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## Supporting Information

### **Dienophile-Modified Mannosamine Derivatives for Metabolic Labeling of Sialic Acids: A Comparative Study**

Jeremias E. G. A. Dold, Jessica Pfozter, Anne-Katrin Späte, and Valentin Wittmann<sup>\*[a]</sup>

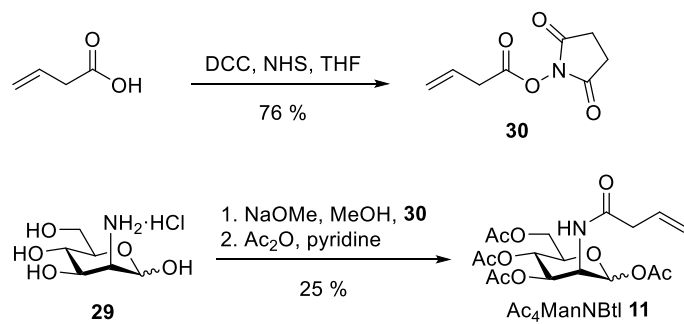
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# Supporting Information

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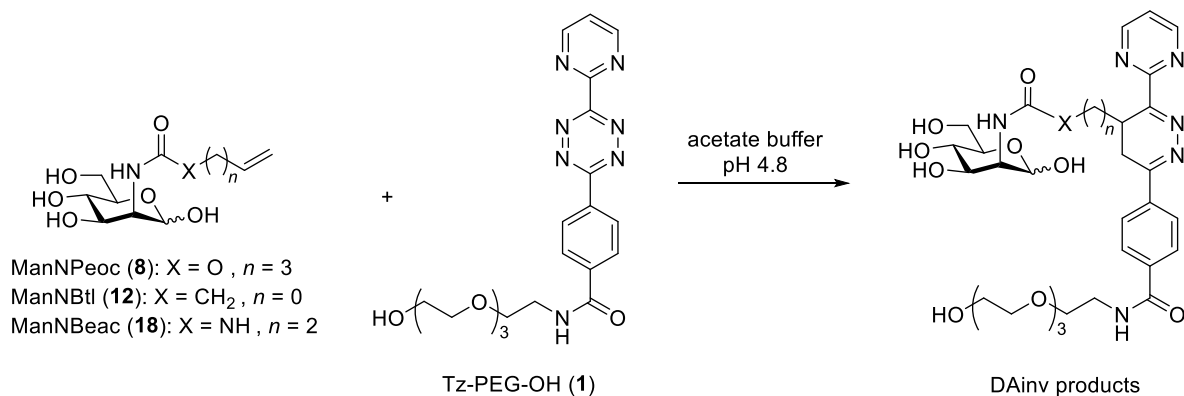
## Synthesis of Ac<sub>4</sub>ManNBtl (**11**)



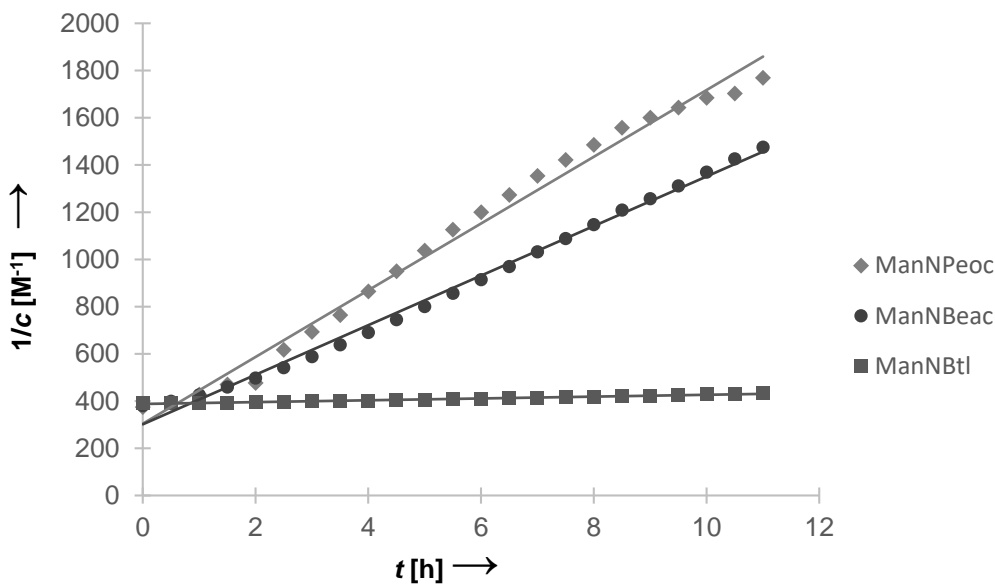
**Scheme S1.** Synthesis of Ac<sub>4</sub>ManNBtl (**11**).

## Determination of Second-Order Rate Constants

A)

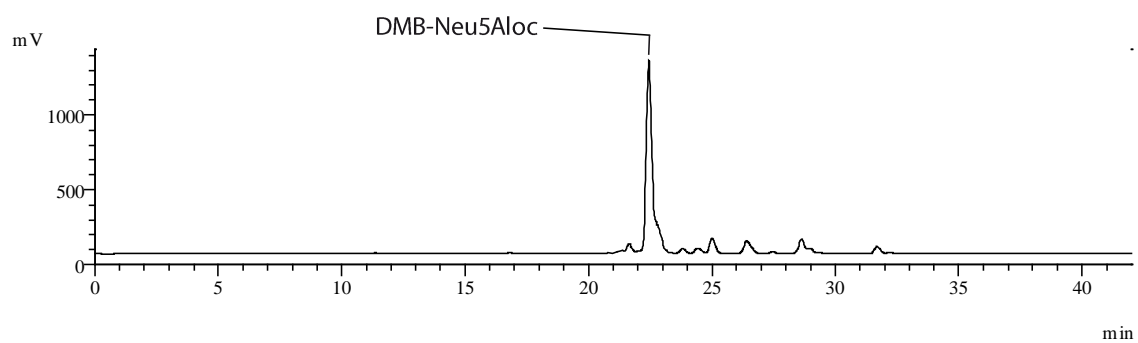
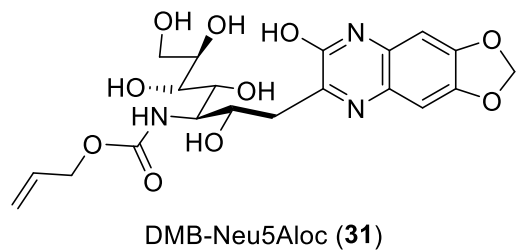


B)

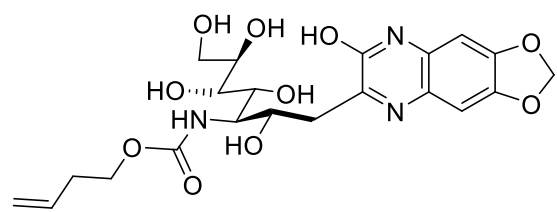


**Figure S1.** A) Reaction of ManNPeoc (**8**), ManNBtl (**12**), or ManNBeac (**18**) with Tz-PEG-OH (**1**) to determine the second-order rate constant of the DAinv reaction. The reaction was followed by observation of the absorbance of the tetrazine at  $\lambda = 522$  nm. B) Plot of the inverse concentration  $c$  of the tetrazine against the reaction time  $t$ . The slope of the lines (with  $t$  in seconds) obtained by linear regression equals the second-order rate constant  $k_2$ .

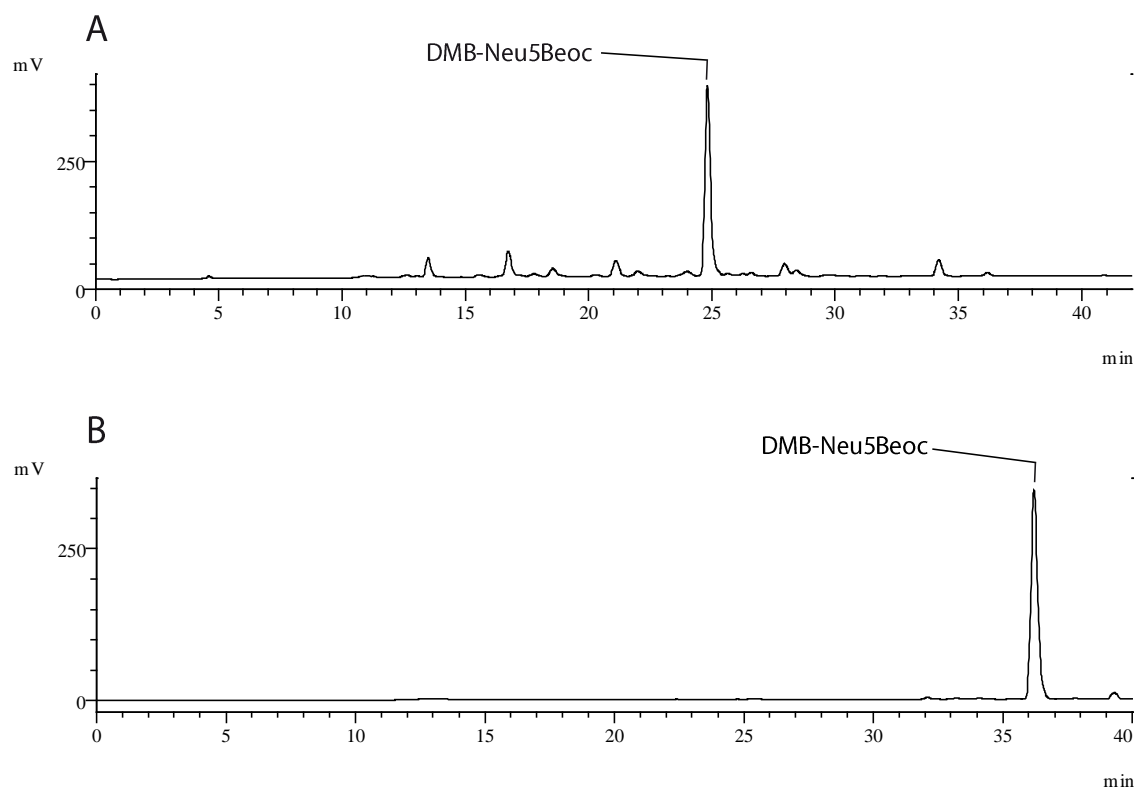
## Characterization of DMB-Labeled Modified Sialic Acids 31–38 by RP-HPLC



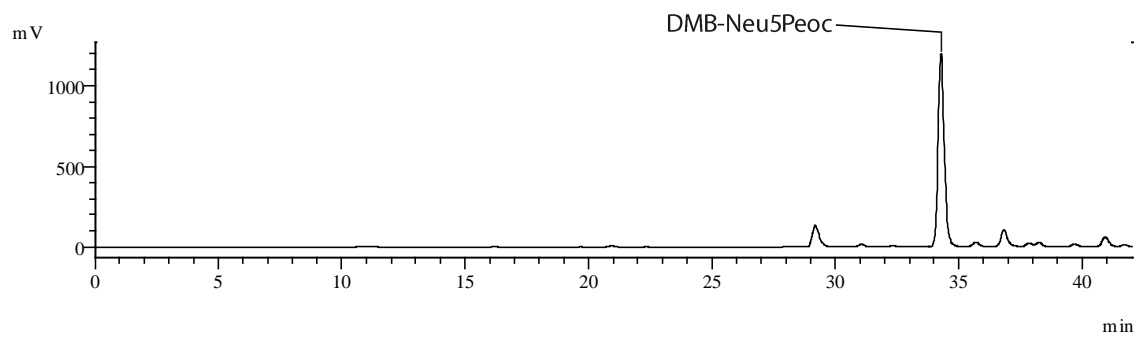
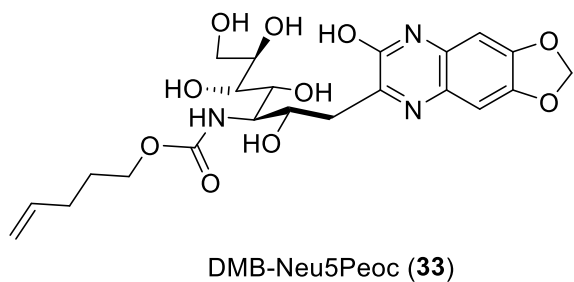
**Figure S2.** Analysis of DMB-Neu5Aloc (**31**) by RP-HPLC (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm).  $R_t = 22.5$  min.



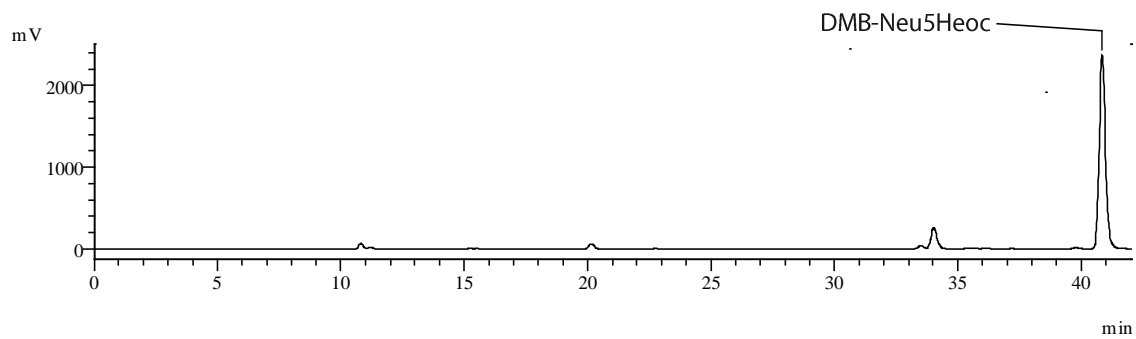
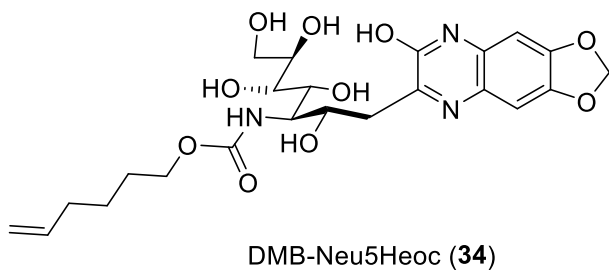
DMB-Neu5Beoc (**32**)



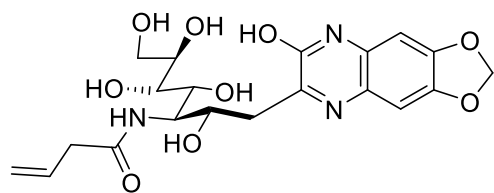
**Figure S3.** Analysis of DMB-Neu5Beoc (**32**) by RP-HPLC with a fluorescence detector (excitation 372 nm, emission 456 nm). A) 10 % – 30 % B in 40 min:  $R_t = 24.9$  min, B) 10 % – 25 % B in 40 min:  $R_t = 36.3$  min.



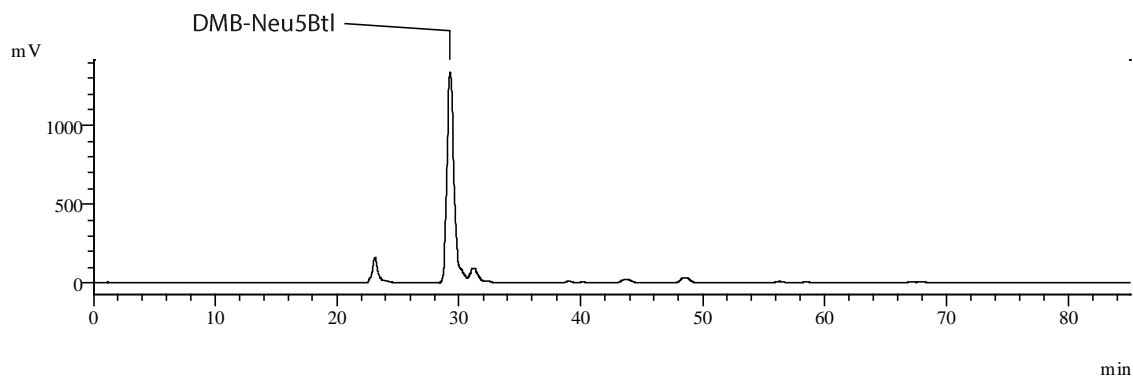
**Figure S4.** Analysis of DMB-Neu5Peoc (**33**) by RP-HPLC (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm).  $R_t = 34.5$  min.



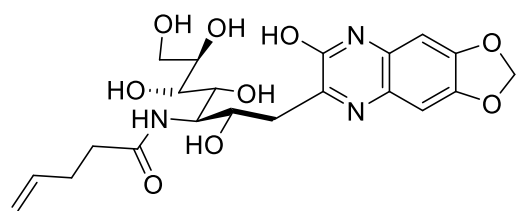
**Figure S5.** Analysis of DMB-Neu5Heoc (**34**) by RP-HPLC (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm).  $R_t = 41.0$  min.



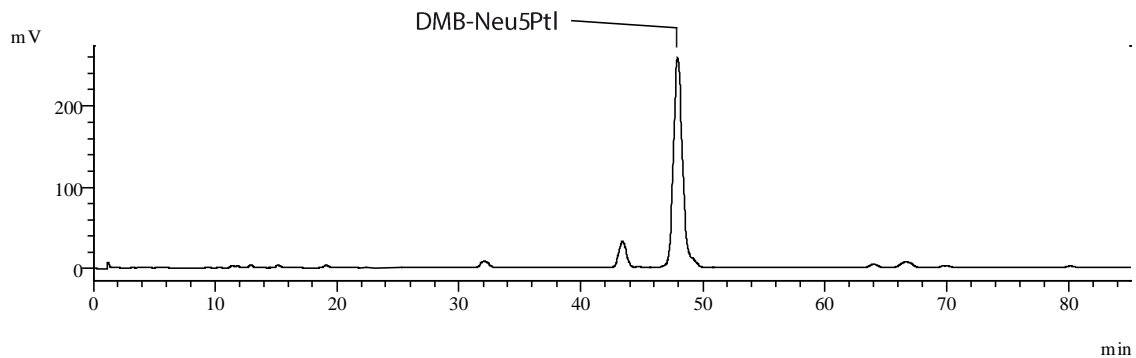
DMB-Neu5Btl (**35**)



**Figure S6.** Analysis of DMB-Neu5Btl (**35**) by RP-HPLC (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm).  $R_t = 29.3$  min.

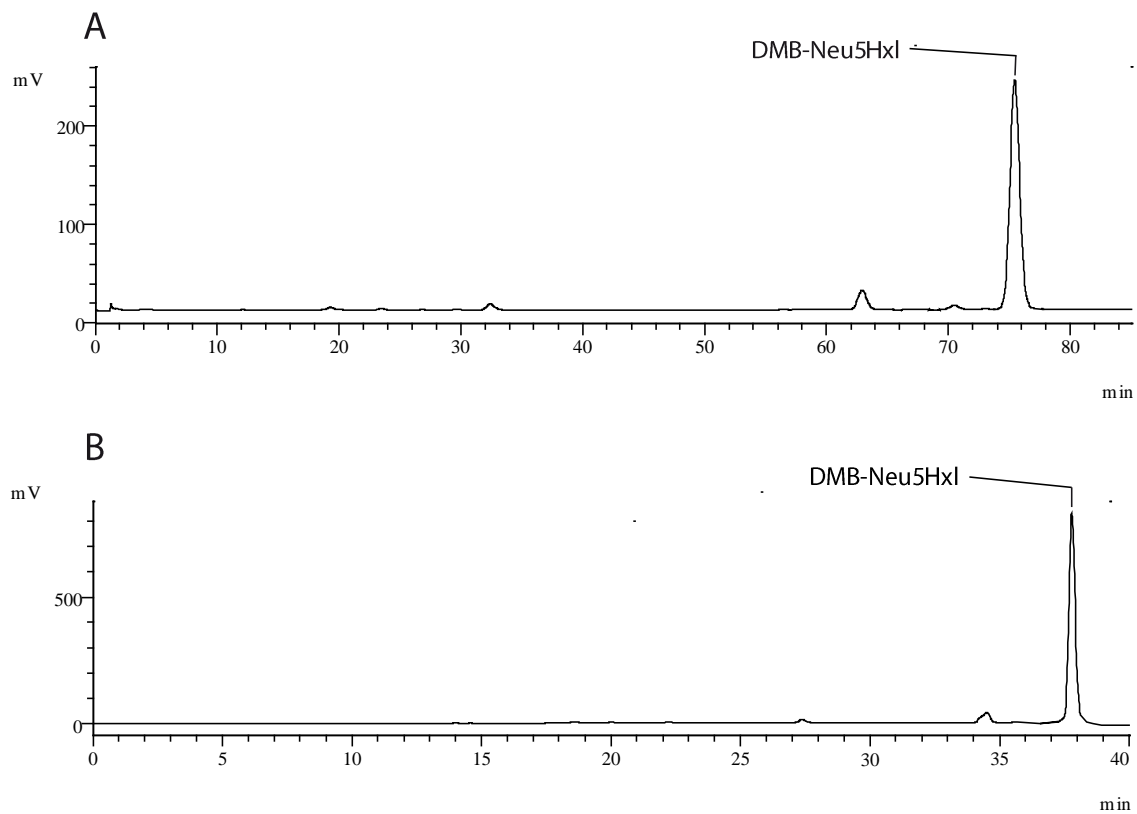
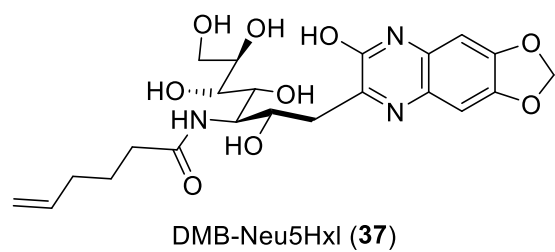


DMB-Neu5Ptl (**36**)

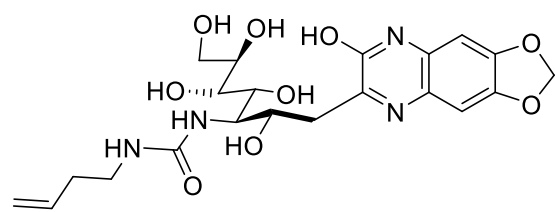


**Figure S7.** Analysis of DMB-Neu5Ptl (**36**) by RP-HPLC (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm).  $R_t = 48.1$  min.

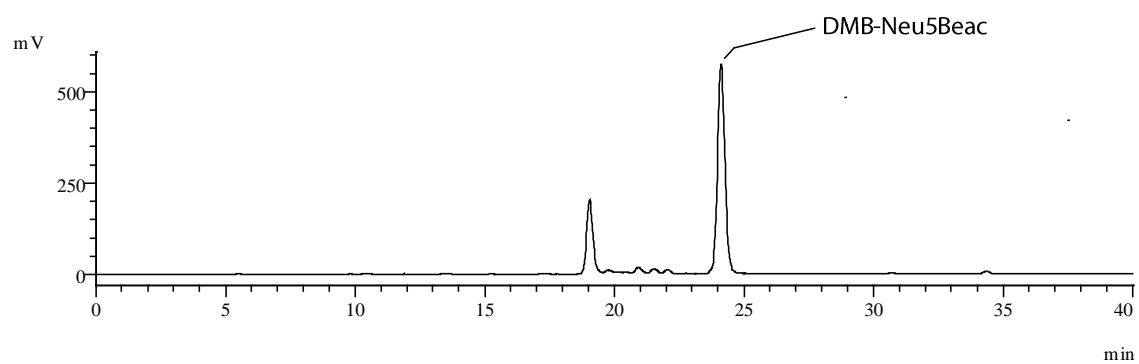




**Figure S8.** Analysis of DMB-Neu5Hxl (**37**) by RP-HPLC with a fluorescence detector (excitation 372 nm, emission 456 nm). A) 10 % – 18 % B in 85 min:  $R_t = 75.5$  min, B) 10 % – 25 % B in 40 min:  $R_t = 37.9$  min.

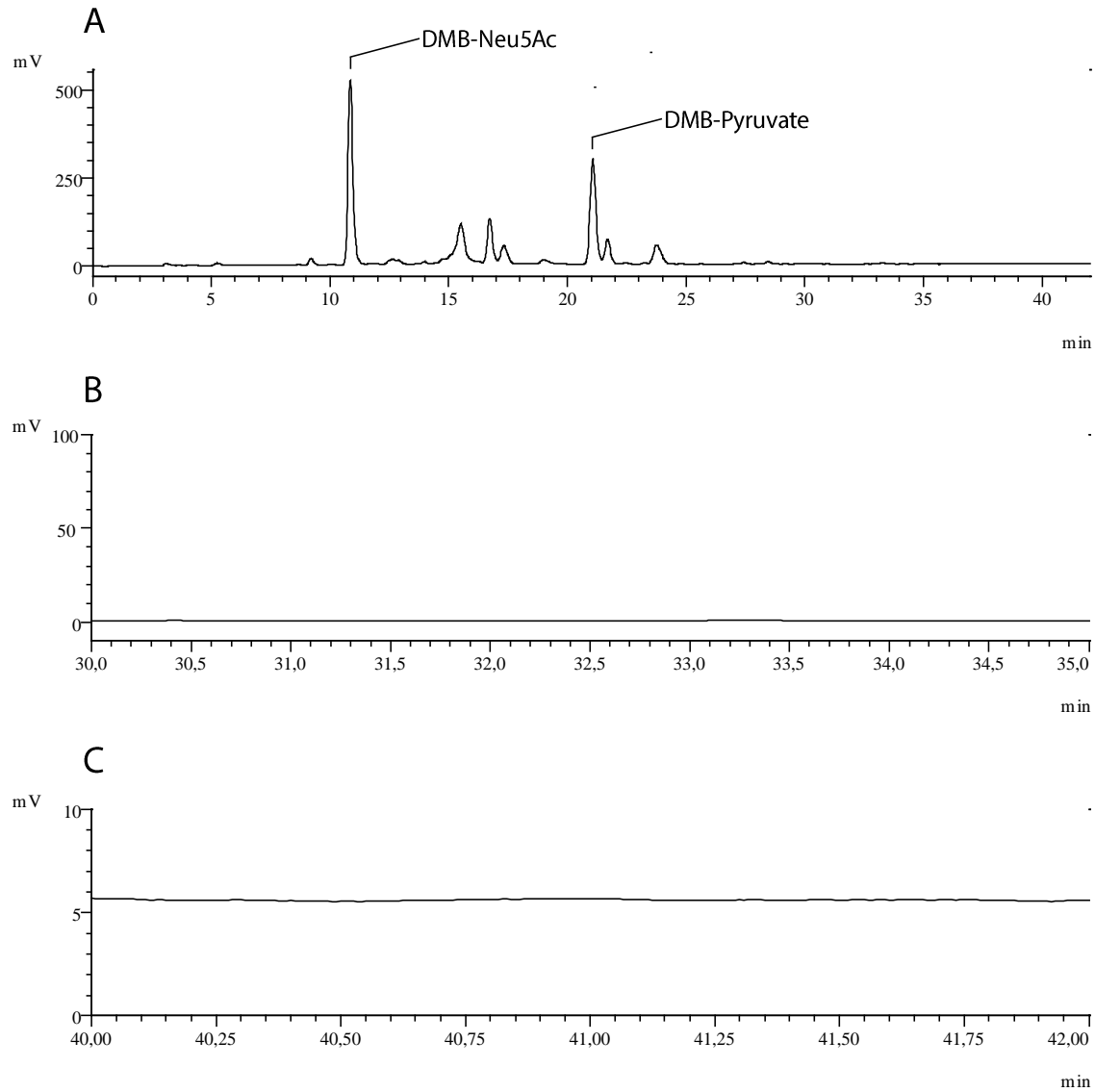


DMB-Neu5Beac (**38**)

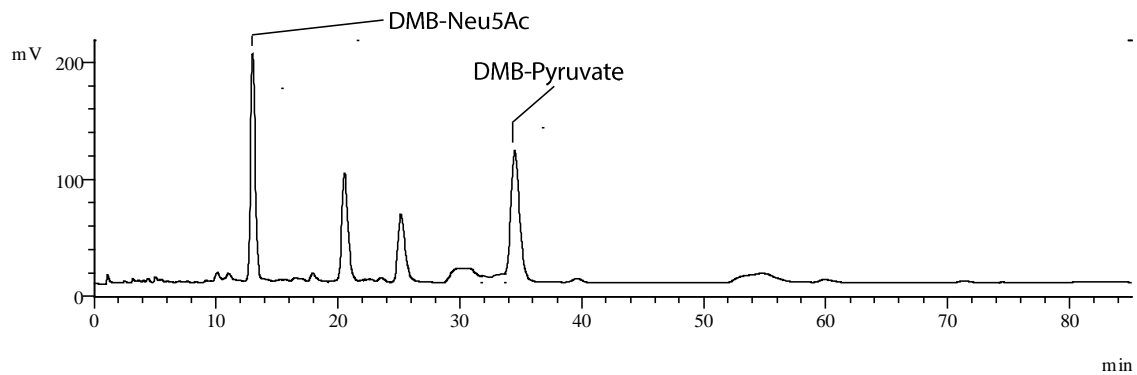


**Figure S9.** Analysis of DMB-Neu5Beac (**38**) by RP-HPLC (10 % – 25 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm).  $R_t = 24.1$  min.

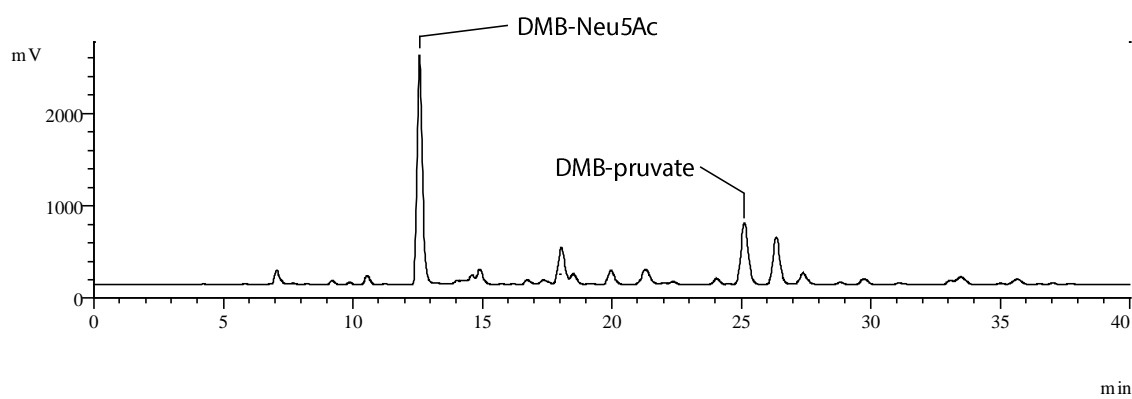
## Analysis of DMB-Labeled Sialic Acids Released from Cells



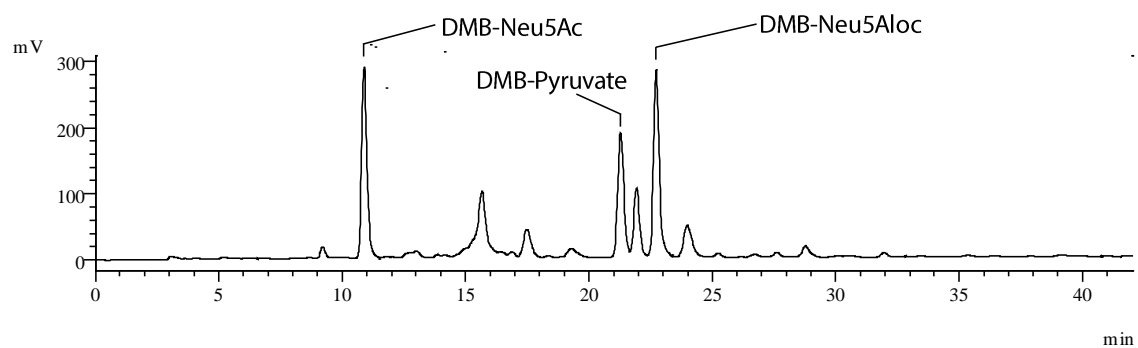
**Figure S10.** RP-HPLC analysis (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown without added sugar (solvent control). A) Complete chromatogram, B) and C) enlarged regions.



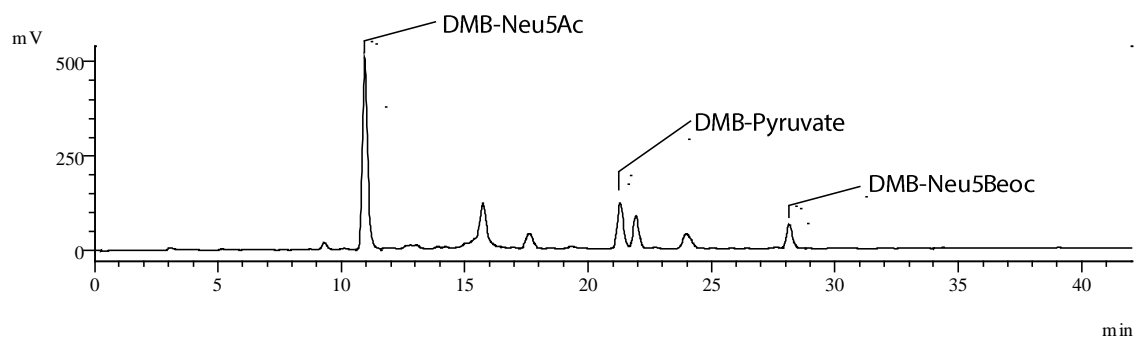
**Figure S11.** RP-HPLC analysis (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown without added sugar (solvent control).



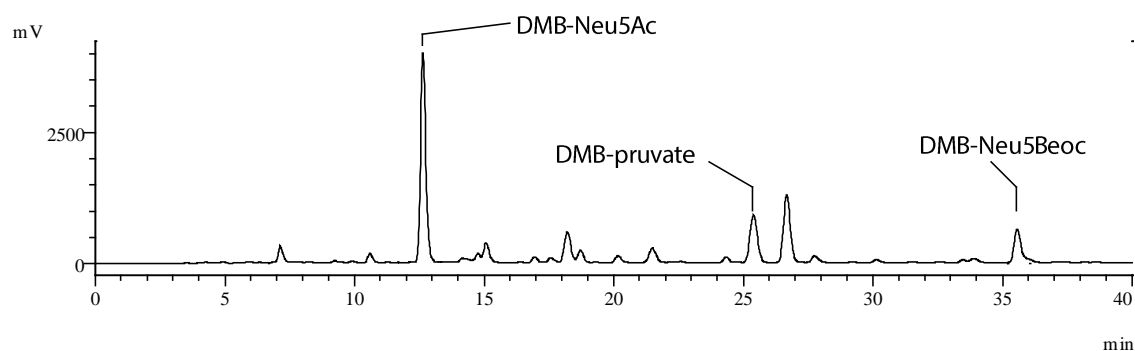
**Figure S12.** RP-HPLC analysis (10 % – 25 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown without added sugar (solvent control).



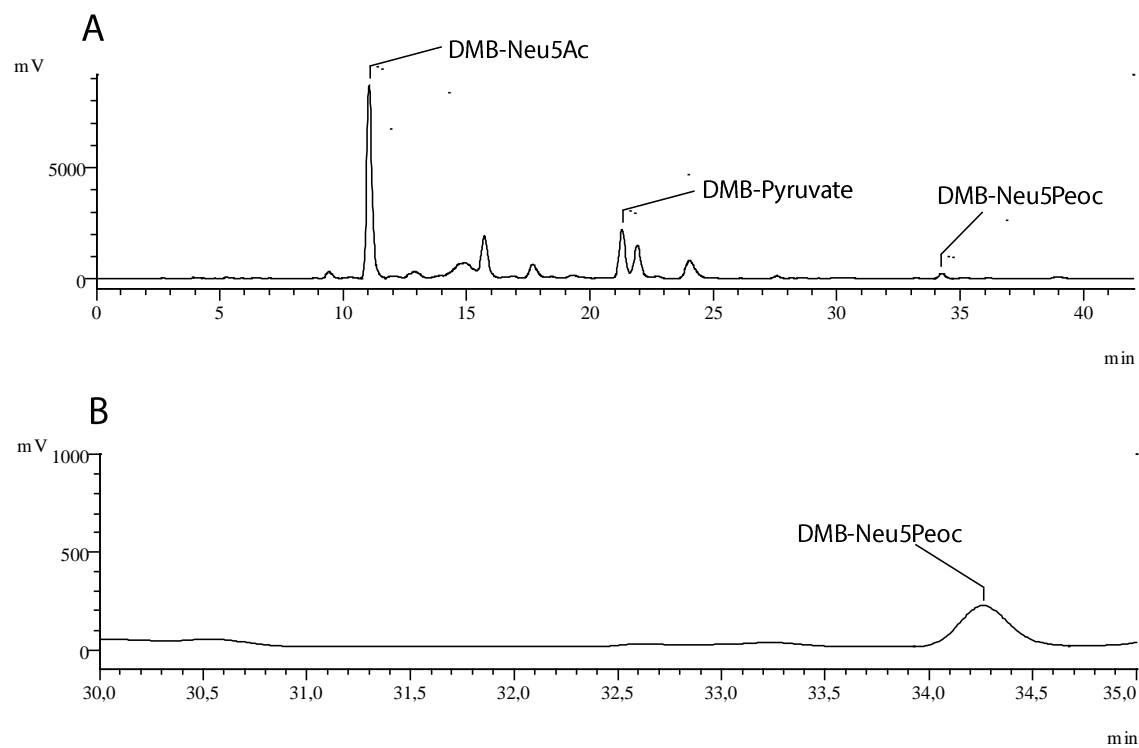
**Figure S13.** RP-HPLC analysis (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac<sub>4</sub>ManNAloc (**3**).



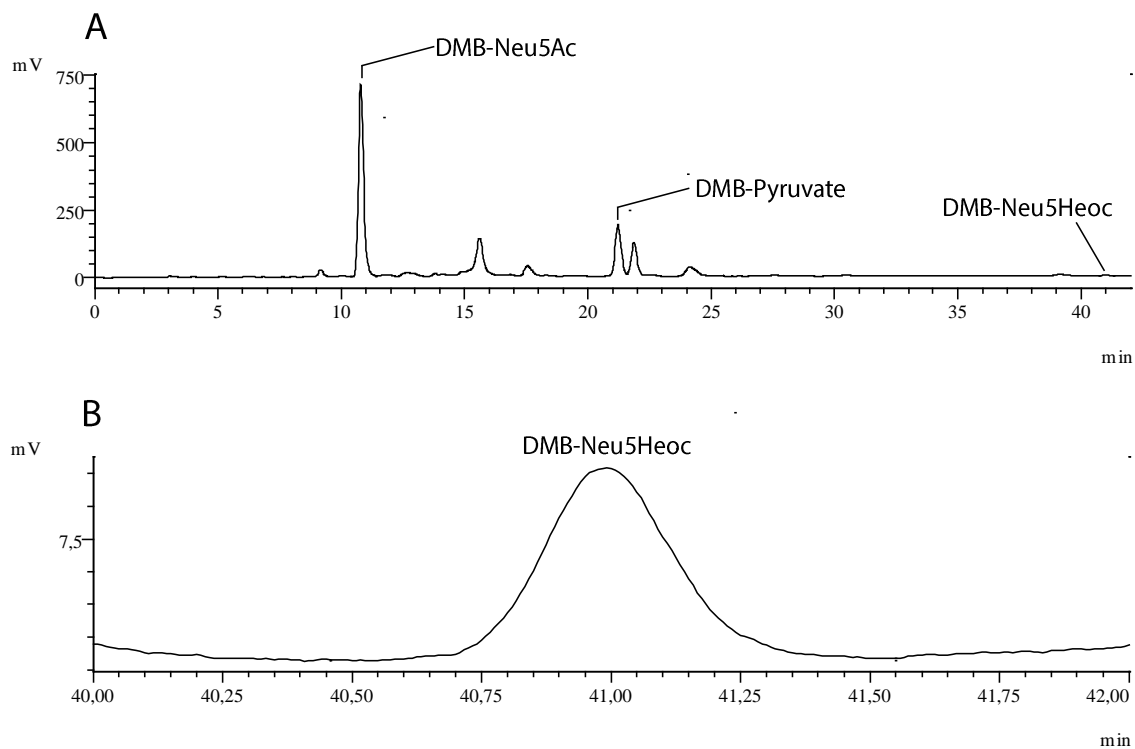
**Figure S14.** RP-HPLC analysis (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac<sub>4</sub>ManNBeoc (**5**).



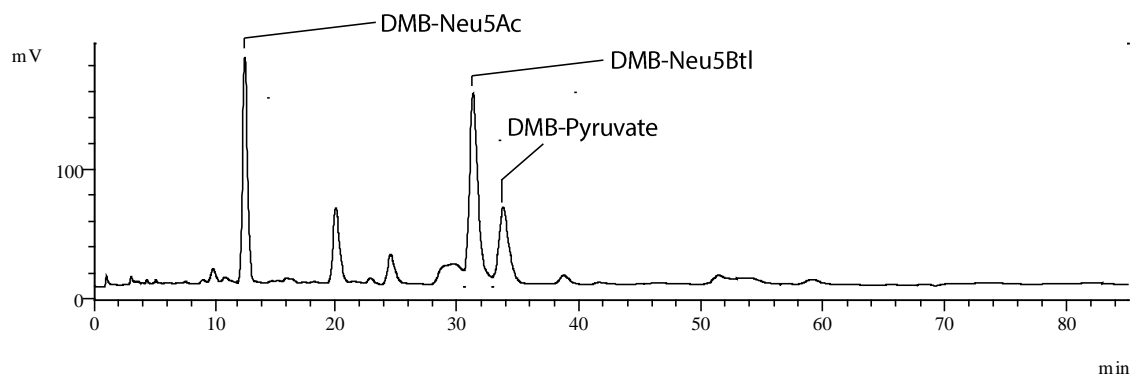
**Figure S15.** RP-HPLC analysis (10 % – 25 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac<sub>4</sub>ManNBeoc (**5**).



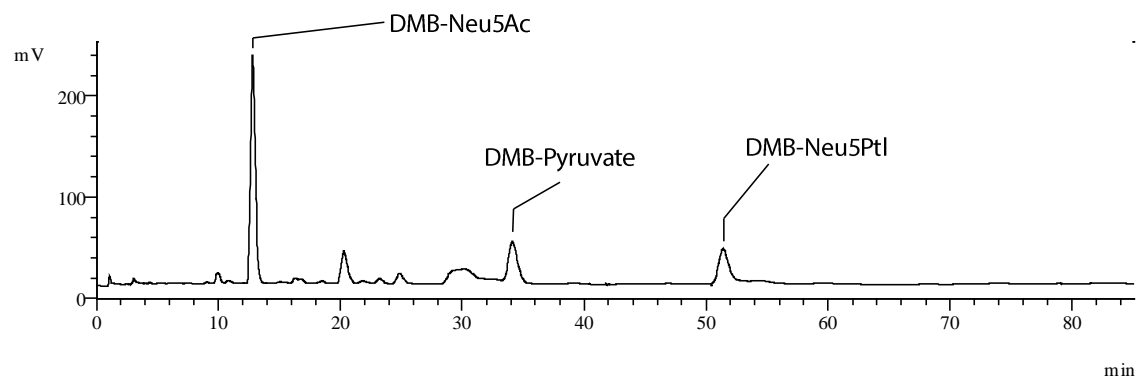
**Figure S16.** RP-HPLC analysis (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac<sub>4</sub>ManNPeoc (**7**). A) Complete chromatogram, B) enlarged region.



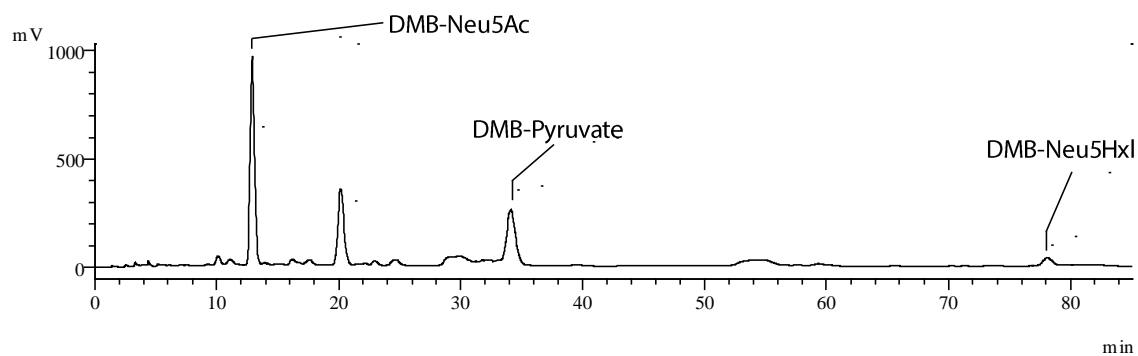
**Figure S17.** RP-HPLC analysis (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with  $Ac_4ManNHec$  (**9**). A) Complete chromatogram, B) enlarged region.



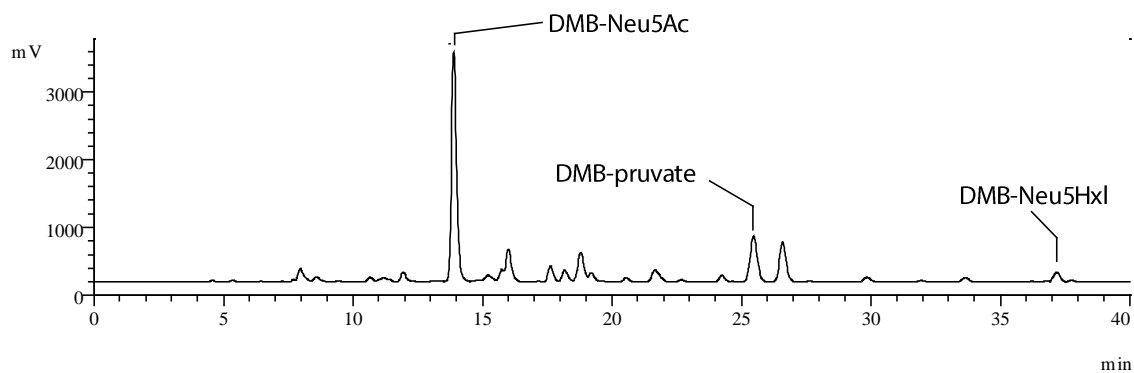
**Figure S18.** RP-HPLC analysis (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with  $Ac_4ManNBtl$  (**11**).



**Figure S19.** RP-HPLC analysis (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with  $Ac_4ManNPtI$  (**13**).

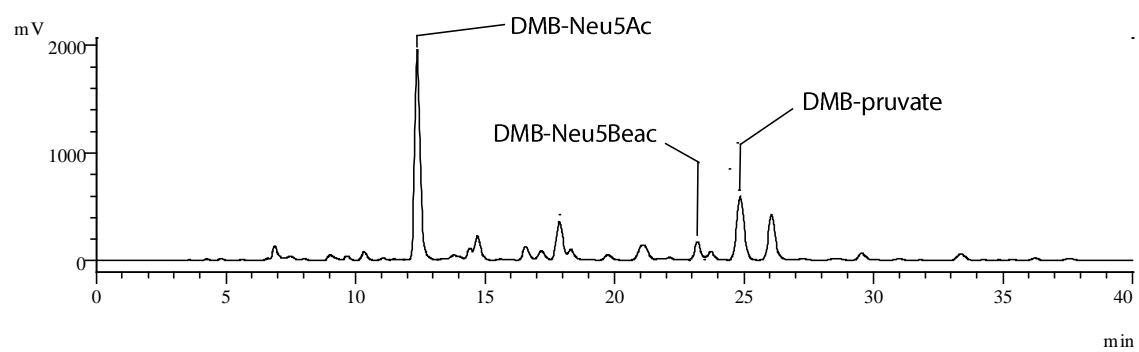


**Figure S20.** RP-HPLC analysis (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with  $Ac_4ManNHxl$  (**15**).



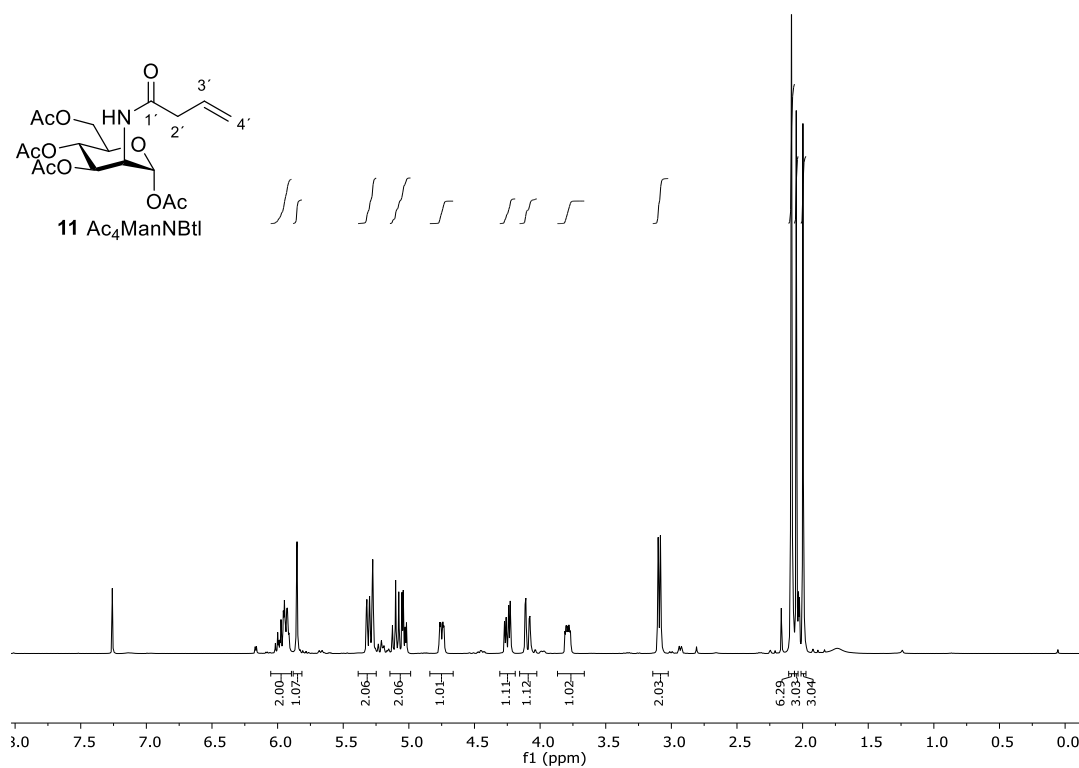
**Figure S21.** RP-HPLC analysis (10 % – 25 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with  $Ac_4ManNHxl$  (**15**).



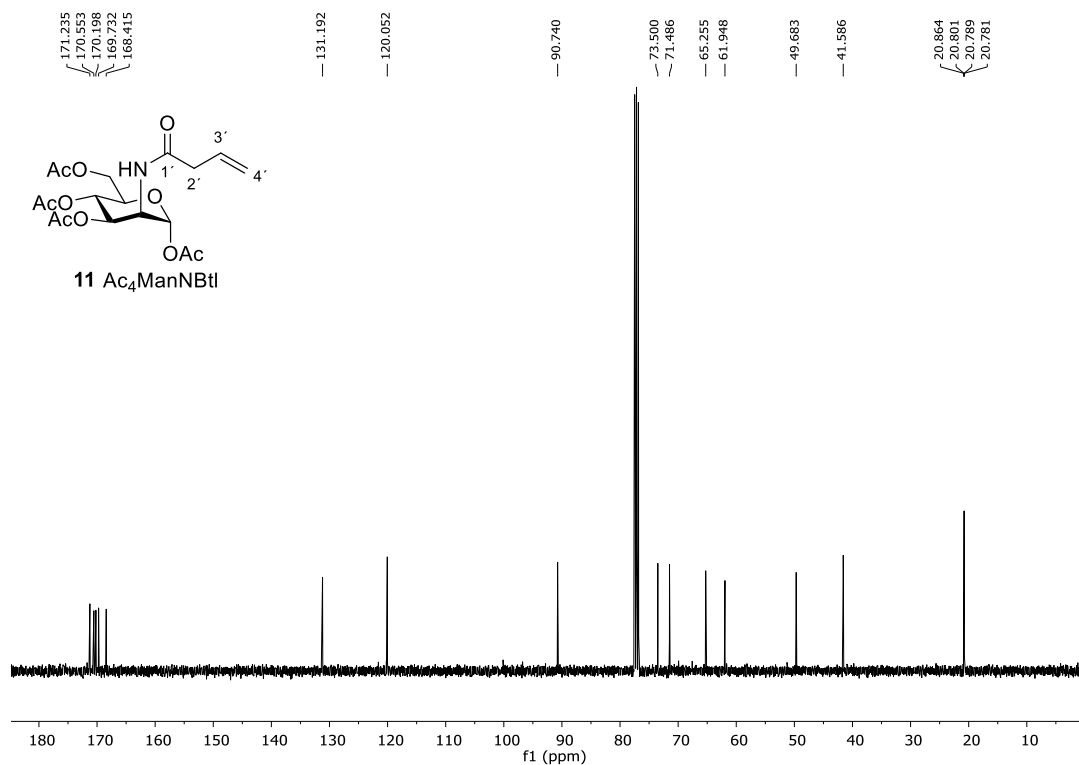


**Figure S22.** RP-HPLC analysis (10 % – 25 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac<sub>4</sub>ManNBeac (**17**).

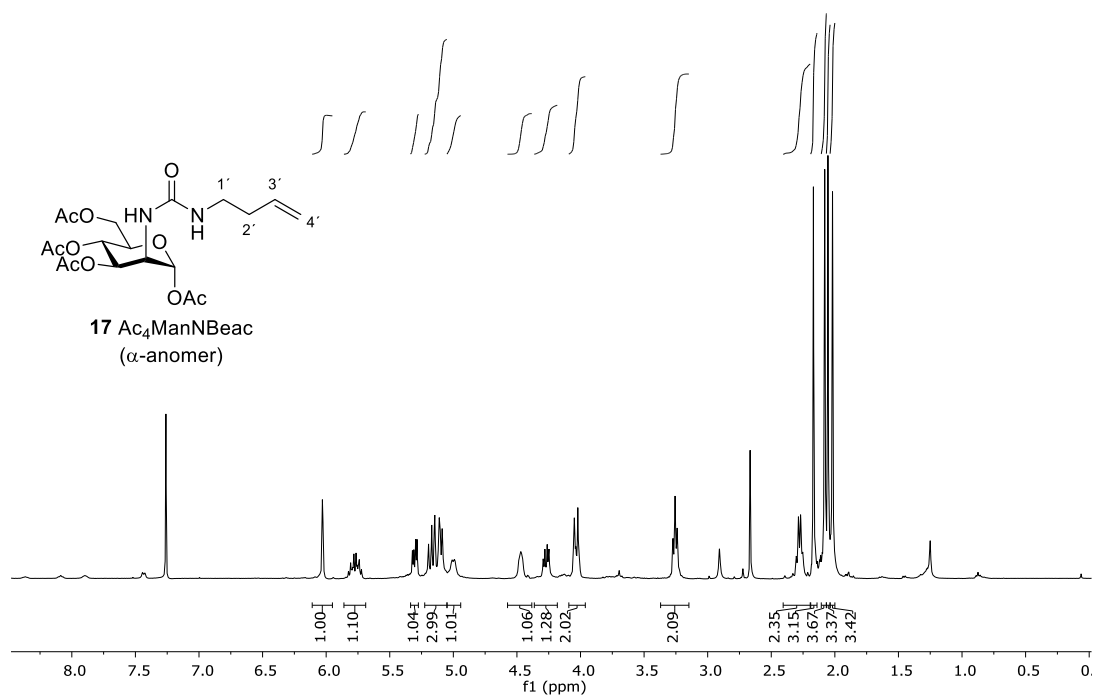
# NMR Spectra



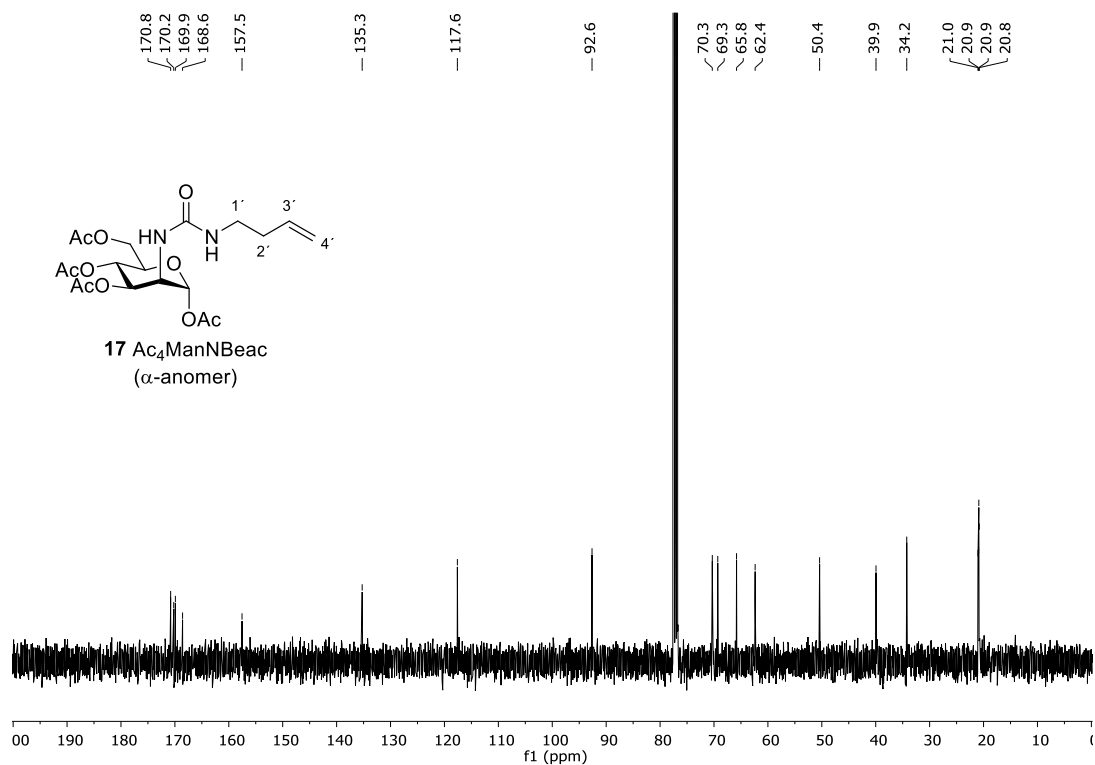
<sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, 400 MHz) of compound **11** (enriched α-anomer).



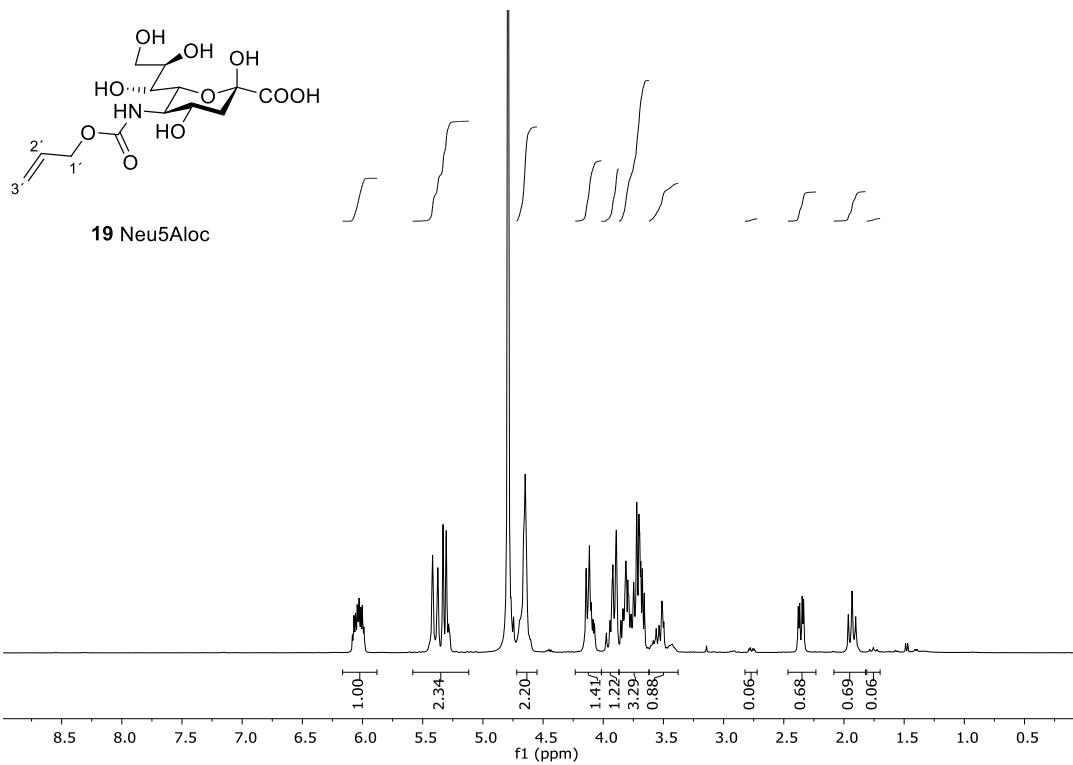
<sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 101 MHz) of compound **11** (enriched α-anomer).



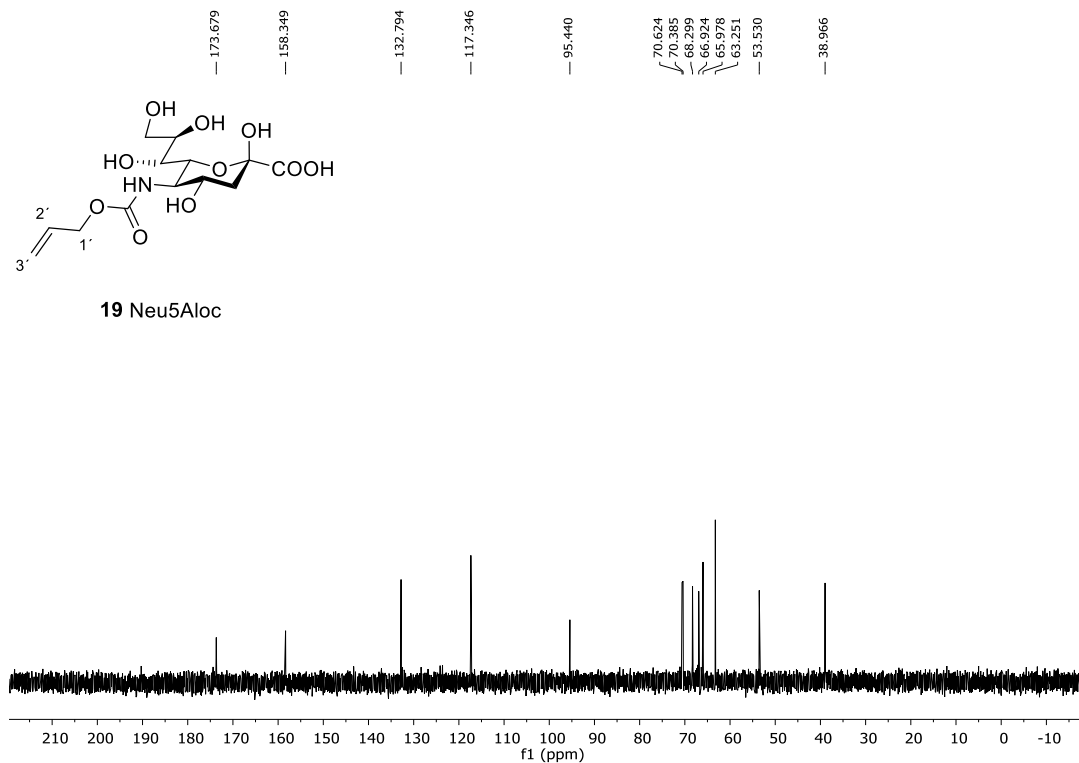
<sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 400 MHz) of the separated  $\alpha$ -anomer of compound **17**.



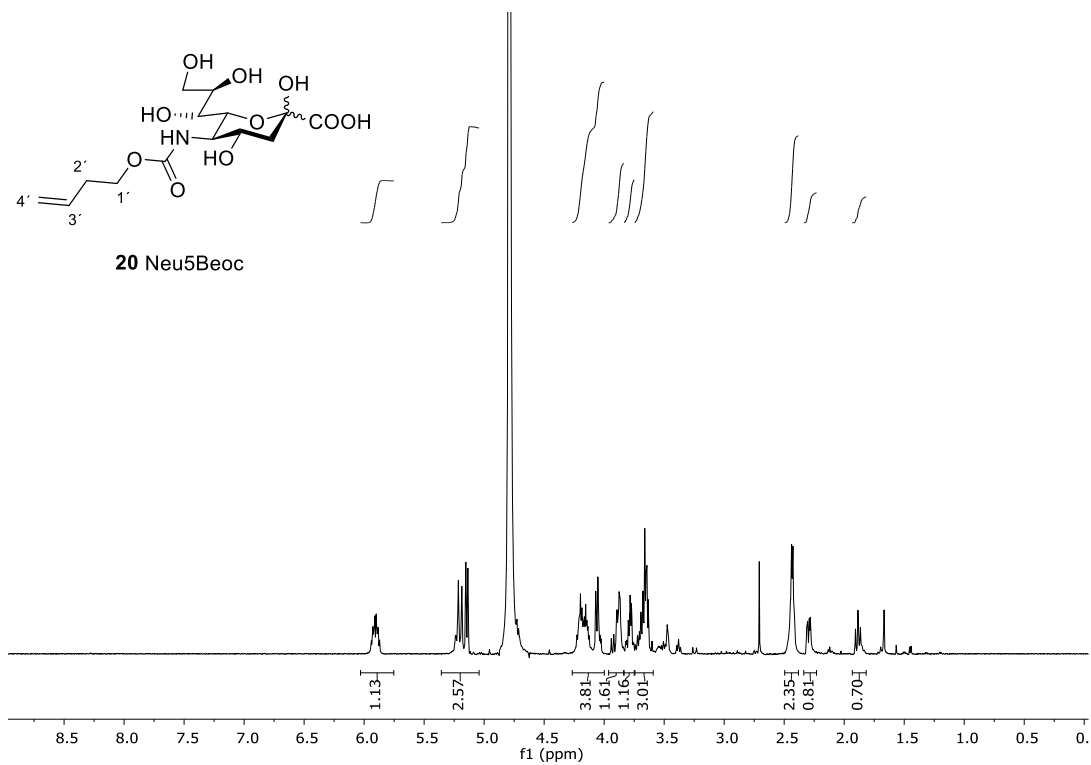
<sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 101 MHz) of the separated  $\alpha$ -anomer of compound **17**.



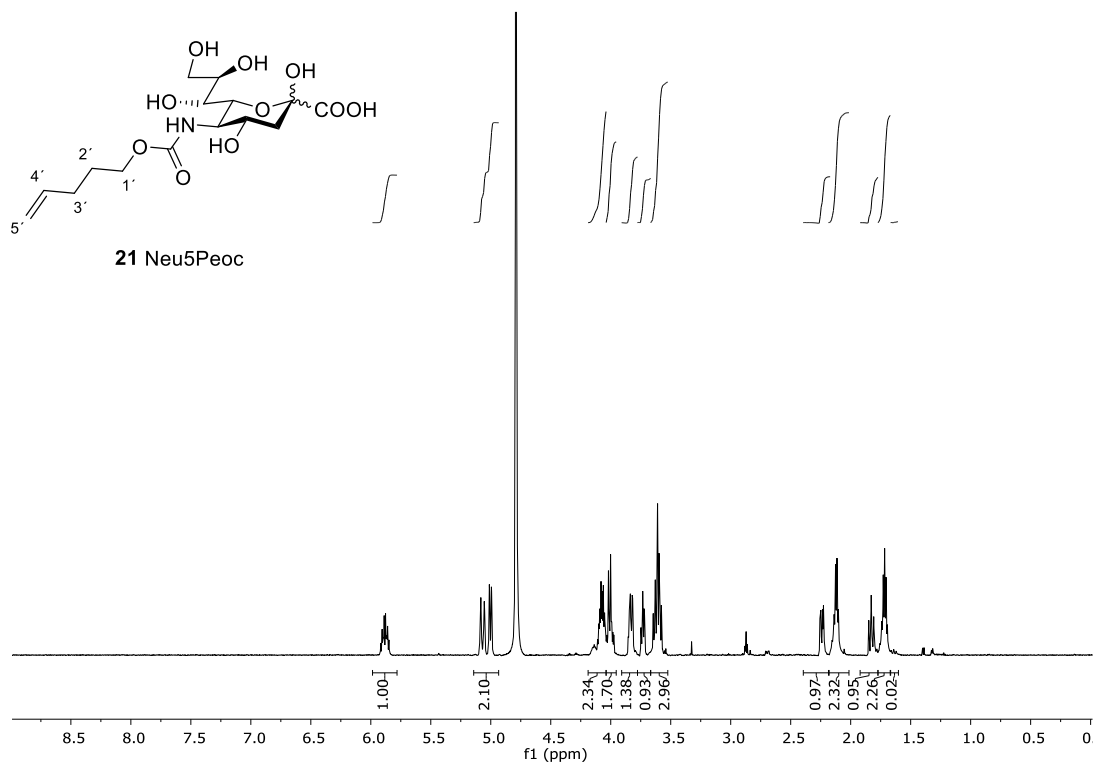
$^1\text{H}$  NMR spectrum ( $\text{D}_2\text{O}$ , 400 MHz) of compound **19**.



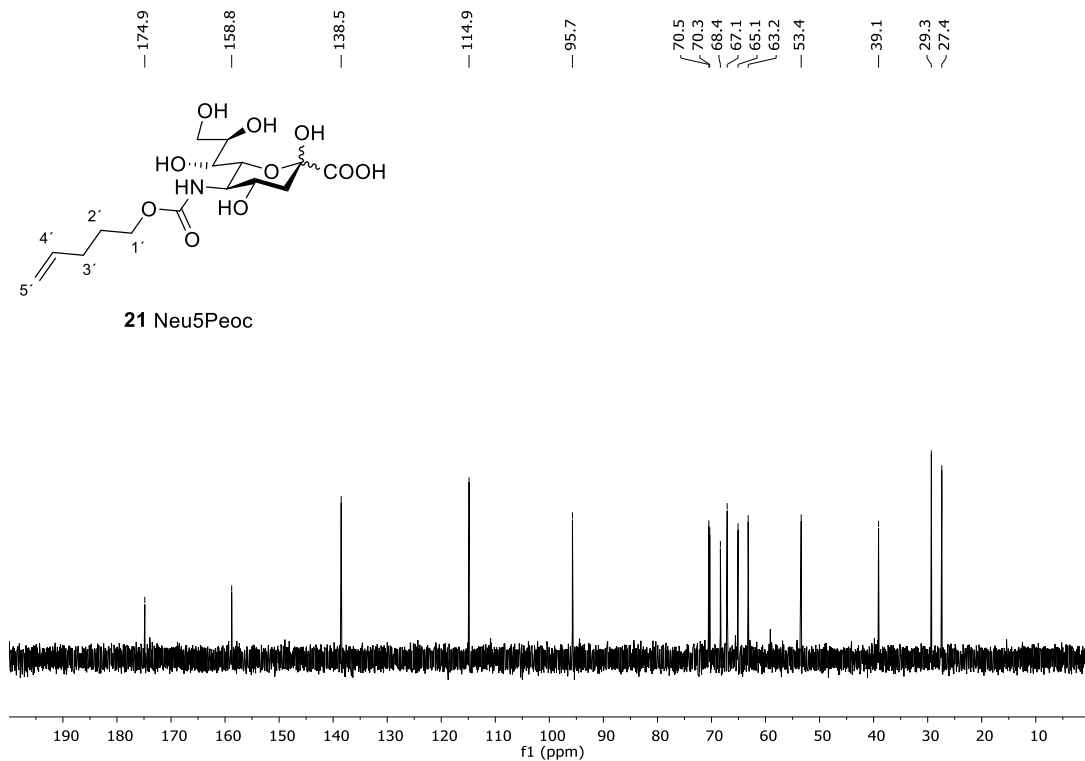
$^{13}\text{C}$ -NMR spectrum ( $\text{D}_2\text{O}$ , 101 MHz) of compound **19**.



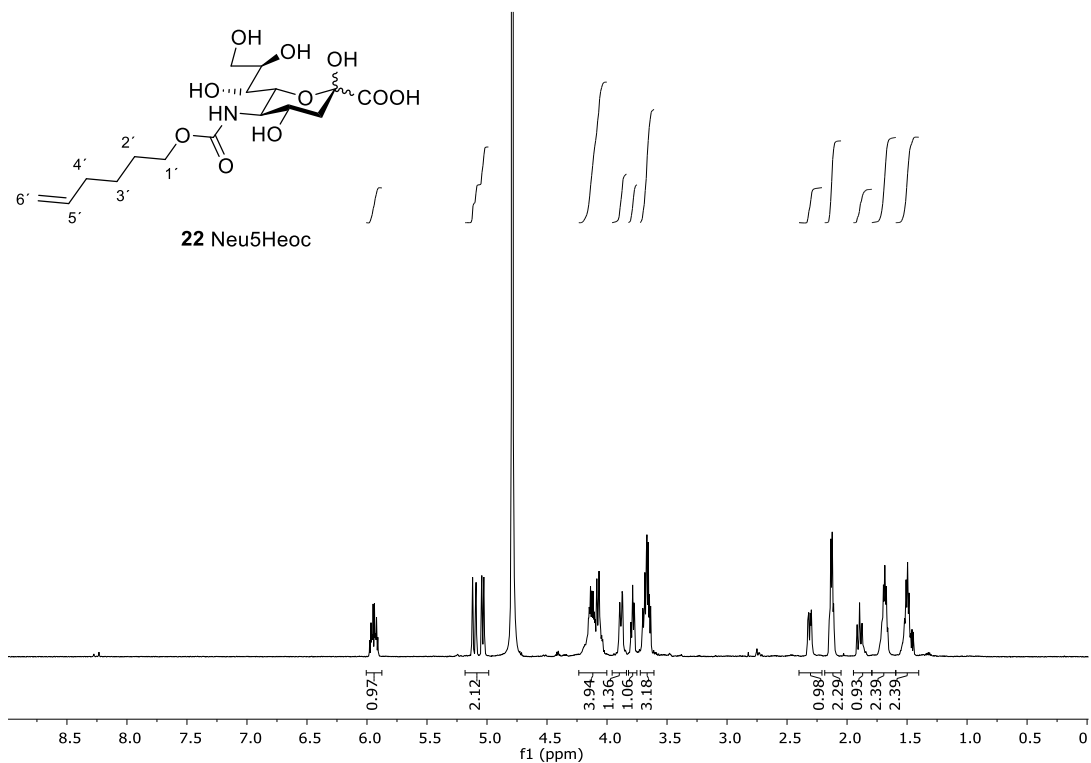
$^1\text{H}$  NMR spectrum (D<sub>2</sub>O, 600 MHz) of compound **20**.



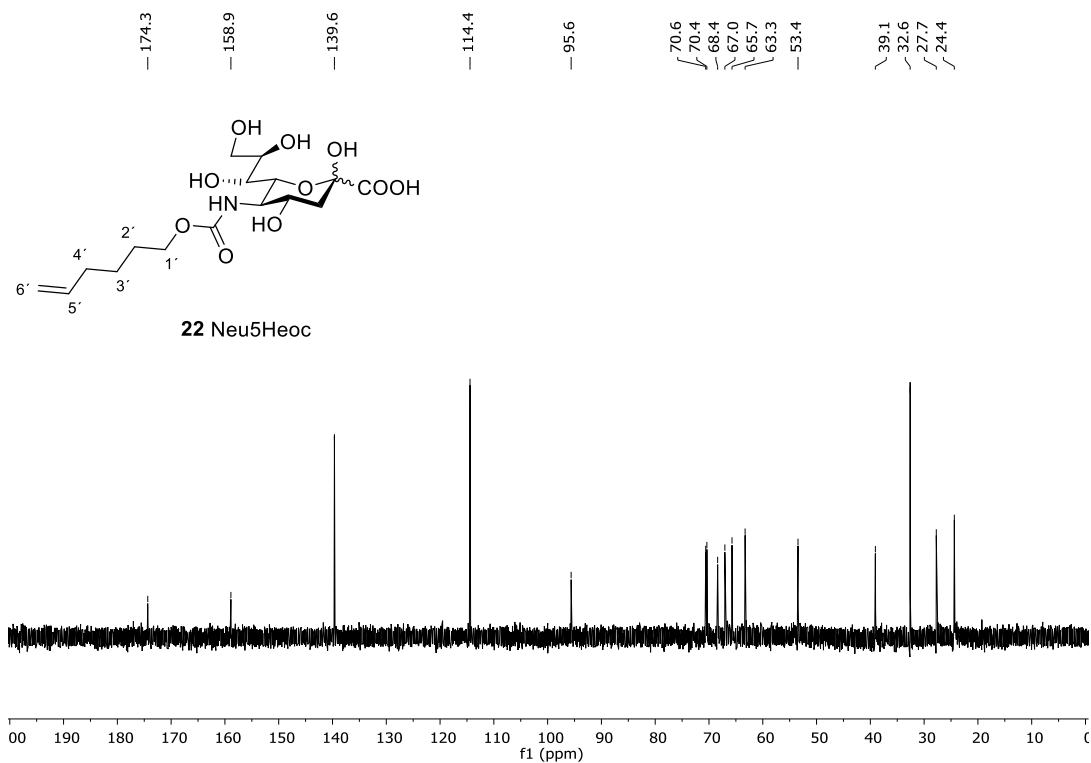
<sup>1</sup>H NMR spectrum (D<sub>2</sub>O, 600 MHz) of compound **21**.



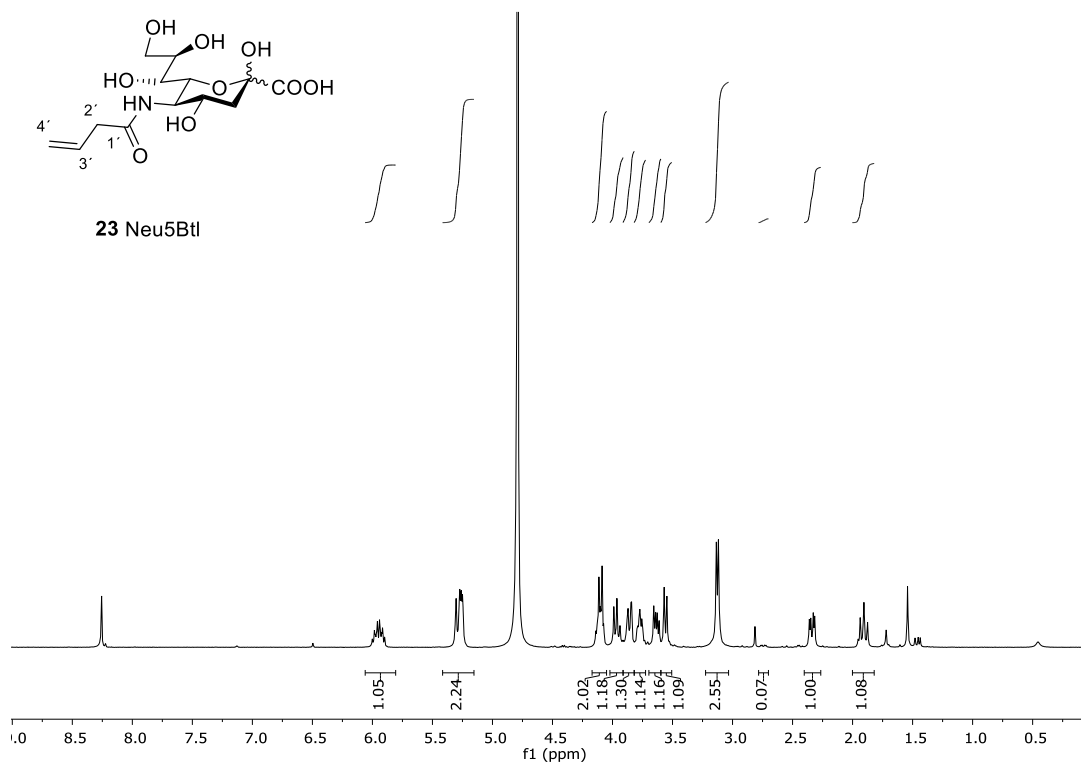
<sup>13</sup>C NMR spectrum (D<sub>2</sub>O, 151 MHz) of compound **21**.



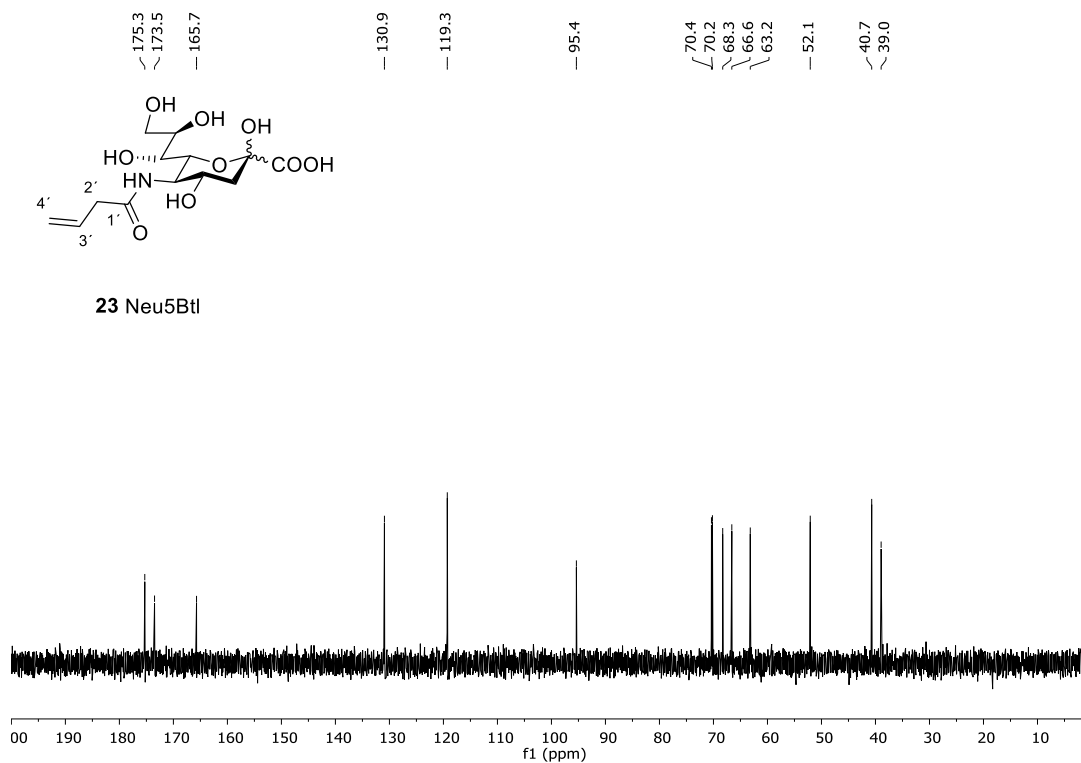
$^1\text{H}$  NMR spectrum ( $\text{D}_2\text{O}$ , 600 MHz) of compound **22**.



$^{13}\text{C}$  NMR spectrum ( $\text{D}_2\text{O}$ , 151 MHz) of compound **22**.

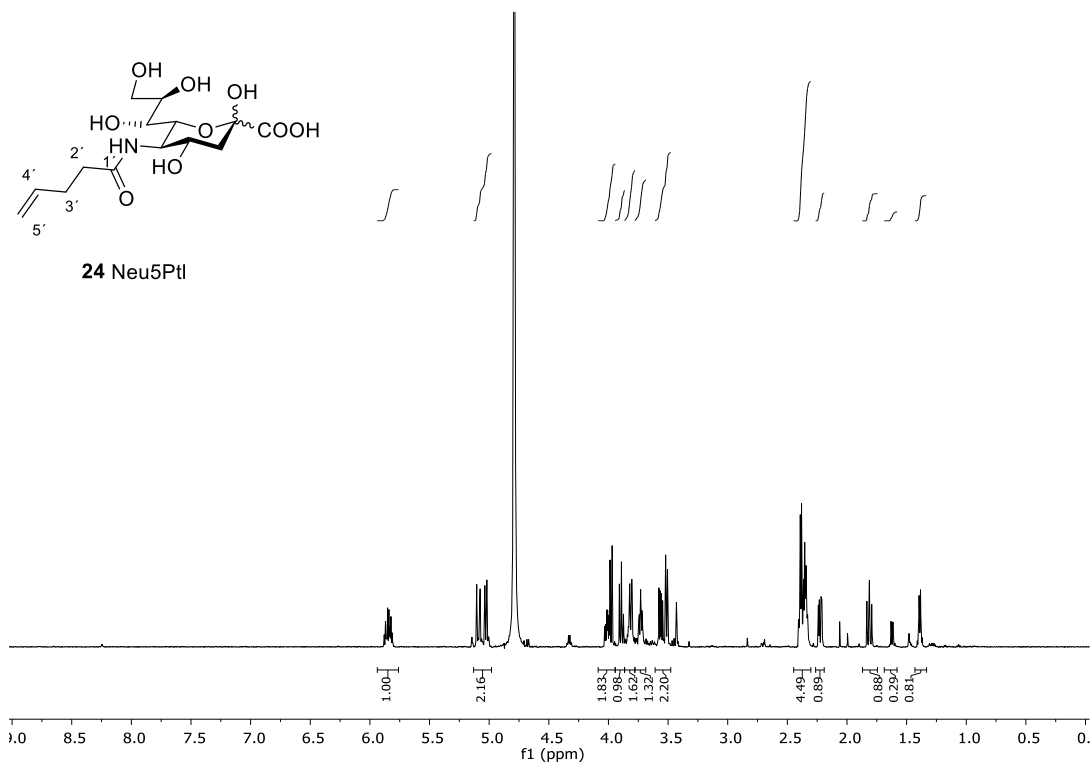


$^1\text{H}$  NMR spectrum ( $\text{D}_2\text{O}$ , 400 MHz) of compound **23**.

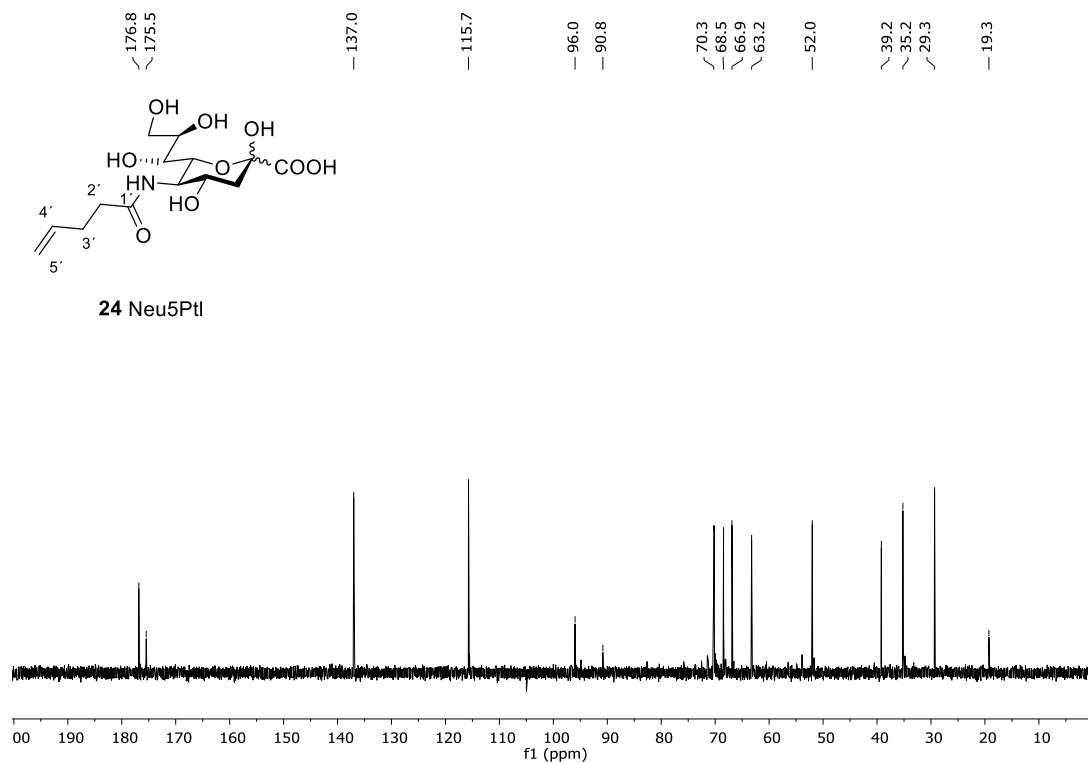


$^{13}\text{C}$  NMR spectrum ( $\text{D}_2\text{O}$ , 101 MHz) of compound **23**.

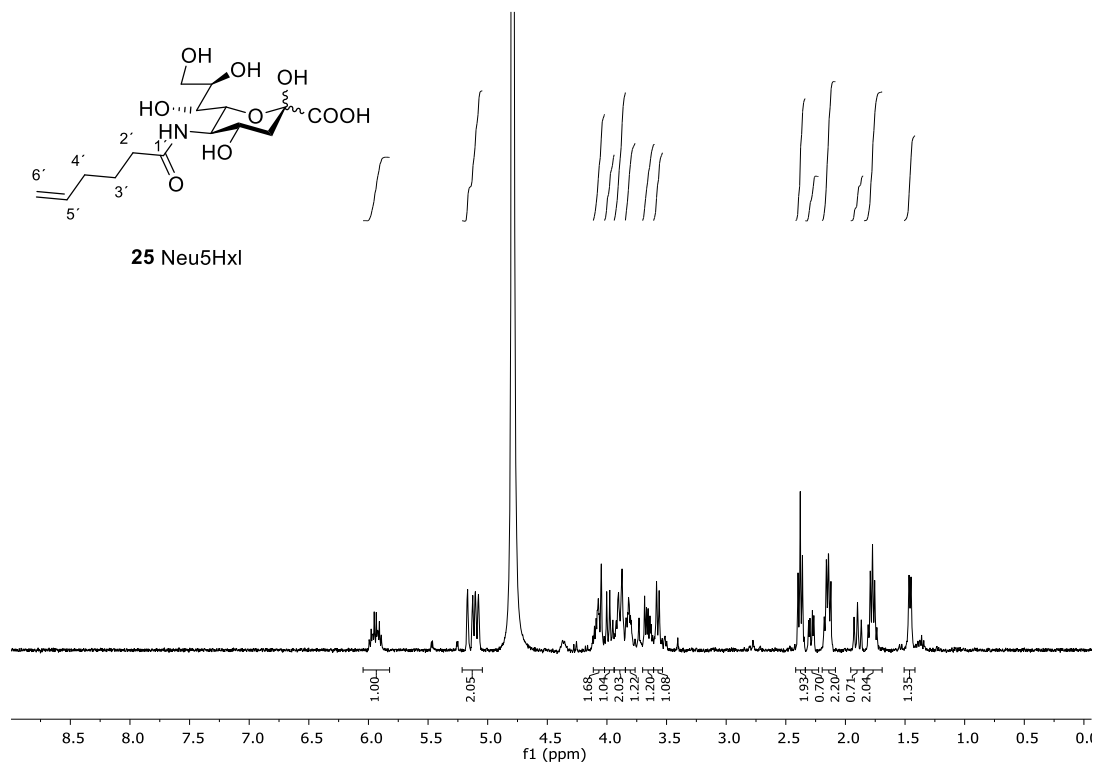




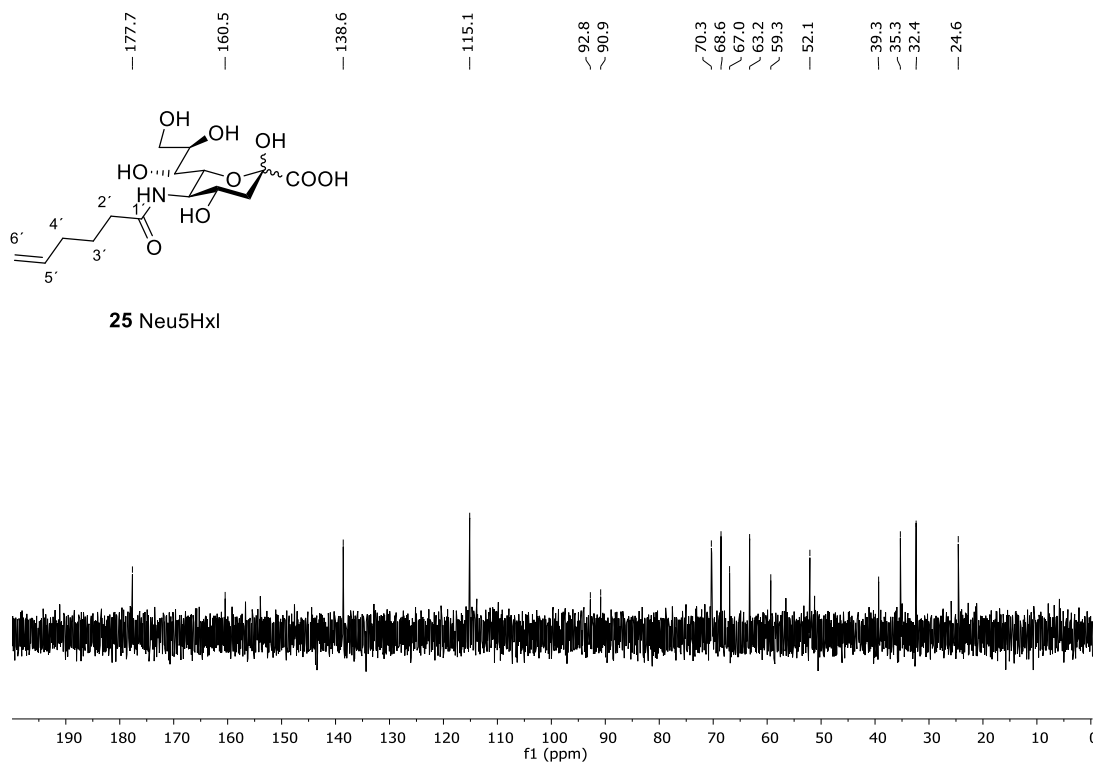
<sup>1</sup>H NMR spectrum (D<sub>2</sub>O, 600 MHz) of compound **24**.



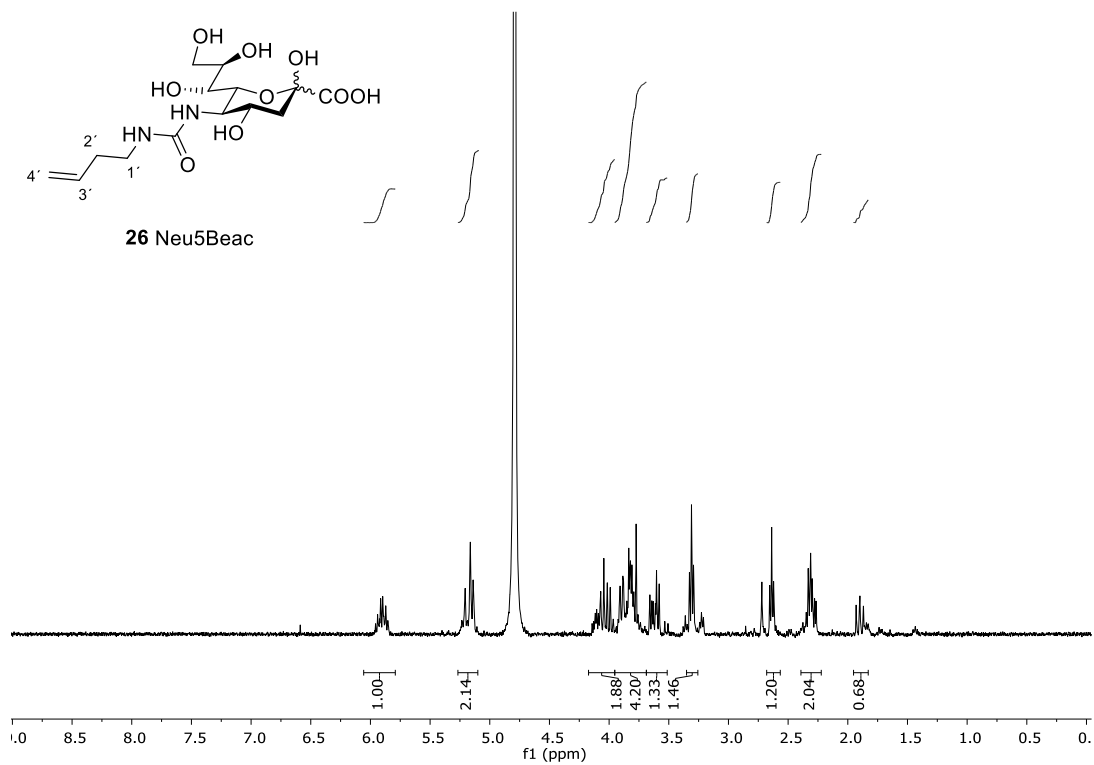
<sup>13</sup>C NMR spectrum (D<sub>2</sub>O, 151 MHz) of compound **24**.



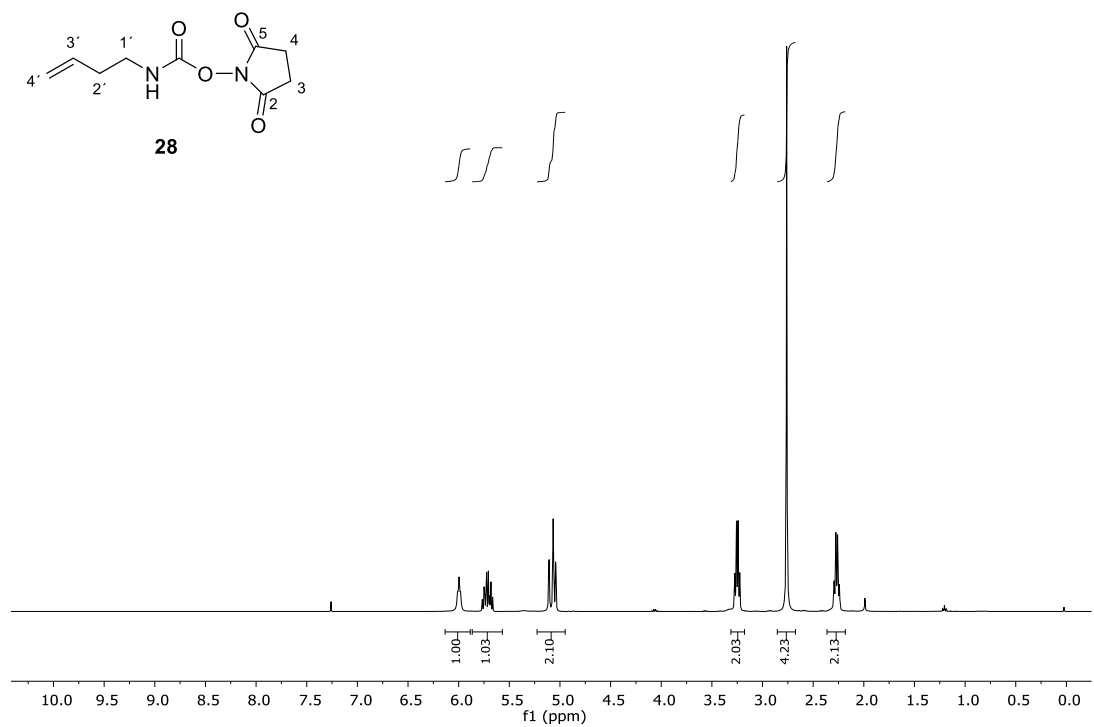
<sup>1</sup>H NMR spectrum (D<sub>2</sub>O, 400 MHz) of compound **25**.



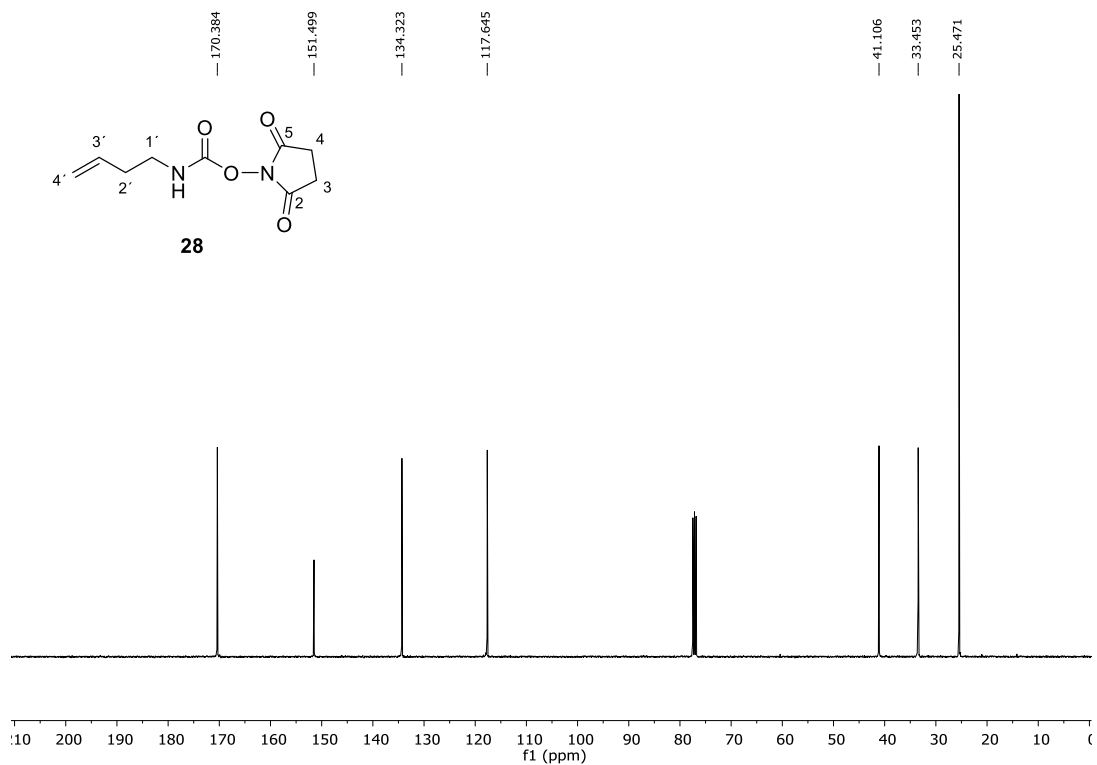
<sup>13</sup>C NMR spectrum (D<sub>2</sub>O, 151 MHz) of compound **25**.



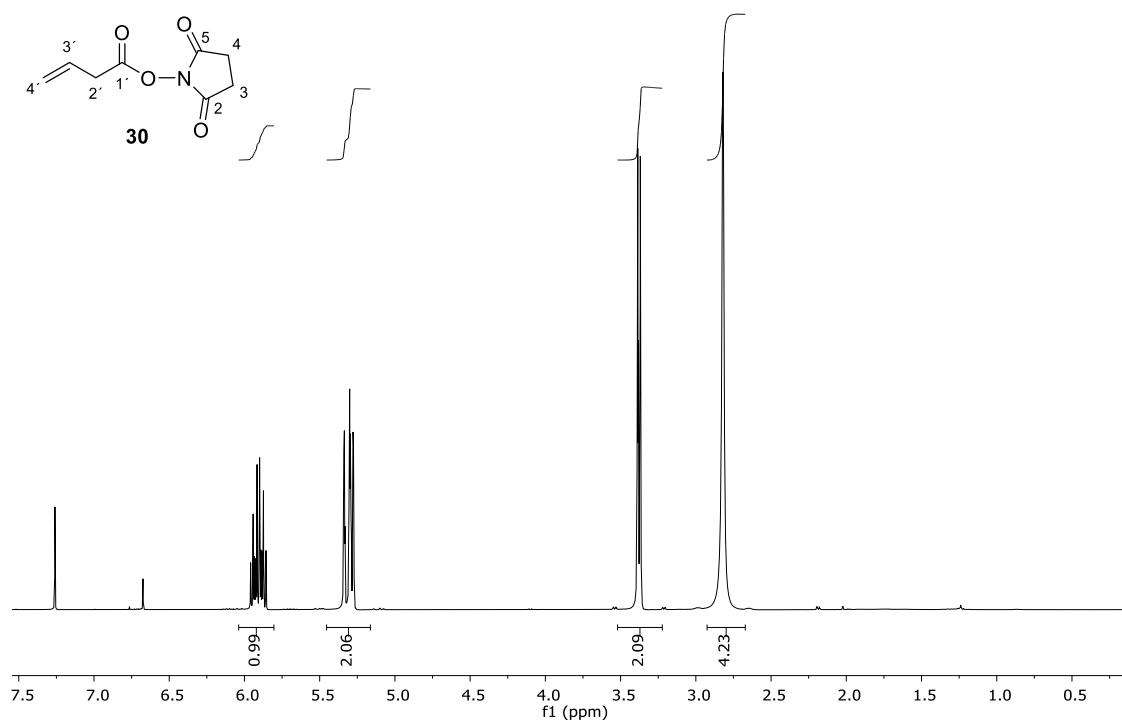
$^1\text{H}$  NMR spectrum (D<sub>2</sub>O, 400 MHz) of compound **26**.



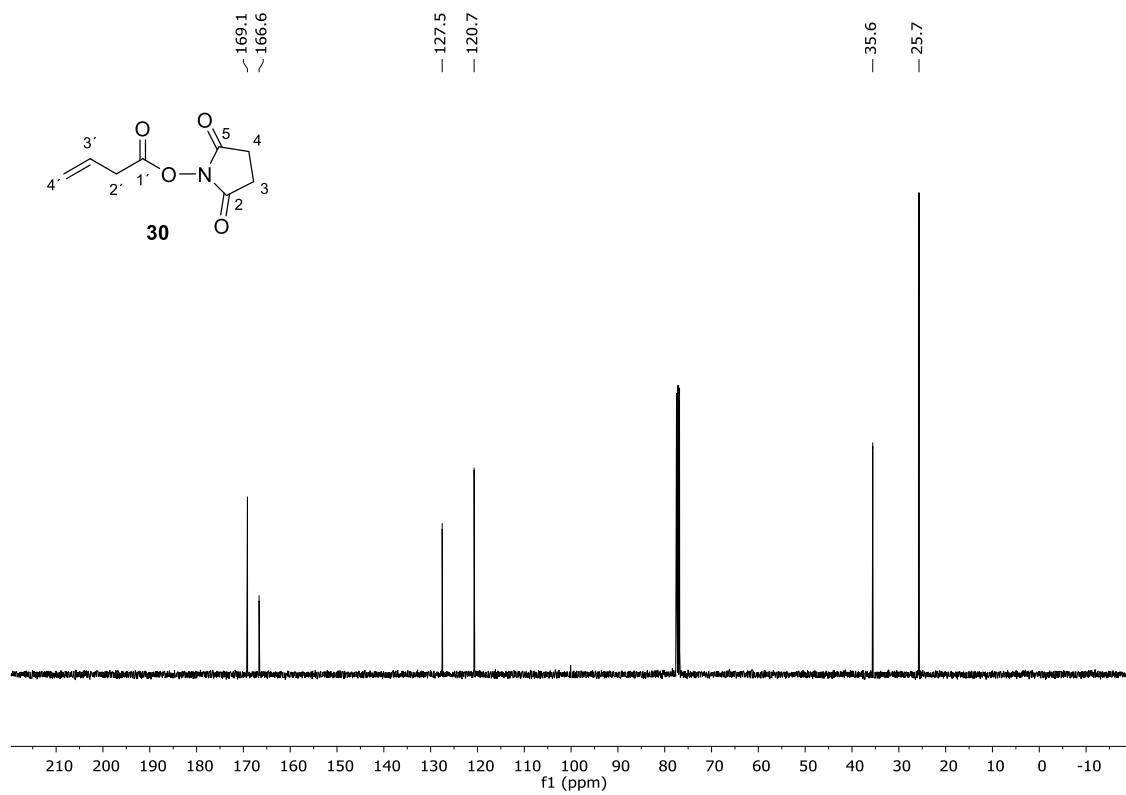
$^1\text{H}$ -NMR spectrum (CDCl<sub>3</sub>, 400 MHz) of compound **28**.



$^{13}\text{C}$ -NMR spectrum (CDCl<sub>3</sub>, 101 MHz) of compound **28**.



$^1\text{H}$ -NMR spectrum (CDCl<sub>3</sub>, 400 MHz) of compound **30**.



$^{13}\text{C}$  NMR spectrum (CDCl<sub>3</sub>, 101 MHz) of compound **30**.