CHEMBIOCHEM

Supporting Information

Exploring the Potential of Norbornene-Modified Mannosamine Derivatives for Metabolic Glycoengineering

Anne-Katrin Späte, Jeremias E. G. A. Dold, Ellen Batroff, Verena F. Schart, Daniel E. Wieland, Oliver R. Baudendistel, and Valentin Wittmann*[a]

cbic_201600197_sm_miscellaneous_information.pdf

Supporting Information

Table of Contents

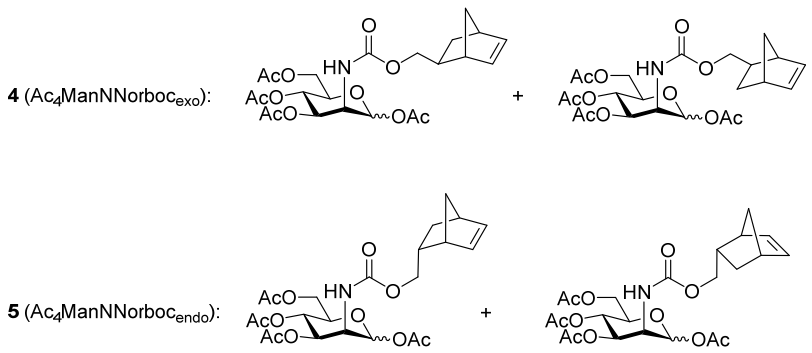


Figure S1. Structures of all diastereoisomers obtained for $Ac_4ManNNorboc_{exo}$ **4** and $Ac_4ManNNorboc_{endo}$ **5**.

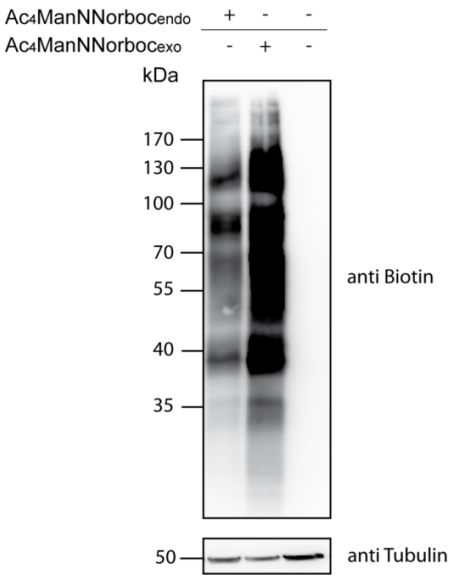


Figure S2. Western blot analysis of soluble glycoproteins. HEK 293T cells were grown with 100 μM sugar **4**, **5**, or without additional sugar. After lysis, the soluble fraction was reacted with Tz-biotin **16** (100 μM, 2 h, r.t.) and proteins were immunoblotted for biotin and tubulin (loading control).

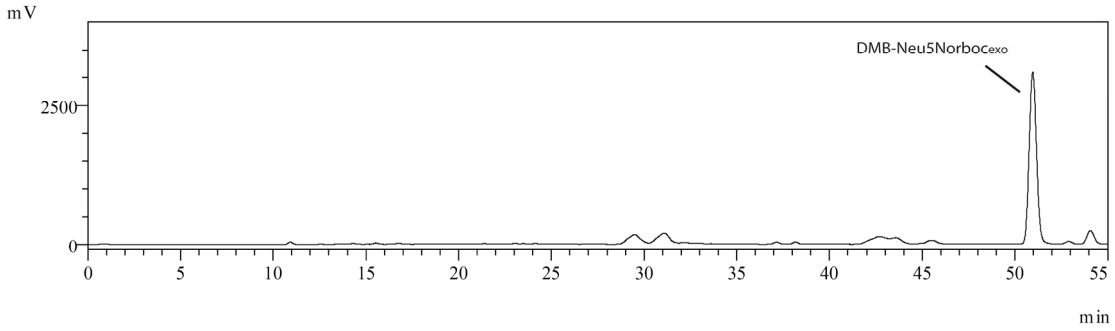


Figure S3. Derivatization of Neu5Norboc$_{exo}$ **18**. Neu5Norboc$_{exo}$ **18** was labeled with DMB as described in the Experimental Section and analyzed by RP-HPLC (10%–30.6% B in 55 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). R_t = 51.0 min.

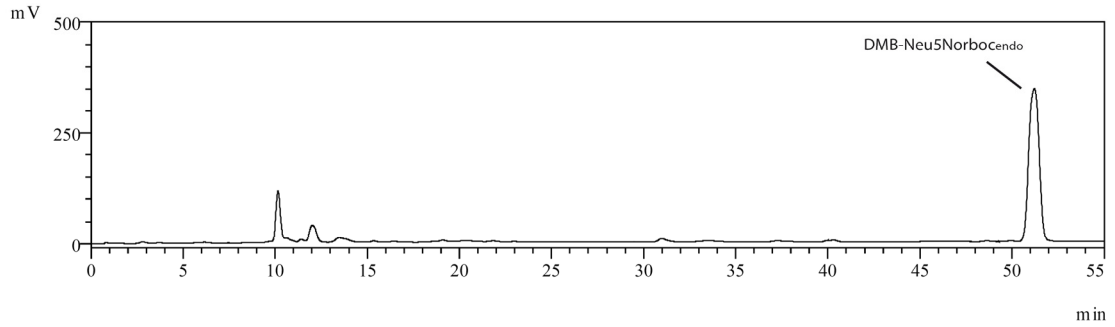


Figure S4. Derivatization of Neu5Norboc$_{endo}$ **19**. Neu5Norboc$_{endo}$ **19** was labeled with DMB as described in the Experimental Section and analyzed by RP-HPLC (10%–30.6% B in 55 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). R_t = 51.2 min.

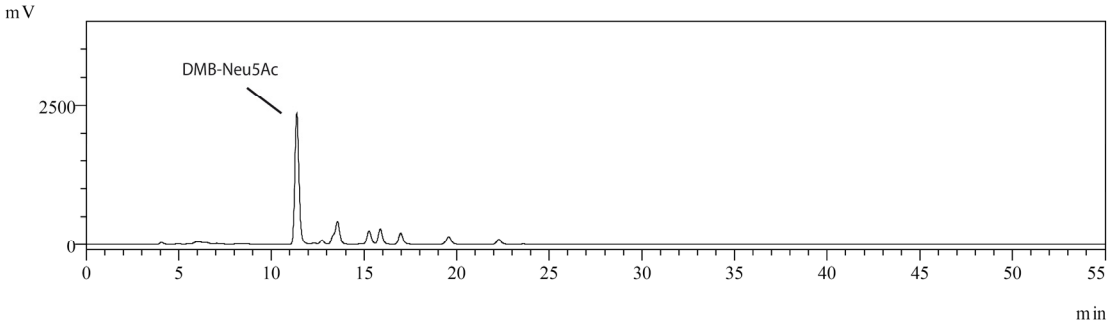


Figure S5. Derivatization of Neu5Ac **20**. Neu5Ac **20** was labeled with DMB as described in the Experimental Section and analyzed by RP-HPLC (10%–30.6% B in 55 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). R_t = 11.3 min.

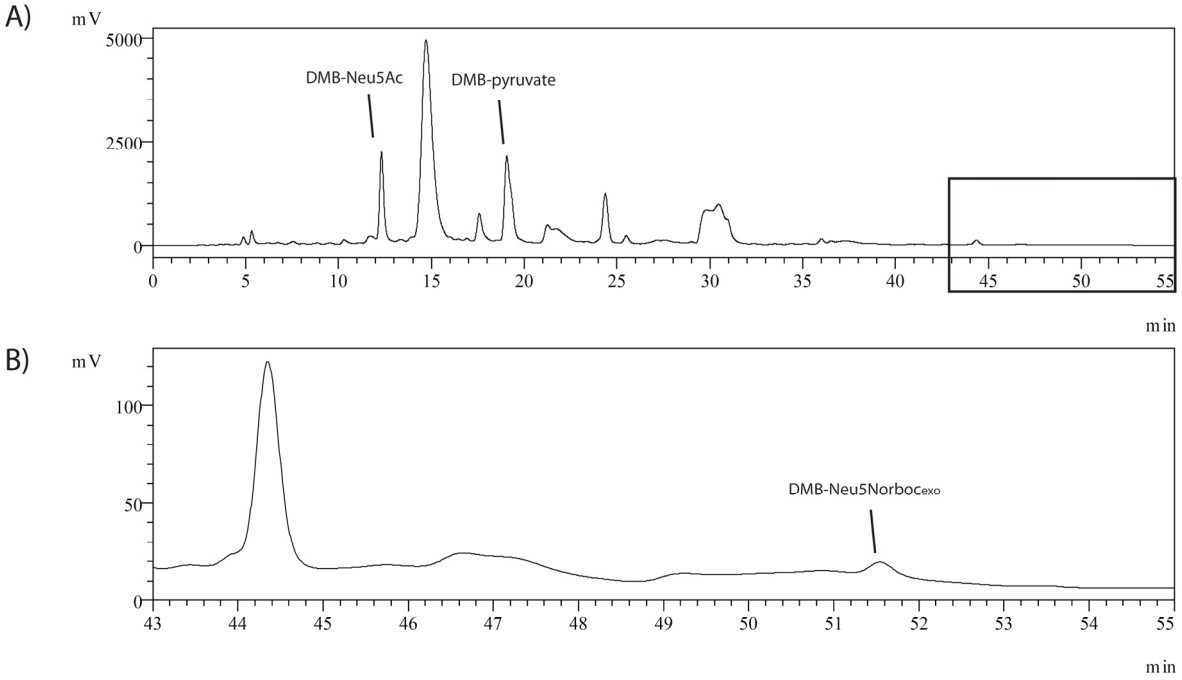


Figure S6. DMB labeling of sialic acids released from engineered cells grown with $Ac_4ManNNorboc_{exo}$ **4**. HEK 293T cells were grown with 250 μM $Ac_4ManNNorboc_{exo}$ **4** for 48 h. Sialic acids were cleaved off by treatment with acetic acid (3M, 80 °C, 90 min) labeled with DMB solution and analyzed by RP-HPLC (10%–30.6% B in 55 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). A) Chromatogram from 0 to 55 min, B) enlarged region from 43 to 55 min.

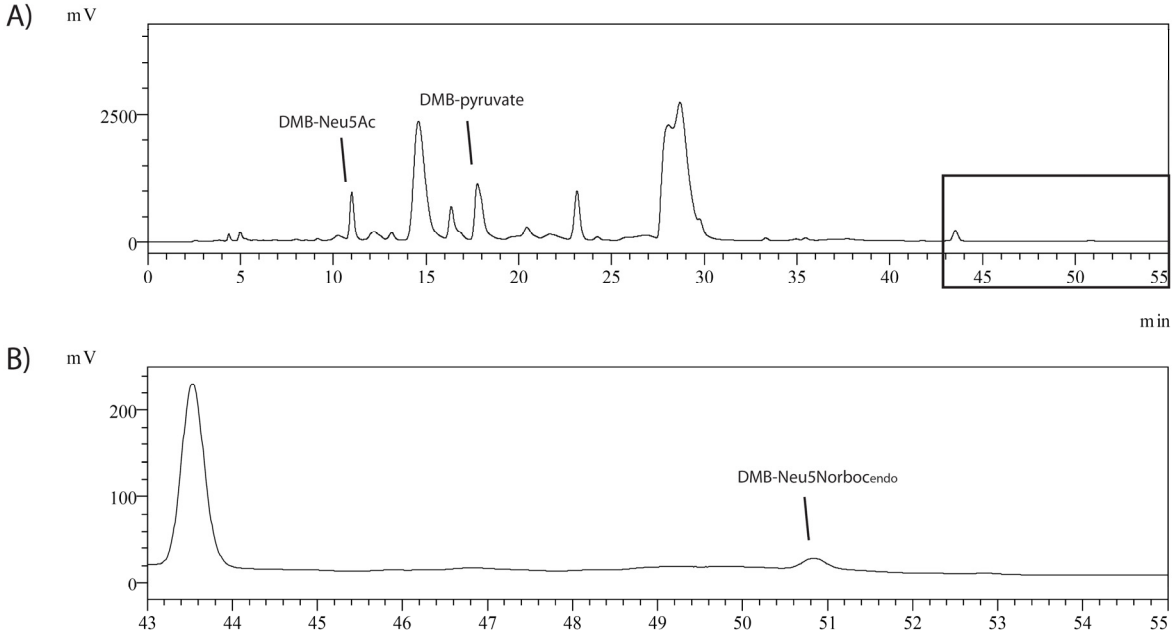


Figure S7. DMB labeling of sialic acids released from engineered cells grown with $Ac_4ManNNorboc_{endo}$ **5**. HEK 293T cells were grown with 250 µM $Ac_4ManNNorboc_{endo}$ **5** for 48 h. Sialic acids were cleaved off by treatment with acetic acid (3M, 80 °C, 90 min) labeled with DMB solution and analyzed by RP-HPLC (10%–30.6% B in 55 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). A) Chromatogram from 0 to 55 min, B) enlarged region from 43 to 55 min.

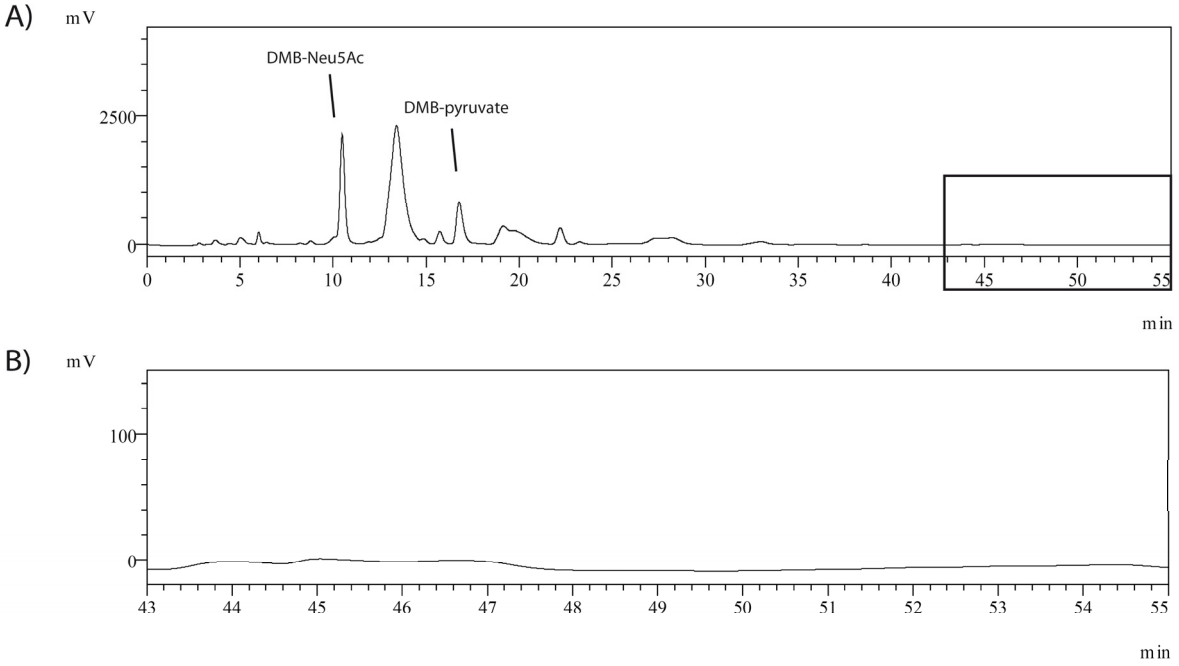


Figure S8. DMB labeling of sialic acids released from engineered cells grown without unnatural sugar. HEK 293T cells were grown without addition of unnatural sugar for 48 h. Sialic acids were cleaved off by treatment with acetic acid (3M, 80 °C, 90 min) labeled with DMB solution and analyzed by RP-HPLC (10%–30.6% B in 55 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). A) Chromatogram from 0 to 55 min, B) enlarged region from 43 to 55 min.

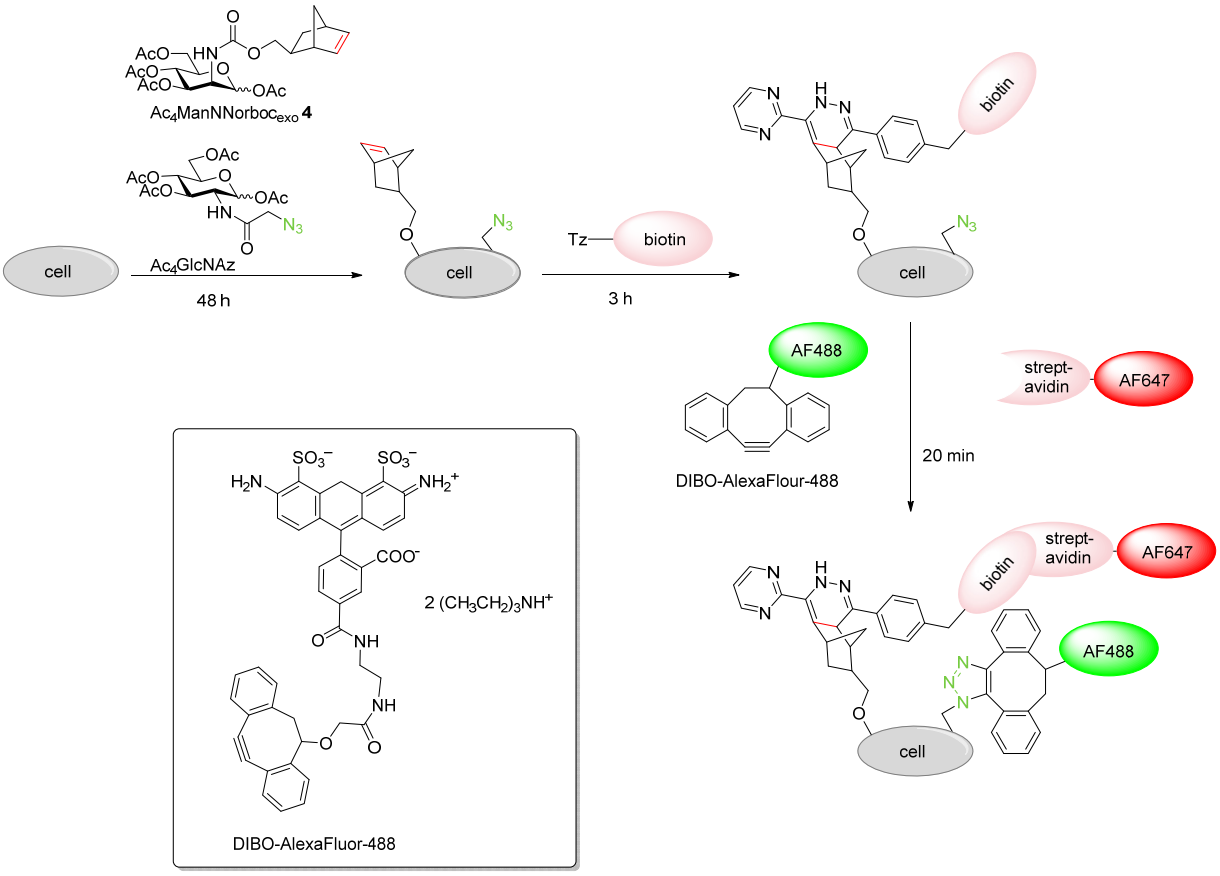
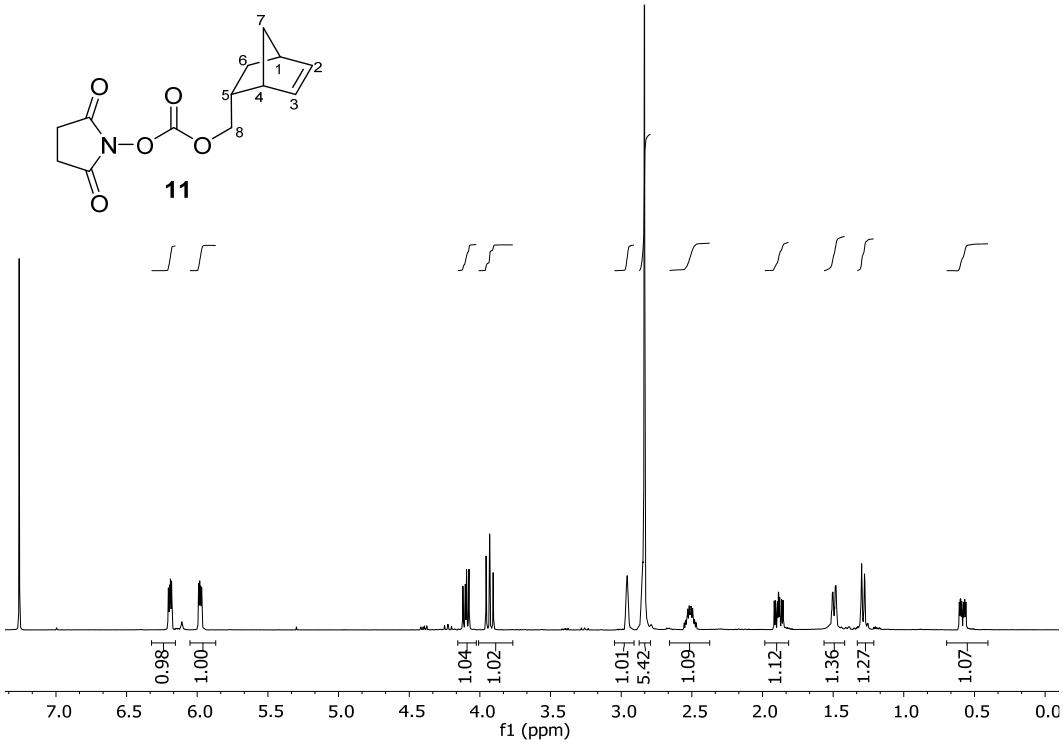
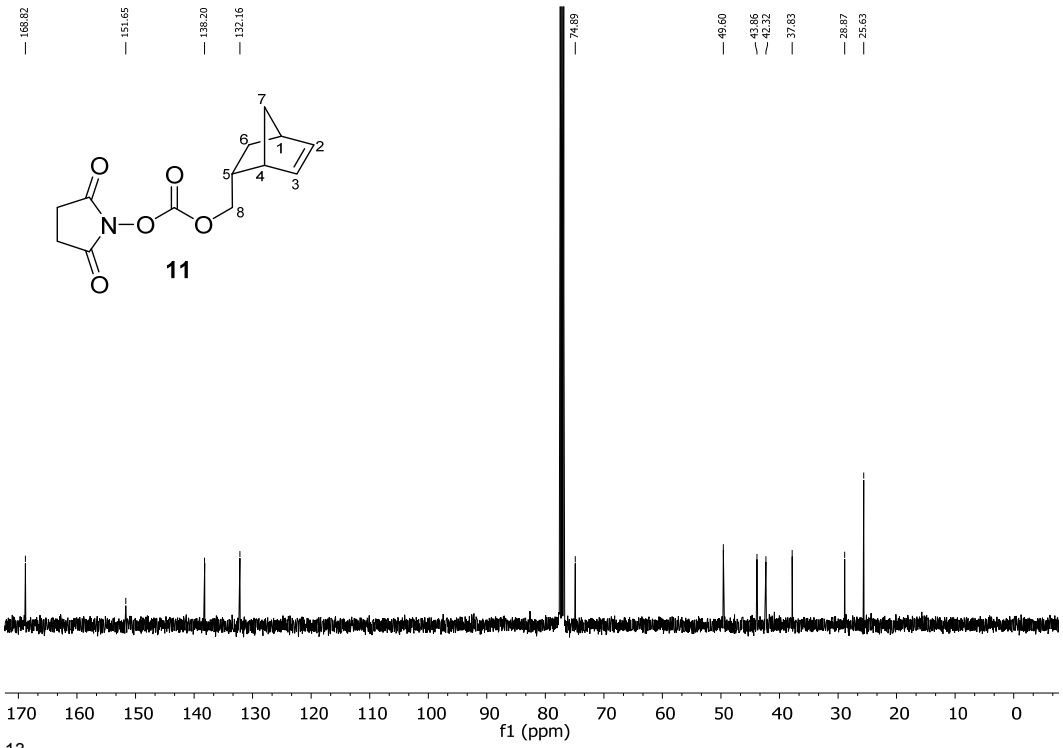


Figure S9. Strategy for dual labeling.

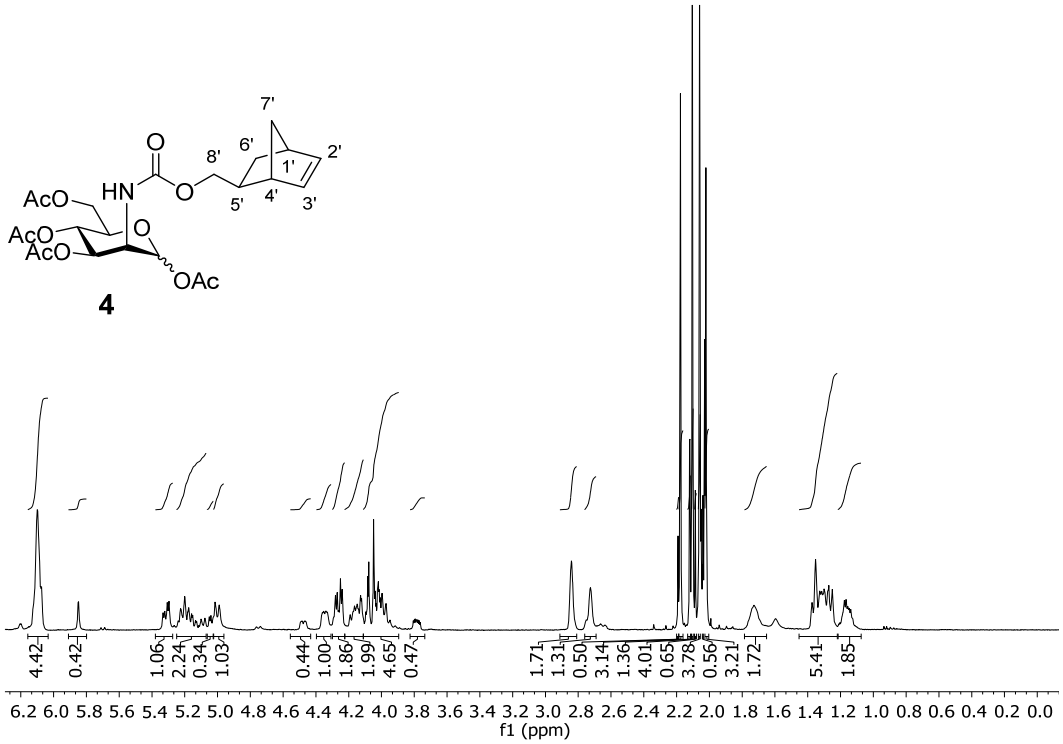
NMR Spectra



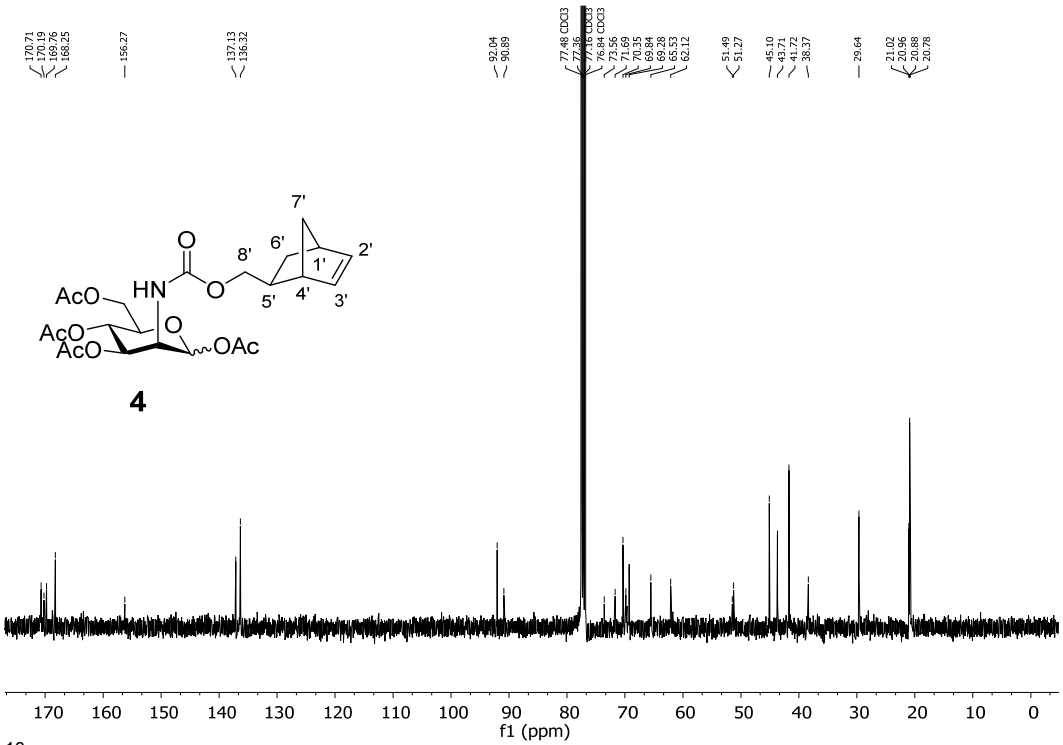
^{1}H NMR Spectrum (400.1 MHz, $CDCl_3$) of compound **11**.



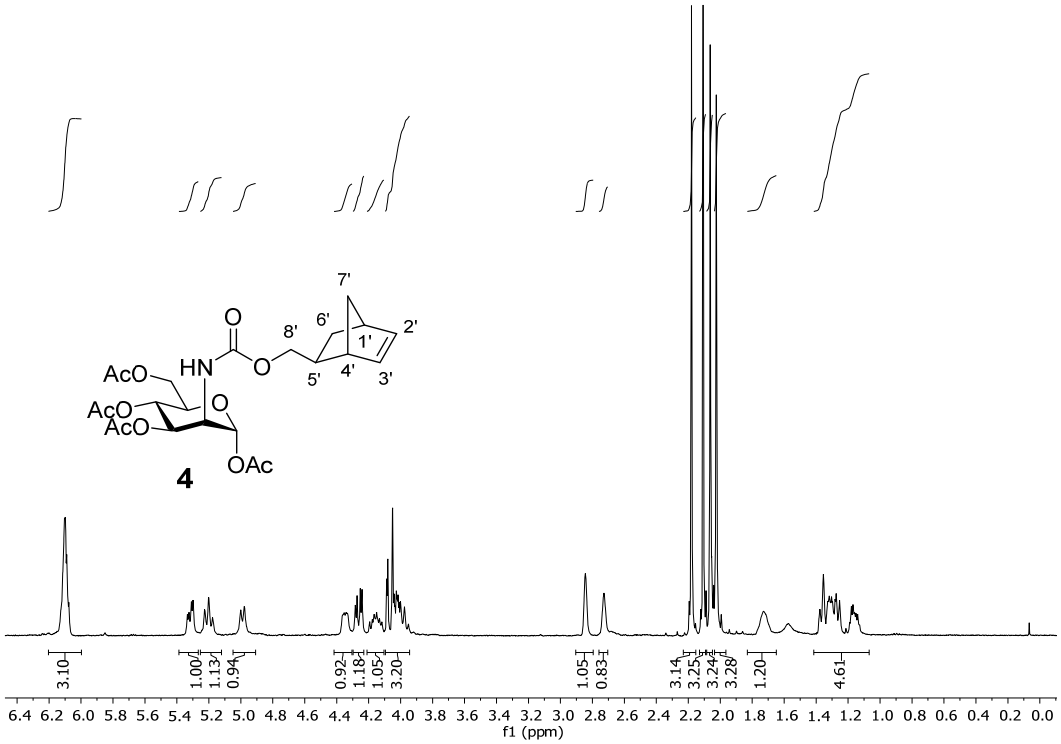
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **11**.



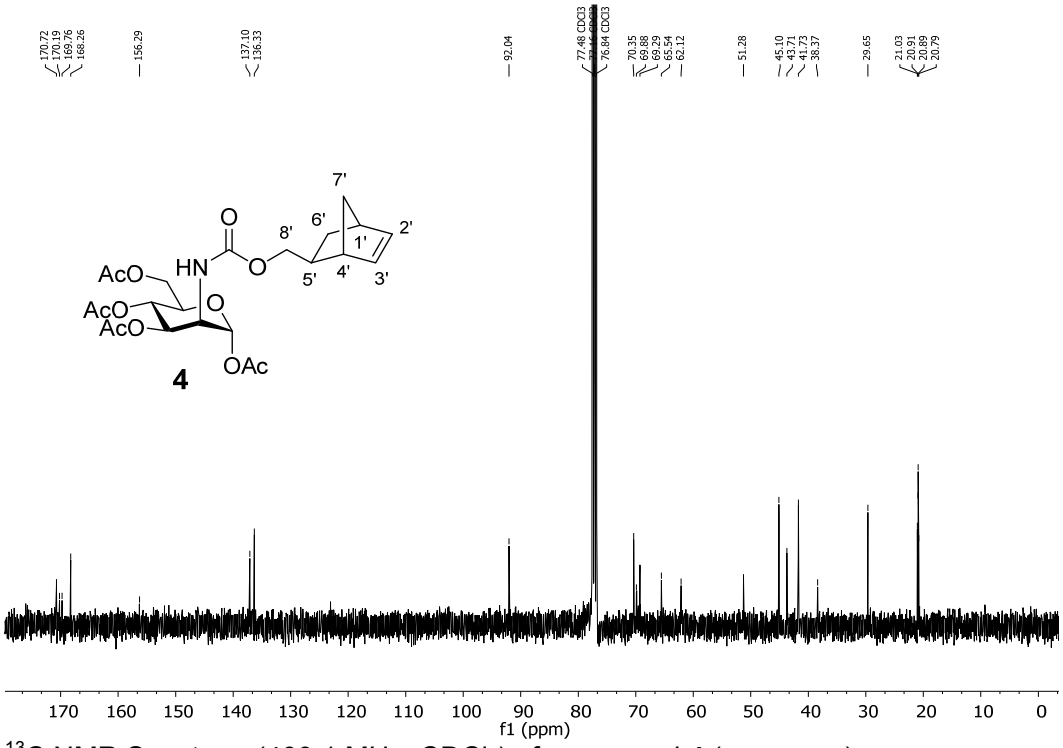
^{1}H NMR Spectrum (400.1 MHz, $CDCl_3$) of compound **4** (α and β).



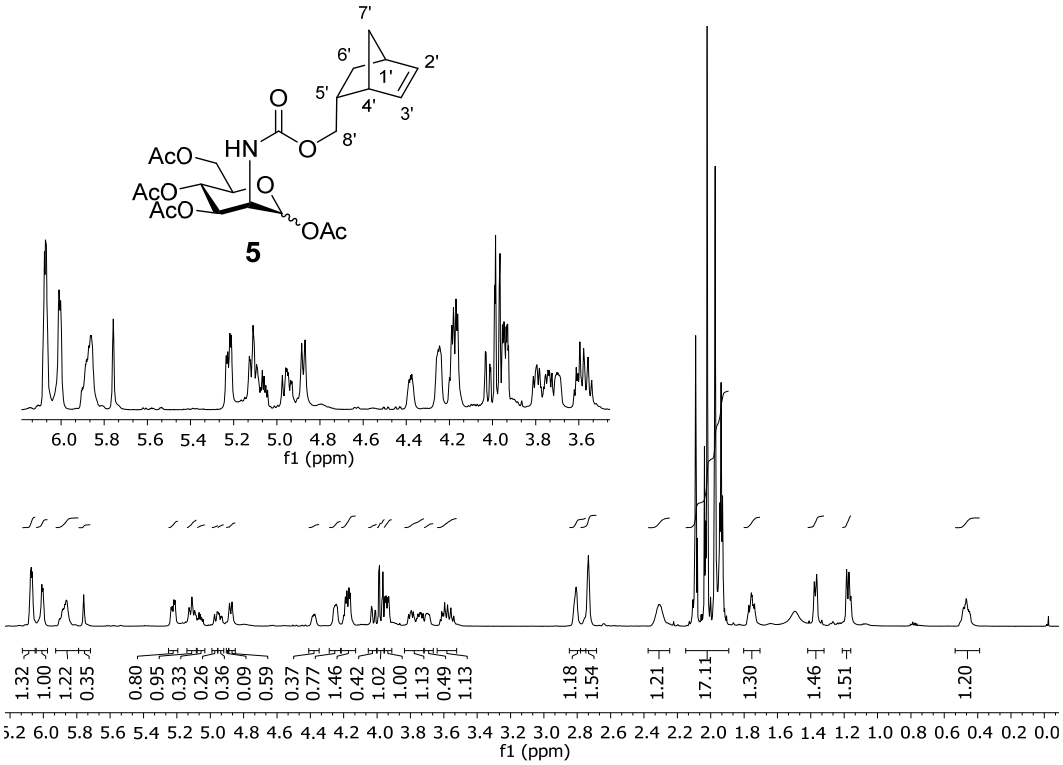
^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of compound **4** (α and β).



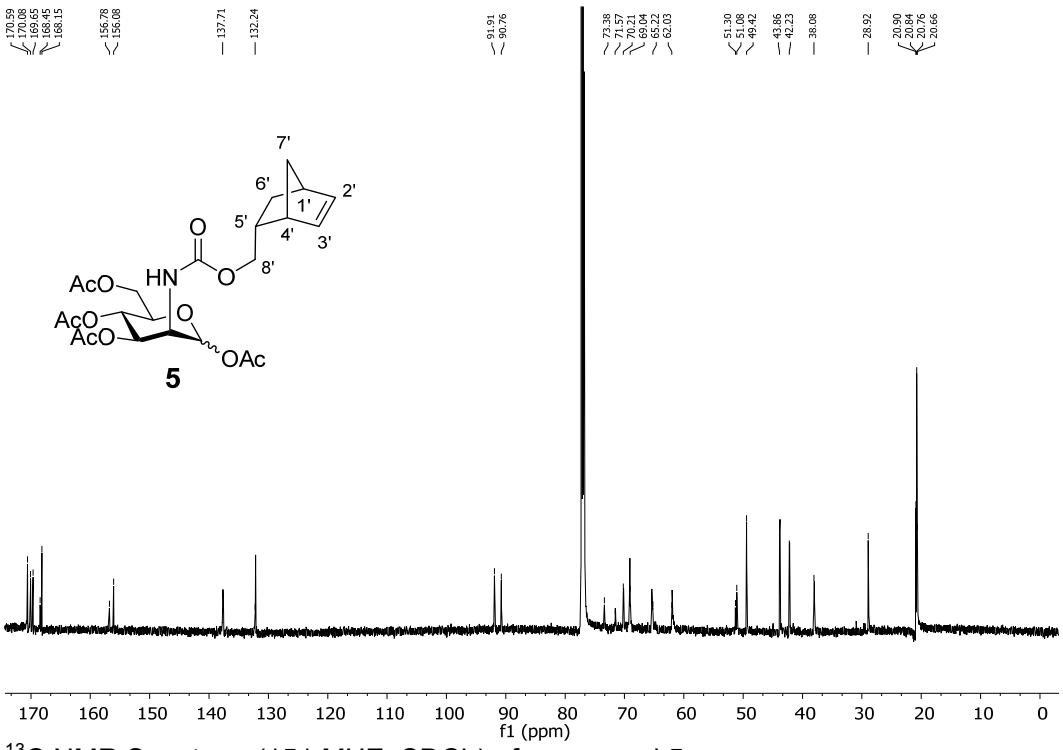
^{1}H NMR Spectrum (400.1 MHZ, $CDCl_3$) of compound **4** (α-anomer).



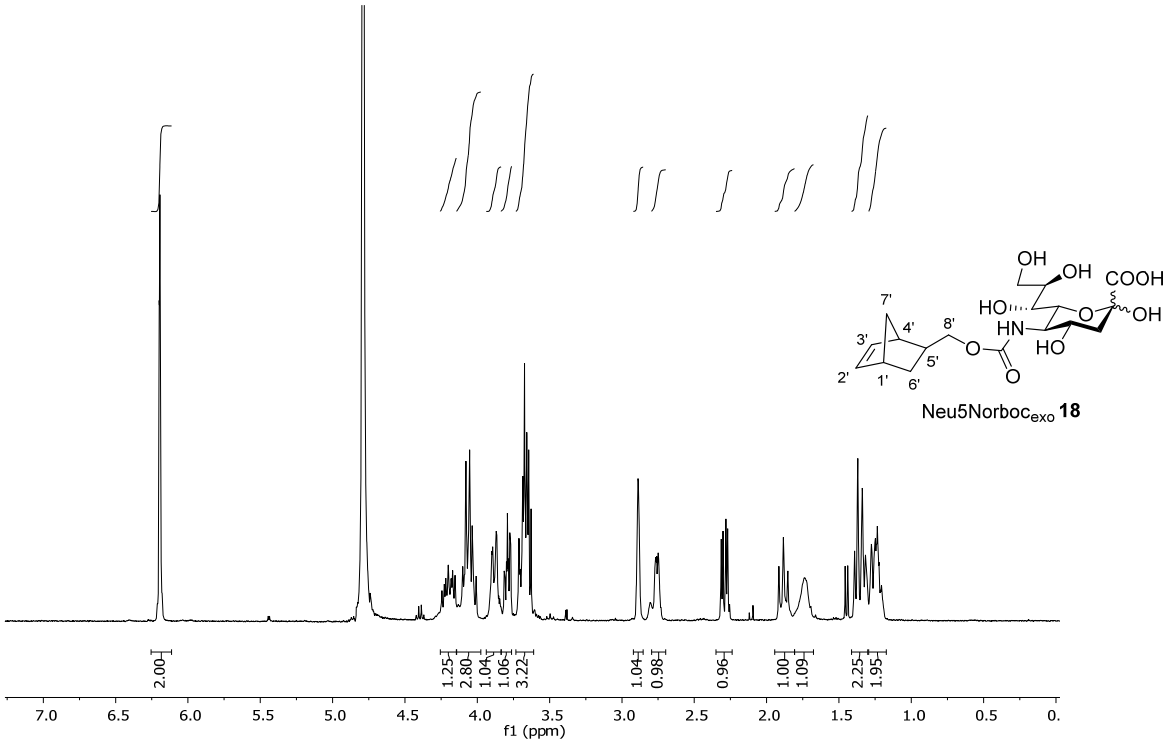
^{13}C NMR Spectrum (400.1 MHz, $CDCl_3$) of compound **4** (α-anomer).



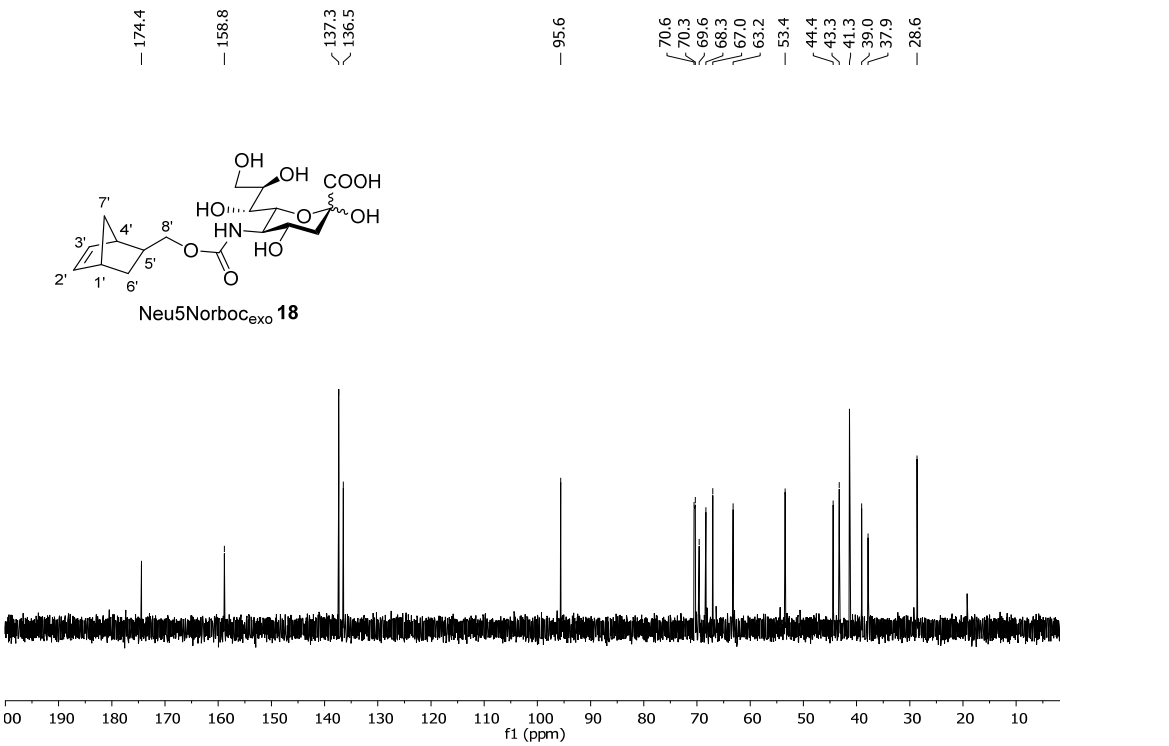
^{1}H NMR Spectrum (600 MHz, $CDCl_3$) of compound **5**.



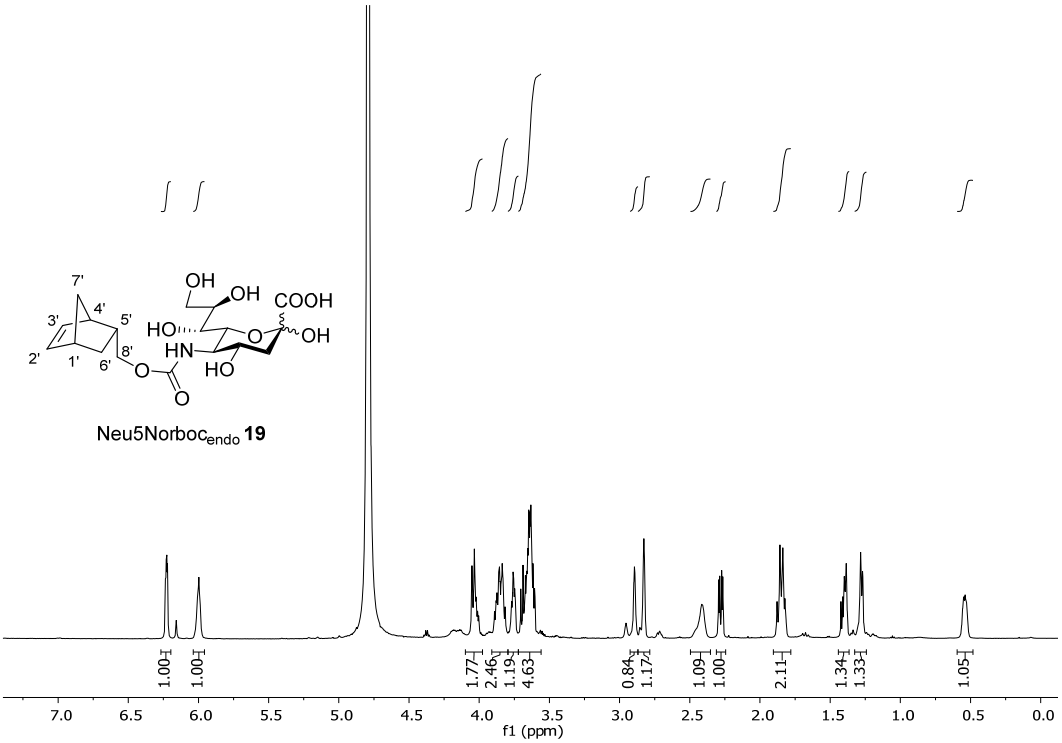
^{13}C NMR Spectrum (151 MHZ, $CDCl_3$) of compound **5**.



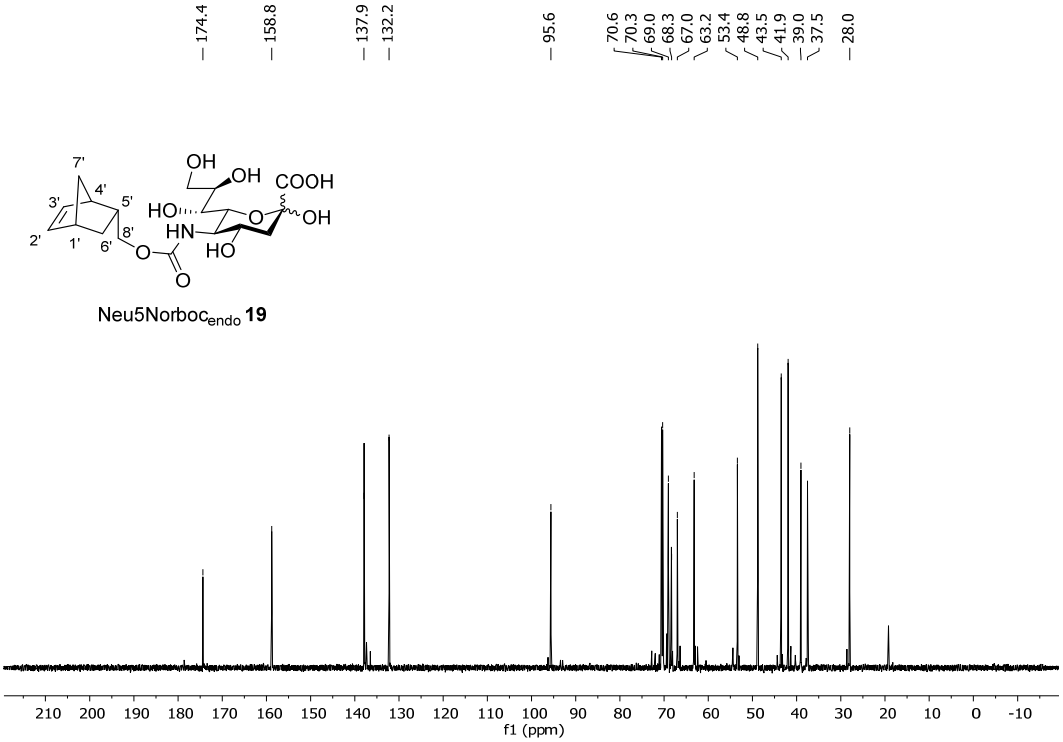
^{1}NMR Spectrum (400.1 MHz, D_2O) of compound **18**.



^{13}C NMR Spectrum (151 MHz, D_2O) of compound **18**.



^{1}NMR Spectrum (600 MHz, D_2O) of compound **19**.



^{13}C NMR Spectrum (151 MHz, D_2O) of compound **19**.