

Synthesis and Characterization of Biotin Derivatives as Multifunctional Oligosaccharide Tags

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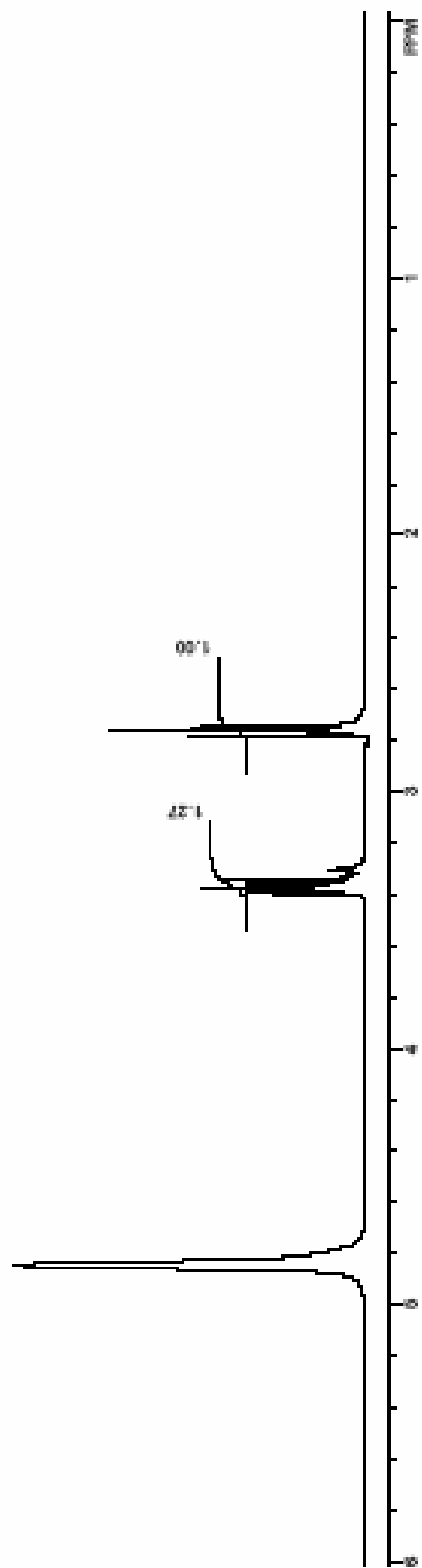
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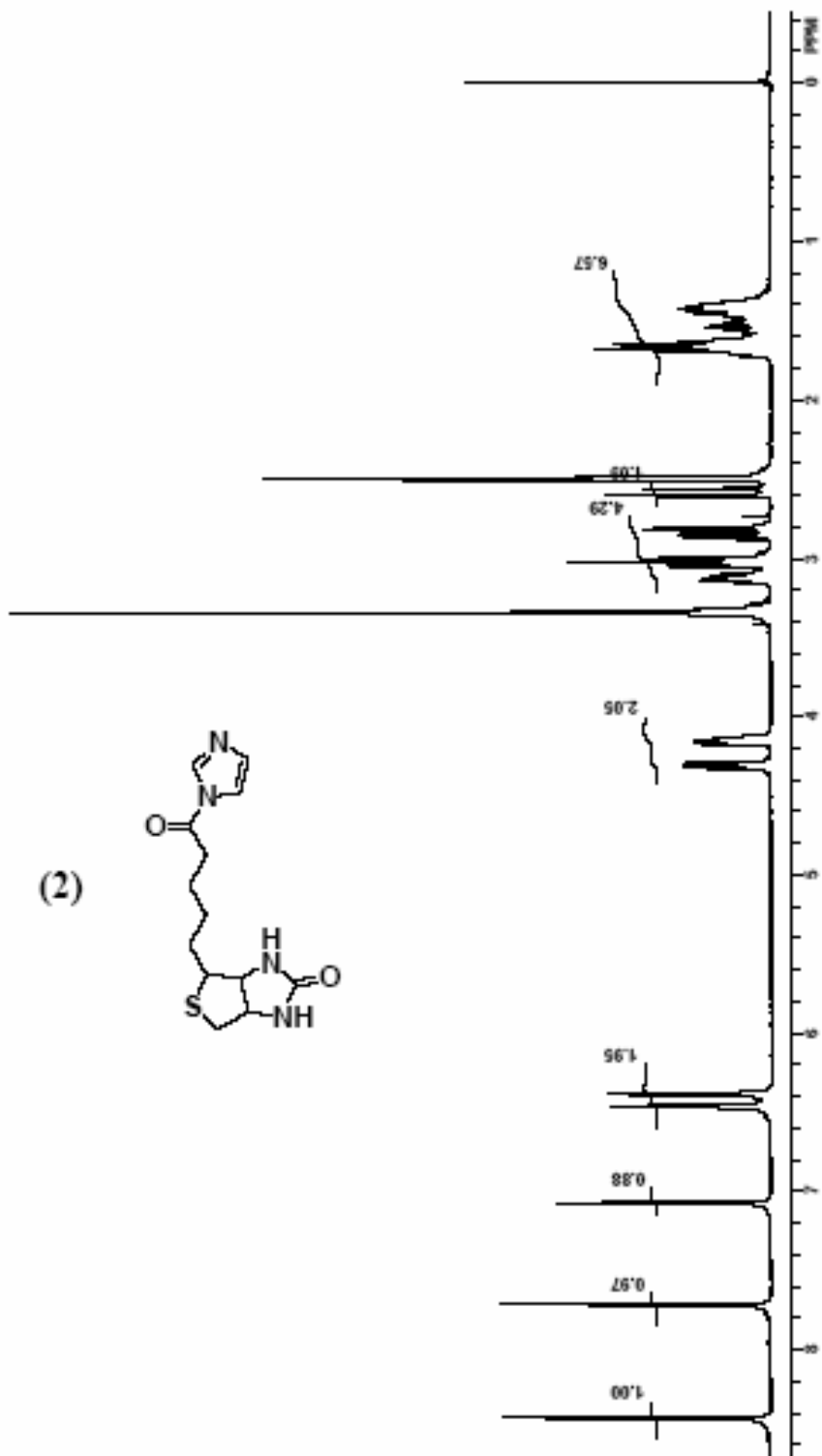
Materials and methods

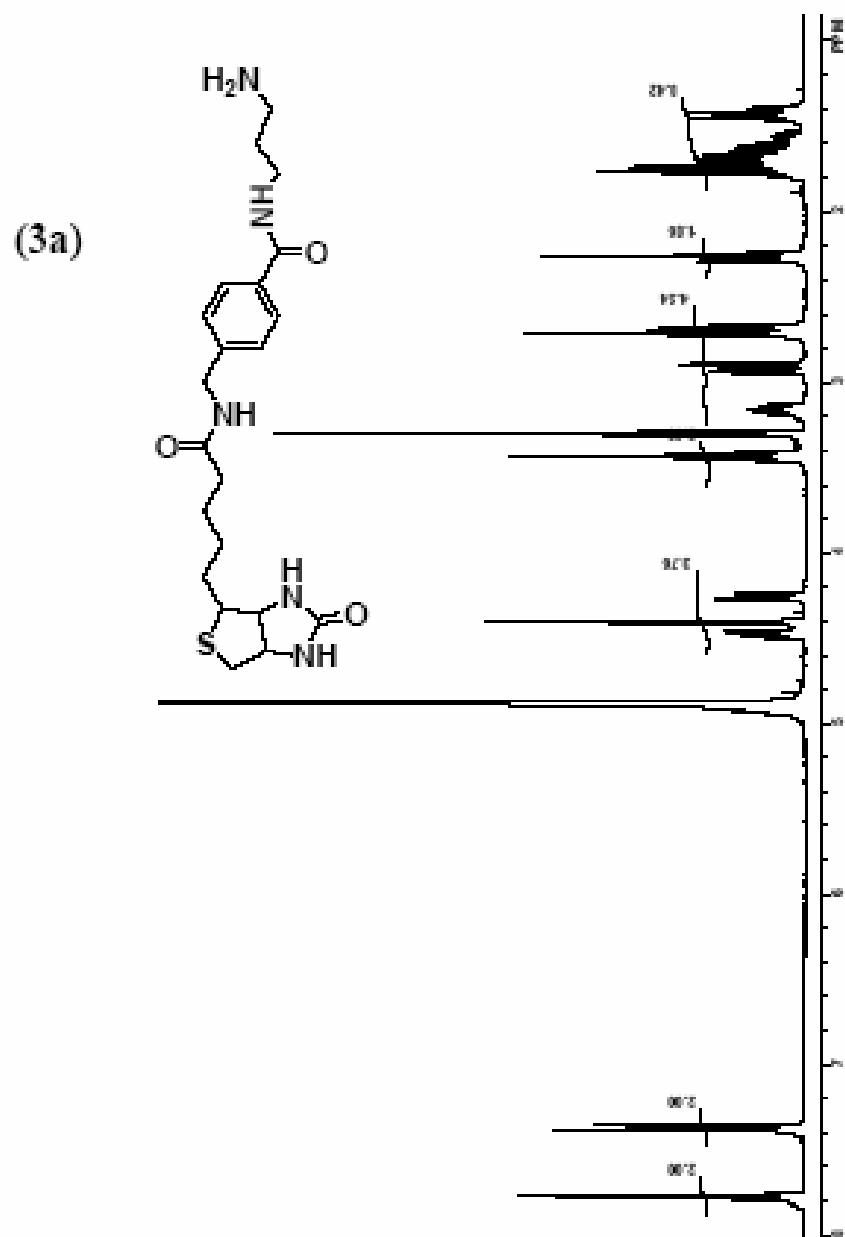
¹H NMR and ¹³C NMR spectra were recorded on at 300 MHz with specified deuterated solvents. The signals were assigned using ¹H NMR, ¹³C NMR, and COSY technique. Chemical shifts are reported in parts per million (ppm) and are referenced to the respective solvent. Coupling constants (J) are reported in 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% Acetonitrile (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

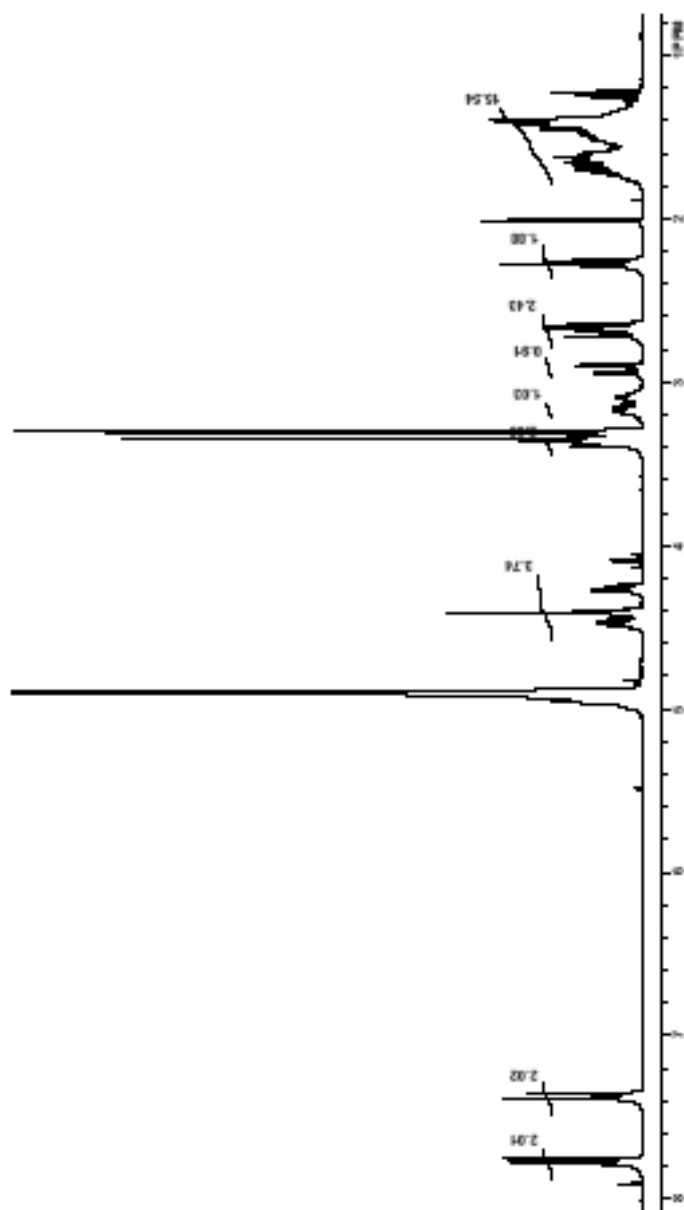
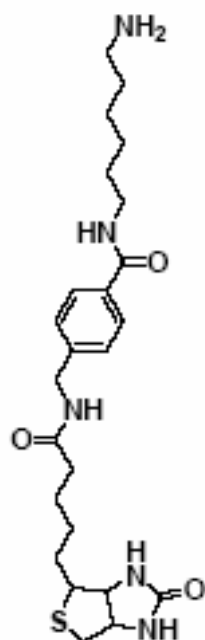
The oligosaccharide was dissolved in 200 µL of methanol (final concentration of ~10⁻⁴ M) in a plastic microcentrifuge vial with lid. Approximately ~ 0.1 mg of tag and 1 µL of glacial acetic acid were then added to the vial. The solution was then incubated for ½ hours at 70 C. After the incubation ~ 10mg 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.

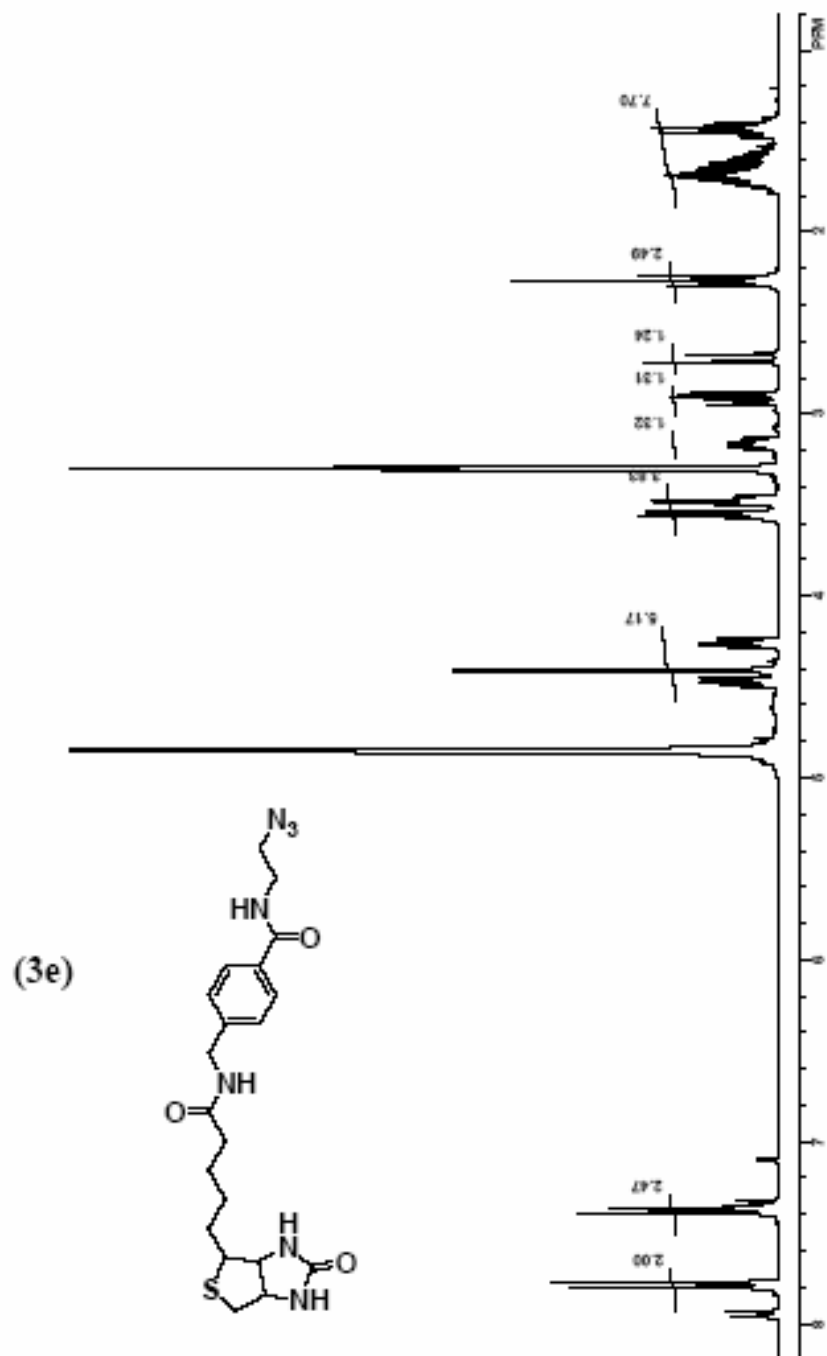


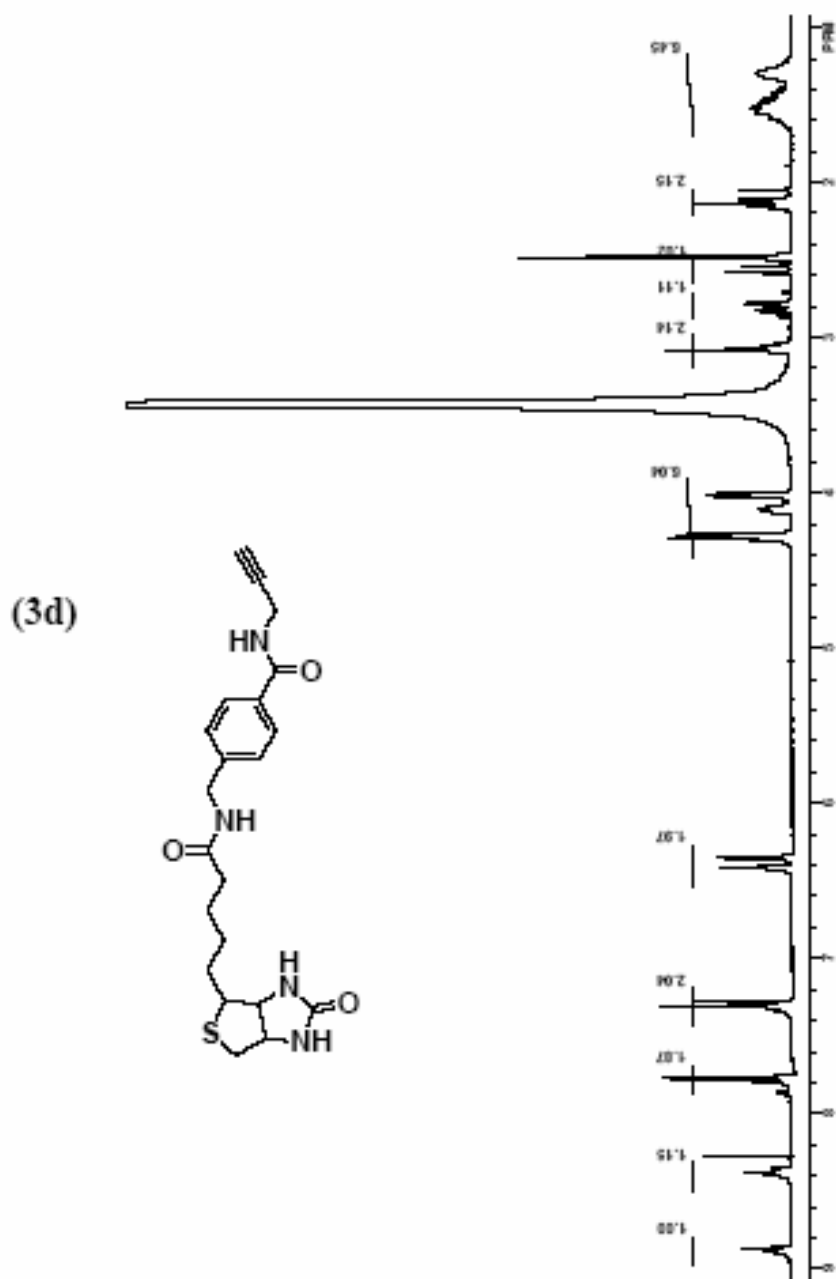




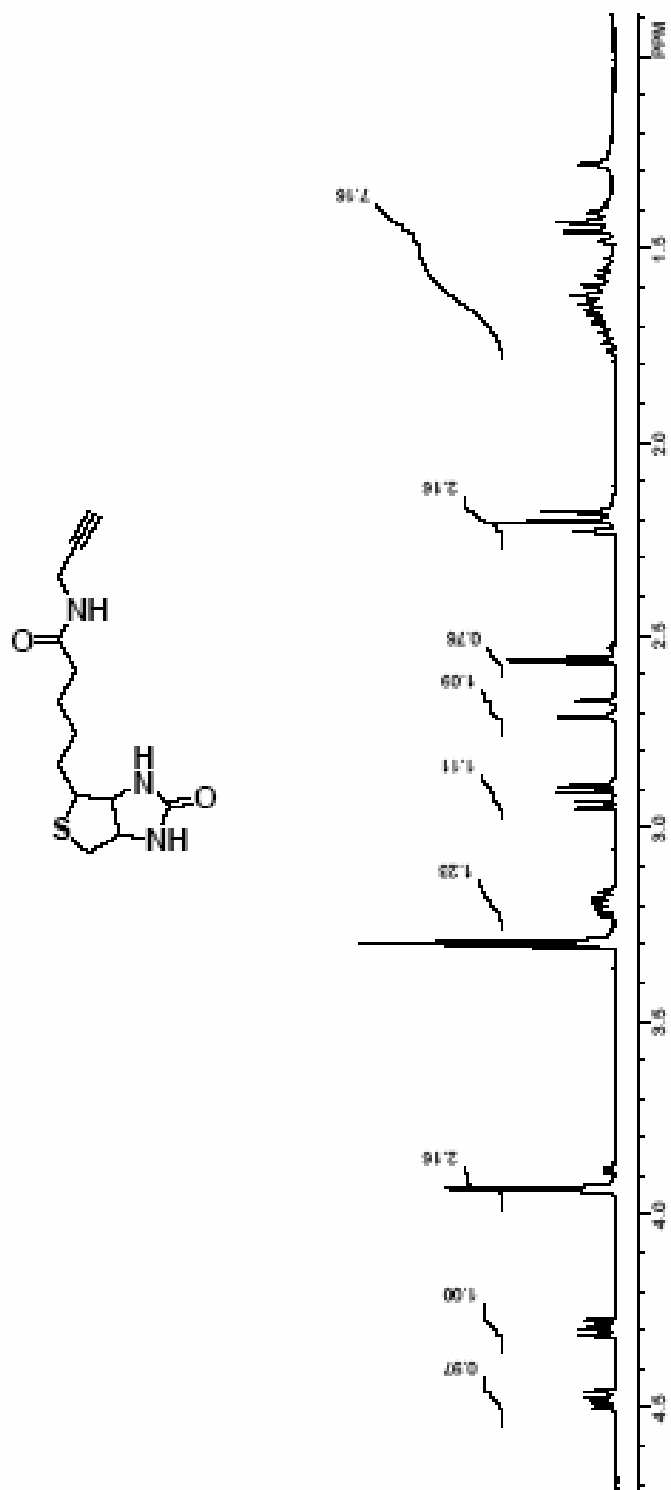
(3b)



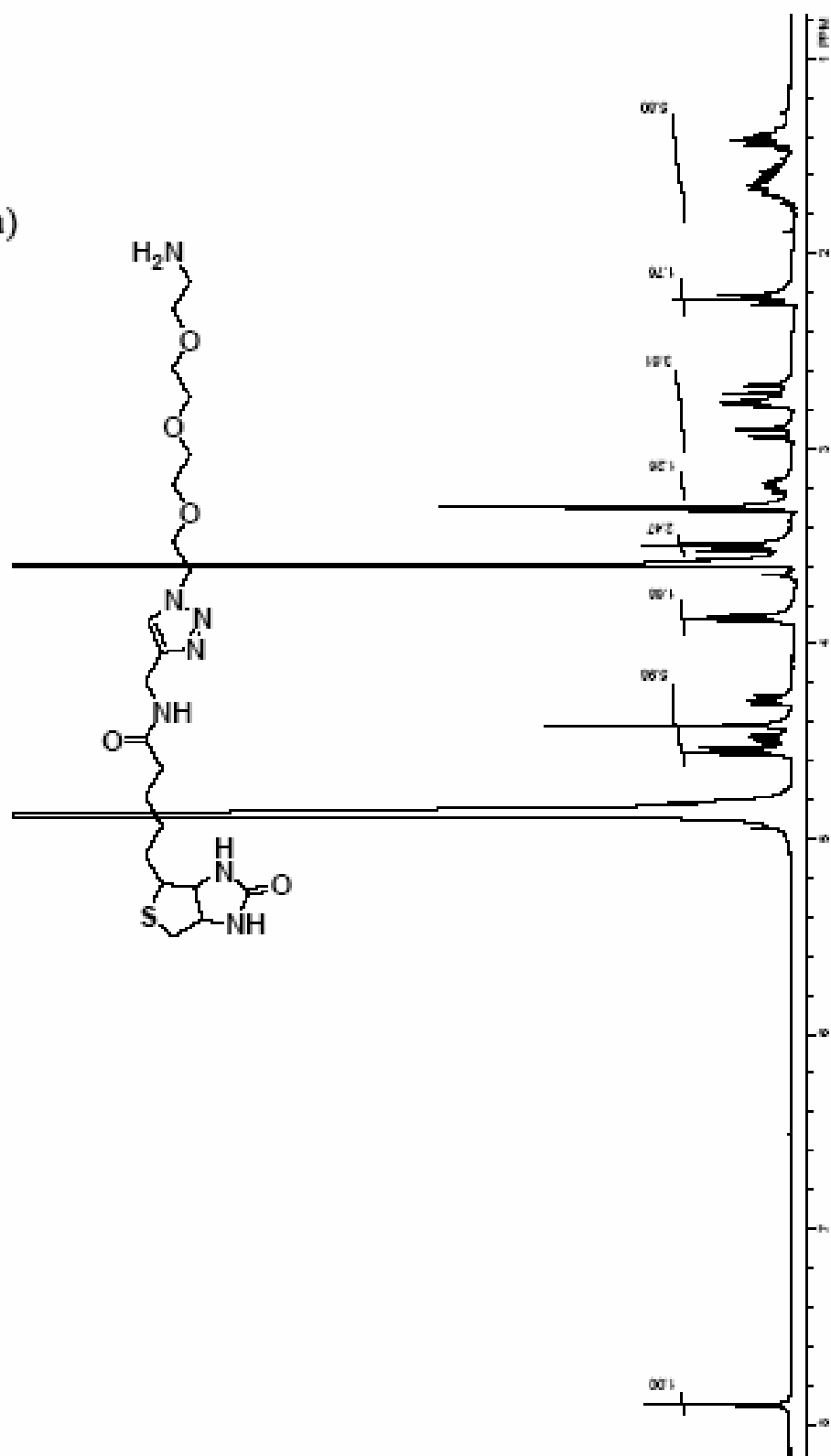


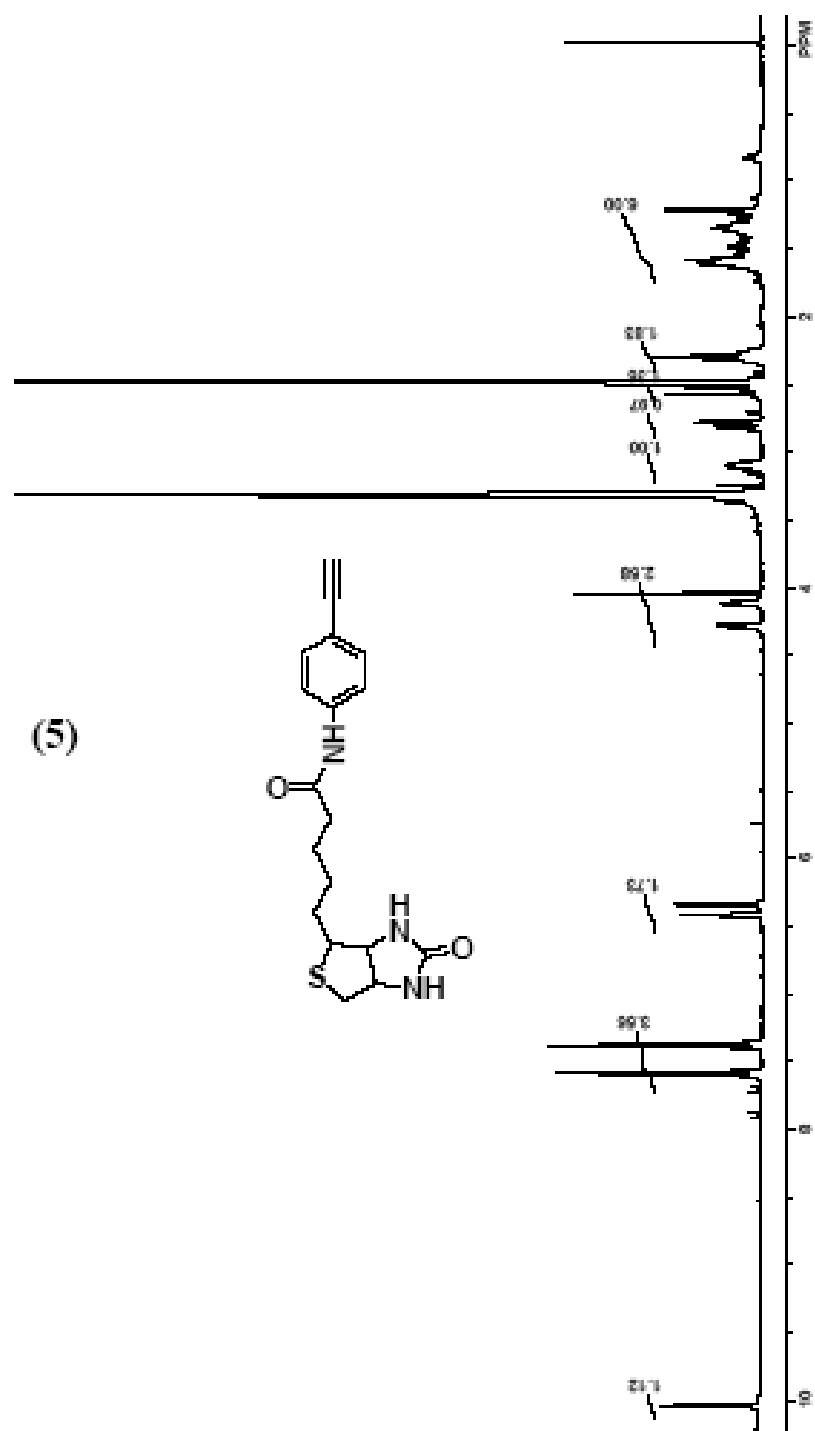


(4)

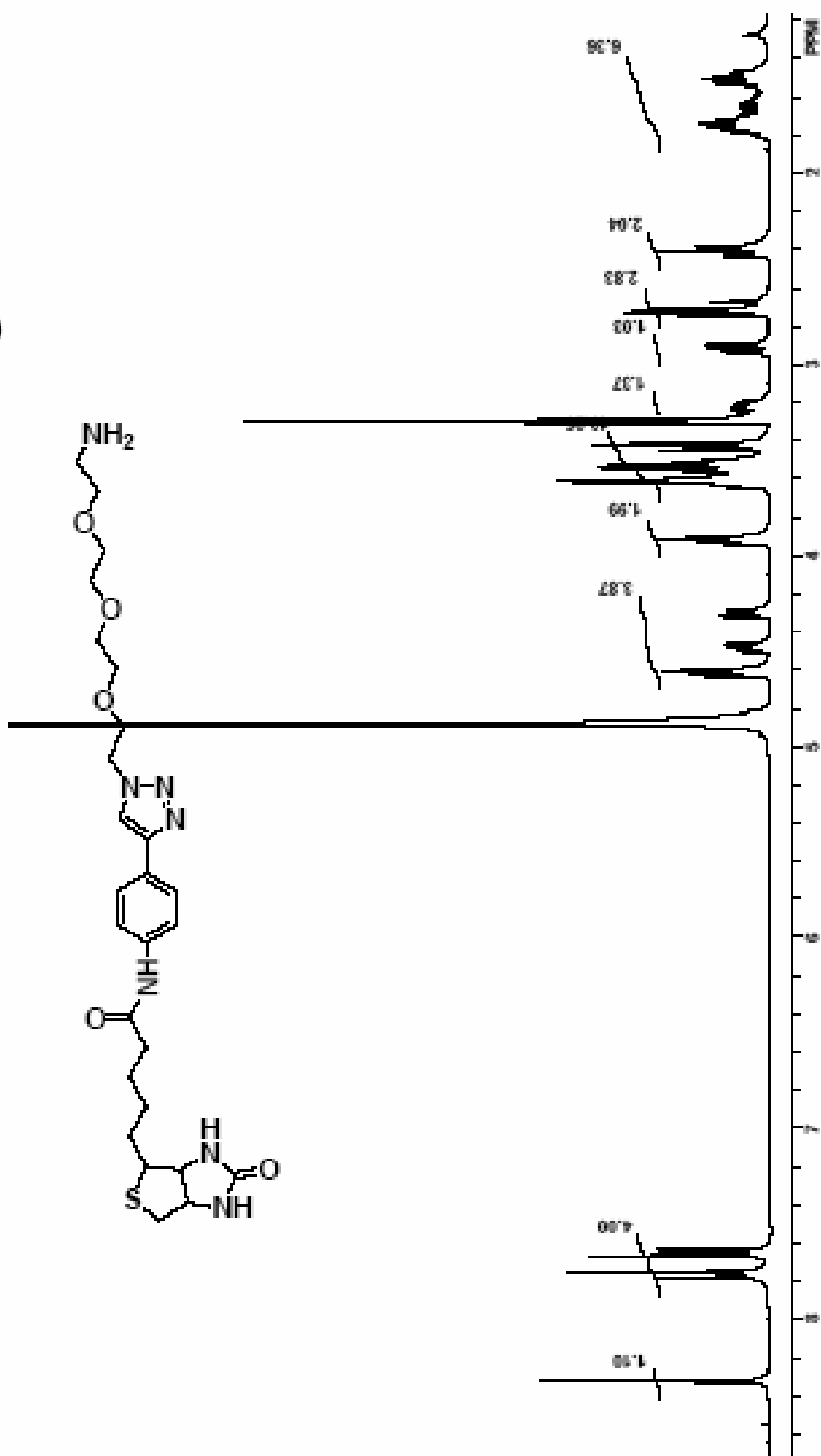


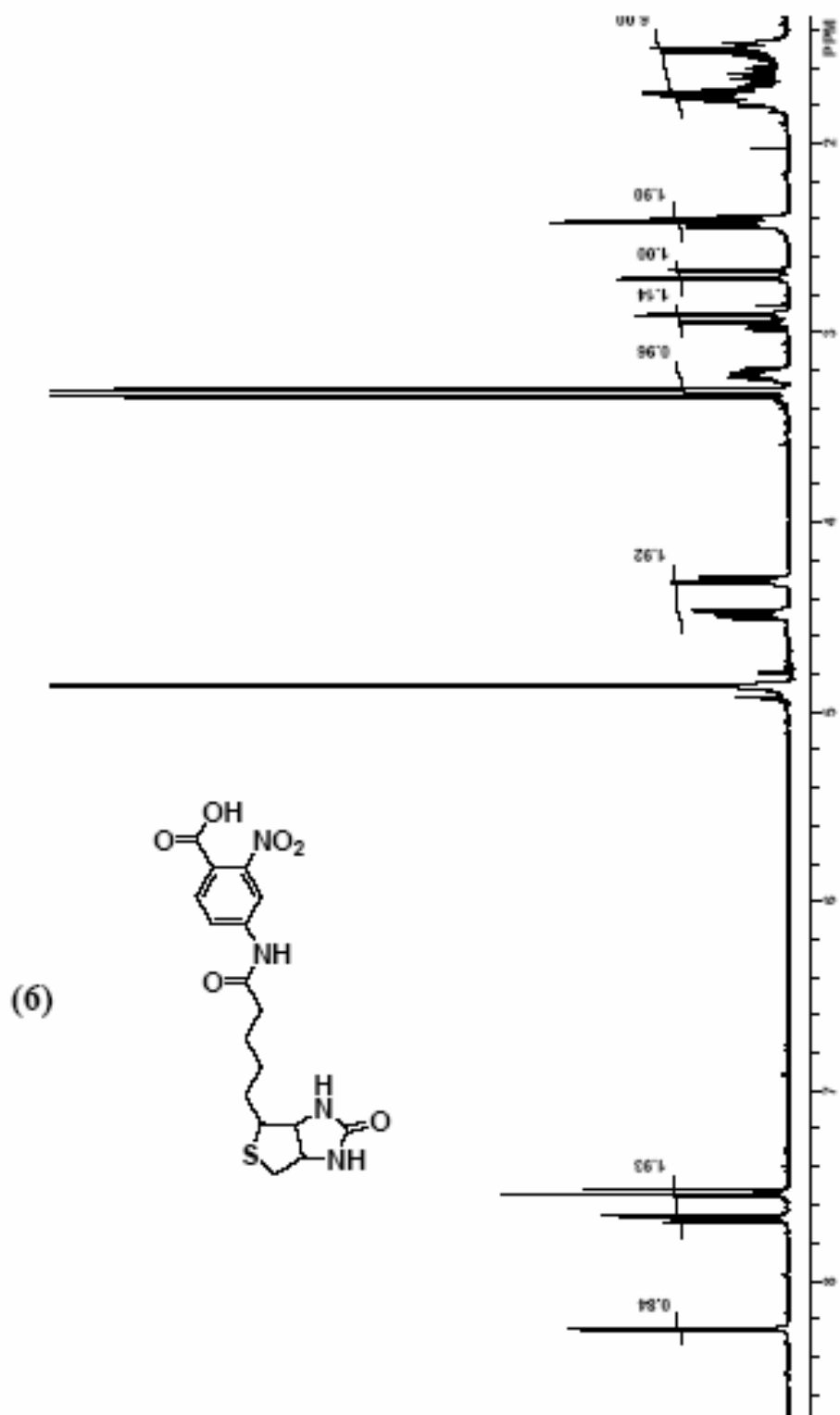
(4a)

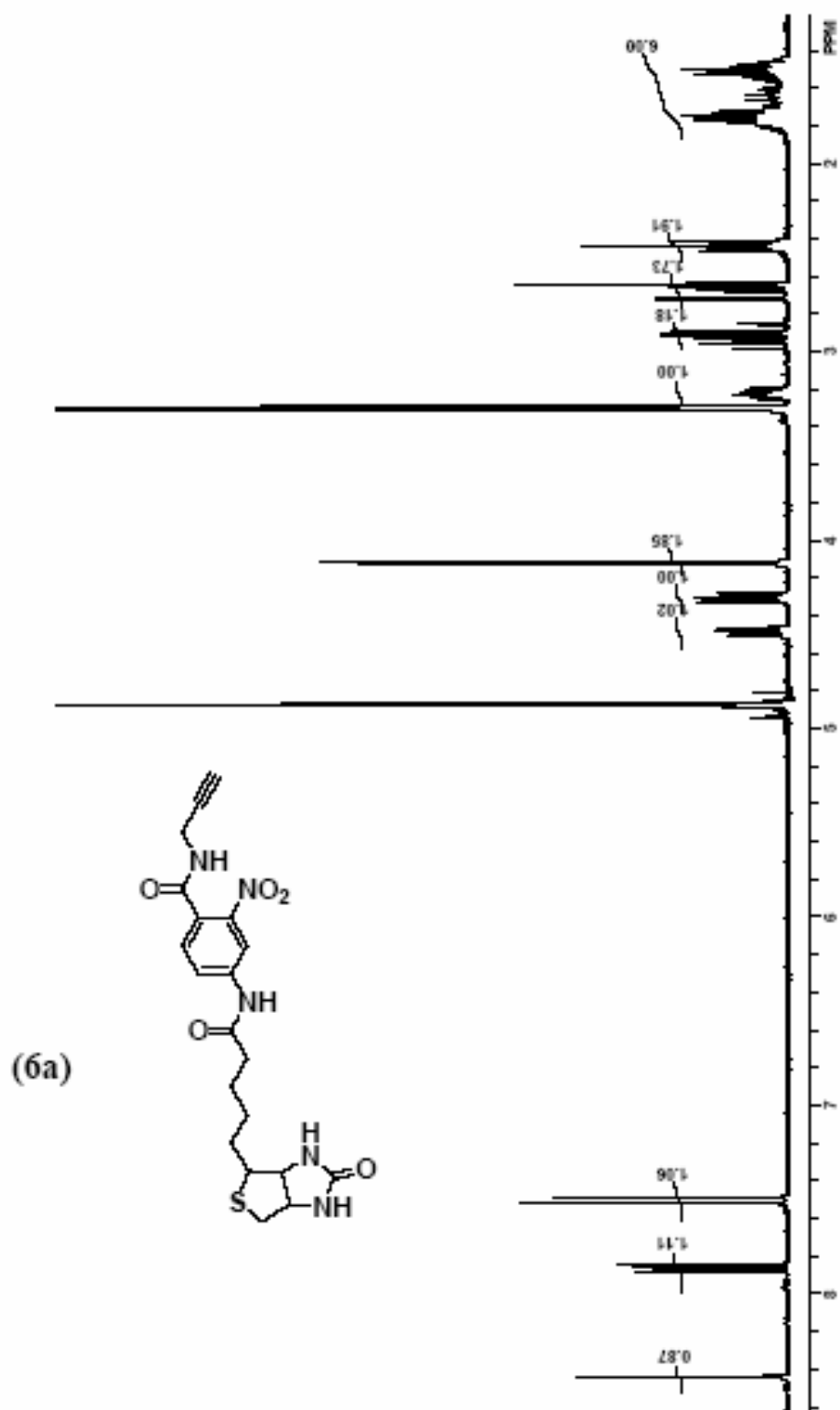


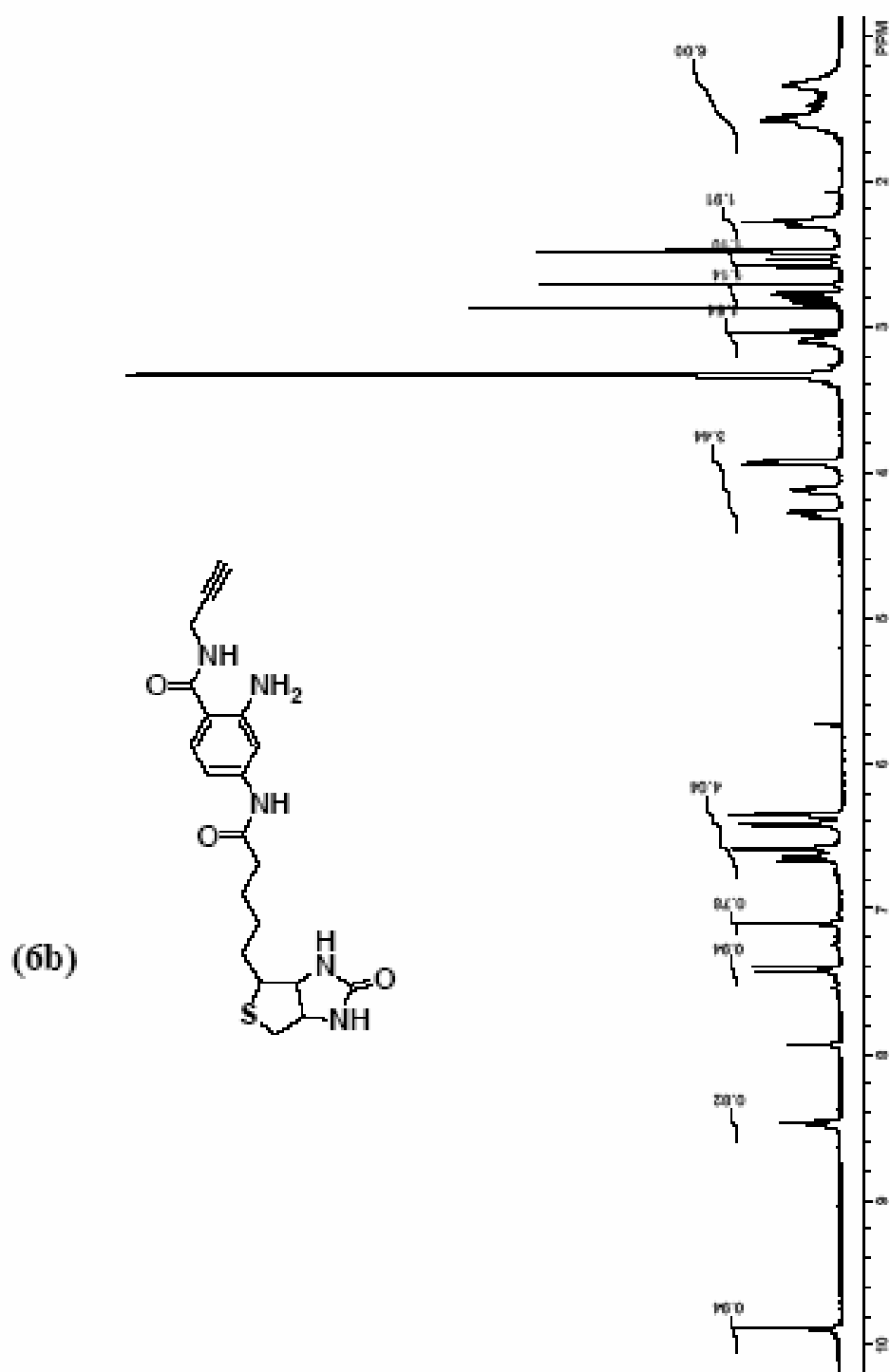


(5a)

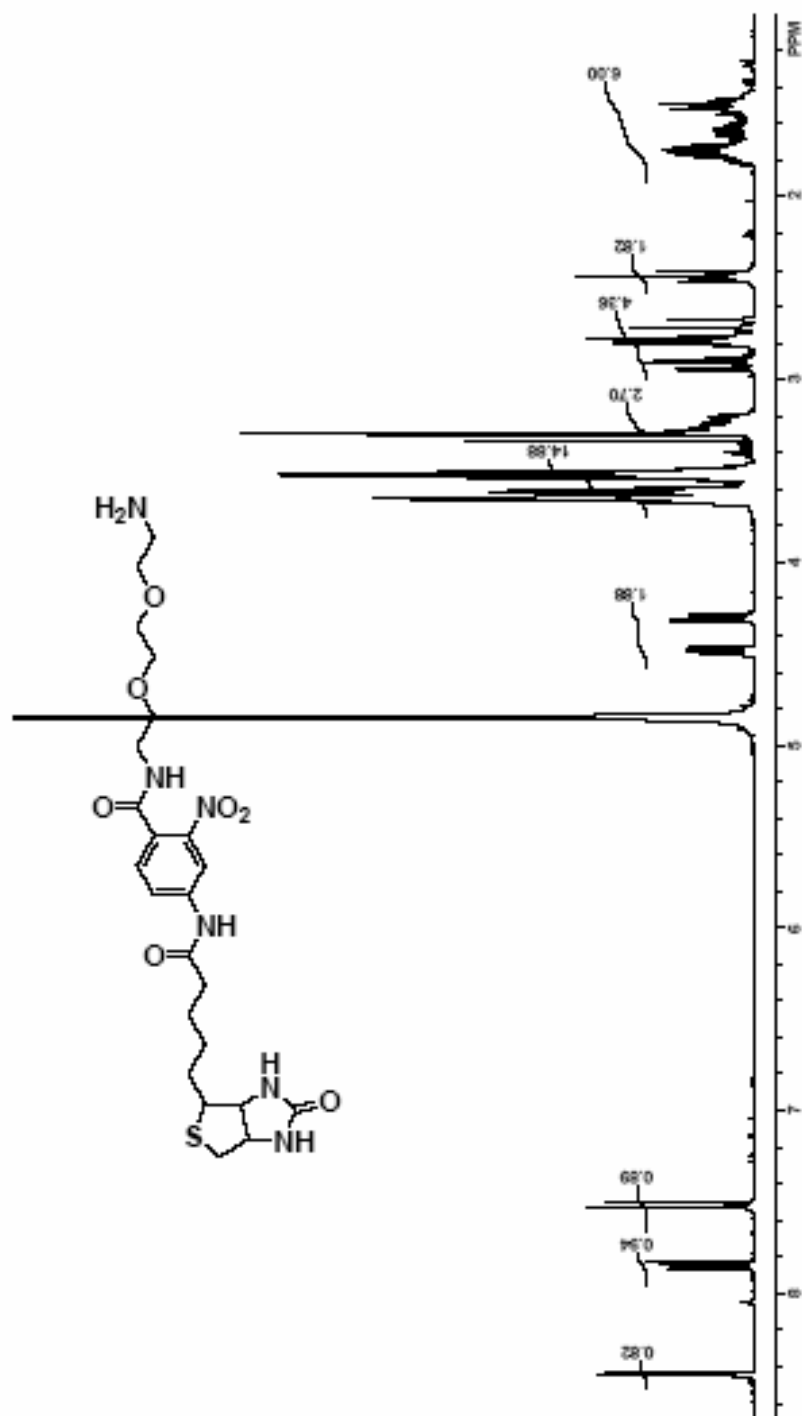




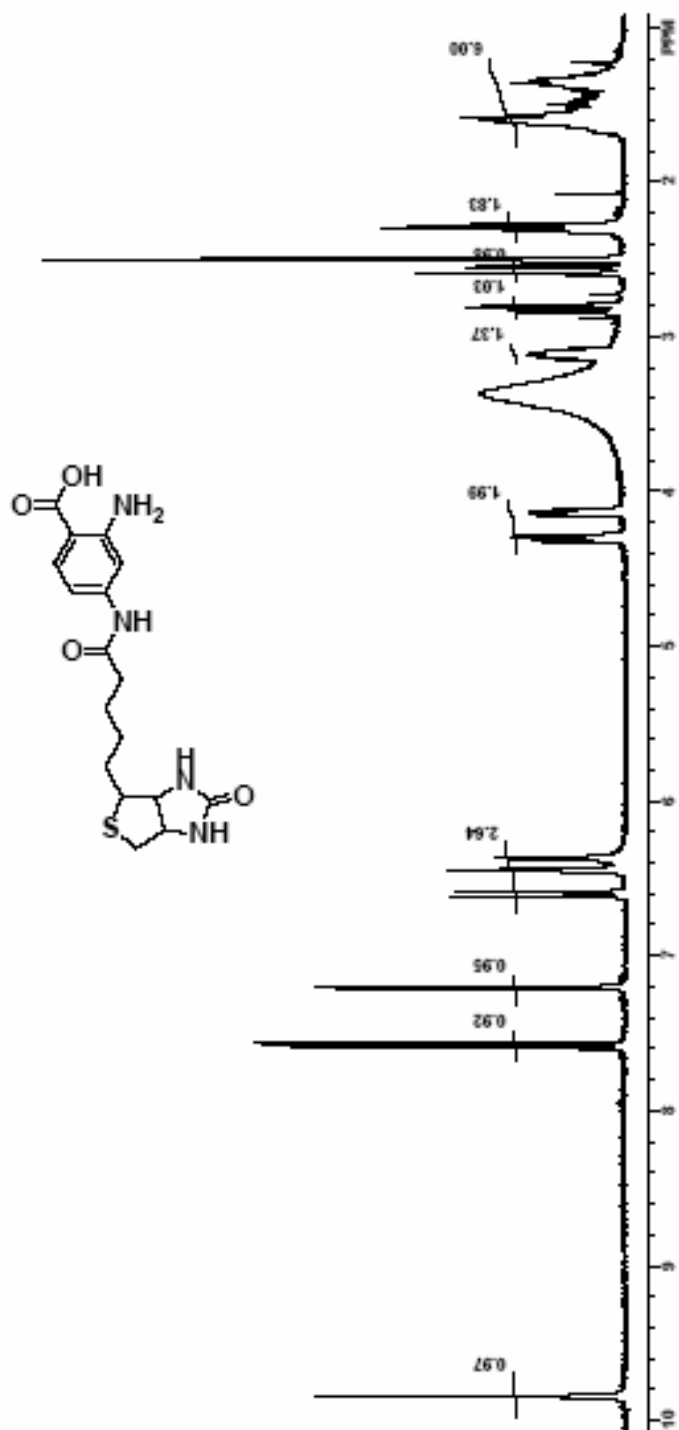


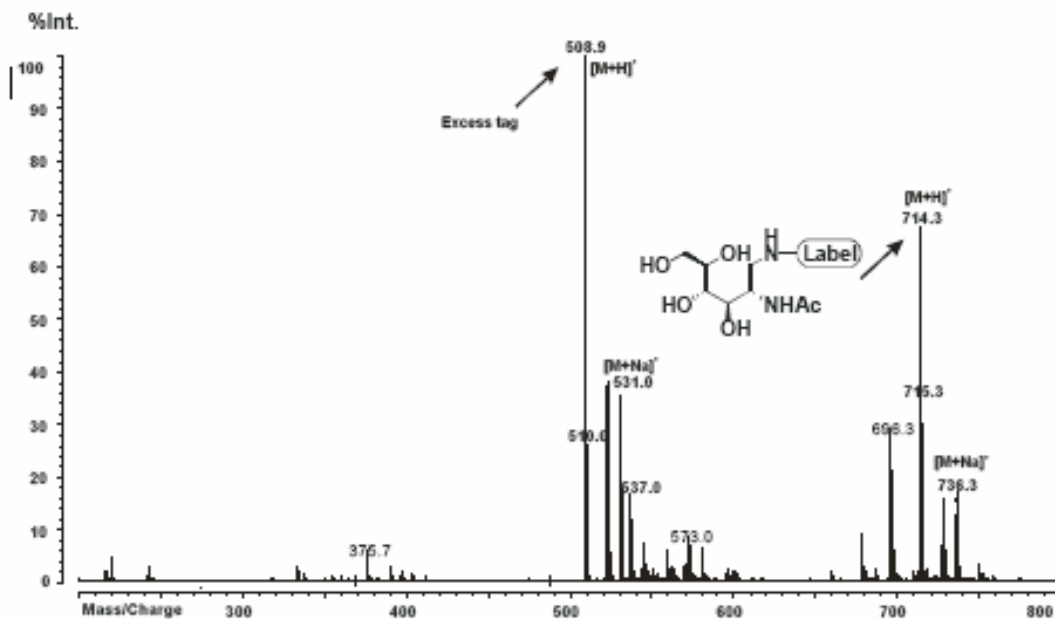


(6c)

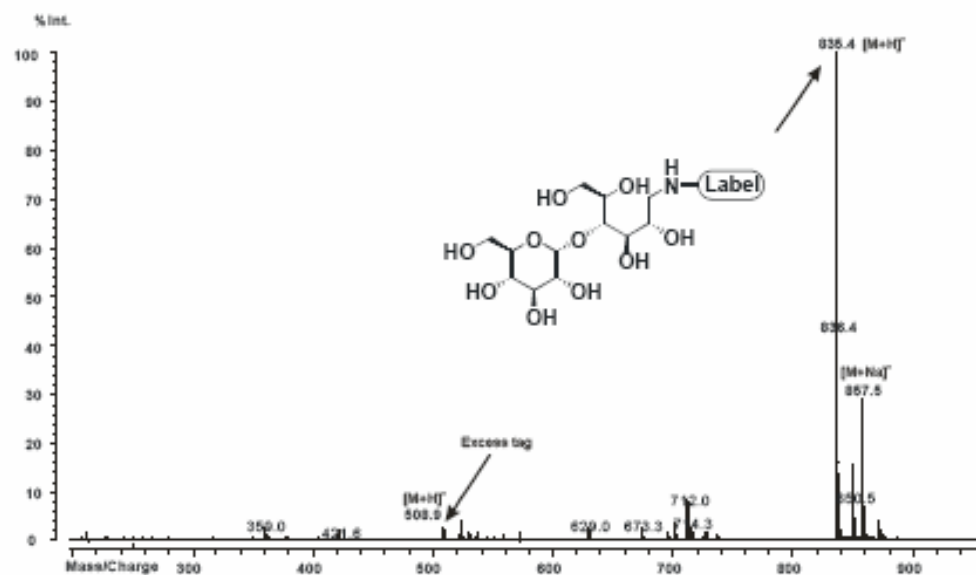


(6d)

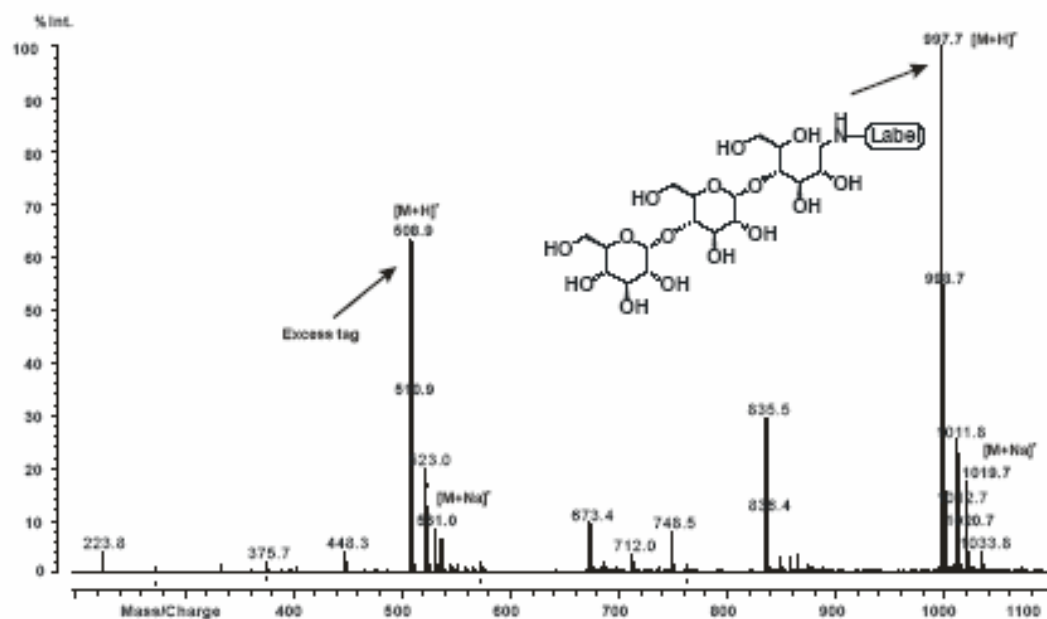




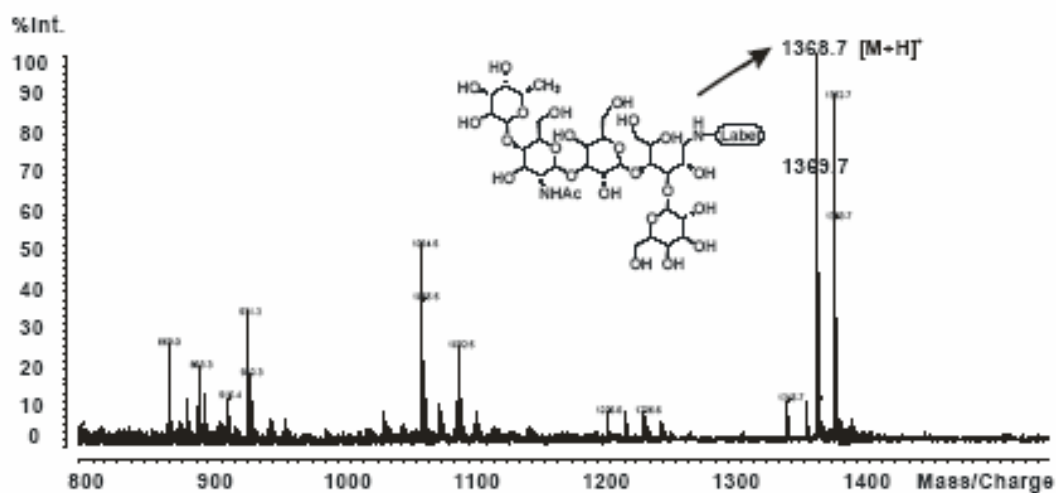
MALDI-TOF spectrum of 2-*N*-acetamido-2-deoxy-glucopyranose (*N*-acetylglucosamine) labeled with compound 3c.



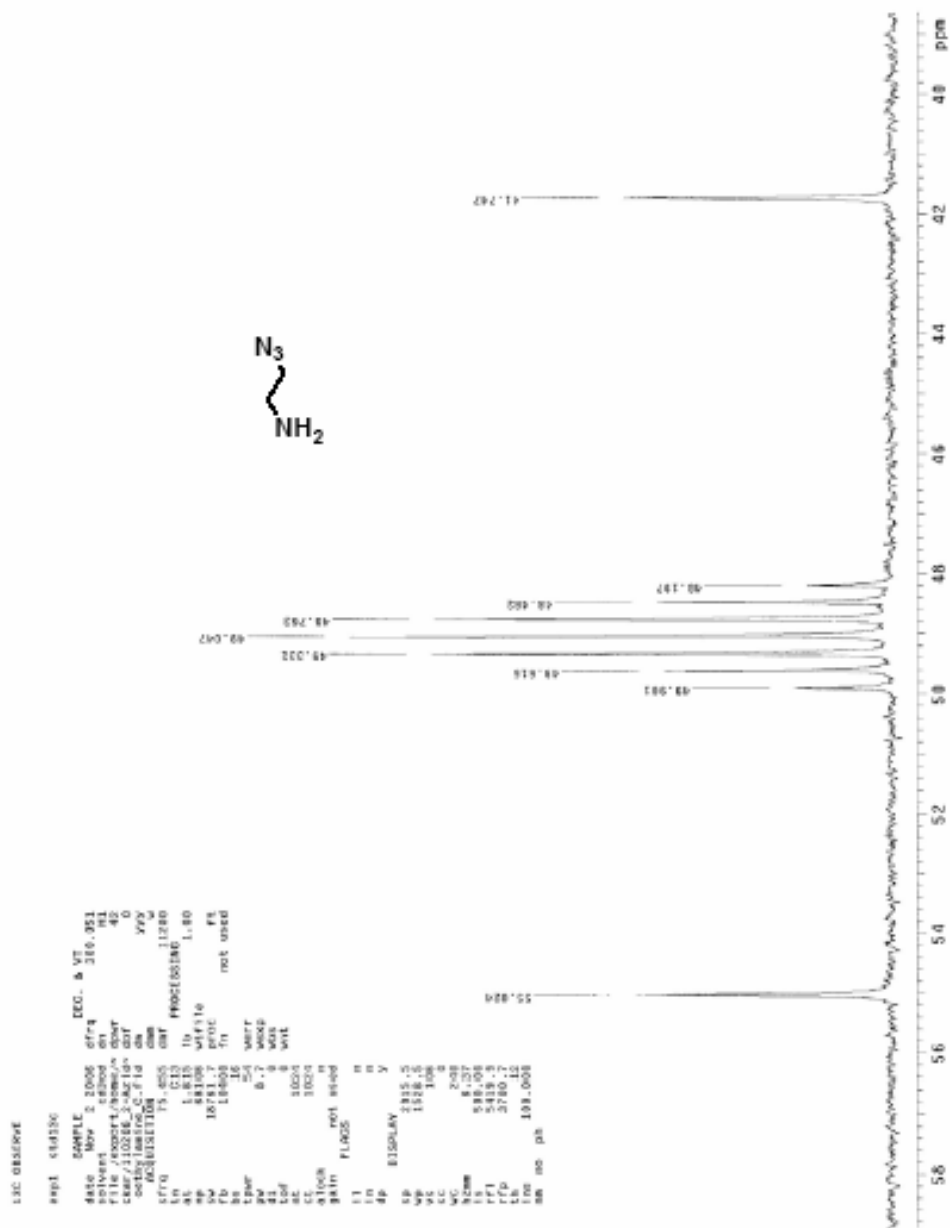
MALDI-TOF spectrum of 4-*O*-(α -glucopyranosyl)-glucopyranose (maltose) labeled with compound 3c.

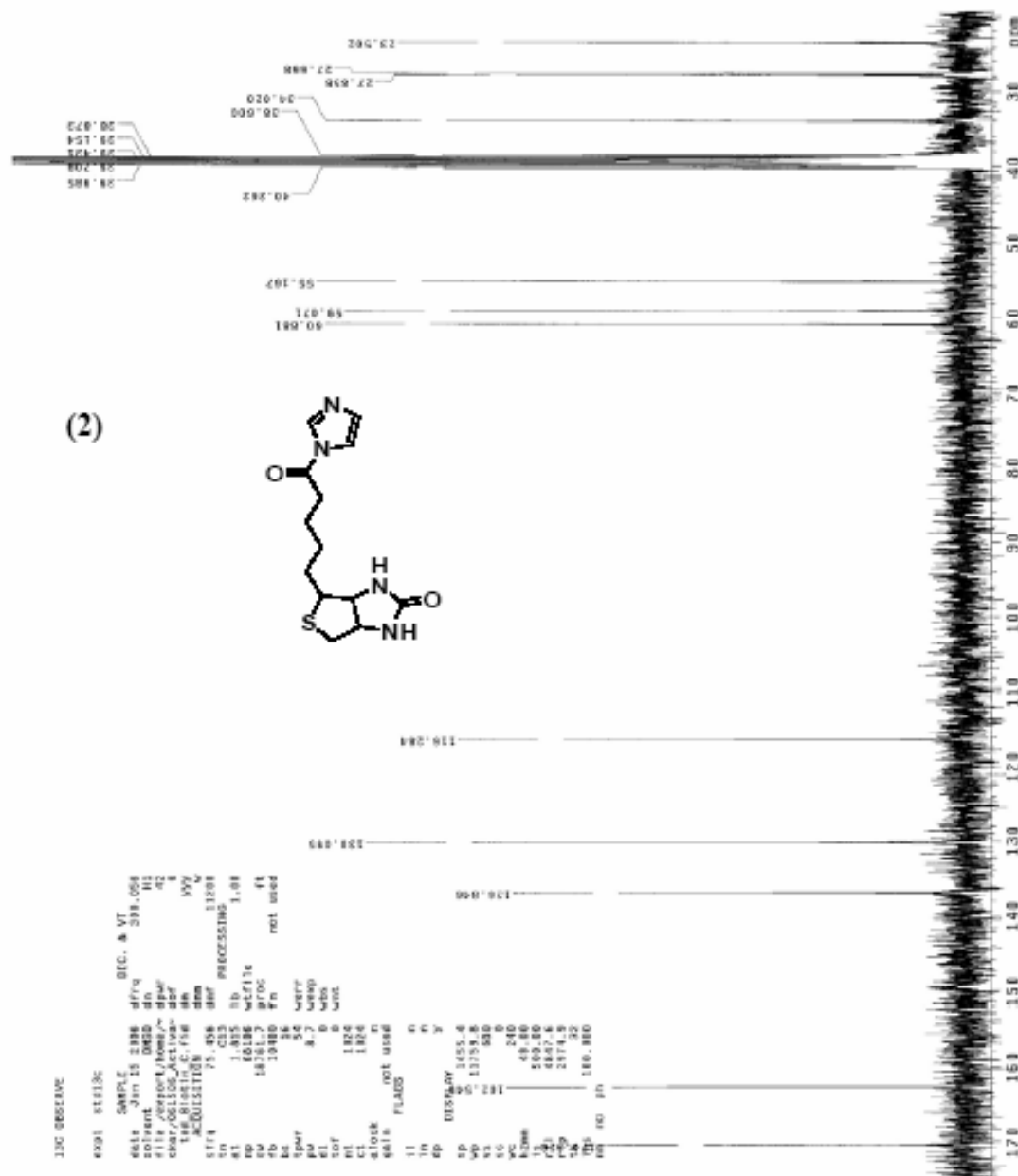


MALDI-TOF spectrum of 4-*O*-[4-*O*-(α -glucopyranosyl)- α -glucopyranosyl]-glucopyranose (maltotriose) labeled with compound 3c.

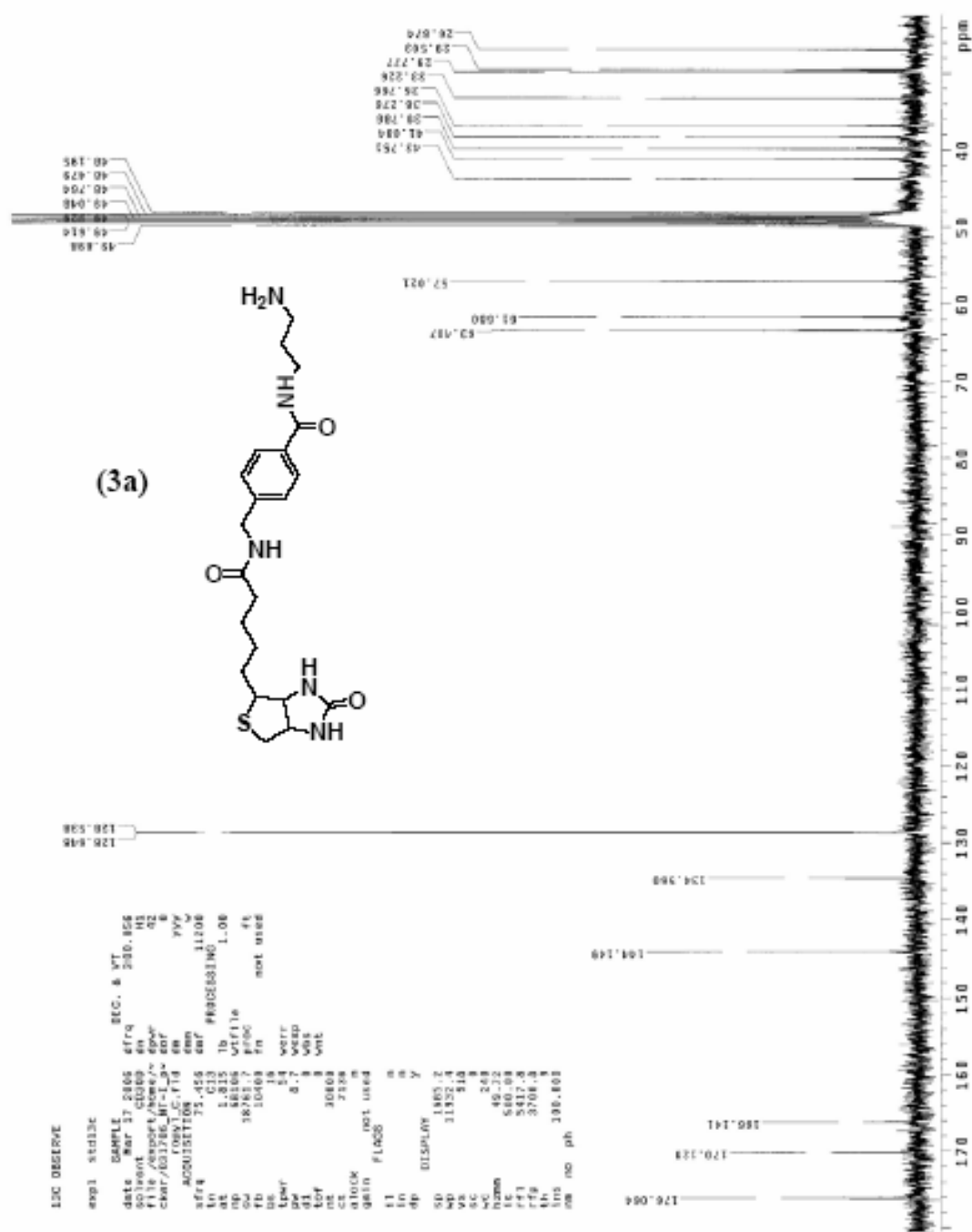


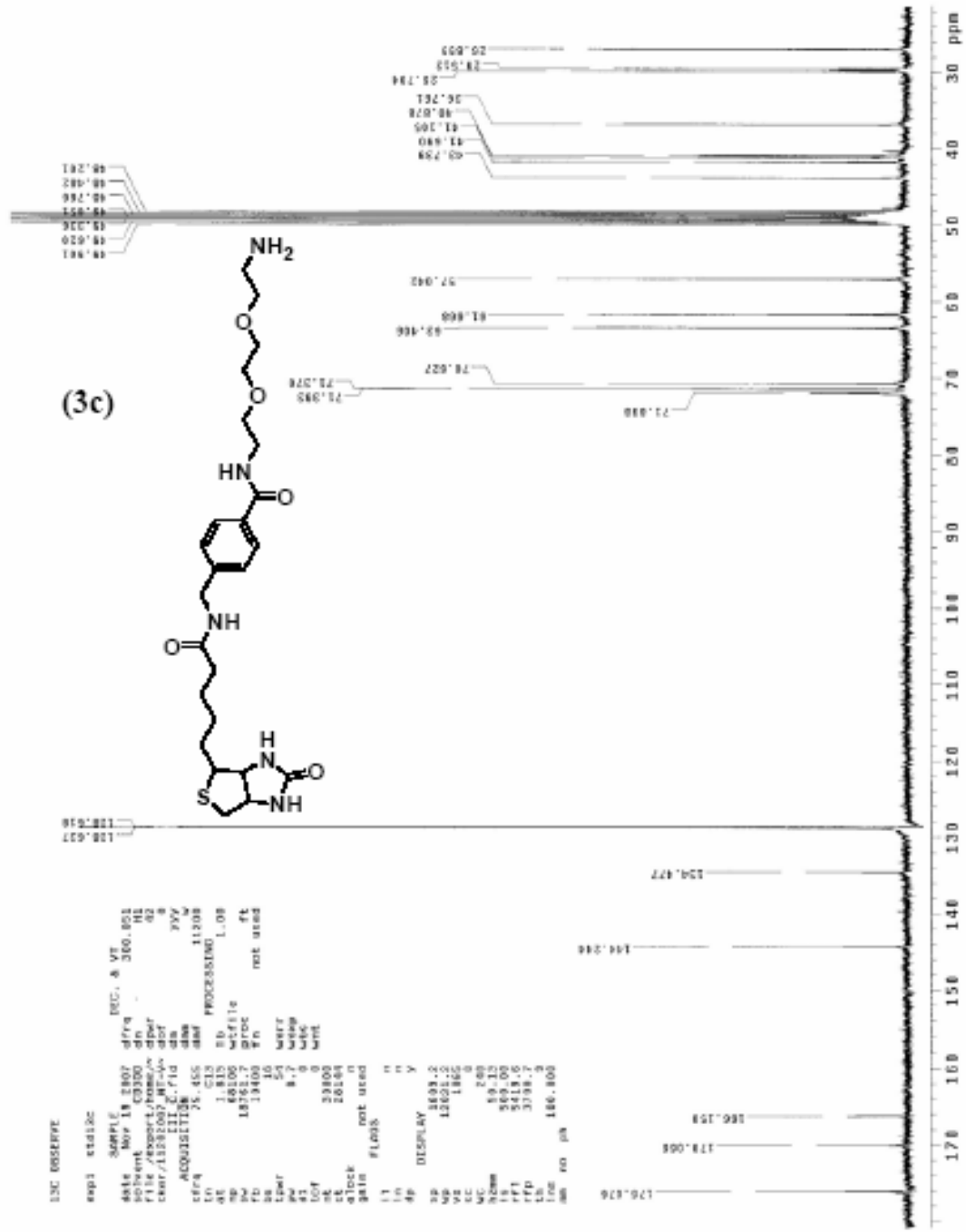
MALDI-TOF spectrum of lacto-*N*-fucopentaose (LNFP-II) labeled with compound 3c.

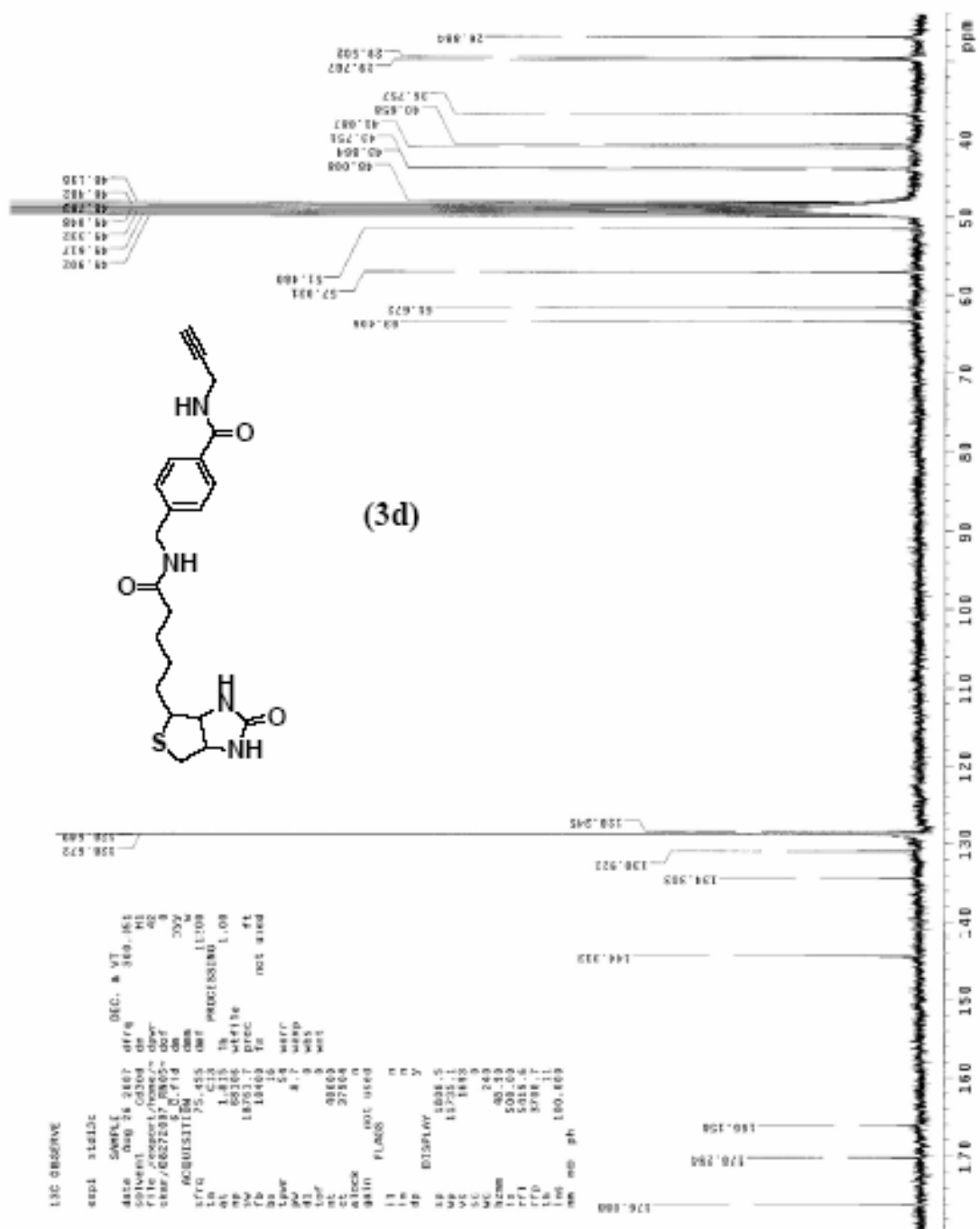


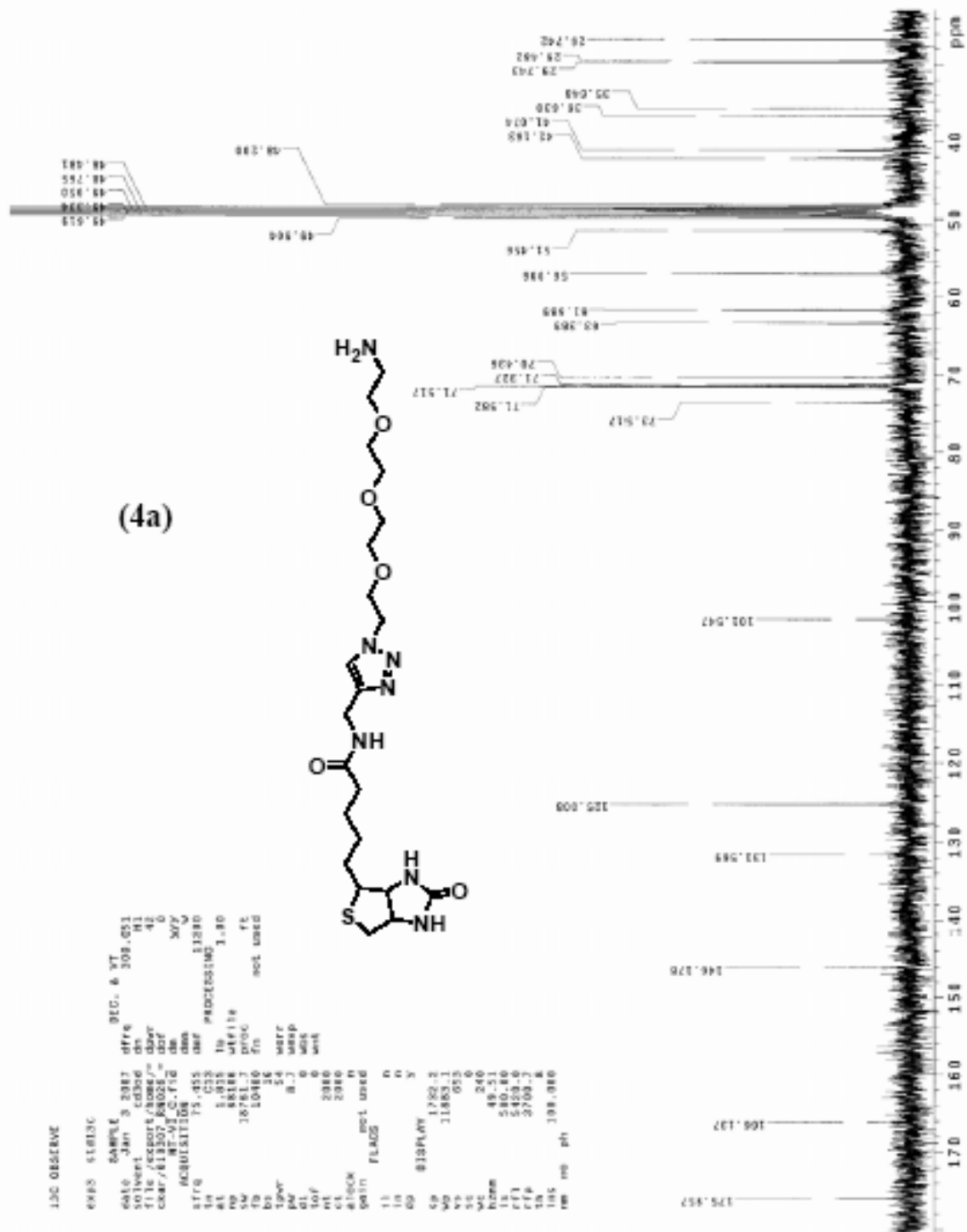


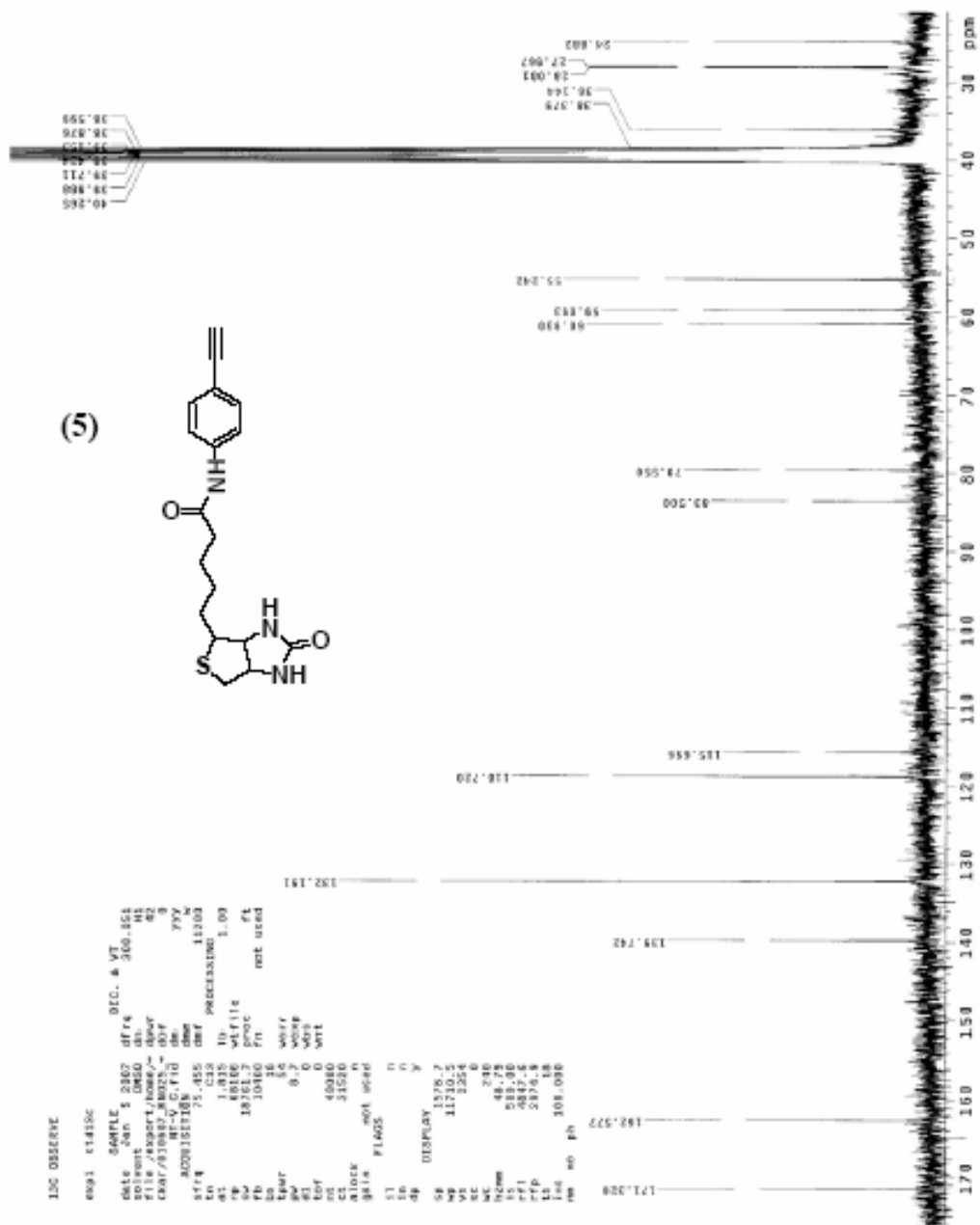
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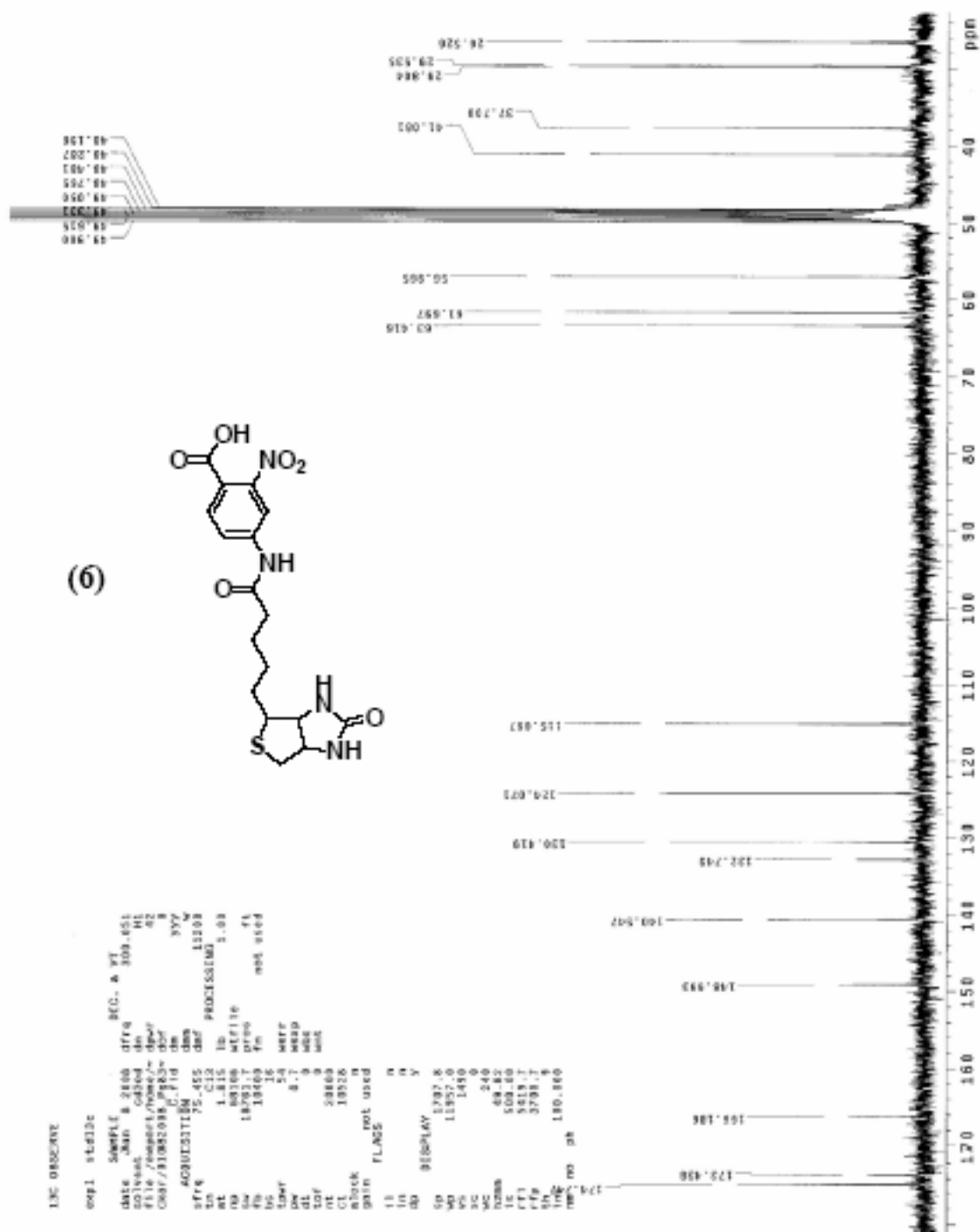












L3C OBSERVE

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(6d)

