

Supplementary Data

Large-scale synthesis of globotriose derivatives through recombinant *E. coli*

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Abbreviations

Sugars used in the text: Gb₃, globotriaosylceramide; Glc, glucose; Gal, galactose; Glc-1-P, glucose-1-phosphate; Gal-1-P, galactose-1-phosphate; Lac, lactose; LacOBn, benzyl β-D-lactoside.

Enzymes in the text: GalK, galactokinase; GalT, galactose-1-phosphate uridylyltransferase; GalU, glucose-1-phosphate uridylyltransferase; PykF, pyruvate kinase.

Additional abbreviations used in the text: STEC, Shiga toxin-producing *E. coli*; HUS, hemolytic-uremic syndrome; UDP, uridine 5'-diphosphate; PEP, phosphoenolpyruvate; UTP, uridine 5'-triphosphate; PPi, pyrophosphate; ATP, adenosine 5'-triphosphate; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; Ni²⁺-NTA, nickel-nitrilotriacetic acid; IPTG, isopropyl-1-thio-β-D-galactospyranoside; rbs, ribosomal binding sites; HPLC, high pressure liquid chromatography; RI, refractive index; HEPES, N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid; DTT, dithiothreitol; PCR, polymerase chain reaction; FAB-MS; fast atom bombardment mass spectra.

General Methods and Materials

Thin-layer chromatography was conducted on Baker Si_{250F} silica gel TLC plates with a fluorescent indicator. For HPLC analysis, Varian ProStar instrument with MICROSORB™-100Å amino 5U 250X4.6 column was used (Varian, Walnut Creek, CA). Carbohydrate analysis was performed with 70:30 acetonitrile:water mobile phase and a RI detector (Varian Star 9040).

¹H and ¹³C NMR spectra were obtained at 25°C using a 400-MHz Varian Mercury-400 NMR or a 500-MHz Varian Unity-500 NMR spectrometer with deuterated water as solvent. Low-resolution and high-resolution fast atom bombardment mass spectra (FAB-MS) were run at the mass spectrometry facility at Wayne State University and University of California at Riverside respectively.

Ni²⁺- NTA agarose (bead size: 45-165 μm; bead structure: cross-linked 6% agarose; support: Sepharose CL-6B; protein capacity: 300-500 nmol/mL), The polymerase chain reaction (PCR) purification kit, QIAEX II gel extraction kit, QIAamp tissue kit and DNA miniprep spin kit were from Qiagen, Santa Clarita, CA. All restriction enzymes, Taq DNA polymerase, 1 kb DNA ladder, and T4 DNA ligase were obtained from Promega, Madison, WI. Sodium chloride and ScintiVerse BD were from Fisher Scientific, Chicago, IL. IPTG, ampicillin, UDP-D-[6-³H]galactose, DOWEX 1X8 anion exchange resin, PEP, ATP, UDP-Glc, Glc-1-P, HEPES hemisodium salt, MgCl₂, MnCl₂, KCl, β-D-lactose, methyl β-D-lactoside, lactulose, lactitol, methyl β-D-galactoside and 3-O-β-D-galactopyranosyl-D-arabinose were obtained from Sigma, St. Louis, MO. Benzyl lactoside and phenyl 1-thio-β-D-lactoside were synthesized previously (Zhang W.; Wang J.; Li J.; Yu L.; Wang P. G. *J. Carbohydr. Chem.* **1999**, *18*, 1009–1017; Fang, J. *Ph.D. Thesis*, Wayne State University, **2000**.). All other chemicals were obtained in reagent grade from commercially available sources.

Plasmid vector pLDR20 (ATCC catalog no. 87205) was purchased from American Tissue Culture Collection. Plasmid vector pET15b and *E. coli* competent cell BL21 (DE3) [*F ompT hsdS_B(r_B⁻m_B⁻) gal dcm (DE3)*] were from Novagen Inc., Madison, WI. Chromosomal DNA of *Neisseria meningitidis* MC58(L3) was a kind gift from Dr. Michael Gilbert (Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada). Plasmid pET15b-lgtC was constructed as described previously.³⁷ Plasmids pET15b-galKT, pET15b-galU, pET15b-pykF were constructed as described previously.^{16, 36} *E. coli* competent cell DH5 α (*lacZ Δ M15 hsdR recA*) was from Gibco-BRL Life Technology, Rockville, MD. Competent cell NM522 {*supE thi-1 Δ (lac-proAB) Δ (mcrB-hsdSM)5(r_K⁻ m_K⁺) [F'proABlacI^qZAM15]*} was from Strategene, La Jolla, CA.

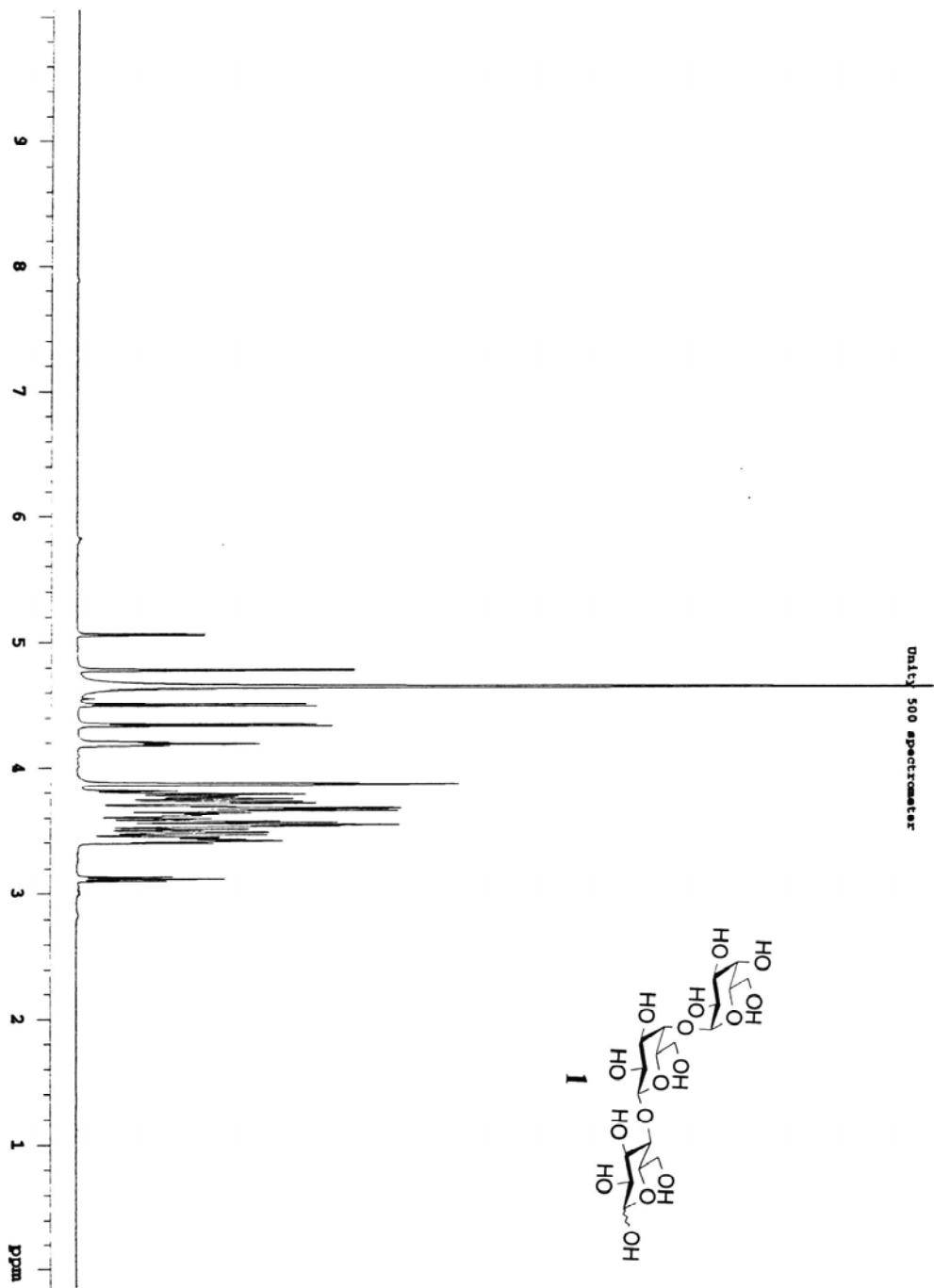
Cloning, overexpression and purification of LgtC.

For construction and overexpression of a recombinant α 1, 4-galactosyltransferase from *N. meningitidis*, the truncated *lgtC* gene (primers 5'-CGGAATTCATATGGACATCGTATTTGCG-3' and 5'-GCCGGATCCTCATCAGTGCGGGACGGCAAGTTTGCC-3') was cloned from *N. meningitidis* MC58 with the deletion of the codon sequence encoding for the 25 amino acid at the C-terminus of the full length LgtC protein. PCR amplified product was purified by QIAquick PCR Purification Kit and QIAEX II Gel Extraction Kit digested with Nde I and BamH I restriction enzymes and inserted into pET15b vector. The resulting plasmid pET15b-lgtC-25aa was transformed into *E. coli* cloning host strain DH5 α and then expression host strain BL21 (DE3) respectively. Selected clones were characterized by restriction mapping. For the expression and purification of LgtC, briefly, overexpression was induced by 0.4 mM IPTG for 3 hr at 37°C in a C25 incubator shaker (New Brunswick Scientific Co., Inc., Edison, NJ). The cells were sedimented, resuspended and lysed

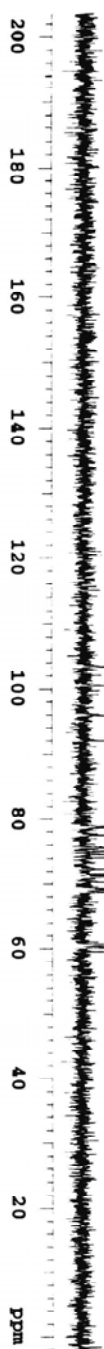
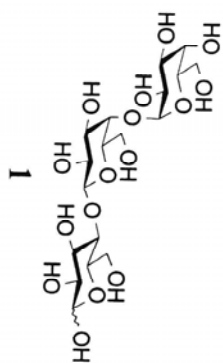
enzymatically (lysozyme to 150 $\mu\text{g/ml}$). The cell lysate and inclusion bodies were separated by centrifugation at 12,000 rpm for 20 min. From cell lysate, the active enzymes were purified using a Ni^{2+} -NTA agarose affinity column. After elution, the fractions containing the purified enzyme (detected by a UV-Vis spectrometry) were combined and dialyzed for enzyme activity assays and enzymatic reactions.

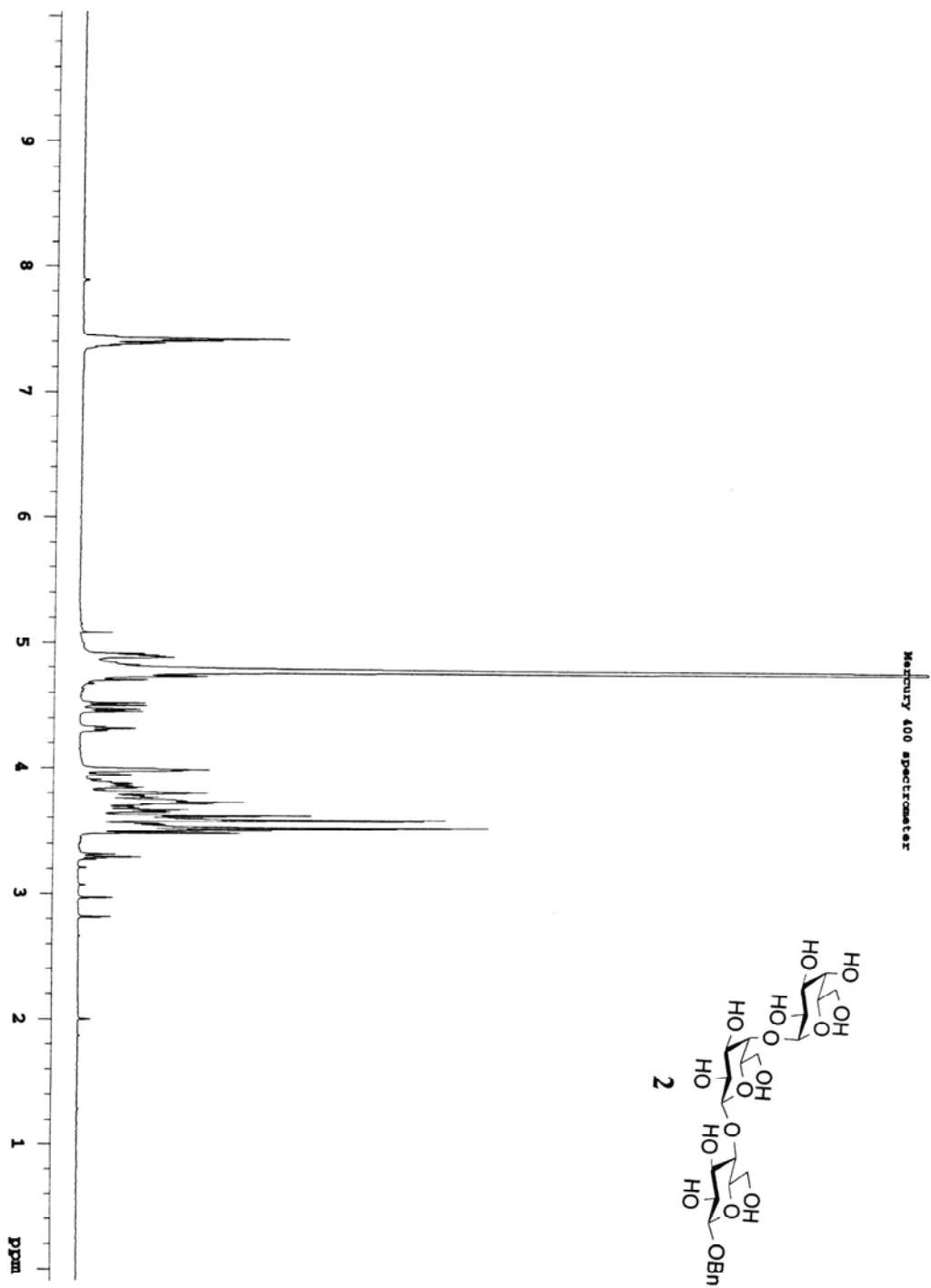
Glycosyltransferase Activity Assays.

Enzymatic assays for LgtC were performed at 37 °C for 15 min in a final volume of 100 μL containing Tris-HCl (10 mM, pH 7.0), MnCl_2 (10 mM), DTT (5 mM), bovine serum albumin (0.1%), UDP-D-[6- ^3H]galactose (0.3 mM) (final specific activity of 1000 cpm/nmol), LgtC (10 μL), and lactose (50 mM). Lactose was omitted for blank. The reaction was stopped by adding 100 μL of ice-cold EDTA (0.1 M). Dowex 1 \times 8-200 chloride anion exchange resin was then added in a water suspension [0.8 mL, 1:1 (v/v)]. After centrifugation, supernatant (0.5 mL) was collected in a 20-mL plastic vial, and ScintiVerse BD (10 mL) was added. The vial was vortexed thoroughly before the radioactivity of the mixture was counted in a liquid scintillation counter (Beckman LS-3801 counter). One unit of enzyme activity is defined as the amount of enzyme that catalyzes the transfer of 1 μmol of galactose from UDP-Gal to lactose per min at 37 °C.

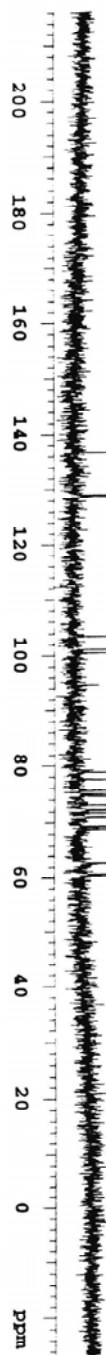
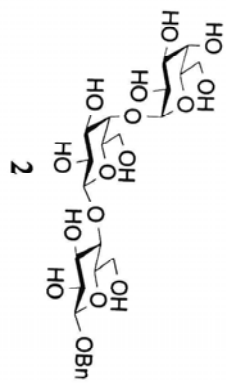


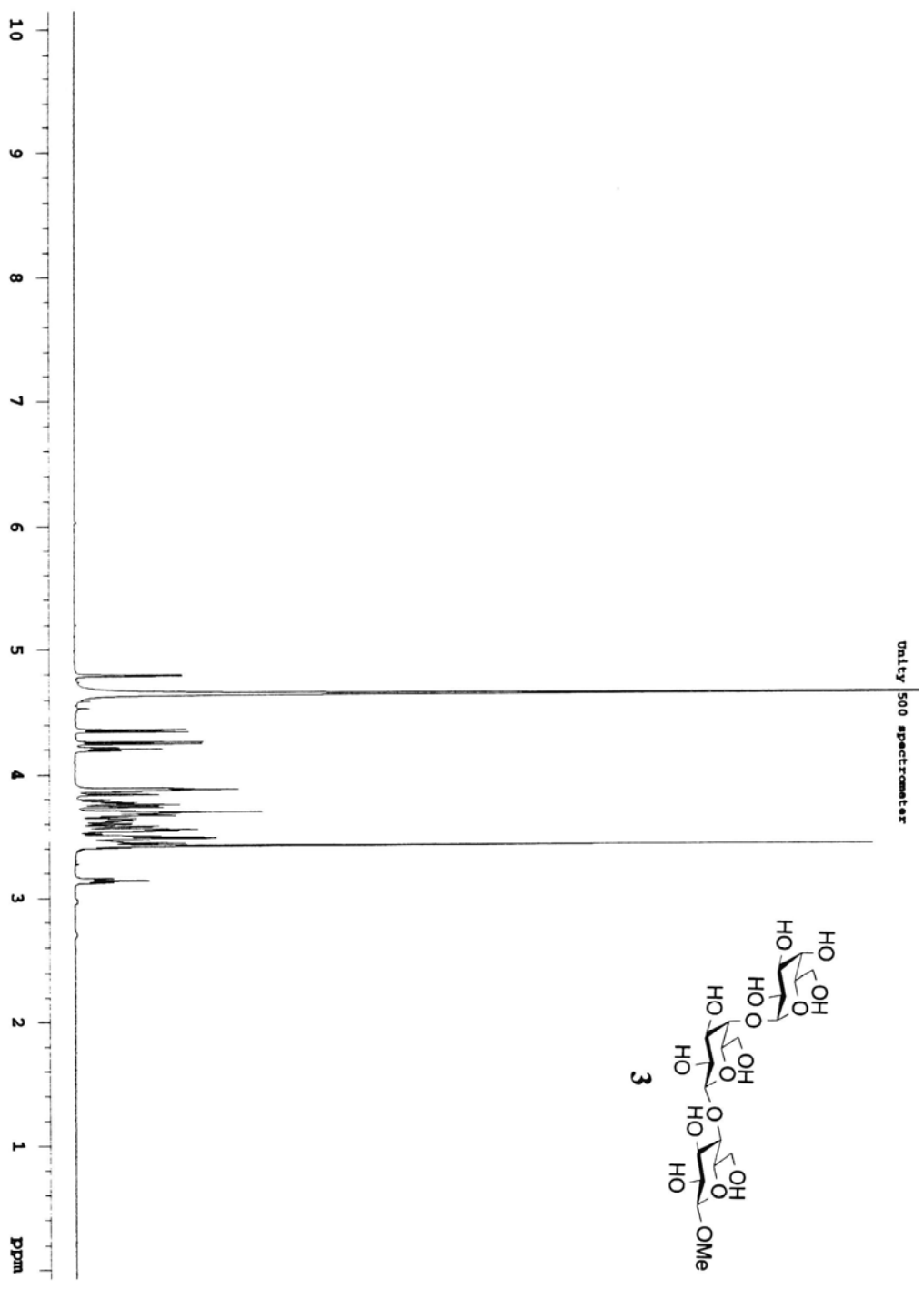
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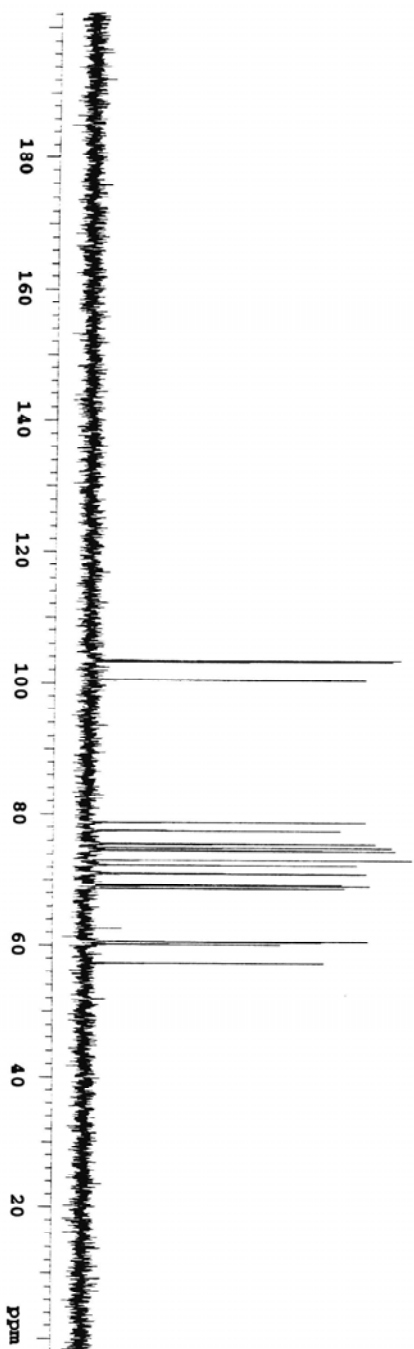
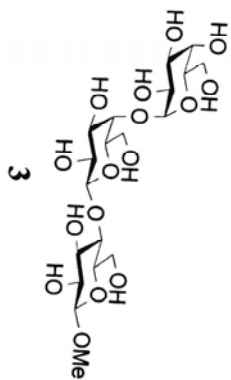


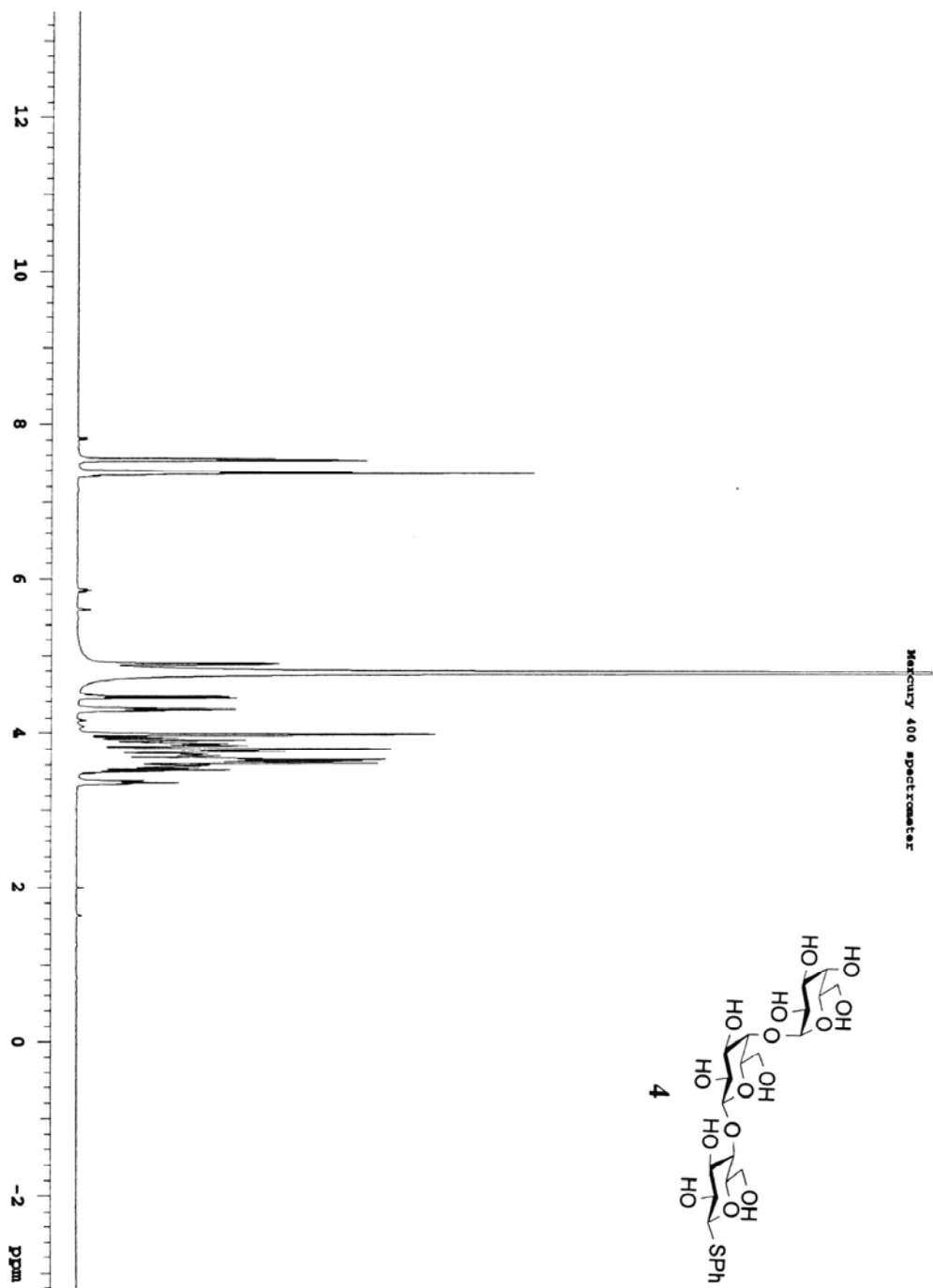
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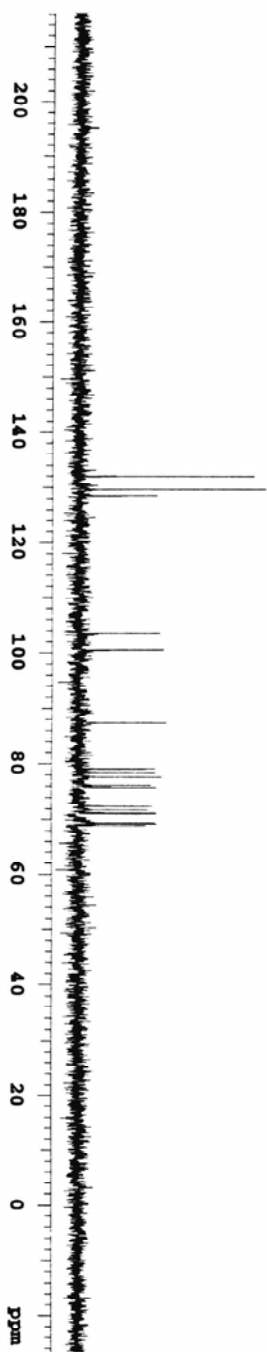
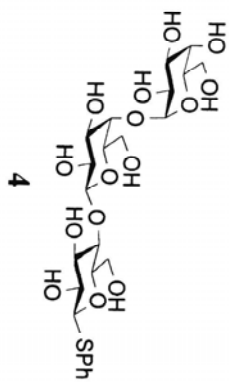


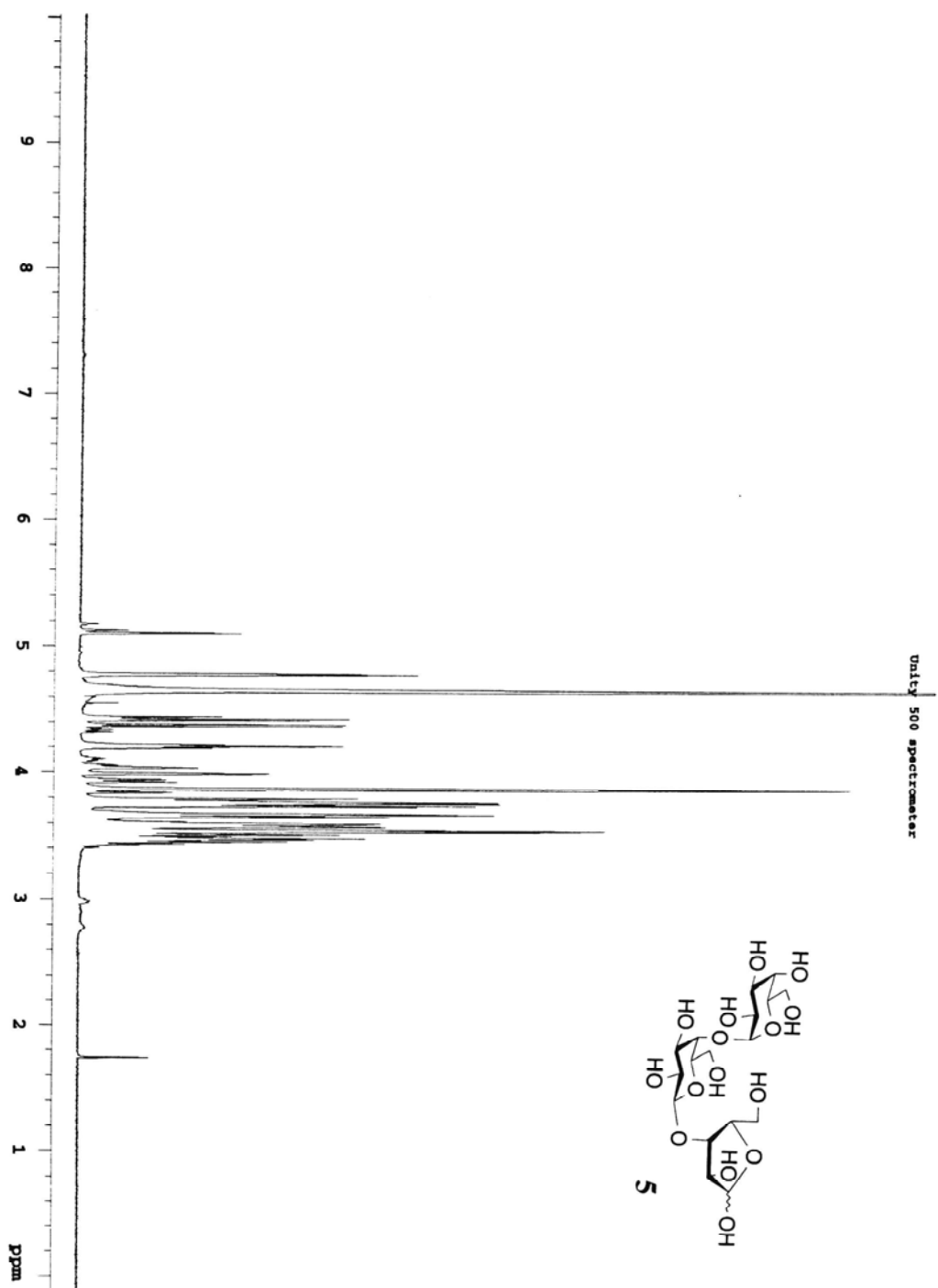
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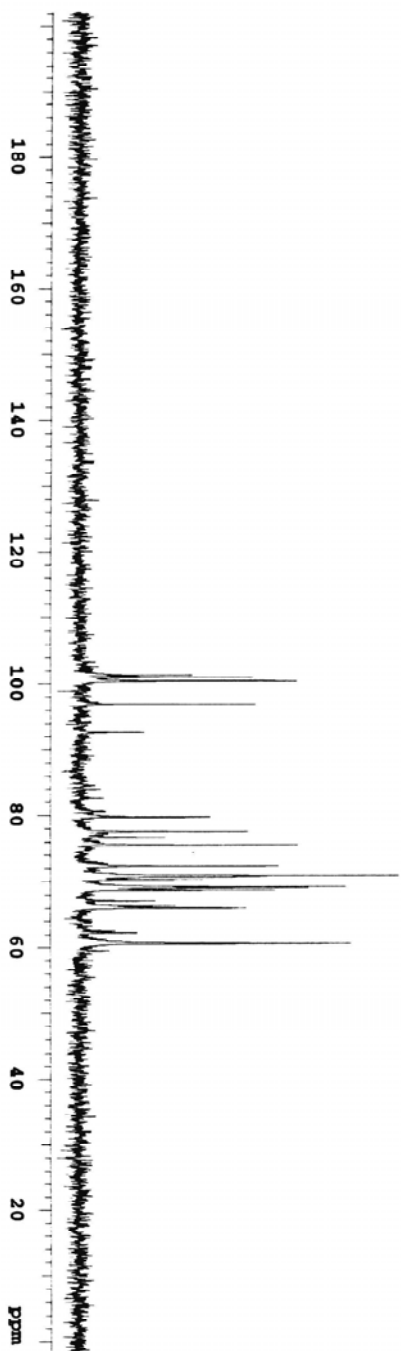
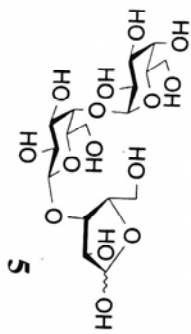


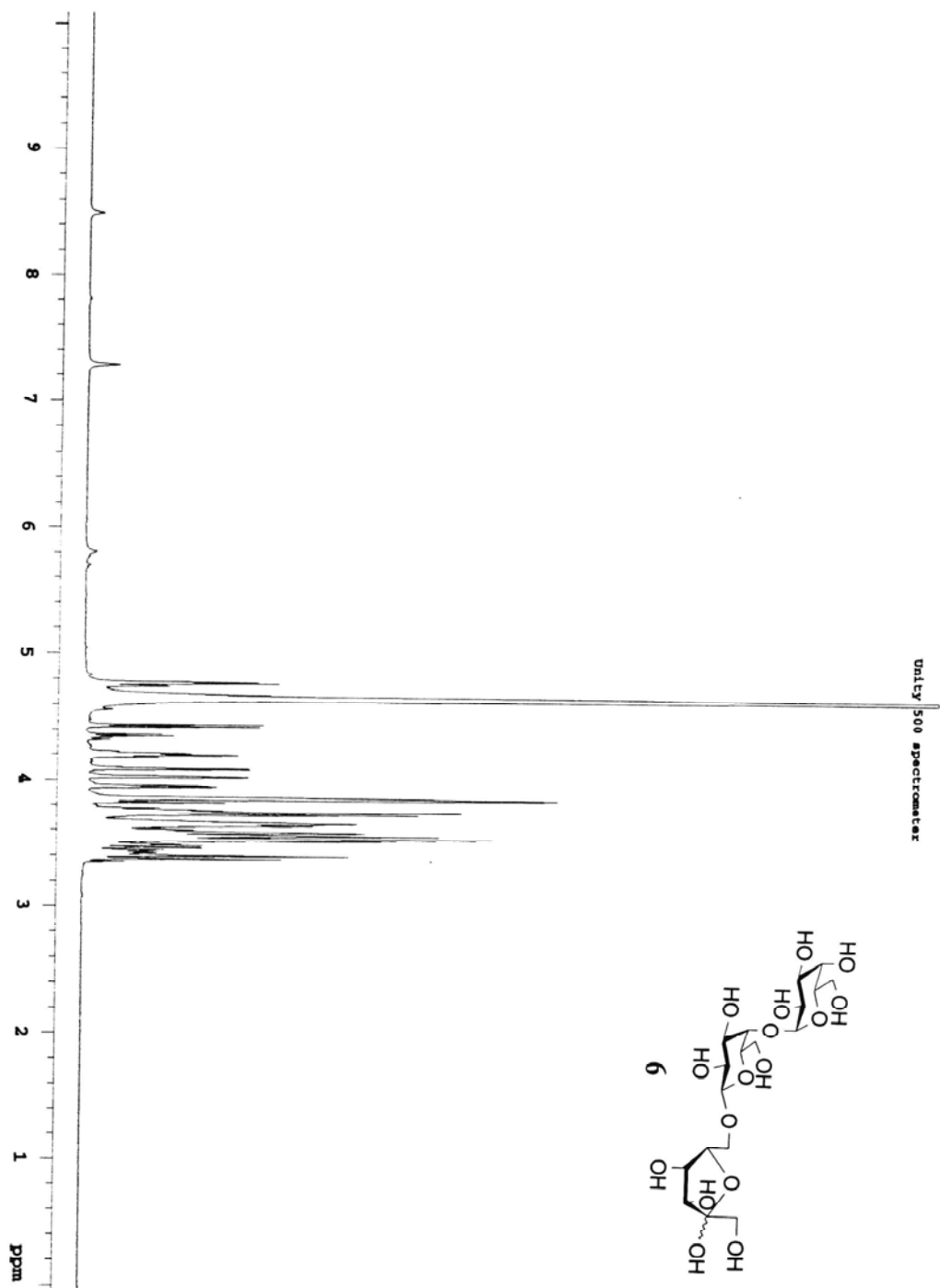
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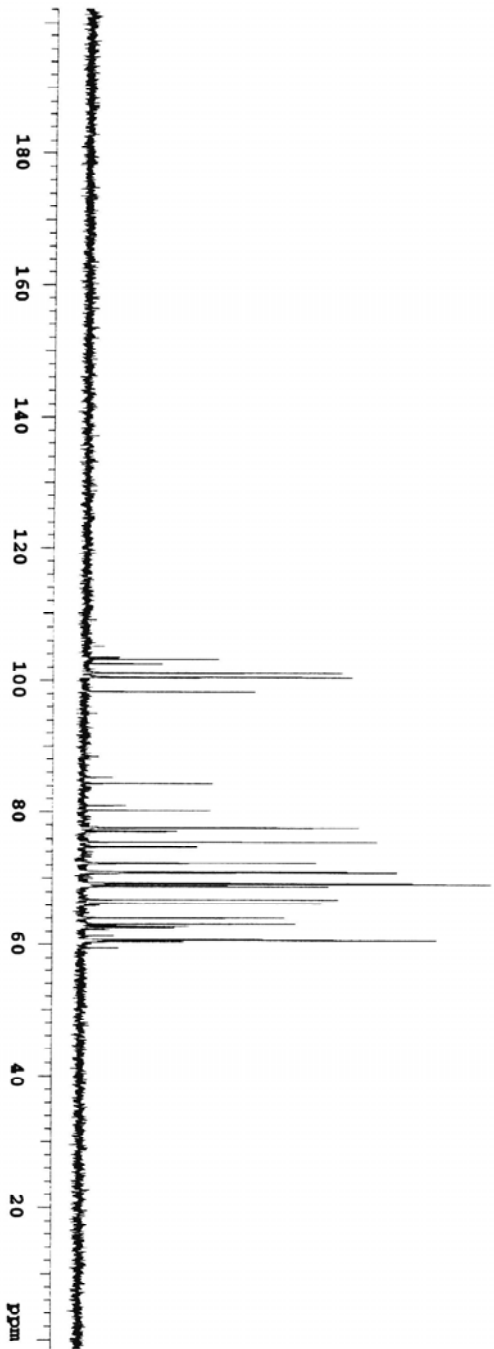
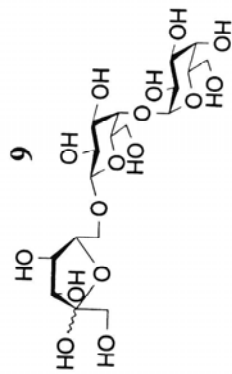


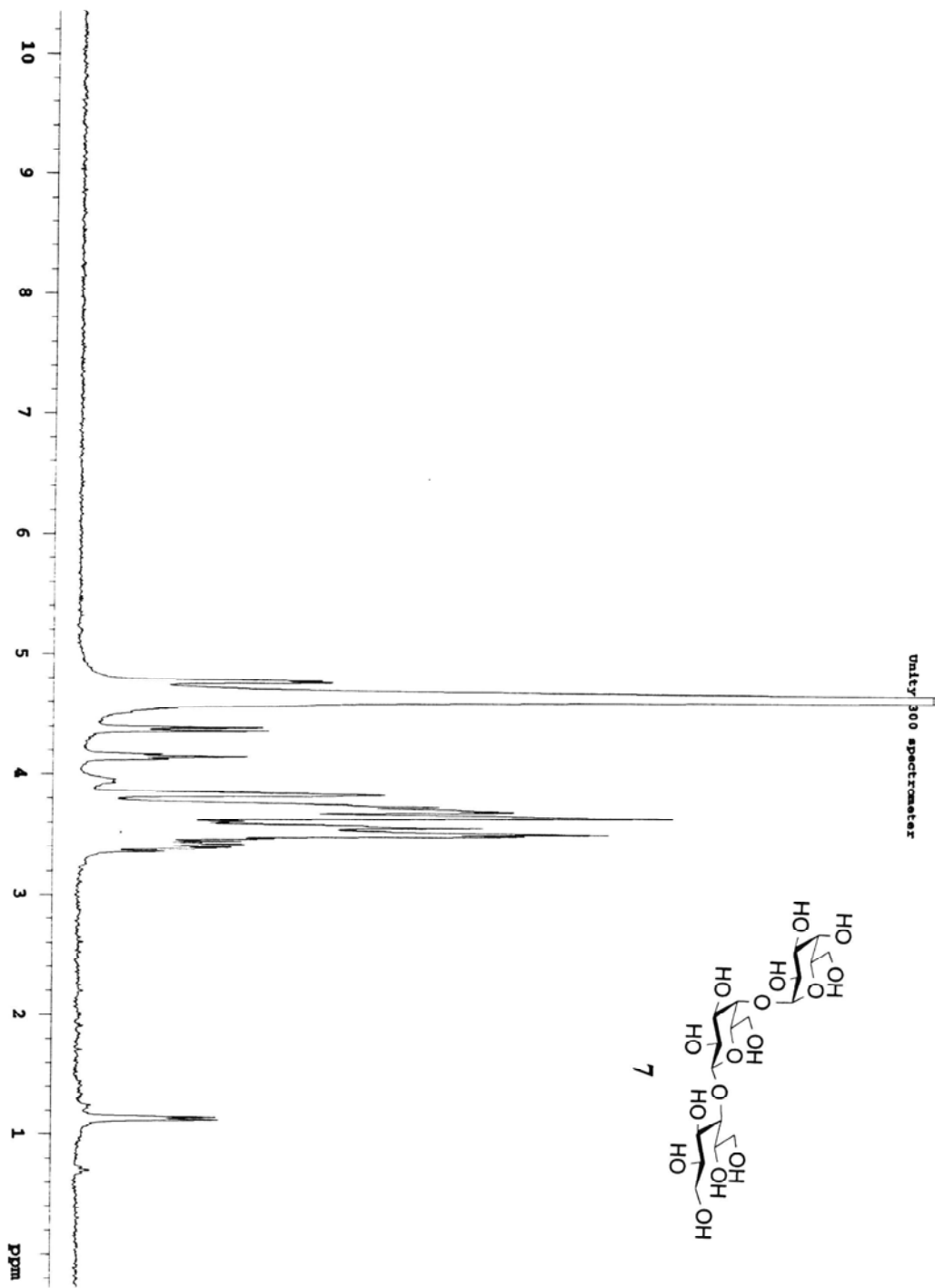
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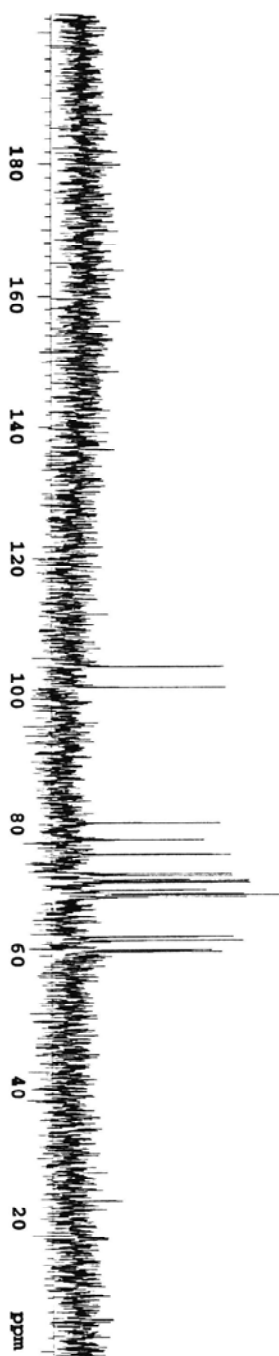
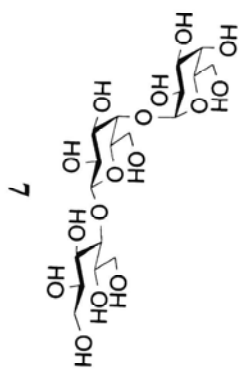


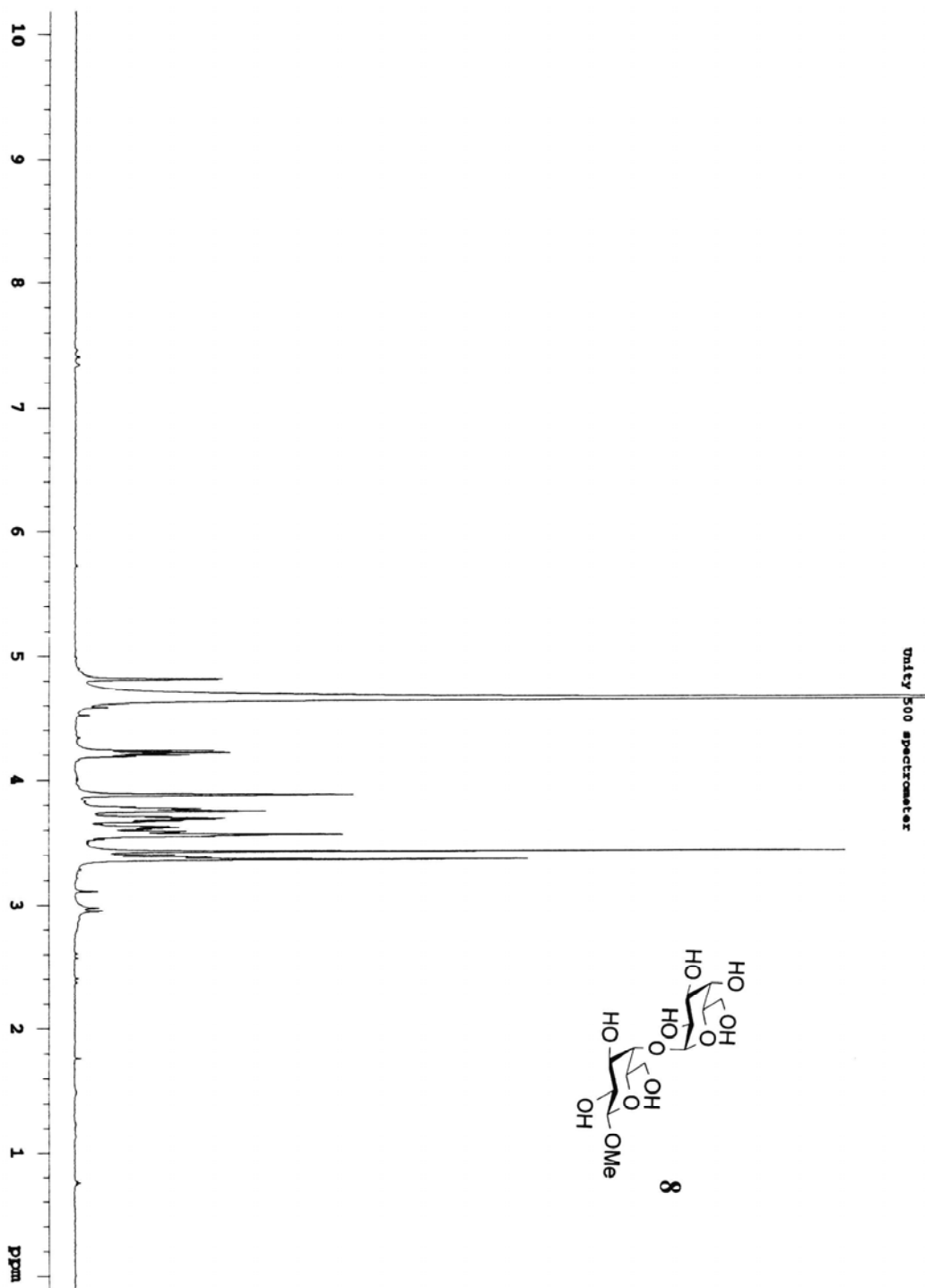
Unity 500 spectrometer





Mercury 400 spectrometer





Unity 500 spectrometer

