# Supplementary Data

## *L. pneumophila* CMP-5,7-di-*N*-acetyllegionaminic acid synthetase (LpCLS)-involved chemoenzymatic synthesis of sialosides and analogues[†]

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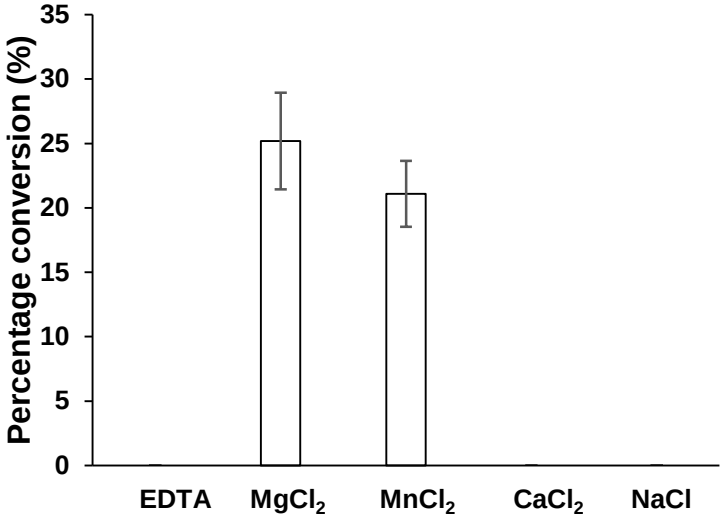



**Figure S1. Metal ion effects of LpCLS.** LpCLS was assayed using Leg5,7Ac$_2$ (1 mM) and CTP (1 mM) as substrates in the presence of a divalent metal cation, sodium chloride, or EDTA (10 mM) at 37 °C for 30 minutes.

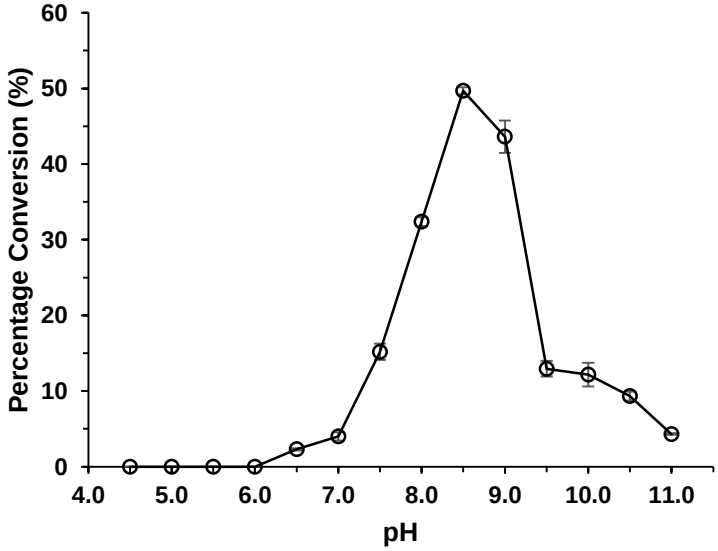
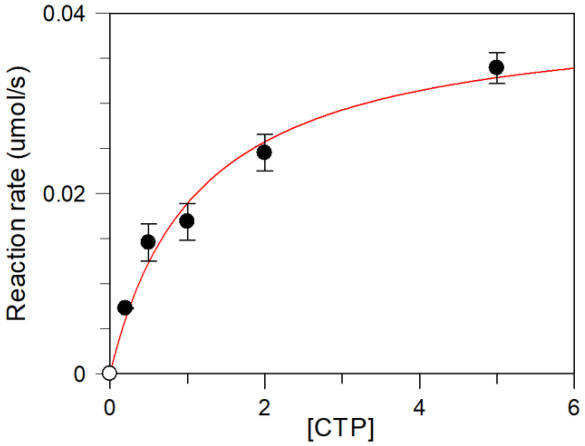



**Figure S2. pH profile of LpCLS.** Reactions were carried out at 37 °C for 30 minutes at pH values ranging from 4.5 to 11.0. MES buffer (100 mM) was used for pH in the range of 4.5–6.5; Tris-HCl (100 mM) was used for the pH range of 7.0–9.0; and CAPS buffer (100 mM) was used for the pH range of 9.5–11.0.




| Parameter | Value | Std. Error |
|---|---|---|
| $V_{max}$ (µmol/s) | 0.0403 | 0.0033 |
| $K_M$ (mM) | 1.1329 | 0.2463 |

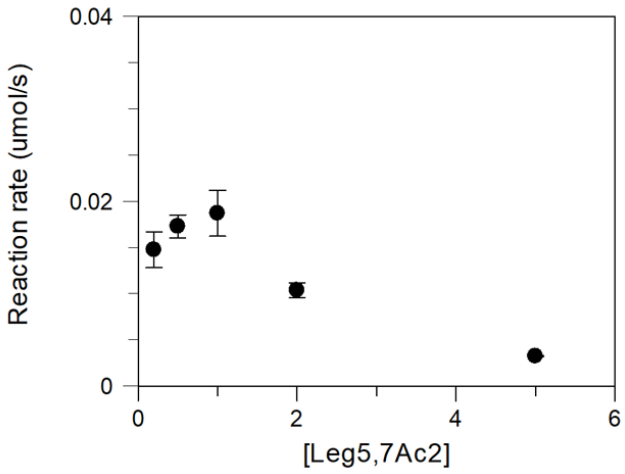



**Figure S3. LpCLS kinetic assay results** catalyzed at 37 °C for 30 minutes with **A)** a fixed concentration (2 mM) of Leg5,7Ac$_2$ and varying CTP concentrations (1.4 nM LpCLS) and **B)** a fixed concentration (2 mM) of CTP and varying Leg5,7Ac$_2$ concentrations (0.7 nM LpCLS). Kinetic parameters for A) were determined in GraFit 5.0 by non-linear regression. The observed substrate inhibition in B) indicates likely an ordered sequential mechanism as discussed in the text.

atgcgtatcctggccgttatcccagcacgcgcgggttccaaacgtcttcctggtaaaaacacccgttt
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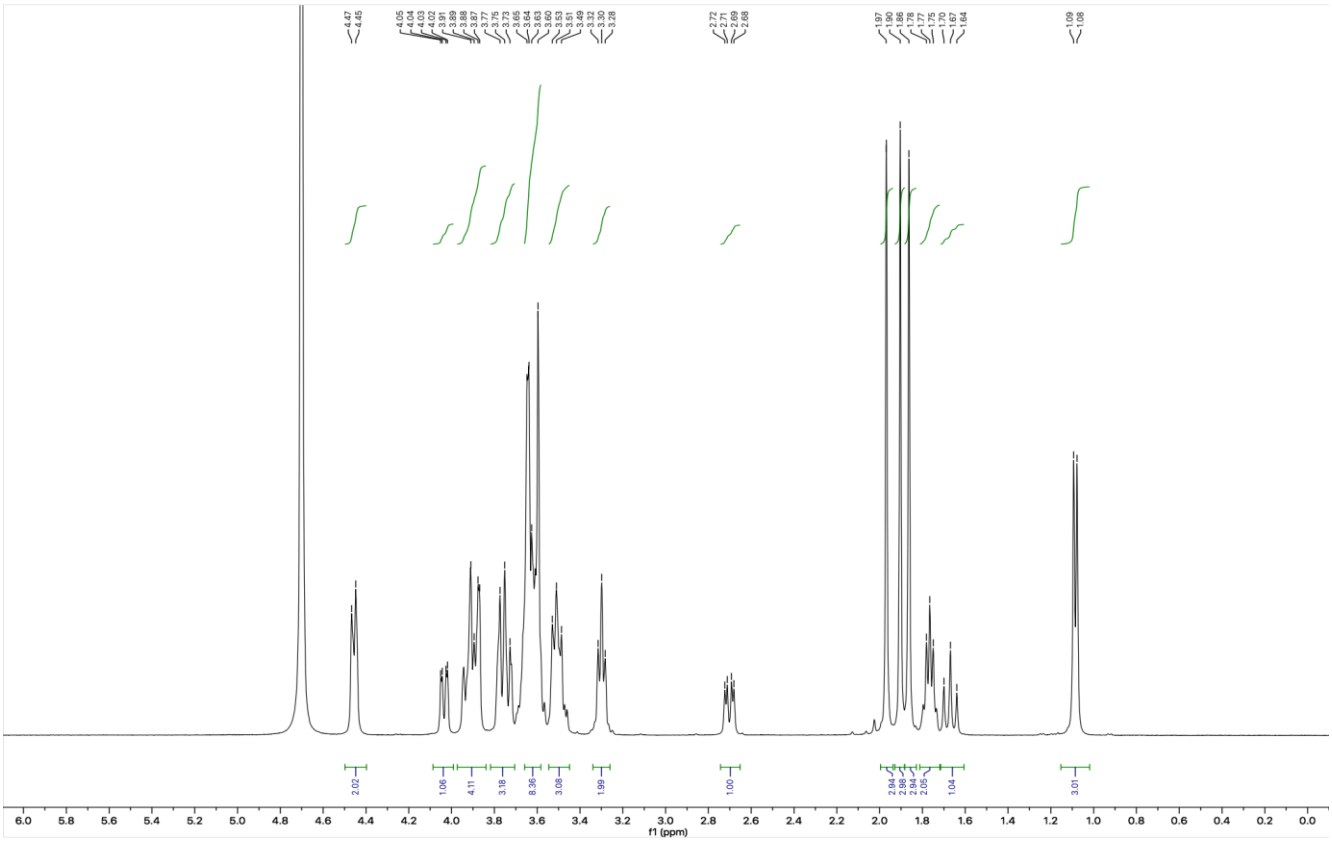
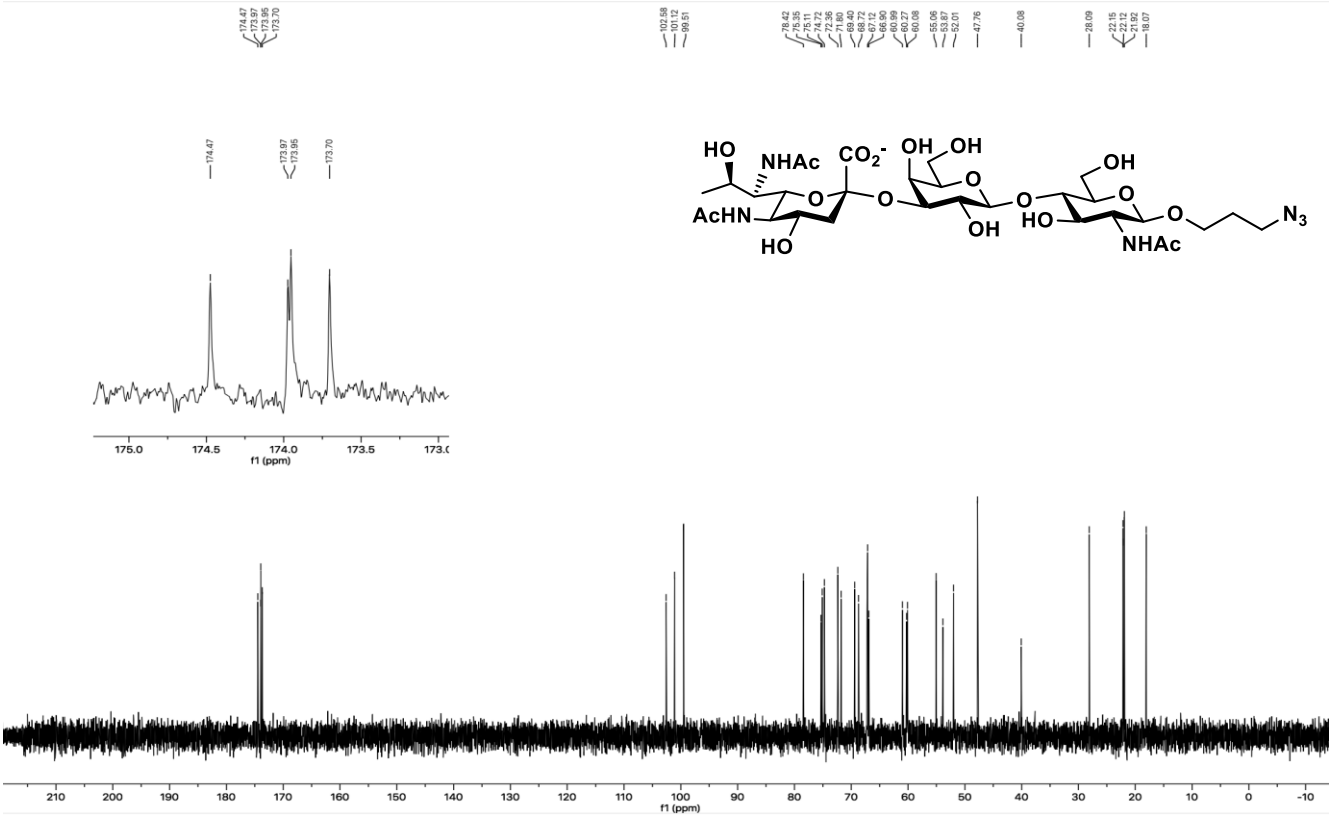
**Figure S4.** DNA sequence of LpCLS.

MRILAVIPARAGSKRLPGKNTRLLAGKPLIAHTIVAALQSSCCEEIVVSTDSKQIADVAVQYGASVPW
LRSEDLATDTSDVIHTVIDLLFKFQQMDVFFDSVLLLQPTSPFRKPETIRHAVEIHKVTGKSVVSVSP
ISLKPSWCRSIDSQGNLVKPELFQDLEIYCNENPIYKLNGSIYIATAKQIIENKSFYSEPTKPLLLNS
ISESIDIDTPIDWALTEKLMELNQEALVVDKLAAALEHHHHHH

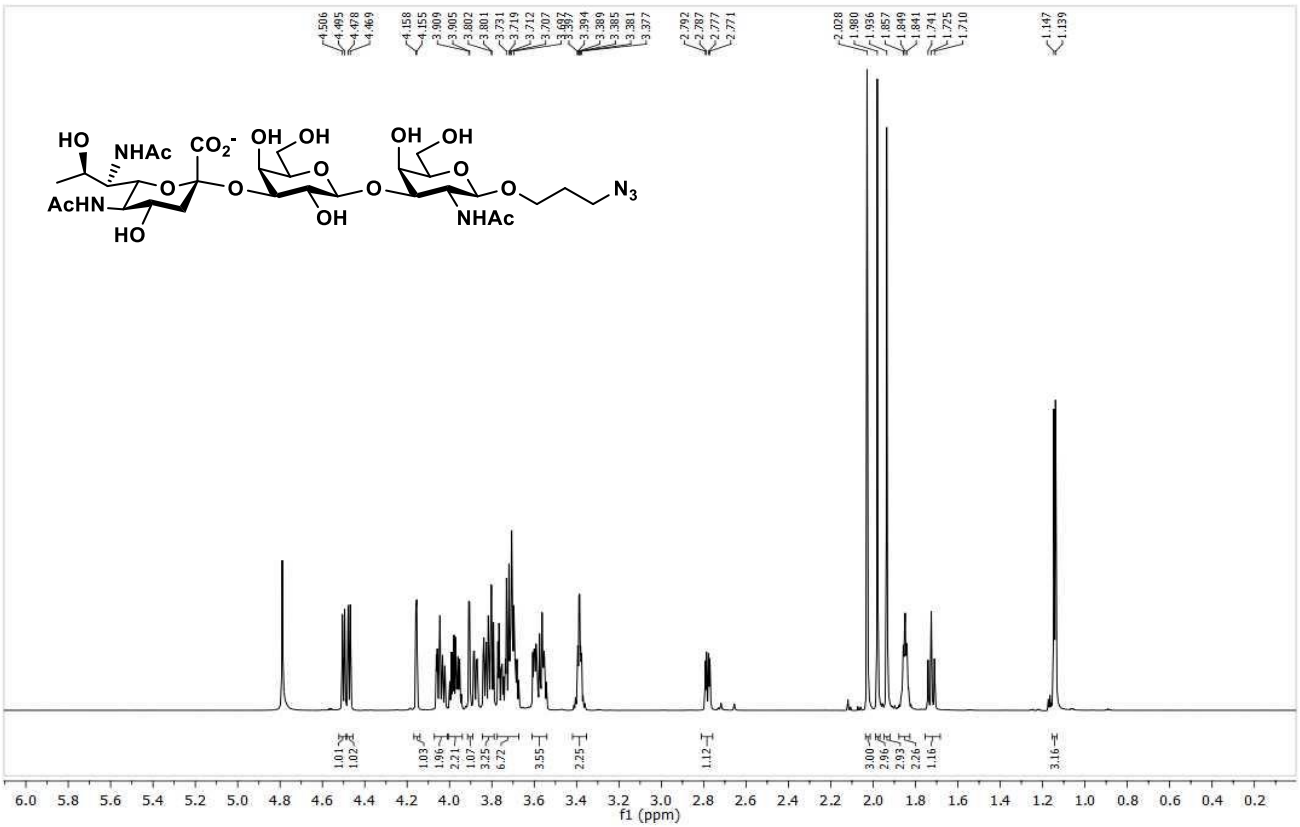
**Figure S5.** Protein sequence of LpCLS.

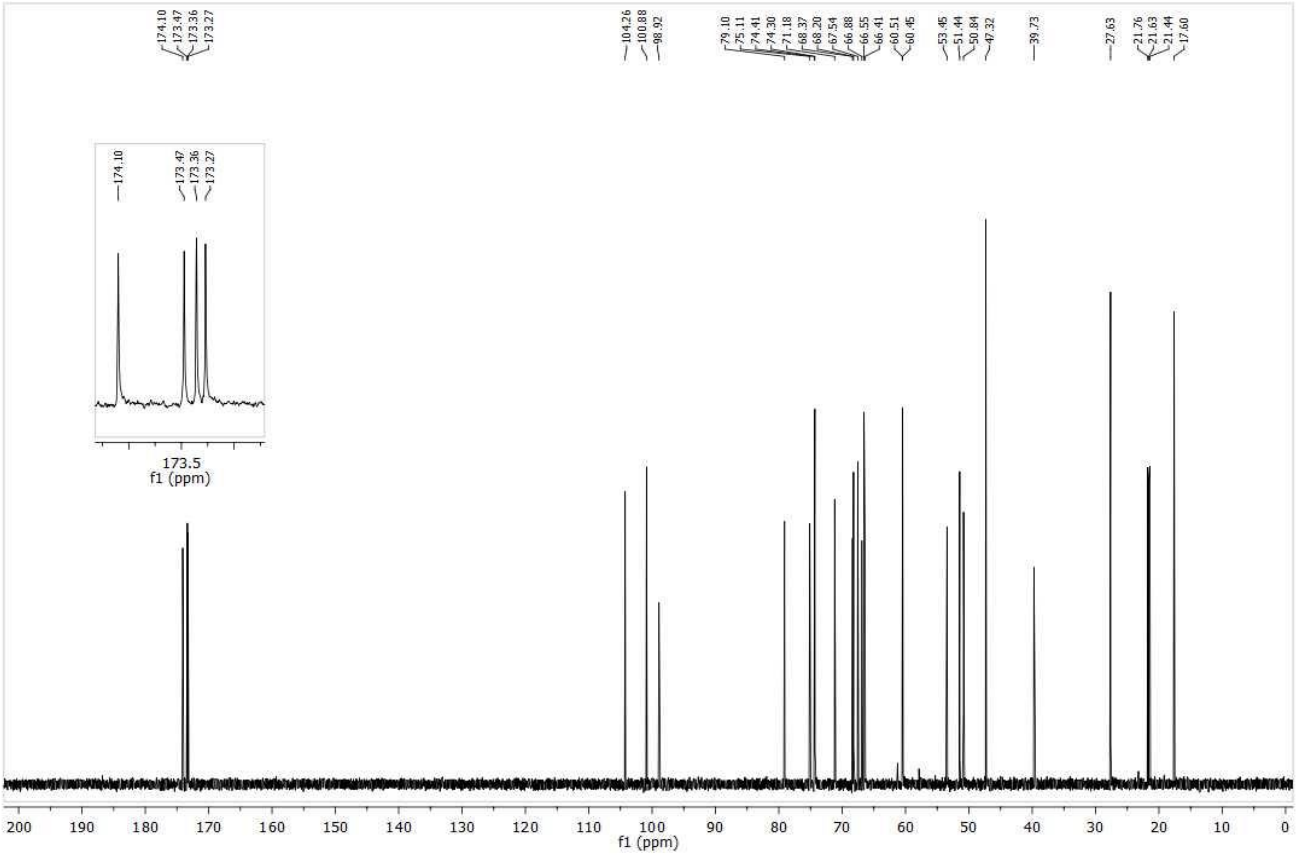
$^{1}H$ and $^{13}C$ NMR spectra of Leg5,7$Ac_2$α2–3LacNAcβPro$N_3$ (**8**)



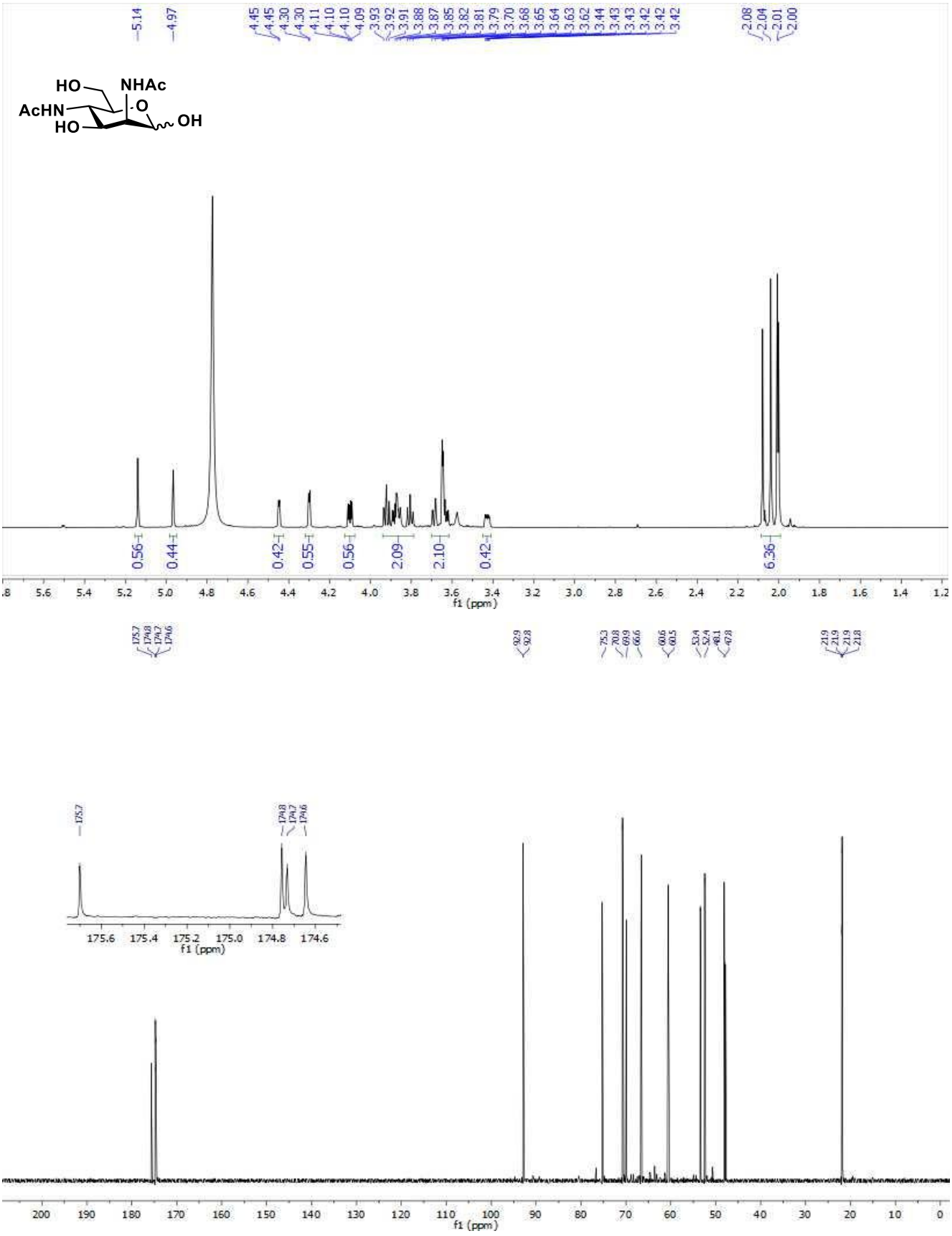


$^{1}H$ and $^{13}C$ NMR spectra of Leg5,7$Ac_2$α2–3Galβ1–3GalNAcβPro$N_3$ (**10**)

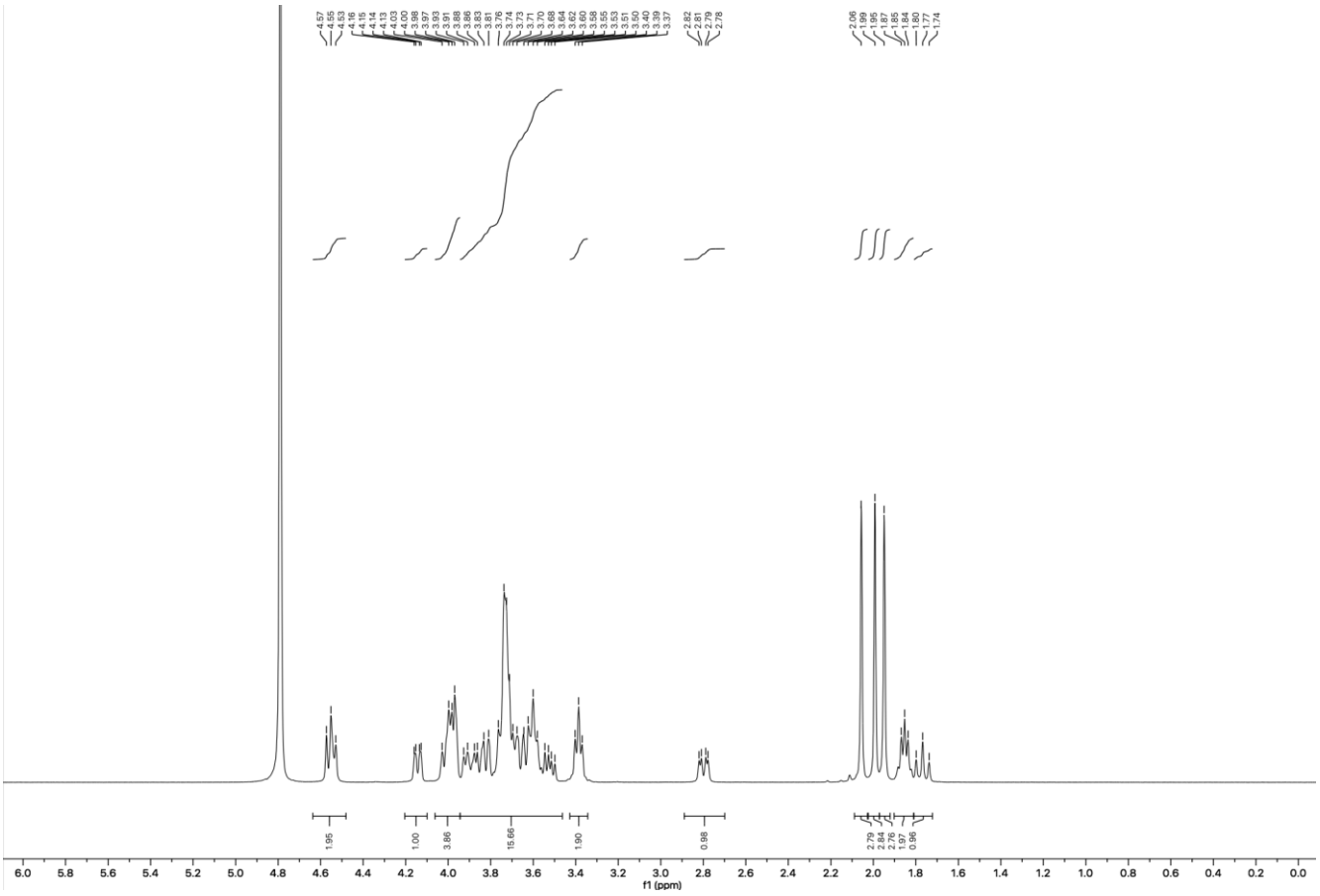


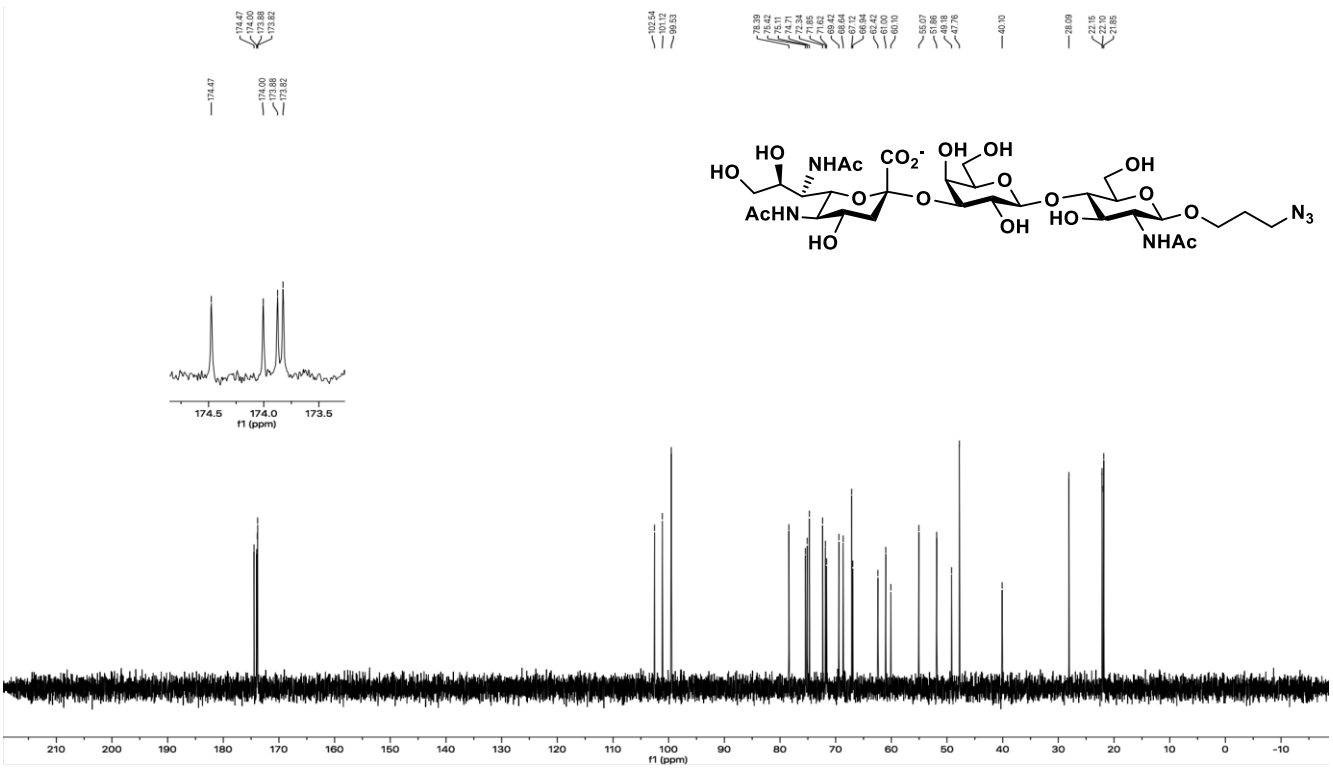



$^{1}H$ and $^{13}C$ NMR spectra of ManNAc4NAc (**5**)



$^{1}H$ and $^{13}C$ NMR spectra of Neu5Ac7NAcα2–3LacNAcβProN$_3$ (**14**)





$^{1}H$ and $^{13}C$ NMR spectra of Neu5Ac7NAcα2–3LacβProN$_3$ (**15**)