 96 well plates (NUNC, Roskilde, Denmark) were incubated in a CO<sub>2</sub> gas incubator at 37° C for 72 h in 200 µl of medium containing various concentrations of the test compounds. Cell growth was measured by using MTT reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, Missouri, U.S.A.). After the addition of 25 µl of MTT solution (2 mg/ml), each well was incubated at 37°C for an additional 4 h. Then the medium was removed and 200 µl of dimethyl sulfoxide (DMSO) were added. After mixing with a mechanical plate mixer for 5 min, absorbance at 540 nm was measured with ImmunoReader NJ-2000 (Nippon InterMed, Tokyo, Japan). The percentage of cell growth inhibition was calculated by the following formula: % inhibition = [1 - OD of sample wells/OD of control wells] x 100. The IC<sub>50</sub> (µM) was given as the concentration at 50% inhibition of cell growth. Its value was determined graphically from the dose-response curve with at least three drug concentration points.

\*Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.*

**1987, 47, 936-942.**



**Figure 7 (The original data of Table 2)**  
**Inhibitory effects of phosmidosine analogues on the growth of KB and L1210 cells**



**Figure 8 (The original data of Table 4)**  
**Inhibitory effects of phosmidosine analogues lacking partial structures on the growth of KB and L1210 cells**

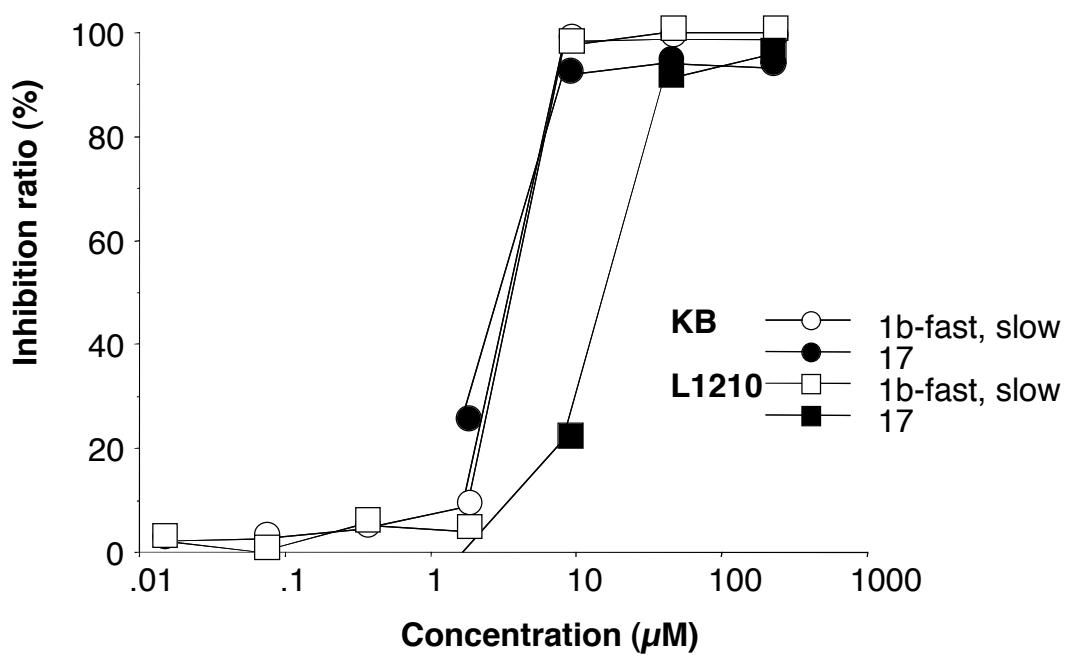


Figure 9 (The original data of Table 5)  
Inhibitory effects of phosphodenosine phosphoramidothioate  
on the growth of KB and L1210 cells

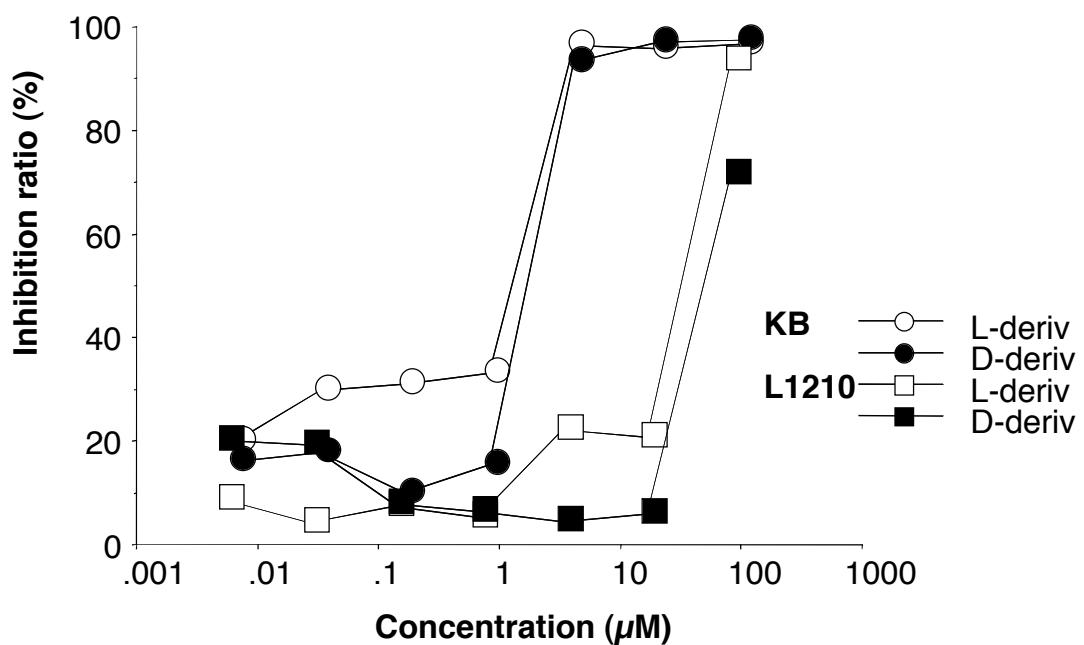
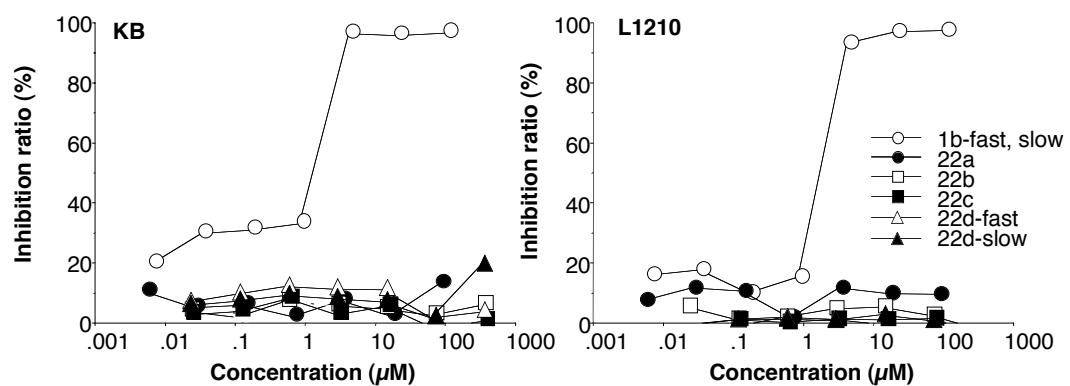


Figure 1 0 (The original data of Table 6)  
Inhibitory effects of phosmidosine analogues having a  
D-proline residue on the growth of KB and L1210 cells



**Figure 11 (The original data of Table 7)**  
**Inhibitory effects of phosmidosine analogues replaced by other amino acid residues on the growth of KB and L1210 cells**