BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Supporting Information

for

Cyclopropene derivatives of aminosugars for metabolic glycoengineering

Jessica Hassenrück and Valentin Wittmann

Beilstein J. Org. Chem. **2019,** *15,* 584–601. doi:10.3762/bjoc.15.54

Additional figures and ^{1}H and ^{13}C NMR spectra of new compounds

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Additional confocal fluorescence microscopy images

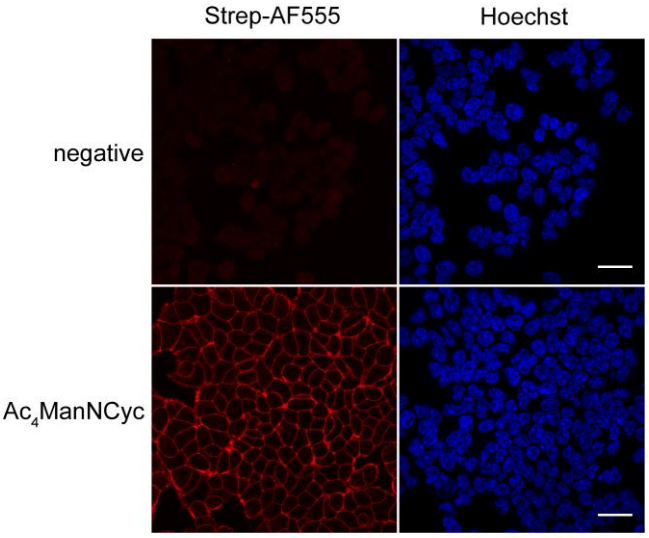
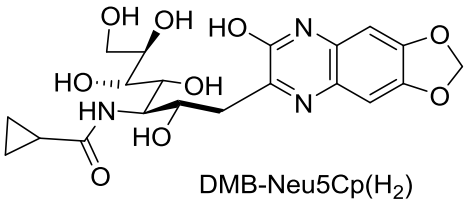


Figure S1: HEK 293T cells were grown with 100 μM Ac_4ManNCyc or DMSO only (negative control) for 48 h. Cells were incubated with Tz-biotin (100 μM) for 1 h at 37 °C followed by incubation with streptavidin-AlexaFluor 555. Nuclei were stained with Hoechst 33342. Scale bar: 30 μm.

RP-HPLC analysis of DMB-labeled sialic acids



DMB-Neu5Cp(H_2)

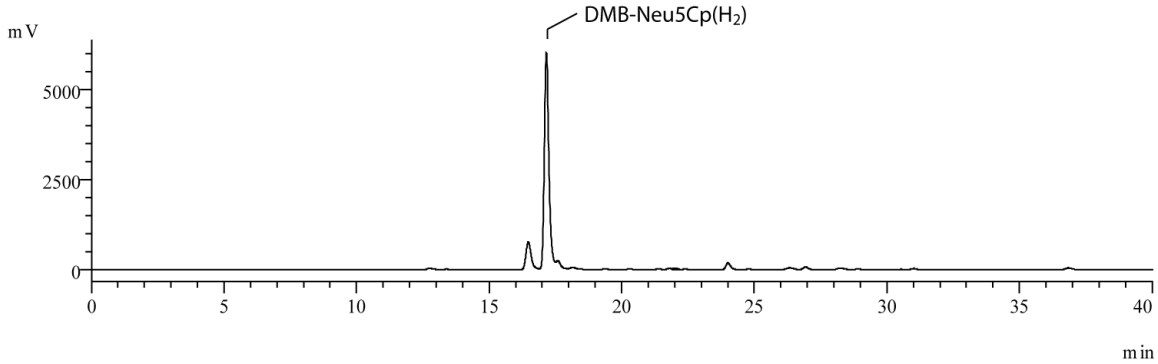
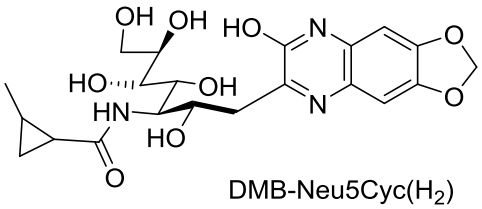


Figure S2: Analysis of DMB-Neu5Cp(H_2) by RP-HPLC (10–25% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). t_R = 17.2 min.



DMB-Neu5Cyc(H_2)

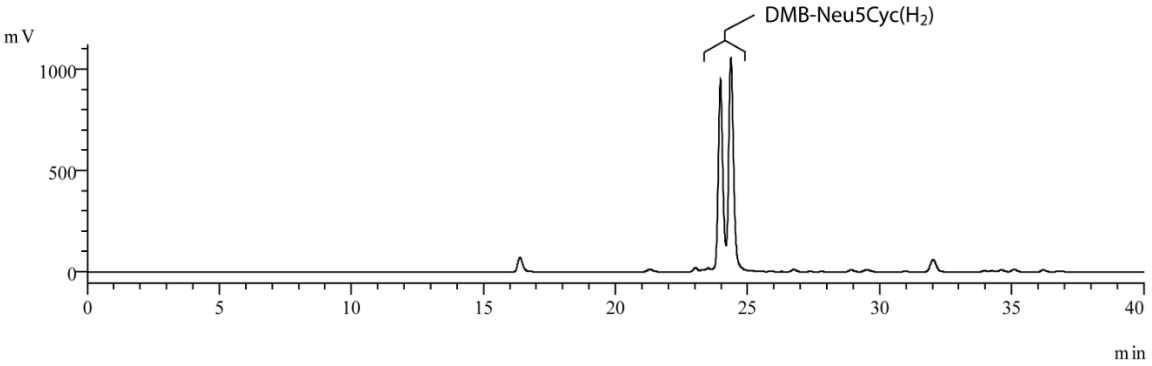


Figure S3: Analysis of DMB-Neu5Cyc(H_2) by RP-HPLC (10–25% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). t_R = 24.0; 24,4 min.

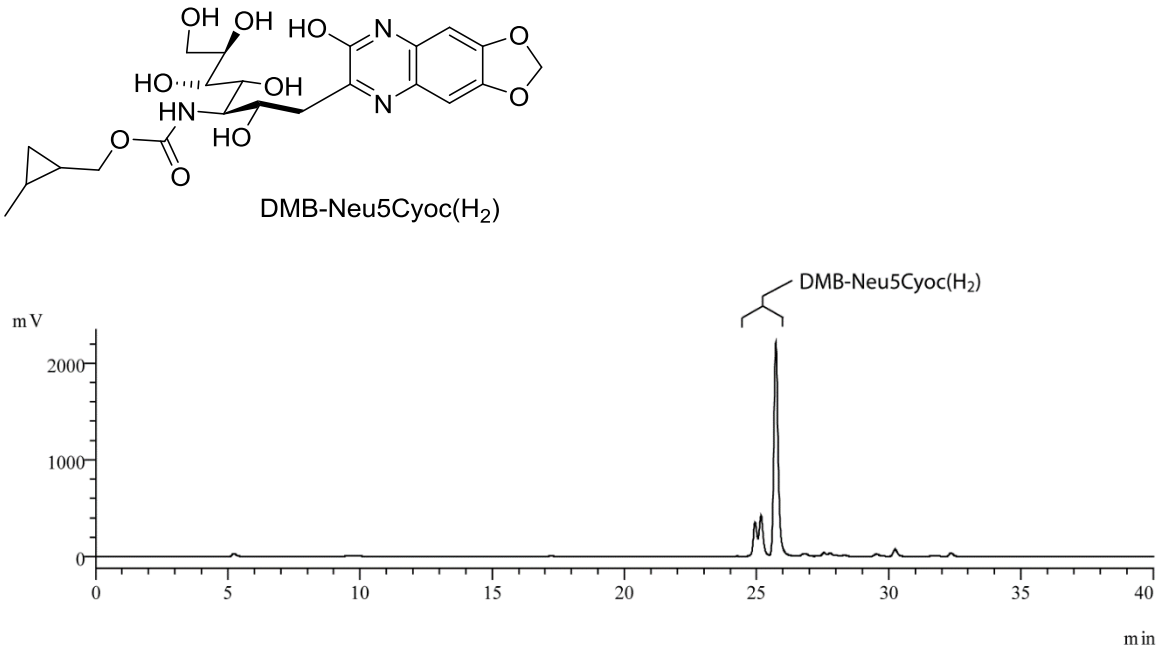


Figure S4: Analysis of DMB-Neu5Cyoc(H_2) by RP-HPLC (10–40% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). t_R = 24.9; 25.2; 25.7 min.

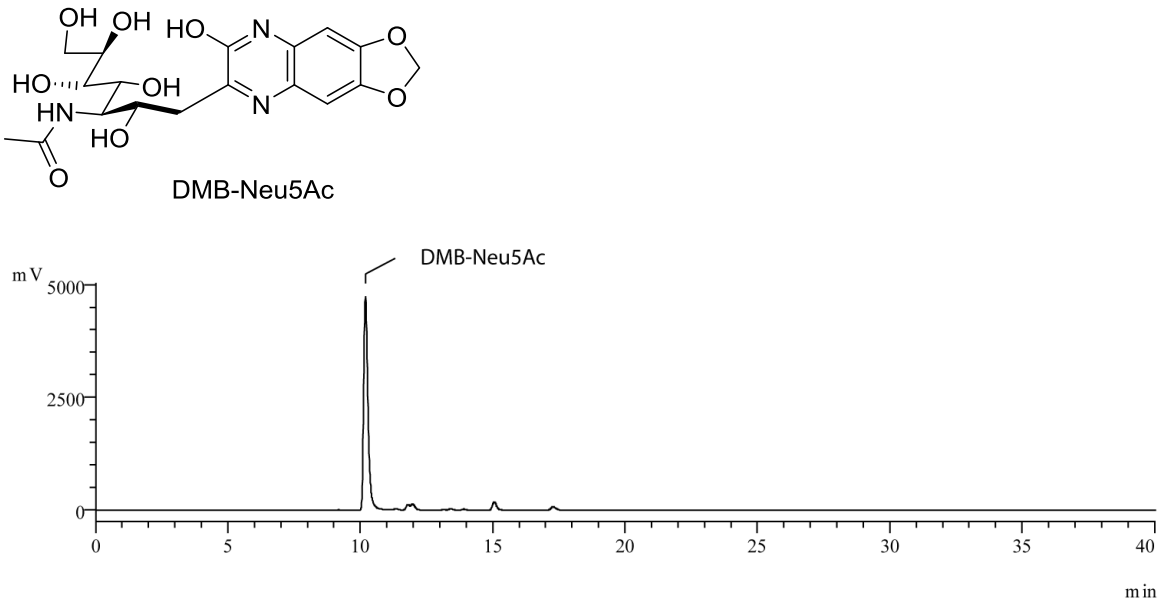


Figure S5: Analysis of DMB-Neu5Ac by RP-HPLC (10–25% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). t_R = 10.2 min.

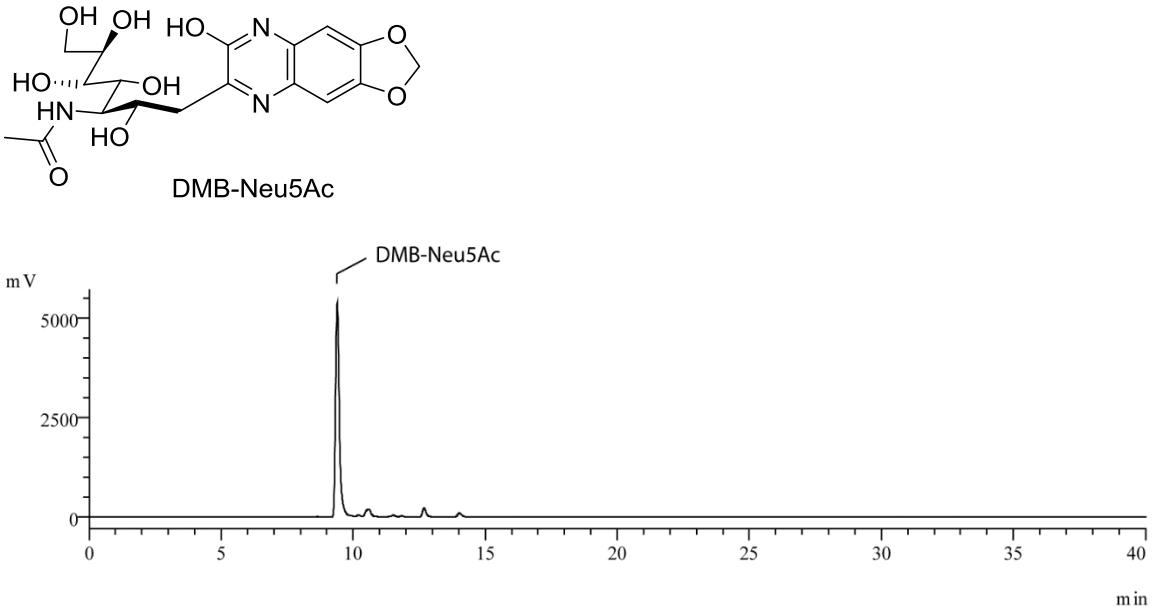
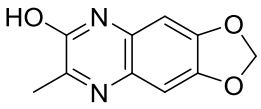


Figure S6: Analysis of DMB-Neu5Ac by RP-HPLC (10–40% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). t_R = 9.4 min.



DMB-Pyruvate

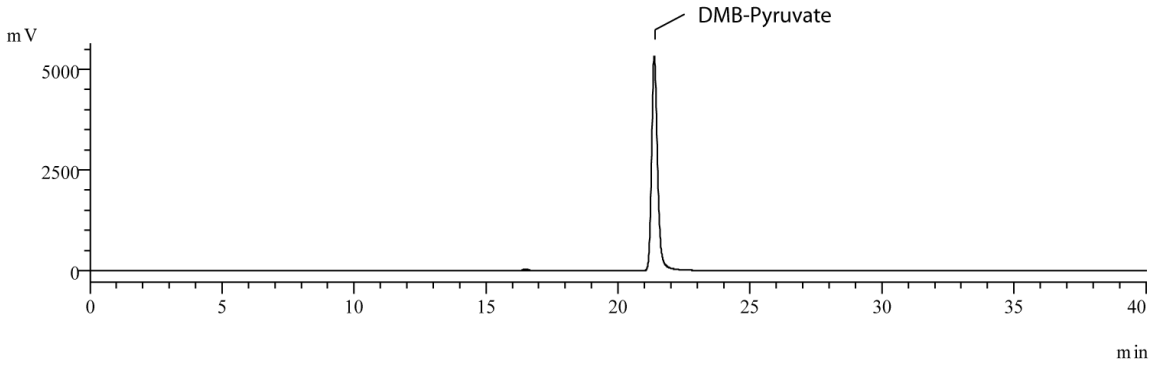
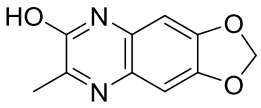


Figure S7: Analysis of DMB-Pyruvate by RP-HPLC (10–25% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). t_R = 21.4 min.



DMB-Pyruvate

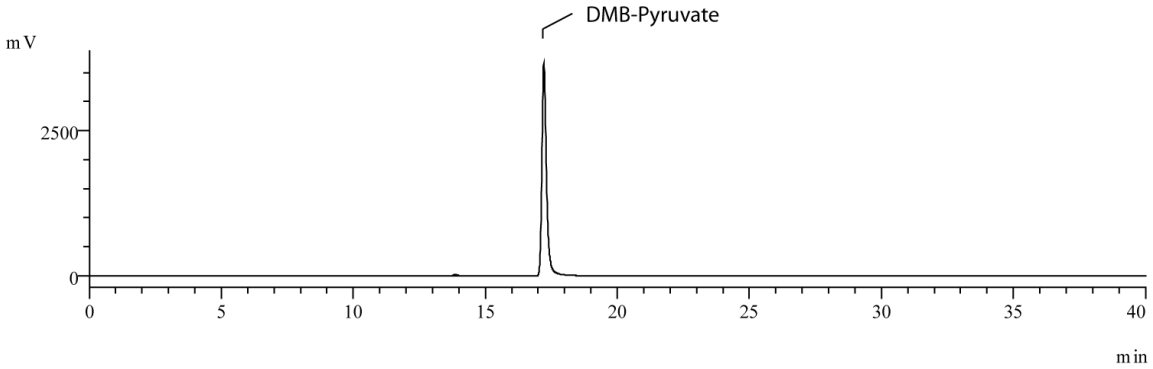


Figure S8: Analysis of DMB-Pyruvat by RP-HPLC (10–40% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). t_R = 17.2 min.

RP-HPLC analysis of DMB-labeled sialic acids released from engineered cells

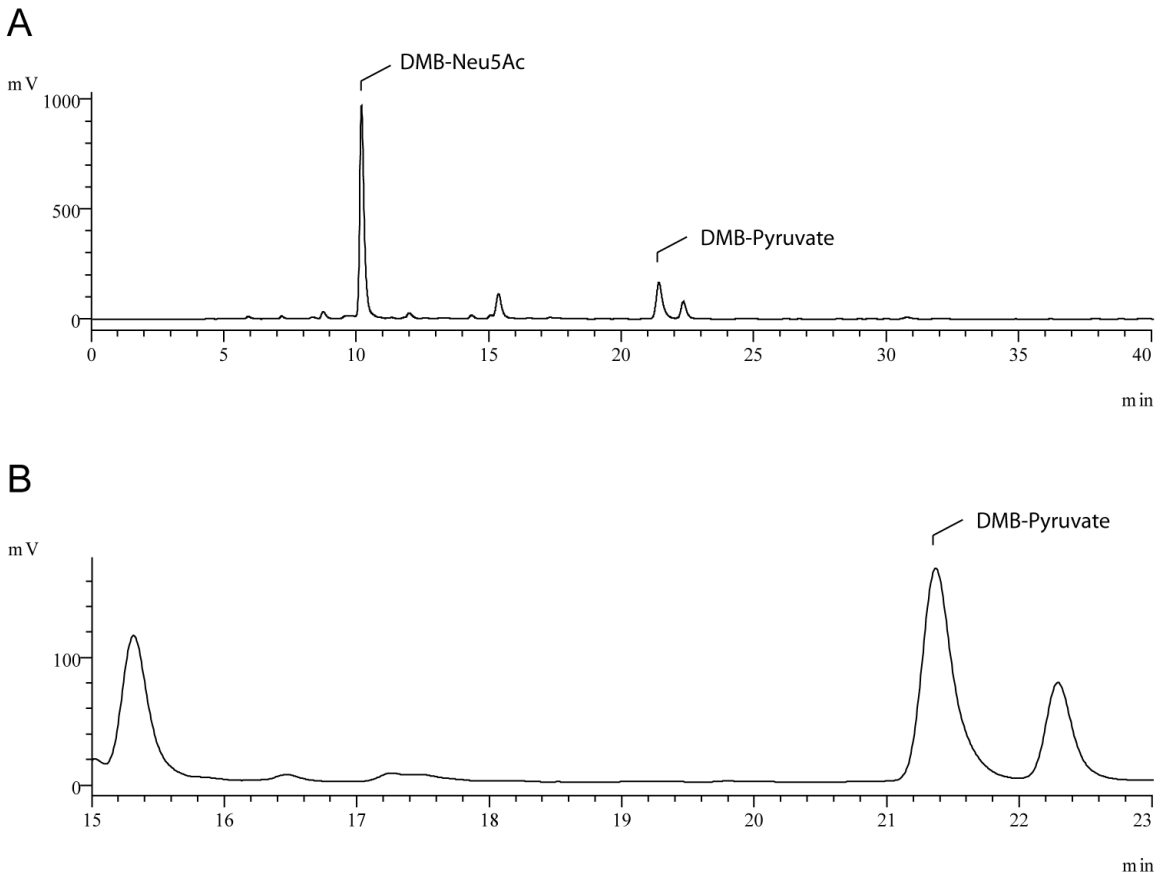


Figure S9: Analysis of DMB-labeled sialic acids released from cells grown without additional sugar (DMSO only, solvent control) by RP-HPLC (10–25% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). A) Complete chromatogram. B) Enlarged region.

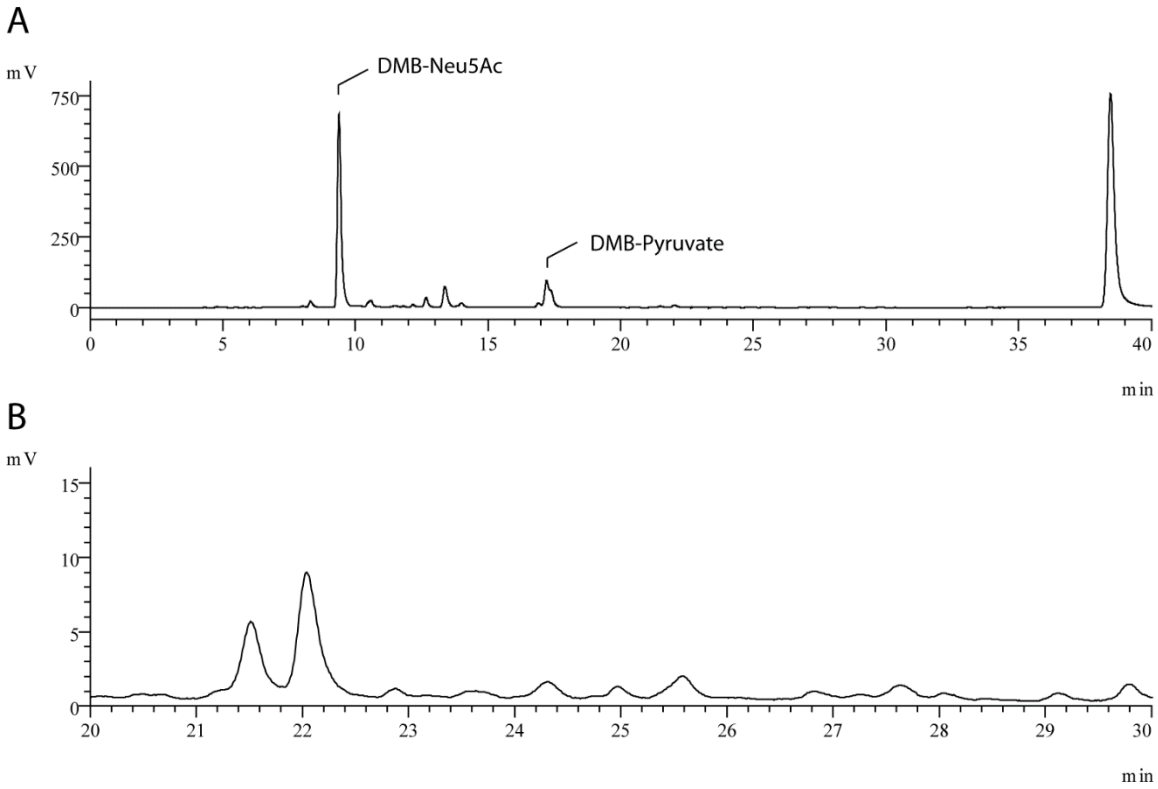


Figure S10: Analysis of DMB-labeled sialic acids released from cells grown without additional sugar (DMSO only, solvent control) by RP-HPLC (10–40% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). A) Complete chromatogram. B) Enlarged region.

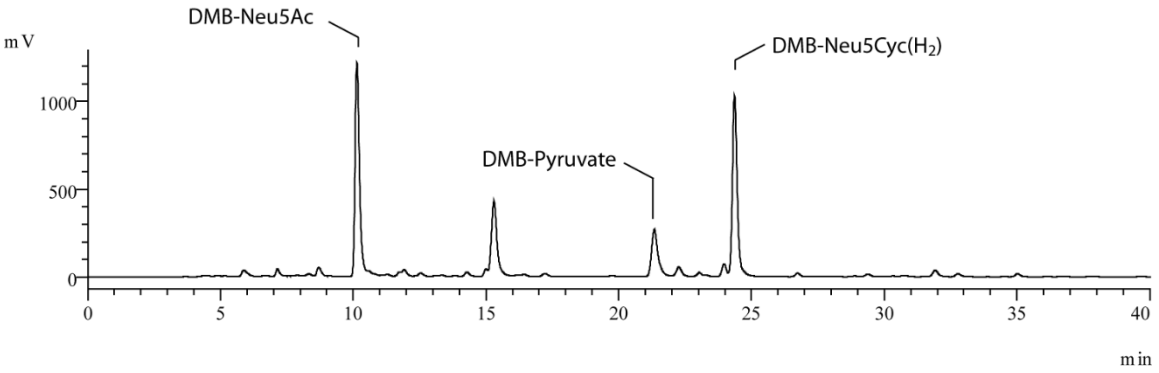


Figure S11: Analysis of DMB-labeled sialic acids released from cells grown with $Ac_4ManNCyc(H_2)$ by RP-HPLC (10–25% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm).

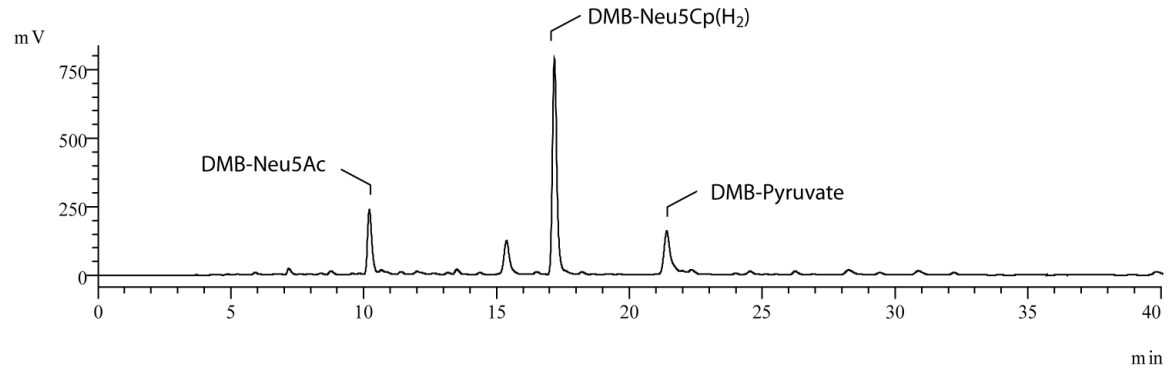


Figure S12: Analysis of DMB-labeled sialic acids released from cells grown with $Ac_4ManNCp(H_2)$ by RP-HPLC (10–25% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm).

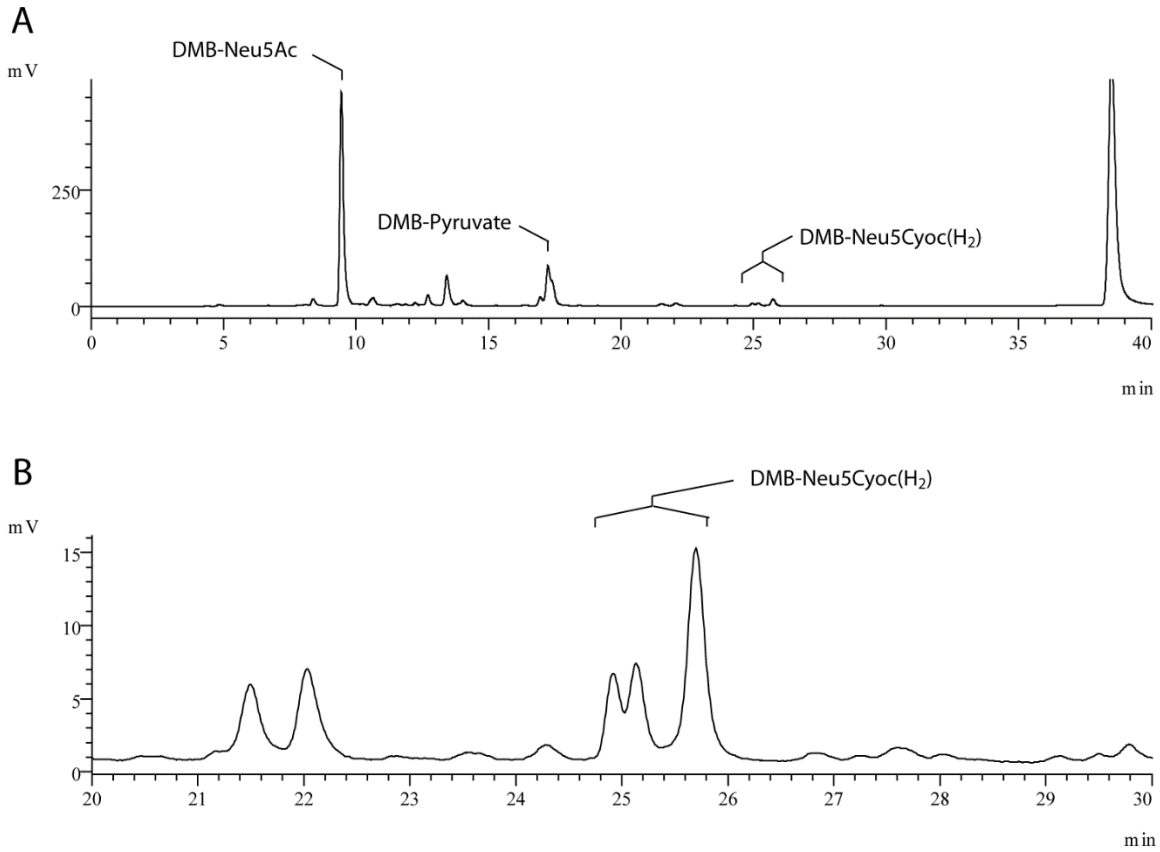


Figure S13: Analysis of DMB-labeled sialic acids released from cells grown with $Ac_4ManNCyoc(H_2)$ by RP-HPLC (10–40% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). A) Complete chromatogram. B) Enlarged region.

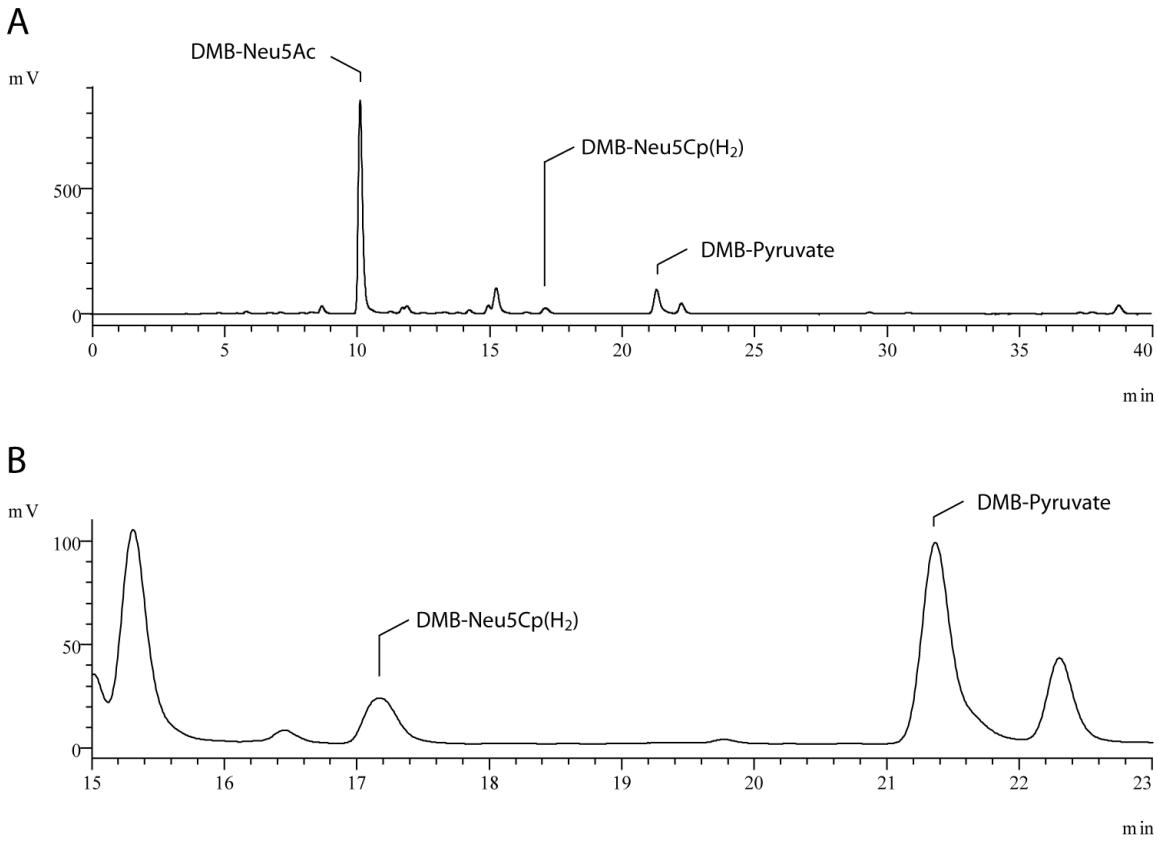


Figure S14: Analysis of DMB-labeled sialic acids released from cells grown with $Ac_4GlcNCp(H_2)$ by RP-HPLC (10–25% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). A) Complete chromatogram. B) Enlarged region.

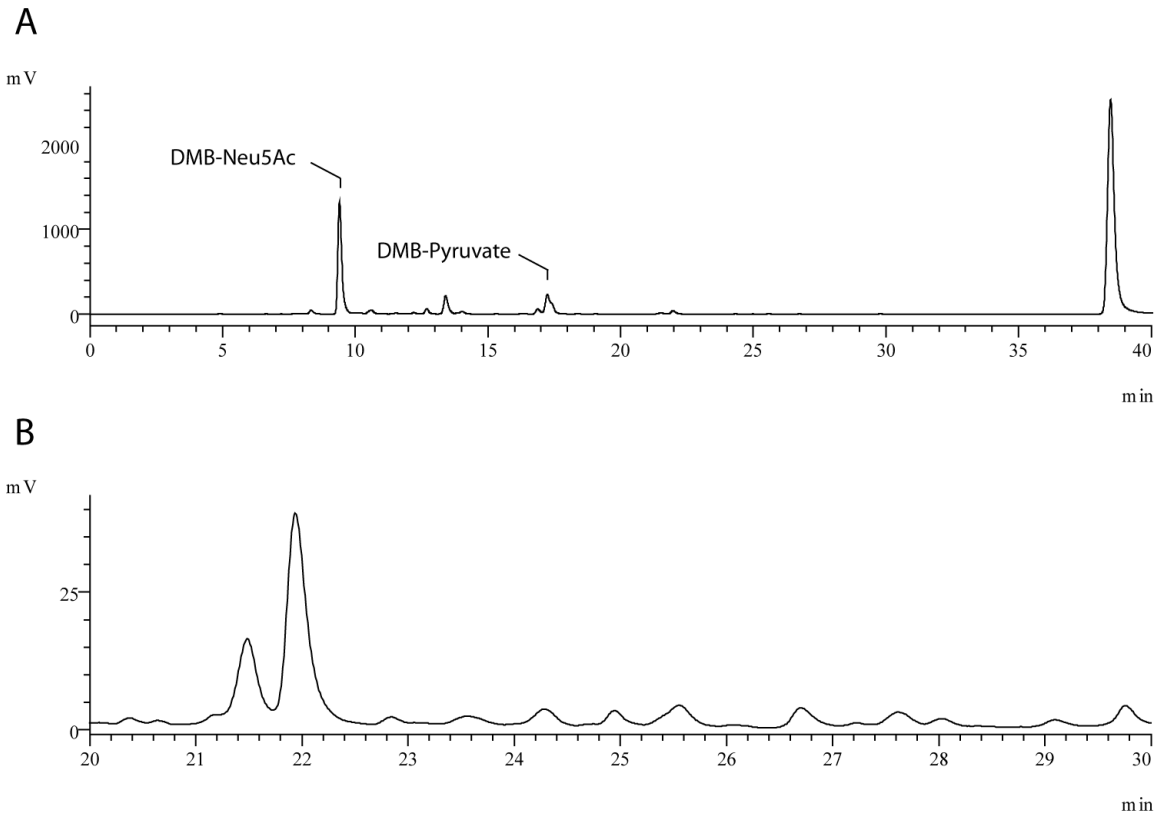
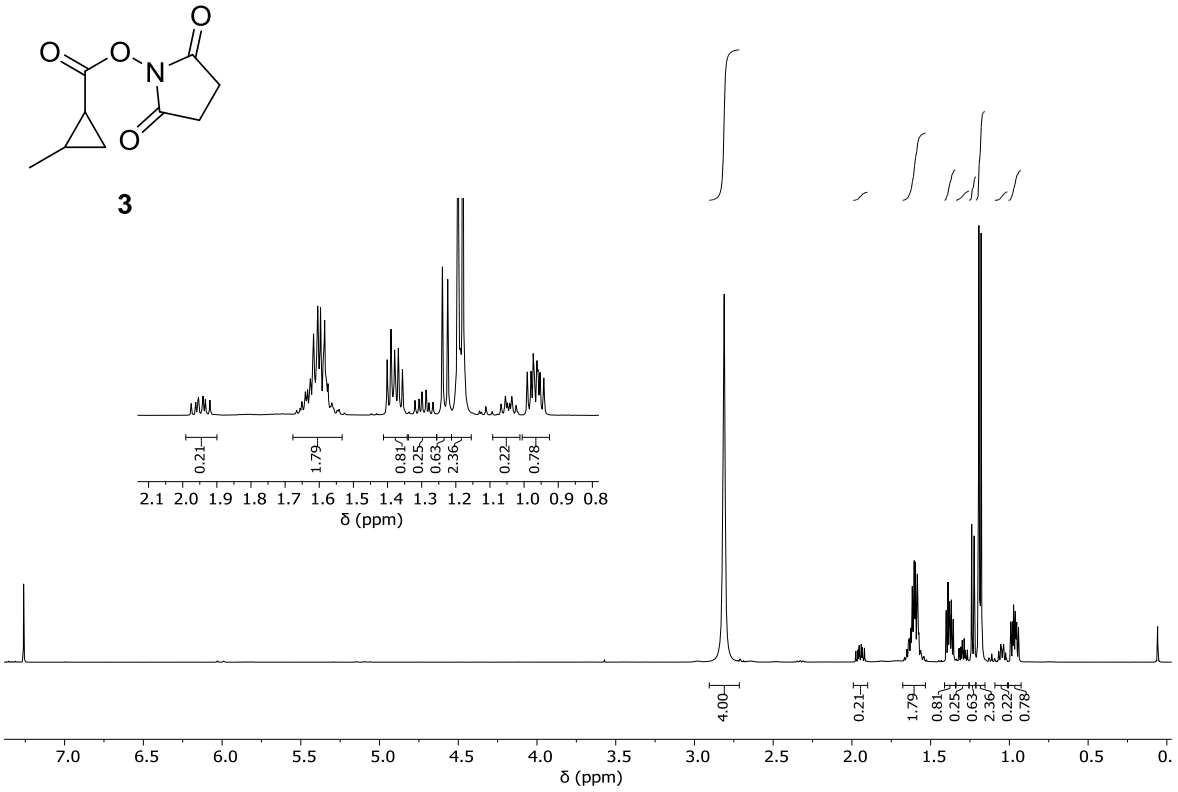
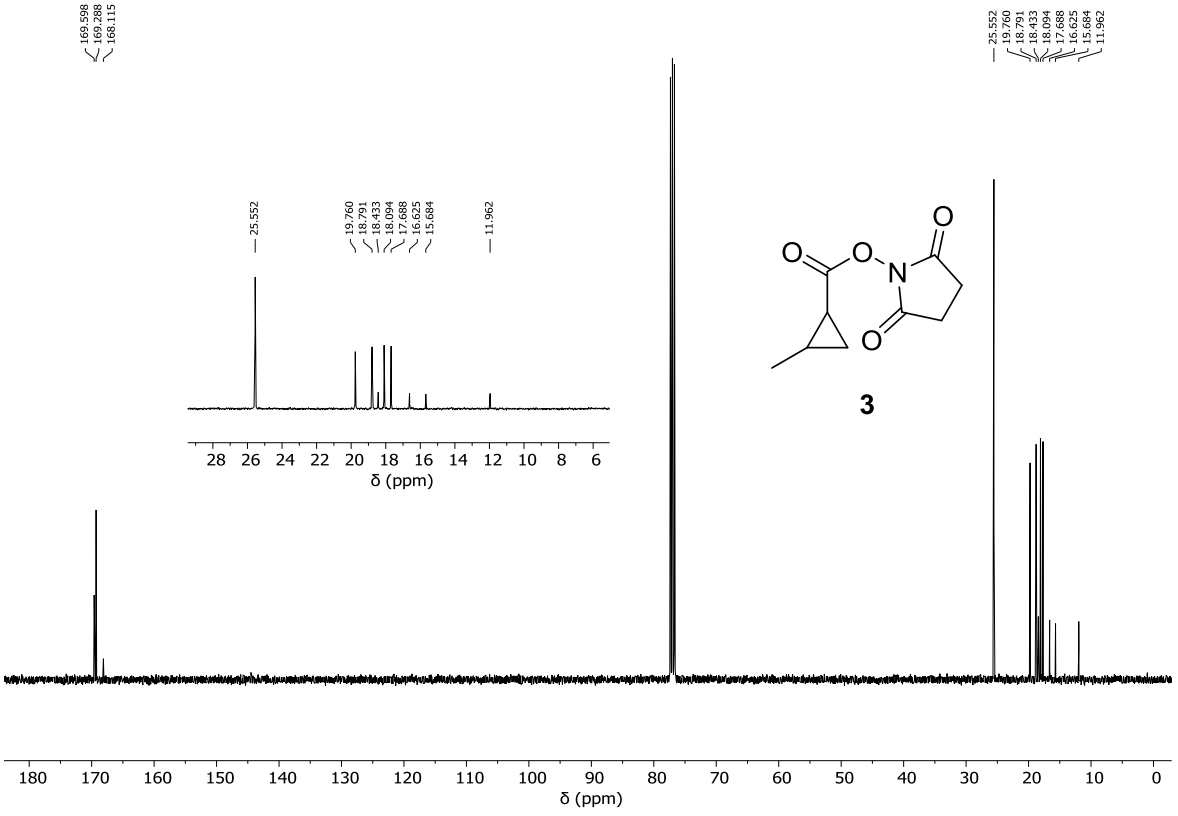


Figure S15: Analysis of DMB-labeled sialic acids released from cells grown with $Ac_4GlcNCyoc(H_2)$ by RP-HPLC (10–40% B in 40 min) with a fluorescence detector (λ_{ex} = 372 nm, λ_{em} = 456 nm). A) Complete chromatogram. B) Enlarged region.

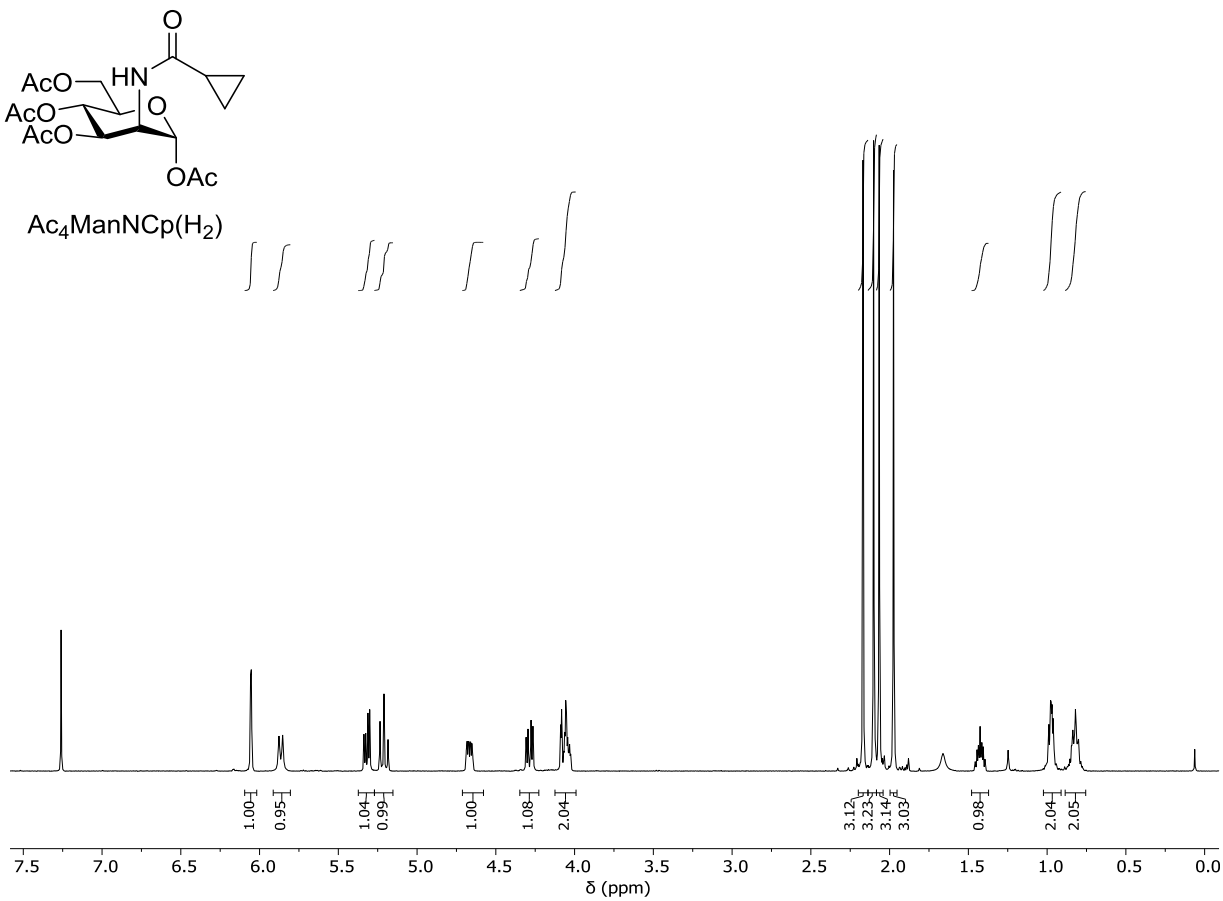
NMR spectra



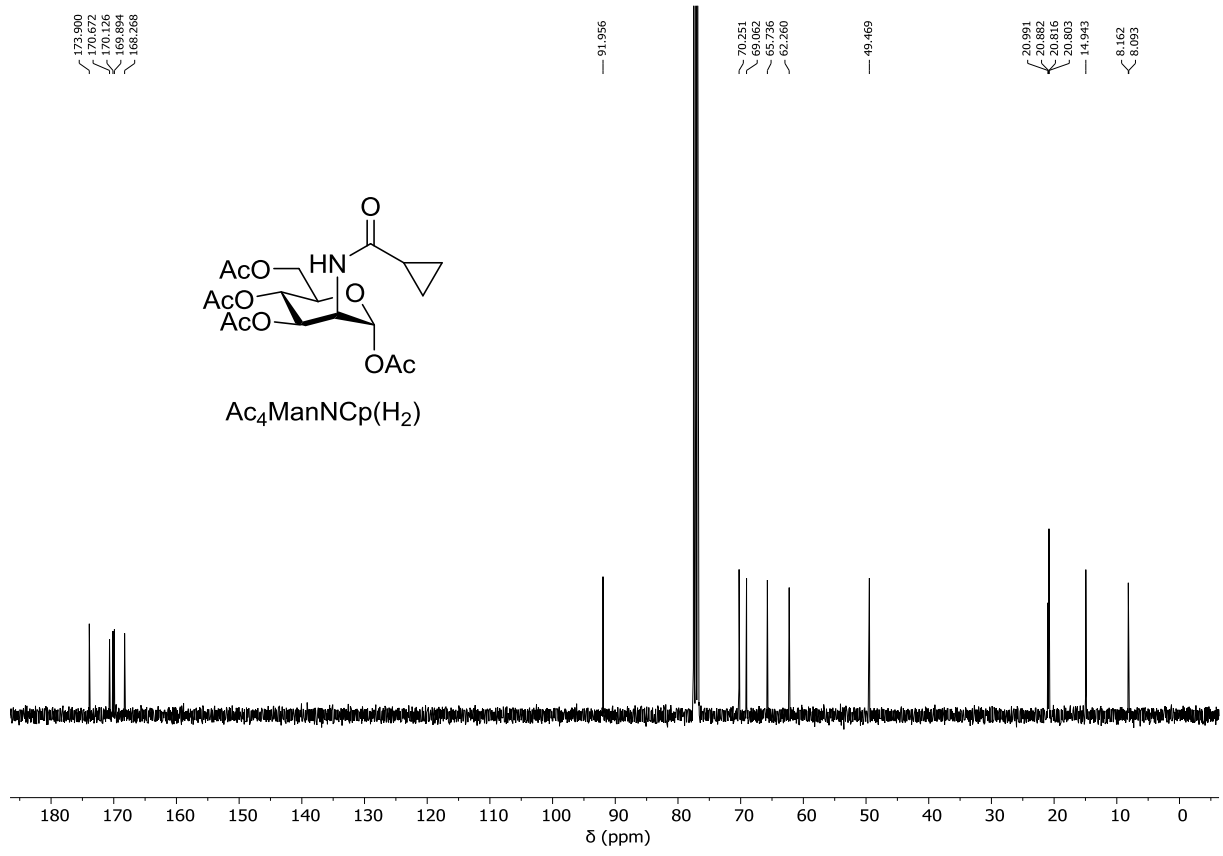
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **3**.



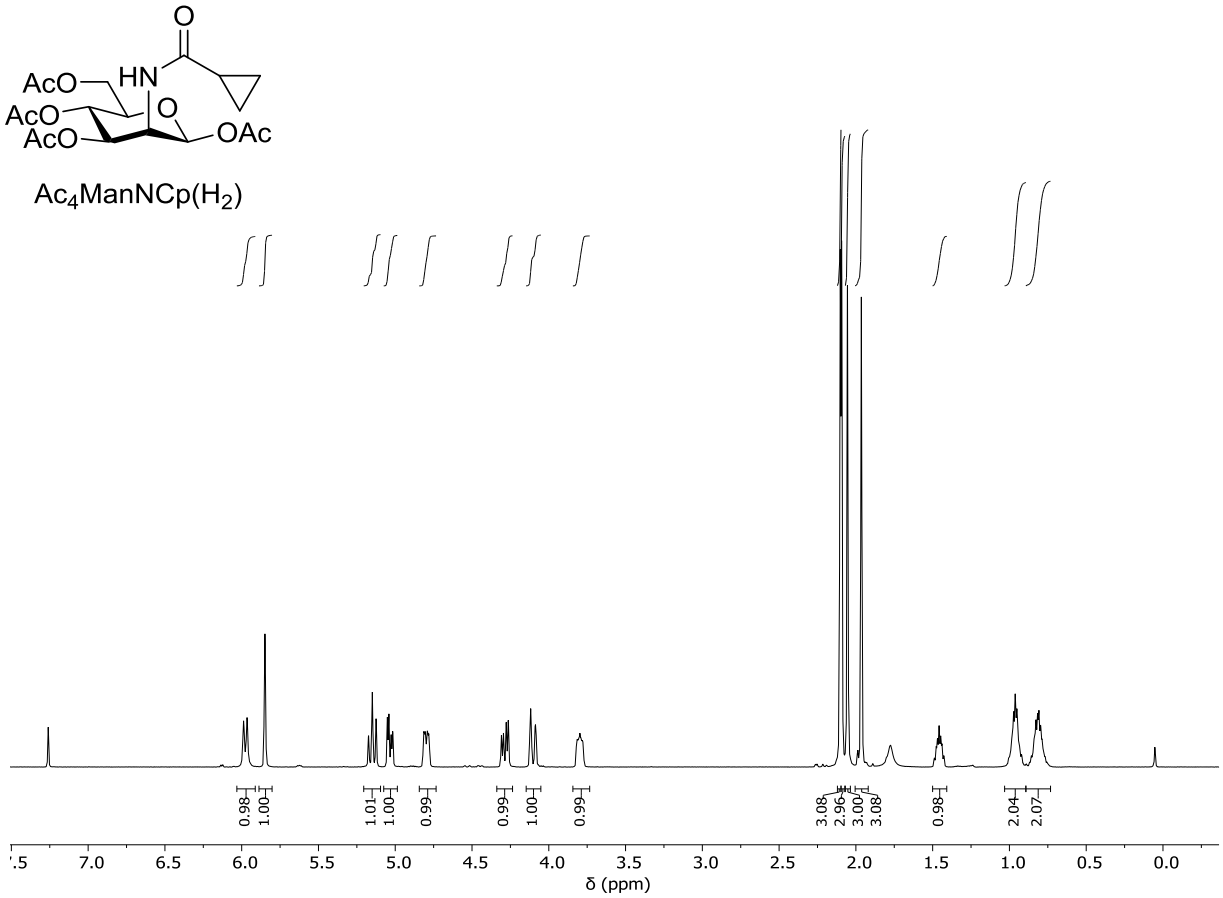
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of **3**.



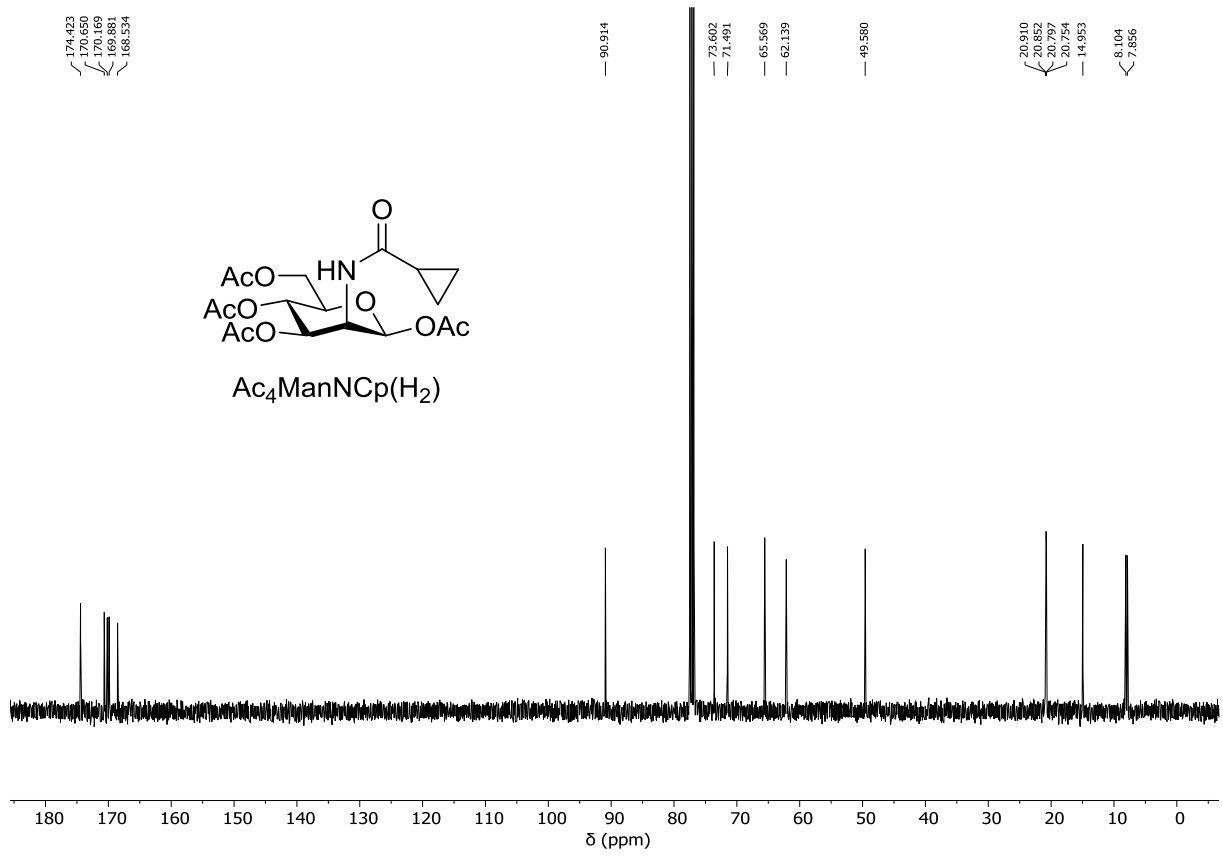
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the α-anomer of $Ac_4ManNCp(H_2)$.



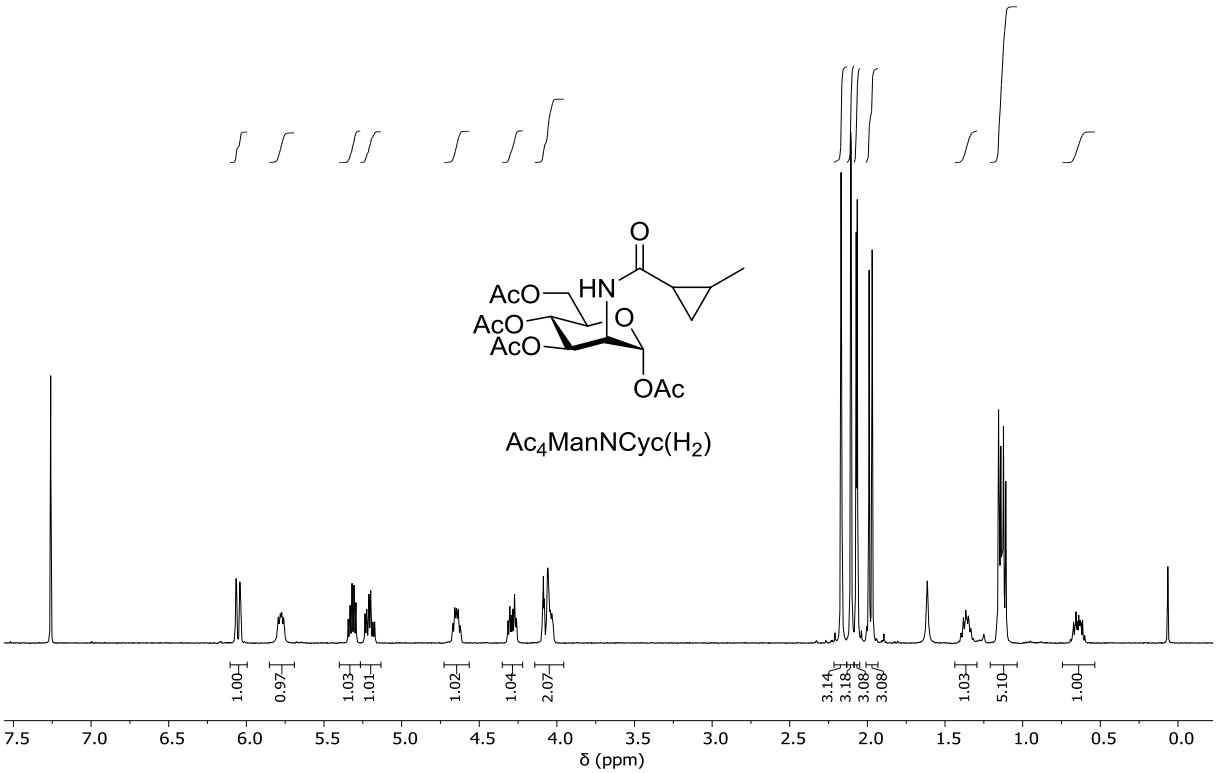
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of the α-anomer of $Ac_4ManNCp(H_2)$.



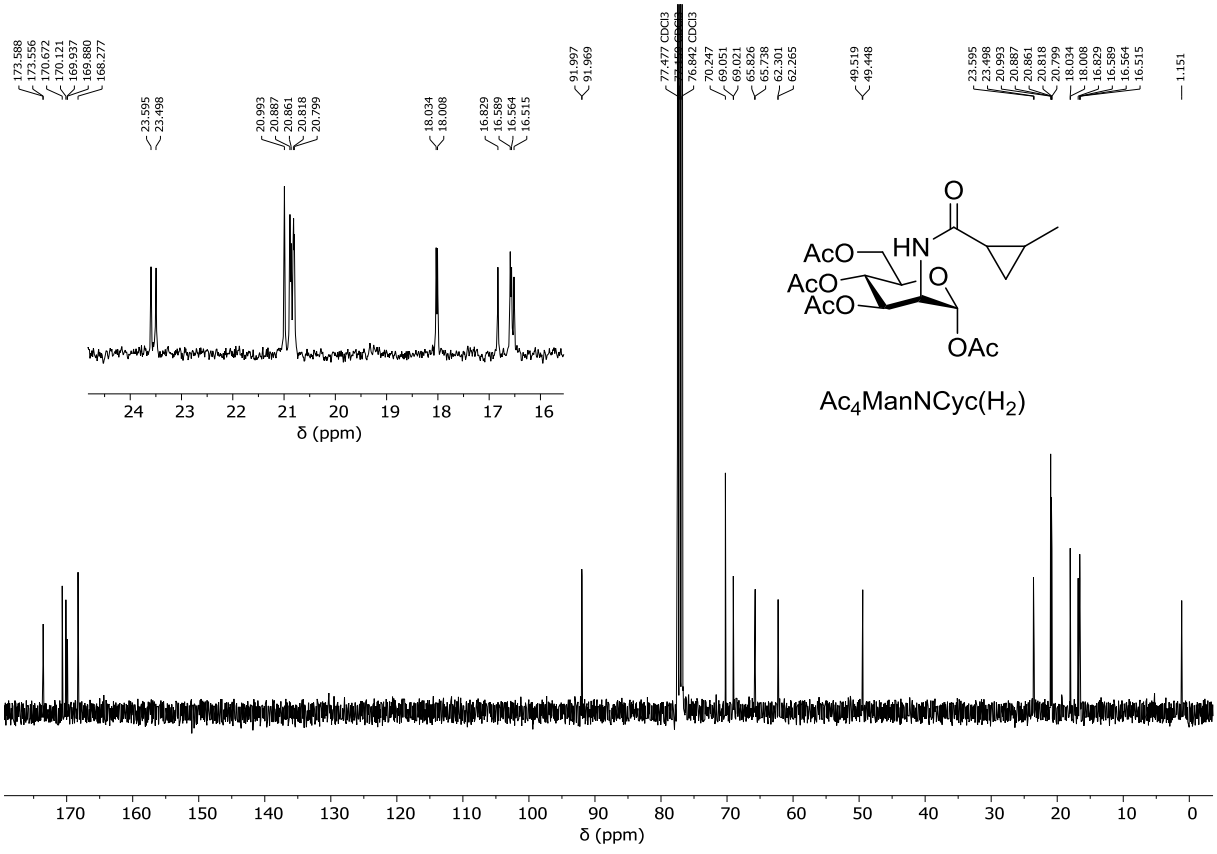
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the β-anomer of $Ac_4ManNCp(H_2)$.



^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of the β-anomer of $Ac_4ManNCp(H_2)$.



^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the α-anomer of $Ac_4ManNCyc(H_2)$.



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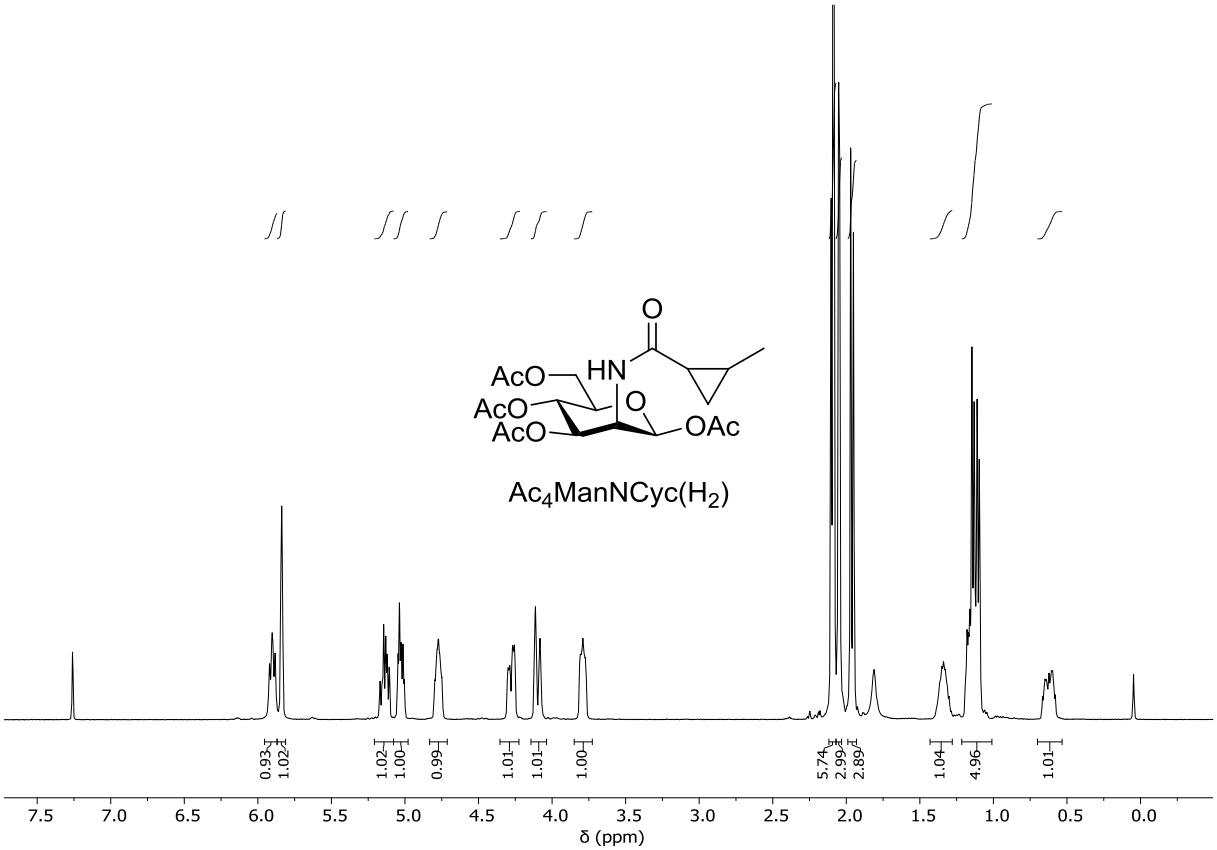

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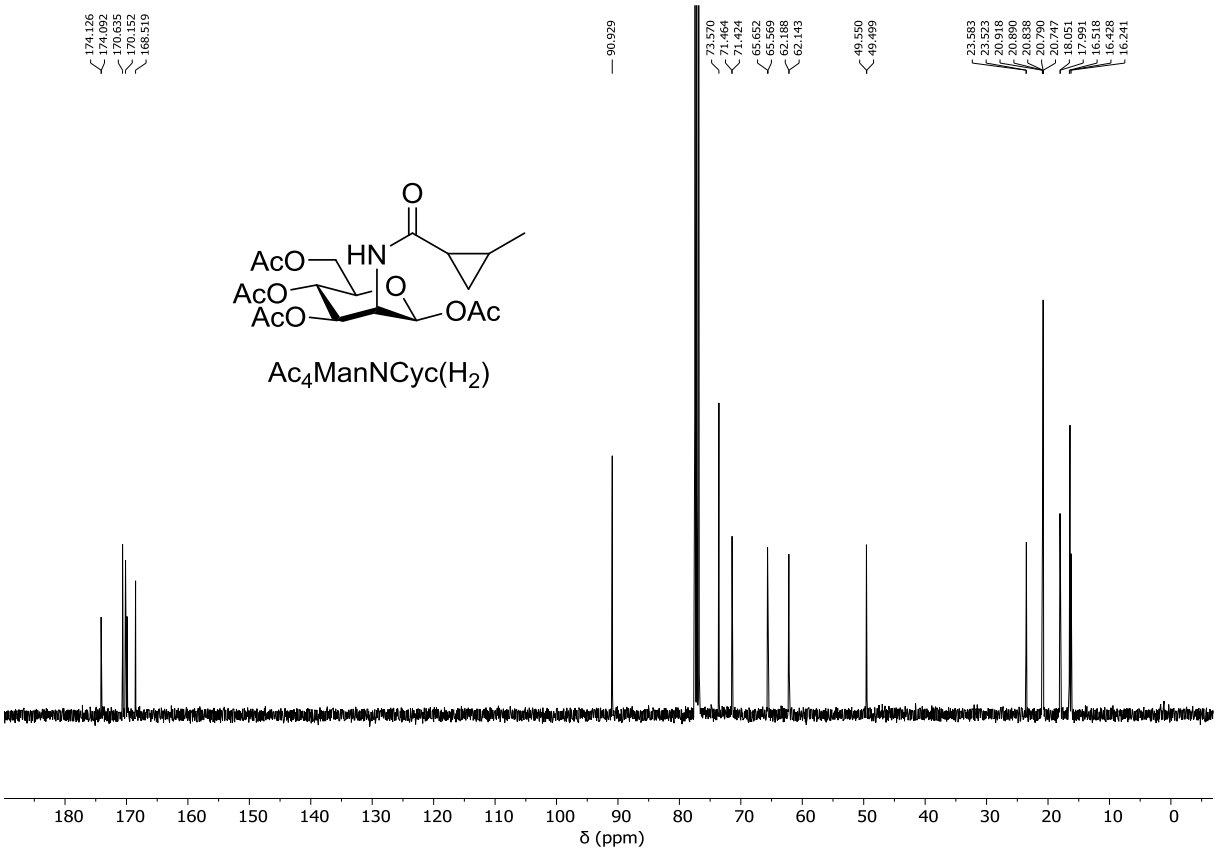

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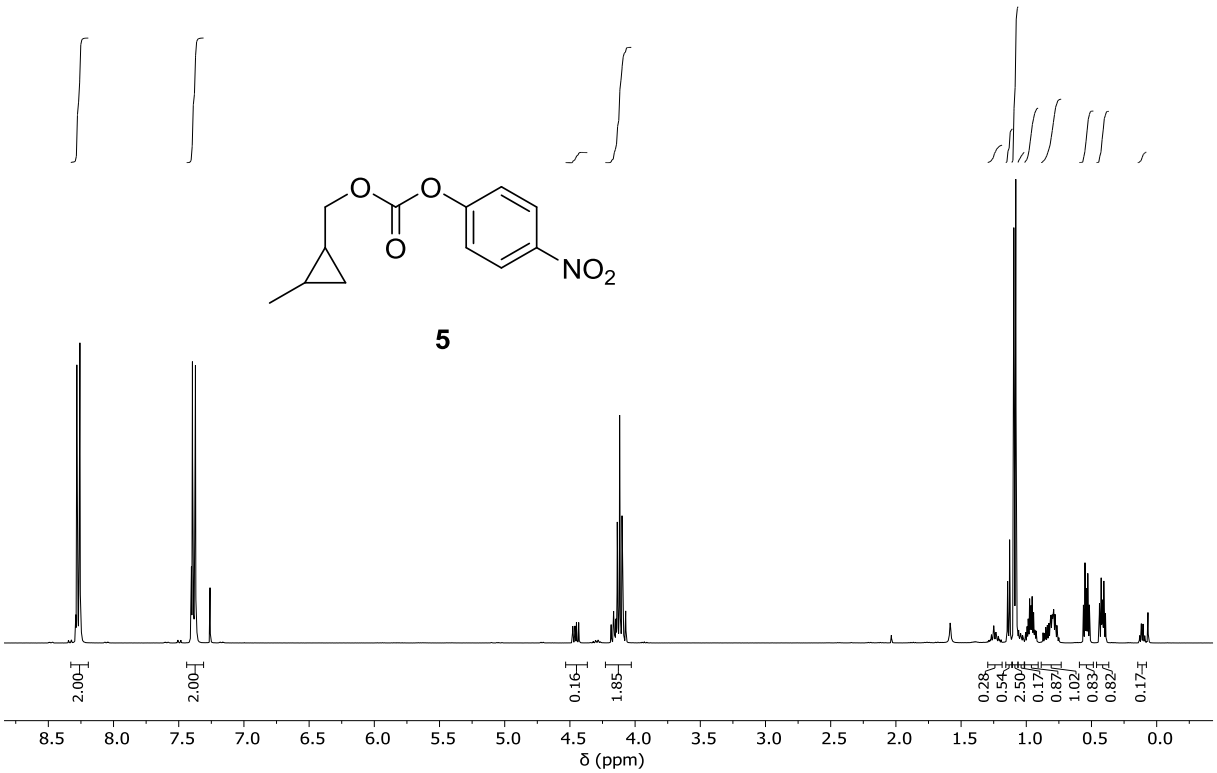
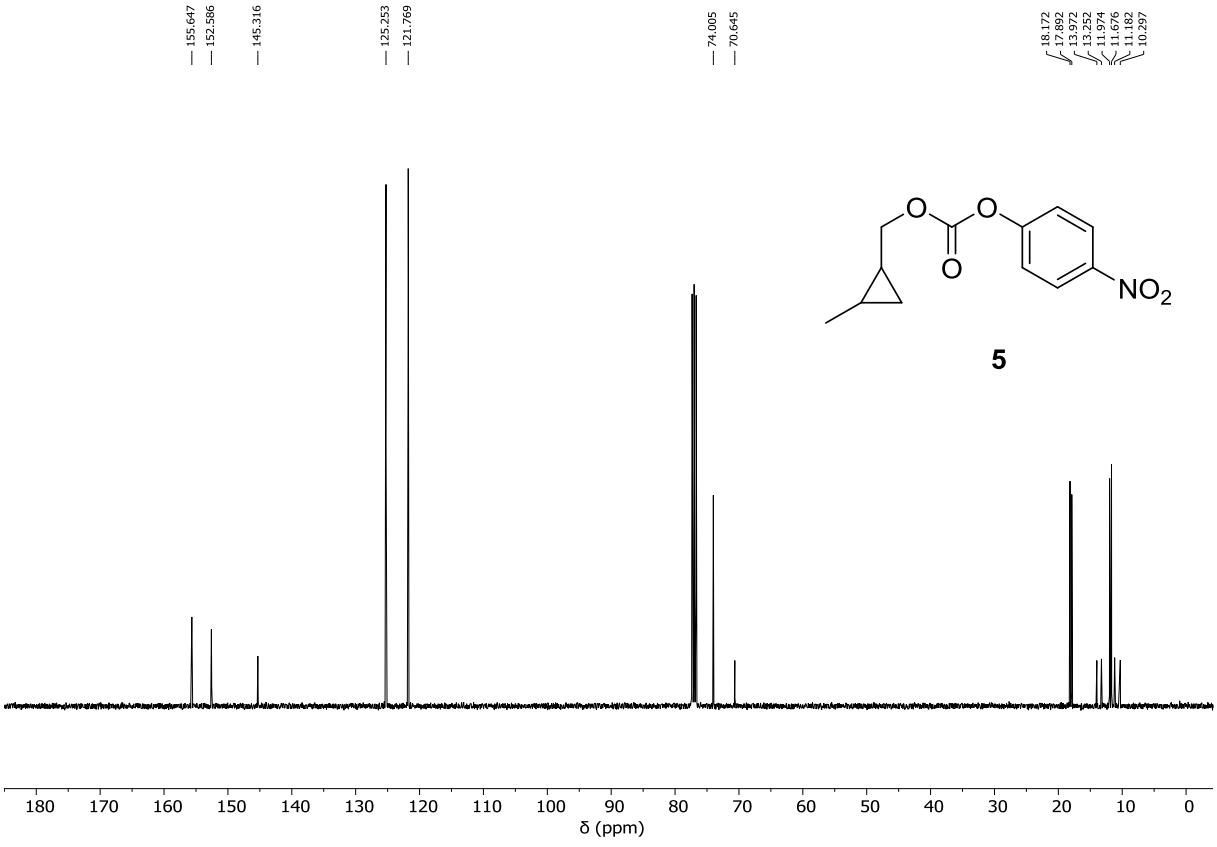


^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **5**.



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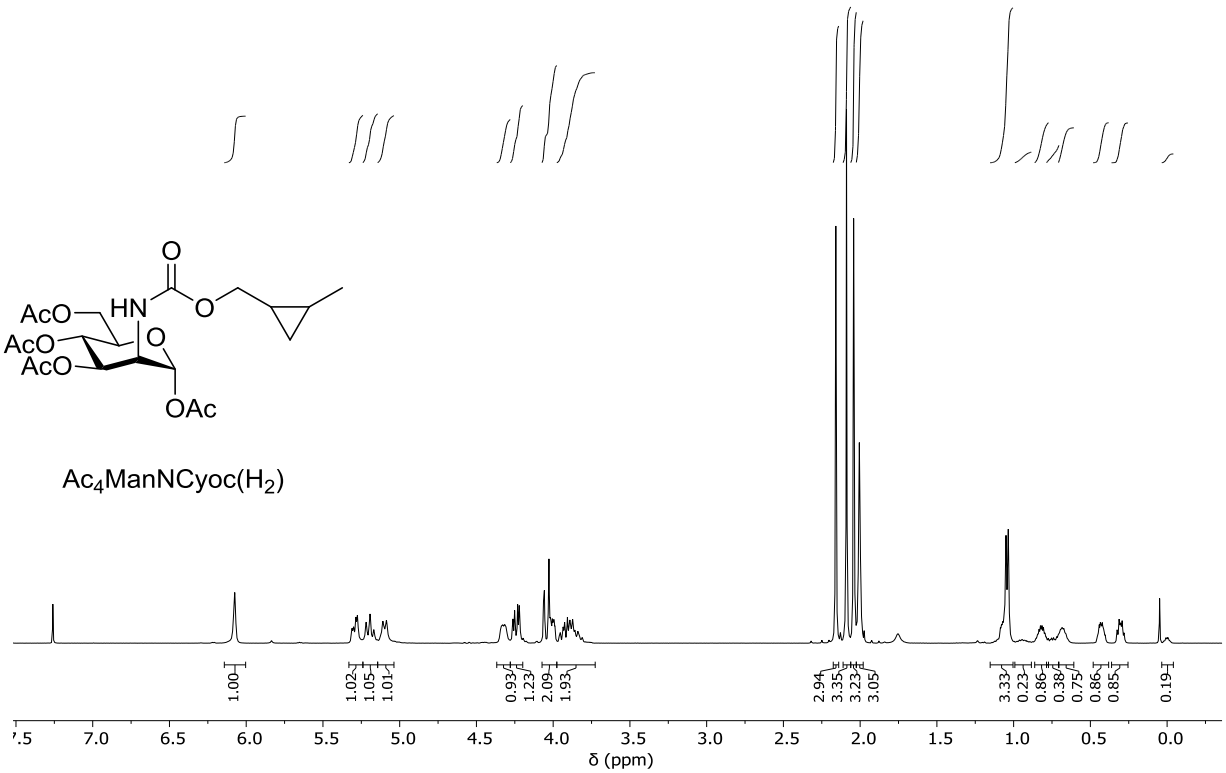

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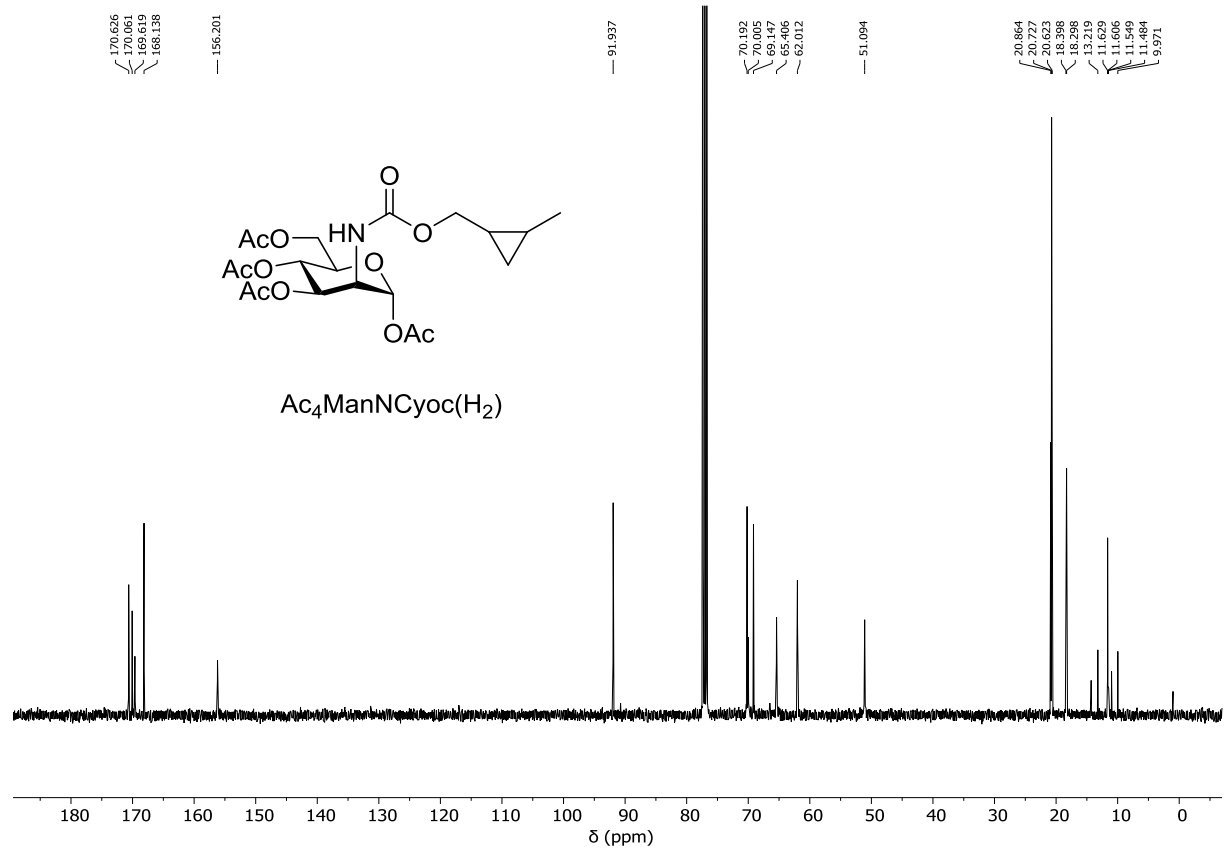

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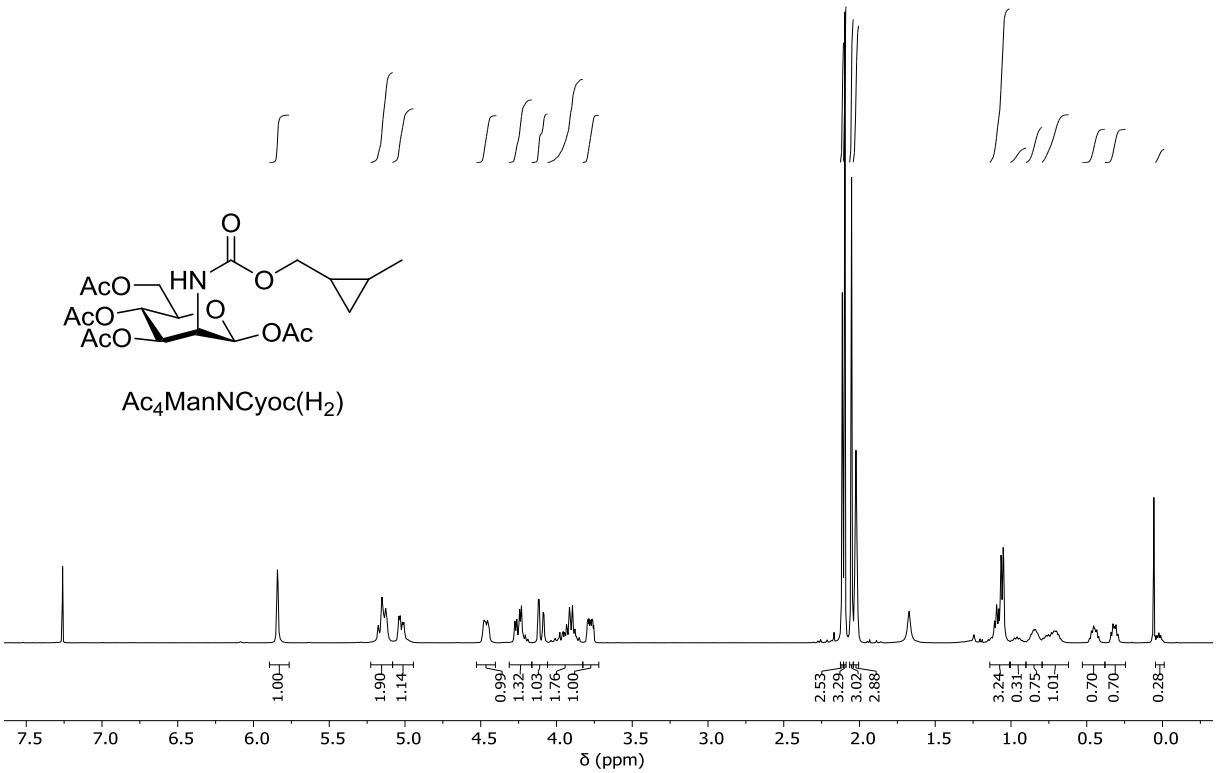

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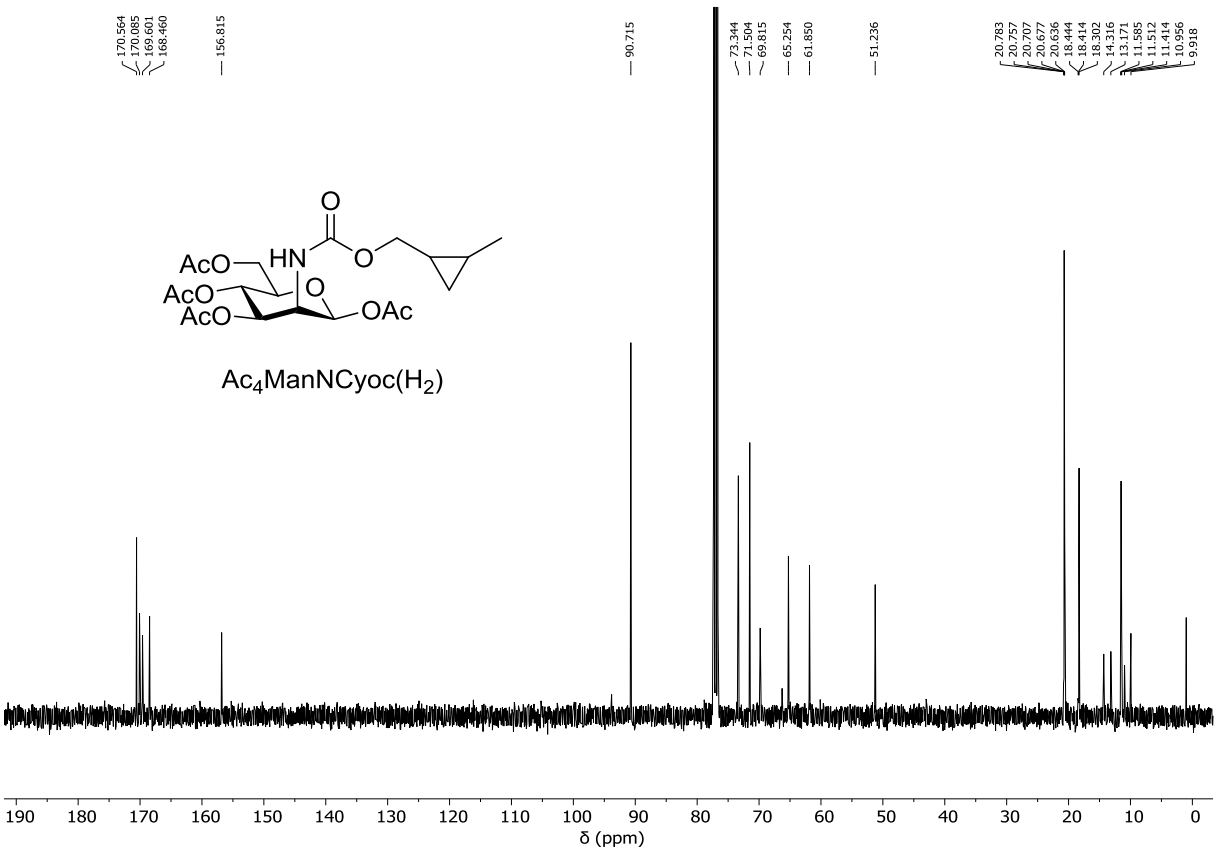
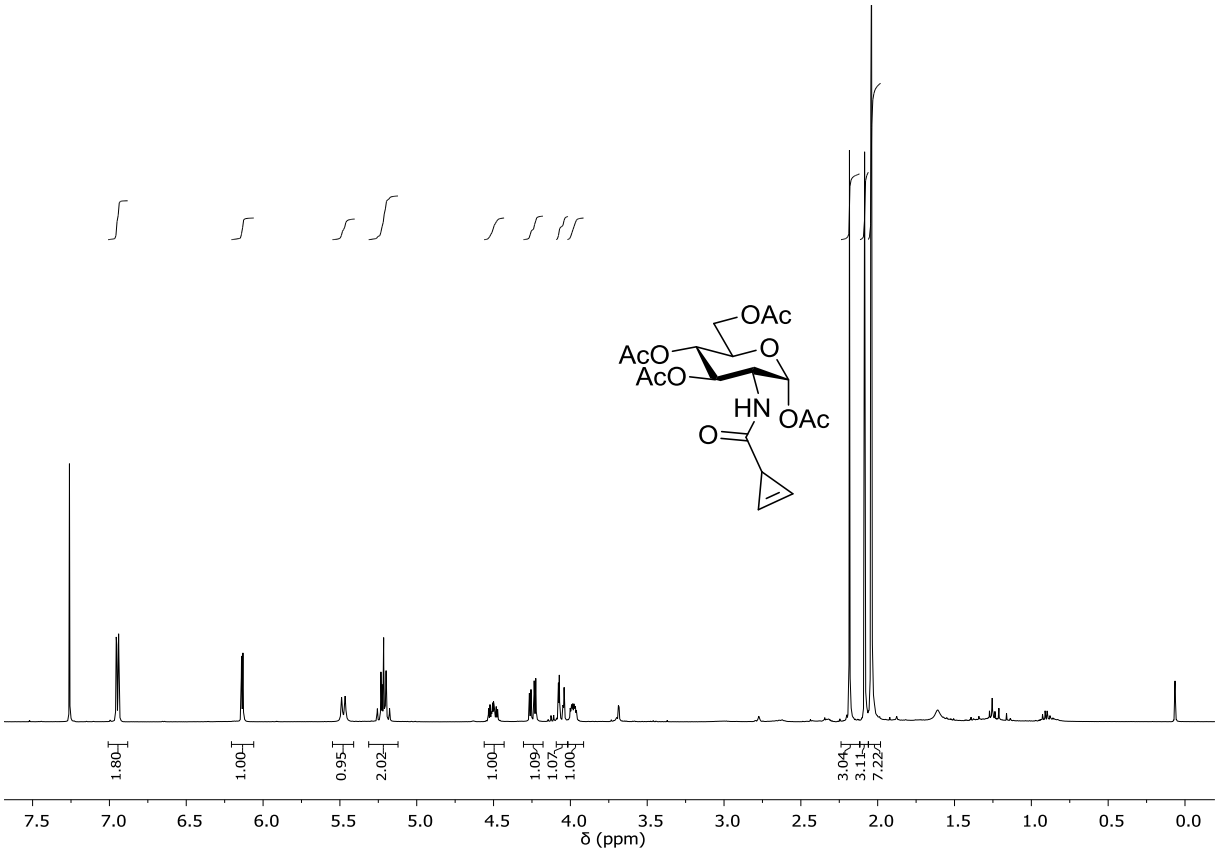


^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of the β-anomer of $Ac_4ManNCyoc(H_2)$.



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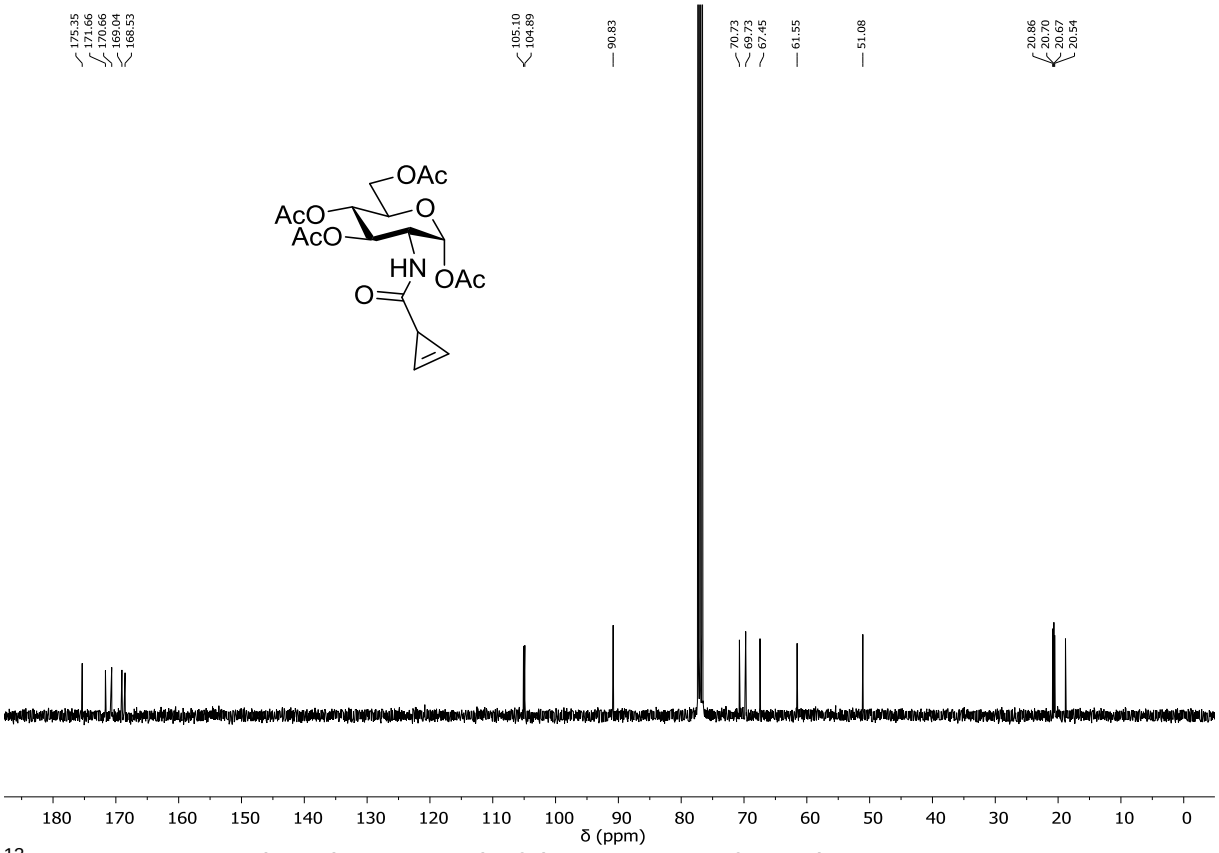

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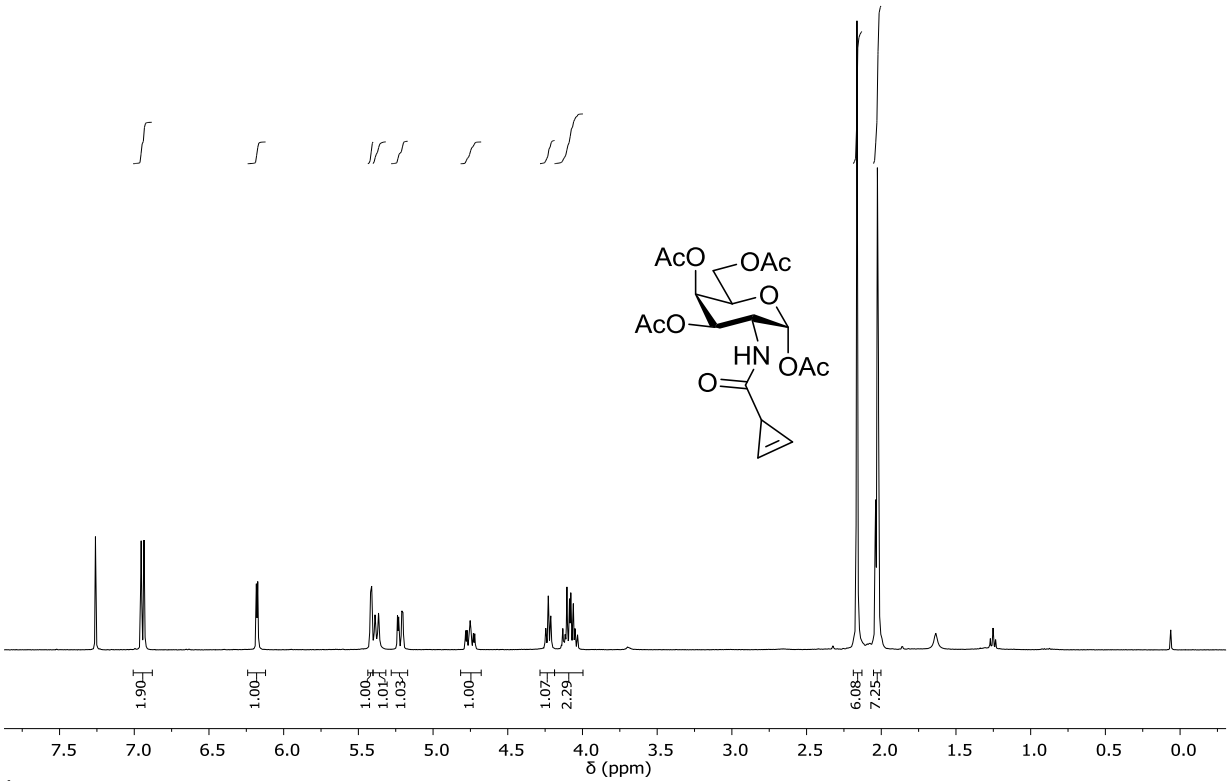

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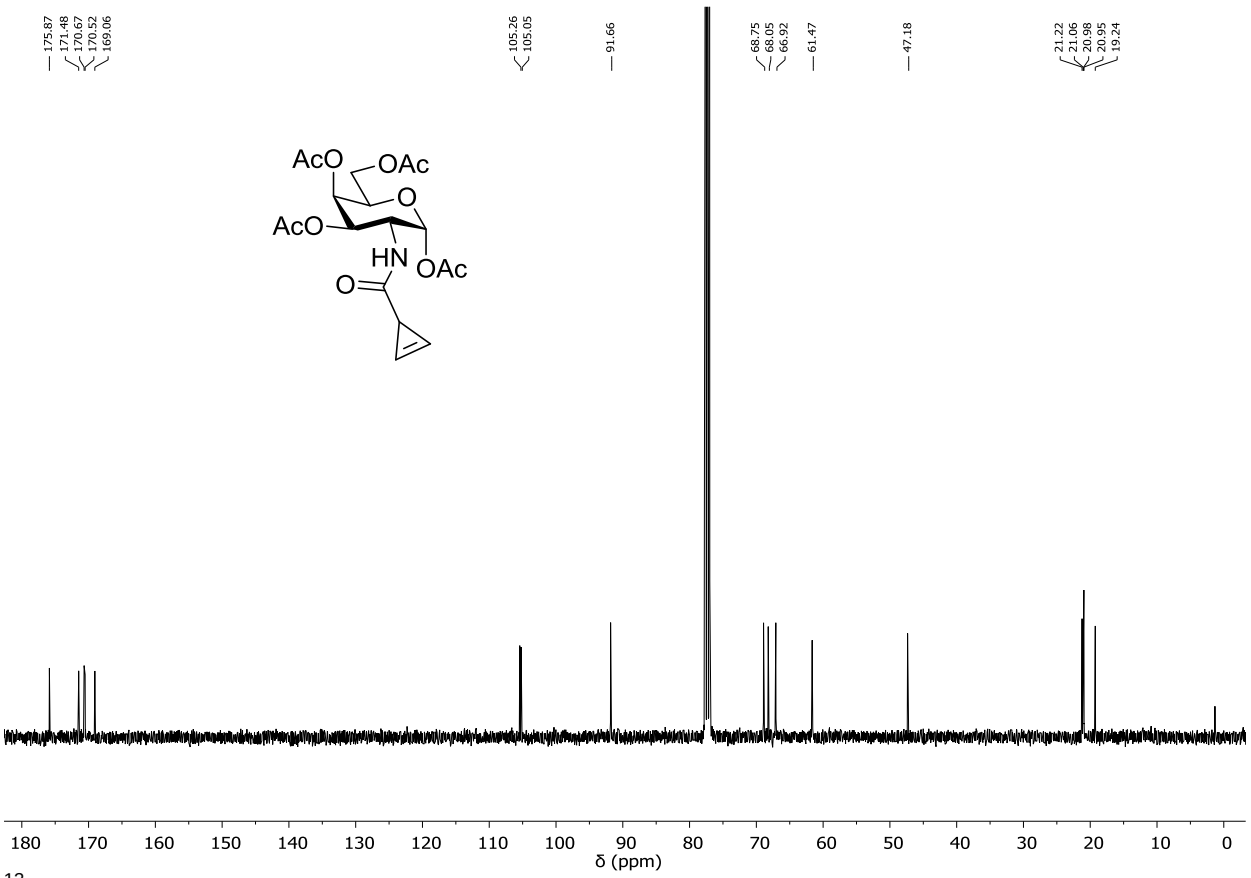

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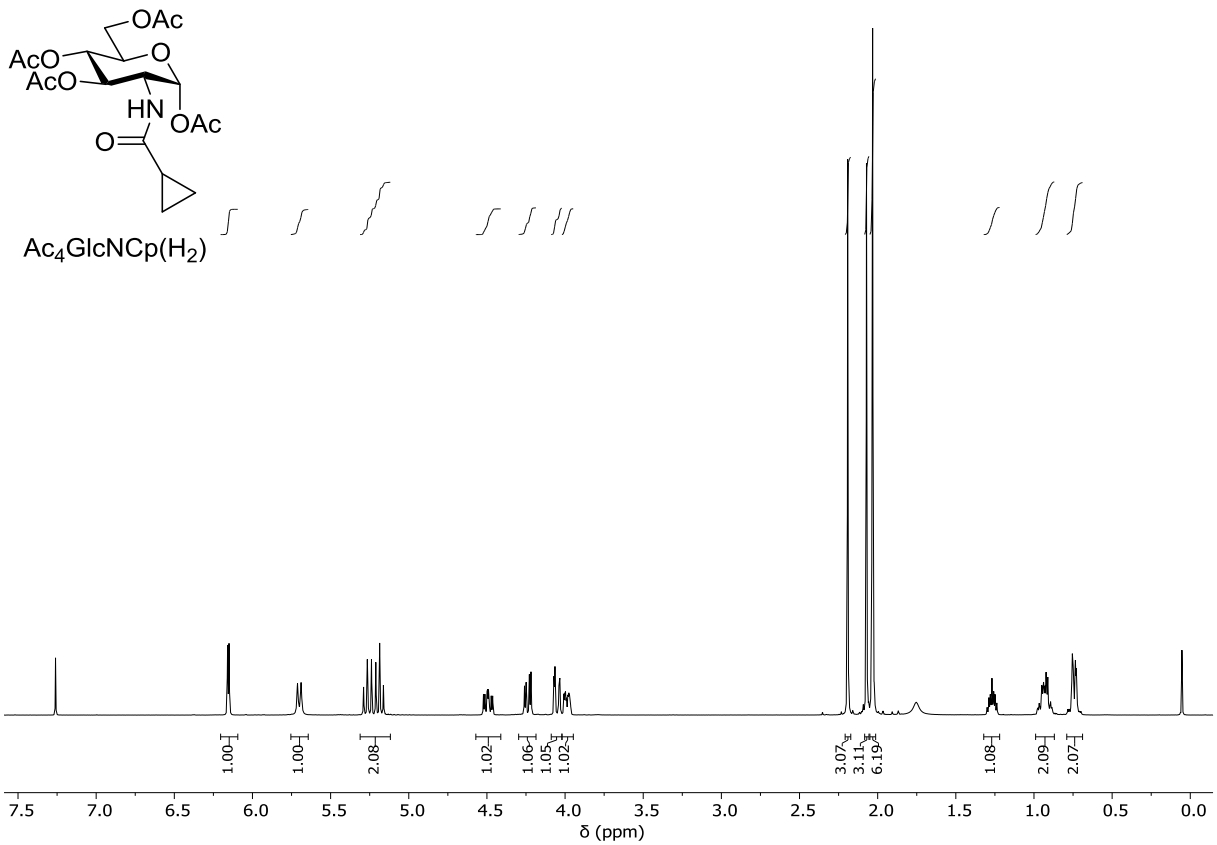

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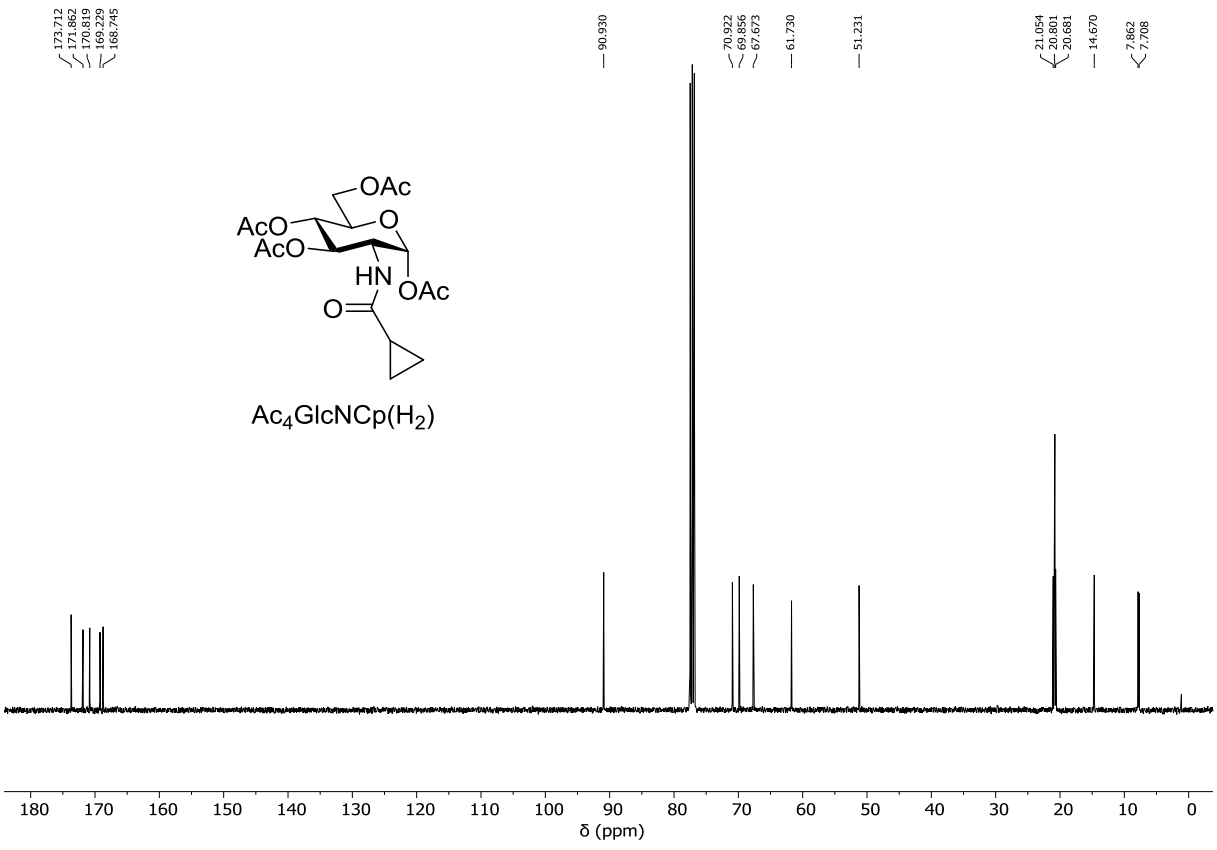
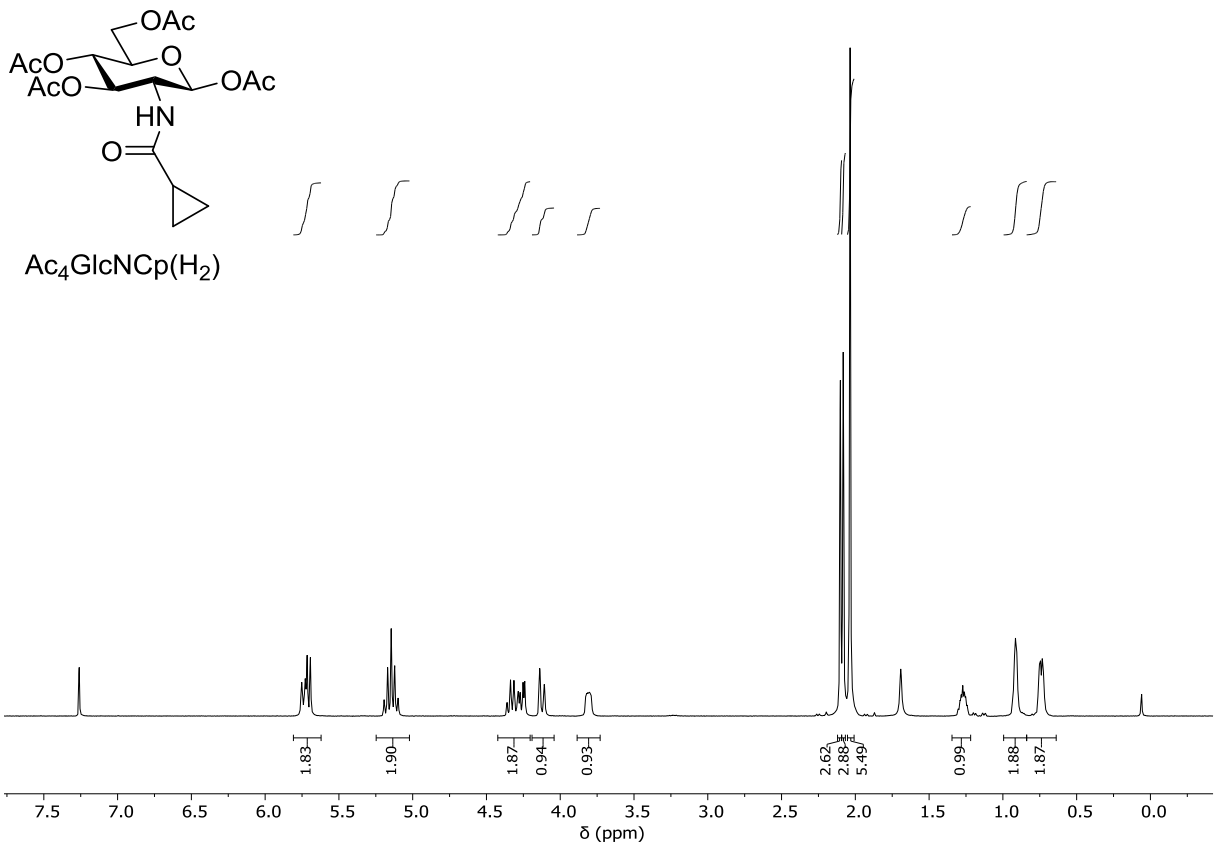


^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of the α-anomer of $Ac_4GlcNCp(H_2)$.



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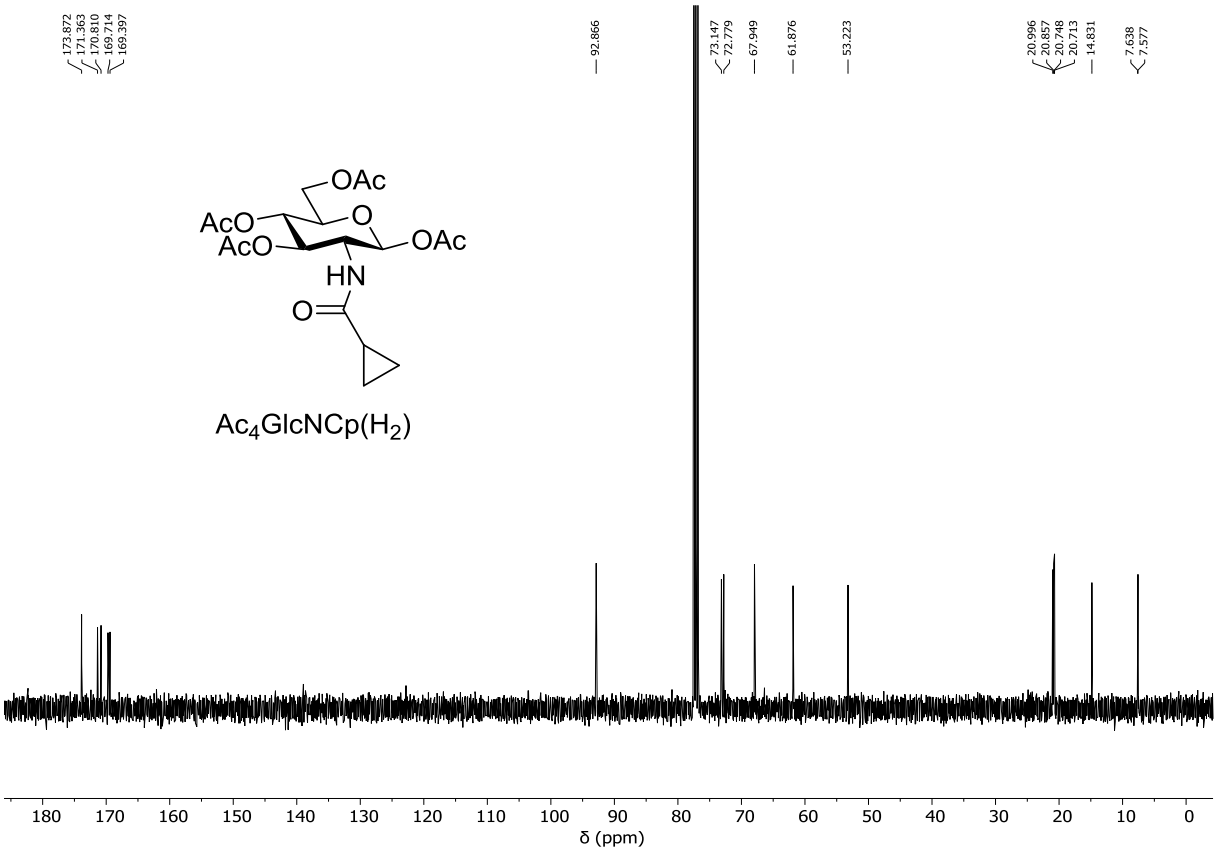
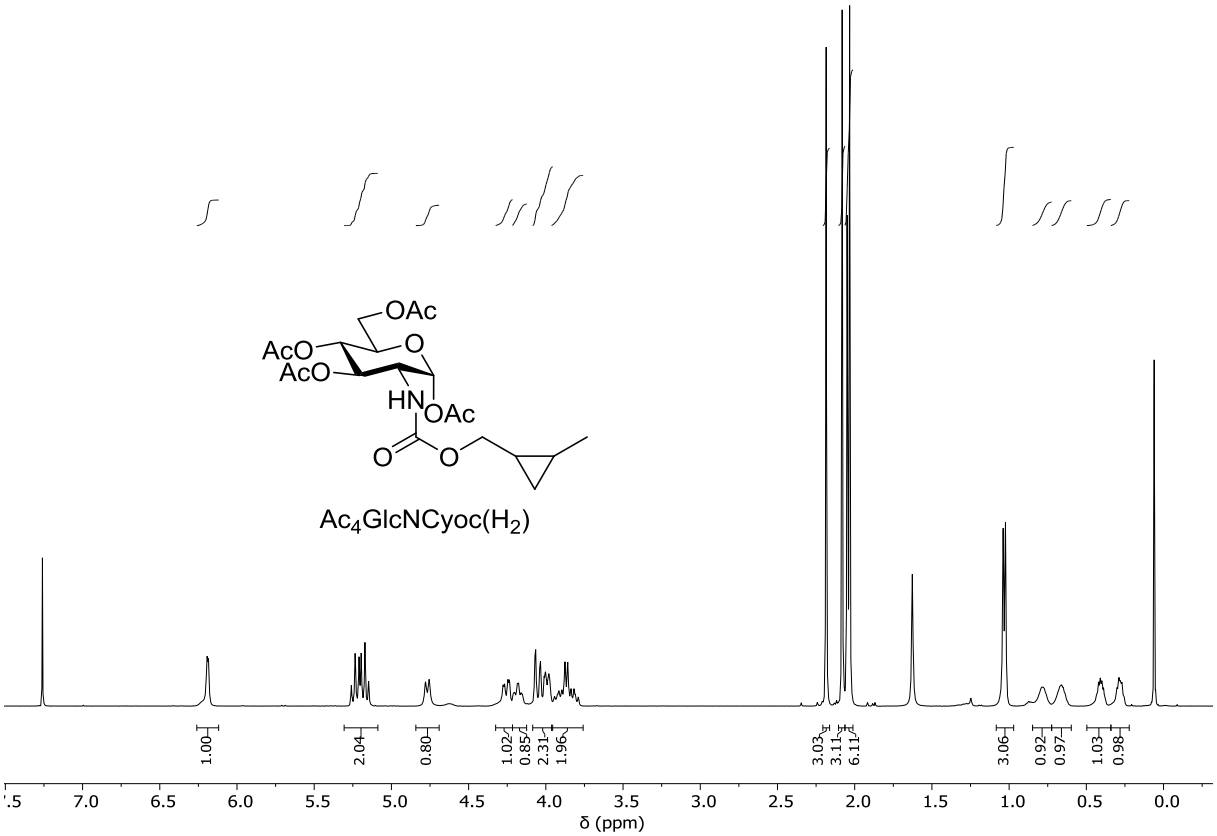
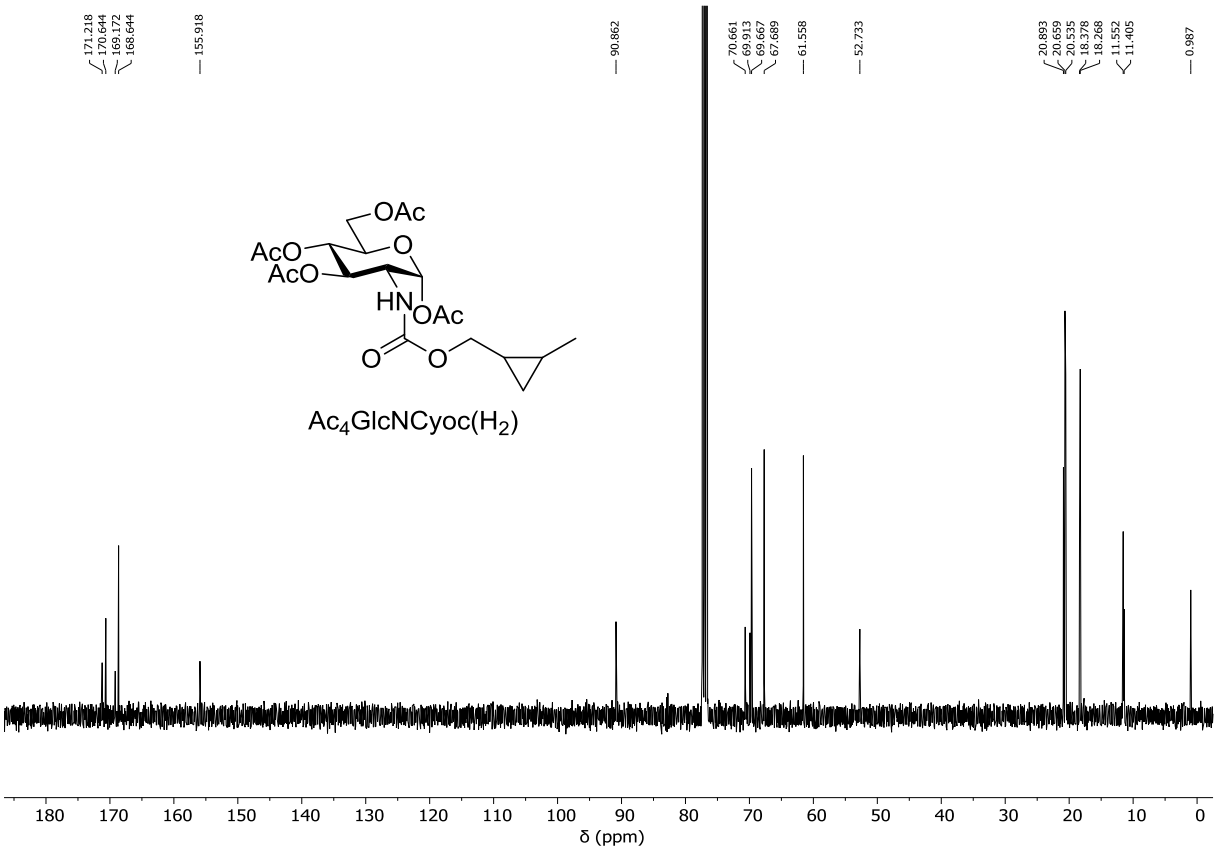


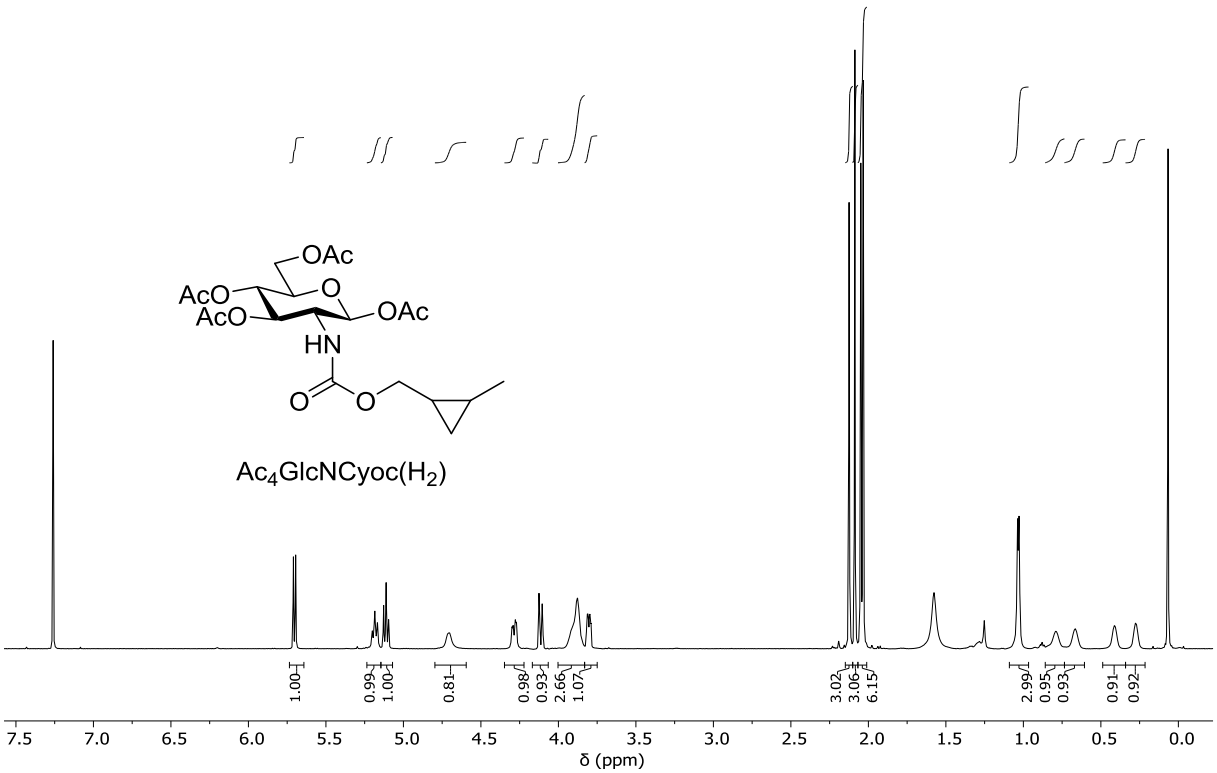
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of the β-anomer of $Ac_4GlcNCp(H_2)$.



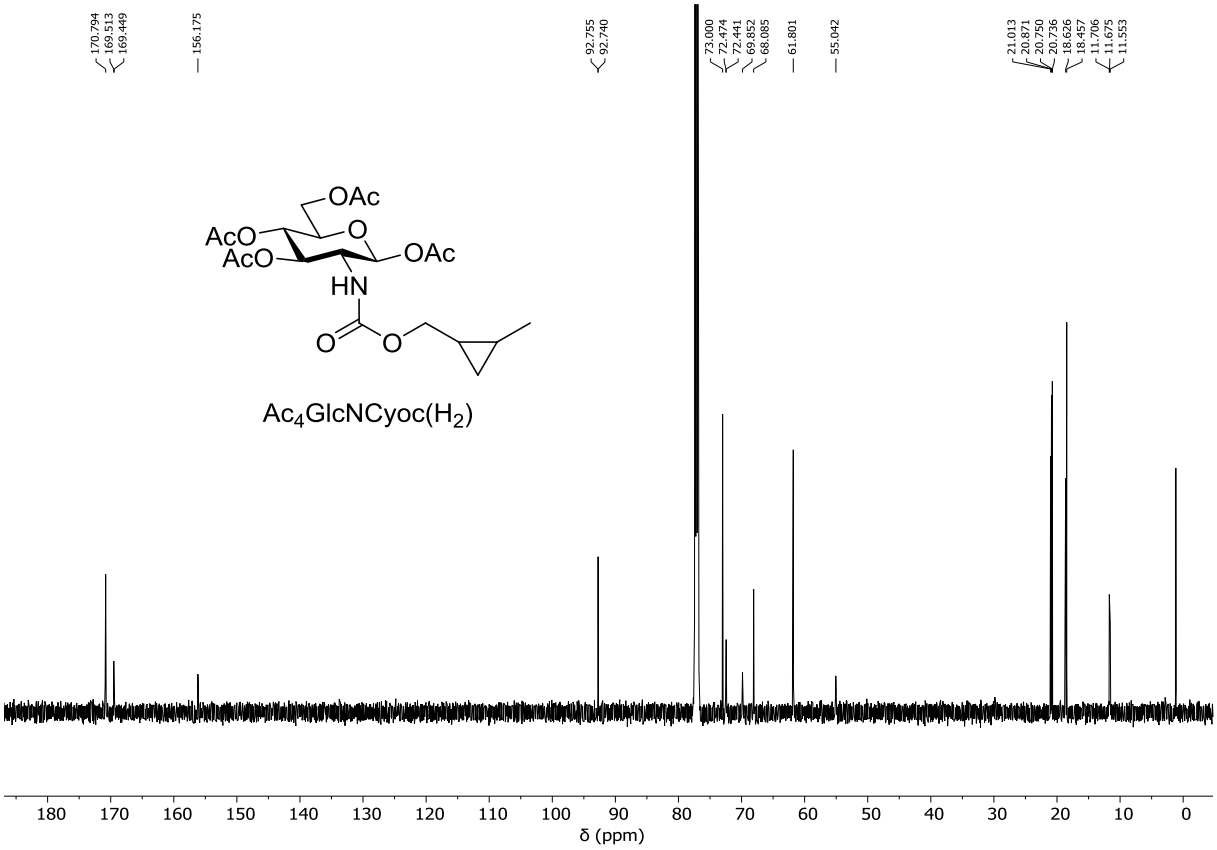
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the α-anomer of $Ac_4GlcNCyoc(H_2)$.



^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of the α-anomer of $Ac_4GlcNCyoc(H_2)$.



^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of the β-anomer of $Ac_4GlcNCyoc(H_2)$.



^{13}C NMR spectrum ($CDCl_3$, 151 MHz) of the β -anomer of $Ac_4GlcNCyoc(H_2)$.