MATERIALS AND METHODS

Metal chelating histidine binding resin was purchased from Novagen. Pyruvate kinase was purchased from Boehringer Mannheim. All other reagents were purchased from Sigma or Aldrich. Unless otherwise noted, all nonaqueous reactions were carried out under dry nitrogen atmosphere in flame dried glassware. CH_2Cl_2 was distilled immediately prior to use over CaH_2. DMF was purchased from Aldrich in sure/seal bottles and used without further purification. Analytical thin-layer chromatrography (TLC) was performed using general purpose 60-Å general-purpose silica gel on glass (Aldrich). TLC plates were visualized with aqueous $KMnO_4$. Flash chromatography columns were prepared with Kieselgel 60-Å silica gel 230–400 mesh (Merck). Proton (^1H), Carbon (^{13}C), and (^{31}P) NMR spectra were recorded on a Bruker ARX300 300 MHz spectrometer. IR spectra were recorded on a Perkin Elmer 1760X FT-IR spectrometer.

EXPERIMENTAL PROCEDURES

Phenylmethyl 5-(6,8-diaza-oxo-3-thiabicyclo[3.3.0]oct-2-yl) pentanoate (2). (+)–Biotin (10.20 g, 42 mmol), DMAP (0.51 g, 4.2 mmol), HOBt (0.64 g, 4.2 mmol) and benzyl alcohol (5.64 g, 52 mmol) were added to 60 mL of DMF in a 500 mL 3 neck flask. The mixture was stirred and heated until a homogenous solution was obtained. DCC (46.2 mL, of a 1 M solution in CH_2Cl_2) was added over 10 min and the reaction mixture was stirred 12 h at rt. The reaction mixture was filtered and the filtrate concentrated *in vacuo*. The resultant solid was dissolved in hot acetone (50 °C) and purified by flash chromatography with 10% MeOH:90% EtOAc to afford a white solid 2 (13.3 g, 95%): mp 79 °C.

Phenylmethyl 5-(6,8-diaza-6-(2-chloroacetyl)-7-oxo-3-thiabicyclo[3.3.0]oct-2-yl)pentanoate (3). **2** (5.6 g, 17 mmol) and Et_3N (7.06 mL, 51 mmol) were added to 40 mL of CH_2Cl_2. Chloroacetylchloride (1.95 mL, 25.2 mmol) was added in three portions. The mixture was stirred and brought to –78°C and (0.65 mL, 8.4 mmol) of chloroacetylchloride was added. The mixture was stirred for 12 hours and allowed

to warm to room temperature. This series of steps was repeated 2 more times. The reaction mixture was filtered and the filtrate was evaporated *in vacuo* to a thick oil, which was dissolved in CH_2Cl_2 and purified by flash chromatography with, 4% MeOH, 48% hexane, 48% EtOAc to afford a yellowish oil 3 (6.9 g, 98%).

Phenylmethyl 5-(6,8-diaza-6-(2-diethoxyphosphono) acetyl)-7-oxo-3-thiabicyclo [3.3.0] oct-2-yl) pentanoate (4). (6.88 g, 17 mmol) of **3** and triethyl phosphite (20 mL) were added to a 100 mL 3 neck flask and heated to 100°C while stirring for 3 hours. The excess triethylphosphite was evaporated *in vacuo*. The resultant viscous oil was dissolved in CH_2Cl_2 and purified by flash chromatography using a gradient of 4% MeOH, 48% hexane, 48% EtOAc to 10% MeOH, 90% EtOAc, to give a yellowish oil **4** (7.6 g, 89%). Crystals were obtained by evaporation of a solution of **4** in EtOAc.

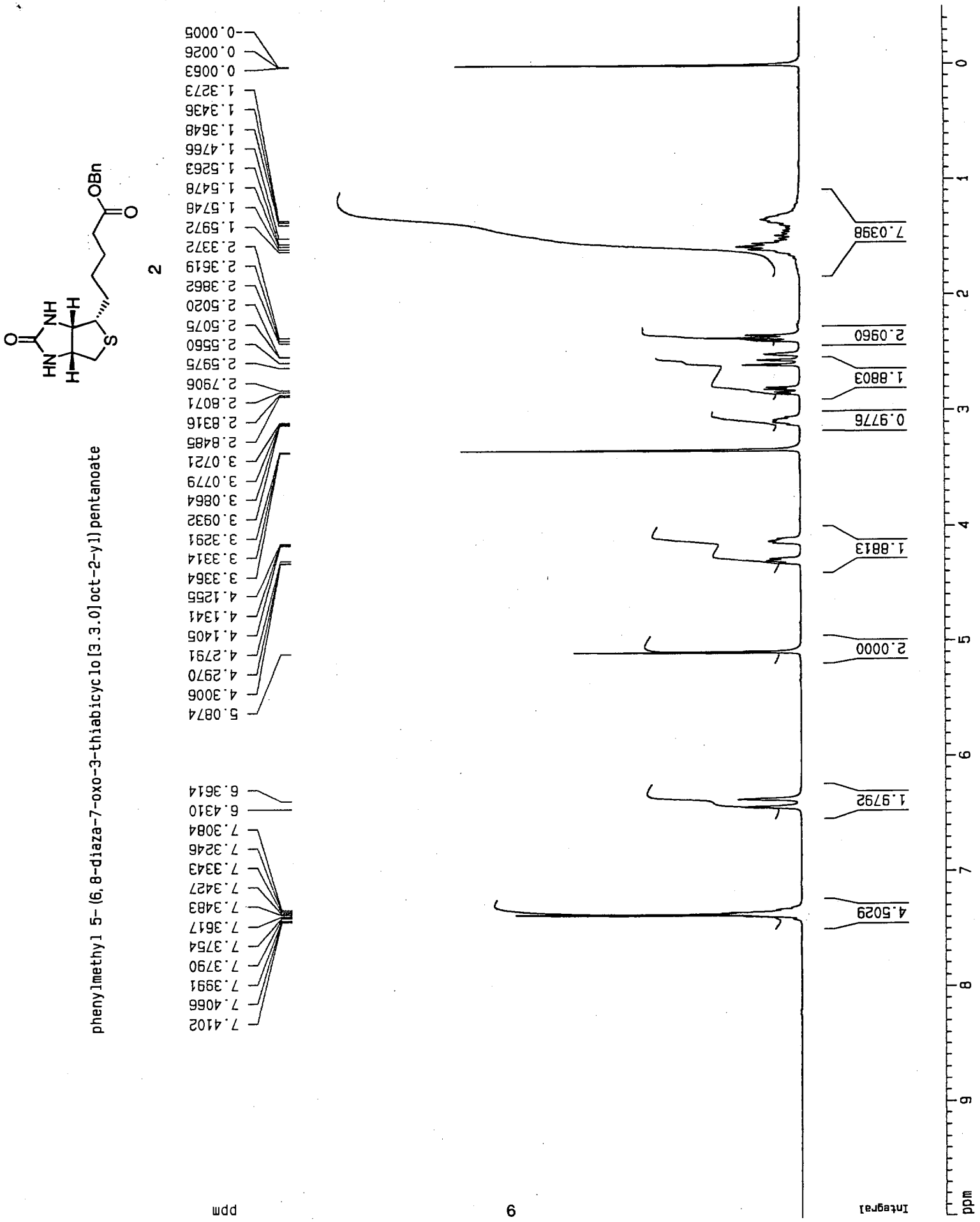
Phenylmethyl 5-(6,8–diaza–7-oxo-6-(2-phosphonoacetyl)–3–thiabicyclo[3.3.0] oct-2-yl) pentanoate (5). To a flask with CH_2Cl_2 (40 mL), (4.75 g, 9.26 mmol) of **4** were added. TMSBr (3.67 mL, 28 mmol) was added and stirred for 2 hours at rt. The reaction was quenched with 5 mL of distilled water and concentrated *in vacuo*. The solid was recrystallized in water / methanol (9:1) to afford a white solid **5**. (3.03 g, 72%); mp 184 °C.

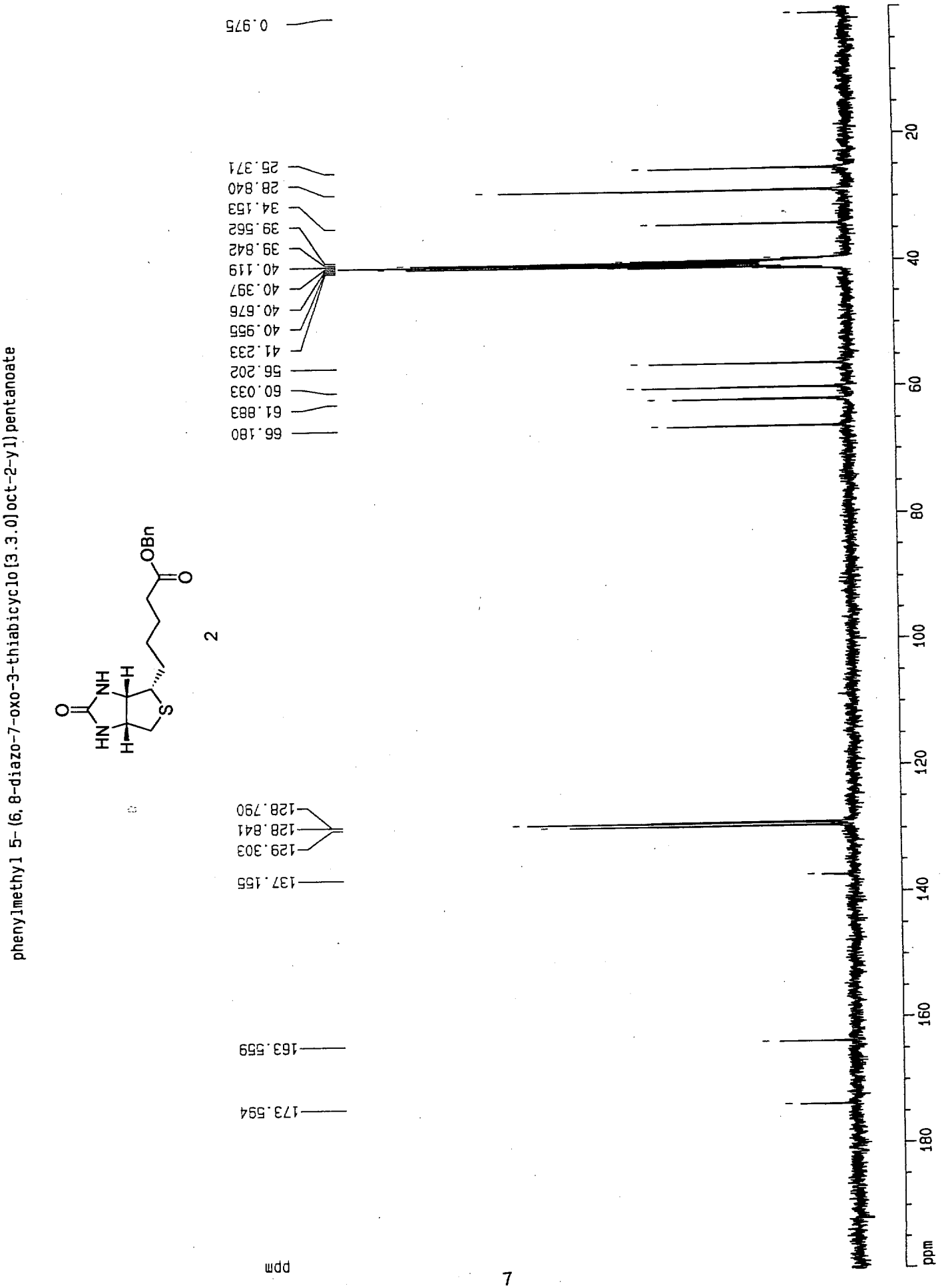
5-(6,8-diaza-7-oxo-6-(2-phosphonoacetyl)-3-thiabicyclo[3.3.0] oct-2-yl) pentanoic acid (1). (0.21g, 4.9 mmol) of LiOH was added to a solution of (0.506 g, 0.225 mmol) of **5** in 40 mL of water. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* and recrystallized in water / acetone (9 : 1) to afford a white solid **1** (0.209 g, 59%).

Purification and Assay of Biotin Carboxylase. Biotin carboxylase was purified from a strain of *E. coli* that overexpresses the gene coding for the enzyme. Purification was accomplished using a histidine-tag

attached to the amino terminus of the mutant form of the enzyme and nickel affinity chromatography as described previously (Blanchard, C.Z.; Lee, Y.M.; Franton, P. A.; Waldrop, G. L.; *Biochemistry* **1999**, in press).

The activity of biotin carboxylase was measured spectrophotometrically by following the production of ADP. The amount of ADP was determined using pyruvate kinase and lactate dehydrogenase and the oxidation of NADH was followed at 340 nm. Each measurement was carried out in a volume of 0.5 mL in 1 cm path length quartz cuvettes. The reaction mixture contained 10 units of pyruvate kinase, 18 units of lactate dehydrogenase, 0.5 mM phosphoenolpyruvate, 0.2 mM NADH, 8 mM $MgCl_2$ and 100 mM HEPES at pH 8.0. For inhibition studies **1** was dissolved in water and the pH was adjusted to 8 with HCl. The concentration of **1** was determined by phosphorus analysis (Ames, B.N.; Dubin, D.T. *J. Biol. Chem.* **1960**, 235, 769-775).


phenylmethyl 5-(6,8-diaza-7-oxo-3-thiabicyclo[3.3.0]oct-2-yl)pentanoate
2
OBn
O
NH
H
S
HN
H
O
-0.0005
0.0026
0.0063
1.3273
1.3436
1.3648
1.4766
1.5263
1.5478
1.5748
1.5972
2.3372
2.3619
2.3862
2.5020
2.5075
2.5560
2.5975
2.7906
2.8071
2.8316
2.8485
3.0721
3.0779
3.0864
3.0932
3.3291
3.3314
3.3364
4.1255
4.1341
4.1405
4.2791
4.2970
4.3006
5.0874
6.3614
6.4310
7.3084
7.3246
7.3343
7.3427
7.3483
7.3617
7.3754
7.3790
7.3991
7.4066
7.4102
7.0398
2.0960
1.8803
0.9776
1.8813
2.0000
1.9792
4.5029
Integral
ppm
0
1
2
3
4
5
6
7
8
9
ppm


phenylmethyl 5-(6,8-diazo-7-oxo-3-thiabicyclo[3.3.0]oct-2-yl)pentanoate
2
OBn
O
NH
H
S
HN
H
O
0.975
25.371
28.840
34.153
39.562
39.842
40.119
40.397
40.676
40.955
41.233
56.202
60.033
61.883
66.180
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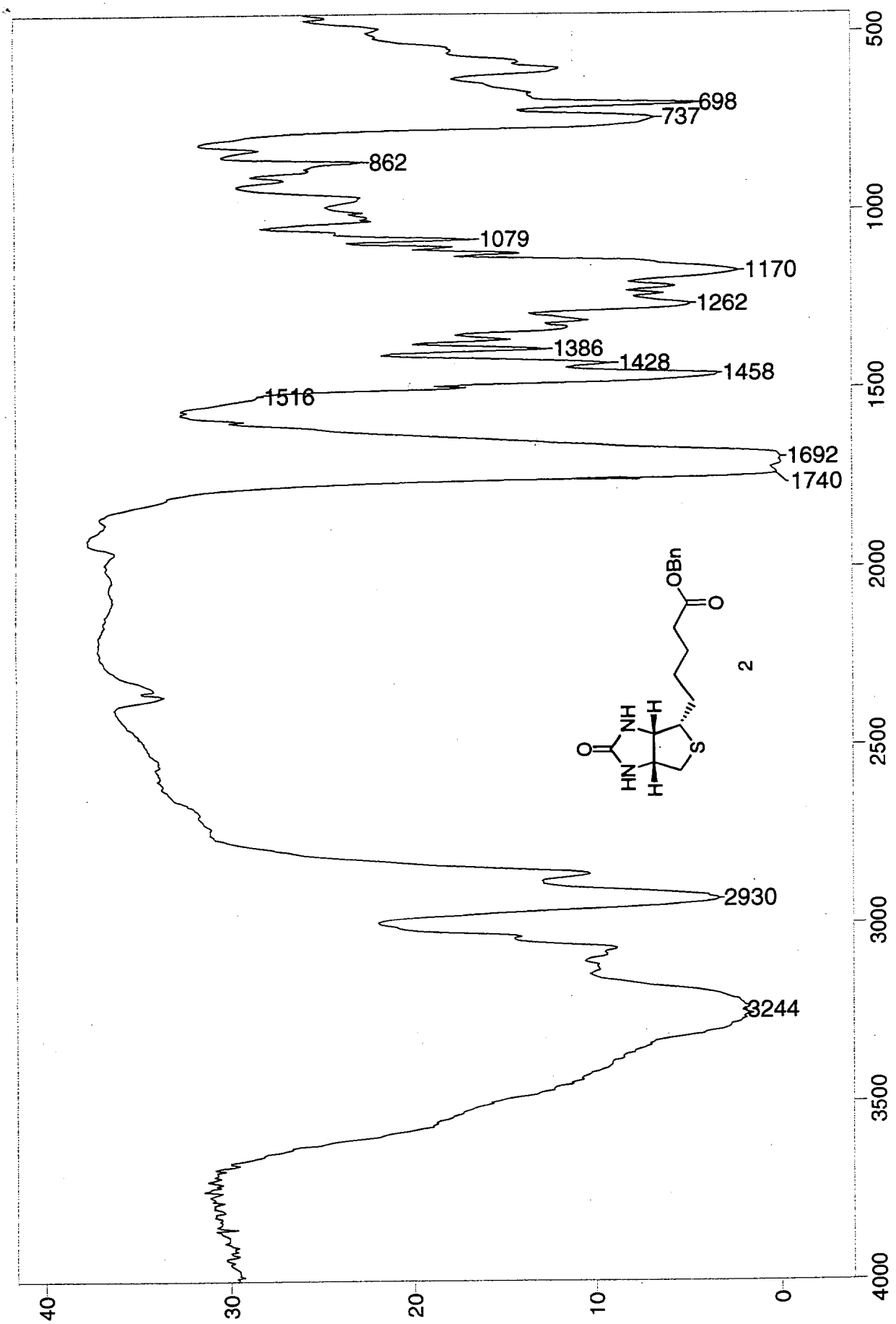
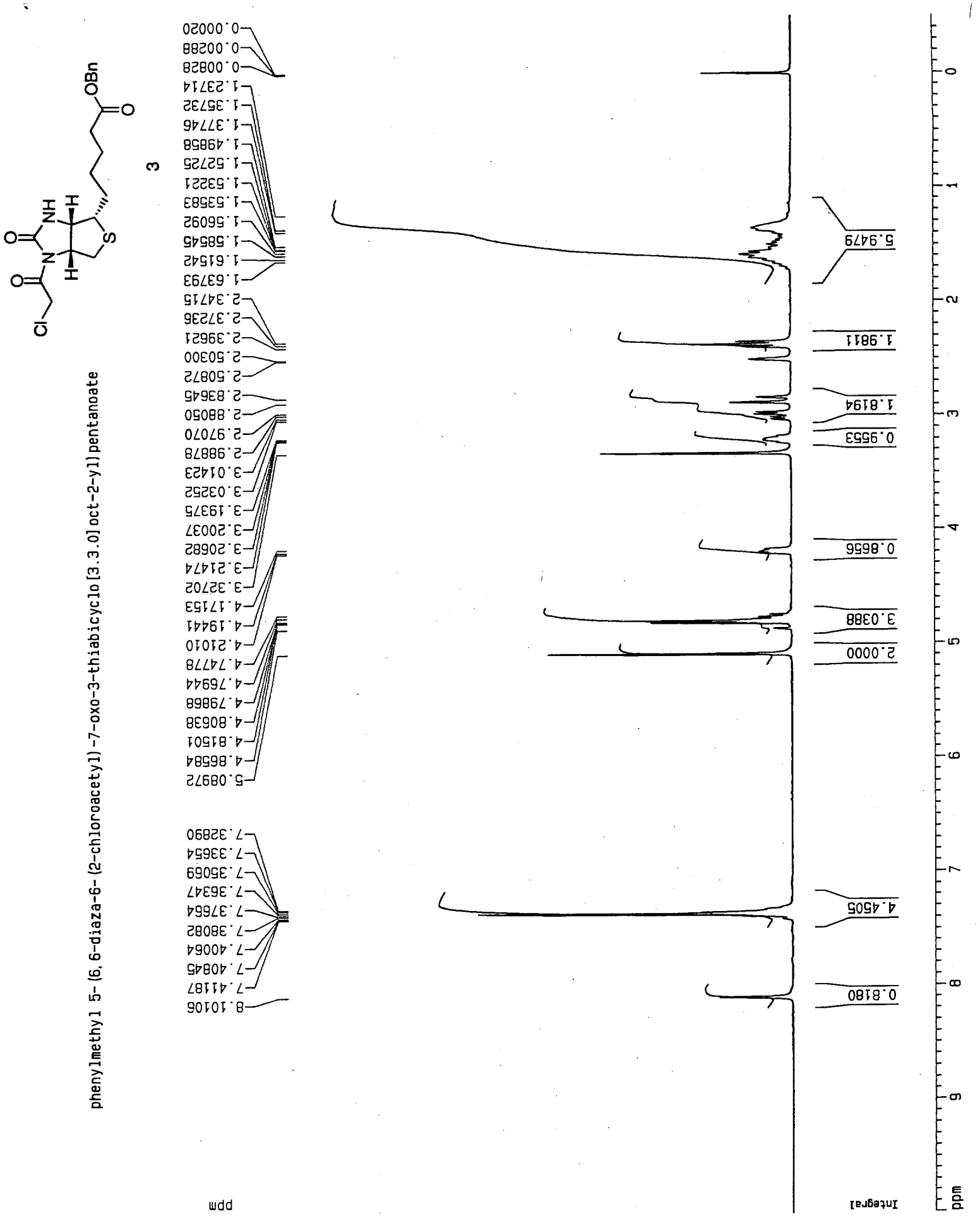
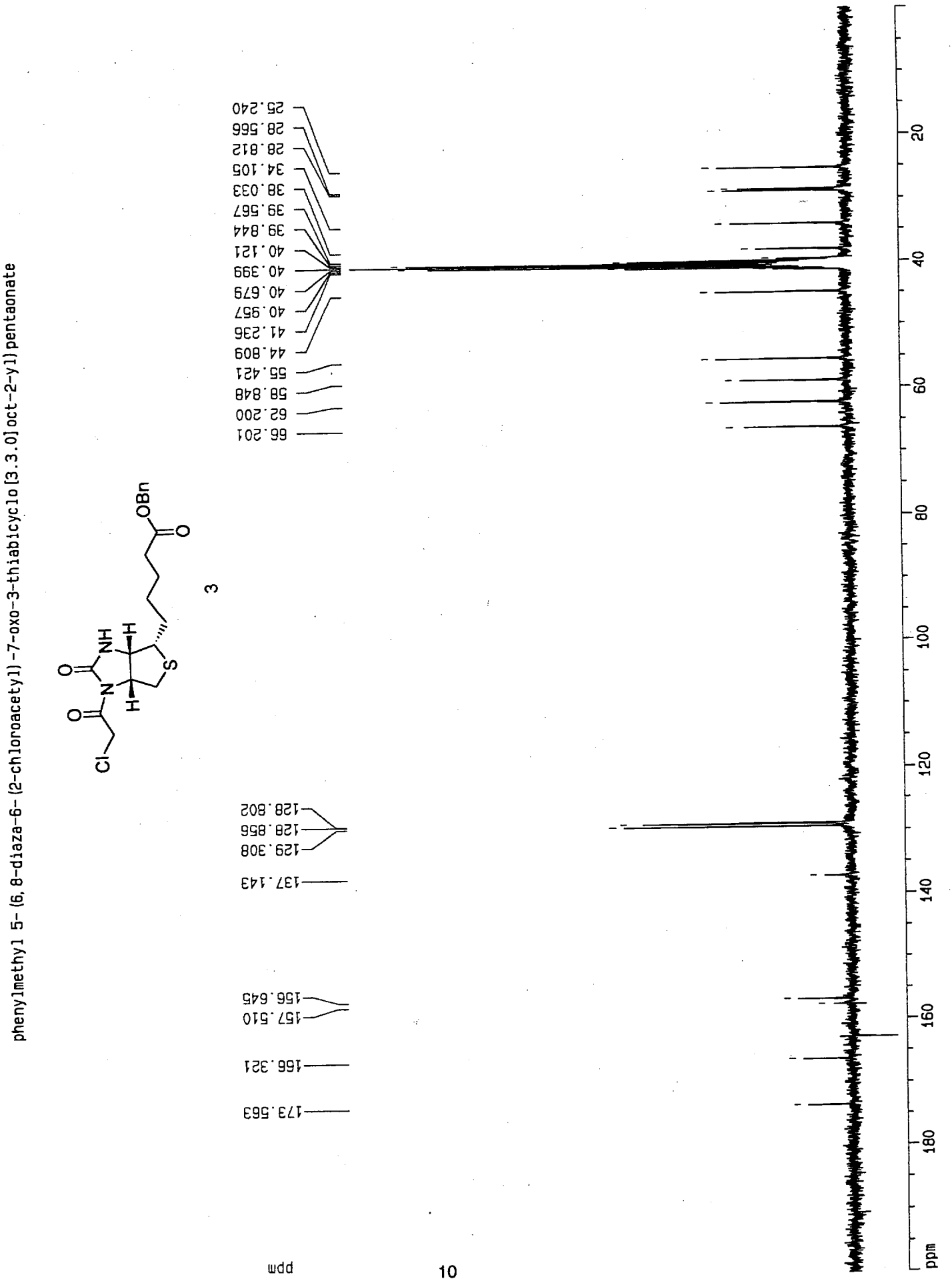
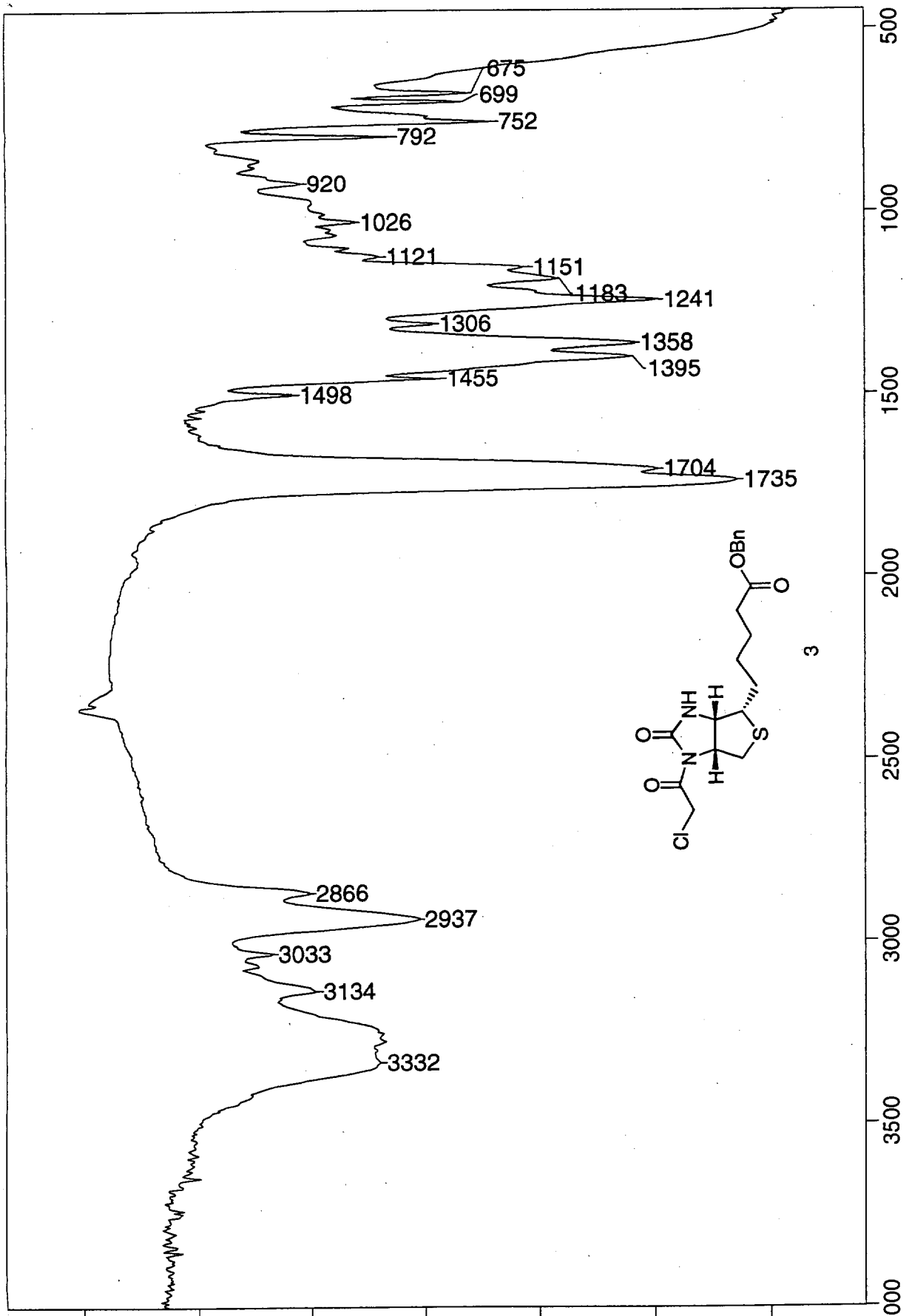
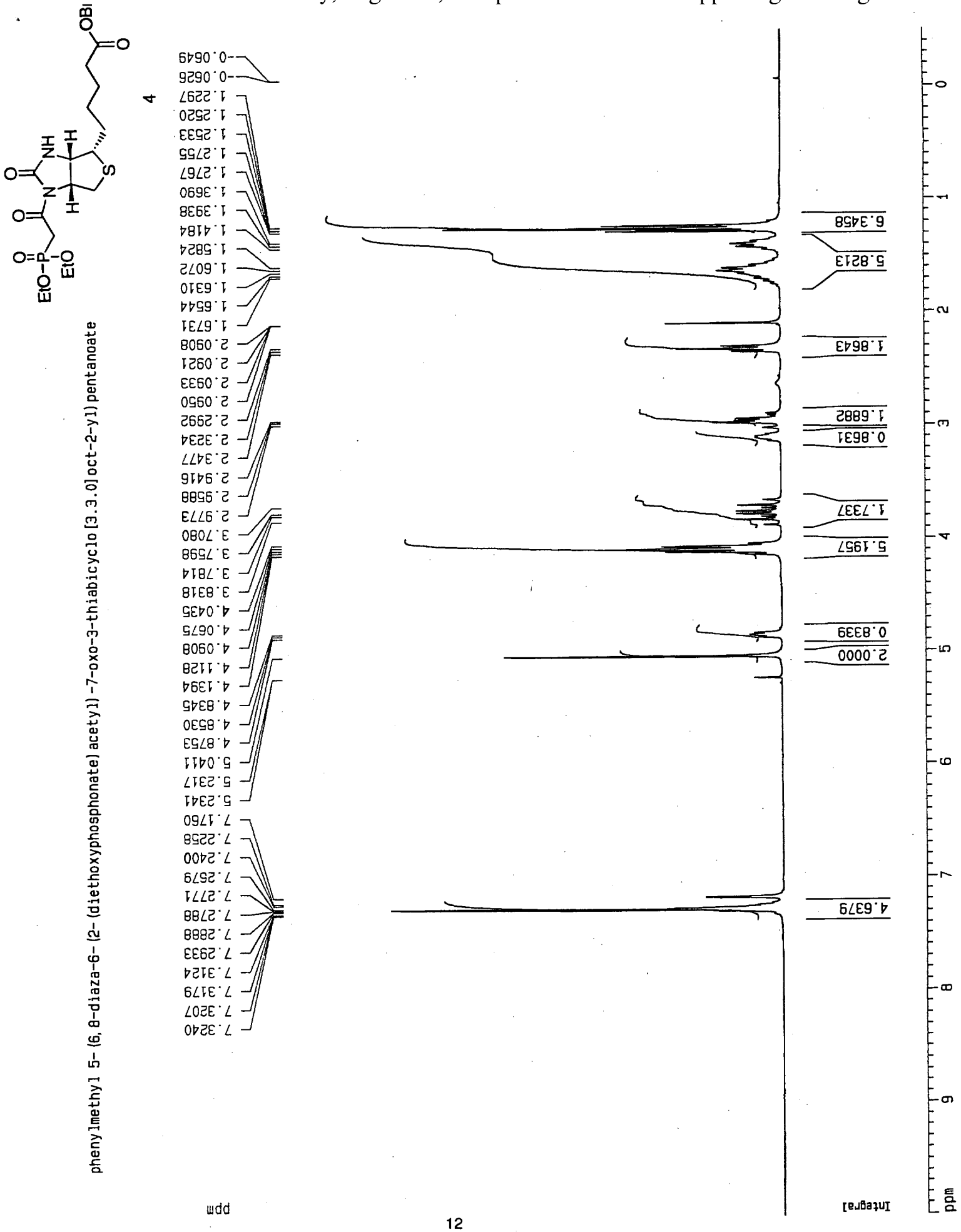
20
40
60
80
100
120
140
160
180
ppm

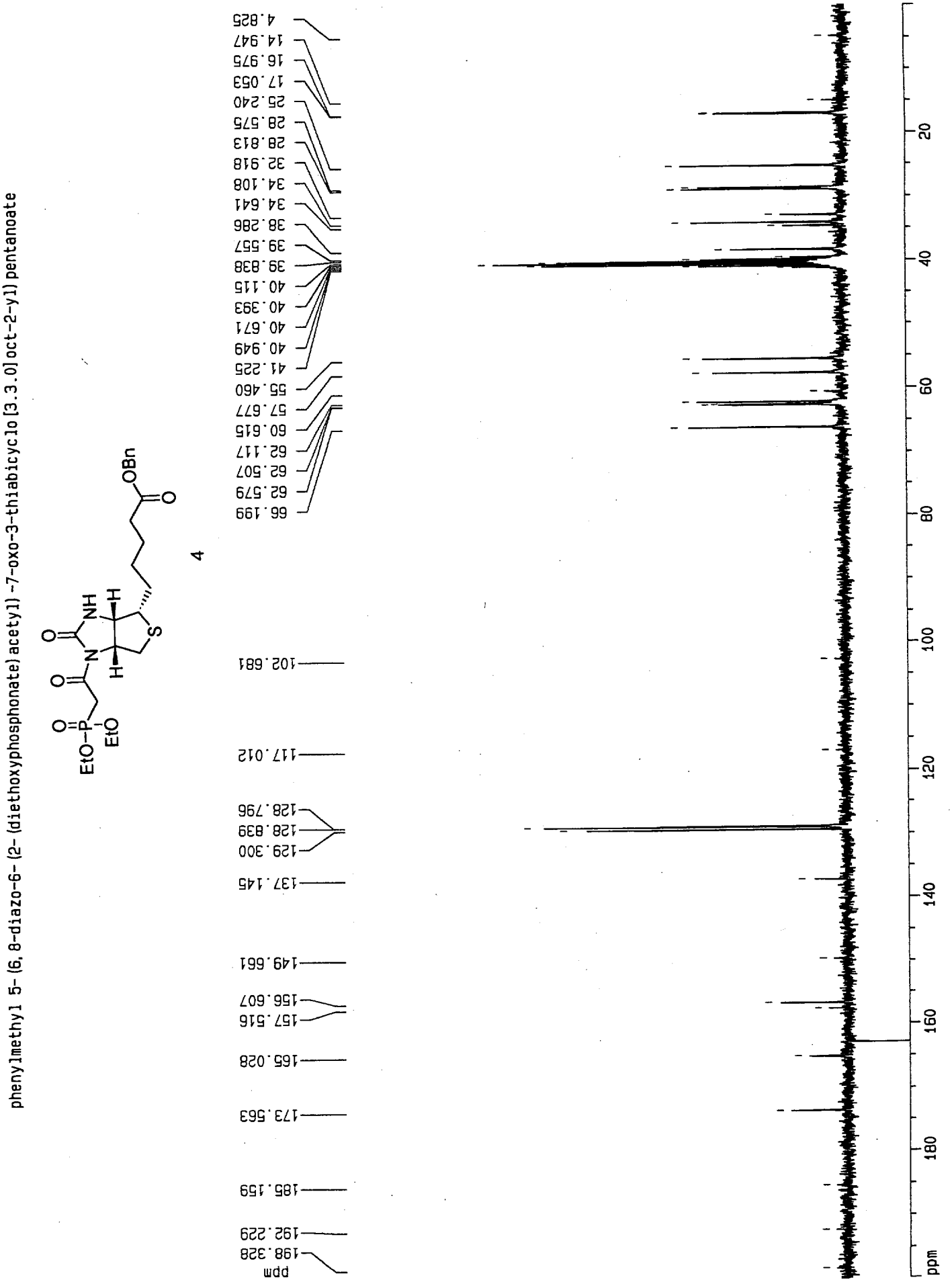



phenylmethyl 5- (6, 6-diaza-6- (2-chloroacetyl) -7-oxo-3-thiabicyclo [3.3.0] oct-2-yl) pentanoate
3
ppm
Integral
5.9479
1.9811
1.8194
0.9553
0.8656
3.0388
2.0000
4.4505
0.8180

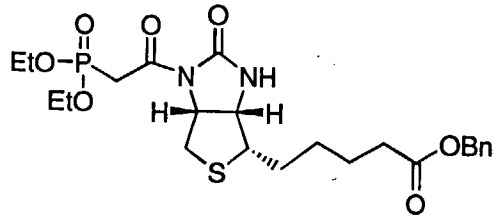







phenylmethyl 5-(6,8-diazo-6-(2-(diethoxyphosphonate)acetyl)-7-oxo-3-thiabicyclo[3.3.0]oct-2-yl)pentanoate
4
OBn
NH
H
S
N
H
O=P
EtO
EtO
4.825
14.947
16.975
17.053
25.240
28.575
28.813
32.918
34.108
34.641
38.286
39.557
39.838
40.115
40.393
40.671
40.949
41.225
55.460
57.677
60.615
62.117
62.507
62.579
66.199
102.681
117.012
128.796
128.839
129.300
137.145
149.661
156.607
157.516
165.028
173.563
185.159
192.229
198.328
ppm
20
40
60
80
100
120
140
160
180
ppm

phenylmethyl 5-(6,8-diaza-6-(2-diethoxyphosphonate)acetyl)-7-oxo-3-thiabicyclo[3.3.0]oct-2-yl)pentanoate



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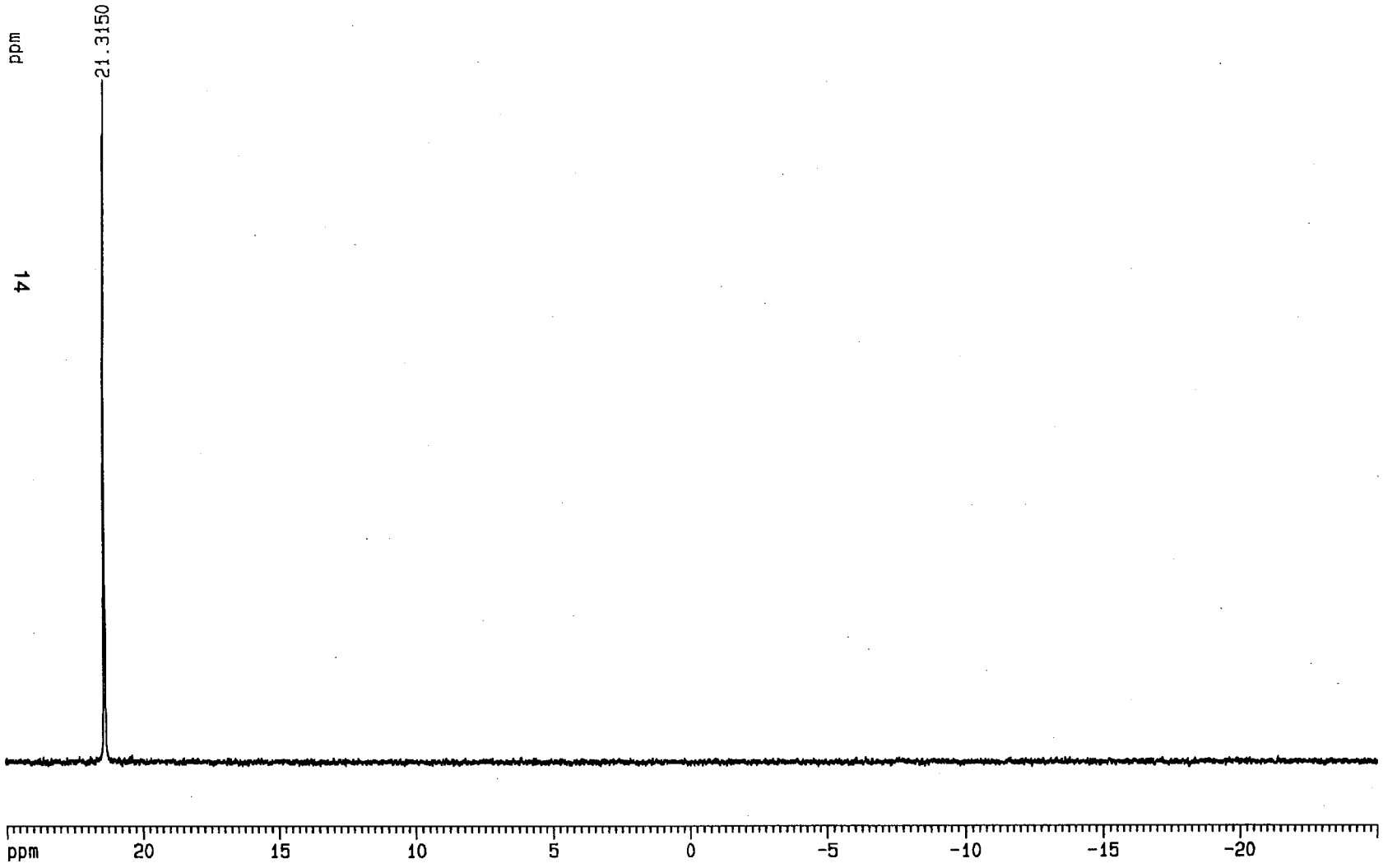

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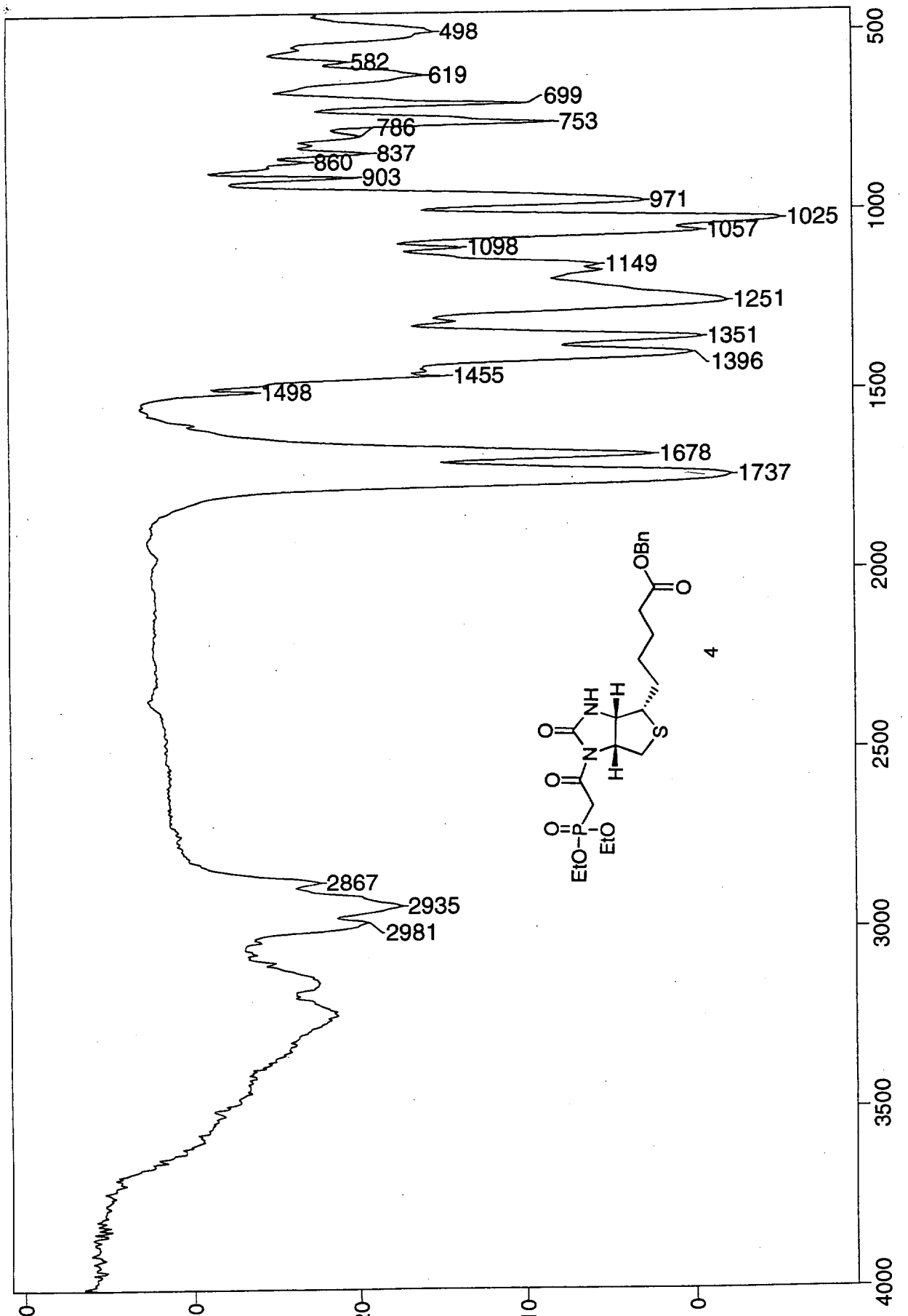
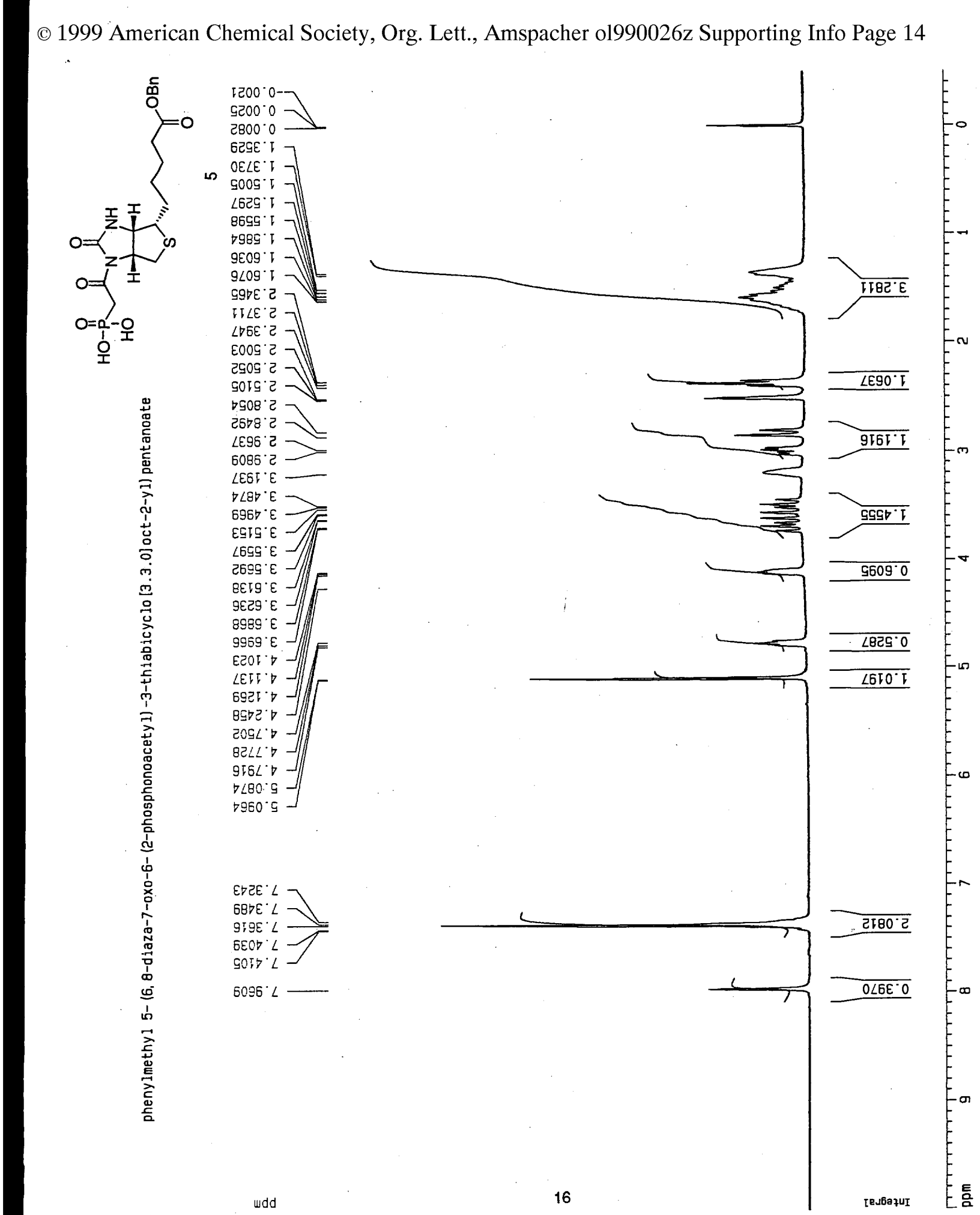
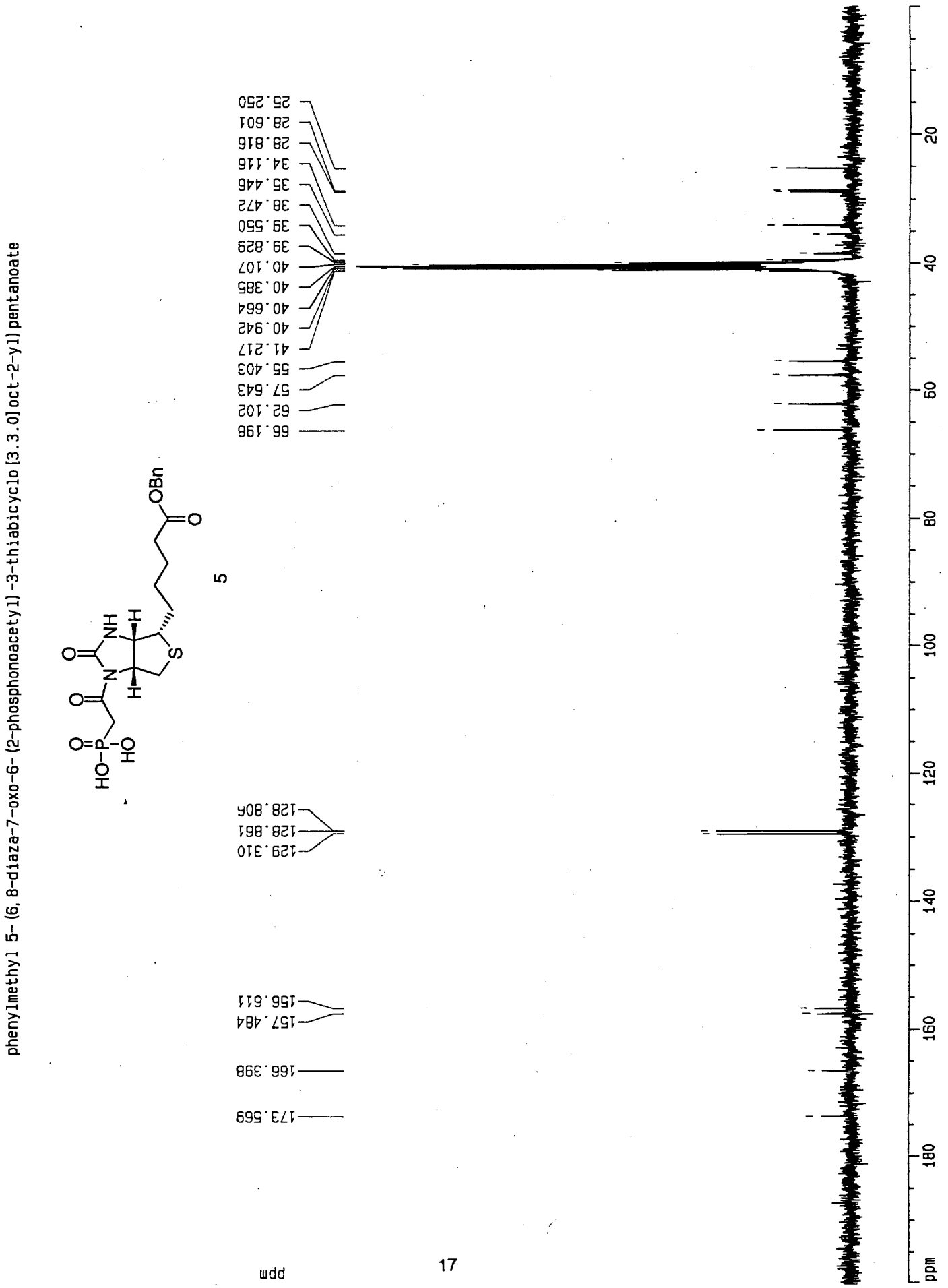
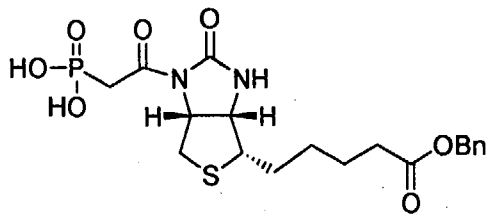






phenylmethyl 5-(6,8-diaza-7-oxo-6-(2-phosphonoacetyl)-3-thiabictclo[3.3.0]oct-2-yl)pentanoate



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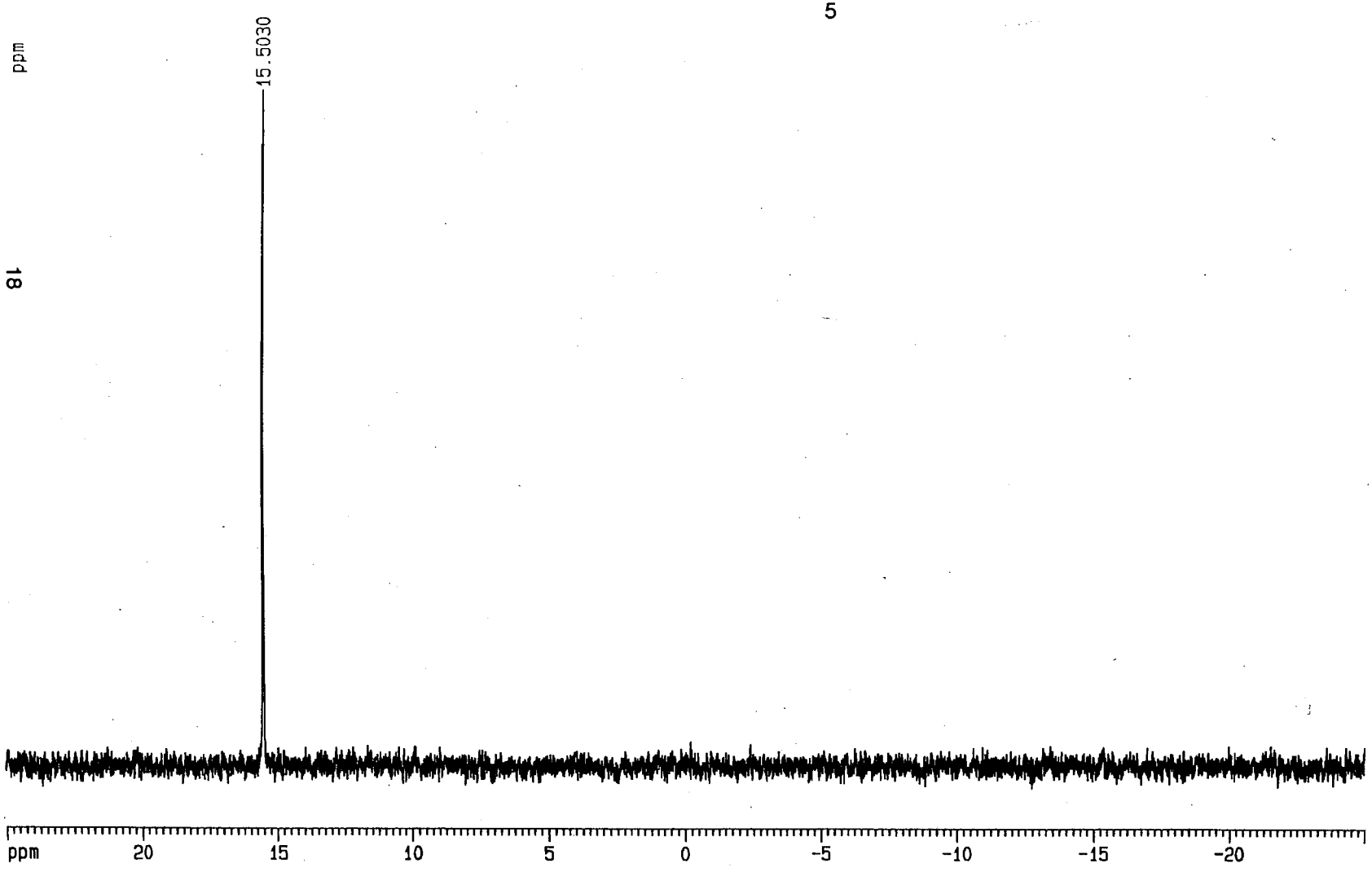
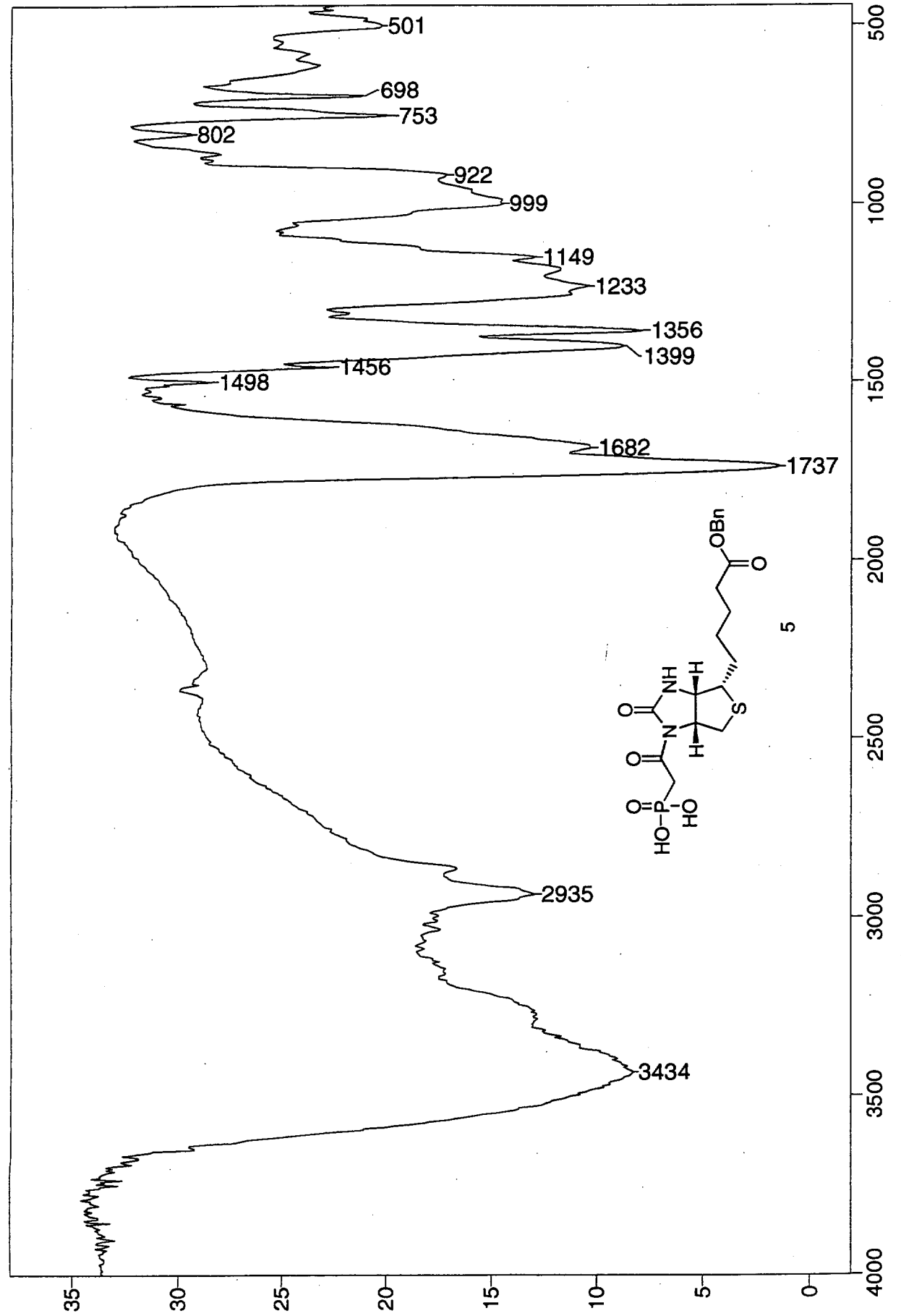
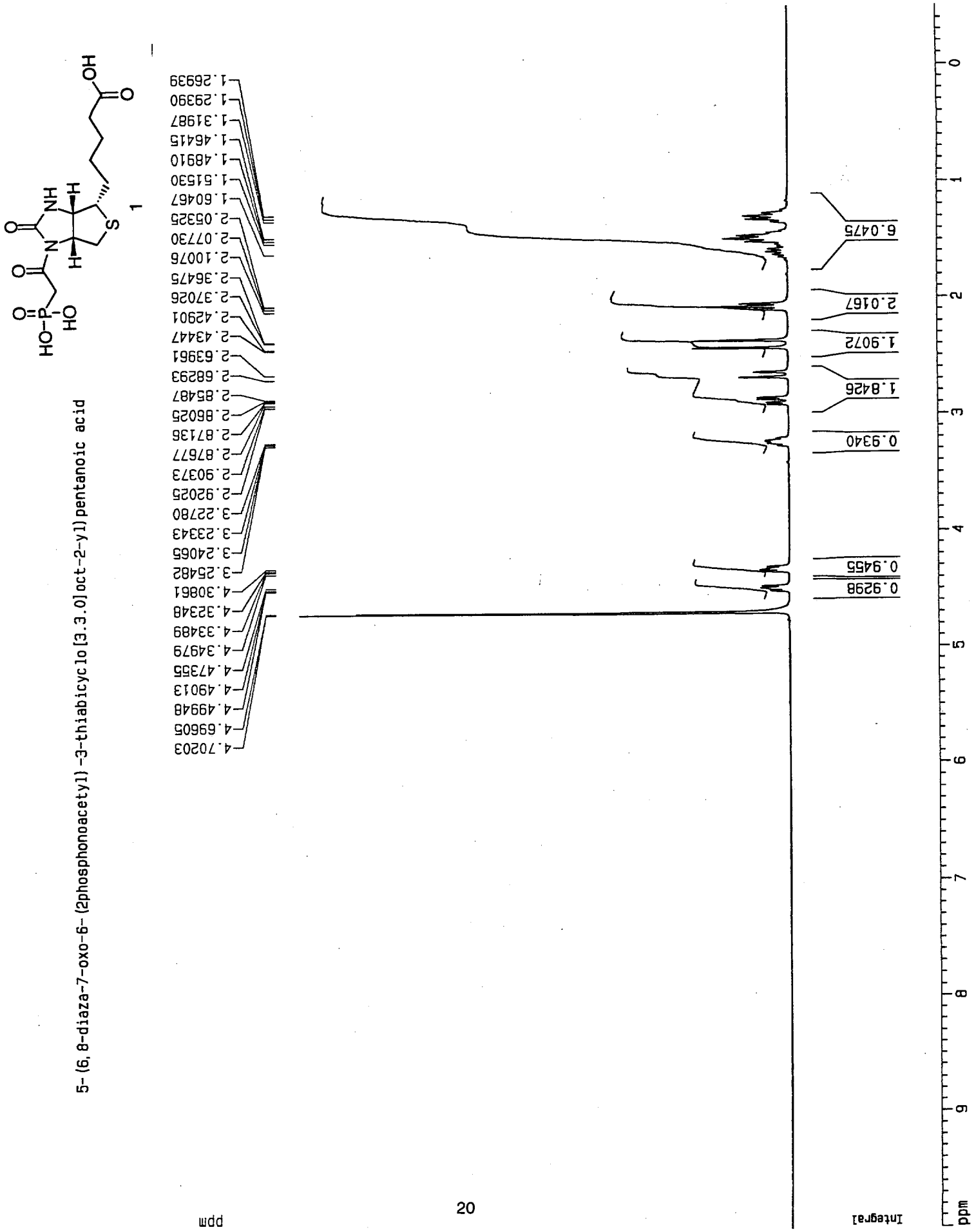
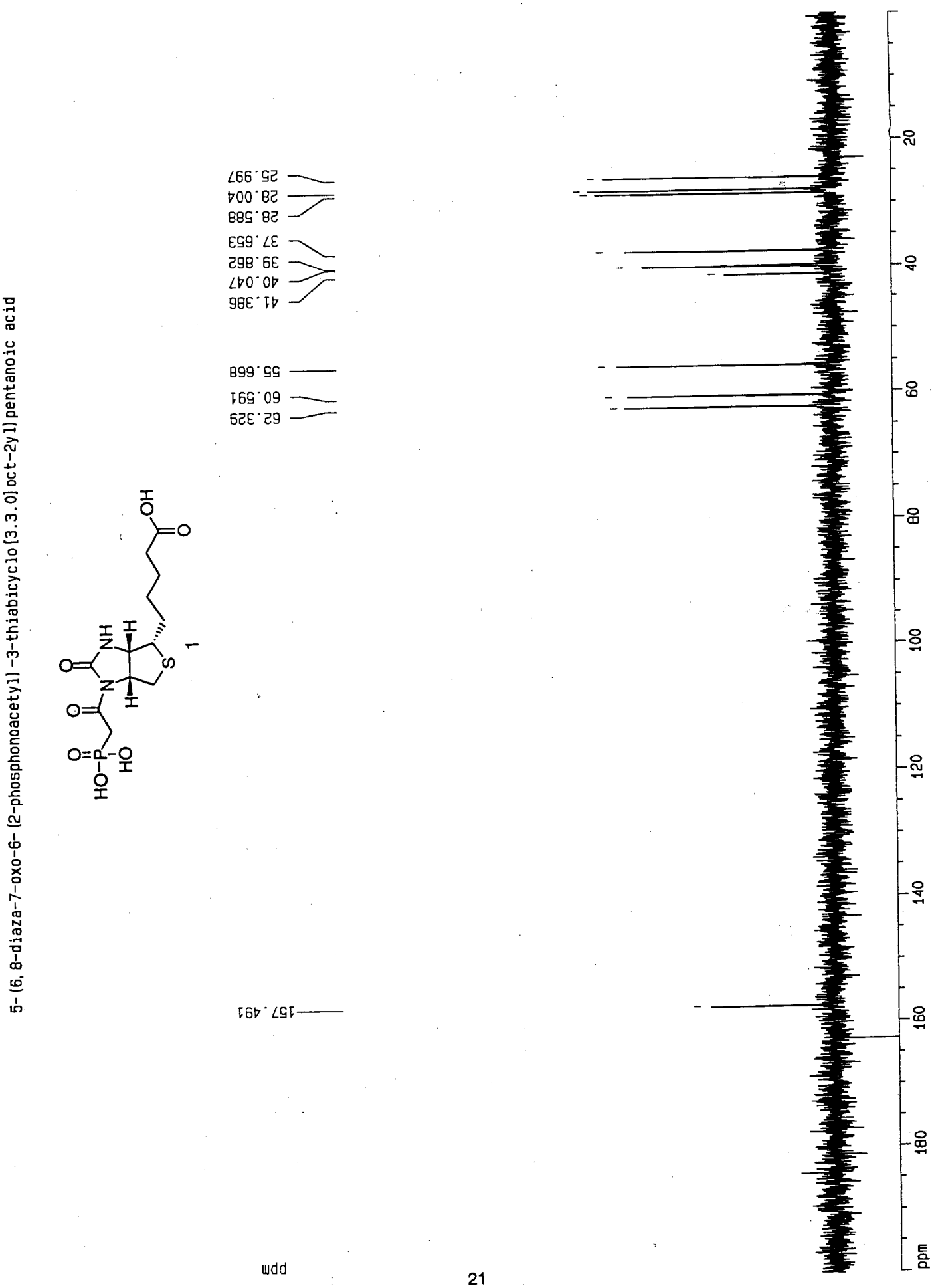
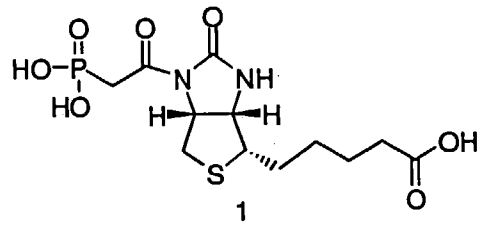








5-(6,8-diaza-7-oxo-6-(2-phosphonoacetyl)-3-thiabicyclo[3.3.0]oct-2-yl)penatnoic acid



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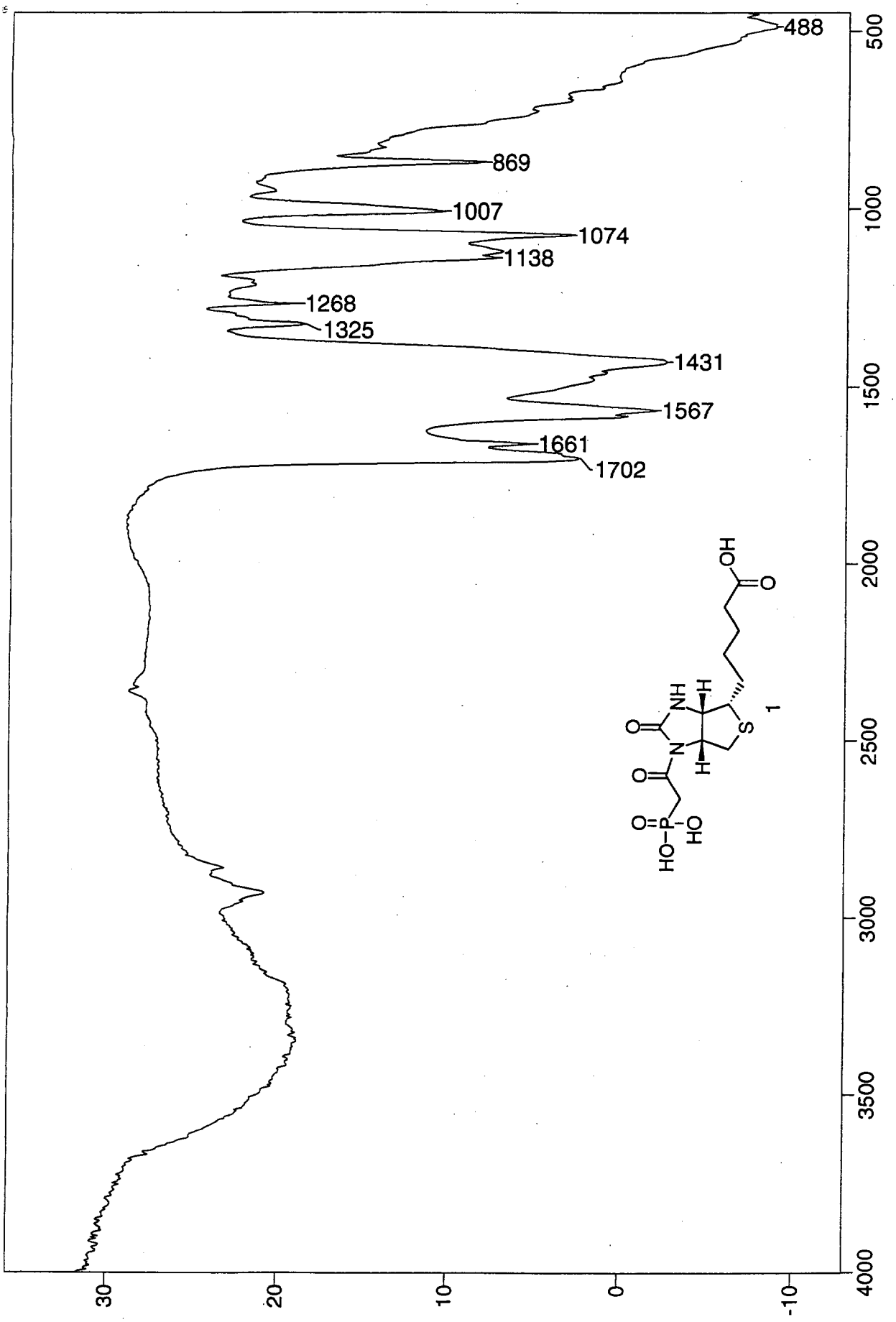

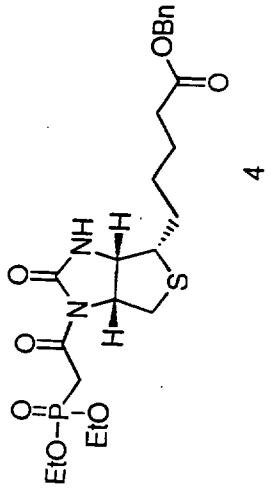
OBn
O
NH
H
O
N
H
S
O
EtO–P–EtO

4

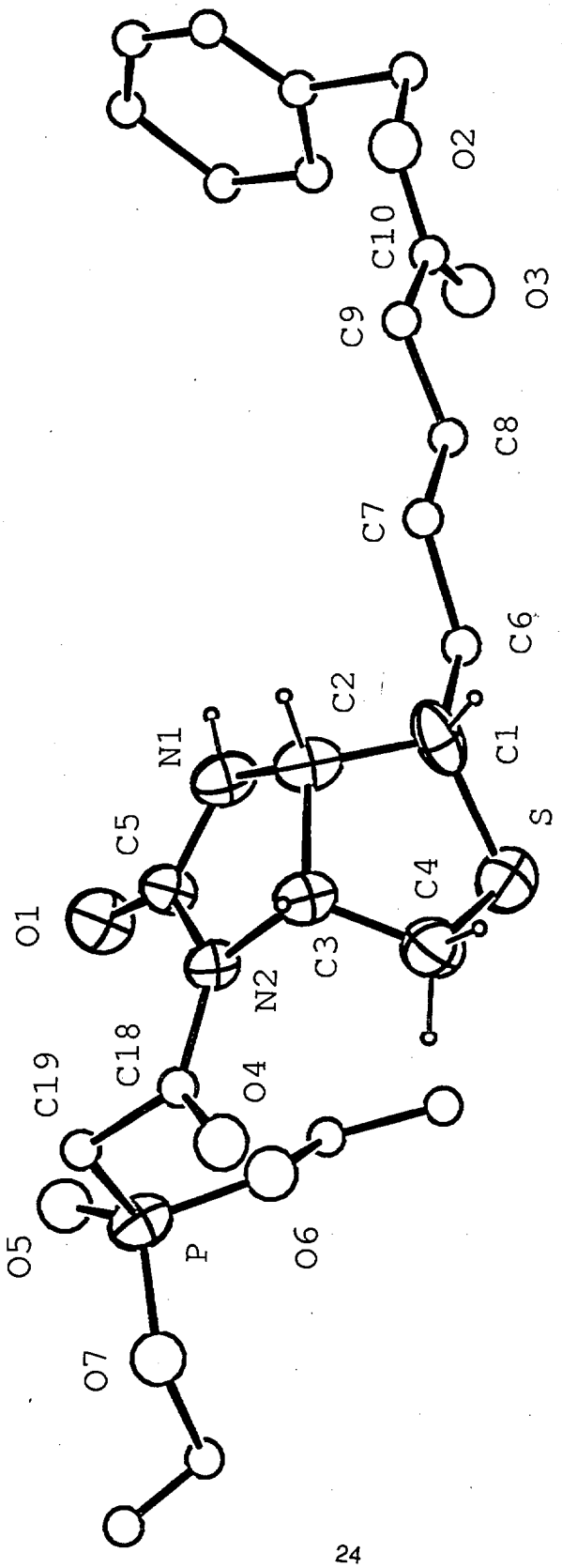
O2
C10
O3
C9
C8
C7
C6
C2
N1
C1
S
C5
C4
O1
C3
N2
C18
C19
O4
O5
P
O6
O7

Table 1. Crystal, Experimental, and Refinement Data for **4**

formula	$C_{23}H_{33}N_2O_7PS$
fw	512.6
crystal system	monoclinic
space group	$P2_1$
temp	299 K
cell constants	
a, Å	10.546(1)
b, Å	12.550(1)
c, Å	10.674(1)
β, °	112.15(1)
V, Å^3	1308.4(5)
Z	2
D_{calcd}, g cm^{-3}	1.301
μ, cm^{-1}	20.25
diffractometer / scan	Enraf-Nonius CAD4 / ω–2θ
radiation	CuKα (λ =1.54184 Å)
cryst dimens, mm	0.48x 0.30 x 0.20
color/shape	colorless fragment
min rel transmissn, %	89.1
decay of standards	27.4%
unique reflections	2824
2θ range, deg	$5 < 2\theta < 150$
range of h,k,l	-13 to 0, 0 to 15, -12 to 13
obsd reflcns [I>1σ(I)]	2104
no. of params refined	287
weights	$4F_o^2[\sigma^2(I)+(0.02F_o^2)^2]^{-1}$
$R = \Sigma \lvert \Delta F \rvert / \Sigma \lvert F_o \rvert$	0.096
$R_W = (\Sigma w(\Delta F)^2 / \Sigma wF^2)^{1/2}$	0.104
GOF	4.753
max. resid density, e Å^{-3}	0.44
min. resid density, e Å^{-3}	-0.28

Table 2. Coordinates and isotropic displacement parameters for **4**

atom	x	y	z	B_{eq} or B_{iso} ($Å^2$)
S	0.5380(3)	0	0.6547(3)	9.56(8)
P	0.8528(2)	0.2816(2)	1.0611(2)	6.67(5)
O1	0.9205(5)	0.0258(6)	1.0550(6)	8.0(2)
O2	0.9253(8)	-0.5890(9)	0.576(1)	17.9(3)
O3	0.9328(9)	-0.4457(9)	0.4880(8)	19.0(3)
O4	0.5523(5)	0.1532(5)	1.0480(5)	6.1(1)
O5	1.0022(5)	0.2874(6)	1.1080(7)	8.4(2)
O6	0.7801(5)	0.2552(6)	0.9094(5)	7.7(2)
O7	0.7772(6)	0.3858(5)	1.0790(7)	8.8(2)
N1	0.7735(5)	-0.1018(6)	0.9292(7)	6.8(2)
N2	0.6825(5)	0.0339(5)	0.9961(6)	5.2(1)
C1	0.5708(7)	-0.132(1)	0.7171(8)	8.0(3)
C2	0.6286(7)	-0.1271(8)	0.8699(9)	6.7(2)
C3	0.5645(6)	-0.0289(7)	0.9109(8)	6.2(2)
C4	0.4763(9)	0.024(1)	0.783(1)	8.6(3)
C5	0.8063(6)	-0.0119(7)	1.0031(7)	5.5(2)
C6	0.6551(8)	-0.1883(9)	0.645(1)	8.3(3)
C7	0.6805(9)	-0.301(1)	0.672(1)	8.8(3)
C8	0.770(1)	-0.349(1)	0.5962(9)	8.8(3)
C9	0.8123(9)	-0.454(1)	0.630(1)	9.2(3)
C10	0.891(1)	-0.493(1)	0.5545(9)	10.2(3)
C11	1.025(1)	-0.637(1)	0.512(2)	15.5(5)
C12	1.1696(8)	-0.622(1)	0.612(1)	9.2(3)
C13	1.227(1)	-0.535(1)	0.580(1)	11.3(4)
C14	1.371(1)	-0.522(2)	0.656(1)	14.5(4)
C15	1.4387(9)	-0.597(1)	0.755(1)	16.5(4)
C16	1.357(2)	-0.670(2)	0.767(2)	17.7(6)*
C17	1.243(1)	-0.681(2)	0.704(2)	14.5(5)*
C18	0.6669(7)	0.1255(7)	1.0590(8)	5.7(2)
C19	0.7910(7)	0.1865(7)	1.1467(9)	6.6(2)
C20	0.835(1)	0.253(2)	0.812(1)	14.9(5)
C21	0.751(2)	0.235(2)	0.681(2)	21.5(6)
C22	0.799(1)	0.494(2)	1.024(2)	16.7(6)*
C23	0.849(2)	0.546(2)	1.119(2)	19.5(7)*

* Refined Isotropically