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Supporting Information

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Supporting Information

for

The Molecular Basis of Inhibition of Golgi a-Mannosidase II by Mannostatin A

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ppm
9
8
7
6
5
4
3
2
1
0
BnO
NHAc
OAm
O
O
7

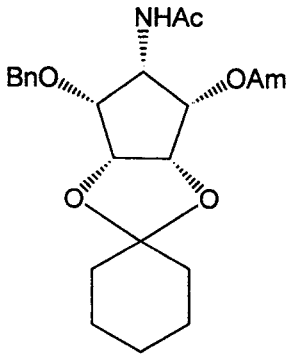
13C OBSERVE

Archive directory: /export/home/wzhong/vnmrsys/data
Sample directory: /export/home/wzhong/vnmrsys/data/wzhong_01Feb2005
File: CARBON

Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Operator: wzhong
Mercury-300BB "merc300"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
5000 repetitions
OBSERVE C13, 75.4552576 MHz
DECOUPLE H1, 300.0819042 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 4 hr, 20 min, 19 sec



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Sample directory:

Pulse Sequence: s2pul

Solvent: cdcl3
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File: 0187_Proton_2006May15_01
UNITYplus-300 "nmr1"

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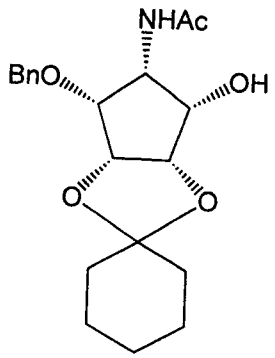

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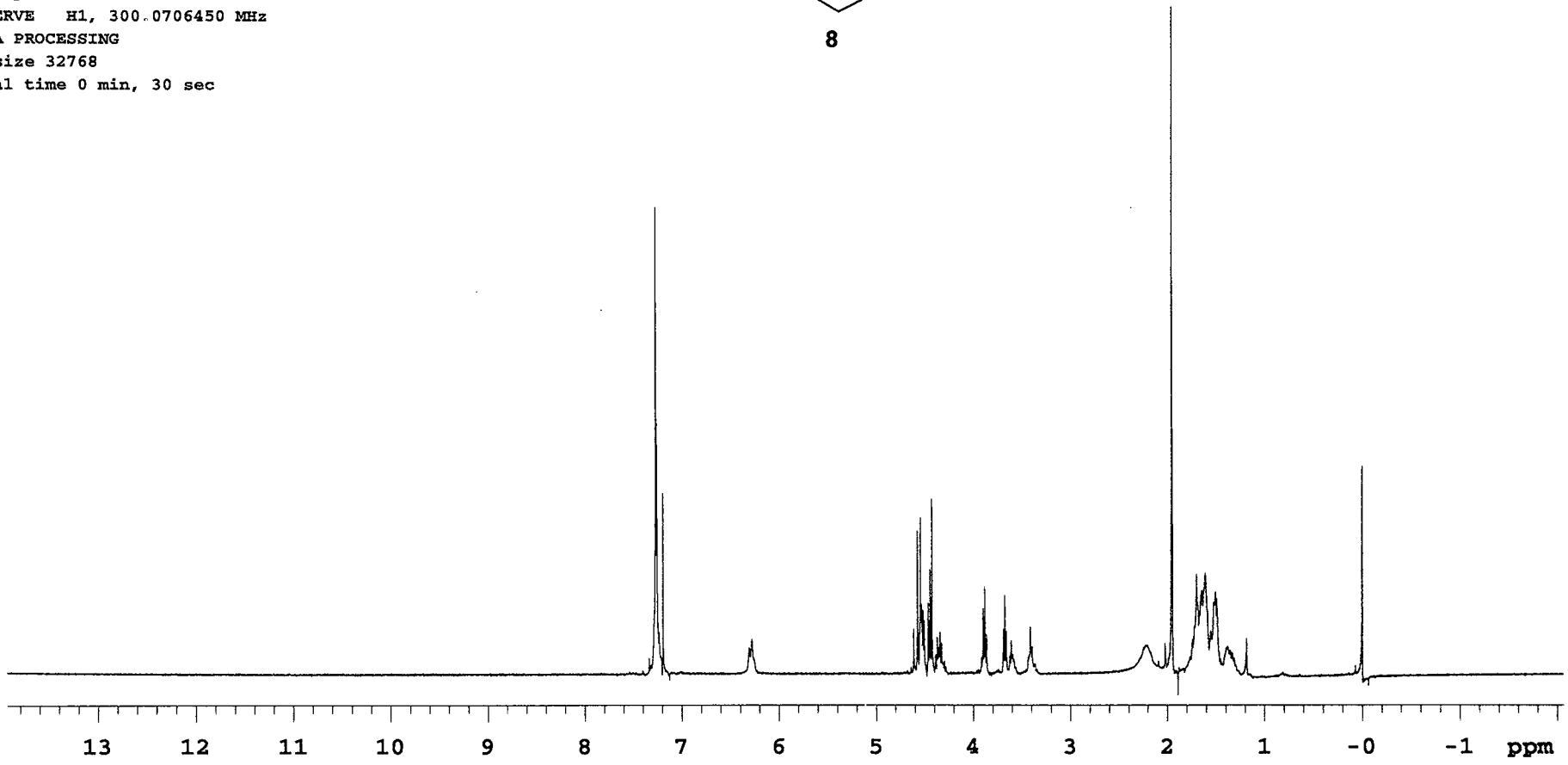

13C OBSERVE

Archive directory: /export/home/wzhong/vnmrsys/data
Sample directory: /export/home/wzhong/vnmrsys/data/wzhong_01Feb2005

Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Operator: wzhong
File: wzhong0187C13
INOVA-600 "nmr1"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
10000 repetitions
OBSERVE C13, 75.4552576 MHz
DECOUPLE H1, 300.0819042 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
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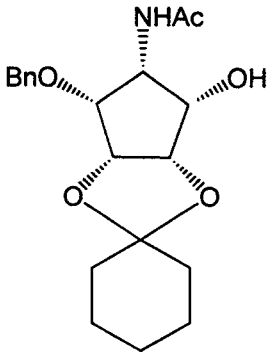
FT size 131072
Total time 7 hr, 51 min, 1 sec



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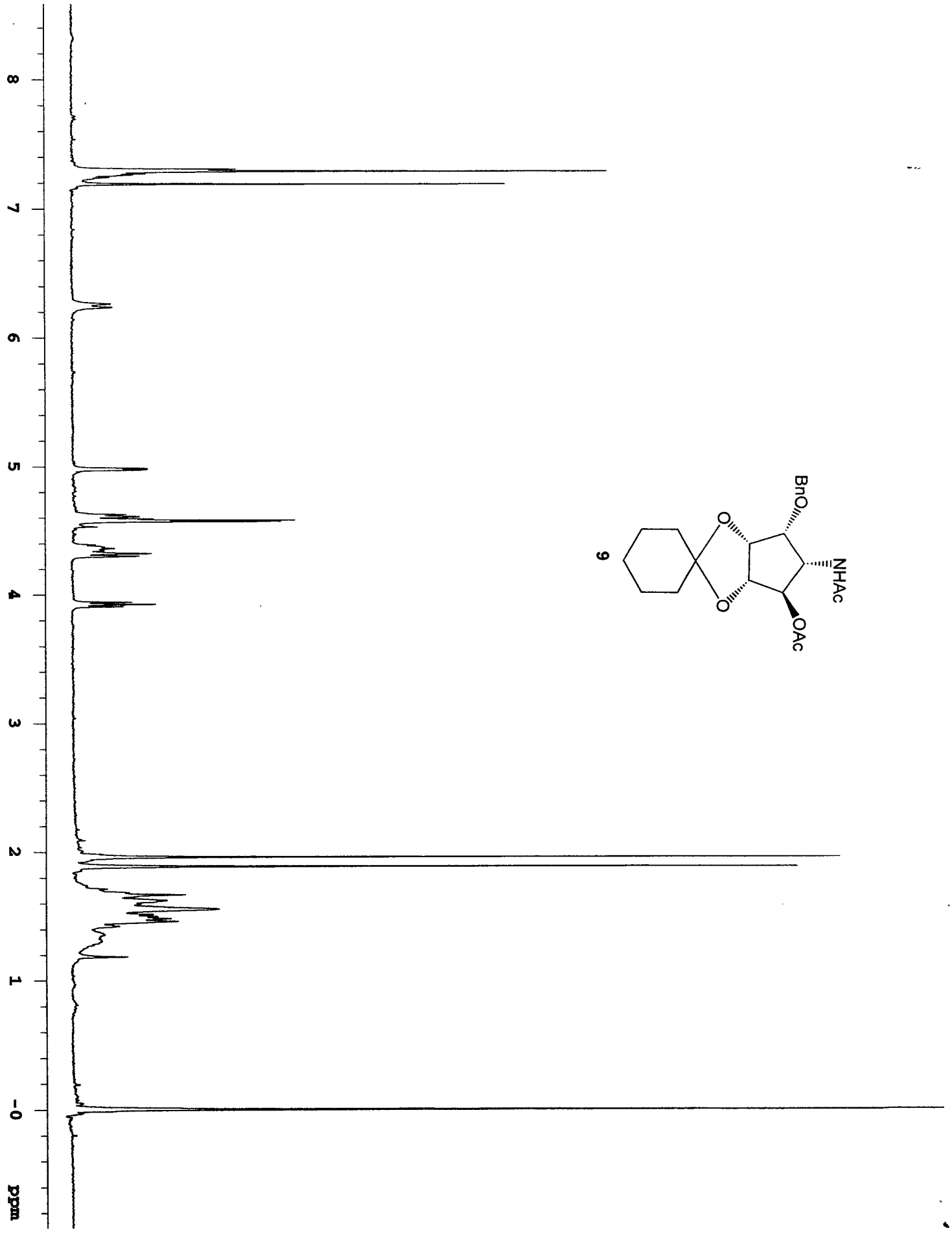
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3
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1
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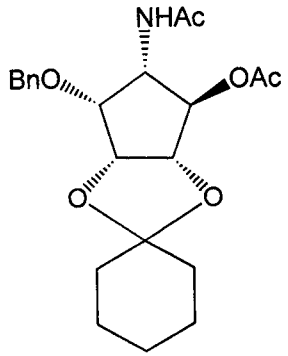
ppm
9
BnO
NHAc
OAc
O
O



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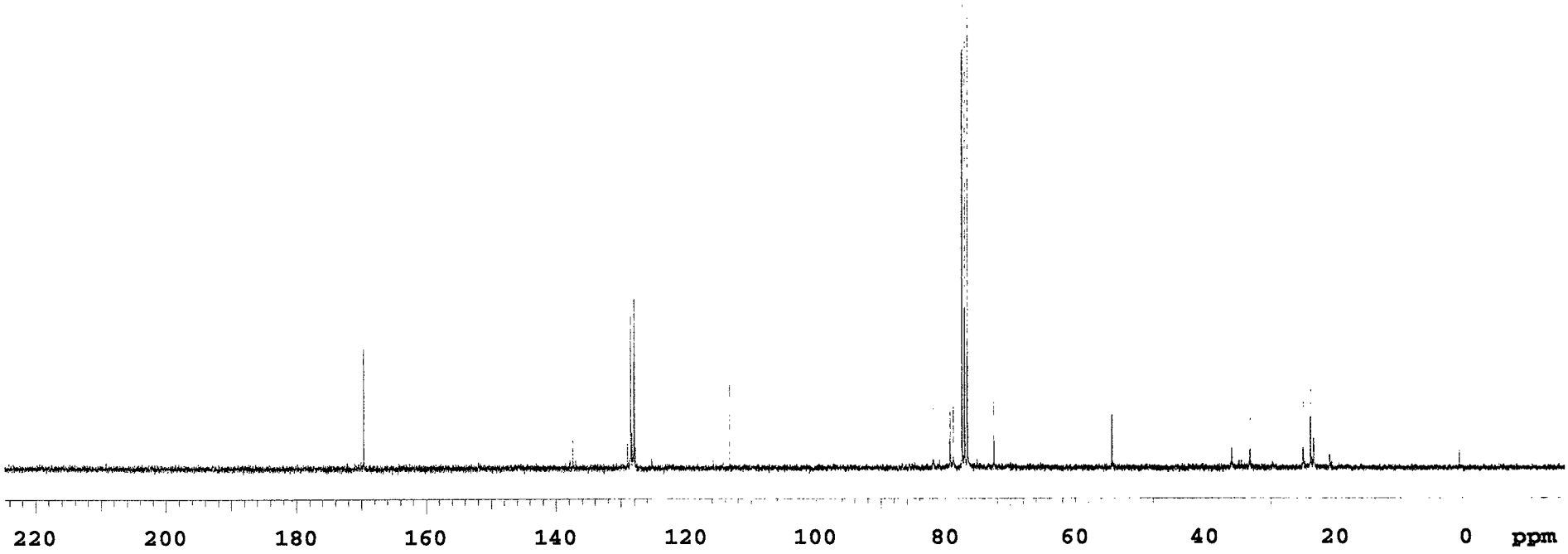

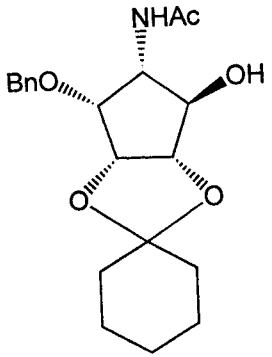

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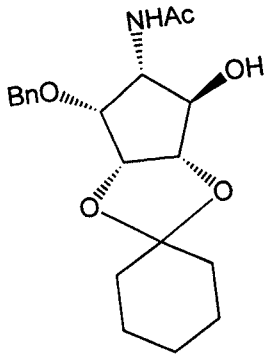

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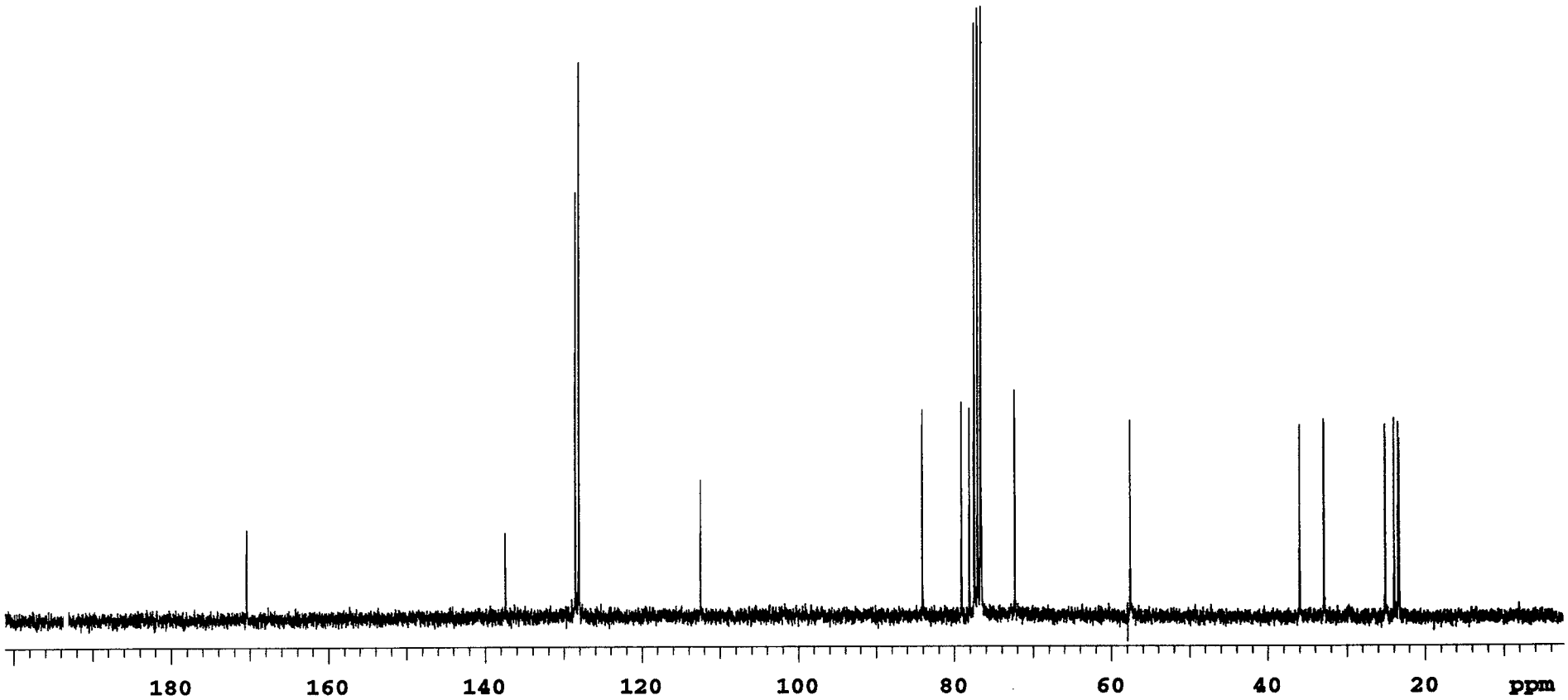

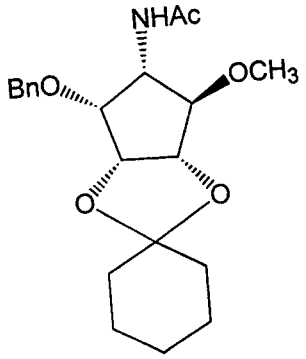

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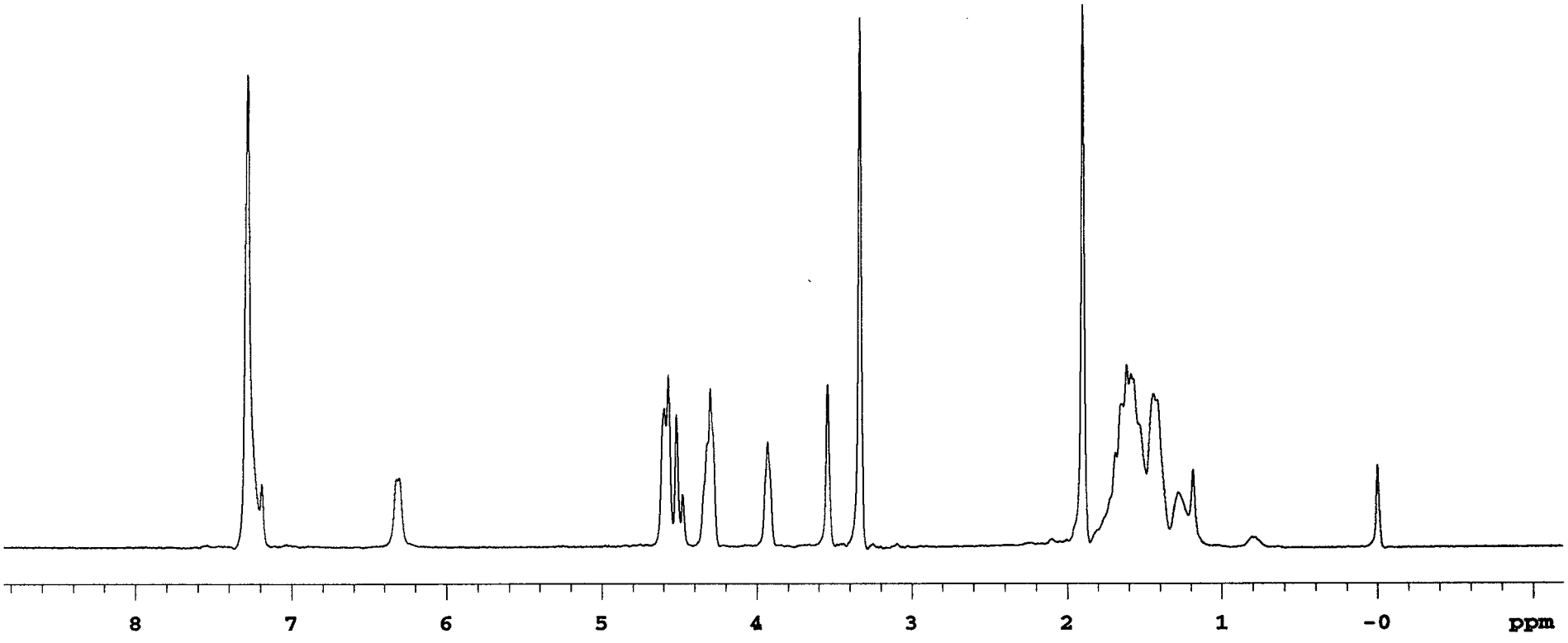

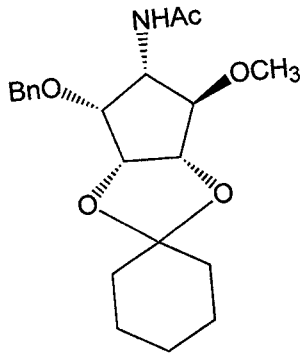

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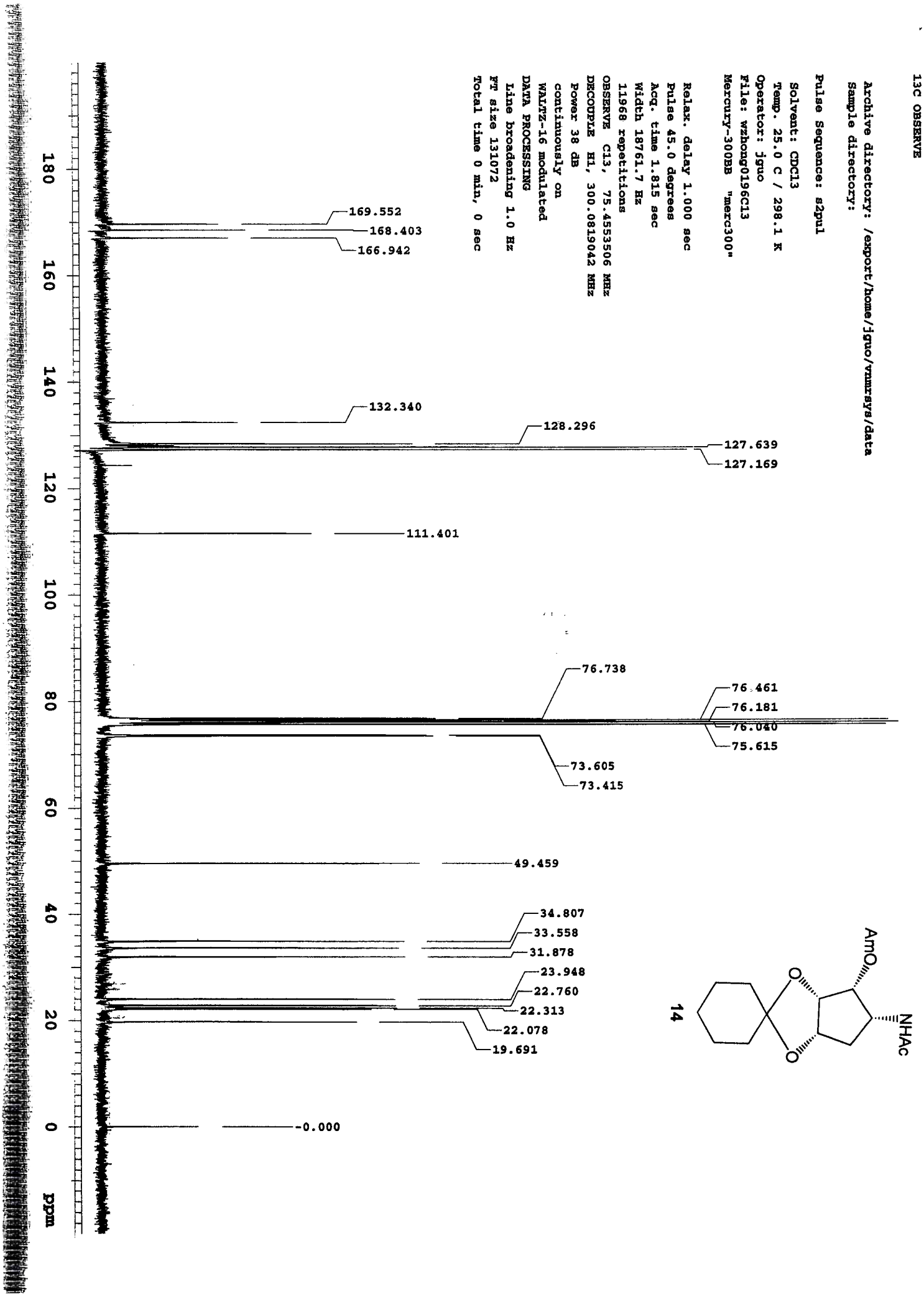
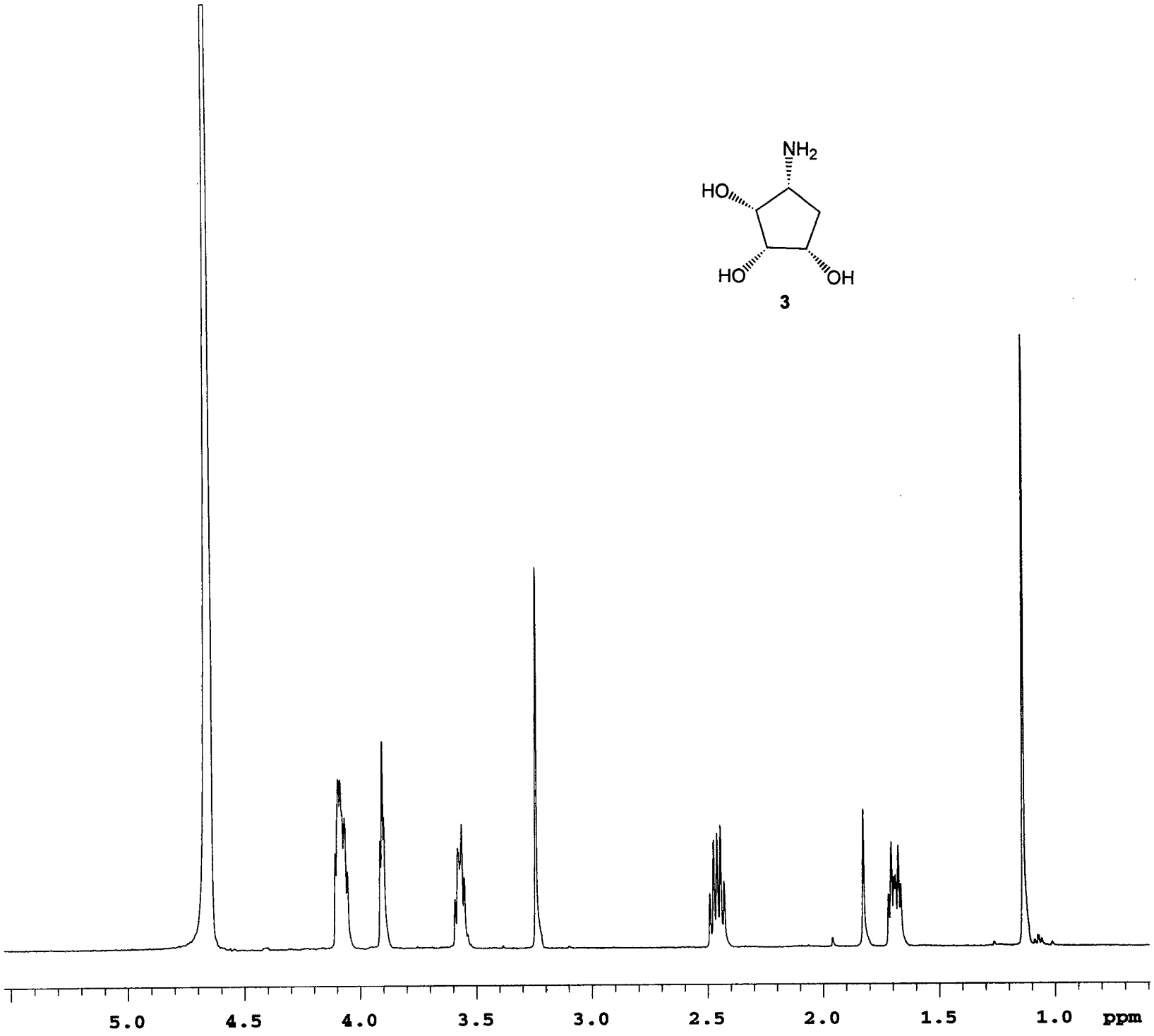

13C OBSERVE
Archive directory: /export/home/jguo/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Operator: jguo
File: wzhong0196C13
Mercury-300BB "merc300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
11968 repetitions
OBSERVE C13, 75.4553506 MHz
DECOUPLE H1, 300.0819042 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 0 min, 0 sec
169.552
168.403
166.942
132.340
128.296
127.639
127.169
111.401
76.738
76.461
76.181
76.040
75.615
73.605
73.415
49.459
34.807
33.558
31.878
23.948
22.760
22.313
22.078
19.691
-0.000
180
160
140
120
100
80
60
40
20
0
ppm
AmO
NHAc
14


NH2
HO
HO
OH
3
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

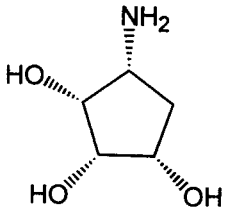
13C OBSERVE

Archive directory: /export/home/wzhong/vnmrsys/data
Sample directory: /export/home/wzhong/vnmrsys/data/wzhong_01Feb2005
File: CARBON

Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Operator: wzhong
Mercury-300BB "merc300"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
928 repetitions
OBSERVE C13, 75.4552576 MHz
DECOUPLE H1, 300.0819042 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 2 hr, 10 min, 40 sec



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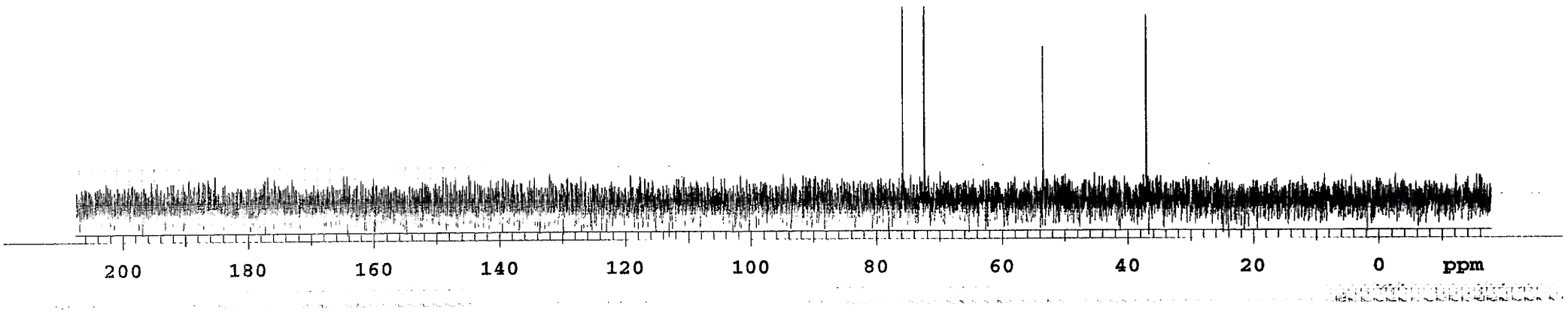

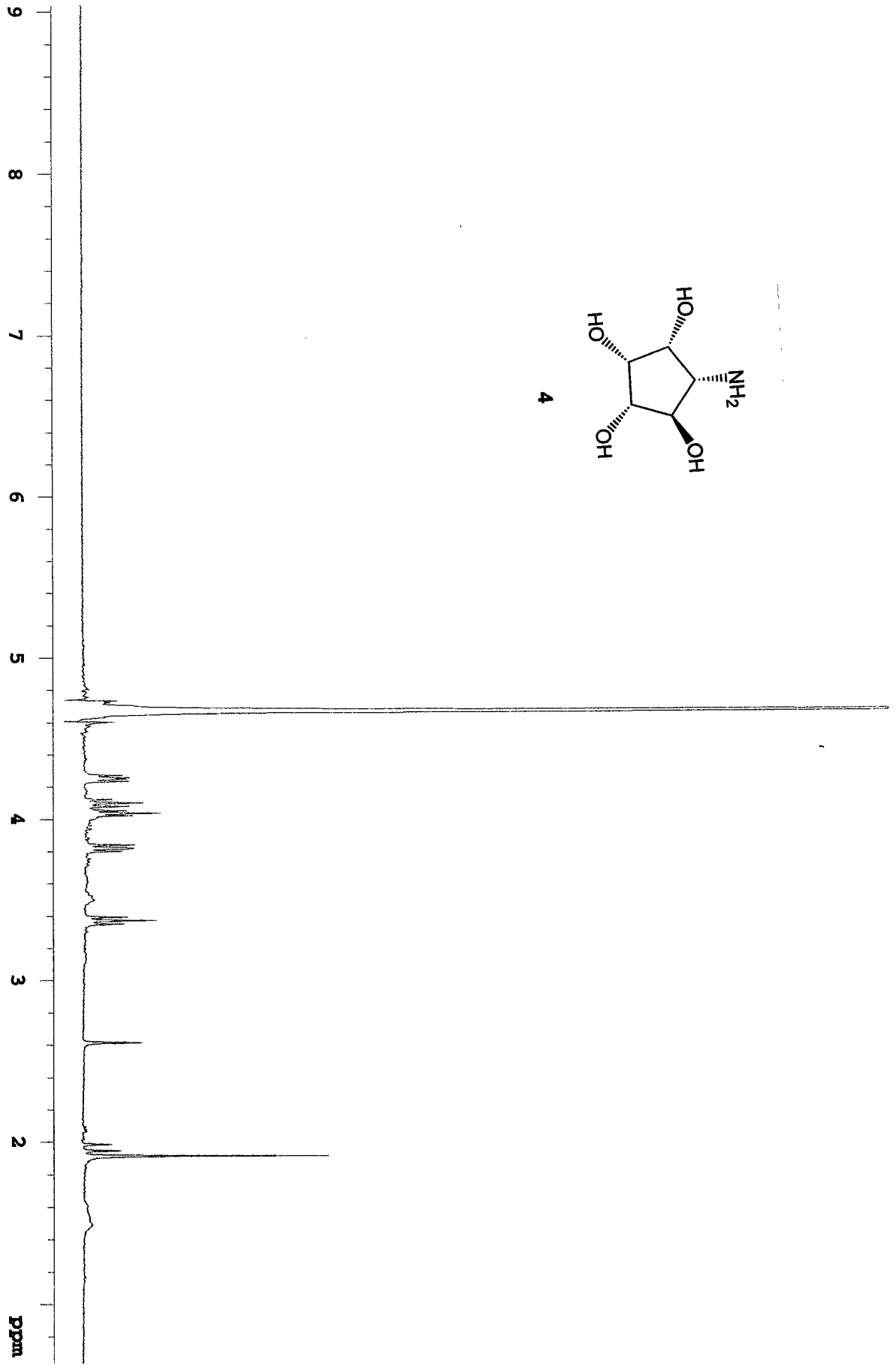

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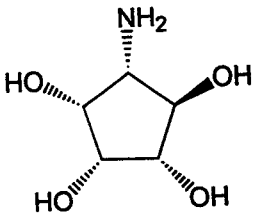

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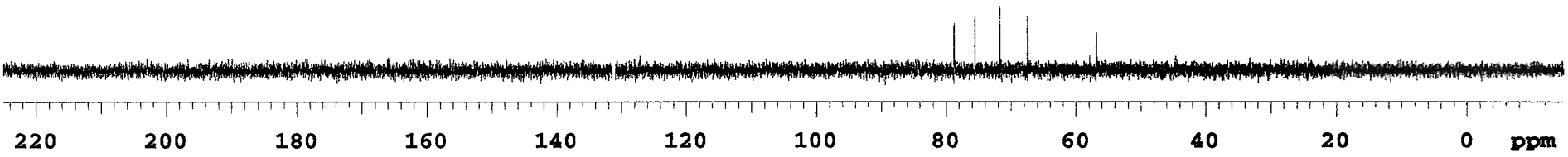

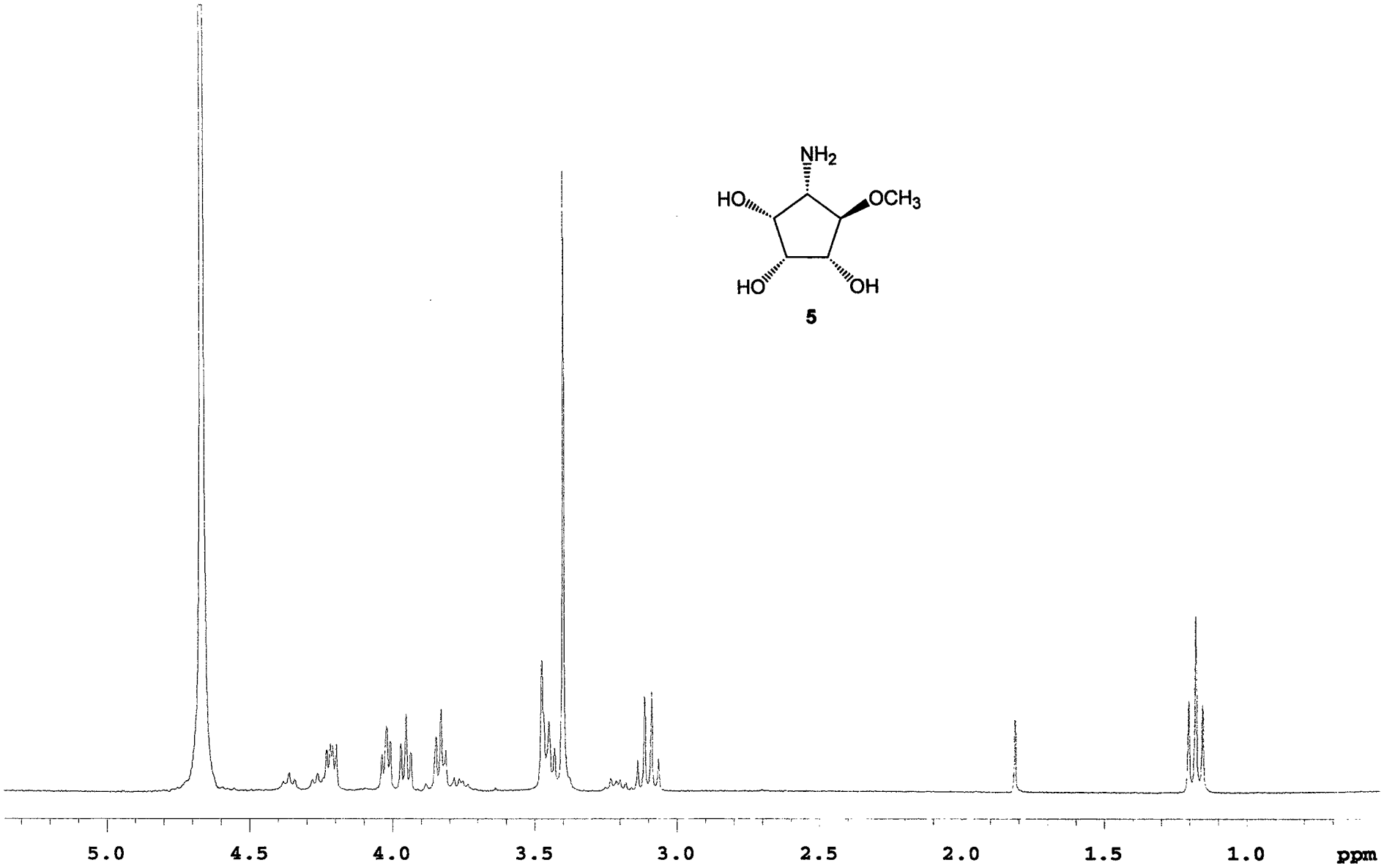

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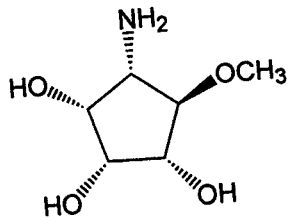

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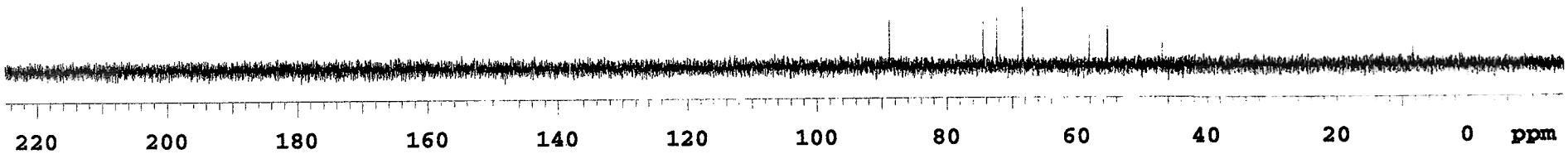

	Mannostatin A pH5.75	Mannostatin B	
Compound complexed with dGMII	**1**	**2**	**3**
PDB Accession Number	**3DXO**	**3DX2**	**3DX1**
PDB Ligand Code	MSN	MZB	YHO
DATA COLLECTION			
Xray Source (λ (Å))	Rotating Anode	CHESS A1	CHESS A1
Cell dimensions (Å)	68.9X109.9 X 139.1	68.7X109.3 X 137.8	68.9X109.5 X138.7
Resolution (Å) overall	20-1.70	25-1.40	20-1.21
Resolution (Å) high resoln. shell	1.73-1.70	1.45-1.40	1.24-1.21
% Completeness (overall/hi_res)	99.6/94.8	96.3/73.8	96.2/87.9
Unique Reflections	116271	196104	305324
Redundancy (overall/hi_res)	4.9/2.3	4.4/2.3	6.9/4
I/sigma (overall/hi_res)	17.3/2.1	16.0/3.8	17.8/3.4
R merge (overall/hi_res)	0.05/0.33	0.05/0.29	0.066/0.54
REFINEMENT			
Program for final refinement	Refmac	Refmac	ShelX
R/R_{free} for all reflections	0.159/0.186	0.142/0.164	0.127/0.163
Atoms in Model	9571	9569	9830
Amino Acids	1015	1015	1015
Alternate Conformers	23	22	29
Water Molecules	1170	1247	1435
rmsd bonds (Å)	0.017	0.012	0.014
rmsd angles (°)	1.56	1.47	2.1
Average BFactor Å^2			
Overall	13.2	18.2	20.5
Protein Main Chain	10.7	15.5	15.2
Protein Side Chain	12.9	17.3	20.1
Water	23.0	29.9	35.5
Bound Compound	8.4	11.1	11.1
Zn	7.9	9.7	9.7

Compound complexed with dGMII	**4a**	**4b**	**5**
PDB Accession Number	**3DX3**	**2F7Q**	**3DX4**
PDB Ligand Code	YTB	AOL	GOO
DATA COLLECTION			
Xray Source (λ(Å))	CHESS A1	Rotating Anode	CHESS A1
Cell dimensions (Å)	69.1X110.0 X 138.7	68.8X109.7 X137.7	68.8X109.7 X 138.3
Resolution (Å) overall	40-1.42	30-1.85	40-1.38
Resolution (Å) high resoln. shell	1.46-1.42	1.89-1.85	1.40-1.38
% Completeness (overall/hi_res)	97.1/78.8	99.0/97.4	99.6/82.8
Unique Reflections	193233	88,637	212071
Redundancy (overall/hi_res)	7.4/5.1	7.5/2.6	14.6/6.7
I/sigma (overall/hi_res)	18.2/6.6	21.1/4.8	24.2/5.2
R merge (overall/hi_res)	0.074/0.26	0.094/0.391	0.078/0.41
REFINEMENT			
Program for final refinement	Refmac	CNS	Refmac
R/R_{free} for all reflections	0.148/0.174	0.15/0.19	0.188/0.203
Atoms in Model	9861	9435	9687
Amino Acids	1015	1014	1015
Alternate Conformers	34	19	20
Water Molecules	1412	1145	1306
rmsd bonds (Å)	0.011	0.024	0.014
rmsd angles (°)	1.47	2.1	1.59
Average BFactor Å^2			
Overall	14.4	20.0	16.0
Protein Main Chain	10.8	17.4	13.0
Protein Side Chain	13.3	19.5	15.2
Water	28.0	31.0	27.4
Bound Compound	7.6	14.9	8.2
Zn	6.3	15.1	7.8

Table S1: Data collection and refinement statistics for *Drosophila* Golgi α-Mannosidase II complexes reported in this study. Space group is $P2_12_12_1$ (all angles are 90°) and there is one molecule per asymmetric unit.

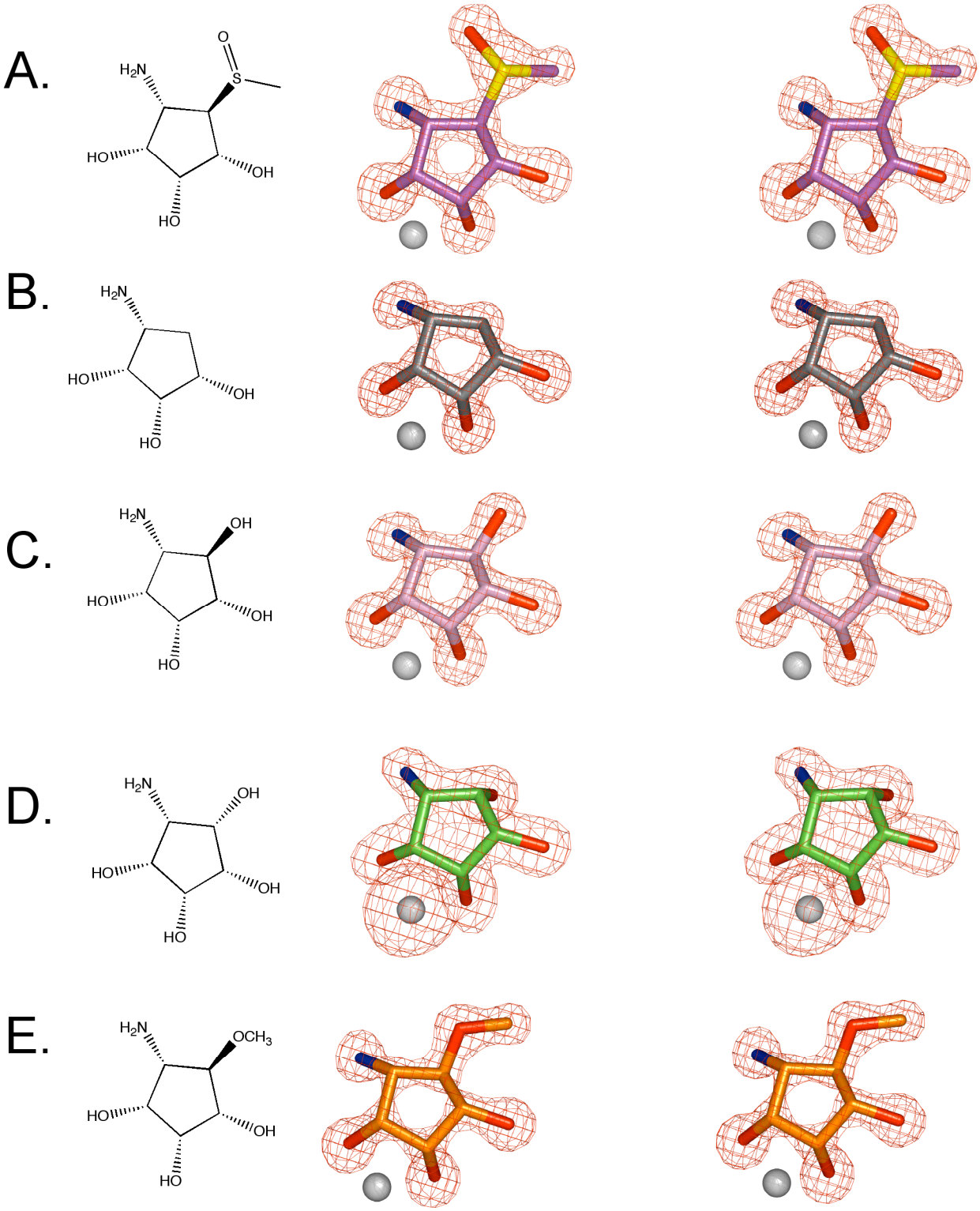

Figure S1: Stereoviews (divergent) of the bound compounds in the active site of dGMII. Simulated annealing (3000 K) Fo-Fc omit maps were generated in CNS. The electron density was contoured at 5 sigma (red). In all cases the compound was removed from the final model before generation of the omit-maps, In D. the zinc was also removed. A. Compound **2** (Mannostatin B), B. Compound **3**, C. Compound **4a**, D. Compound **4b**, E: Compound **5**,

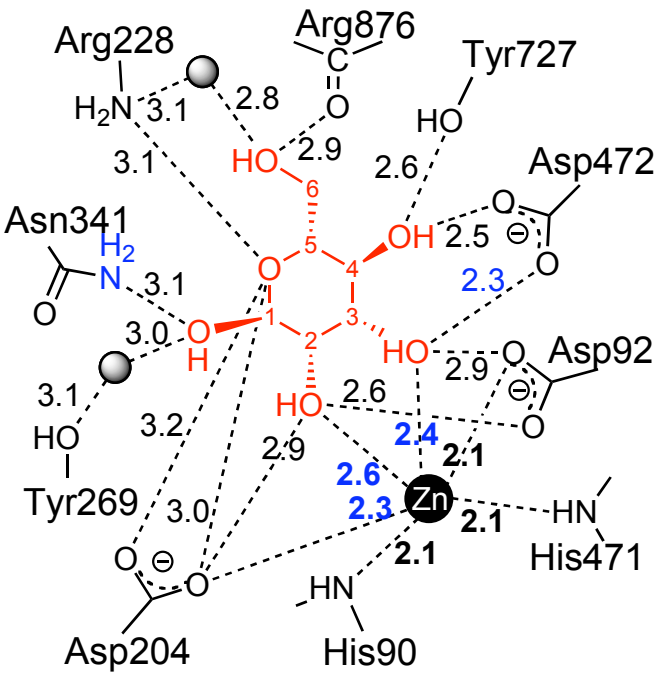

Figure S2. Close interactions between dGMII D341N mutant and mannose. Data from PDB: 3BUD. Zinc is shown as a black sphere and waters are shown as grey spheres. Zinc interactions are shown in bold. Obvious differences between the bound mannose and bound Mannostatin A interaction distances are highlighted in blue.