Synthesis and Characterization of Biotin Derivatives as Multifunctional Oligosaccharide Tags

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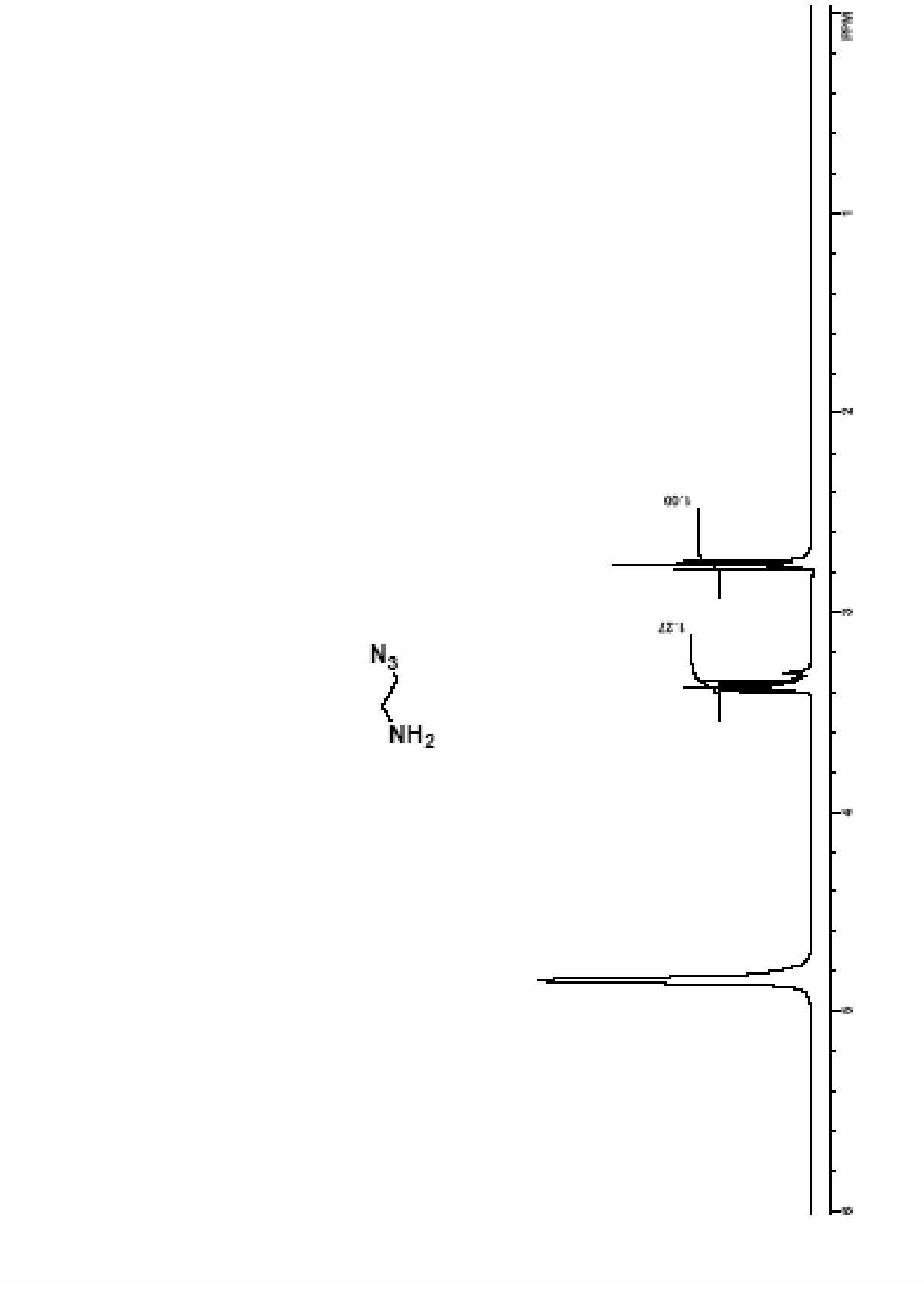
Contents

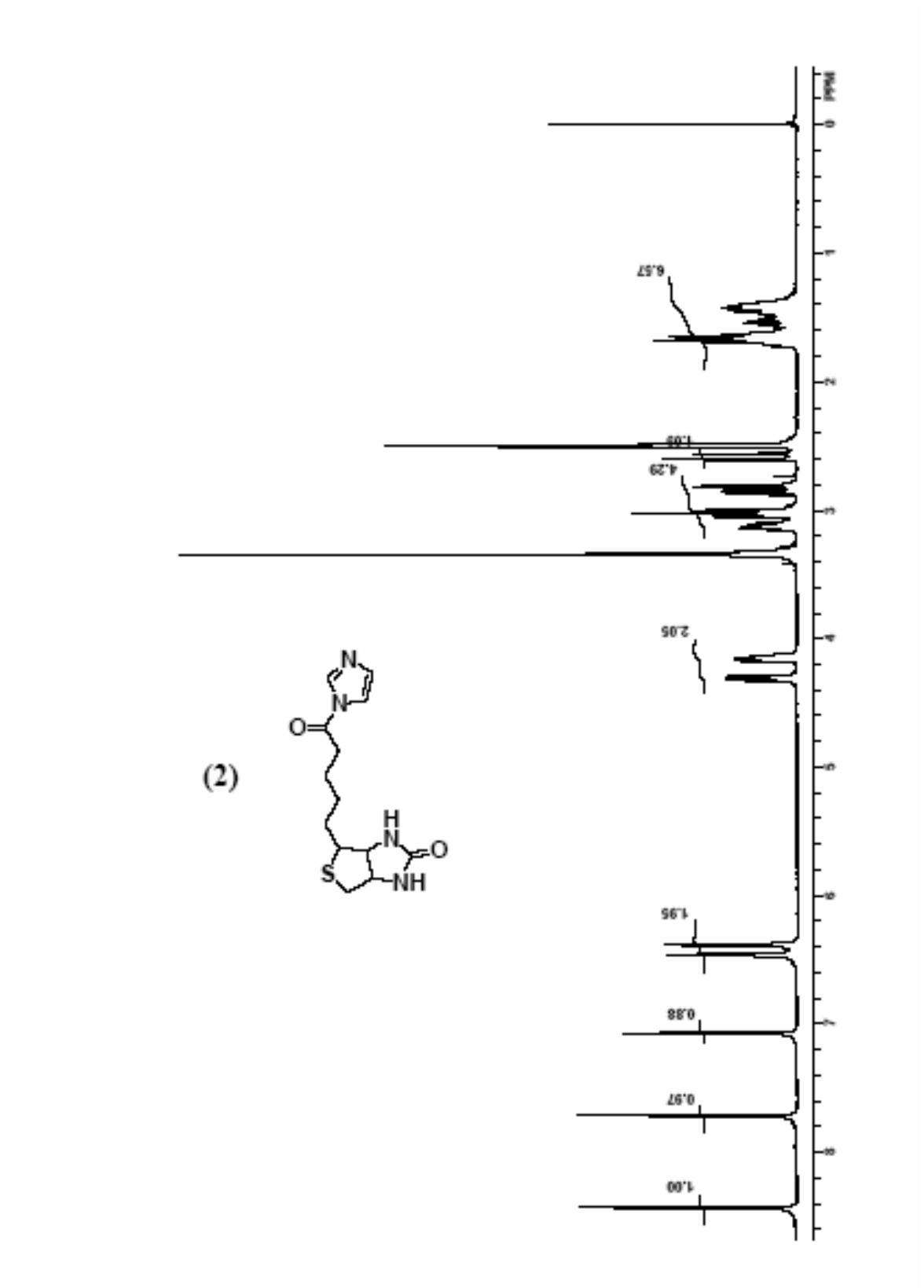
Materials and methods

1H NMR and 13C NMR spectra were recorded on at 300 MHz with specified deuterated solvents. The signals were assigned using 1H NMR, 13C NMR, and COSY technique. Chemical shifts are reported in parts per million (ppm) and are referenced to the respective solvent. Coupling constants (J) are reported in Hertz (Hz). During purification of labeled oligosaccharides, a heated CentriVap Concentrator (LABCONCO) was used to remove solvent and concentrate the samples. Aliquots of sample were desalted using the Porous Graphitized Carbon (PGC) cartridges (Supplier-Thermo Electron Hypersil Keystone, UK). The cartridges were washed with 60% Aetonitrile (MeCN) and DI water (H2O) prior to use. Micro liter volumes of solvent were removed in a heated (40 °C) CentriVap (Labconco, Kansas City, MO) with spinning at reduced pressure (12 mTorr).

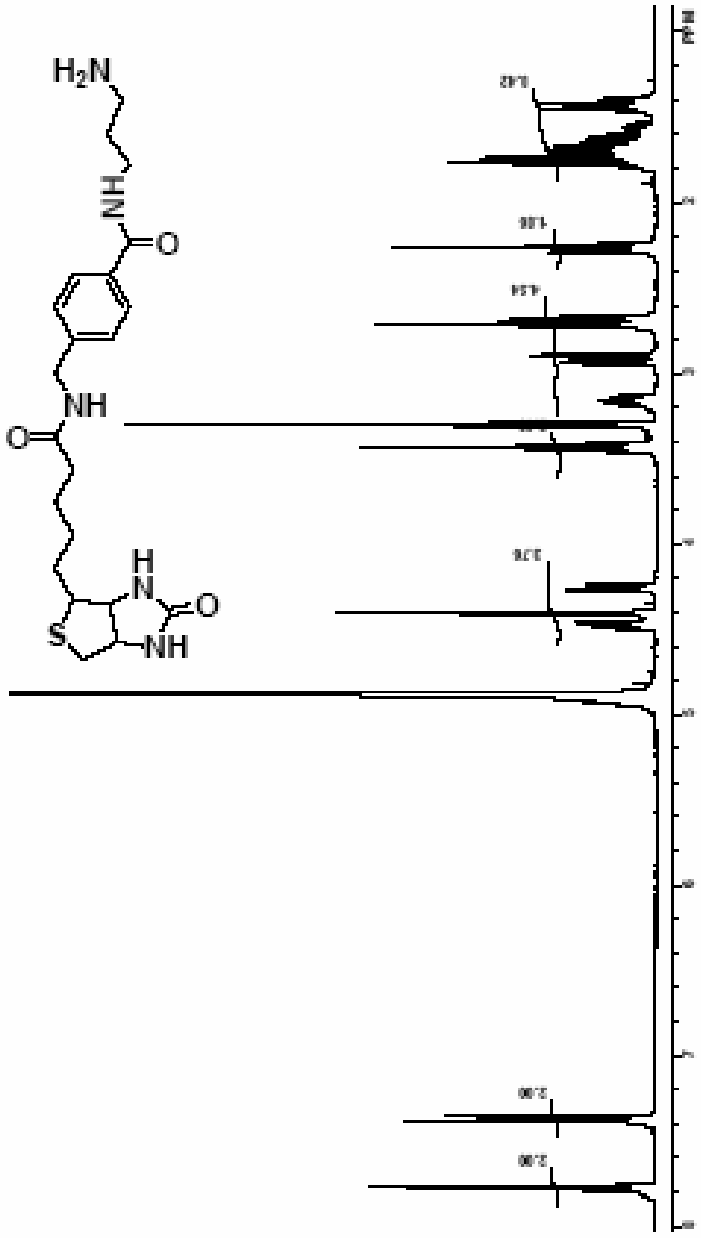
General procedure for labeling mono- and oligosaccharides with the tag

The oligosaccharide was dissolved in 200 µL of methanol (final concentration of ~10-4 M) in a plastic microcentrifuge vial with lid. Approximately ~ 0.1 mg of tag and 1 µL of glacial acetic acid were then added to the vial. The solution was then incubated for ½ hours at 70 C. After the incubation ~ 10mg of NaCNBH3 was added and the tube was sealed and heated at 70o C for 2-2.5 hours. The solution was evaporated to dryness using CentriVap concentrator. The residue was dissolved in 500 µL of deionized water and loaded on [preconditioned with 60.0 % acetonitrile (2 x 1 mL) and DI water (2 x 1 mL)] PGC cartridge. The cartridge was washed with DI water (2 x 1 mL). The desalted sample was eluted off the cartridge with 60% MeCN (1 mL). 1.0 mL fraction of the eluent was collected in microcentrifuge vials. Excess solvent was removed by CentriVap concentrator and the sample analyzed using MALDI-TOF mass spectrometer.

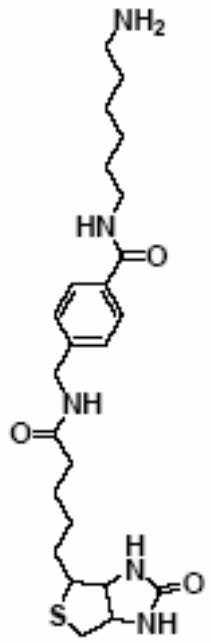

N3
NH2
1.00
1.27
PPM

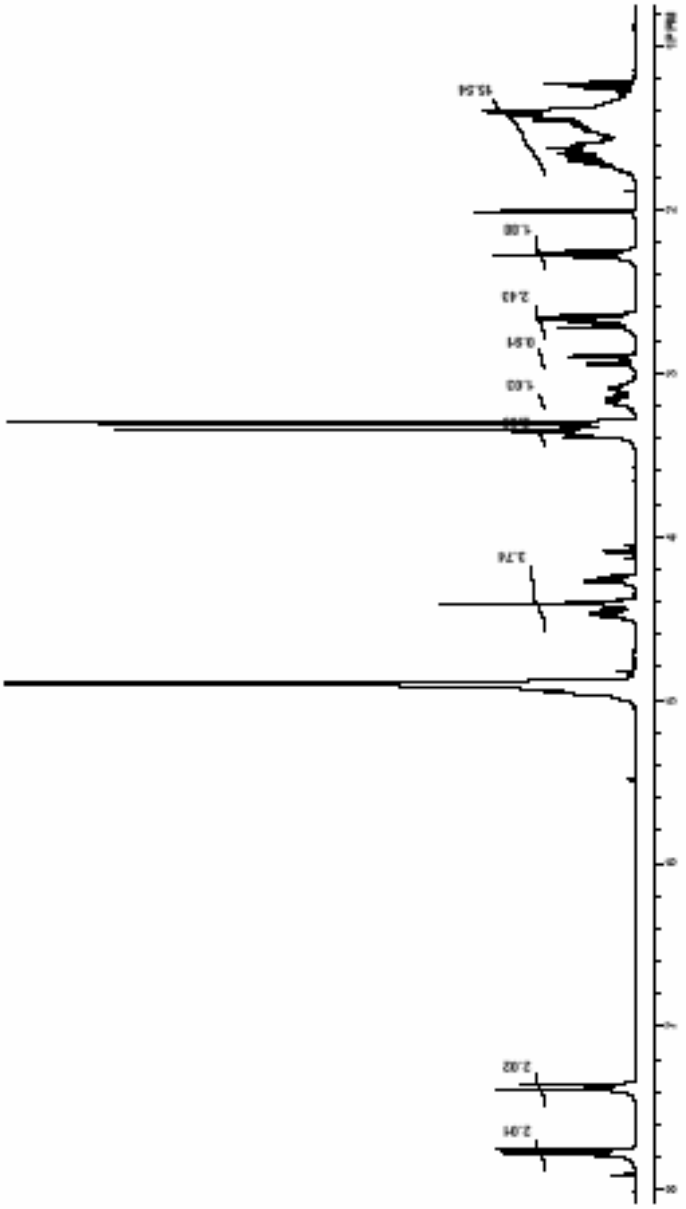

(2)
N
N
O
H
N
O
S
NH
6.57
4.29
2.05
1.95
0.88
0.97
1.00
PPM

(3a)

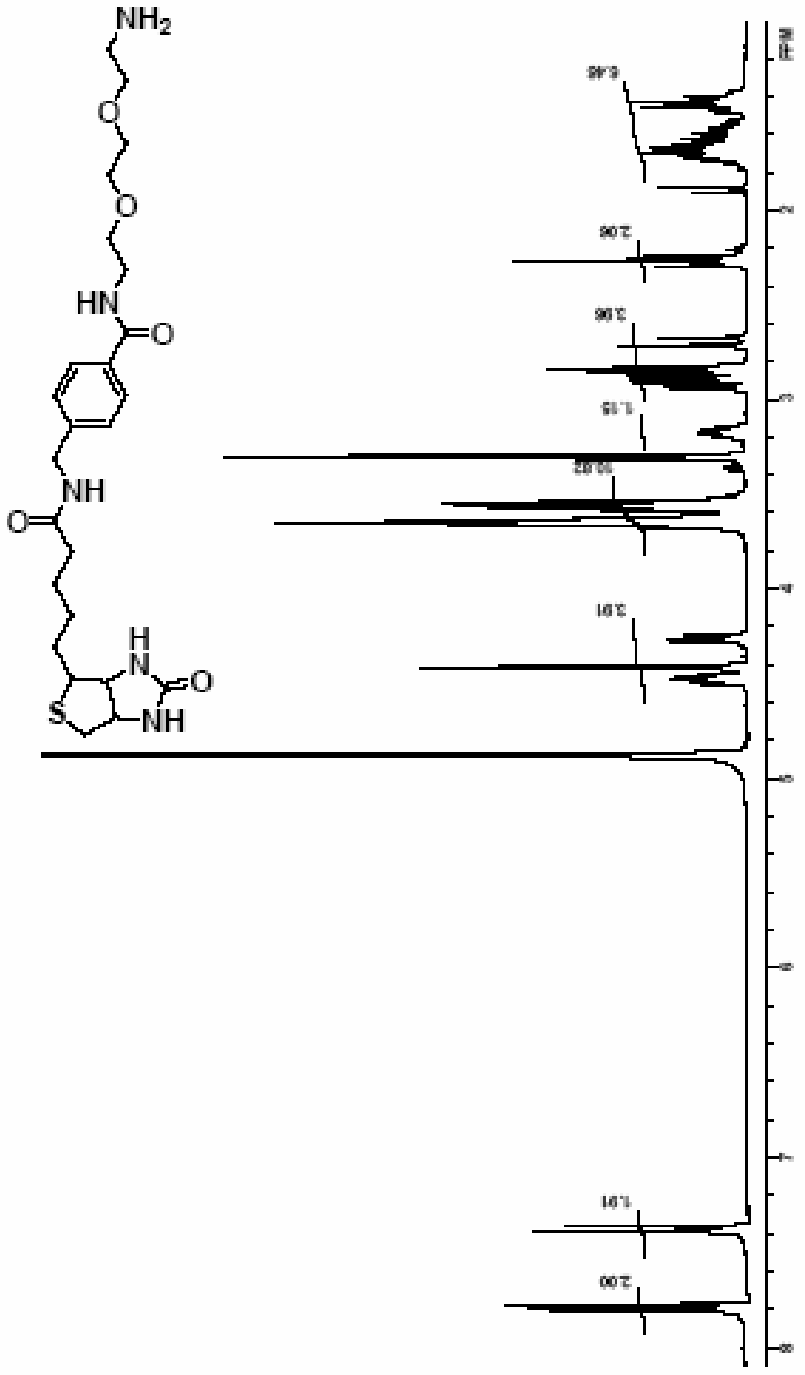


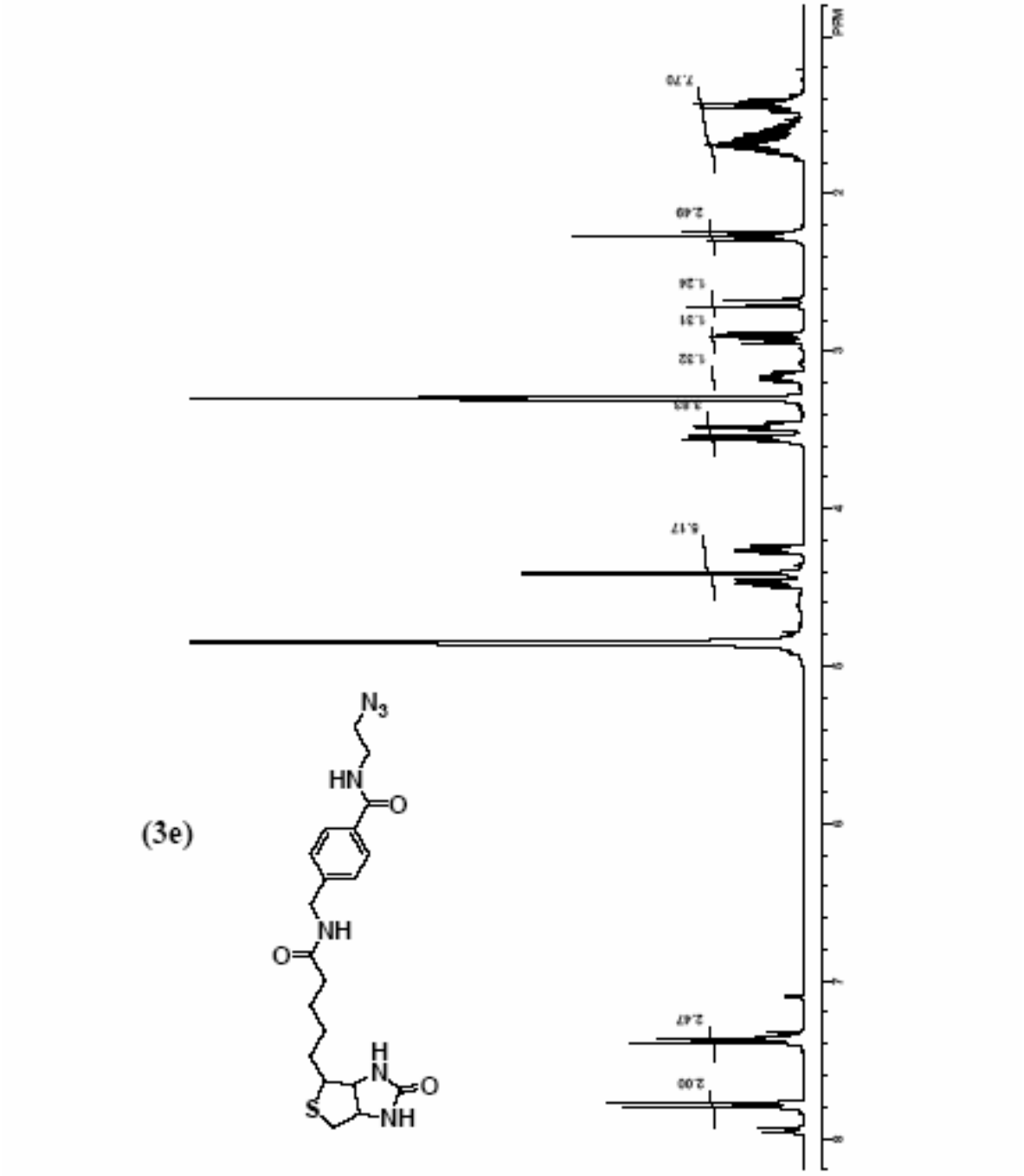
(3b)



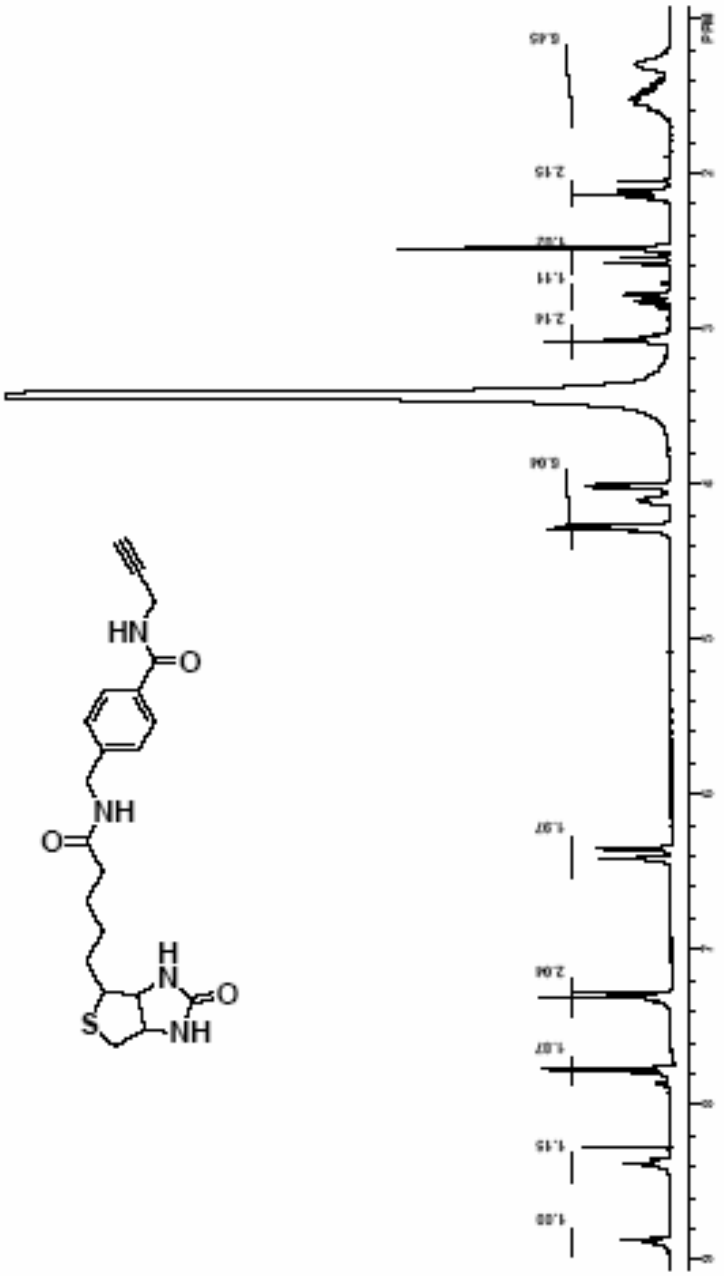


(3c)

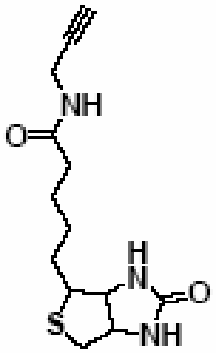


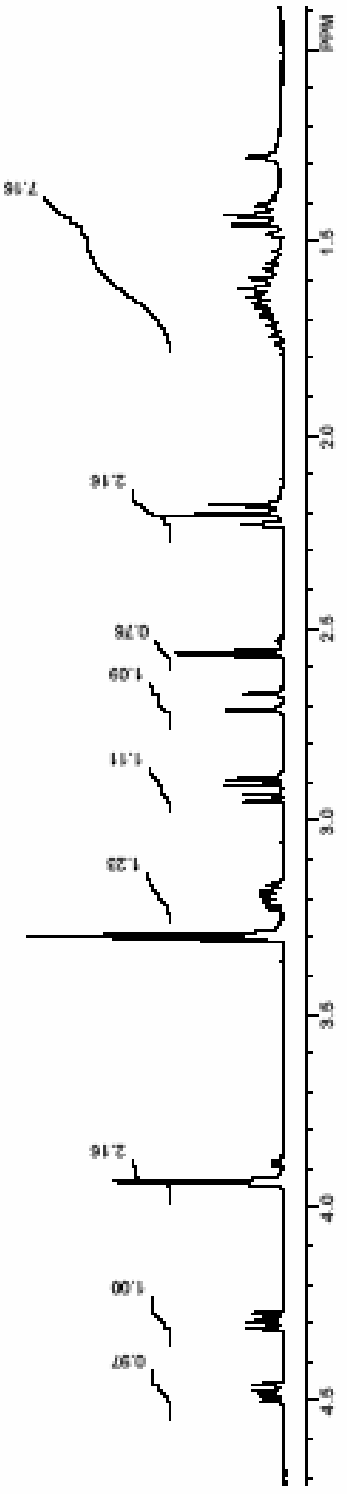

7.70
2.49
1.34
1.31
1.32
3.89
5.17
2.47
2.00
PPM
(3e)
N3
HN
O
NH
O
H
N
O
S
NH

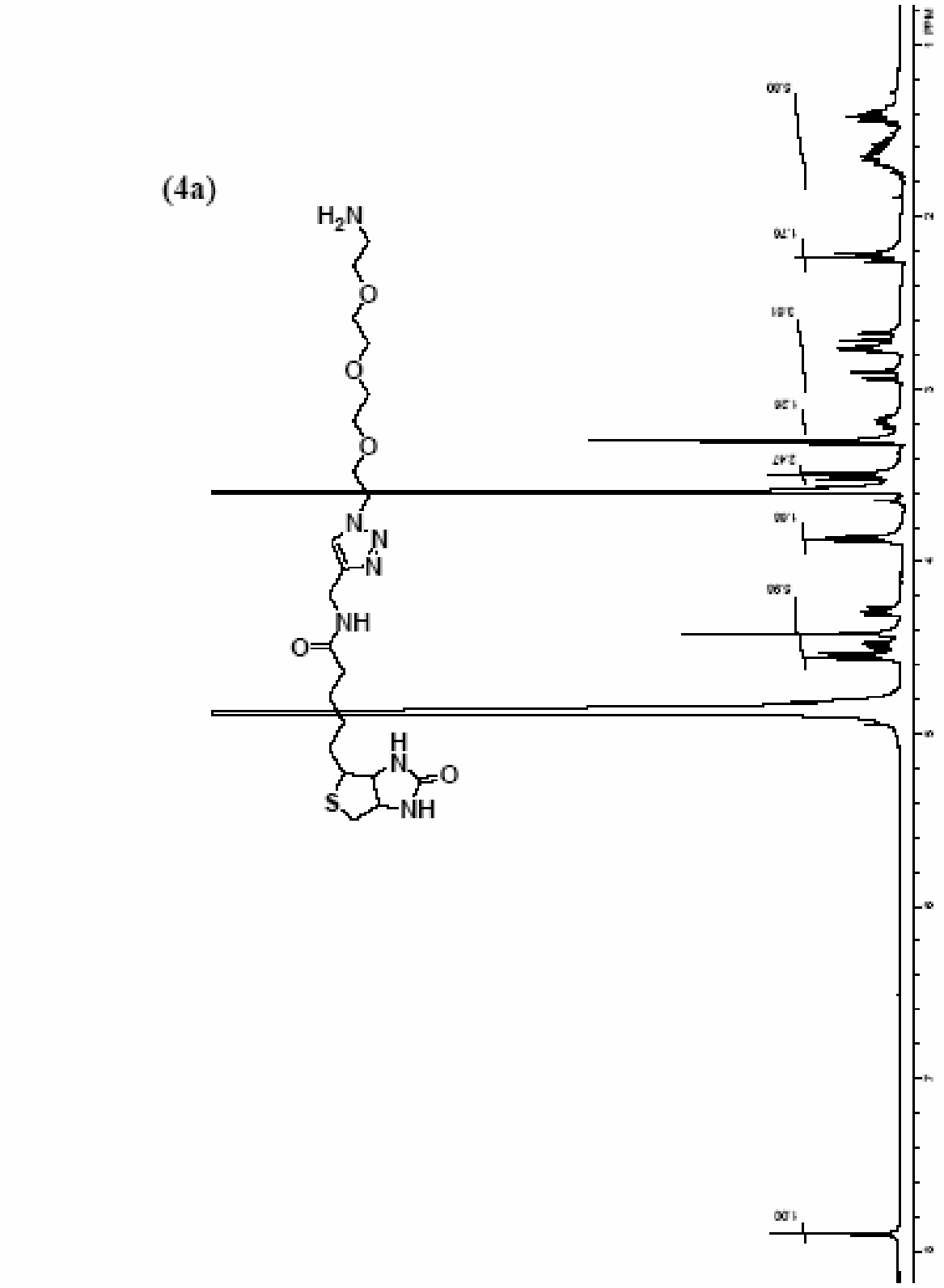
(3d)

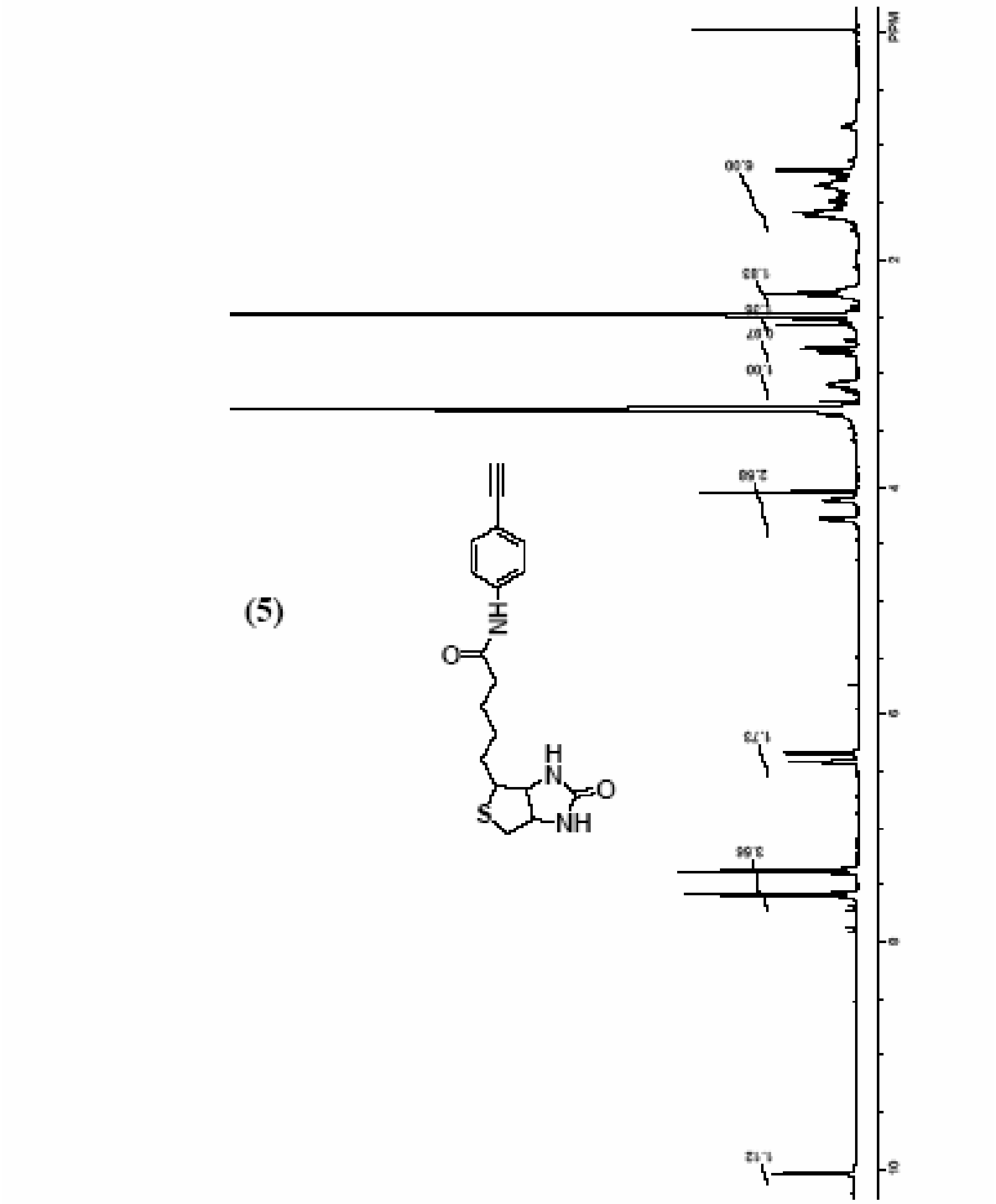


(4)

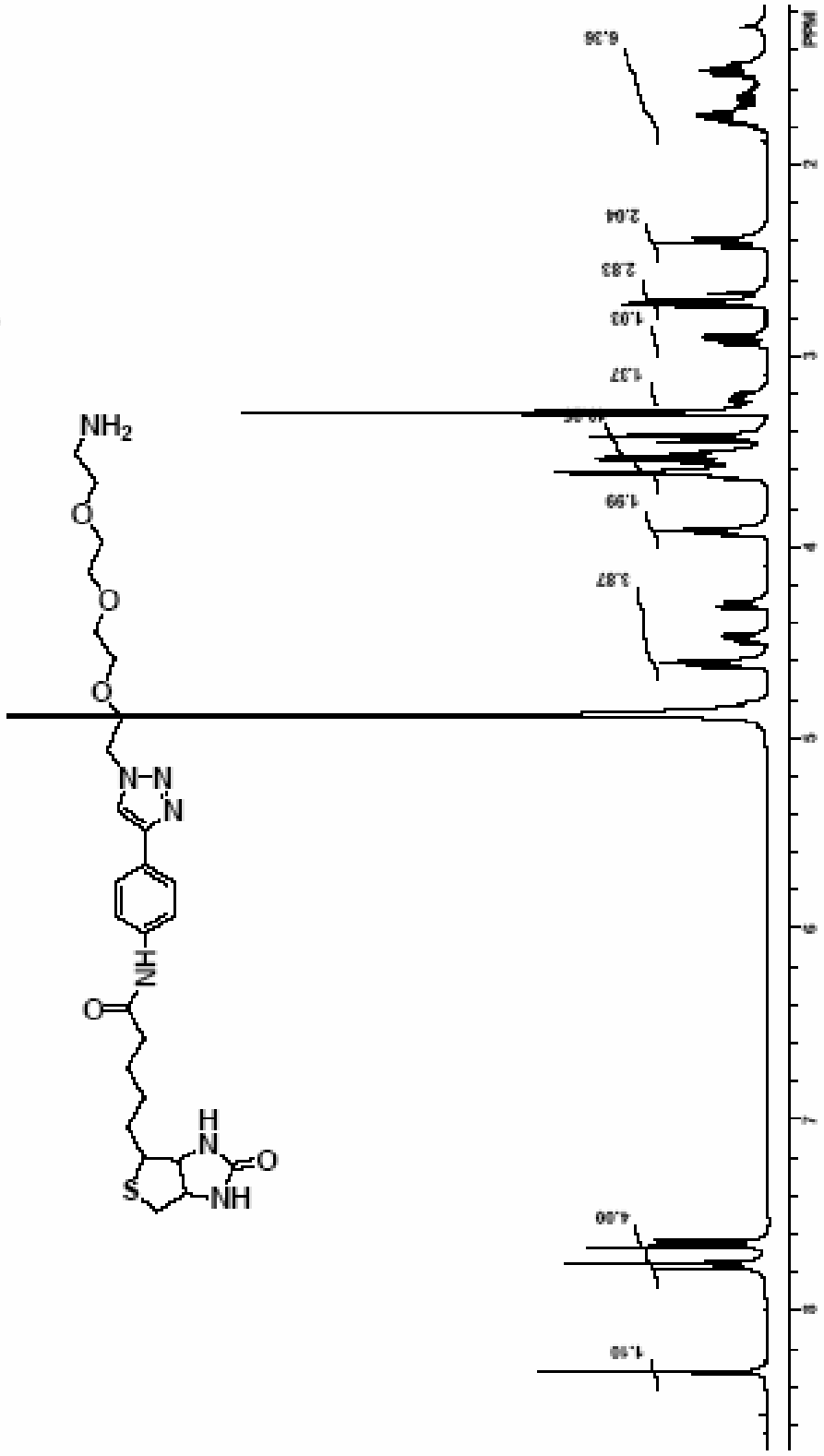

NH
O
H
N
O
S
NH


7.16
2.16
0.76
1.09
1.11
1.23
2.16
1.06
0.97
PPM
1.5
2.0
2.5
3.0
3.5
4.0
4.5

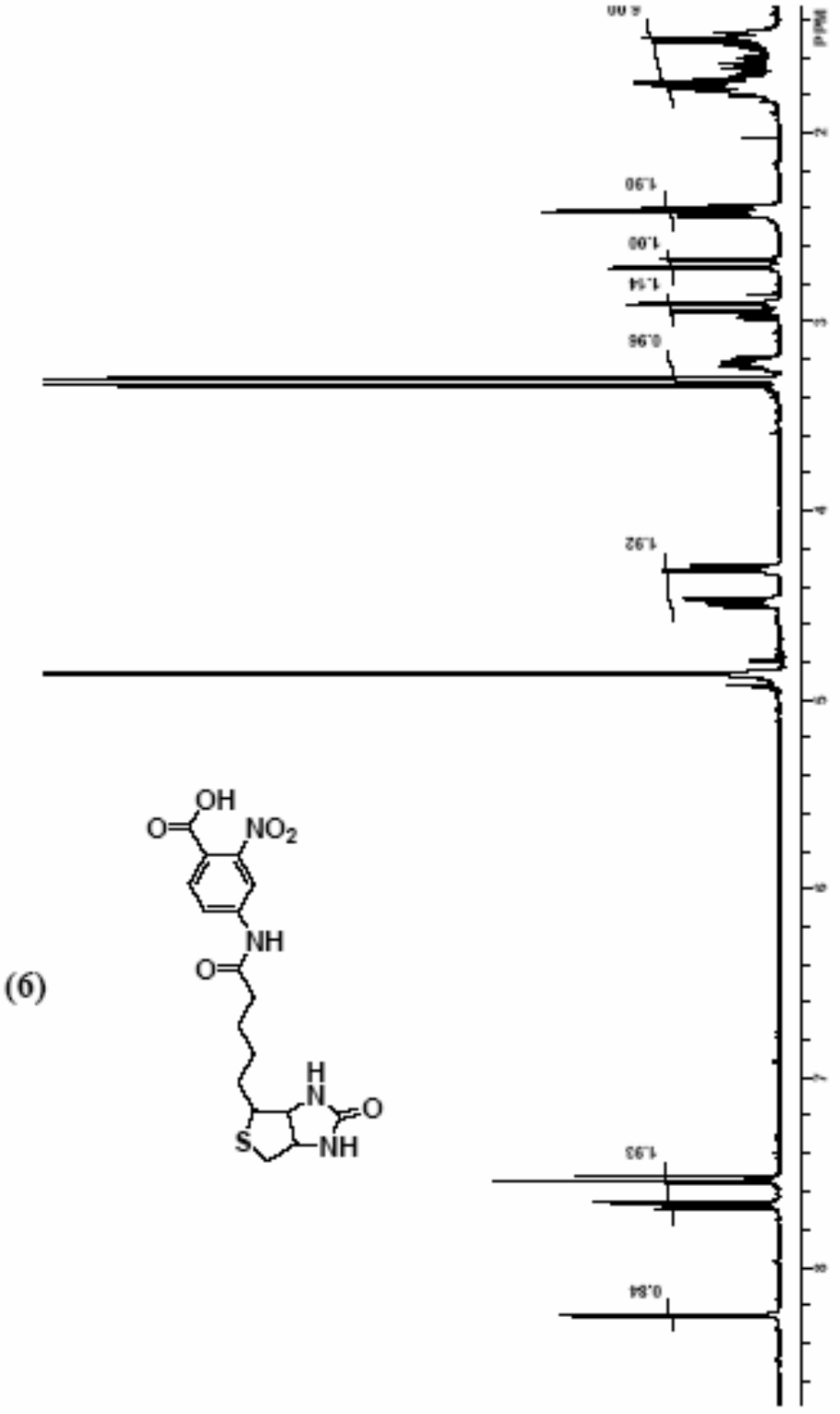

(4a)
H2N
O
O
O
N
N
N
NH
O
H
N
O
S
NH

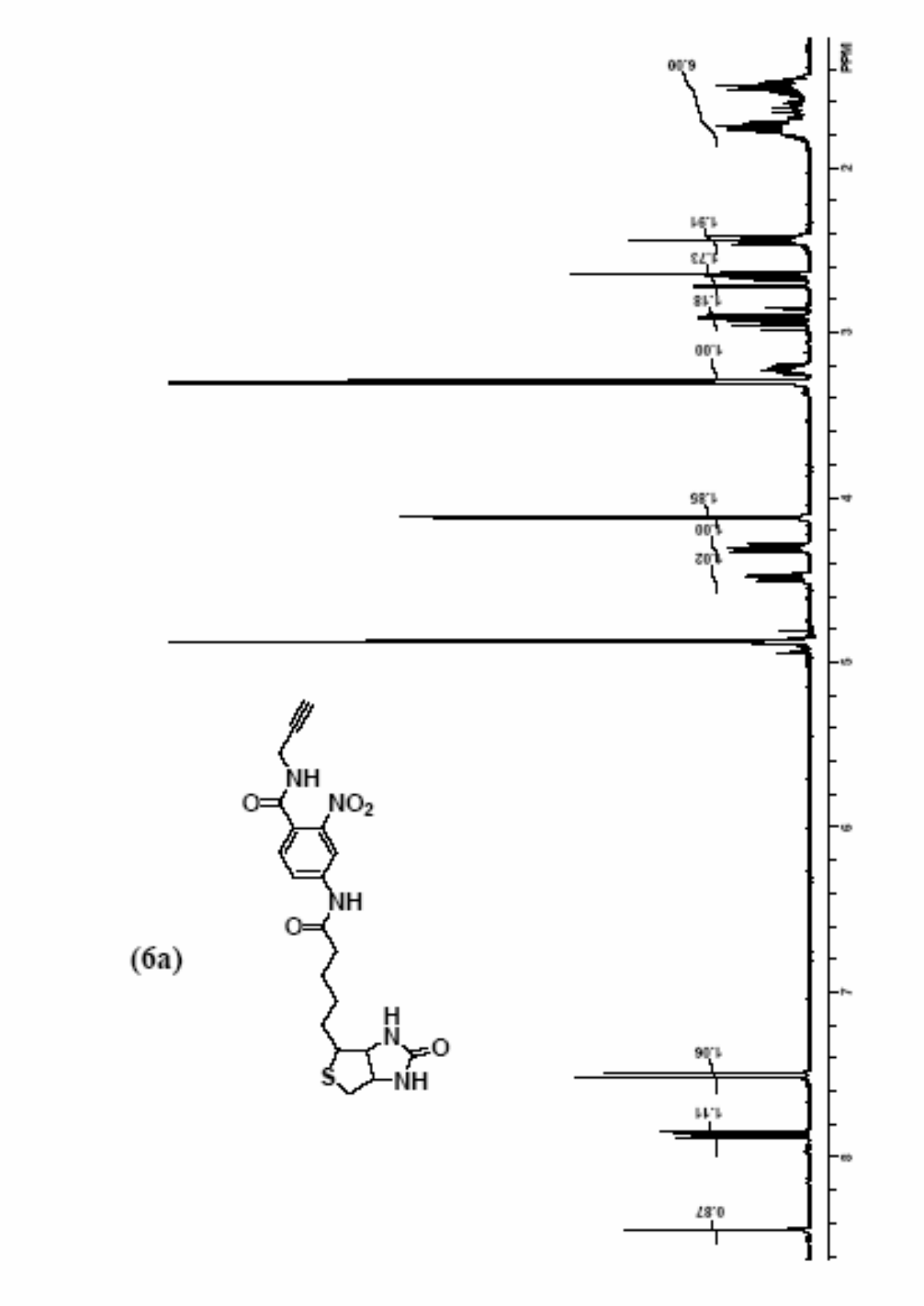

(5)
NH
O
H
N
O
S
NH
PPM
6.00
1.33
0.36
0.07
1.00
2.69
1.79
3.66
1.12

(5a)



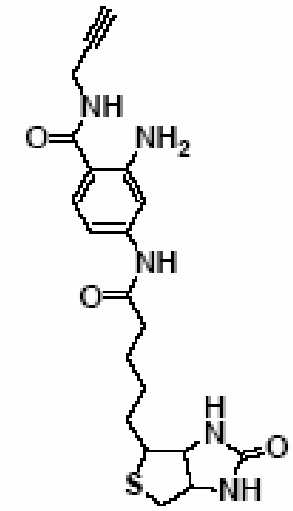
(б)



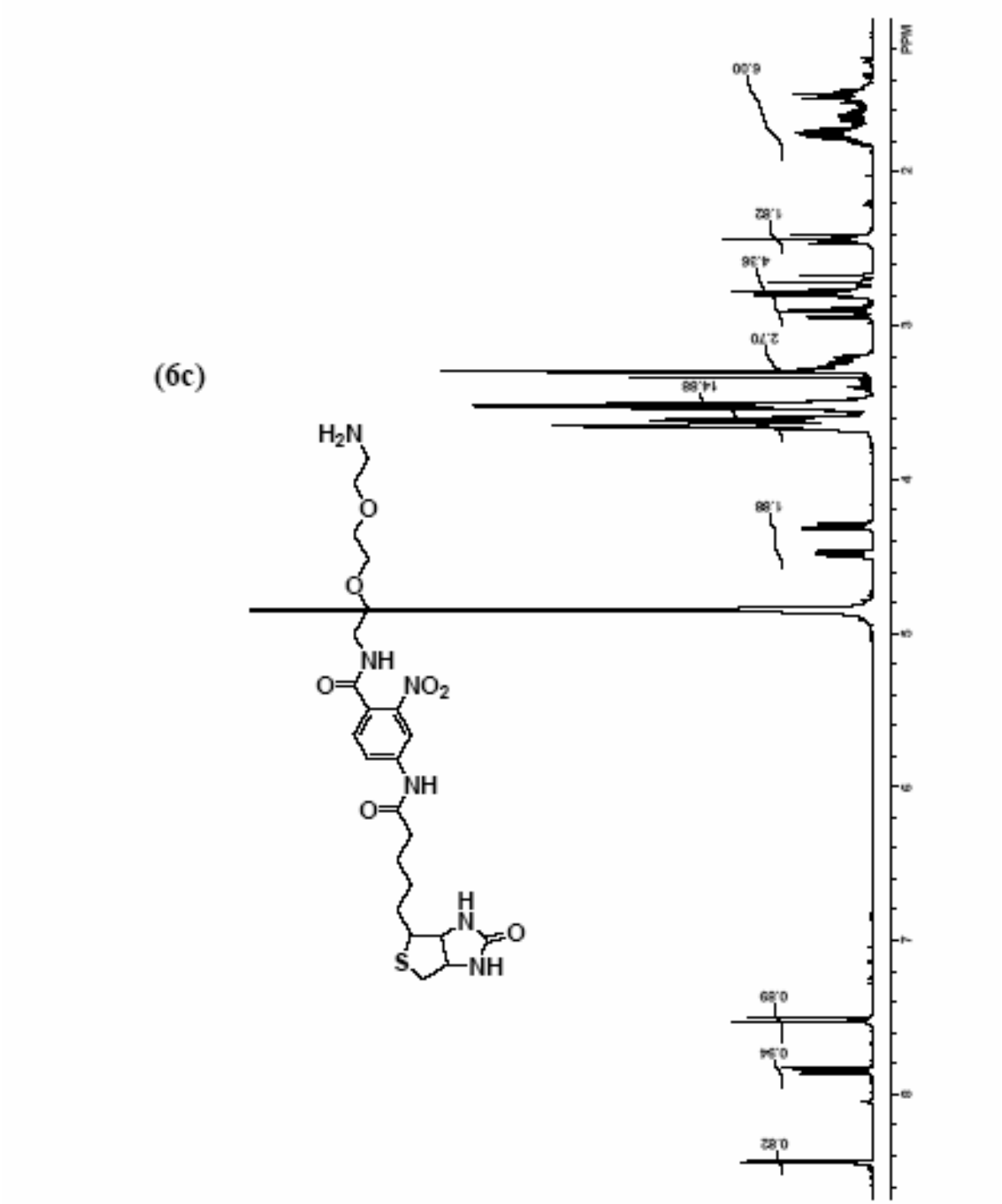

6.00
1.91
3.73
1.18
1.00
1.85
1.00
1.02
1.06
1.11
0.87
PPM
NH
O
NO2
NH
O
H
N
O
S
NH

(6a)

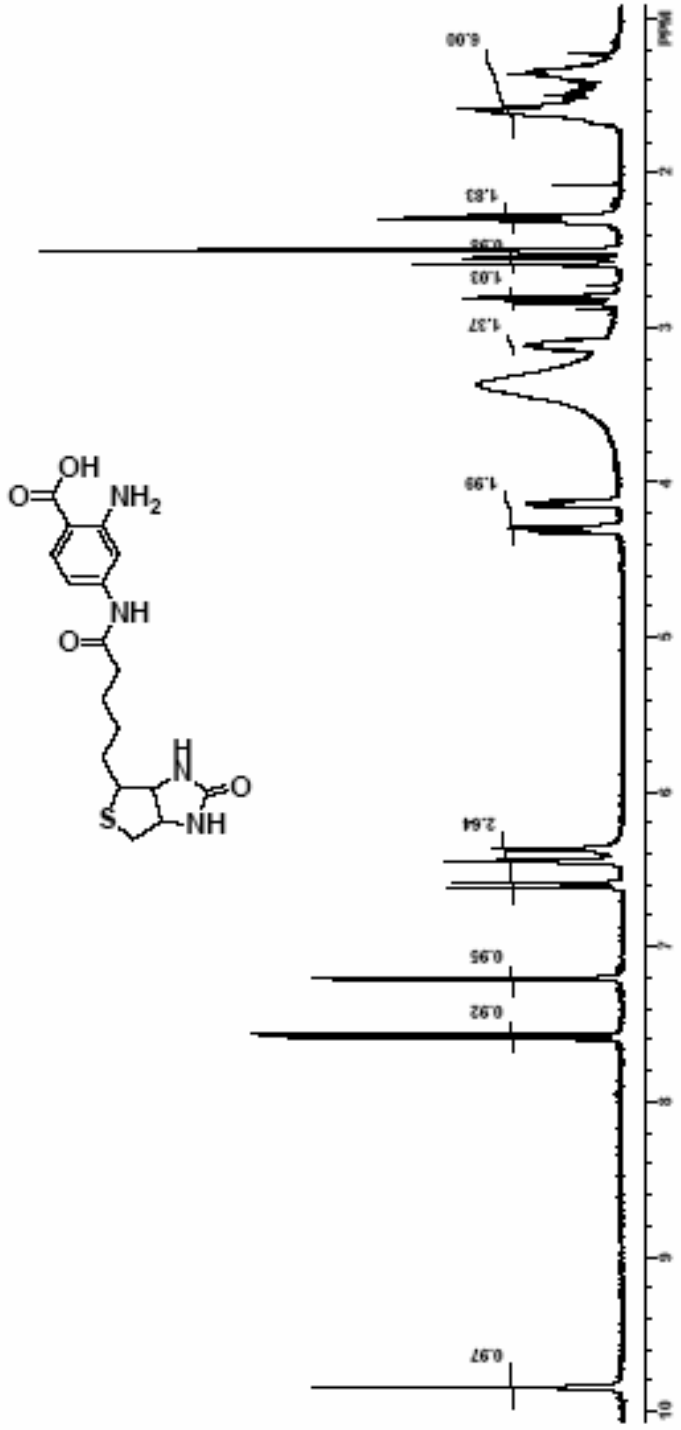
(6b)

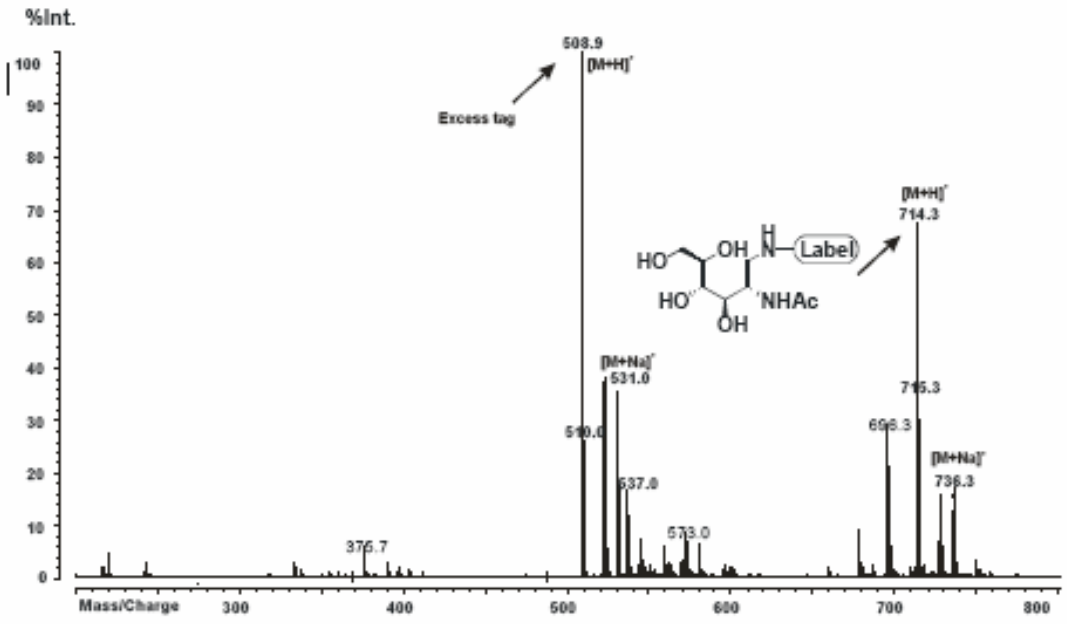


(6c)

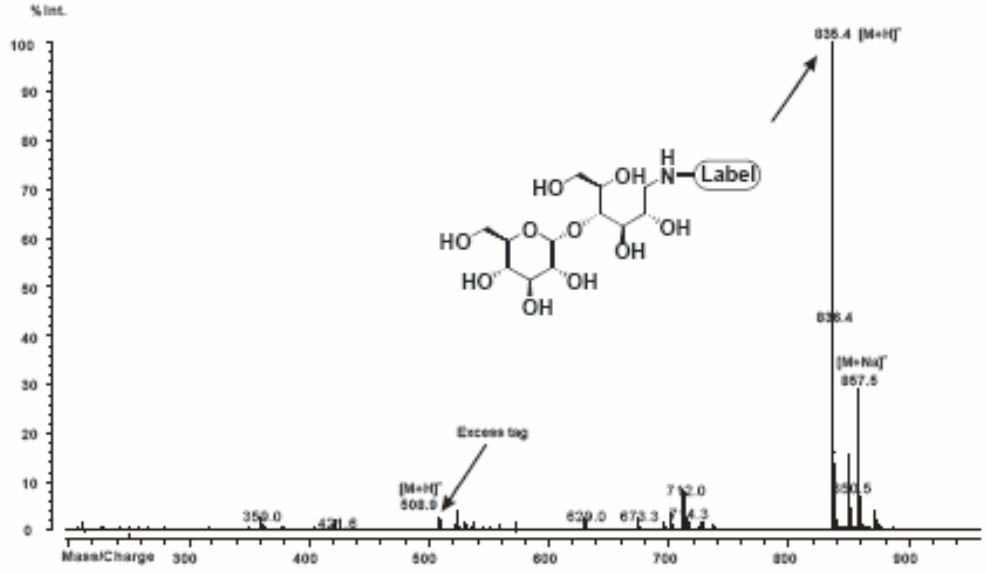

H2N
O
O
NH
O
NO2
NH
O
H
N
O
S
NH
6.00
1.82
4.36
2.70
14.88
1.88
0.89
0.94
0.82
PPM

(6d)

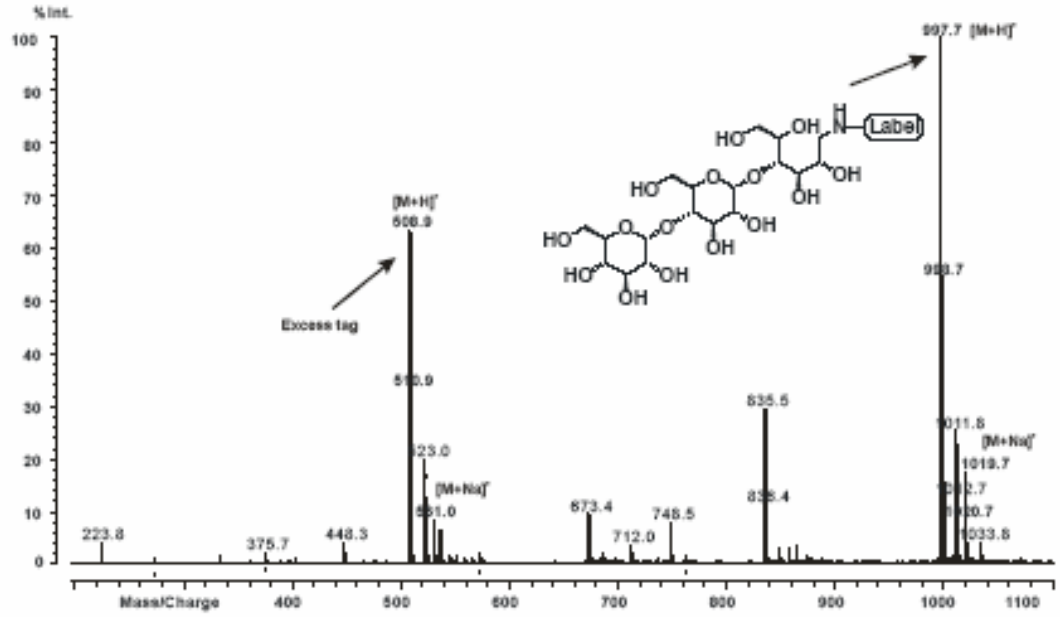




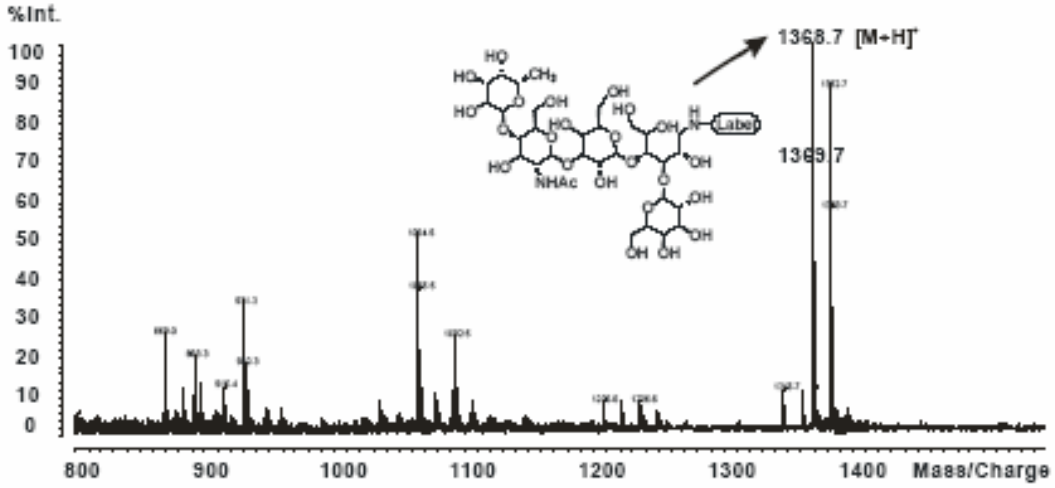
MALDI-TOF spectrum of 2-*N*-acetamido-2-deoxy-glucopyranose (*N*-acetylglucosamine) labeled with compound **3c**.



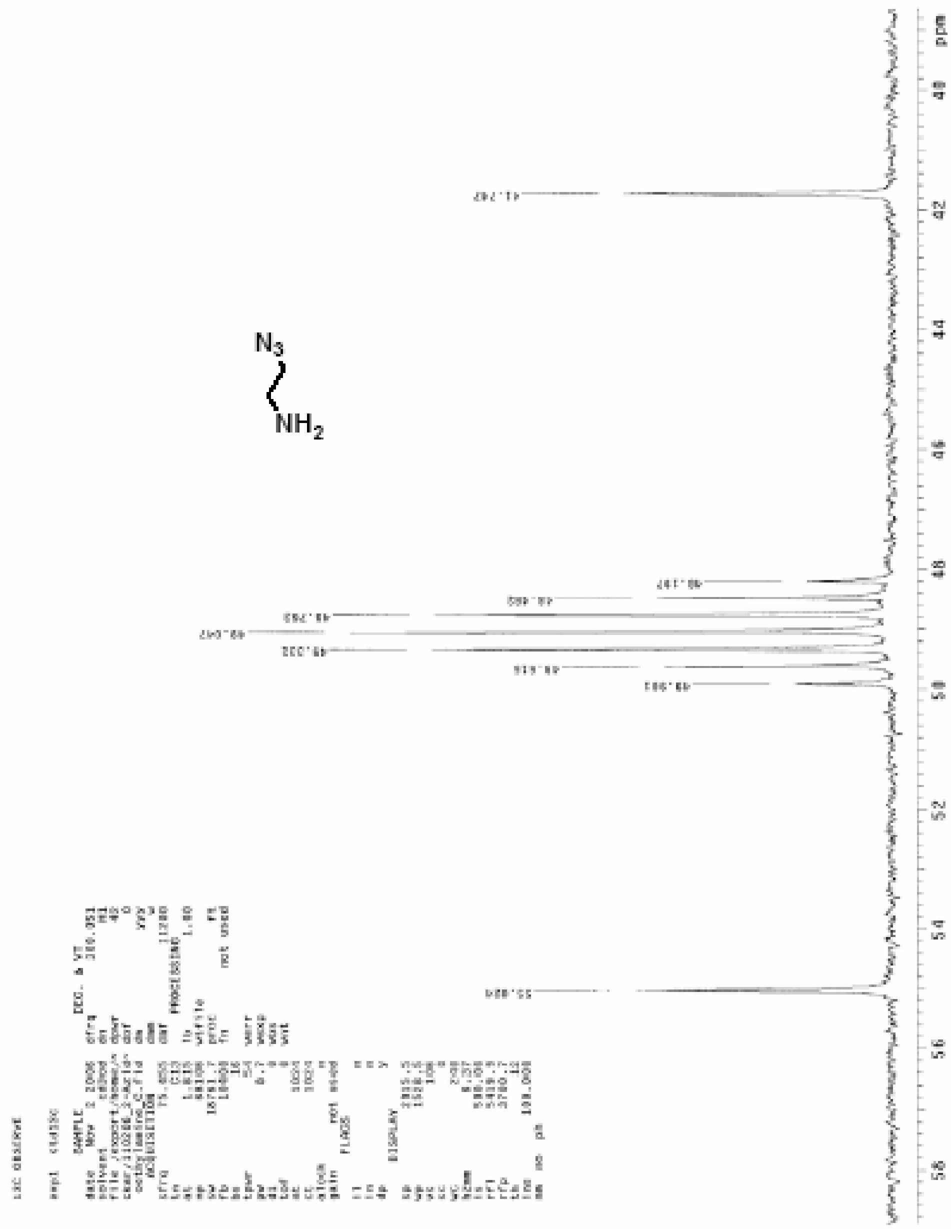
MALDI-TOF spectrum of 4-*O*-(α-glucopyranosyl)-glucopyranose (maltose) labeled with compound **3c**.

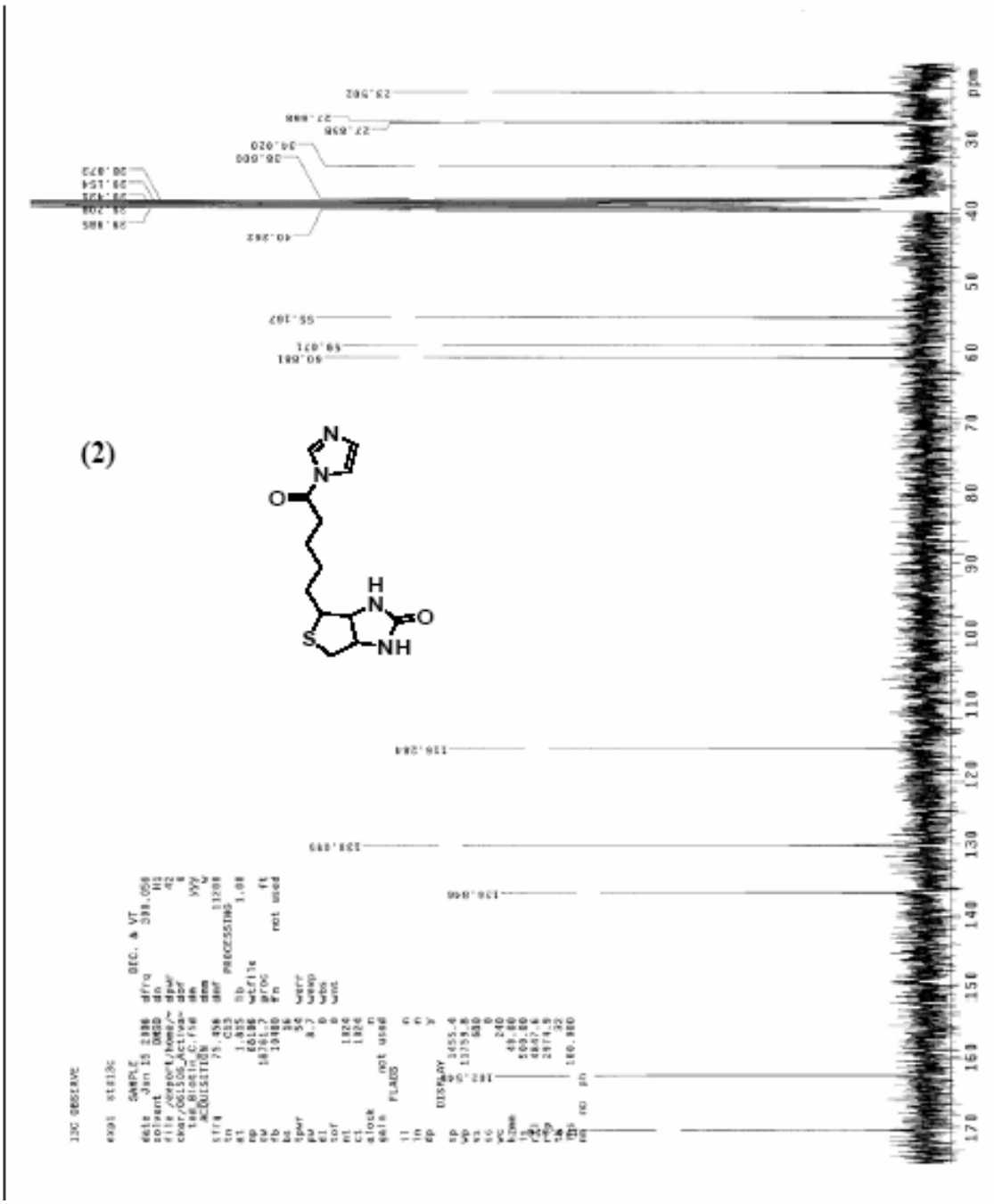


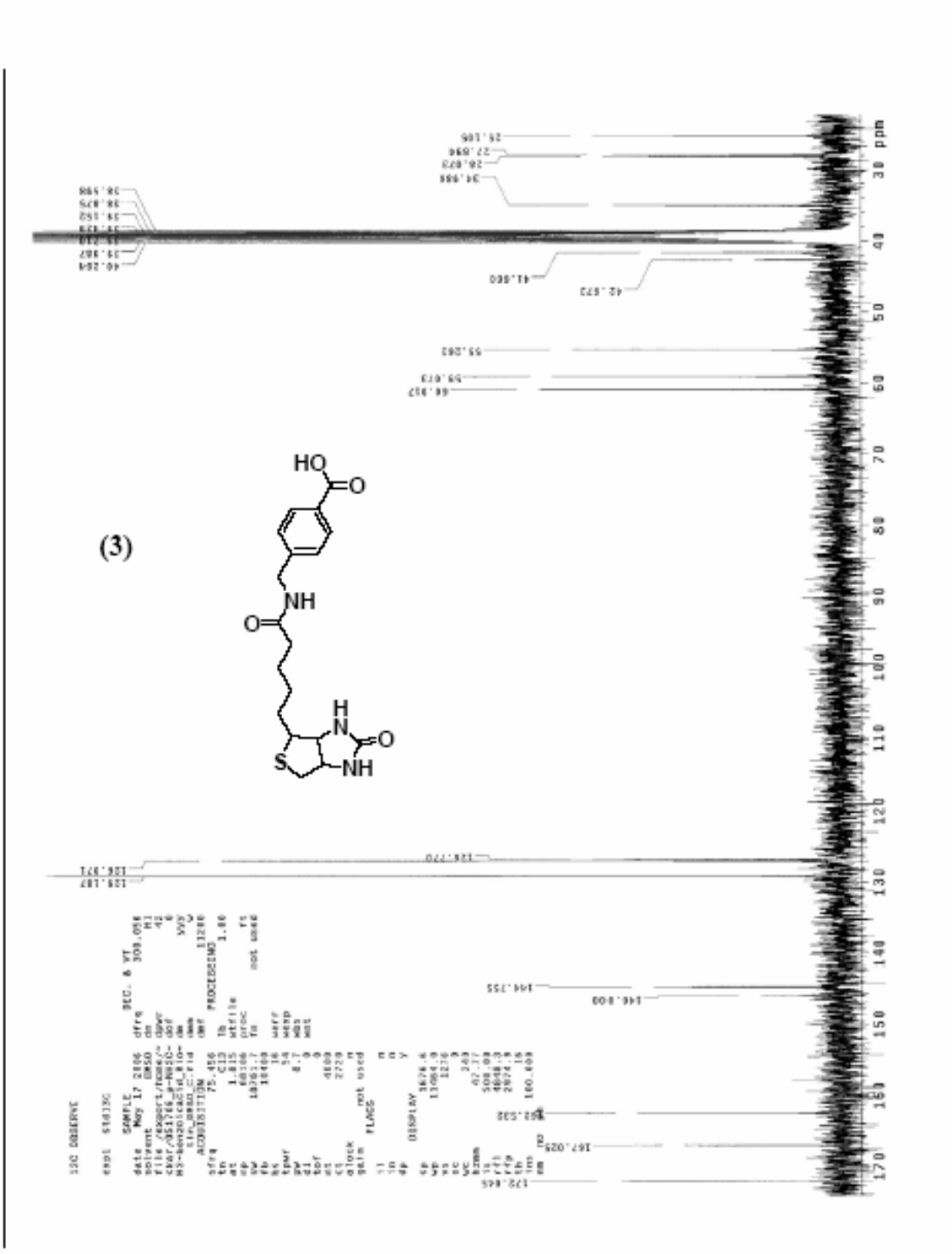
MALDI-TOF spectrum of 4-*O*-[4-*O*-(α-glucopyranosyl)-α-glucopyranosyl]-glucopyranose (maltotriose) labeled with compound **3c**.

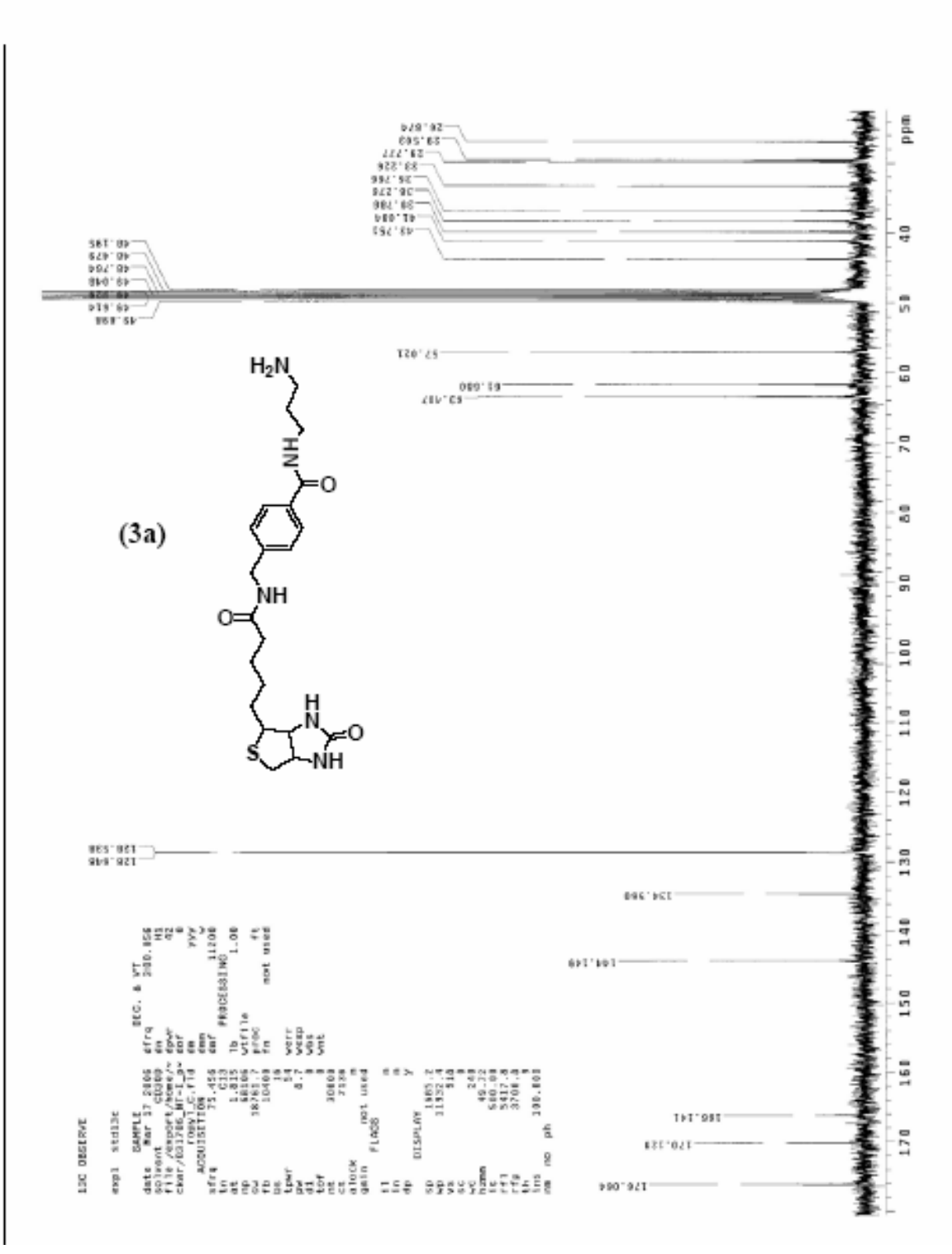


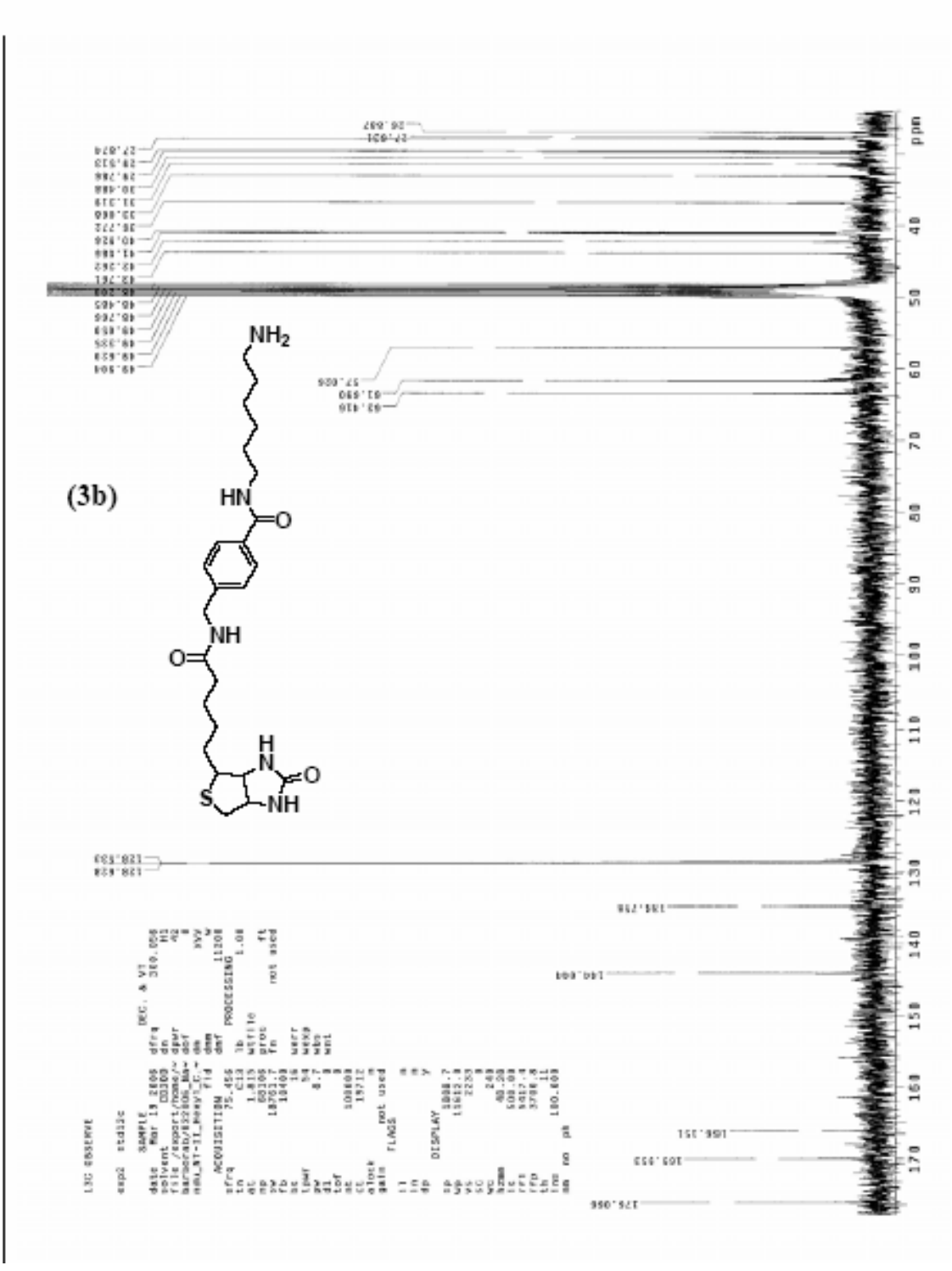
MALDI-TOF spectrum of lacto-*N*-fucopentaose (LNFP-II) labeled with compound **3c**.

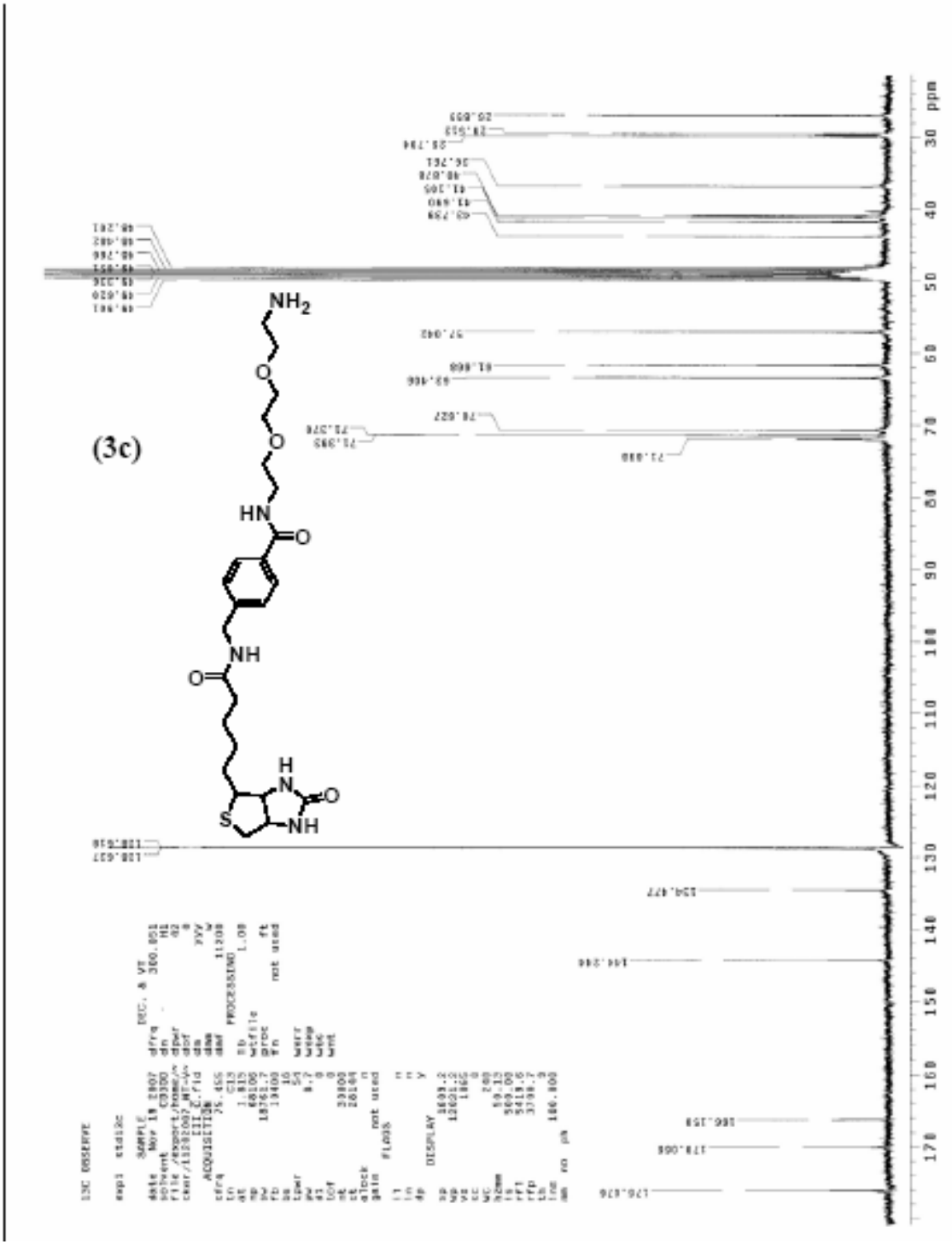

N3
NH2
ppm

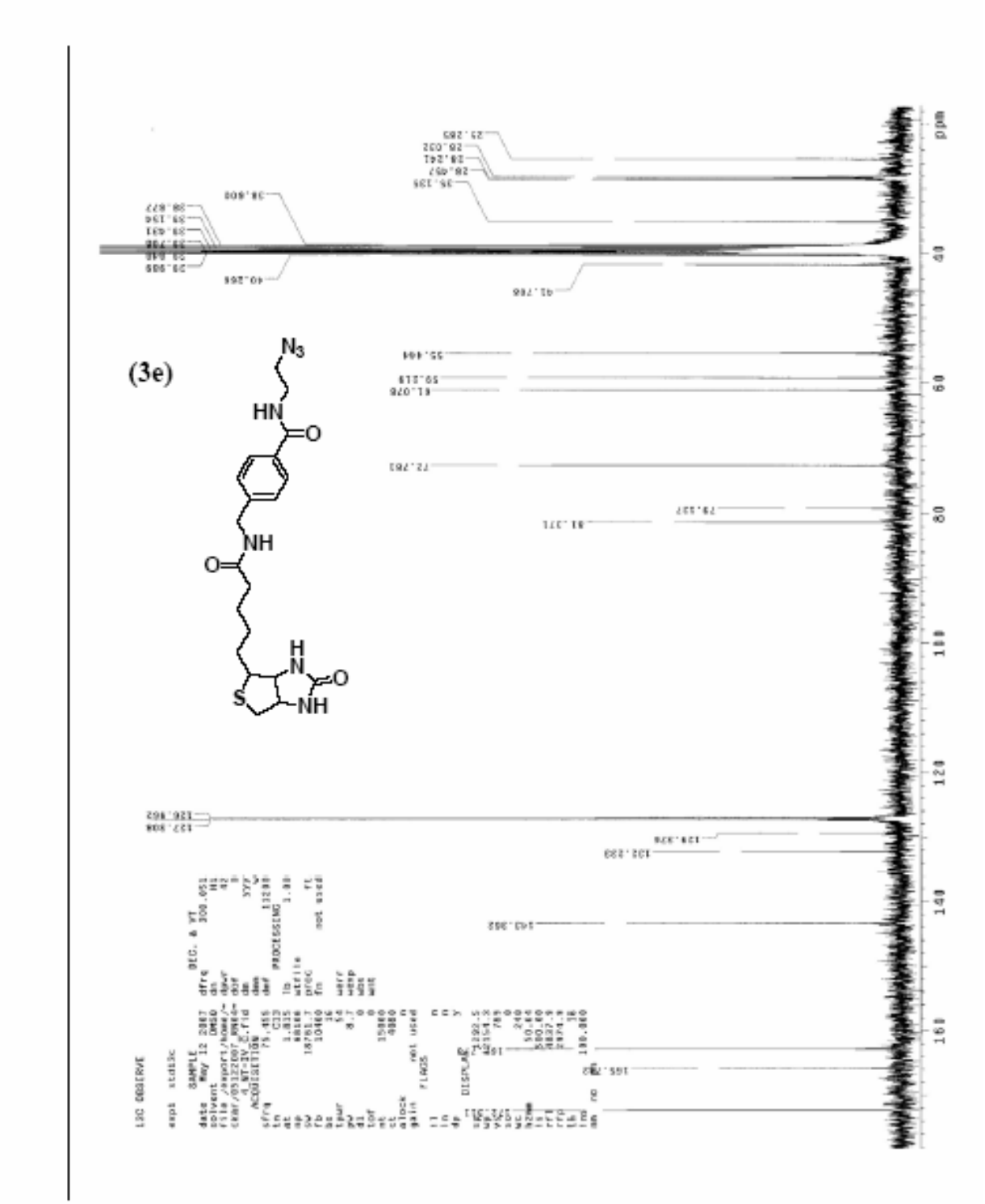

(2)
N
N
O
H
N
O
S
NH
ppm

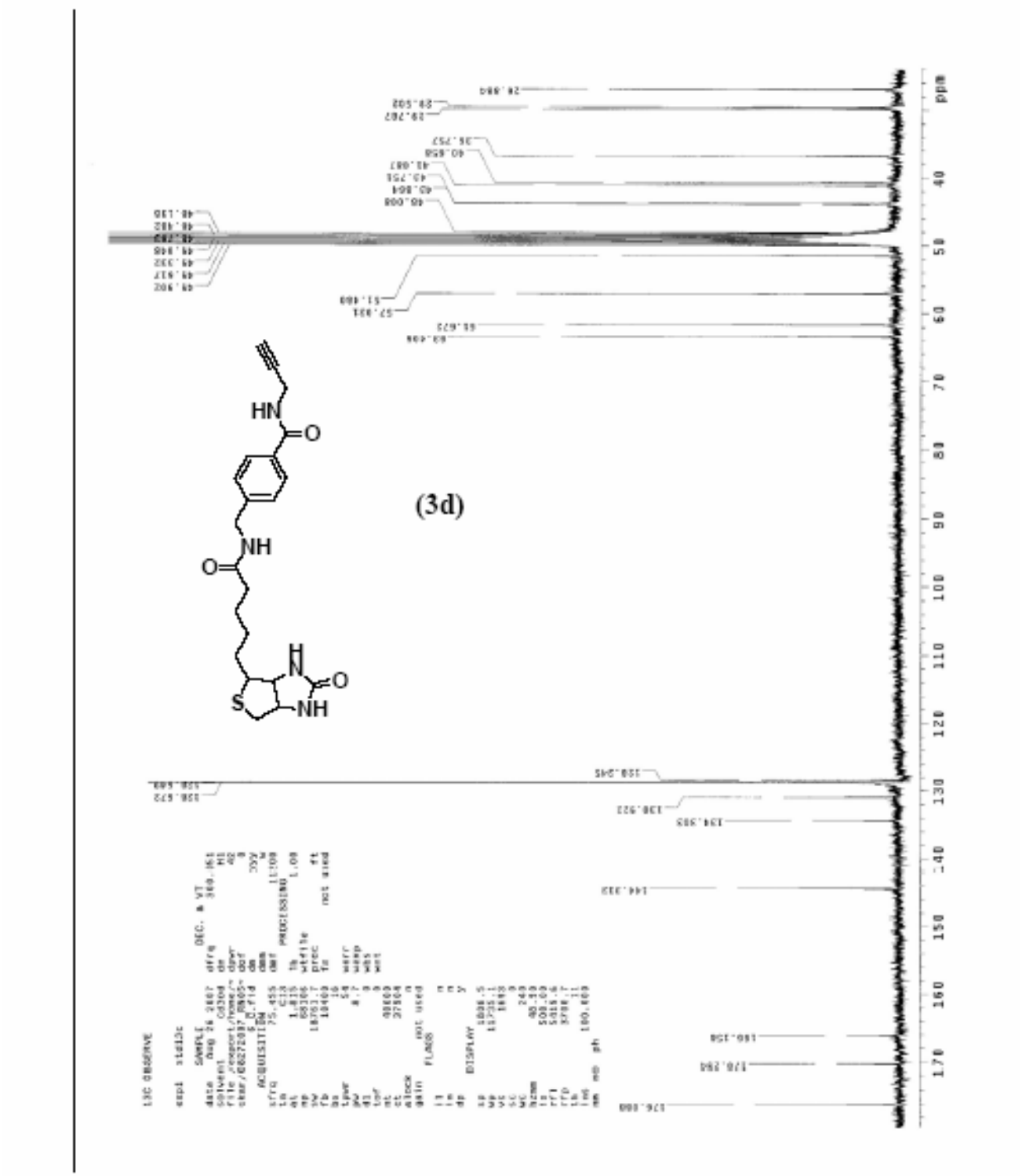

(3)
HO
O
NH
O
H
N
O
S
NH

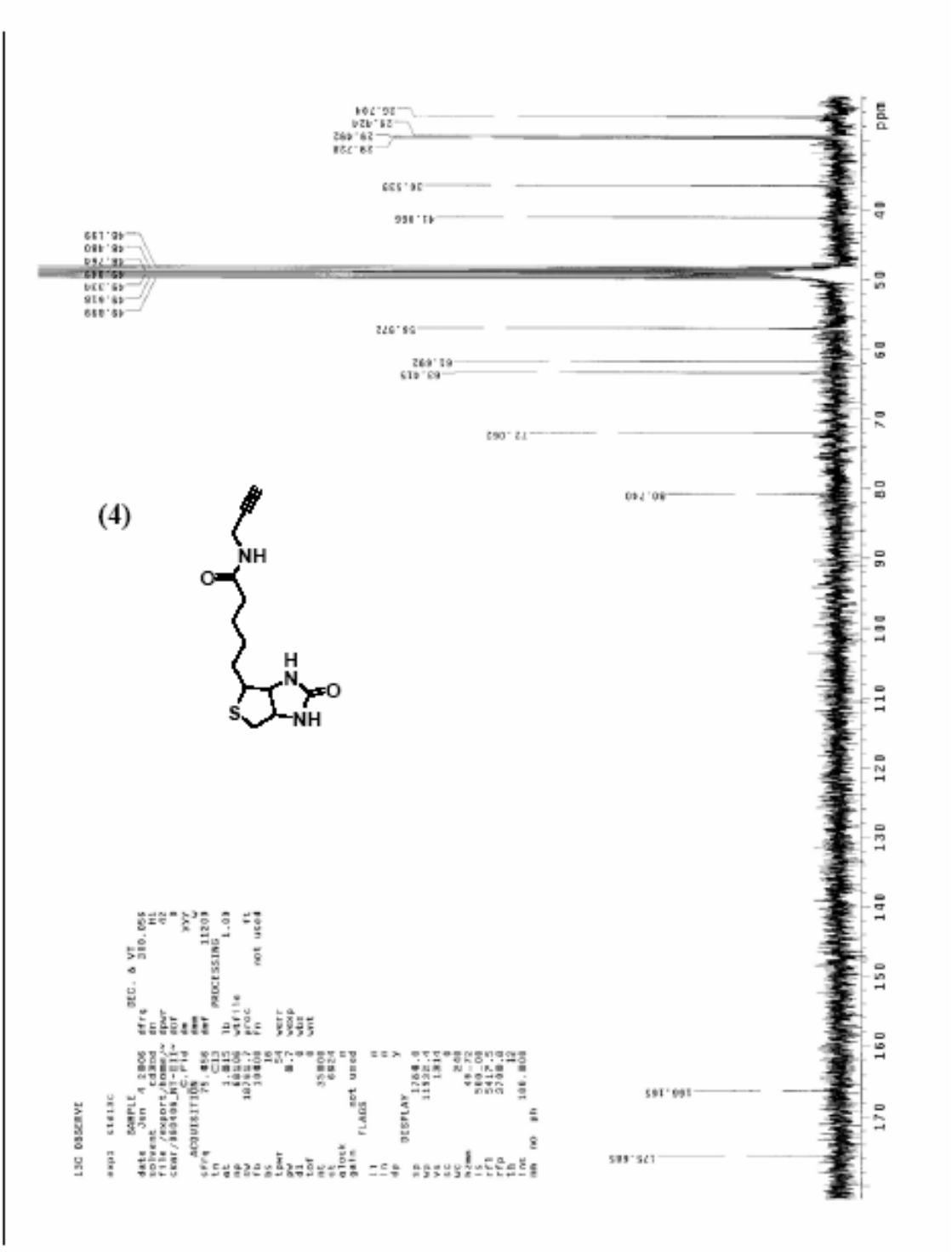

(3a)
H2N
NH
O
NH
O
H
N
O
S
NH
ppm

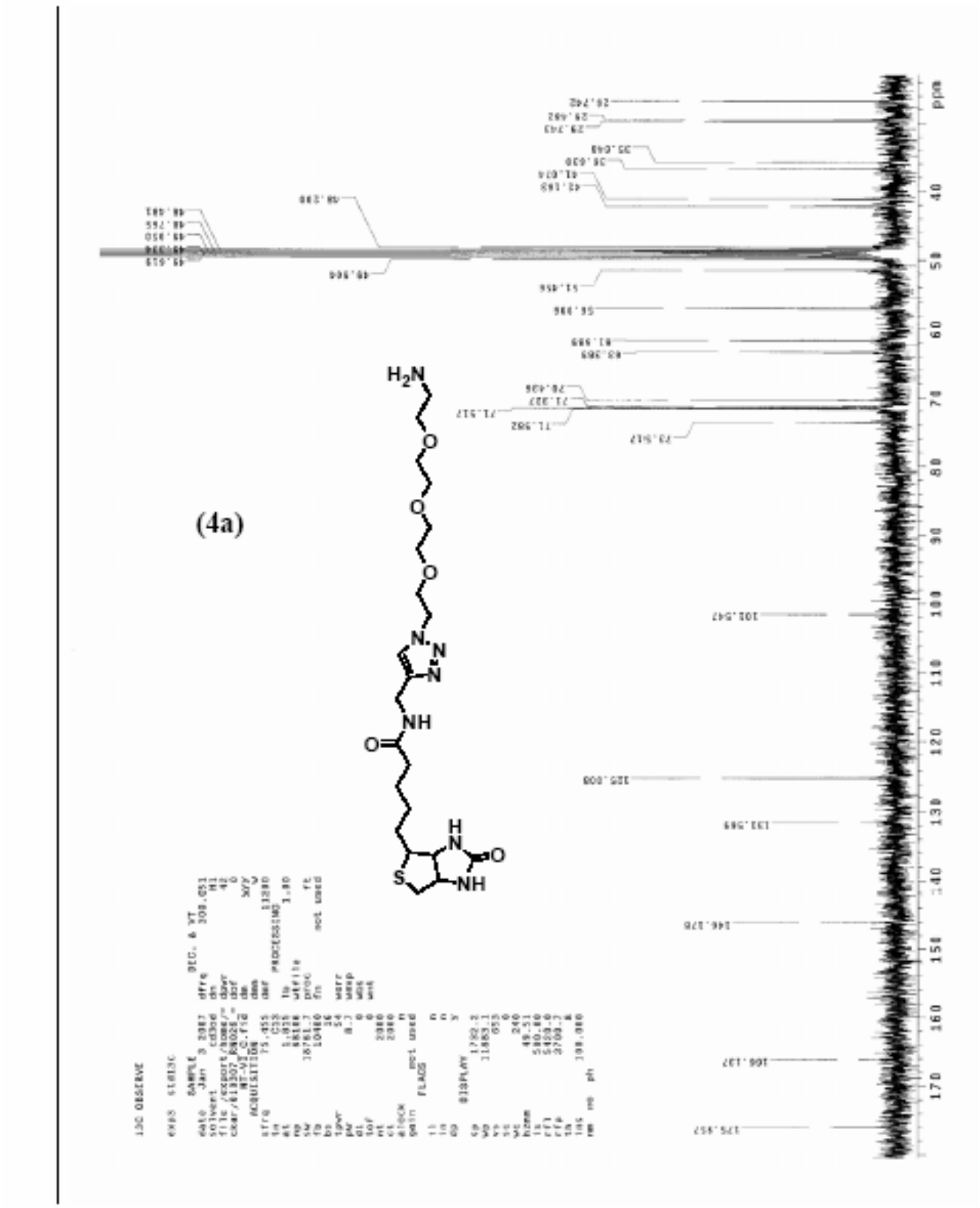

(3b)
NH2
HN
O
NH
O
H
N
O
S
NH
ppm

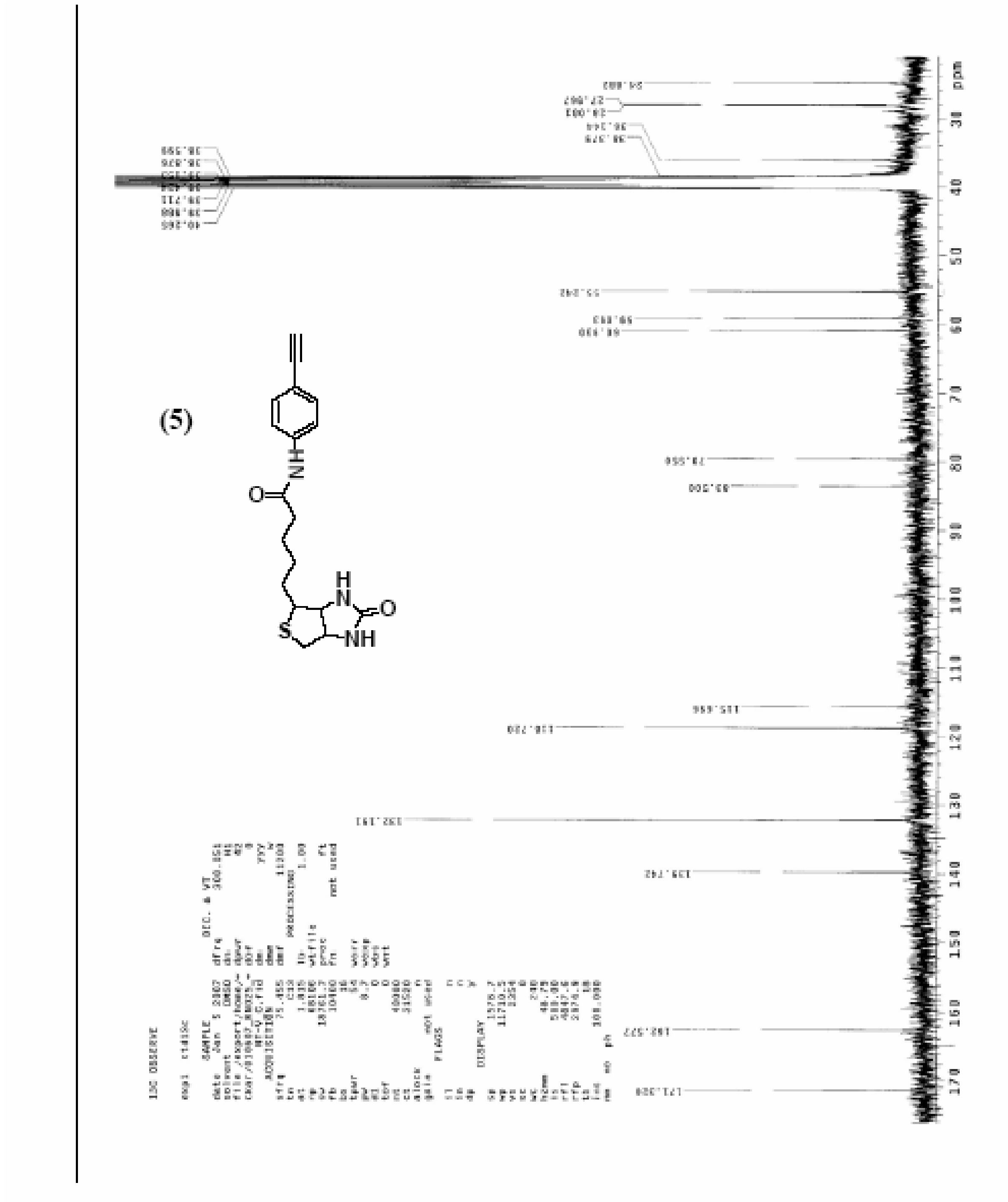

(3c)
NH2
O
O
HN
O
NH
O
H
N
O
S
NH
ppm


(3e)
N3
HN
O
NH
O
H
N
O
S
NH
ppm


(3d)
HN
O
NH
O
N
H
O
S
NH


(4)
NH
O
H
N
O
S
NH
ppm


(4a)
H2N
O
O
O
O
N
N
N
NH
O
H
N
O
S
NH
ppm



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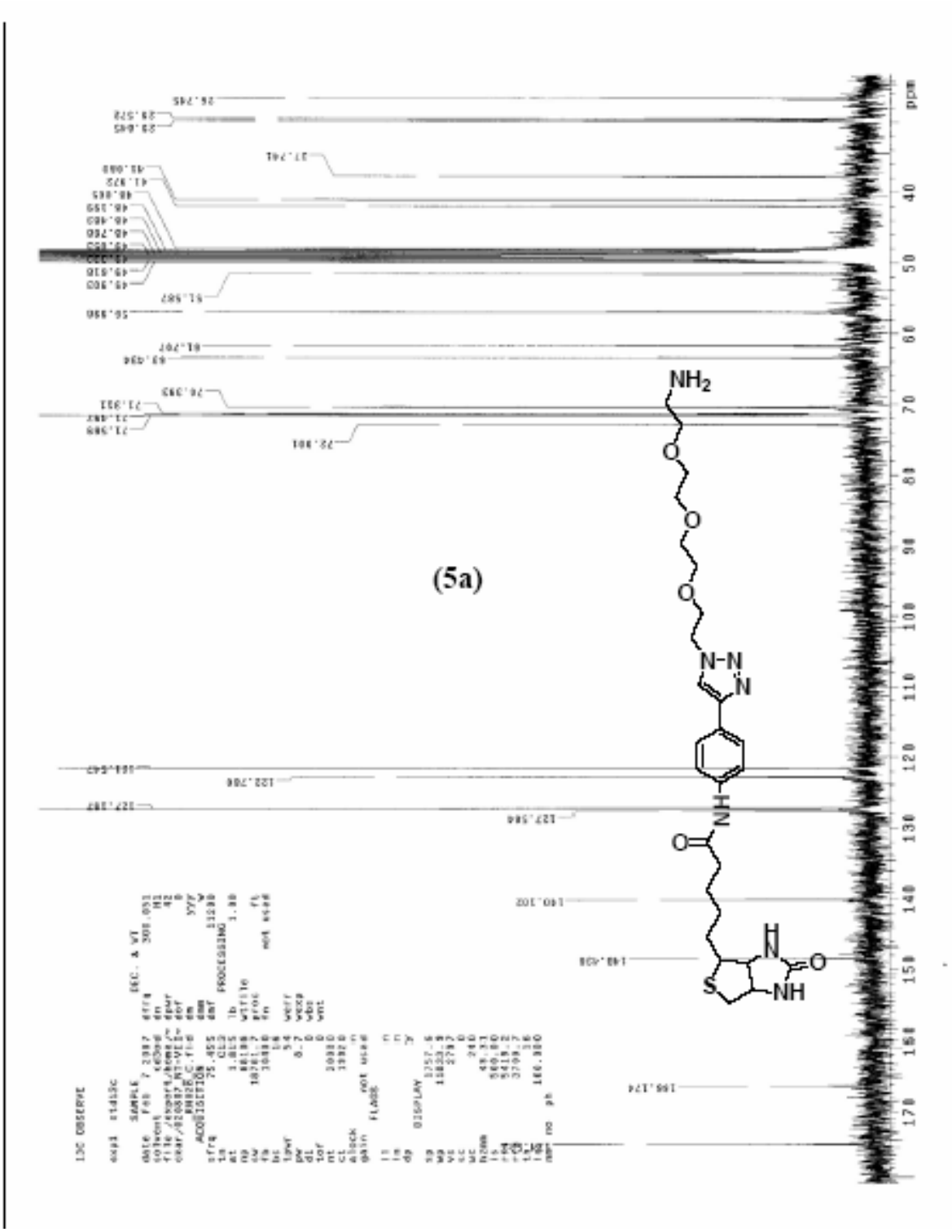
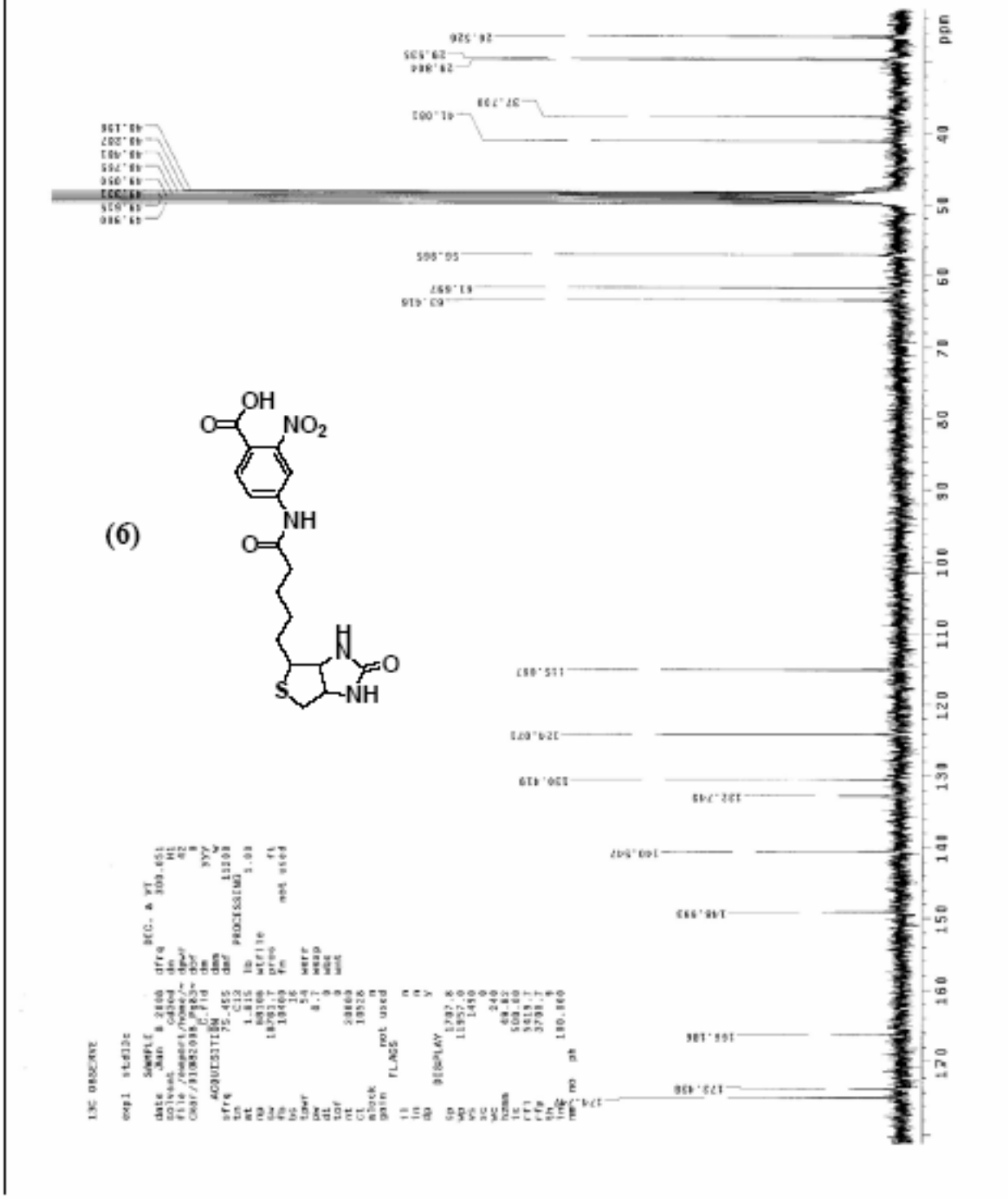
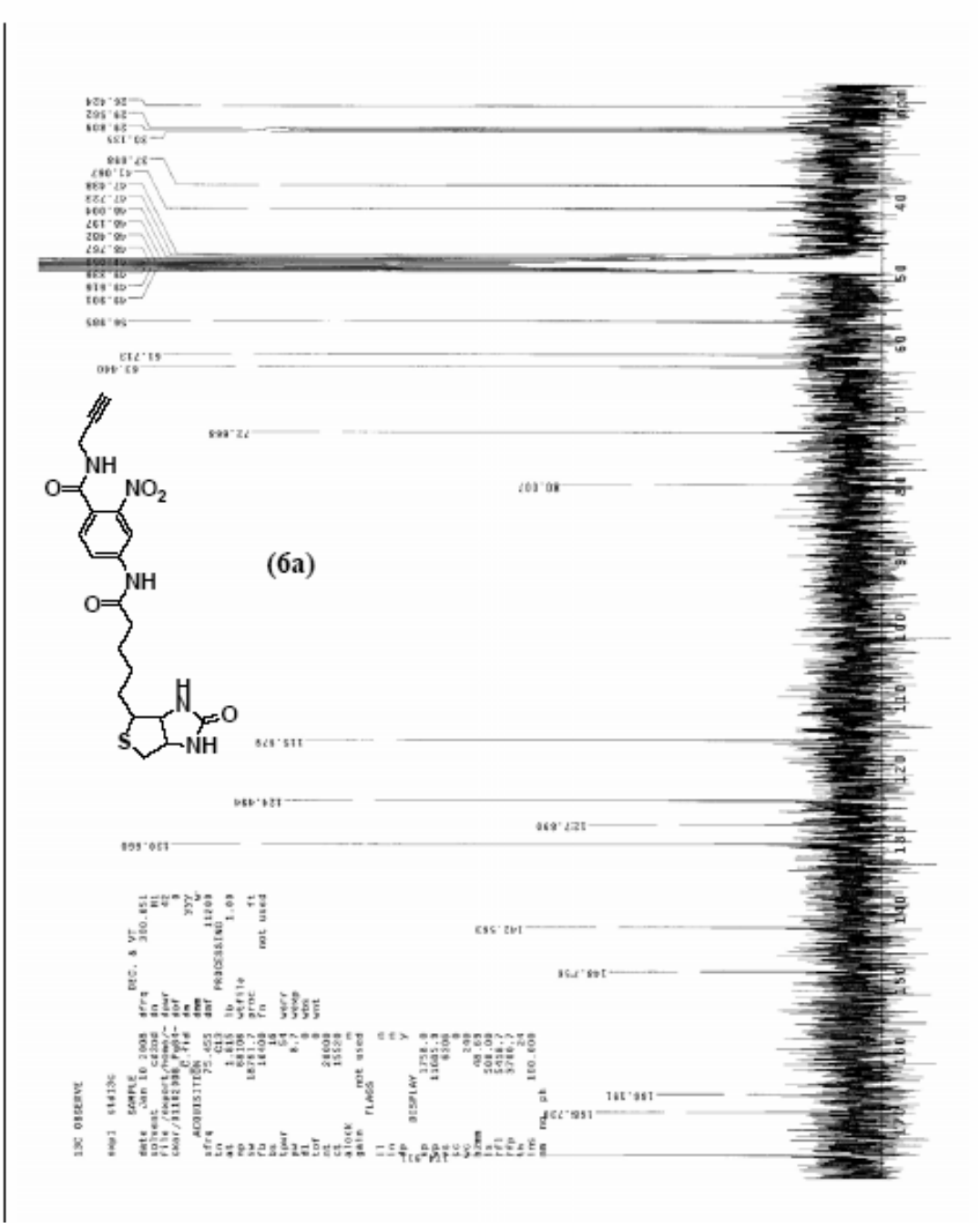
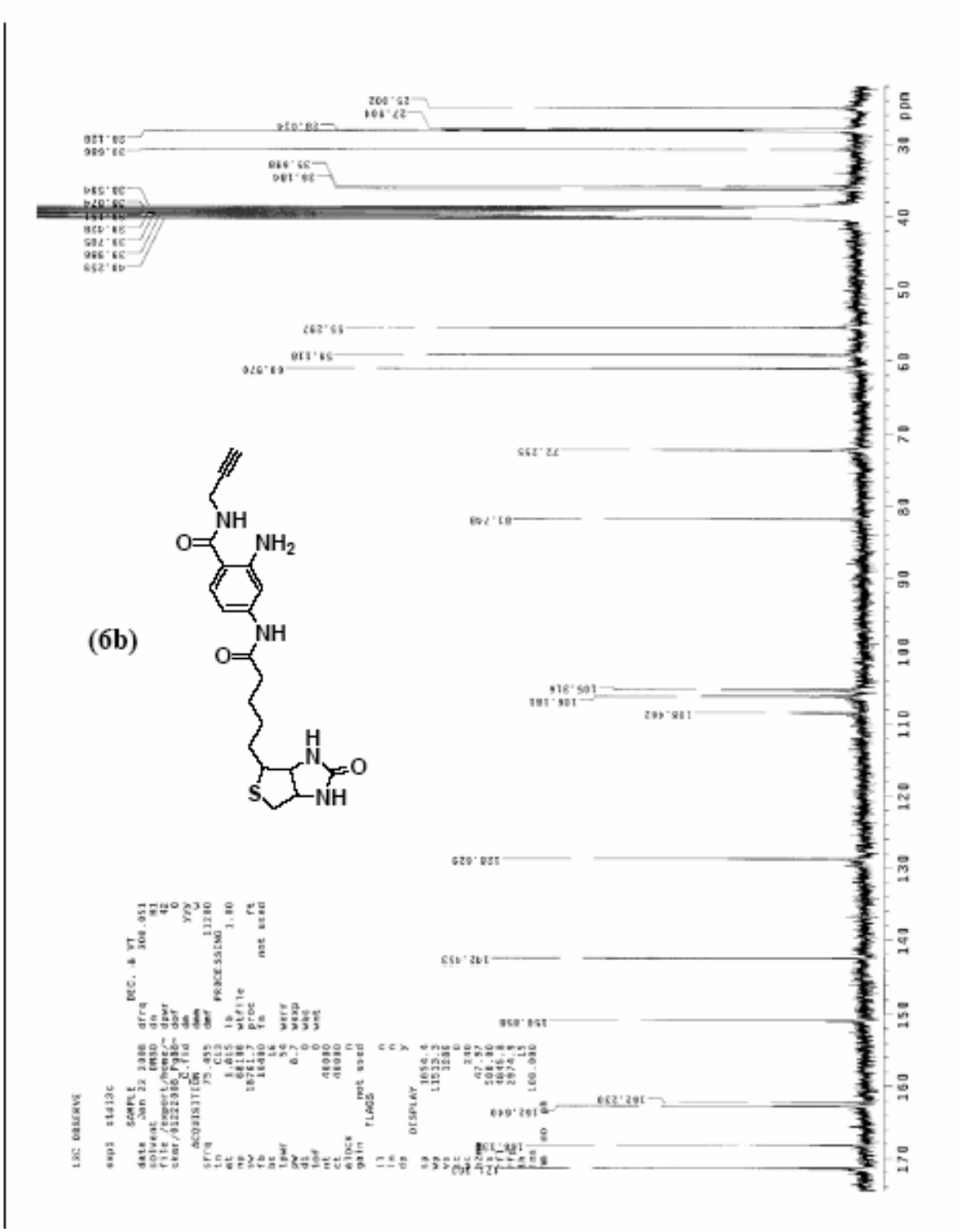
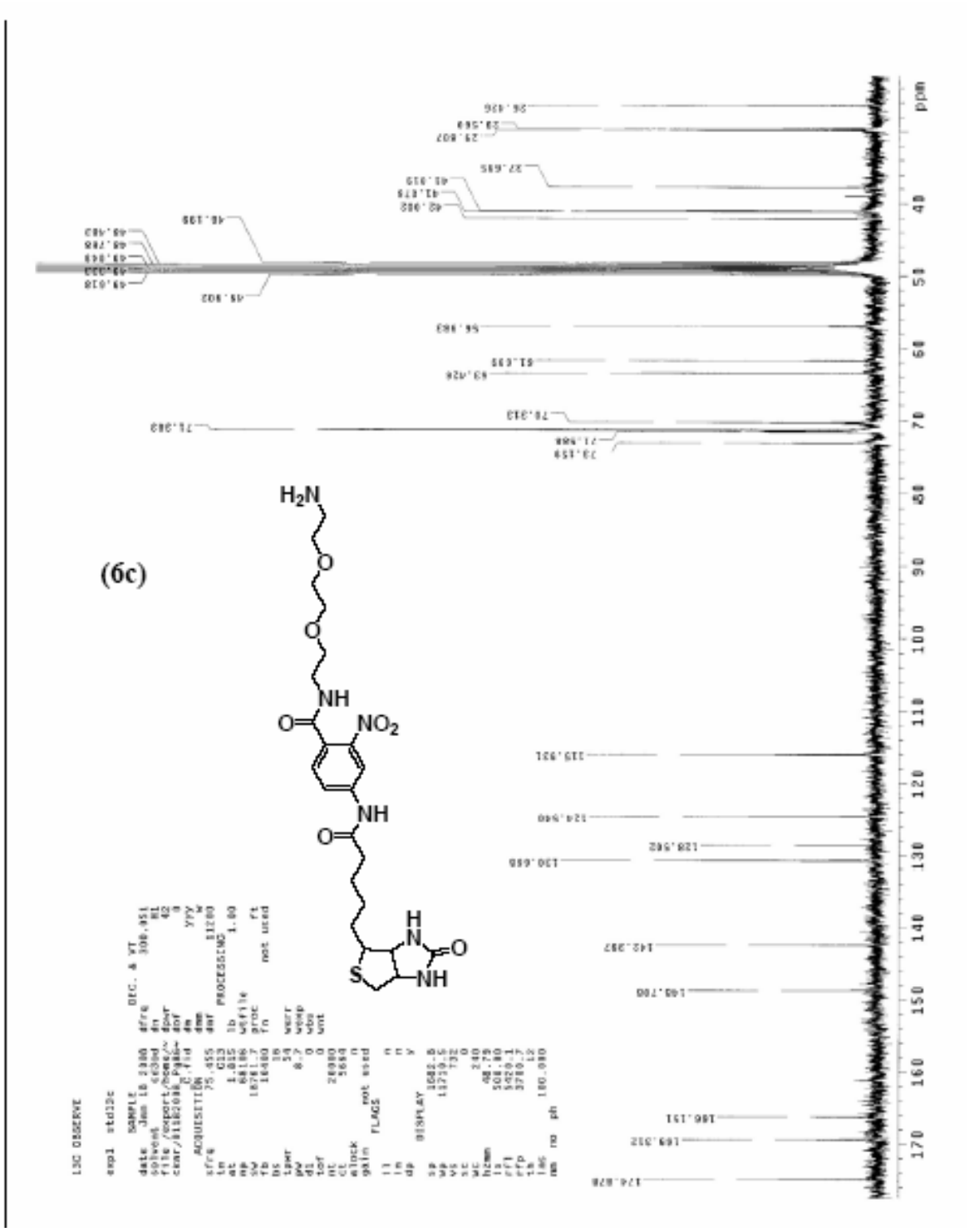
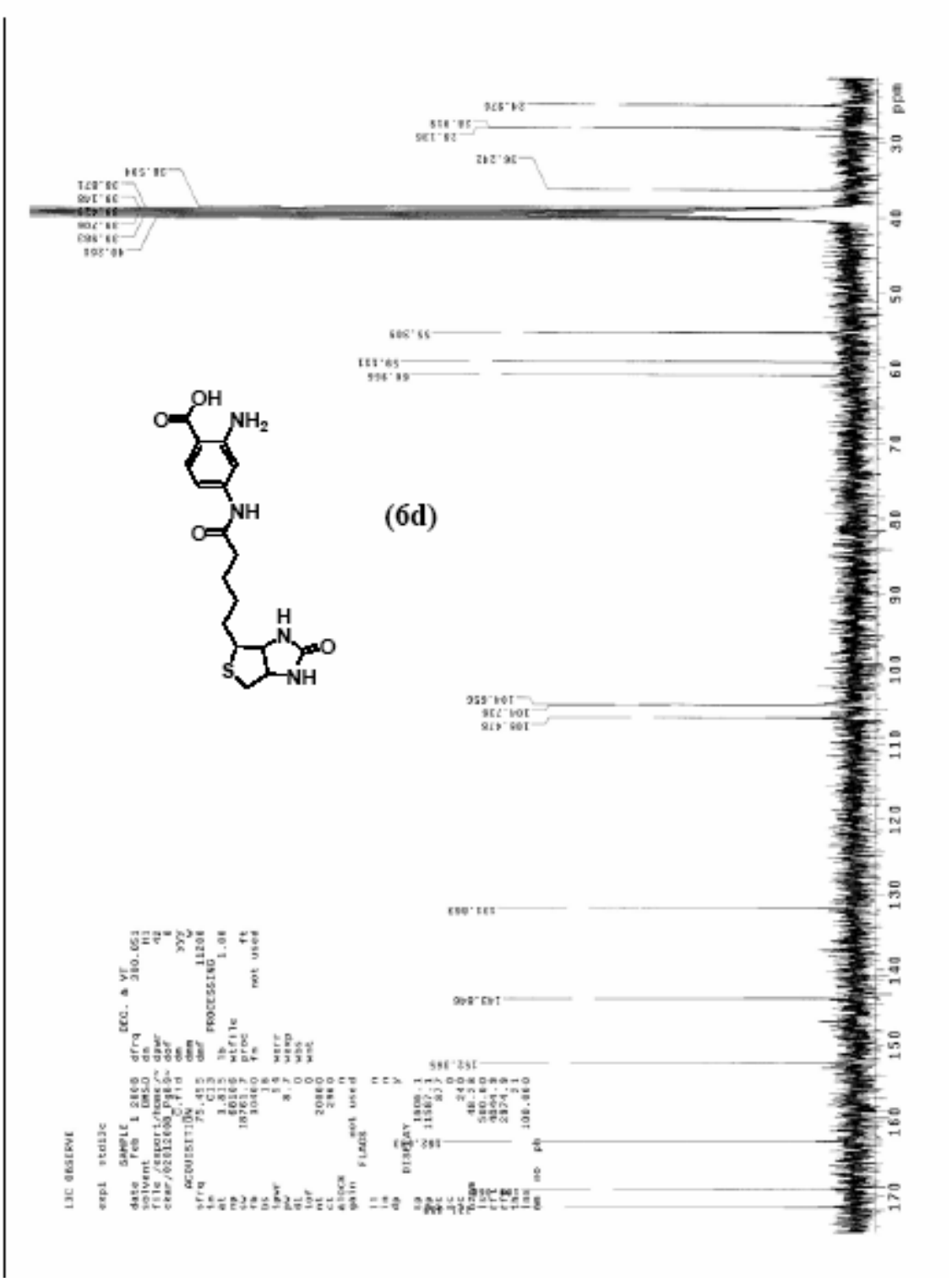

(5a)
NH2
O
O
O
O
N-N
N
NH
O
N
H
O
S
NH
ppm


(б)
OH
O
NO2
NH
O
N
H
O
S
NH
ppm


NH
O
NO2
NH
O
(6a)
NH
O
S


(6b)
NH
O
NH2
NH
O
H
N
O
S
NH
ppm


(6c)
H_2N
O
O
NH
O
NO_2
NH
O
H
N
O
S
NH
ppm


OH
O
NH2
NH
O
H
N
O
S
NH
(6d)