Supporting Information

Streptococcus pneumoniae Sialidase SpNanB-Catalyzed One-Pot Multienzyme (OPME) Synthesis of 2,7-Anhydro-Sialic acids as Selective Sialidase Inhibitors

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Table S1. Substrate specificity of SpNanB. Acceptable substrate is shown with "+" and nonacceptable substrate is shown with "−".

Substrate	Siaα2–3Galβ*p*NP	Siaα2–6Galβ*p*NP
Neu5Ac–R	+	−
Neu5AcF–R	+	−
Neu5AcOMe–R	+	−
Neu5AcN_3–R	+	−
Neu5Ac9F–R	+	−
Neu5Ac9OMe–R	+	−
Neu5Ac9deoxy–R	+	−
Neu5Ac9N_3–R	+	−
Neu5Ac9NAc–R	+	−
Neu5Ac7F–R	+	−
Neu5Ac7OMe–R	+	−
Neu5Ac7deoxy–R	+	−
Neu5Ac7N_3–R	+	−
Neu5Gc–R	+	−
Neu5Gc9F–R	+	−
Neu5Gc9OMe–R	+	−
Neu5Gc9deoxy–R	+	−
Neu5Gc9N_3–R	+	−
Kdn–R	−	−
Kdn5F–R	−	−
Kdn5N_3–R	−	−
Kdn5deoxy–R	−	−
Kdn5OMe–R	−	−
Kdn9F–R	−	−
Kdn9OMe–R	−	−
Kdn9deoxy–R	−	−
Kdn9N_3–R	−	−
Kdn7F–R	−	−
Kdn7OMe–R	−	−
Kdn7deoxy–R	−	−
Kdn7N_3–R	−	−

Figure S1. Substrate specificity study of SpNanB using Siaα2–3Galβ*p*NP (white columns) or Siaα2–6Galβ*p*NP (black columns) as substrates.

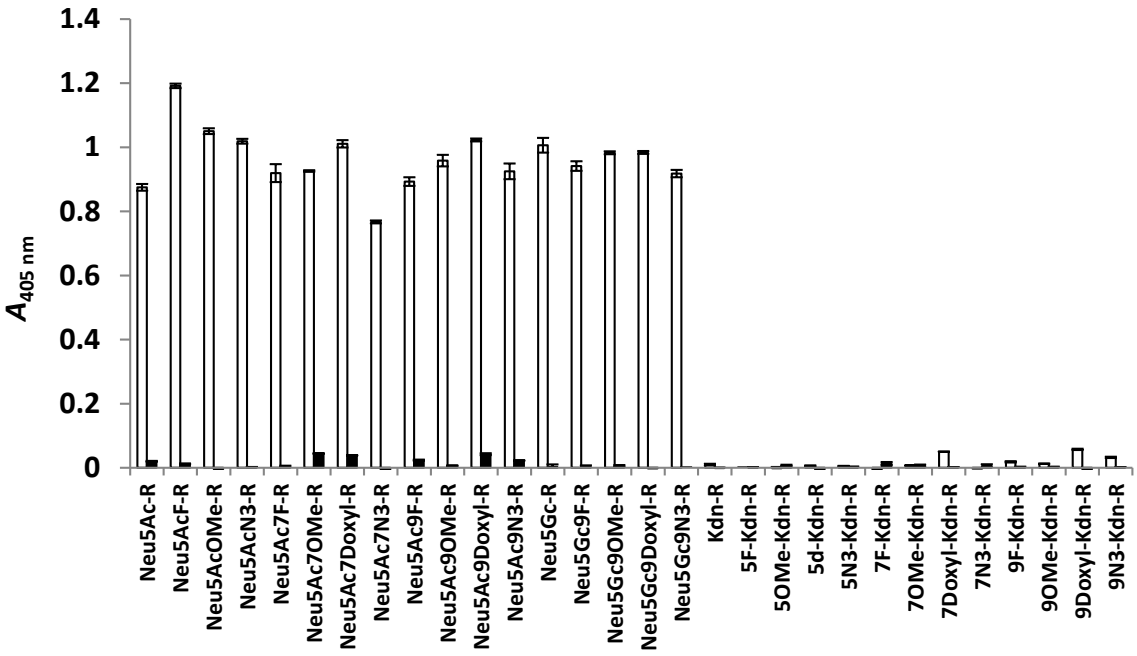


Figure S2. Mass spectrometry result of the reaction mixture of Neu5Ac7N_3α2–3Galβ*p*NP treated with SpNanB.

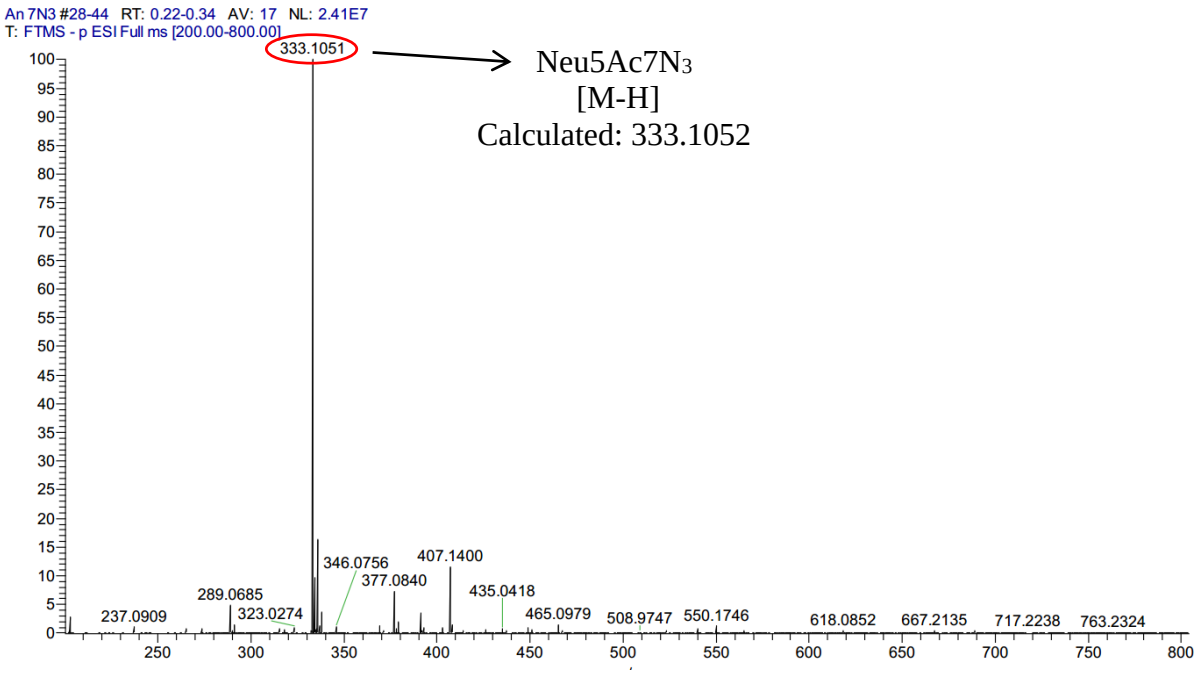
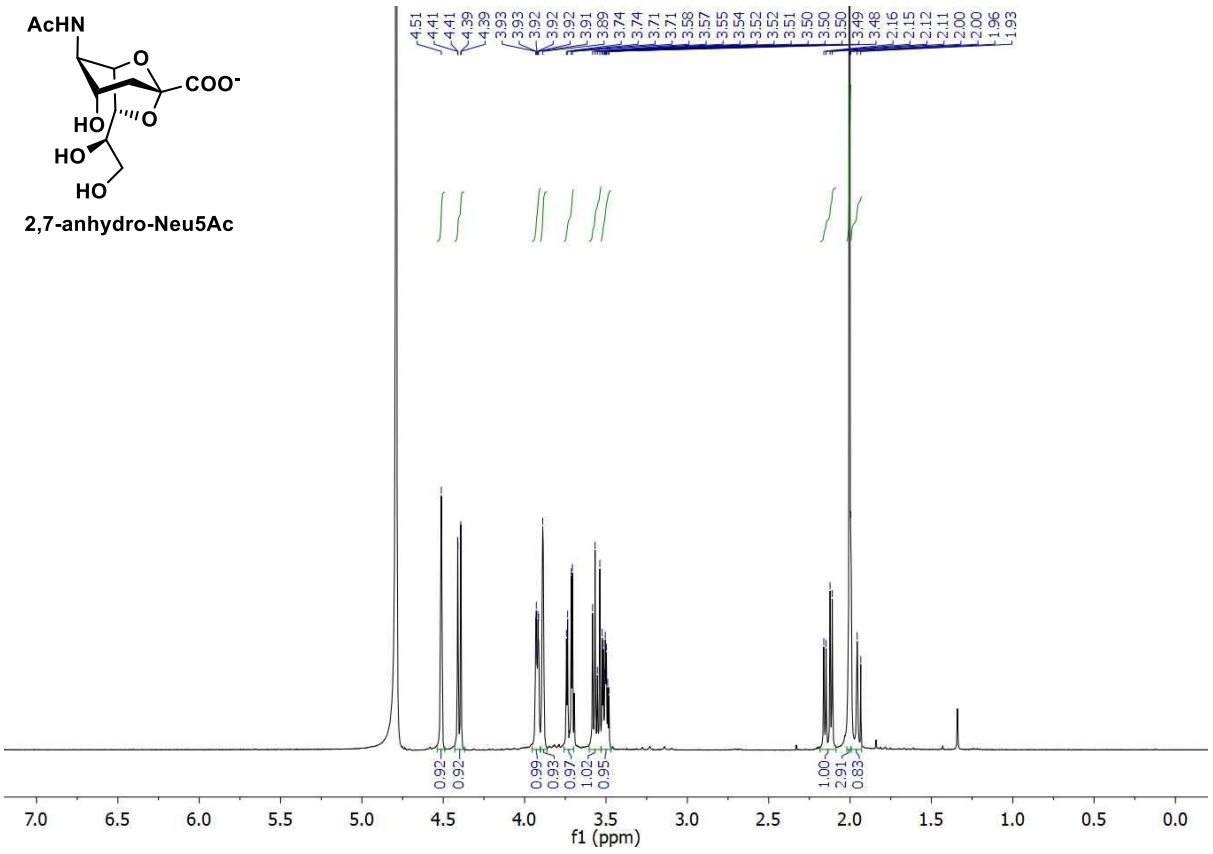
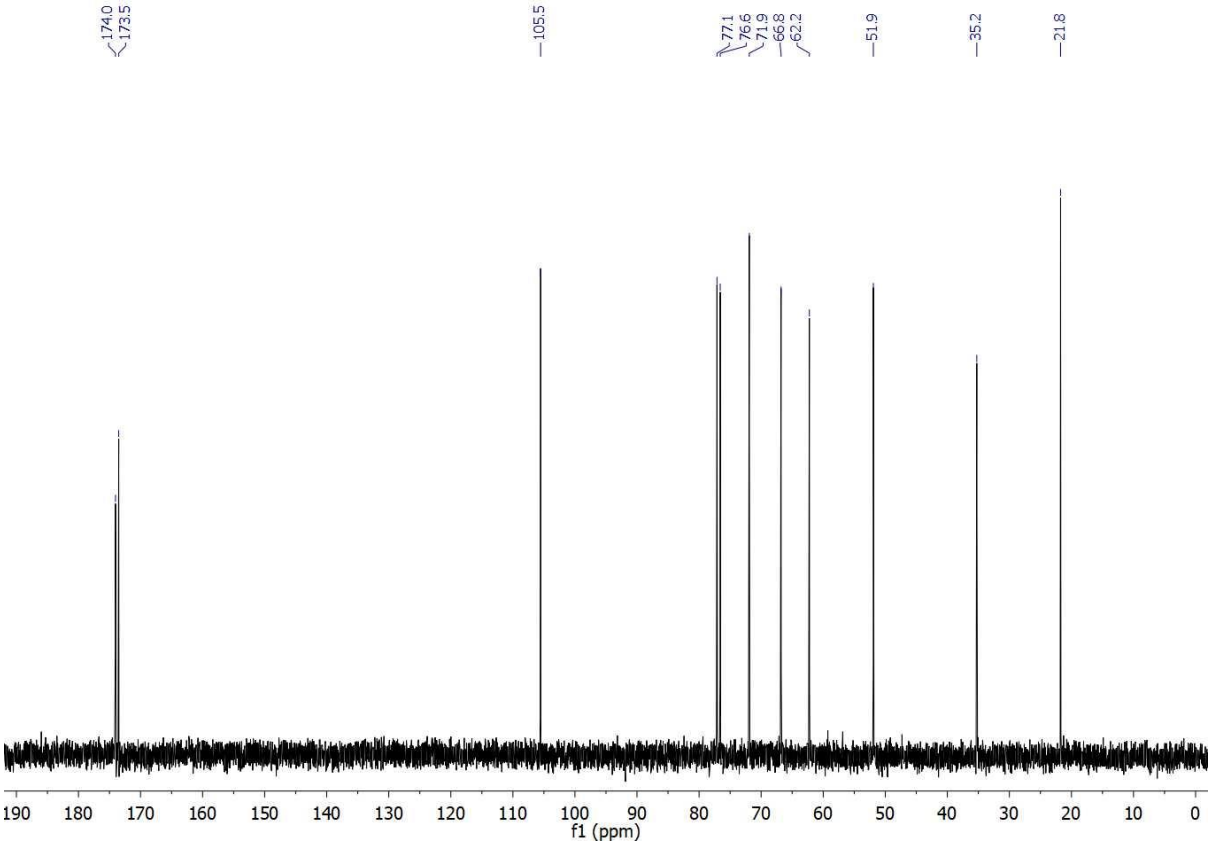


Table S2. Percentage inhibition (%) of 2,7-anhydro-sialic acids (**1**–**4**) (1 mM of each inhibitor was used) against bacterial and human sialidases. Neu5Ac2en was used as a control. Those with more than 50% inhibition are highlighted in bold.

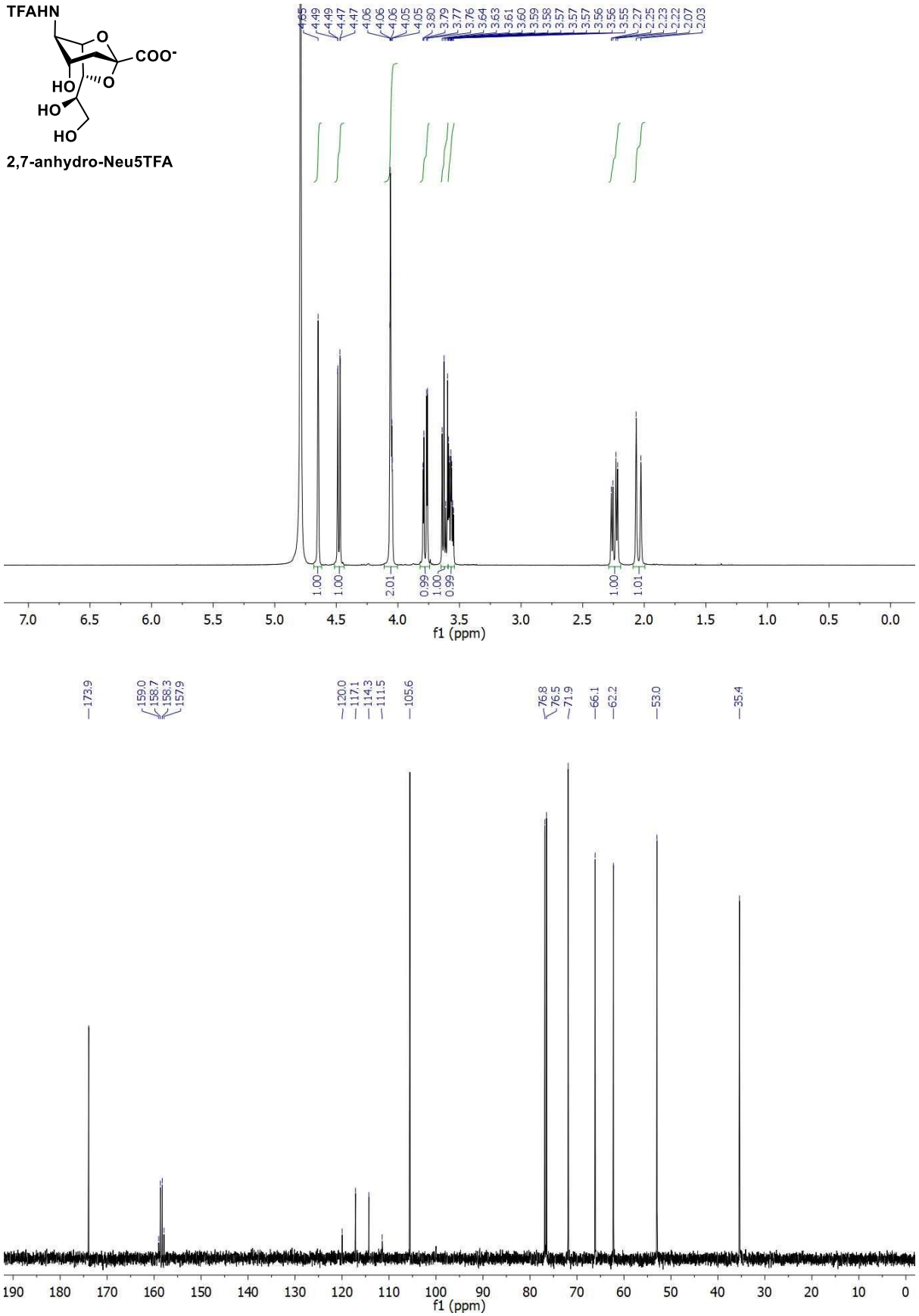
Sialidases	**Inhibitors**				
	Neu5Ac2en	**(1)**	**(2)**	**(3)**	**(4)**
SpNanA	**98.9** ± 1.8	36.4 ± 0.1	**83.8** ± 0.3	19.3 ± 4.2	**61.2** ± 0.6
SpNanB	26.1 ± 1.0	30.0 ± 1.7	**68.2** ± 2.6	11.7 ± 2.6	**86.6** ± 0.3
SpNanC	9.0 ± 3.5	3.0 ± 1.9	4.4 ± 2.0	14.9 ± 3.0	**94.2** ± 0.1
AuSialidase	**99.6** ± 0.6	49.4 ± 1.3	**85.1** ± 0.3	1.3 ± 2.2	16.8 ± 2.4
CpNanI	**93.3** ± 3.8	26.7 ± 3.7	44.1 ± 1.0	10.1 ± 5.7	13.6 ± 0.1
VcSialidase	**98.8** ± 0.1	22.7 ± 1.2	**62.1** ± 0.6	1.3 ± 2.0	1.2 ± 0.7
PmST1	15.8 ± 0.1	10.2 ± 2.8	11.0 ± 2.7	13.8 ± 3.3	12.3 ± 1.9
BiNanH2	**95.3** ± 0.2	6.6 ± 1.8	3.8 ± 2.6	3.2 ± 3.2	1.2 ± 0.9
hNEU2	**99.8** ± 0.2	4.6 ± 1.1	2.5 ± 2.1	1.4 ± 2.4	3.6 ± 0.1

^{1}H and ^{13}C NMR spectra of 2,7-anhydro-Neu5Ac (**1**)

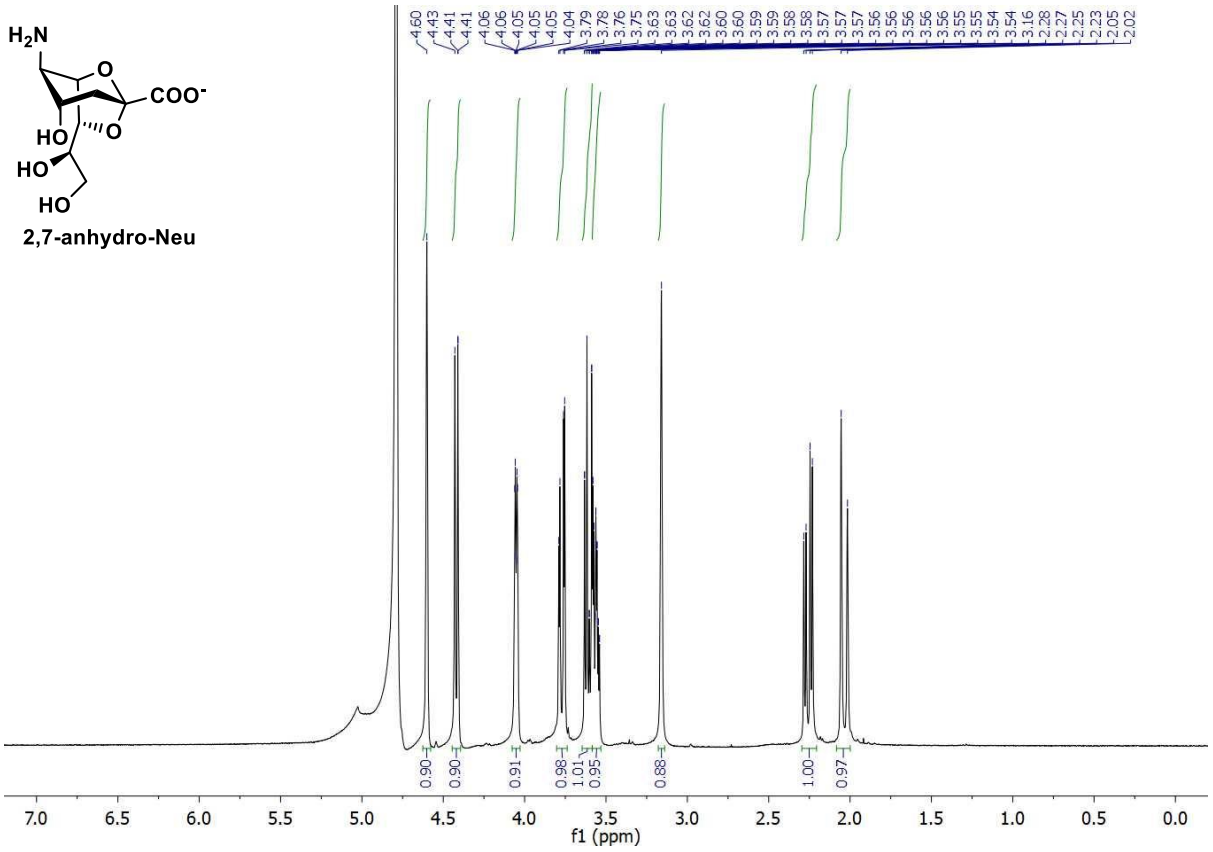


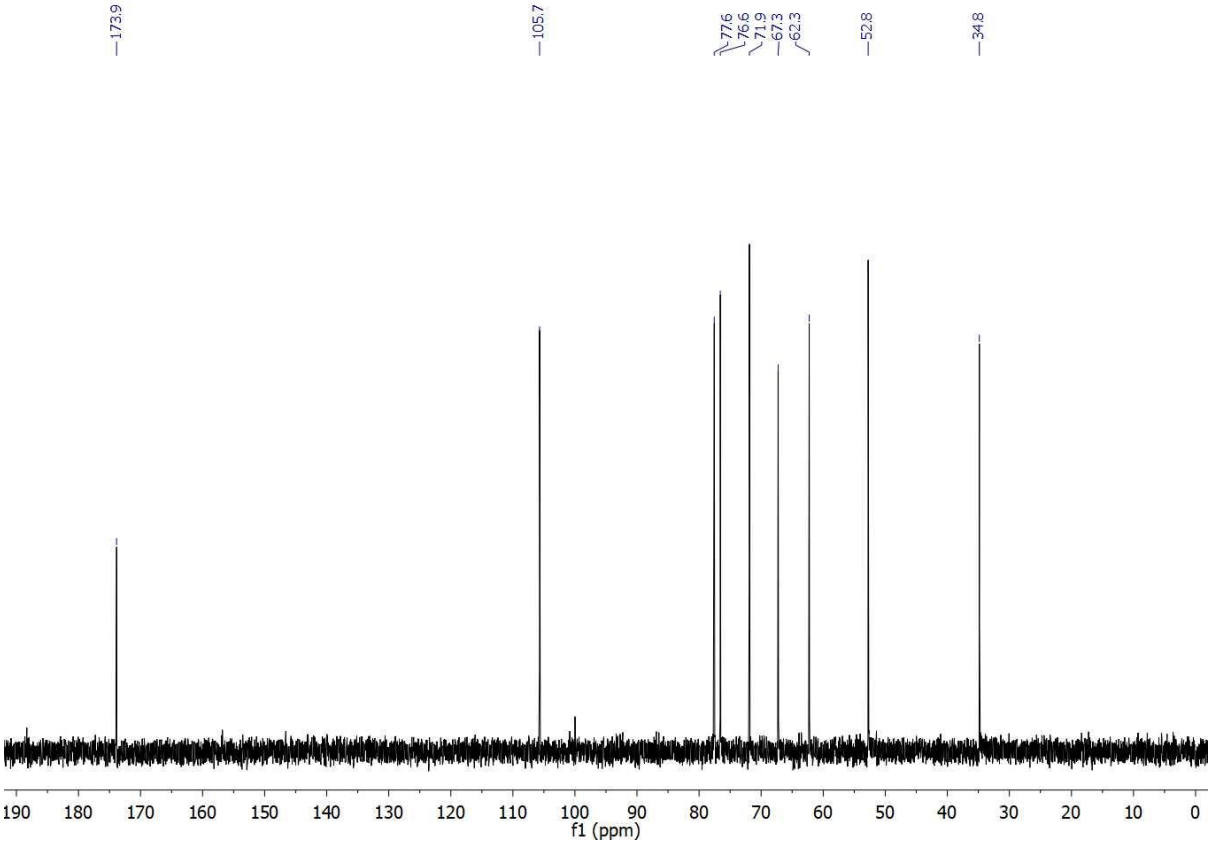


^{1}H and ^{13}C NMR spectra of 2,7-anhydro-Neu5TFA (**2**)



^{1}H and ^{13}C NMR spectra of 2,7-anhydro-Neu (**3**)





^{1}H and ^{13}C NMR spectra of 2,7-anhydro-Neu5Cyclohexyl (**4**)