Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPLEMENTARY INFORMATION

Profiling of *Haemophilus influenzae* strain R2866 with carbohydrate-based covalent probes

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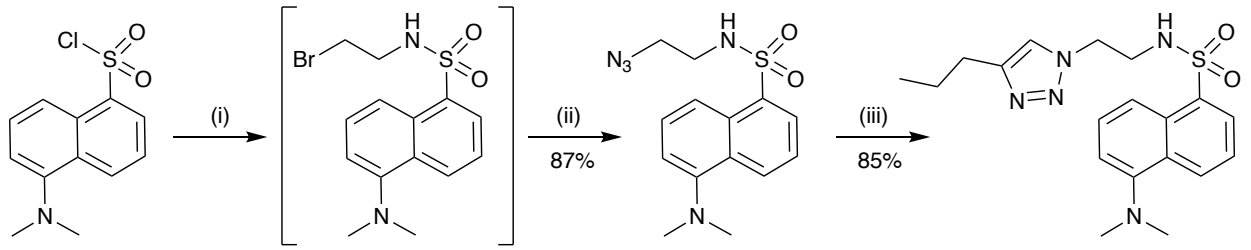
Table of Contents

(1) Synthesis of fluorophore controls 9 and 10.

The synthesis of **9** followed the protocol described by Yan et al., *J. Am. Chem. Soc.* **2013**, 135, 703-709.



11 **9** **10**

Scheme S1 (i) Dansyl chloride **11** (1 equiv.), 2-bromoethylamine hydrobromide (1 equiv.), Et_3N (2 equiv.), DCM, rt, 4 hours; (ii) NaN_3 (2.5 equiv.), MeCN, reflux, overnight, 87% (over both steps); (iii) pentyne (1.5 equiv.), $CuSO_4$-$5H_2O$ (1 equiv.), sodium ascorbate (1.5 equiv.), DIPEA (3 equiv.), THF : water 10:1, rt, 3 hours, 85%.

(2) Additional figures S1-S6.

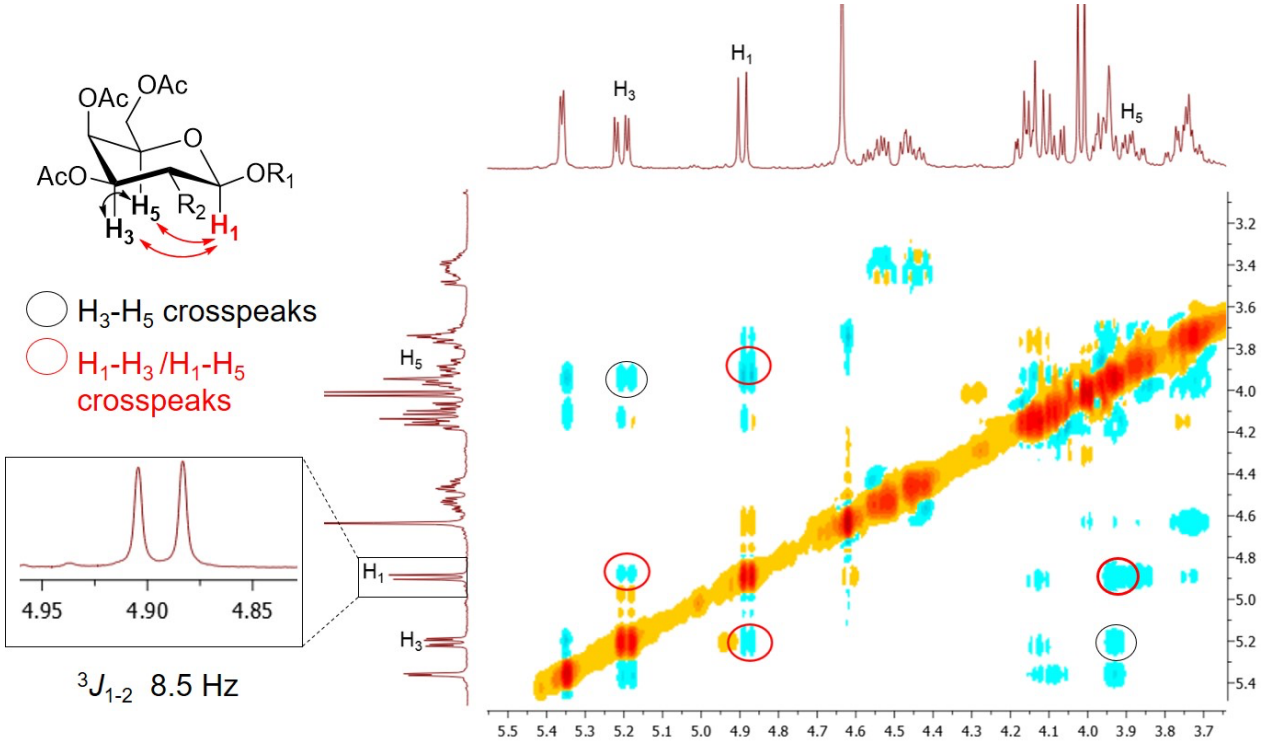


Figure S1. Assignment of the anomeric configuration of **7-β** by ^{1}H NMR and 2D ROESY NMR (in $CDCl_3$).

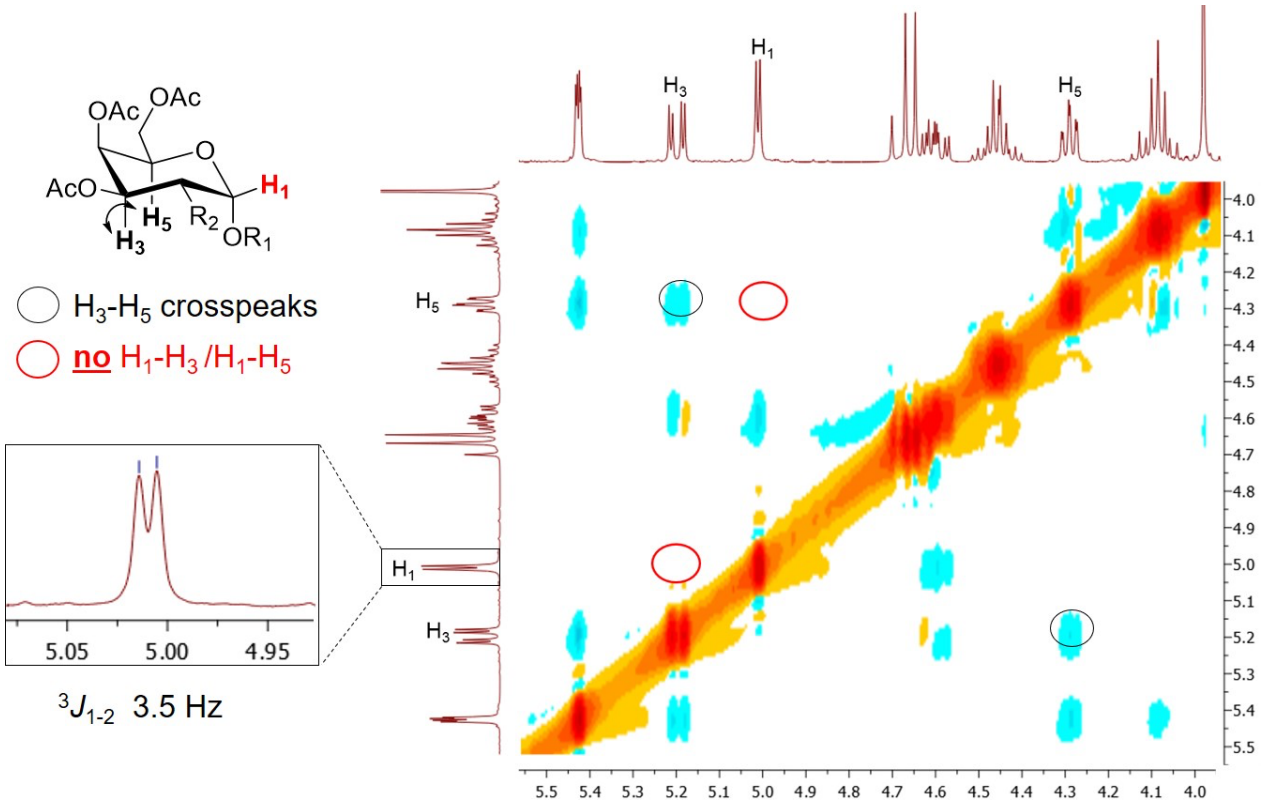


Figure S2. Assignment of the anomeric configuration of **7-α** by ^{1}H NMR and 2D ROESY NMR (in $CDCl_3$).

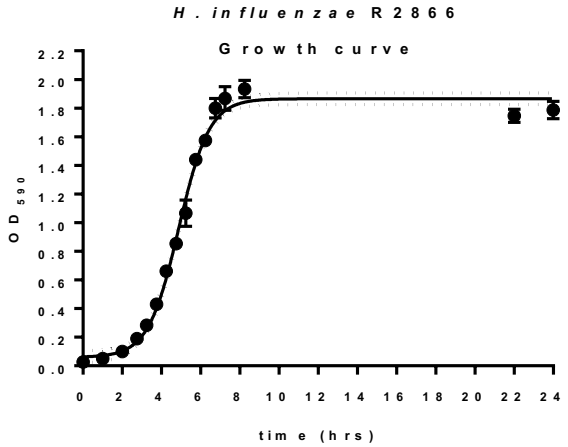


Figure S3. Growth curve of *H. influenzae* R2866. Cells were grown in supplemented BHI broth over 24 hours at 37°. OD_{590} was measured at specific time points. Stationary growth phase was reached after 8-9 hours. The exponential growth phase, indicating healthy and performant cells, occurred over a period of two hours, between 5 and 7 hours after inoculation. The experiment was carried out in triplicate (technical). Error bars represent standard deviation.

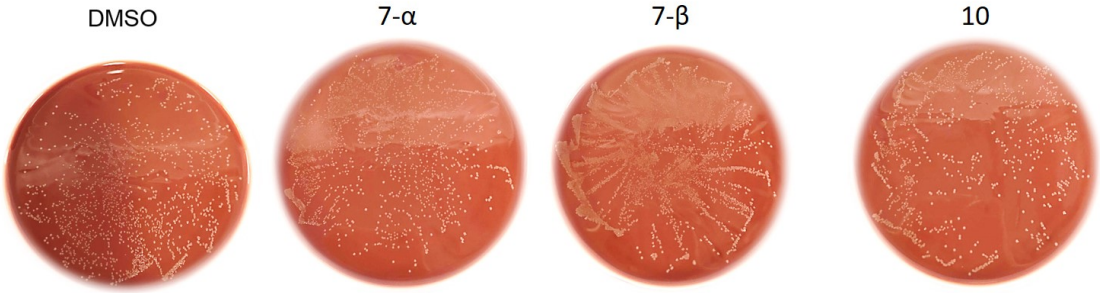


Figure S4. Viability of *H. influenzae* R2866 in the presence of carbohydrate-based probes. Cells were incubated with **7-α**, **7-β**, **10** (fluorophore control), or DMSO (control) for 2h. Bacterial growth on Agar chocolate plates was inspected visually after 48hrs incubation at 37°C.

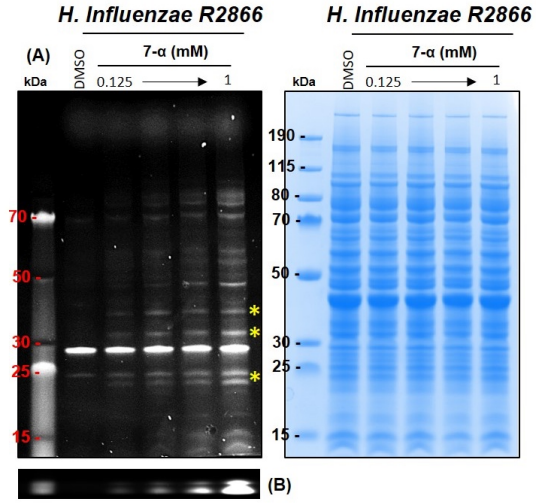


Figure S5. Effect of probe concentration (**7-α**) on protein labelling in intact *H. influenzae* R2866 cells. See Fig. 3 for general conditions. Cell pellets were suspended in PBS (900µL) and incubated with stock solutions of **7-α** (100µL, 1.25-10mM stock). (A) Full SDS-page gels. Proteins detected at the lowest probe concentration (125µM) are denoted with an asterisk. (B) Gel front, indicating an increased uptake of probe.

(3) LC-MS/MS analysis of SDS-page gel bands.

Samples were obtained by incubation of *H. influenzae* R2866 lysates or intact cells with **8-α** (1mM) for 1 hour (lysates) or 2 hours (intact cells) and SDS-page analysis, as described. Selected bands from the intact cell experiment (Fig. S6, A-G), and the band for the low-abundance protein at 50kDa from the lysate experiment (Fig. 2, asterisk), were excised from the respective gel and analysed by LC-MS/MS.

LC-MS/MS analysis successfully identified candidate proteins from excised gel band samples after searching against the Uniprot *Haemophilus influenzae* (HI) Taxonomy database. The database generated files were uploaded into Scaffold 4 (v4.9.0) software (www.proteomesoftware.com) to create a .sfd file.

The raw data was searched at a stringency threshold of 5% false discovery rate (FDR) for protein and peptide and a minimum of one peptide per protein as determined by Mascot and Sequest in the Proteome Discoverer method. Any protein that is above this identity threshold is deemed significant. Peptides below this threshold are manually verified to determine correct assignment.

The stringency threshold parameters were therefore set to 95% protein, minimum 1 peptide and 95% peptide. In total, 102 proteins were identified from the intact cell experiment at this stringency after searching against the HI Taxonomy database. The total number of potential protein assignments for each band was 30 (A), 22 (B), 13 (C), 17 (D), 32 (E), 12 (F) and 13 (G), respectively. High probability assignments based on the largest number of significantly matched peptide identifications are shown in Fig. S6.

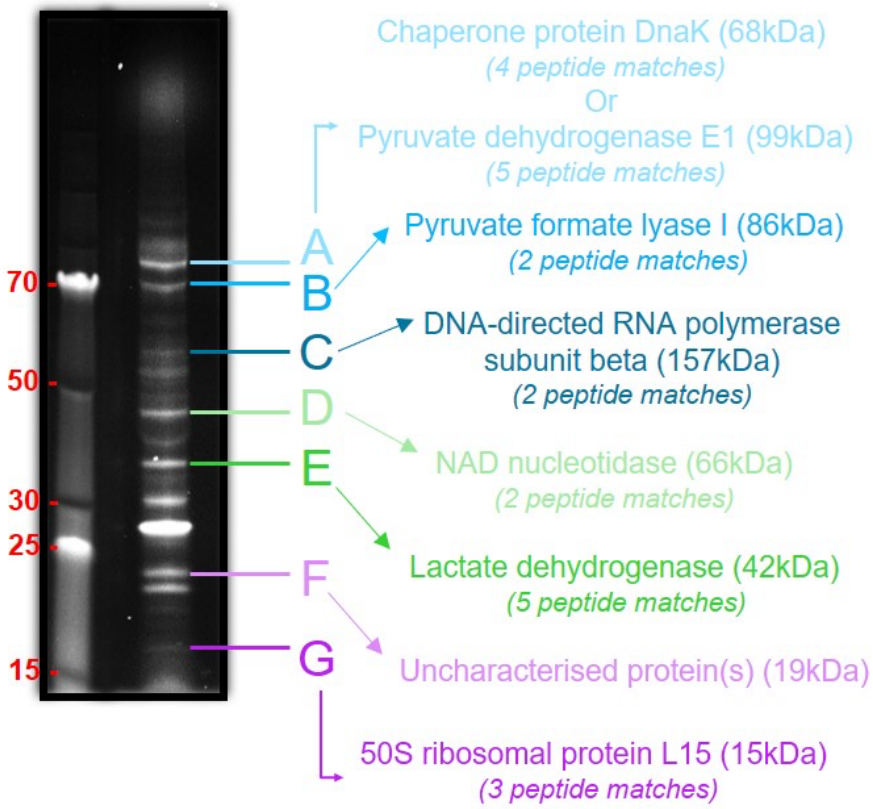
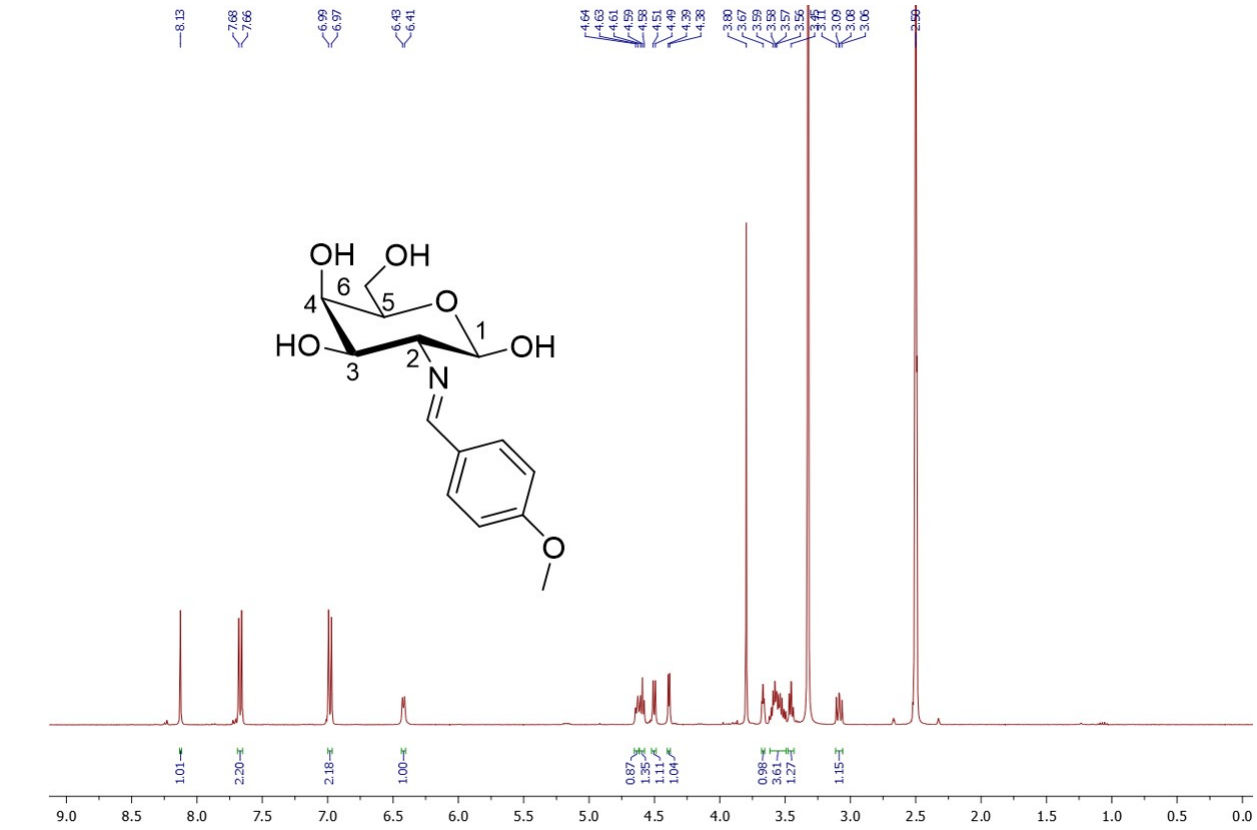
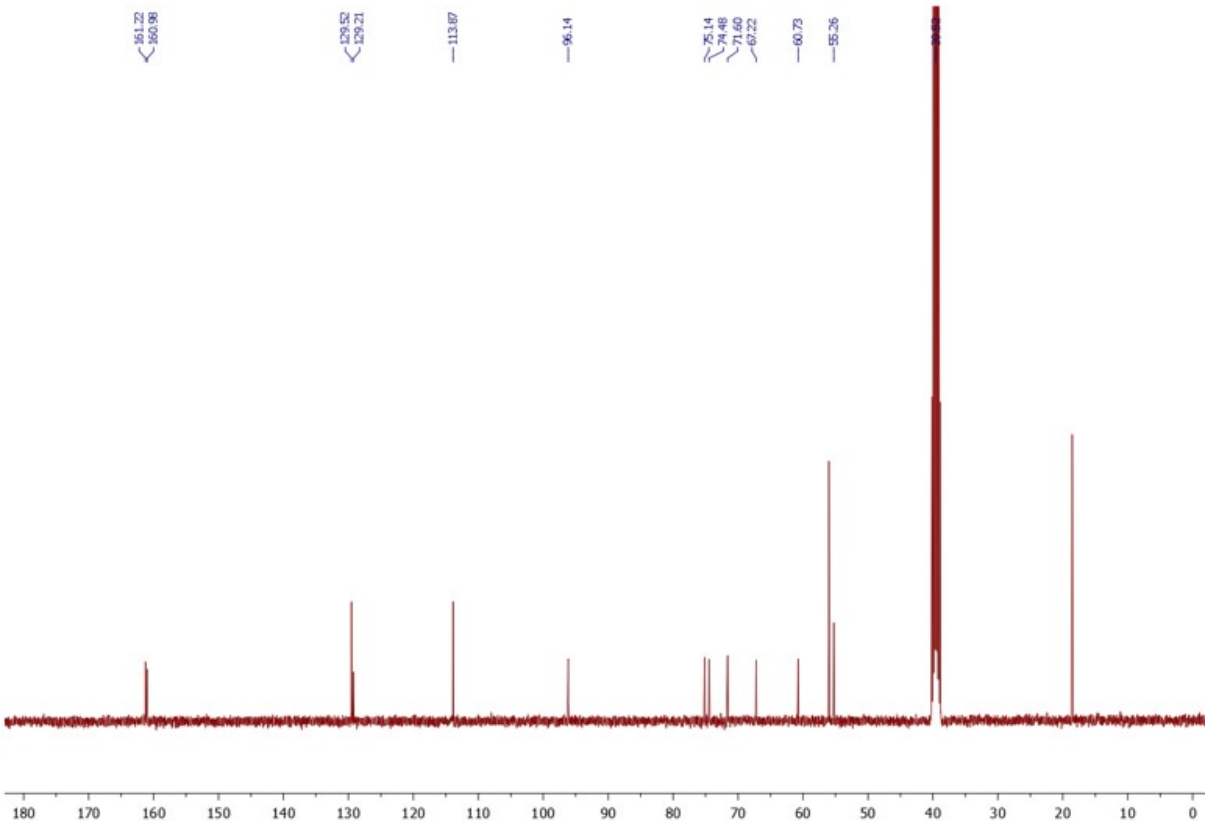


Figure S6. SDS-page gel from incubation of intact *H. influenzae* R2866 cells with **8-α** (1mM). Selected bands A-G for LC-MS/MS analysis and corresponding high probability assignments are shown.

(4) Spectroscopic data for compounds 2, 3, 4, 5, 6-α, 6-β, 7-α, 7-β, 10 and 12.

Compound **2**





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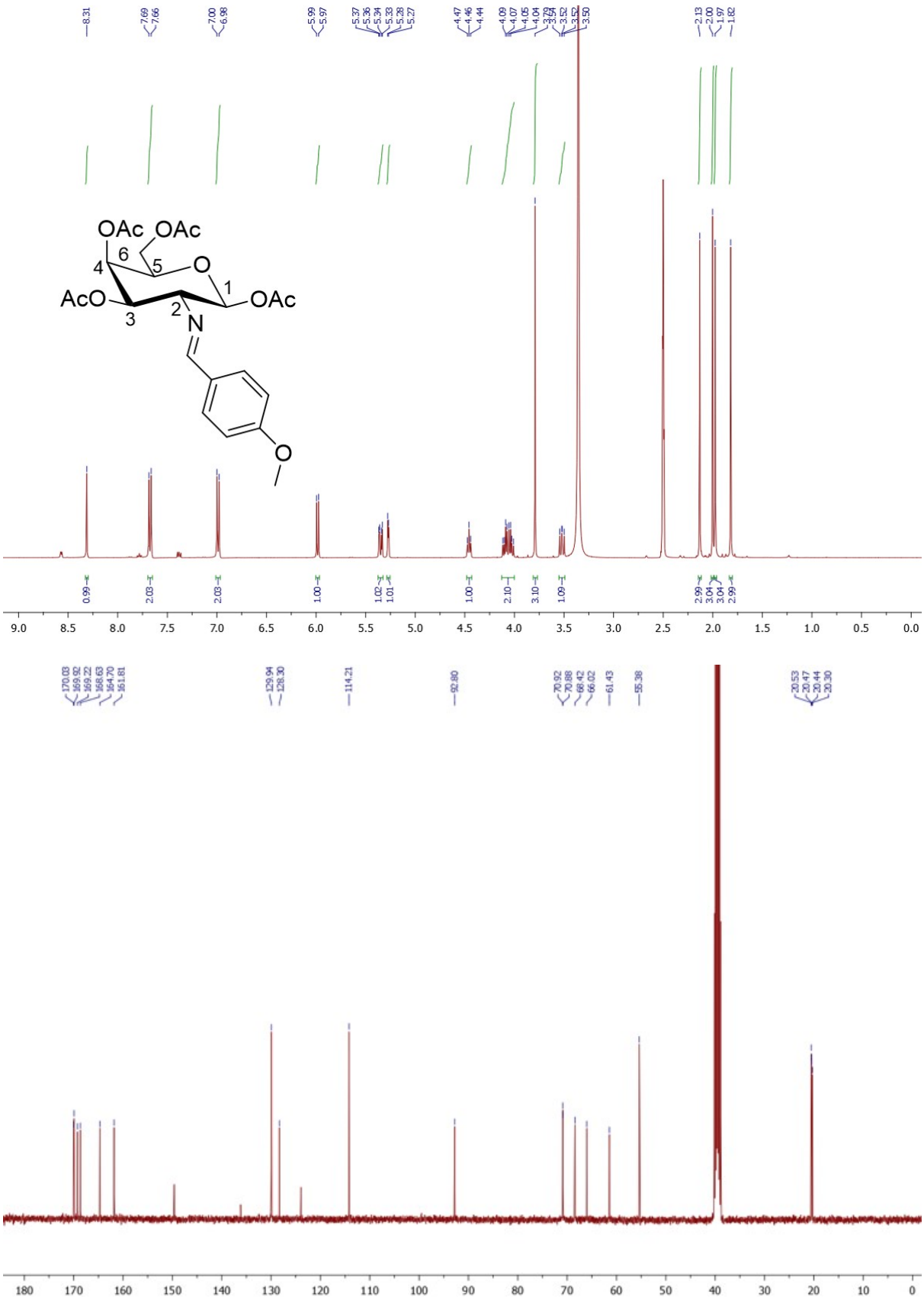

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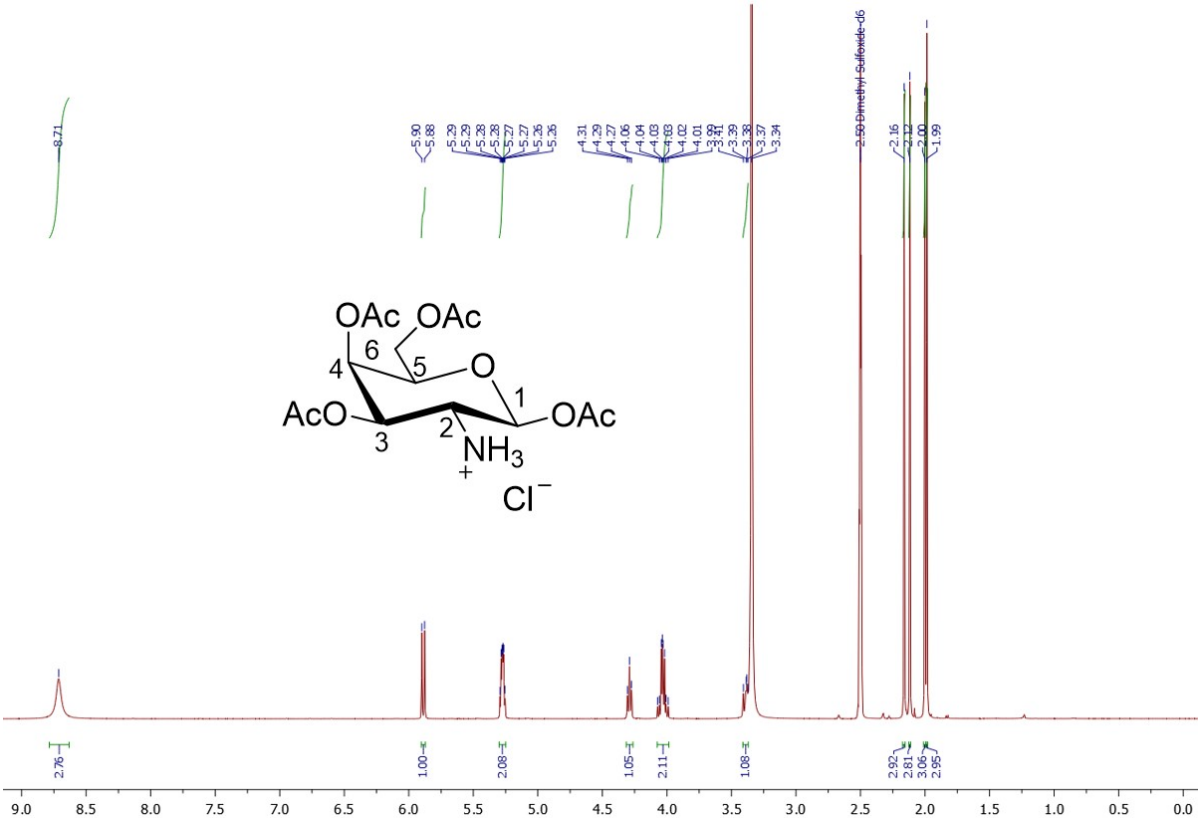

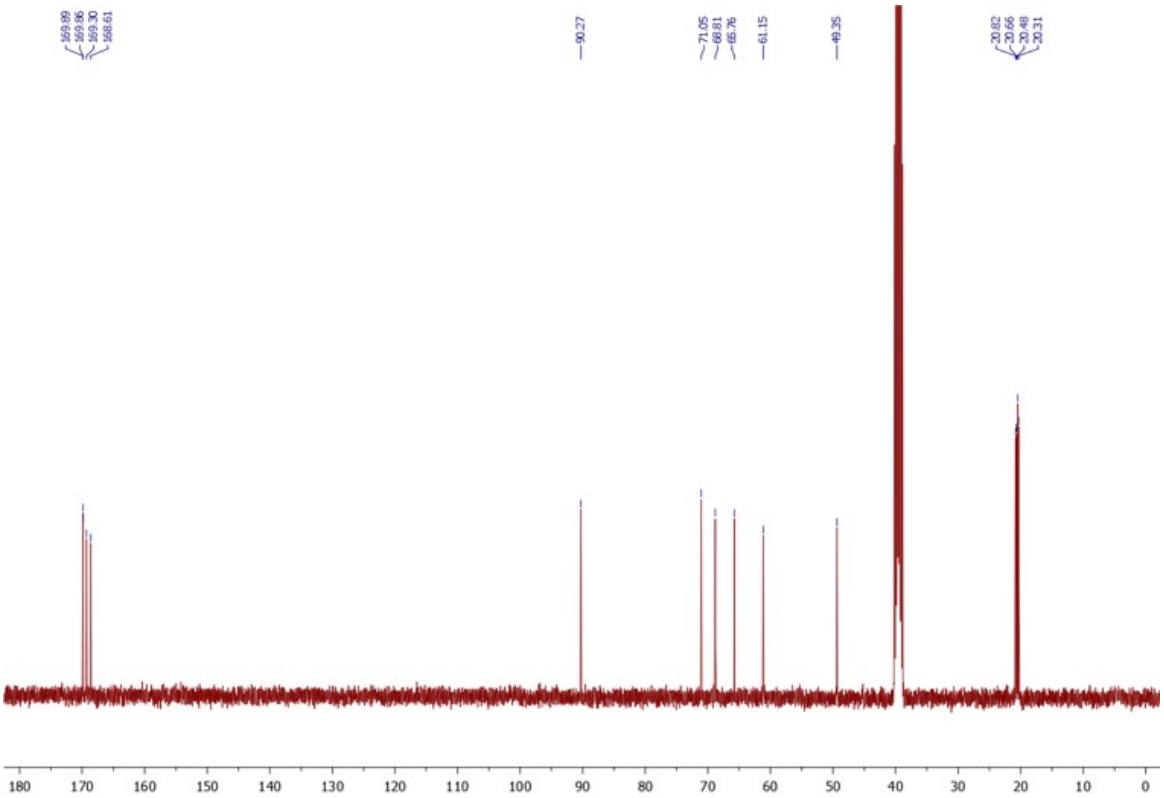

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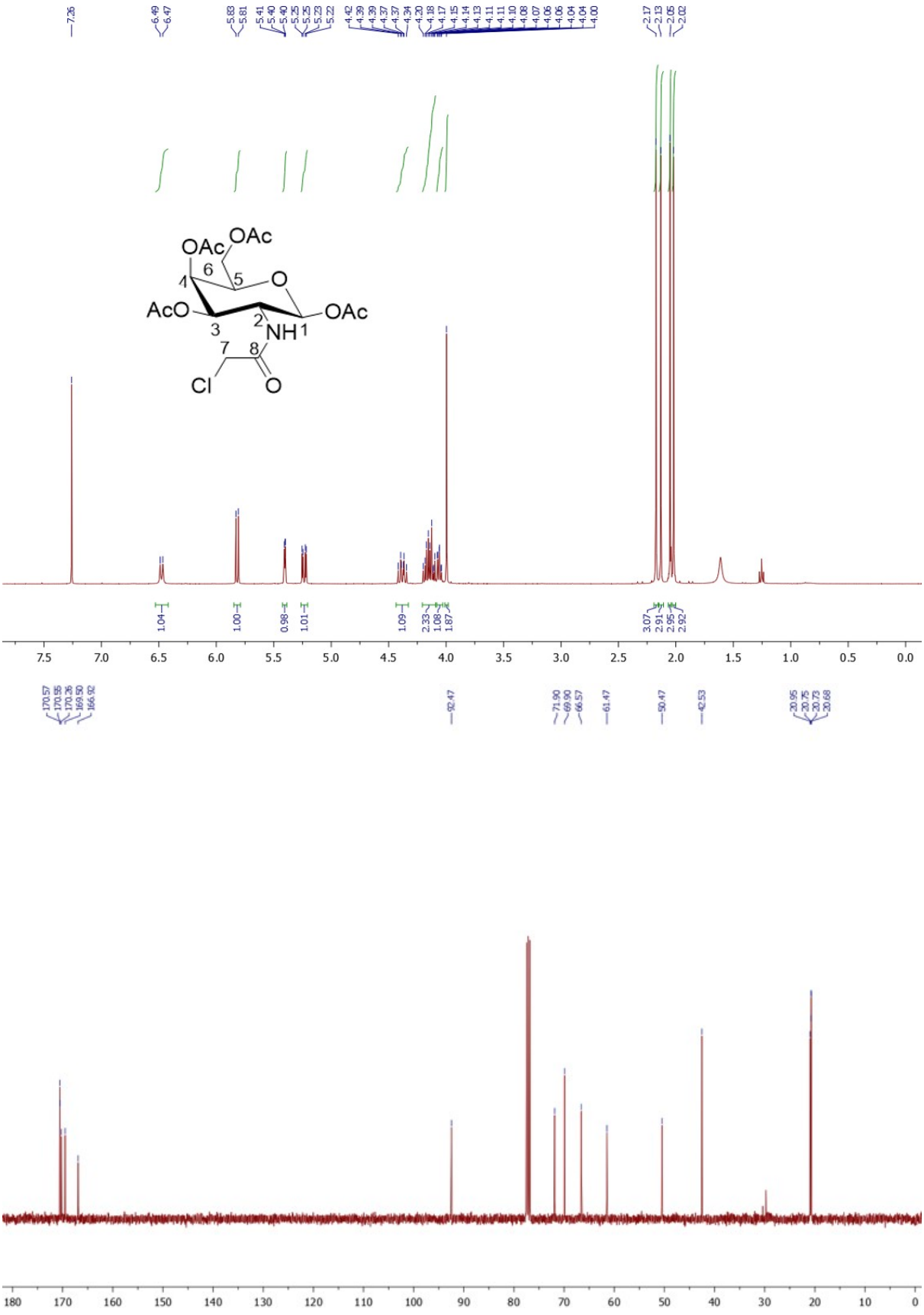

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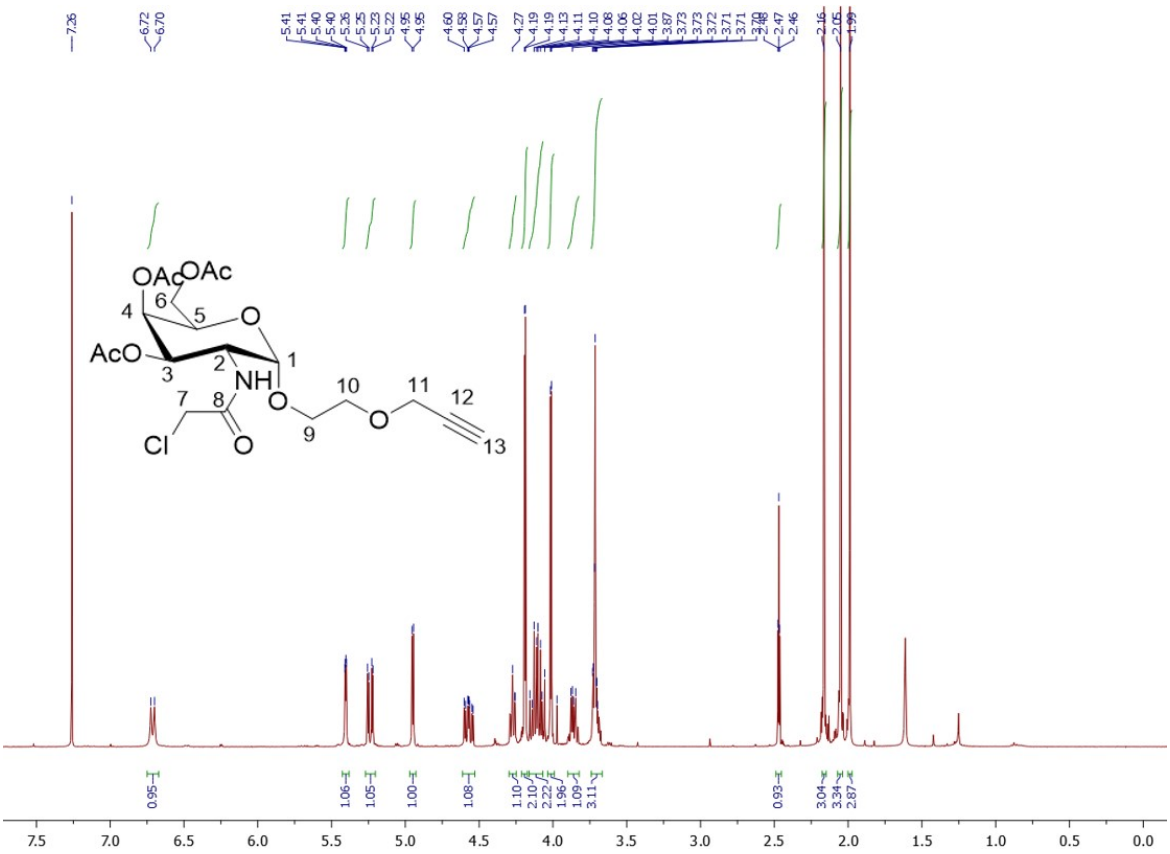
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5.22
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4.27
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4.13
4.11
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4.08
4.06
4.02
4.01
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3.72
3.71
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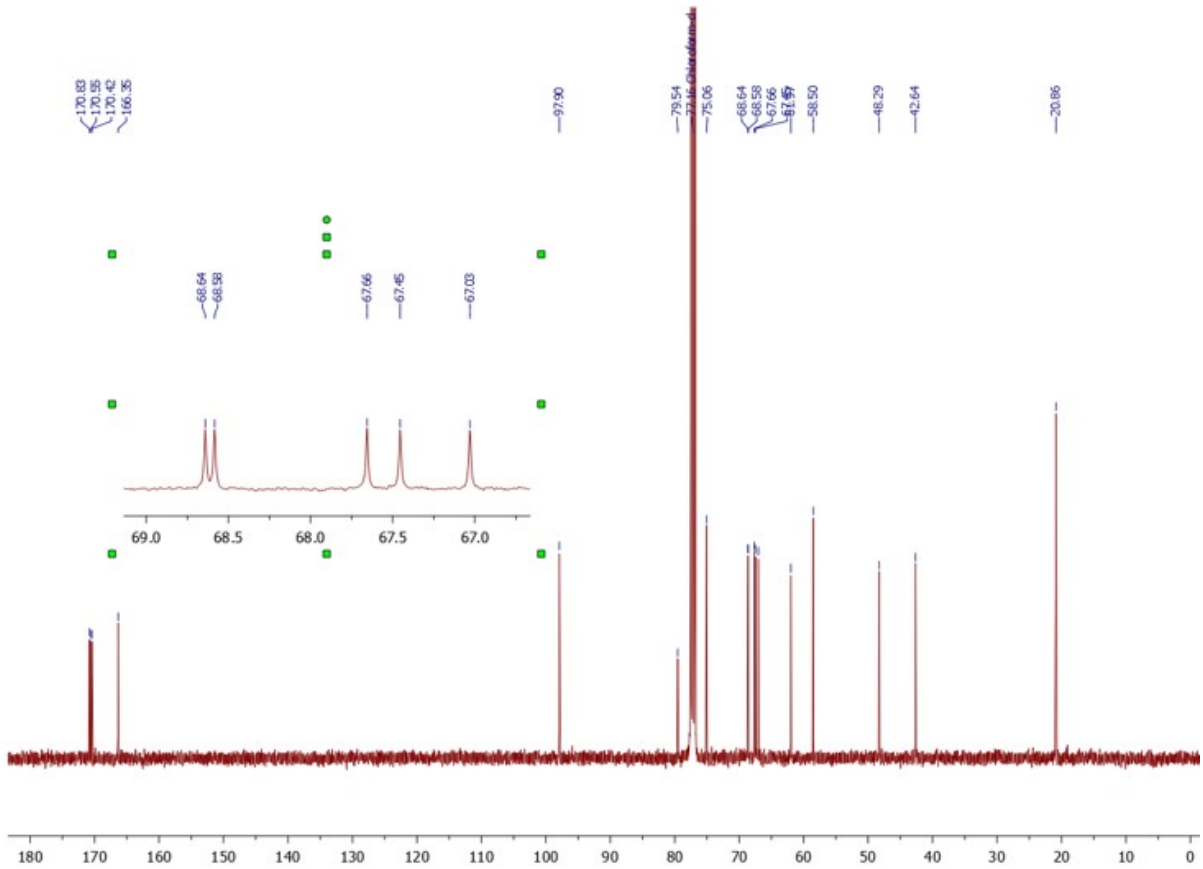
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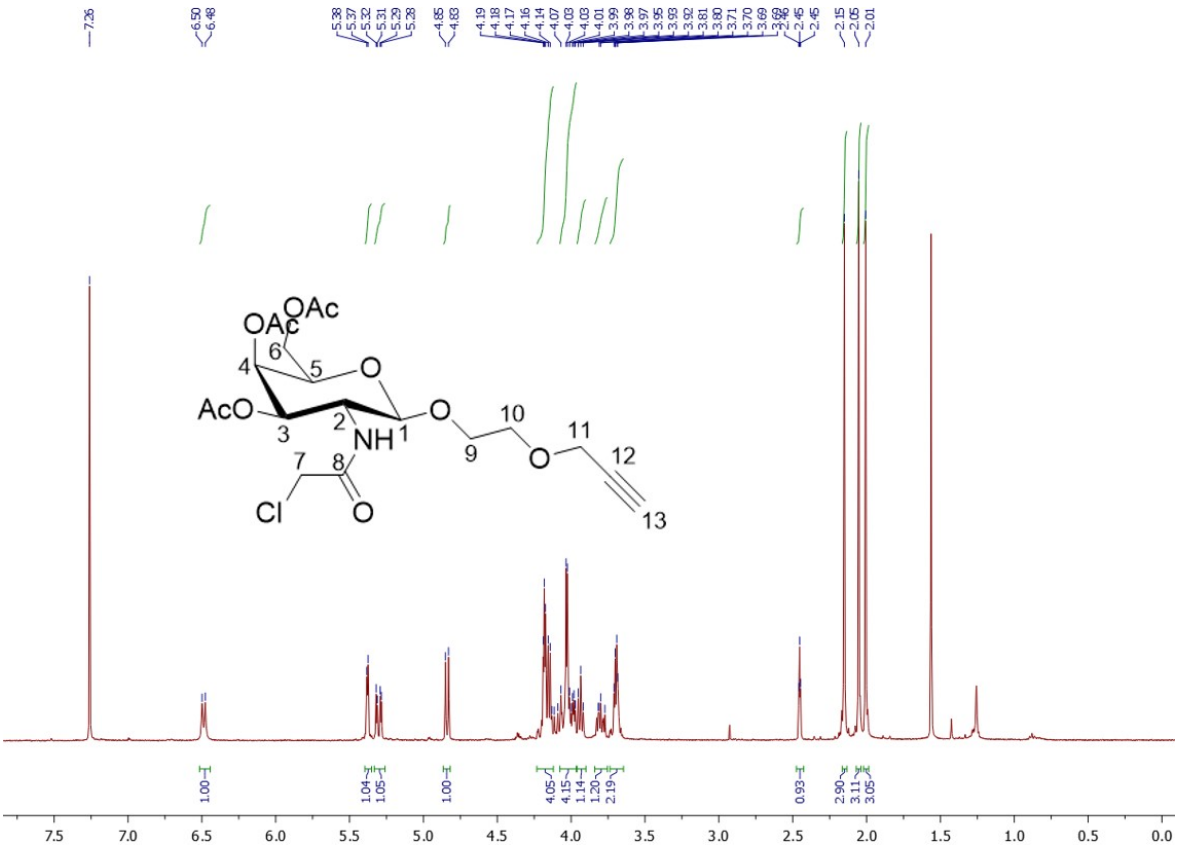
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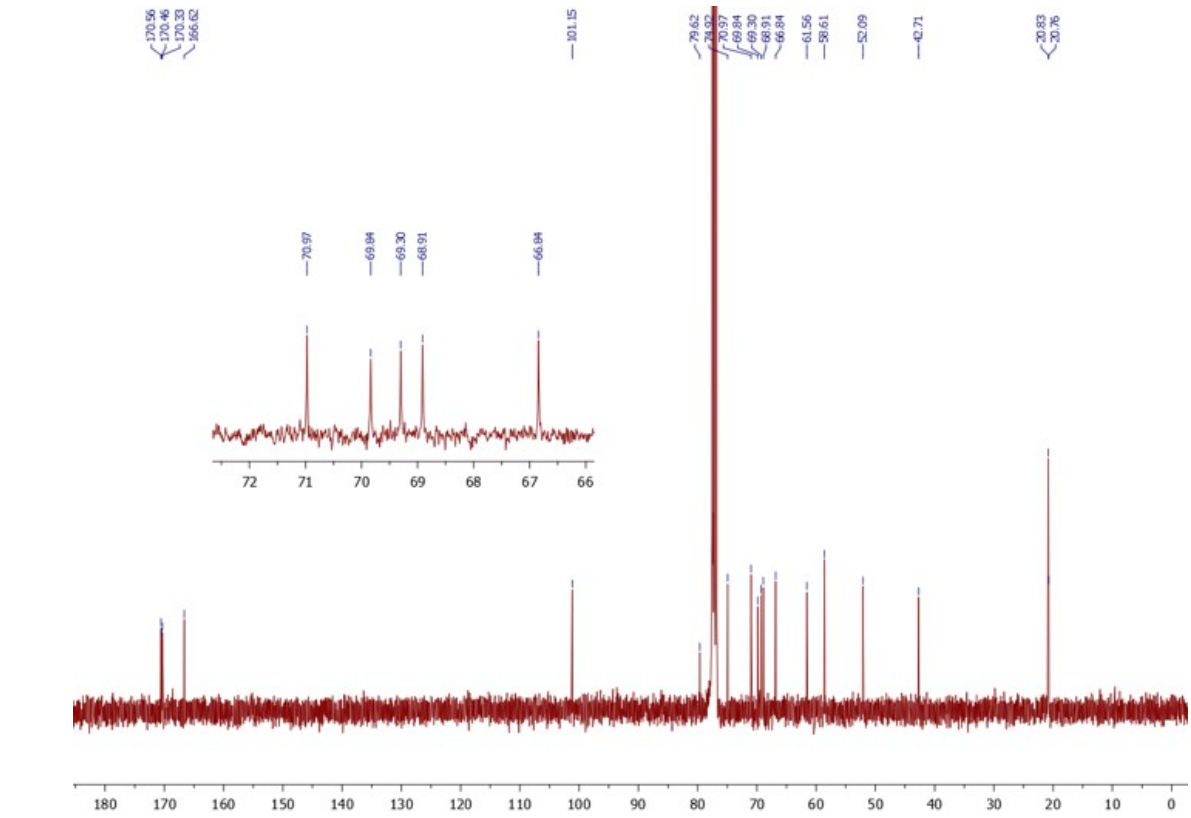
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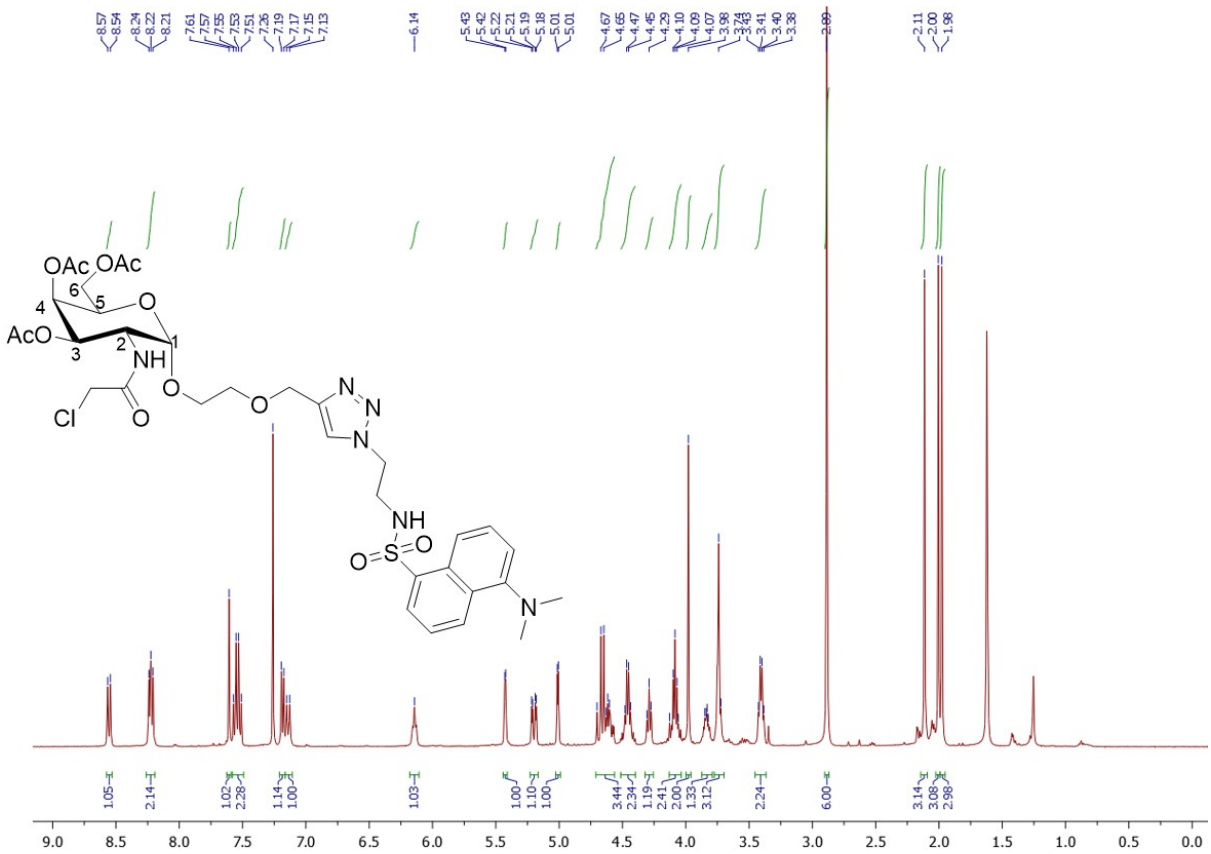
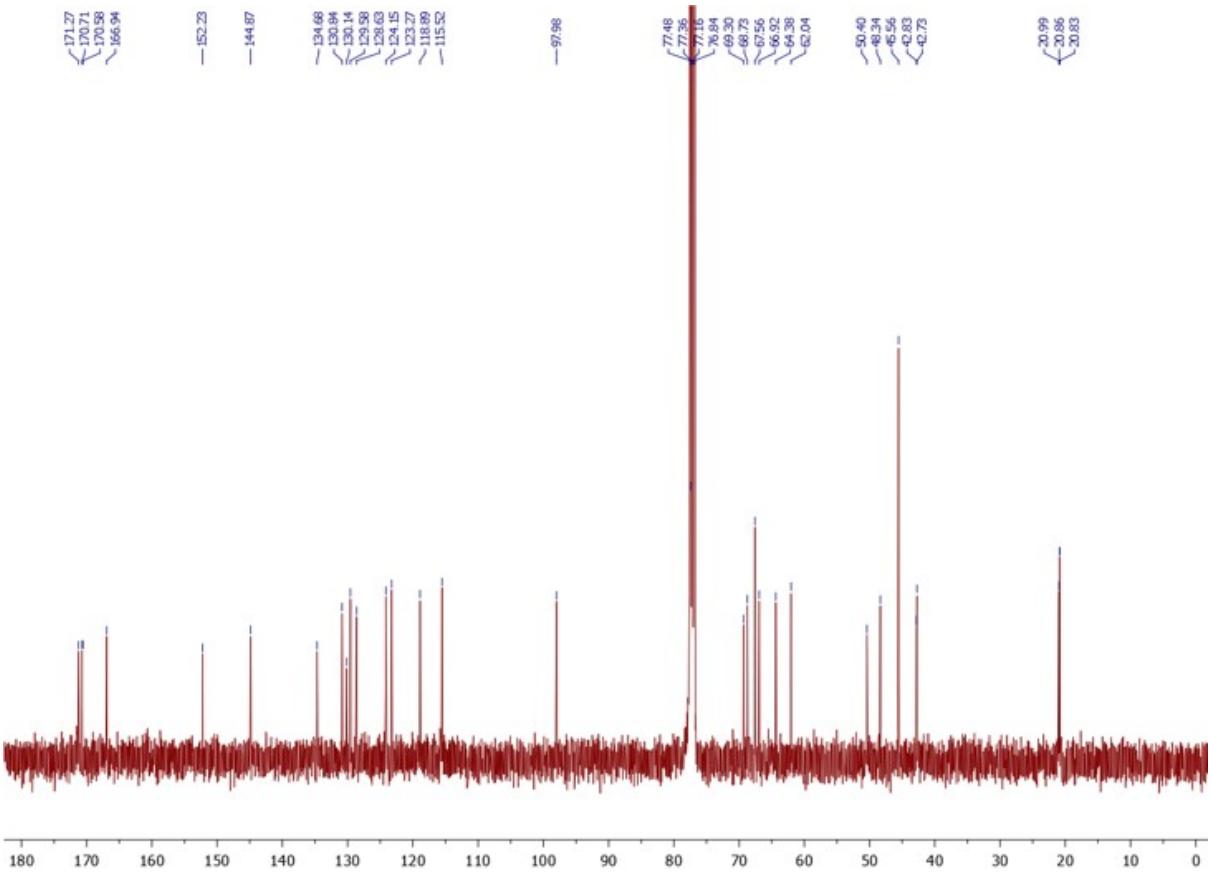
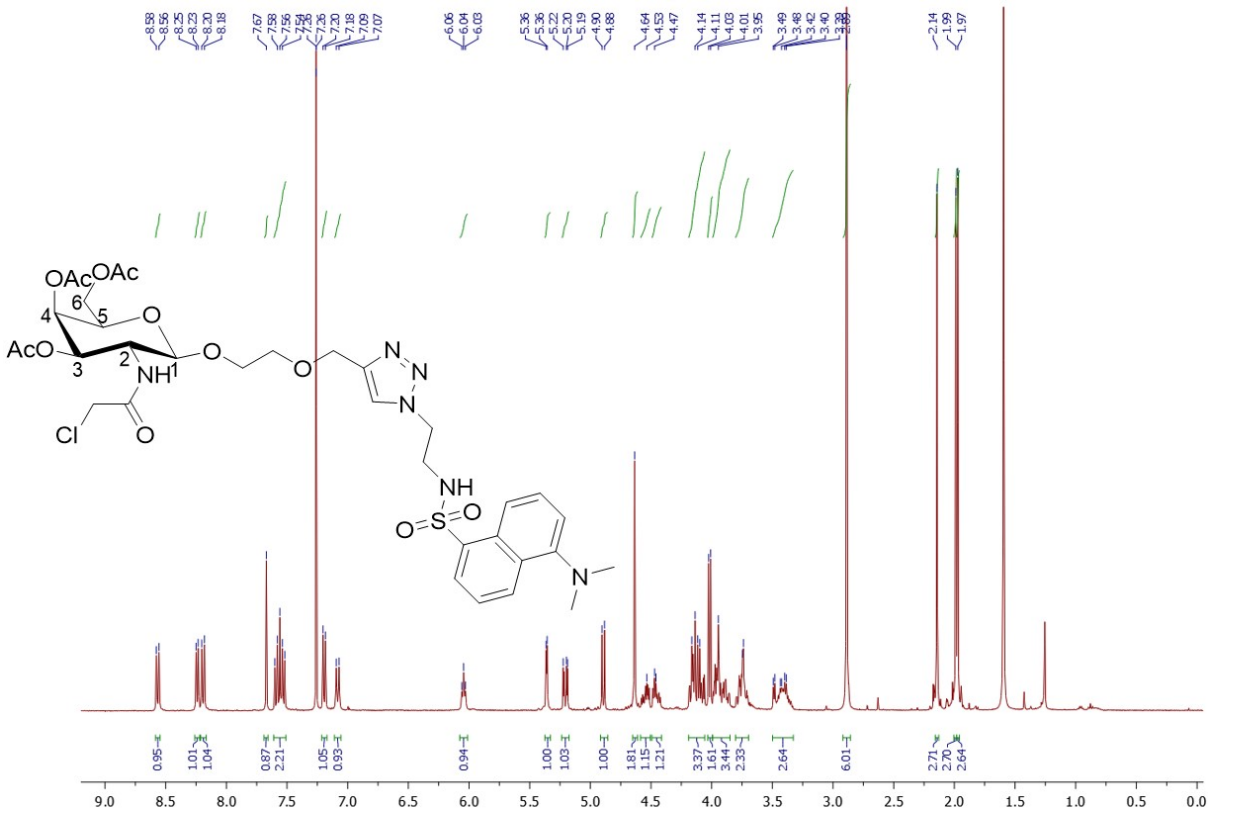
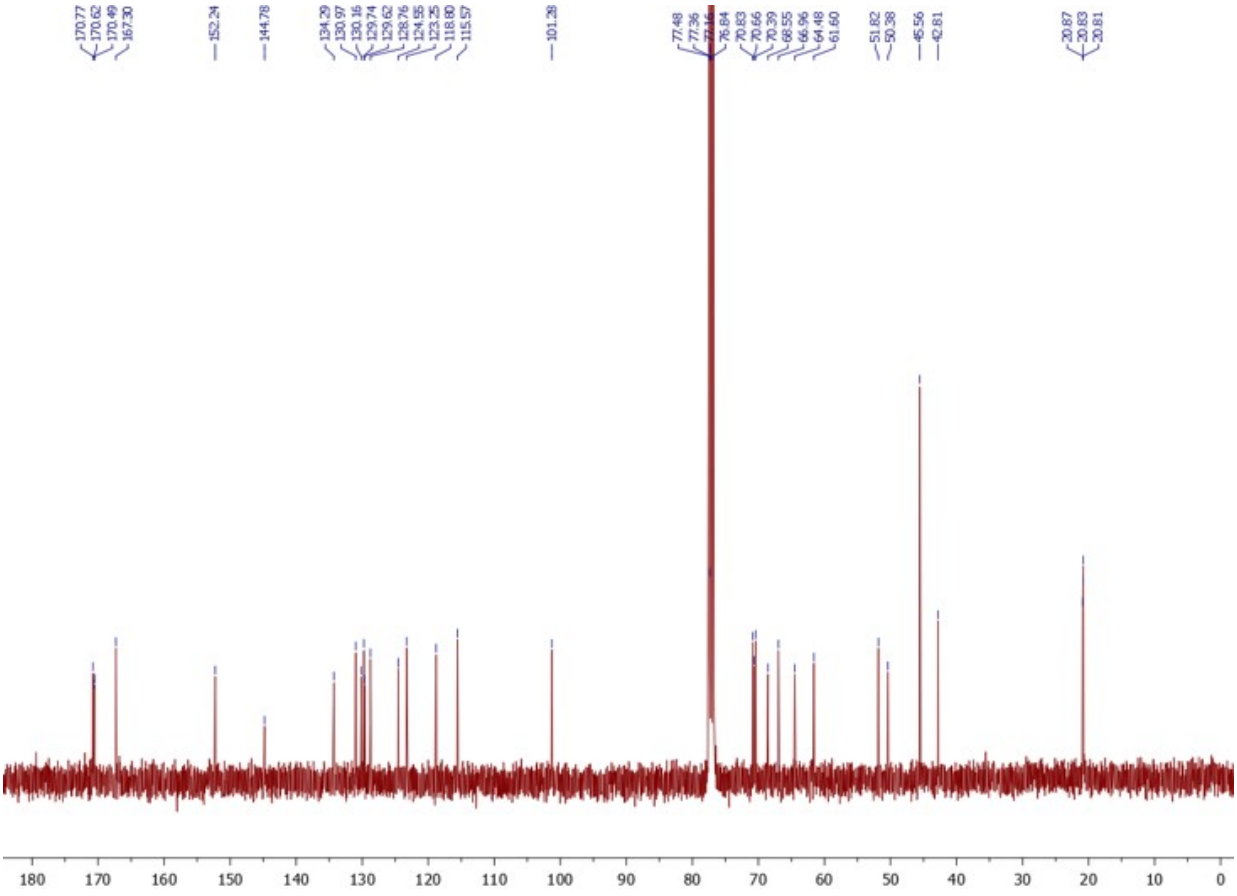
Compound **7-α**





Compound **7-β**





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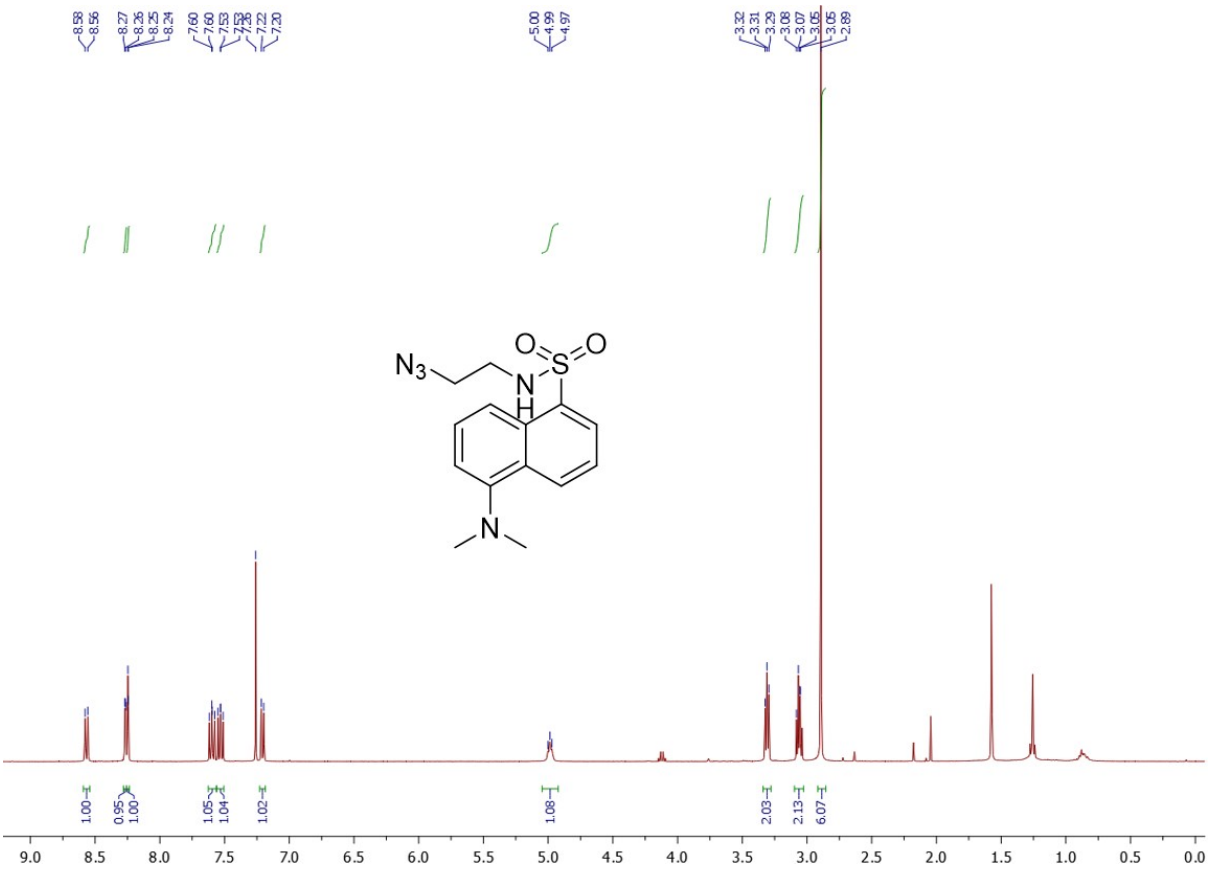
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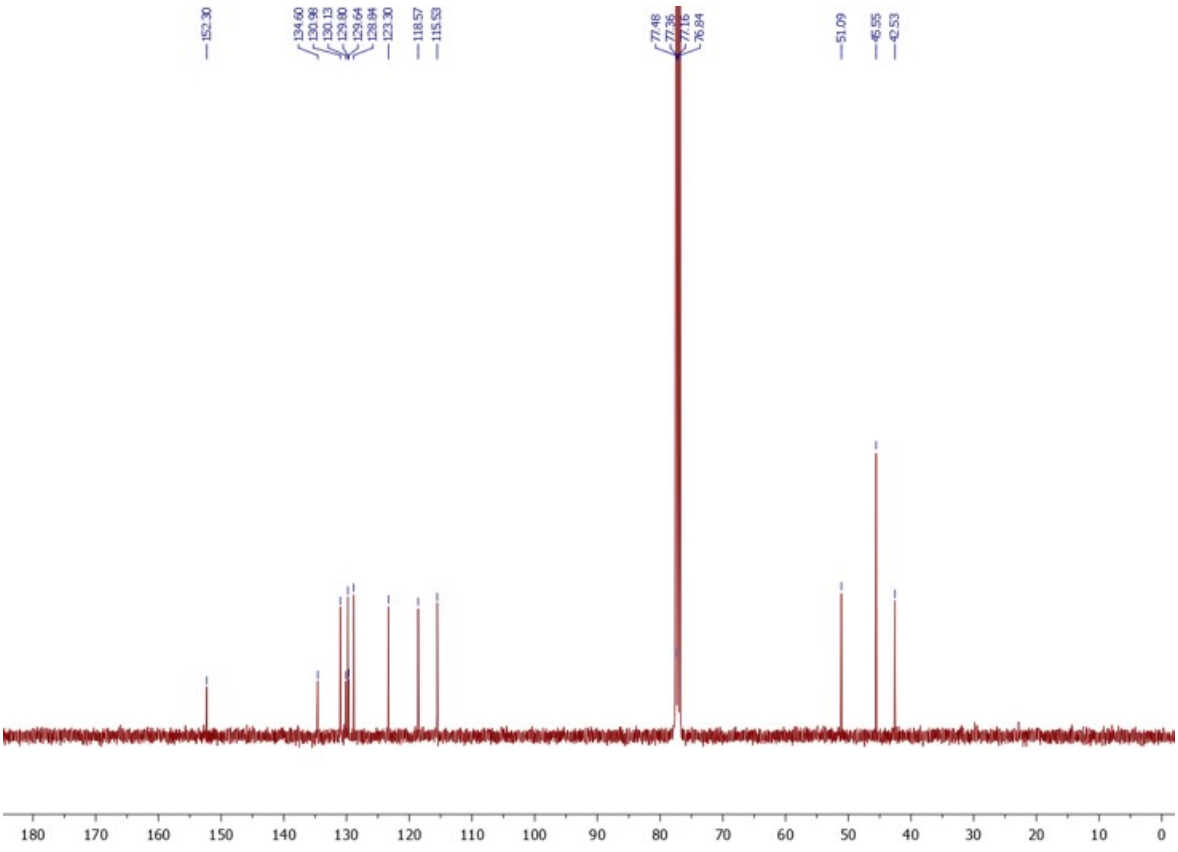
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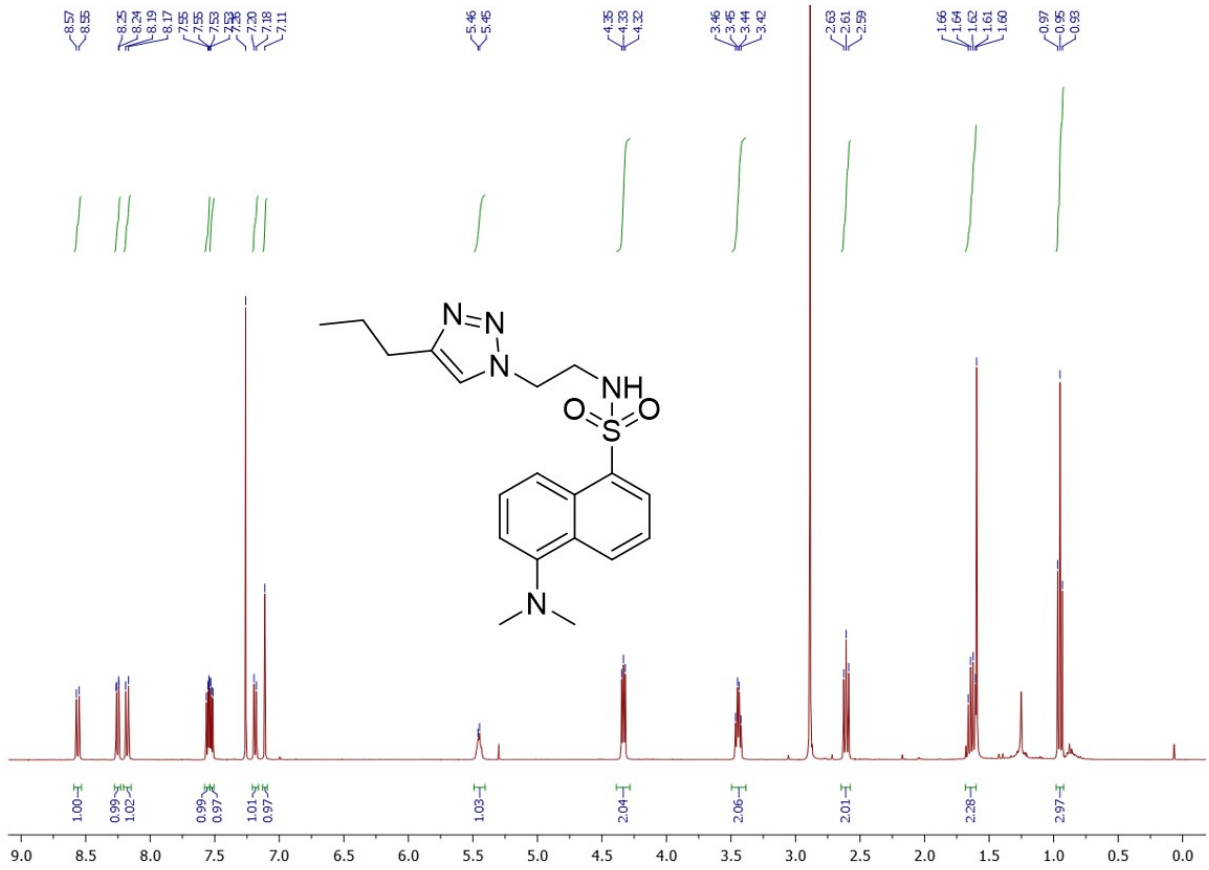
Compound **10**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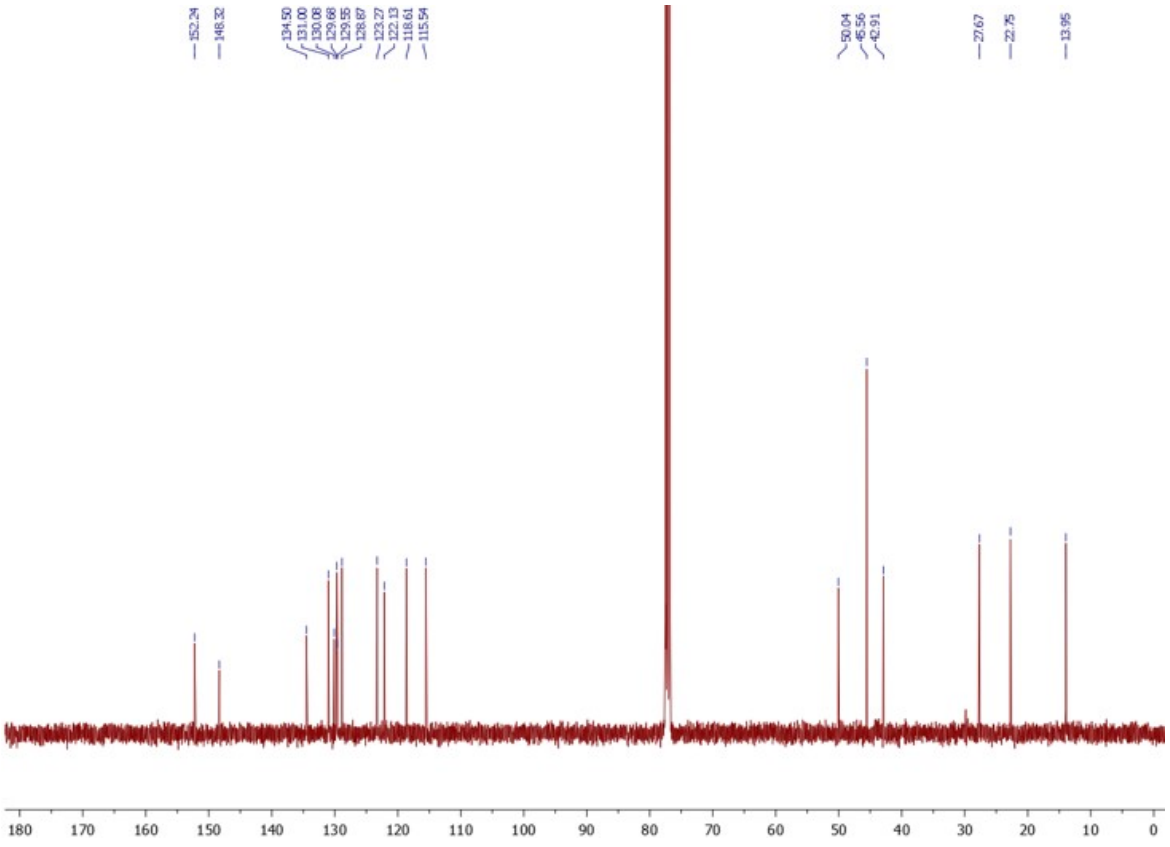
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