Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Vinyl sulfone: A versatile function for simple bioconjugation and inmobilization

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Crysyalliztion and determination of the tridemensional structure of glycosylated HEW lysozyme with glucopyranosyl vinyl sulfone (conjugate 16). Needle crystals of the glycosylated lysozyme (conjugate **16**) were grown by the hanging-drop vapour-diffusion method at 293ºK. Typically, drops contained 1.5 µl 25 mg/mL glycosylated lysozyme in 20 mM acetate pH 4.5 and 1.5 µl reservoir solution consisting of 100 mM Bis-Tris buffer pH 6.9 and 0.75-1.25 M $MgSO_4$ were equilibrated against 500 µl reservoir solution. Data collection was carried out using a MAR CCD at ESRF beamline BM16 (λ=0.98 Å), placed at a distance of 105 mm. The oscillation angle was 0.3 and the exposure time was 10 s per frame. The data set consisted of 441 images that were processed with the XDS software package.[1,2] The diffraction parameters and unit cell were indexed in $P2_12_12$ and $P2_12_12_1$, which were the two space groups with the highest likelihood of being correct. Further details can be found in López-Jaramillo et al (2005).[3]

Molecular replacement was carried out with CNS[4] using as a search model the atomic resolution structure at 0.94 Å of the HEW lysozyme (PDB code 1iee), omitting the solvent molecules. The rotation search was carried out as a direct rotation search from 15 to 2.5 Å. For the translation searches the resolution range was 10-2 Å. At this stage, the space group was confirmed to be $P2_12_12_1$. The structure was refined with CNS using torsion-angle molecular dynamics and a cross-validated maximum-likelihood target function,[5] 10% of the data set being randomly selected to estimate the free R value. Rebuilding of the model was carried out with XtalView on the basis of σ A-weighted maps.[6] No cut-off was applied and a solvent mask was automatically determined and updated by CNS to use the resolution range 10.0-1.6 A. Inclusion of solvent molecules and ligands was based on peaks of at least 3σ and 1σ in the σ A- weighted Fo-Fc and 2Fo-Fc maps, respectively.

Summary of the main features of the data set and refinement of the glycosylated lysozyme (PDB entry 2b5z). Values in parentheses correspond to the highest resolution shell.

Data collection	
X-ray source	BM16, ESRF
Wavelength (Å)	0.98
No. of crystals	1
Temperature of data collection (K)	100
Space group	$P2_12_12_1$
Unit cell parameters (Å)	$a = 30.58$ $b = 32.97$ $c = 120.27$
Data set	
Resolution range (Å)	15.0 – 1.6 (1.7 – 1.6)
Measured reflections	85461 (14050)
Unique reflections	16442 (2657)
Completeness [I/σ(I)>0](%)	97.9 (97.0%)
Completeness [I/σ(I)>3] (%)	91.6.0% (78.1%)
Rmerge (%)	3.8 (15.3)
Mean (I/σ(I)	24.93 (10.71)
Refinement	
Resolution range (Å)	10.0 – 1.6
Free R value, random 10%	0.225
R value	0.212
No. water molecules	80
No. glycopyranosyl vinyl sulfone	4
Rmsd covalent bonds[a] (Å)	0.014
Rms bonds angles[a] (º)	1.9
Overall coordinate errors (DPI)[b]	0.083
Correlation coefficient[c]	0.873
Optical resolution[d]	1.34
Ramachandran Plot	
Residues in the most favoured regions (%)	89.1
Residues in additional allowed regions (%)	10.9

[a]Deviation from the standard dictionary.[7]
[b] Diffraction-data precision indicator.[8]
[c] Correlation coeffcient between calculated and observed structure-factor amplitude output by SFCHECK.[9]
[d]Expected minimum distance between two resolved peaks in the electron-density map as implemented in SFCHECK.

References

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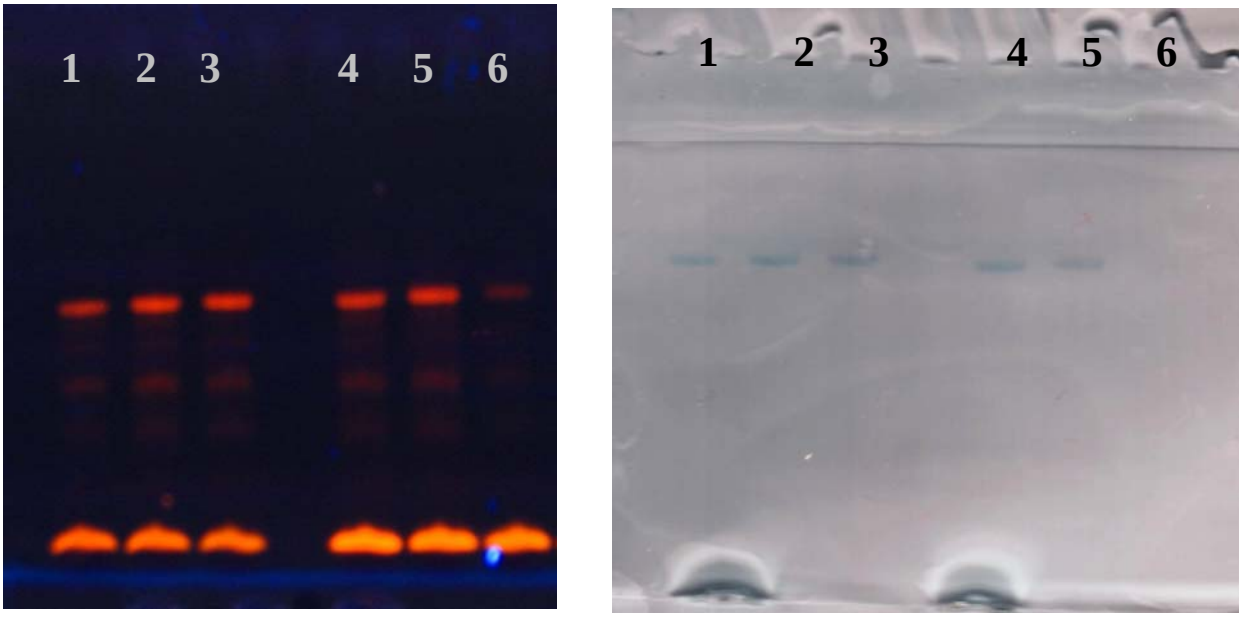


Fig. S1. *Influence of the stoichiometry and time of reaction on the labeling of concanvalin A with compound 4.* SDS-PAGE of the samples labeled with protein:compound **4** stoichiometries 1:5 (lanes 1-3) and 1:10 (lanes 4-6) and reaction time of 3 h (lanes 1 and 4), 8 h (lanes 2 and 5) and 24 h (lanes 3 and 6) visualized with a standard transilluminator at 365 nm (left) and later stained with coomassie blue (right).

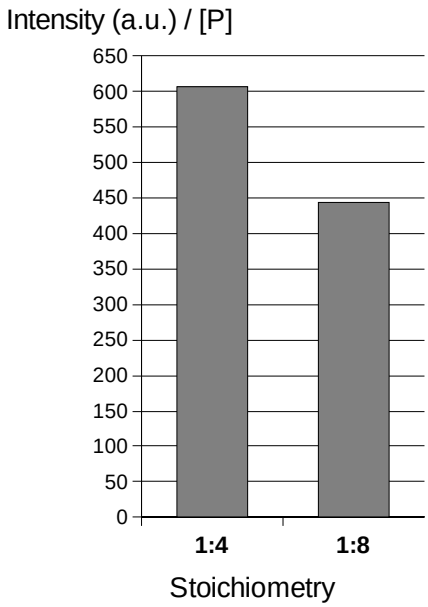


Fig. S2. *Influence of the stoichiometry on the fluorescence intensity per micromol of protein of the tetrameric avidin labeled with compound 5***.** Over-labeling of avidin with product 5 does not lead to precipitation but causes quenching.

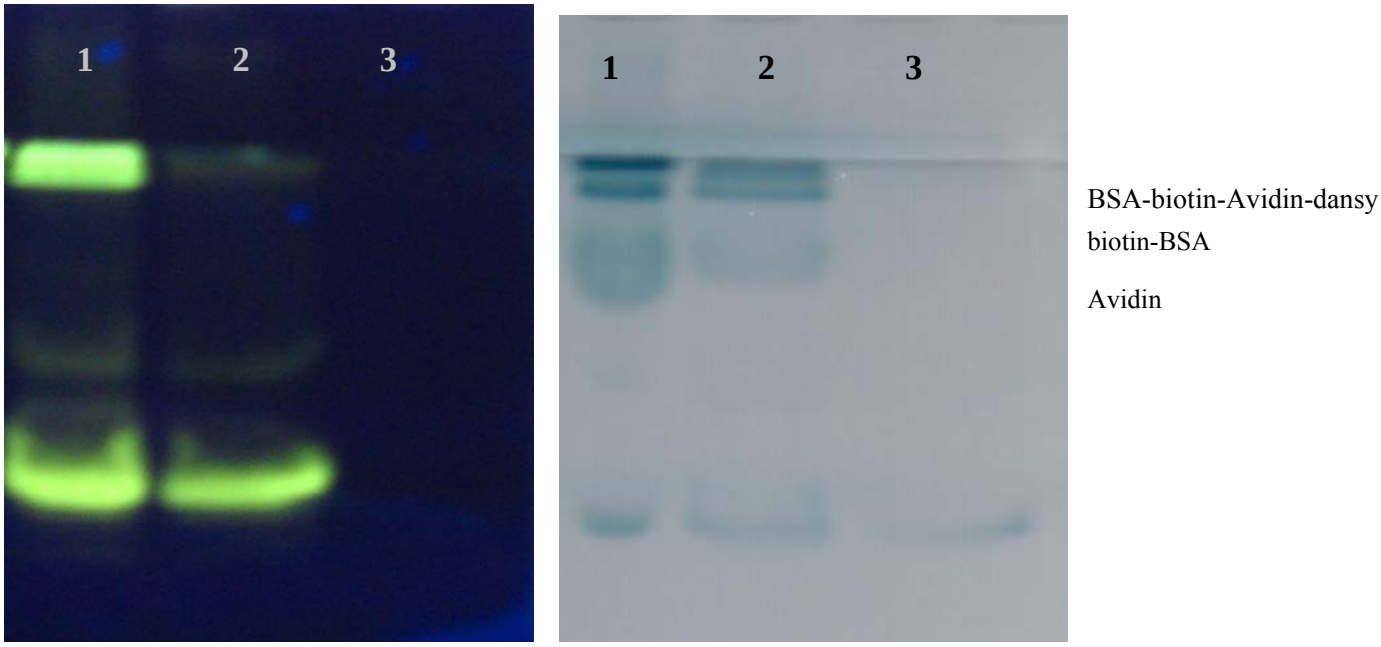


Fig. S3. *Electrophoresis of the complex BSA-biotin-avidin-dansyl denaturized in mild conditions.* Left fluorescence and right Coomassie. Lanes are: 1)BSA-biotin-avidin-dansyl stoichiometry 4:4; 2) BSA-biotin-avidin-dansyl stoichiometry 1:4; 3) avidin (control)

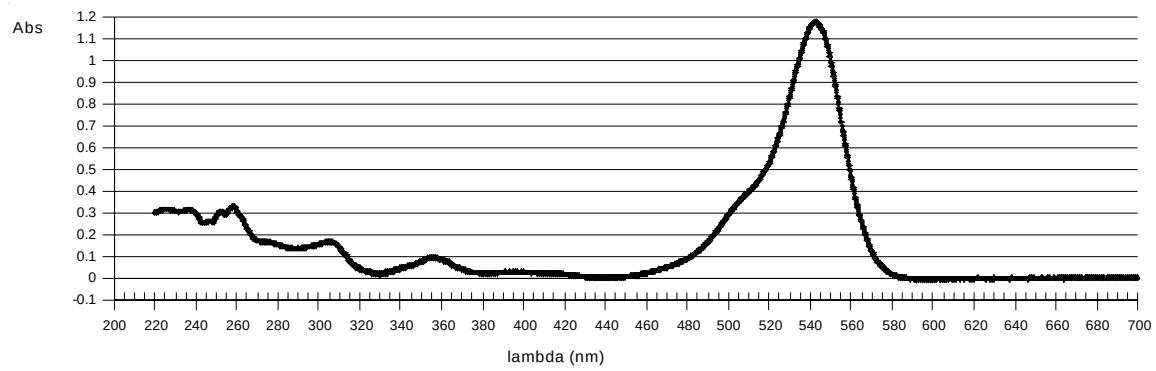
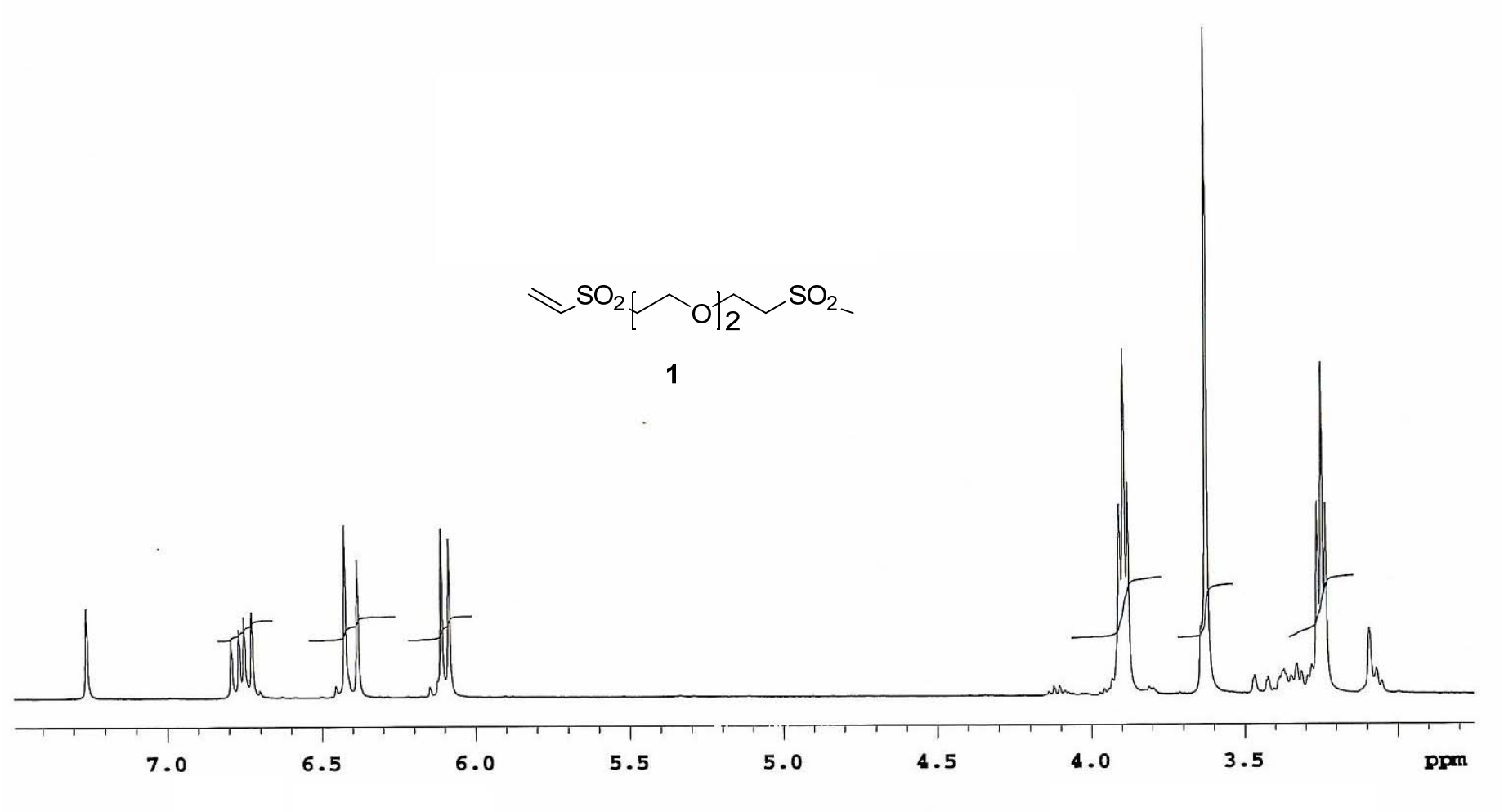
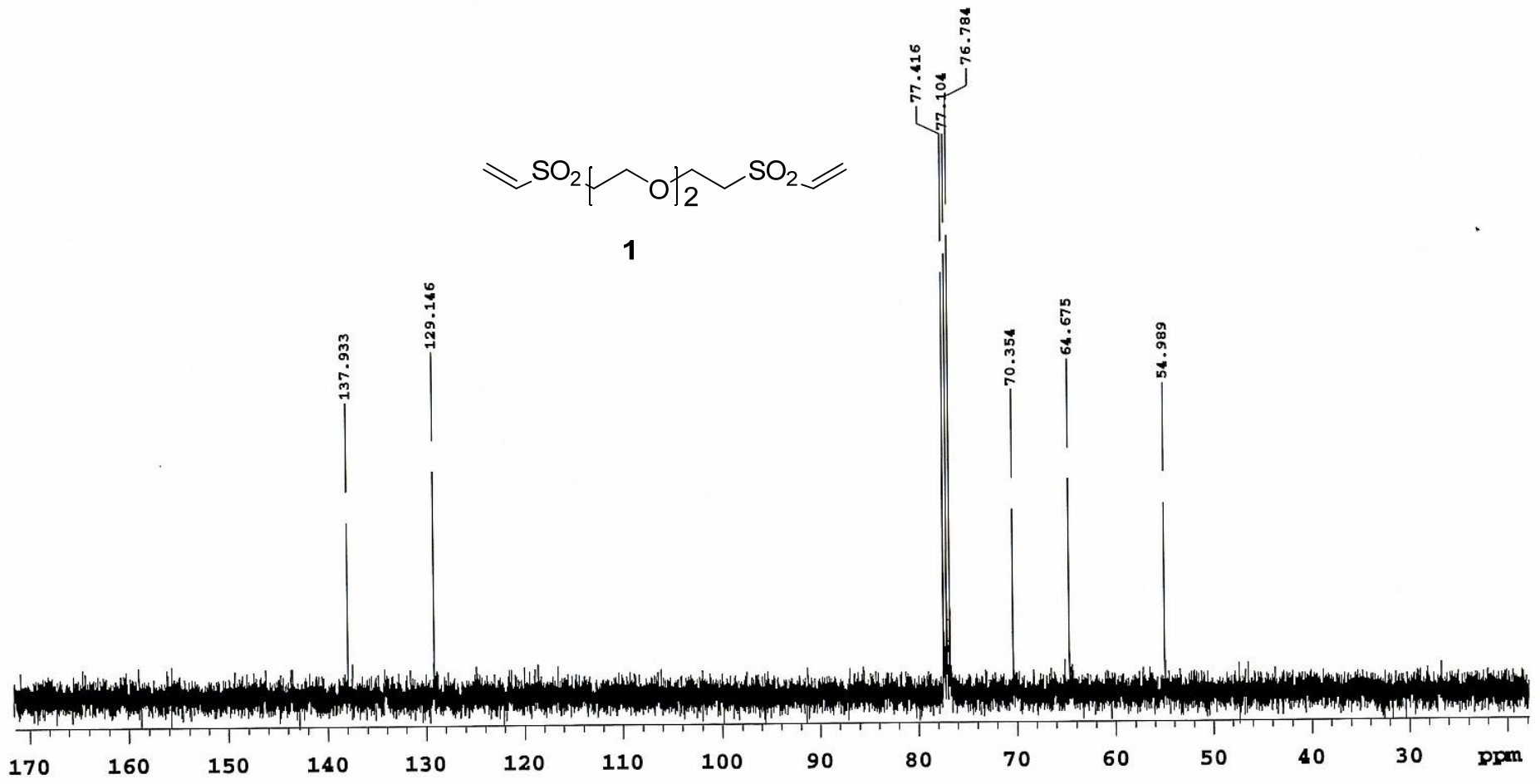


Fig. S4.- *Absorption and emission spectrum of vinyl sulfone rhodamine B.* (A)Absorption spectrum. Arrow shows the unsuitable wavelength of excitation of the conventional transilluminator (365 nm). (B)Emission spectrum excited at 365 nm and the optimum weighlength (*** nm)



^{1}H-NMR Spectra for compound **1**



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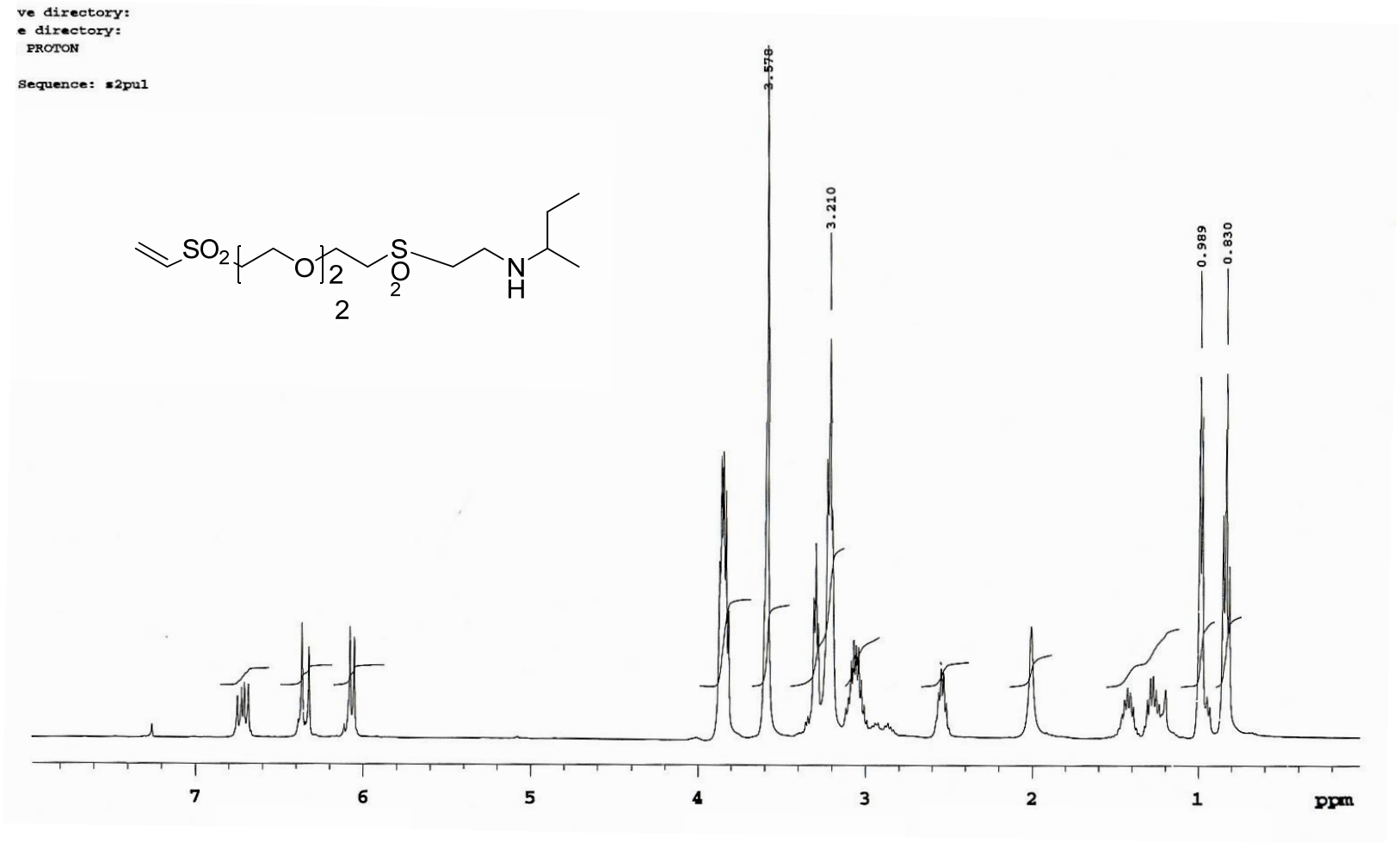

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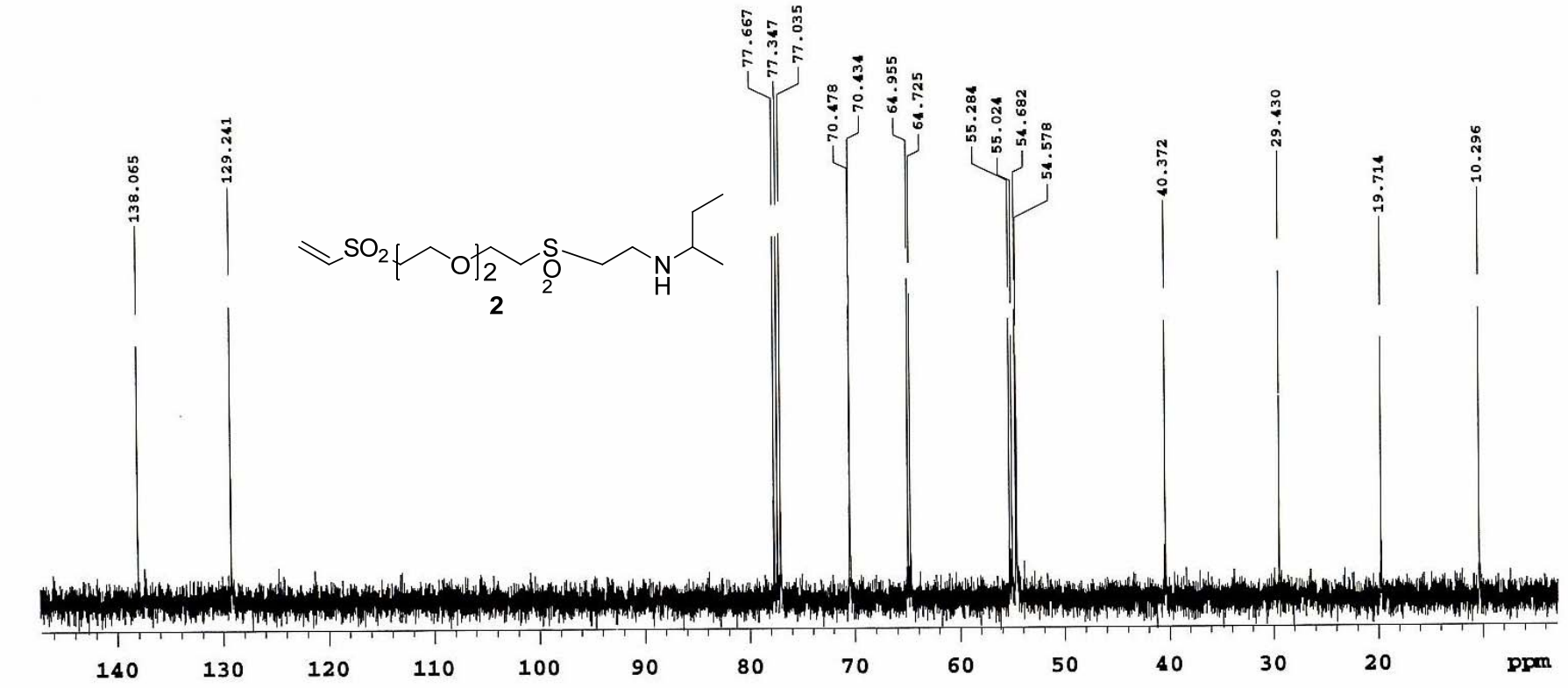

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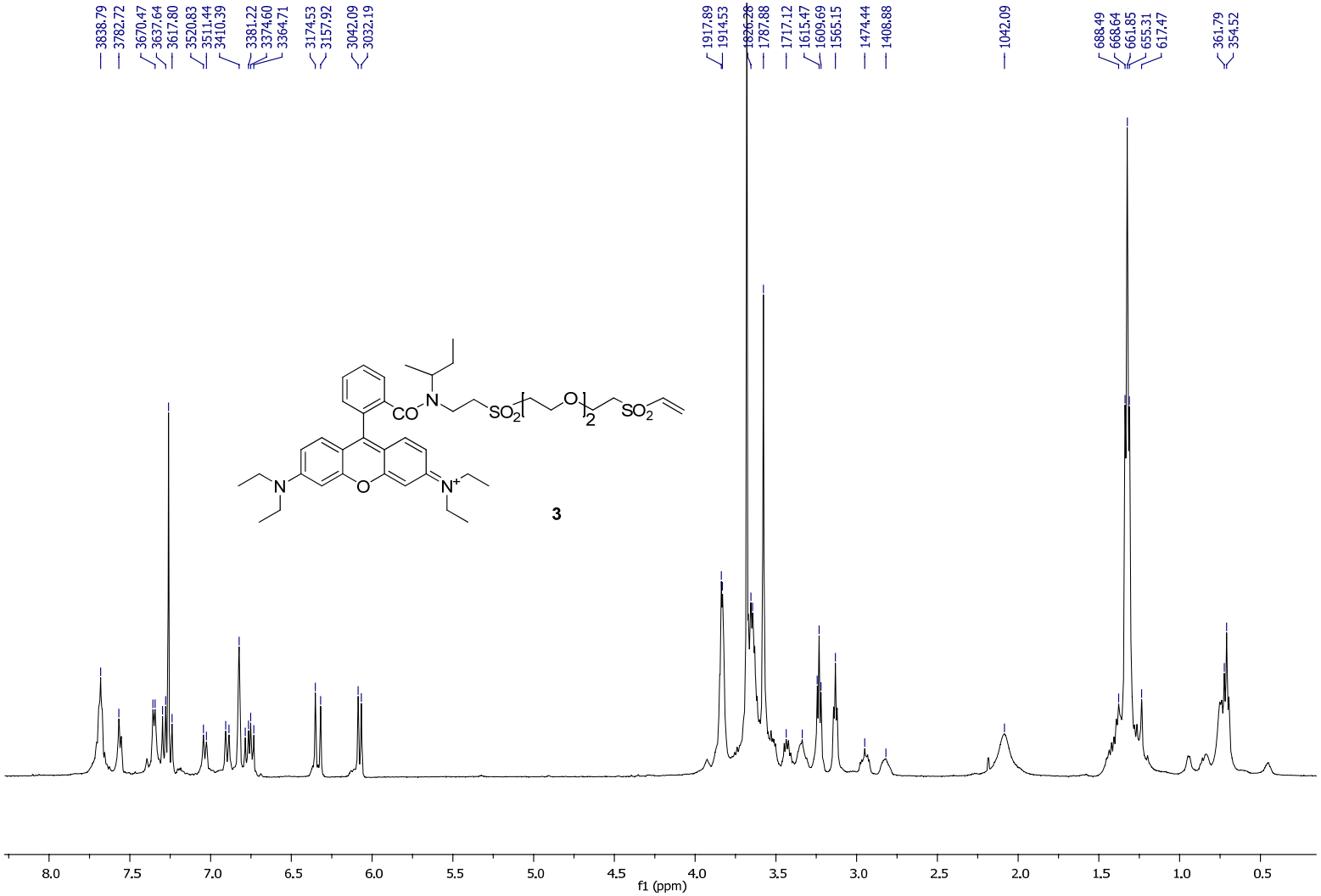

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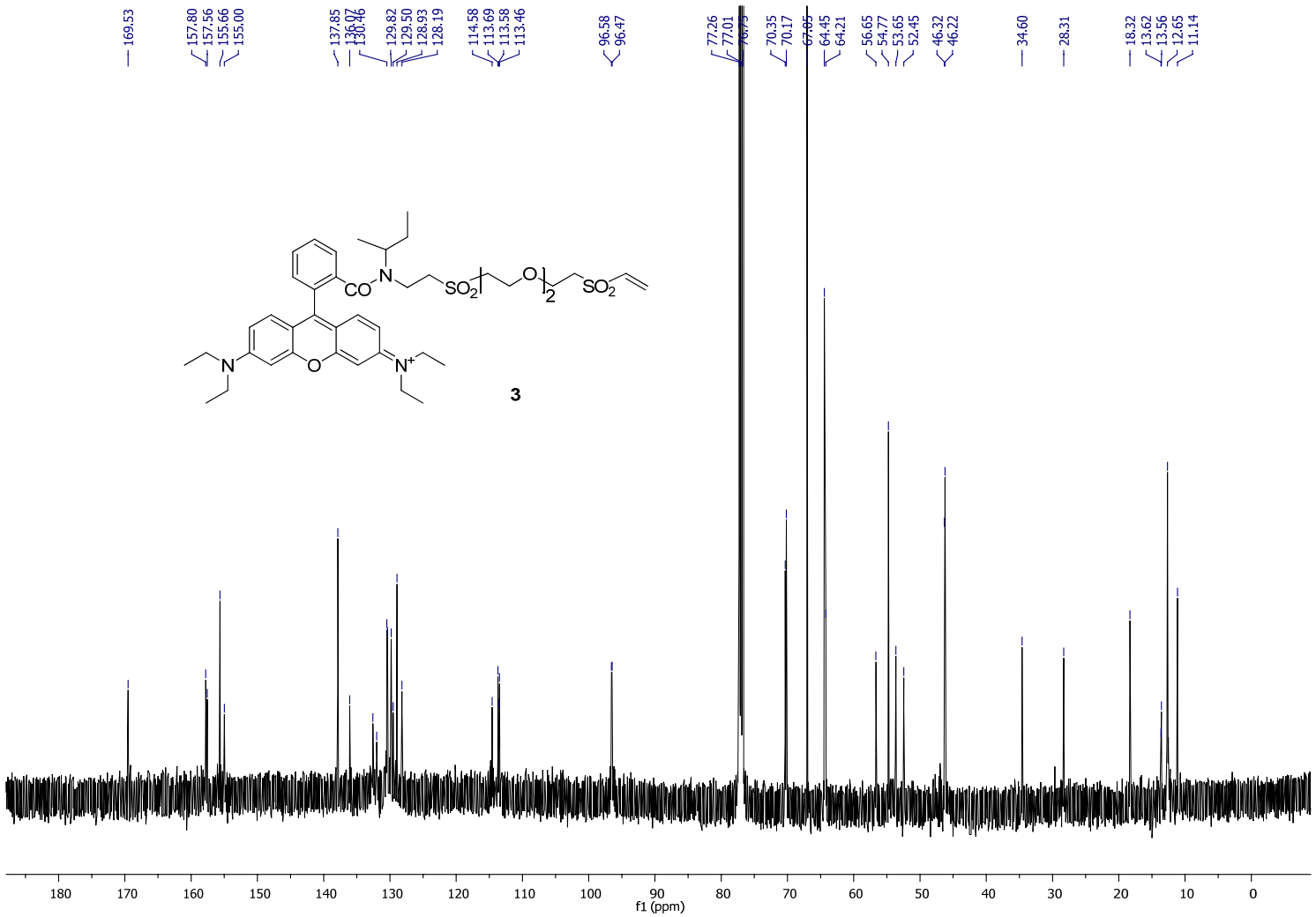

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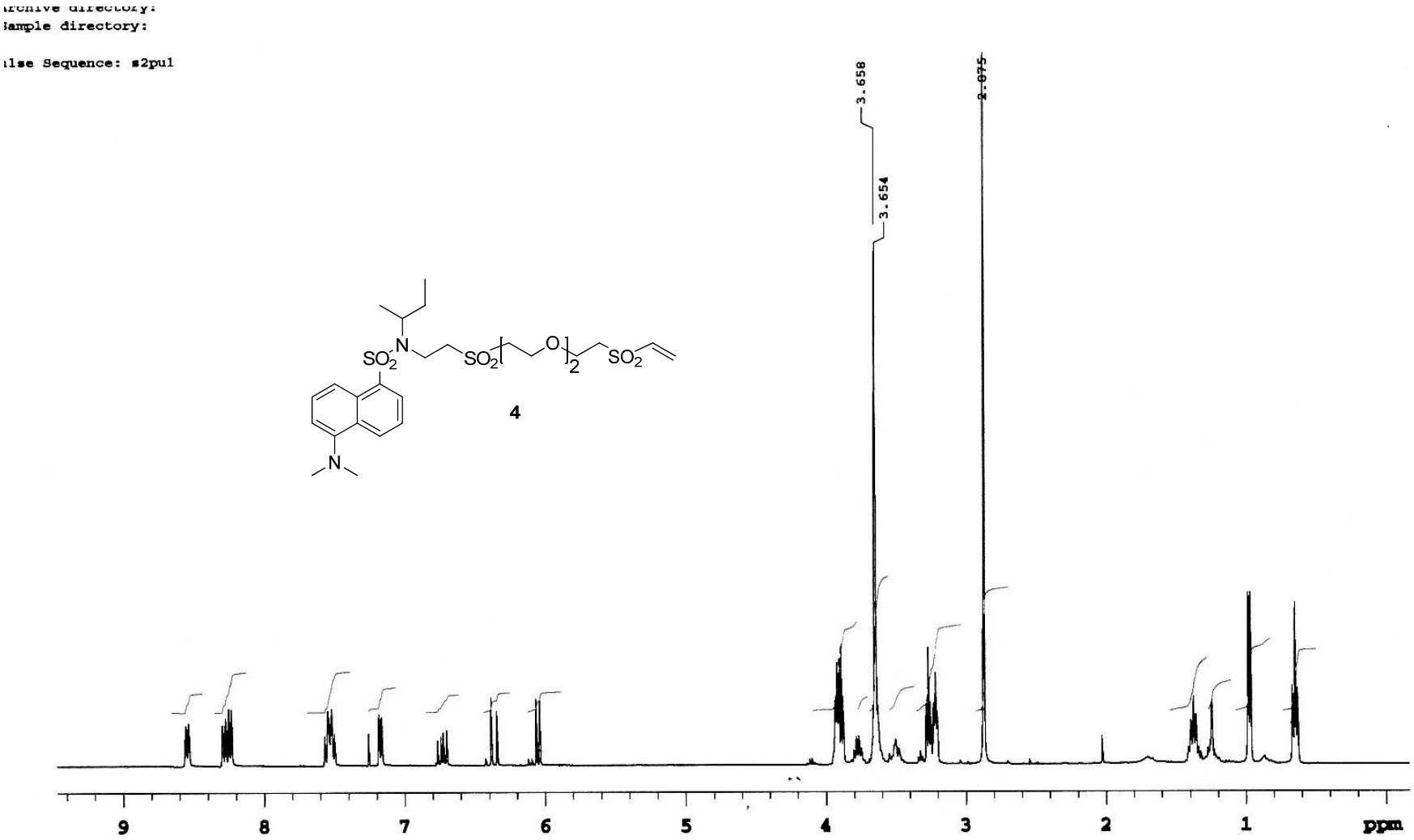

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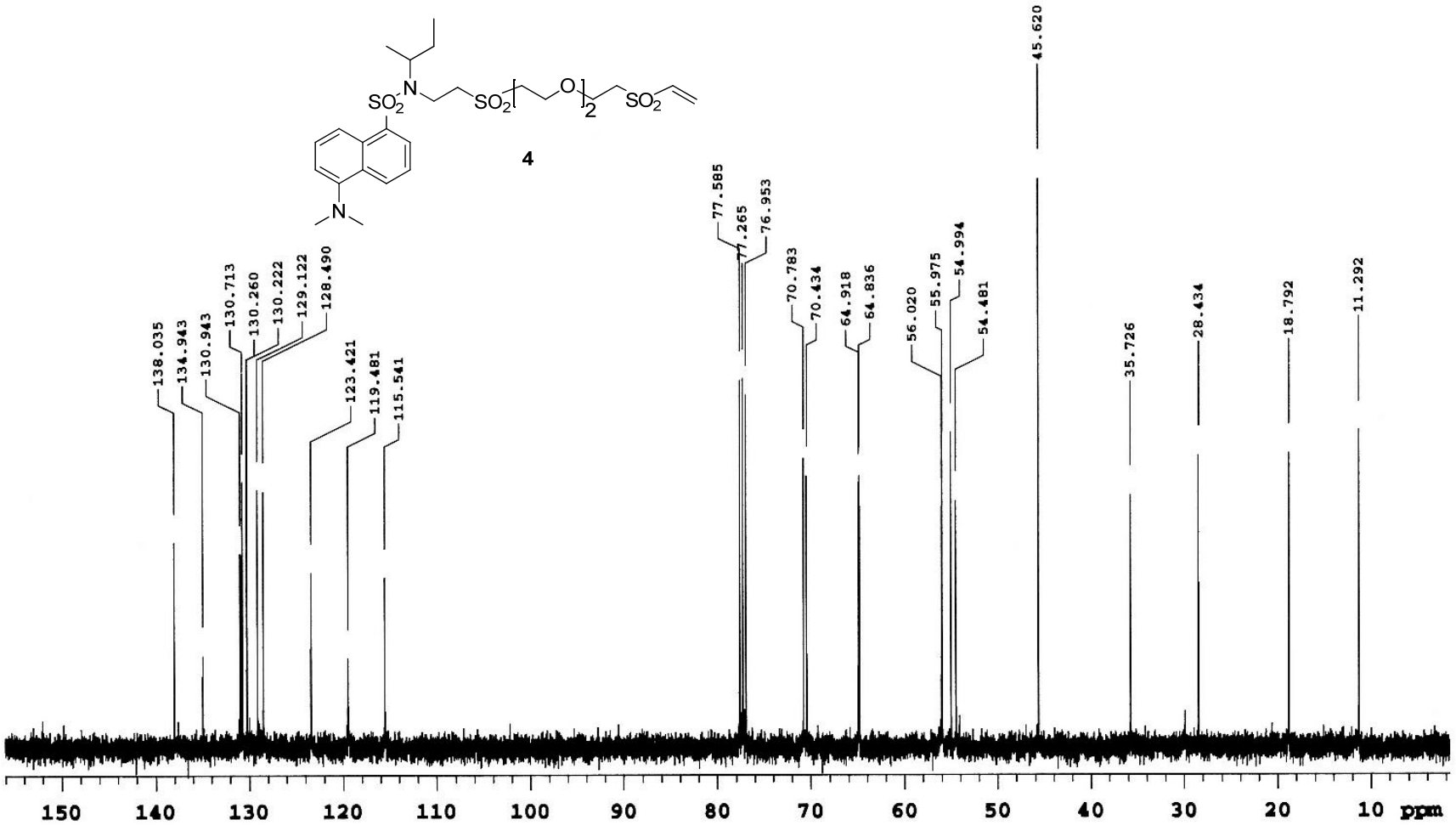

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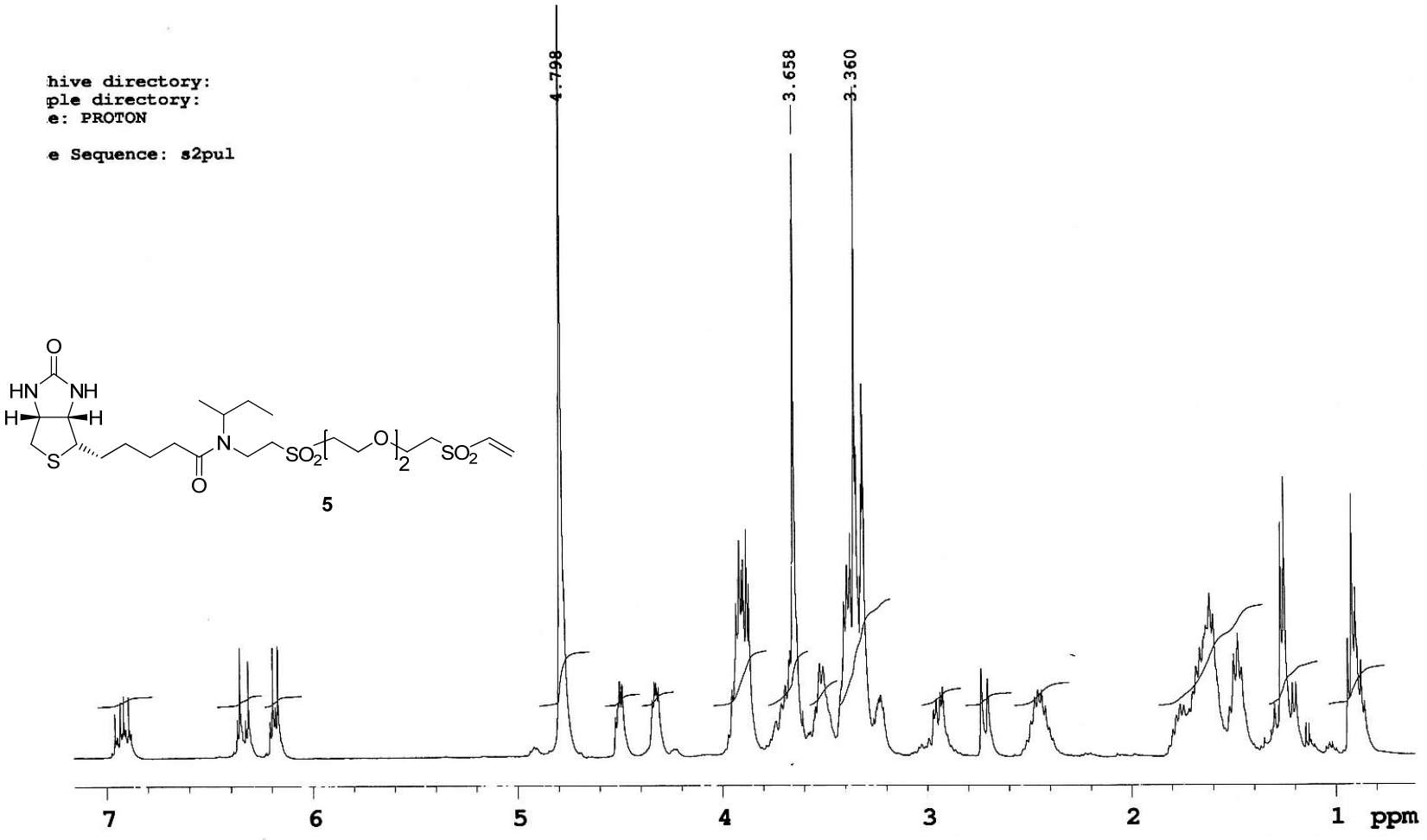

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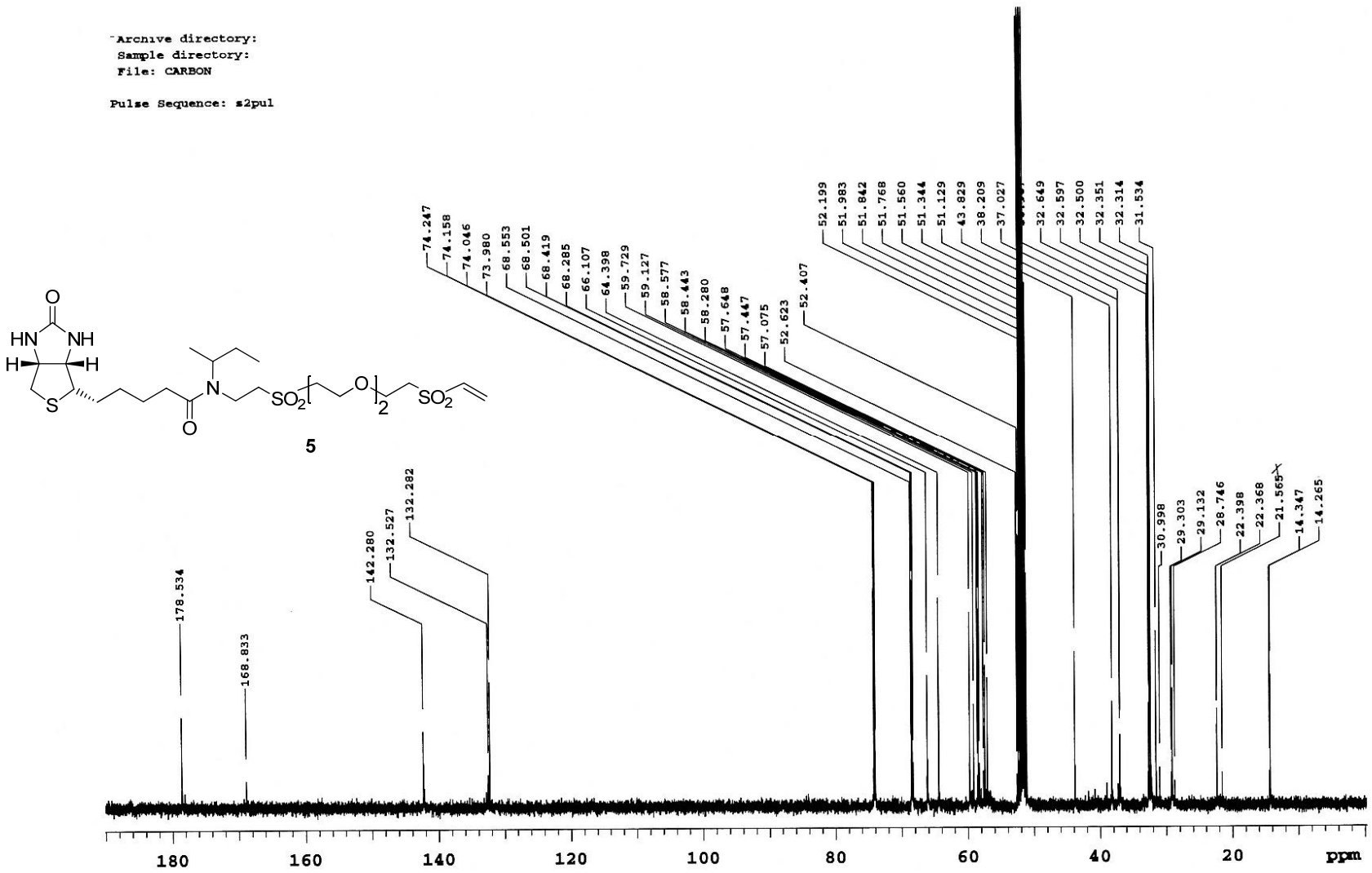

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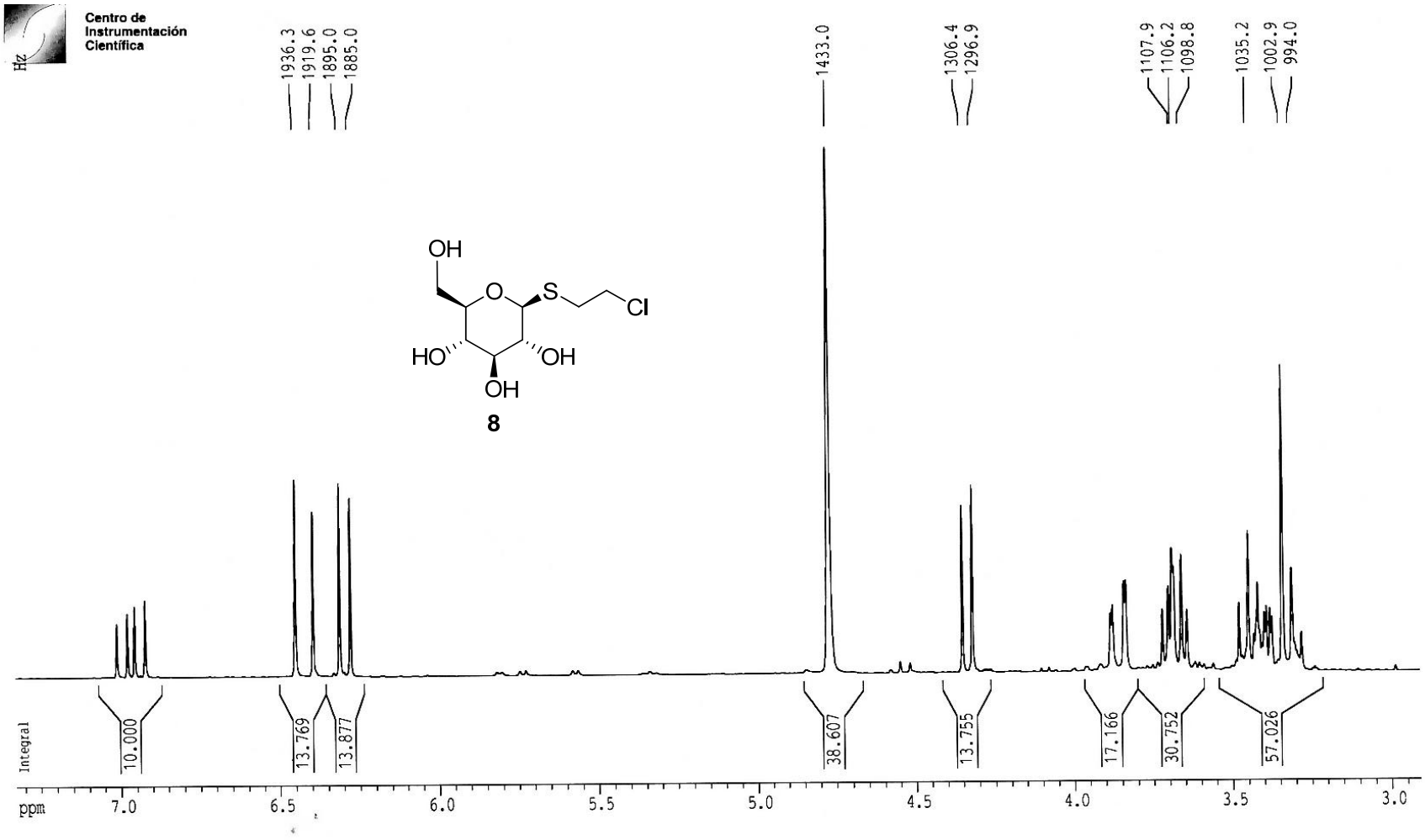

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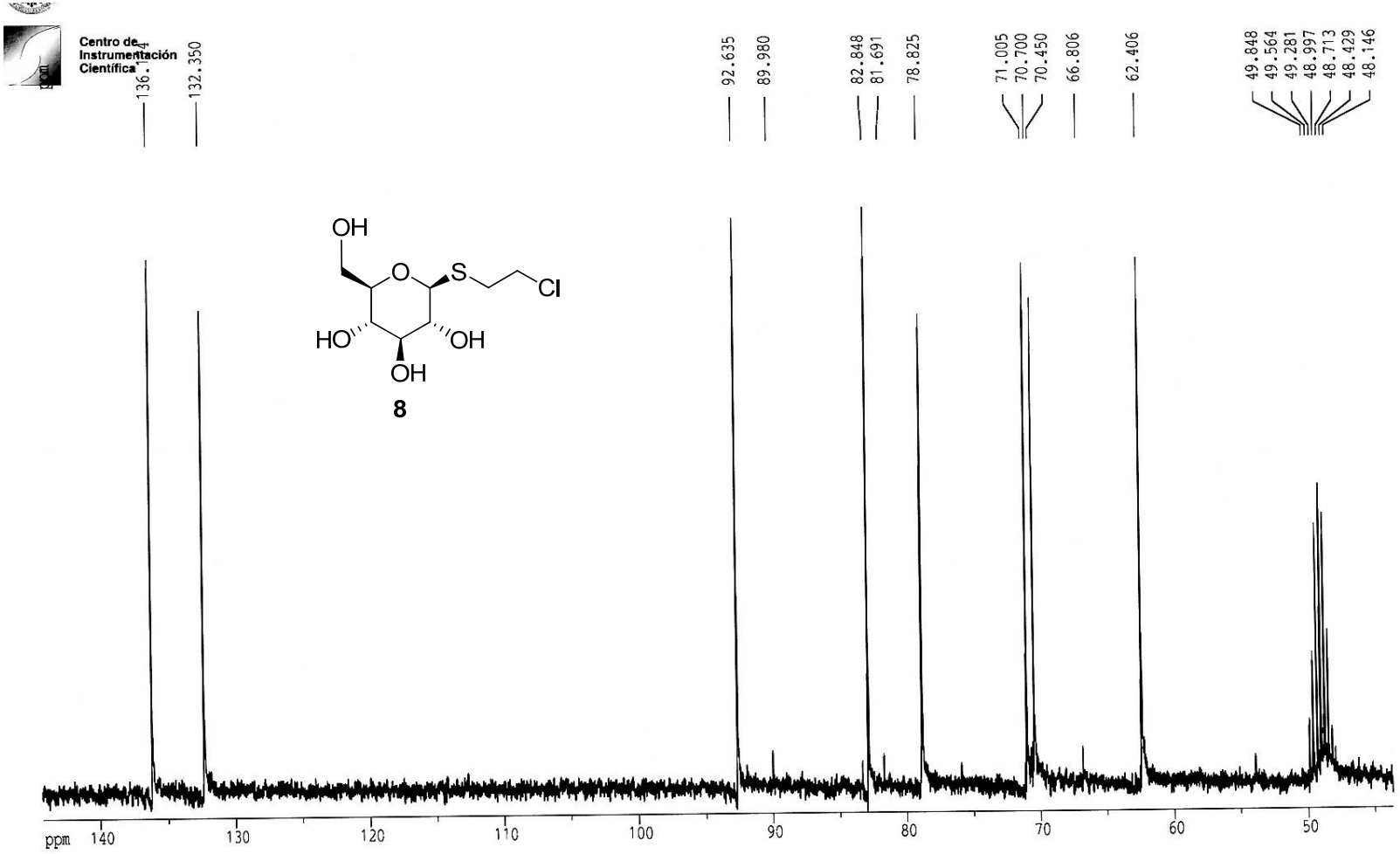

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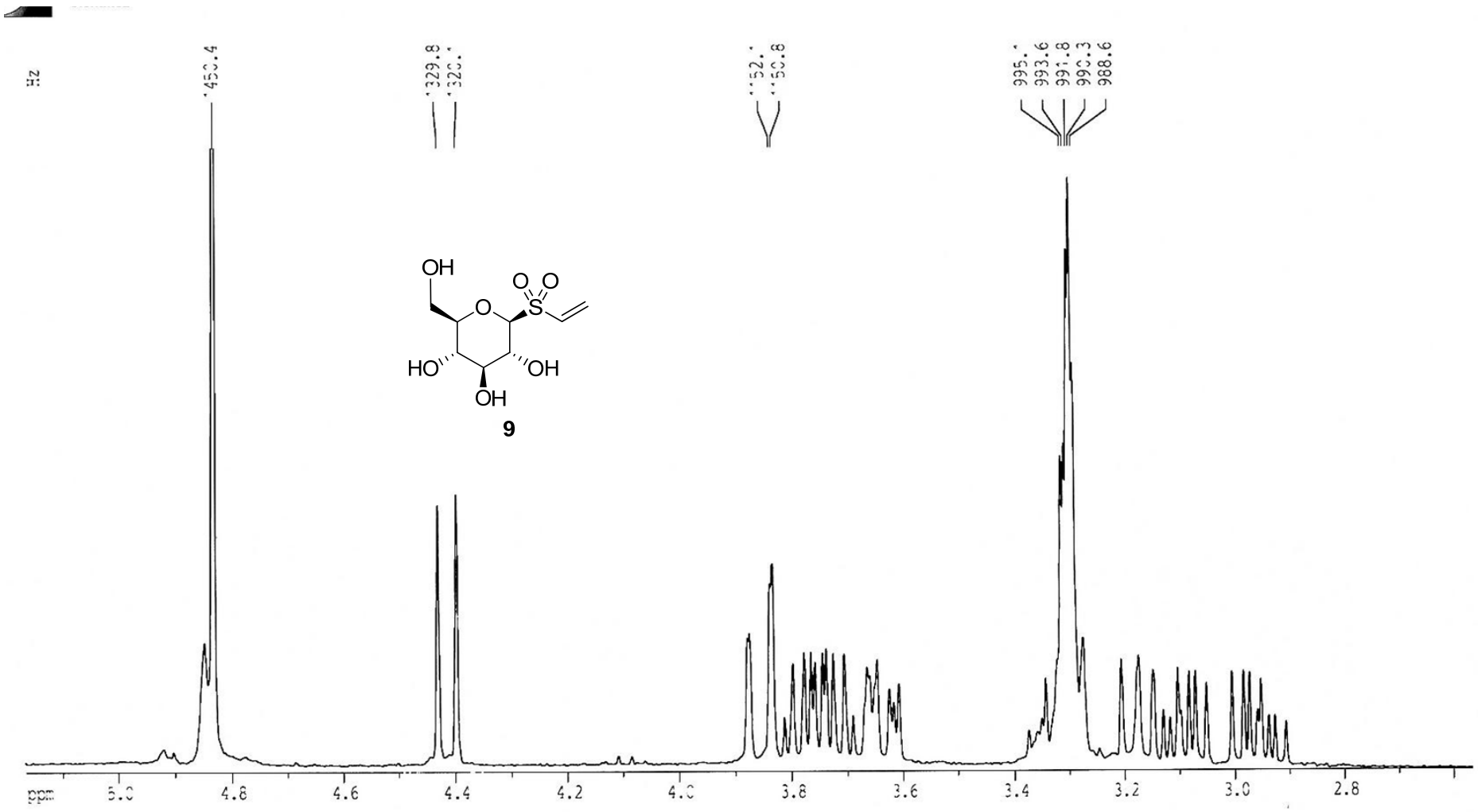

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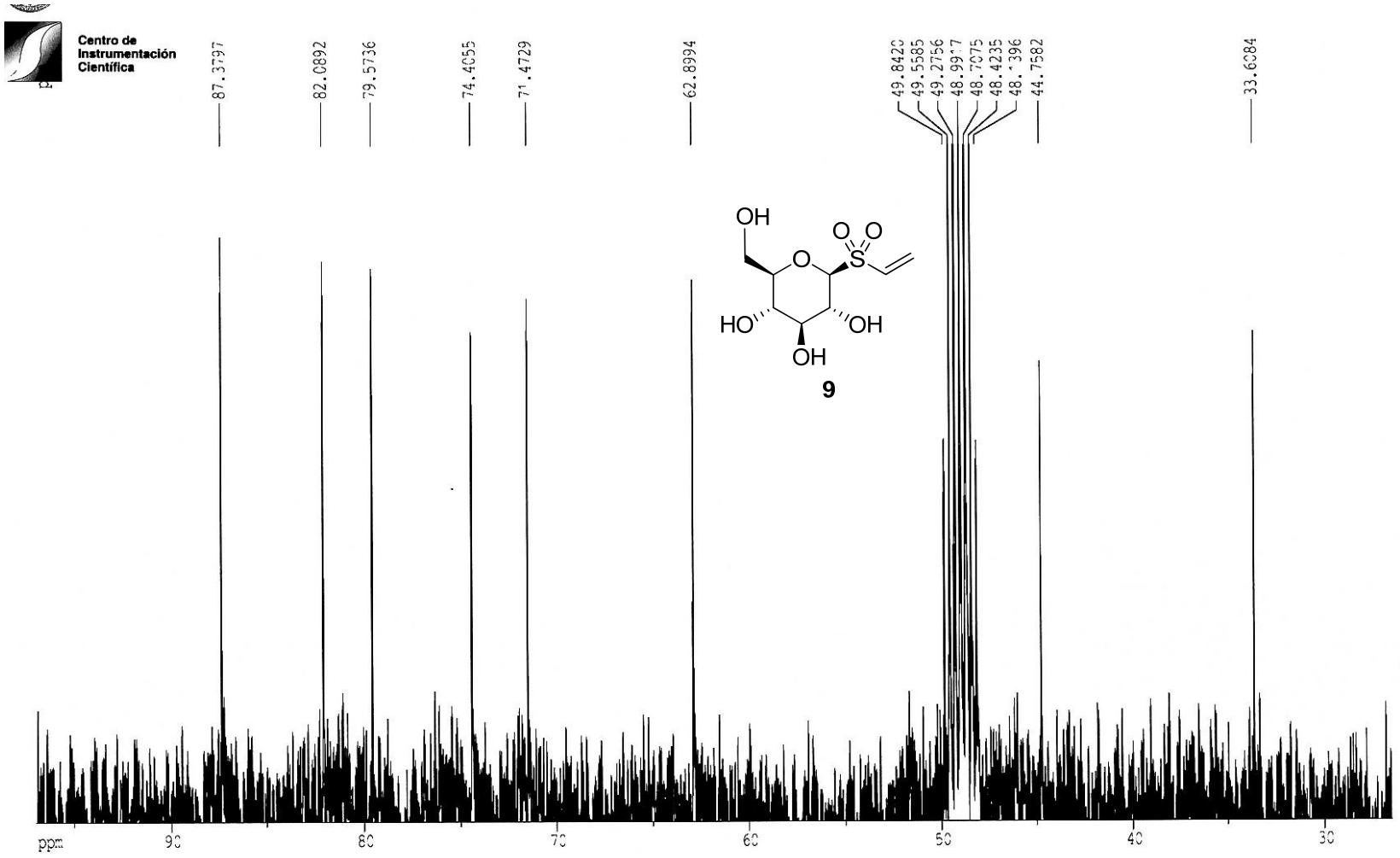

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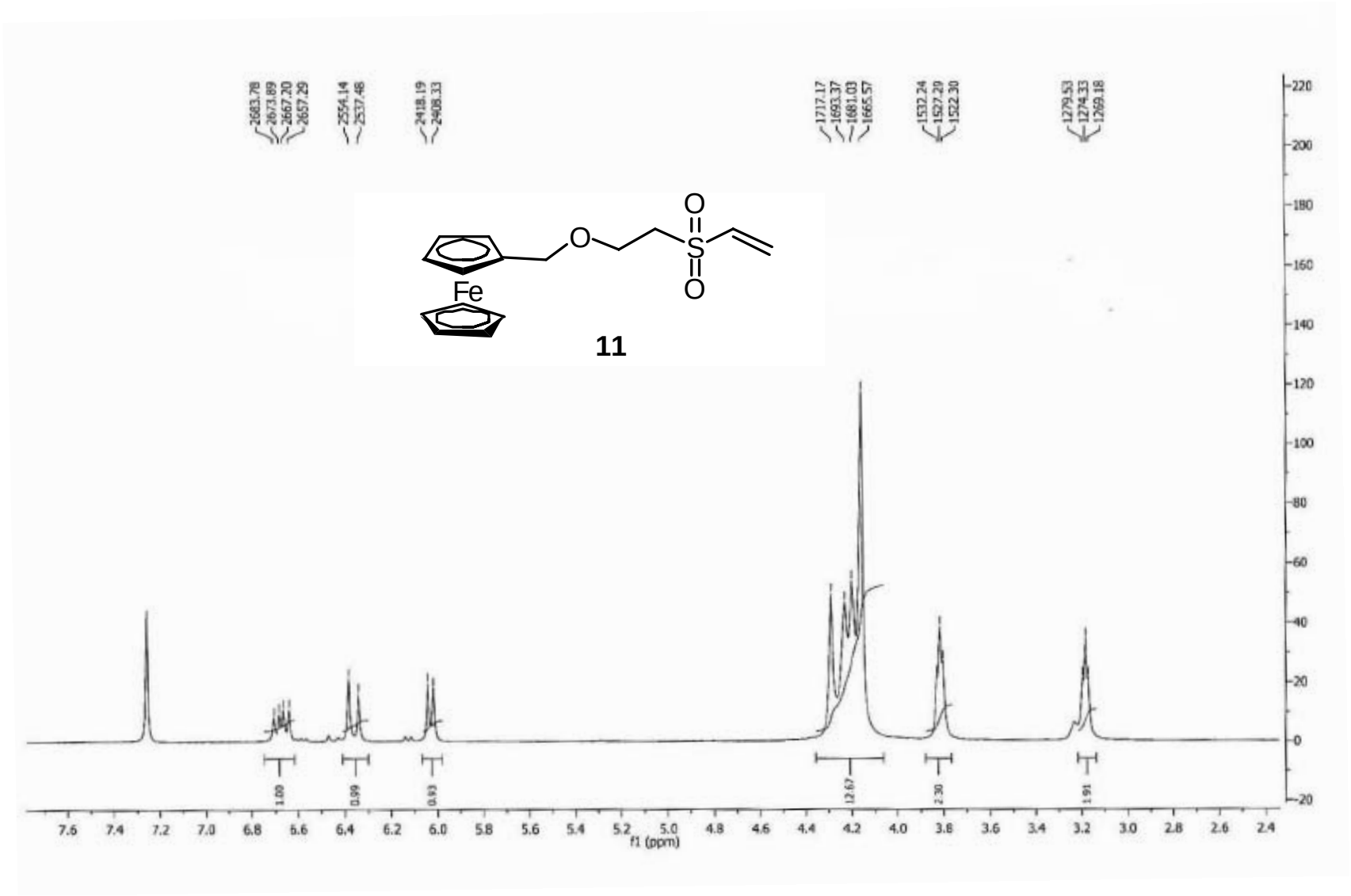

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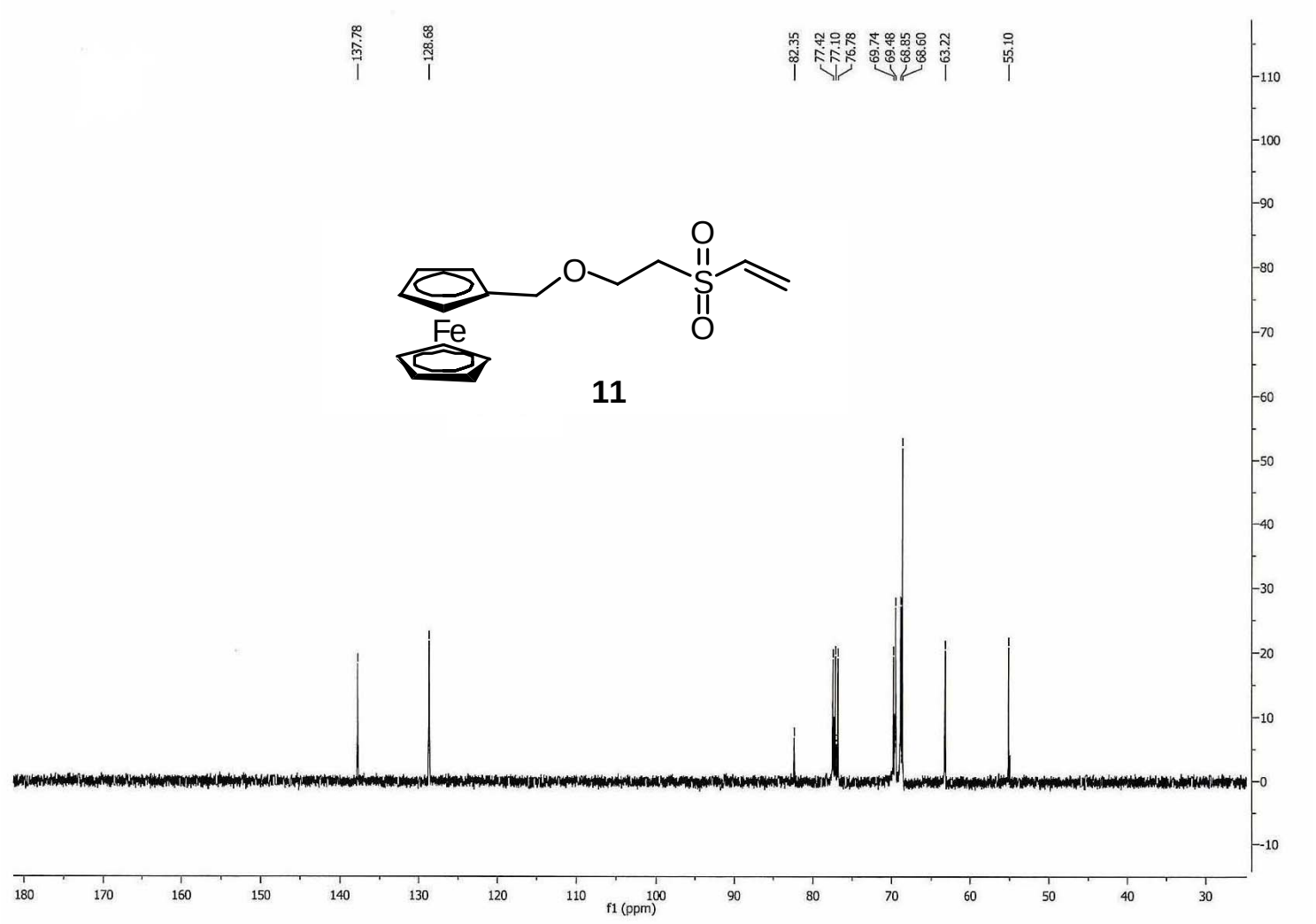

^{13}C-NMR Spectra for compound **11**