Intrinsic proteolytic activities from cancer cells are sufficient to activate alkoxyamine prodrugs and induce cell death

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Characterization of alkoxyamines alkoxyamines 1.c-a and 2.c-a:

Figure S1: 1-c (^{1}H NMR)

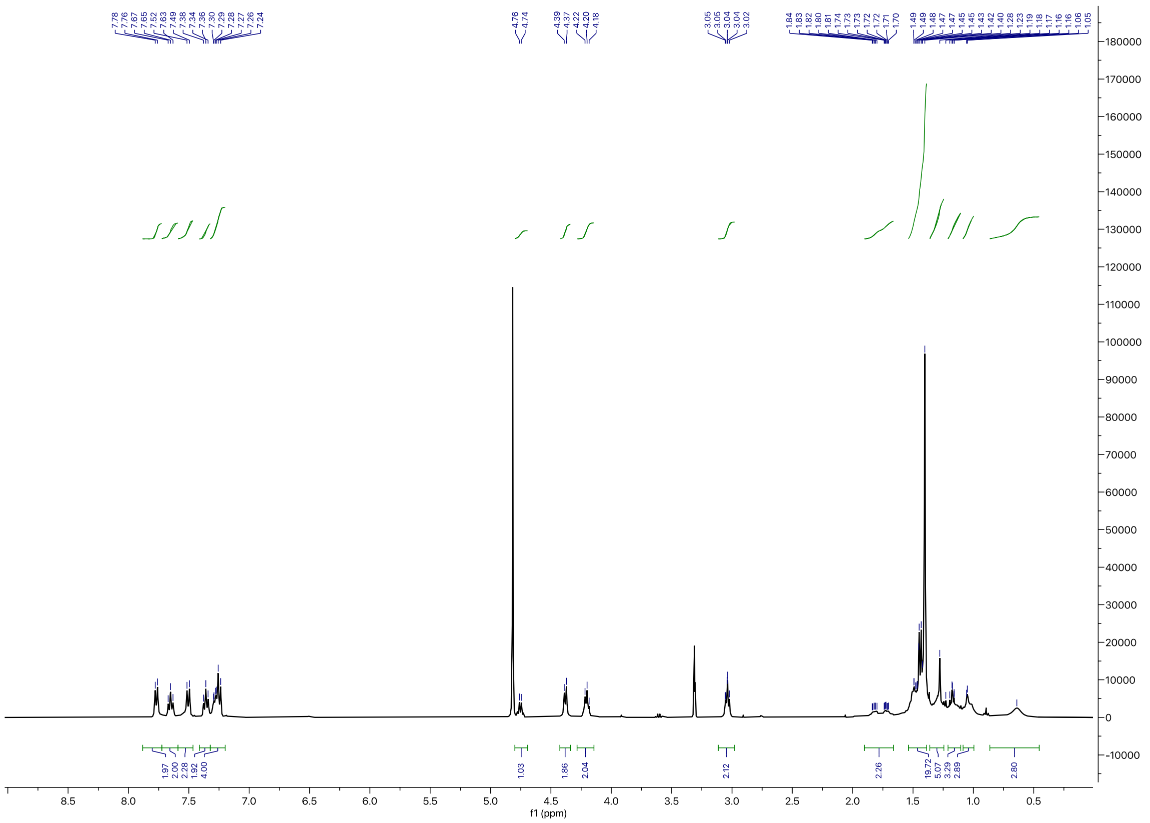


Figure S2: 1-c (^{13}C NMR)

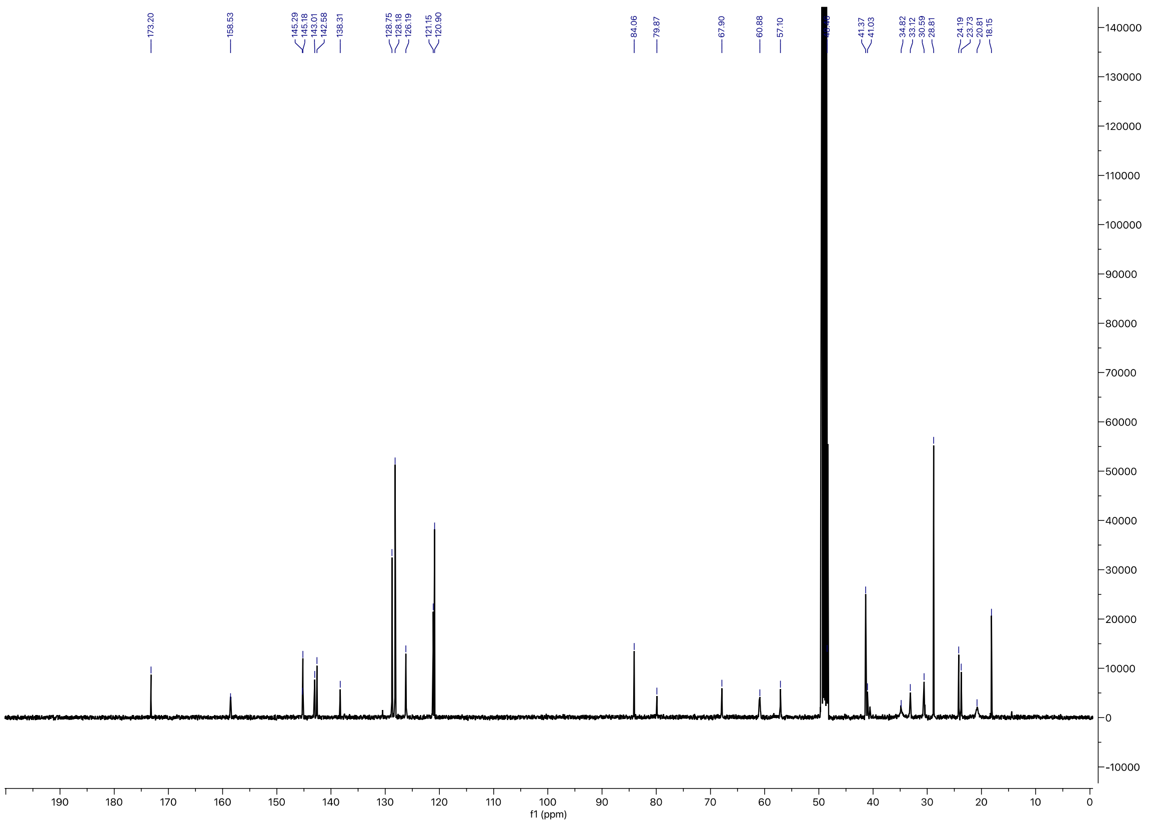


Figure S3: 1-c (^{13}C DEPT NMR)

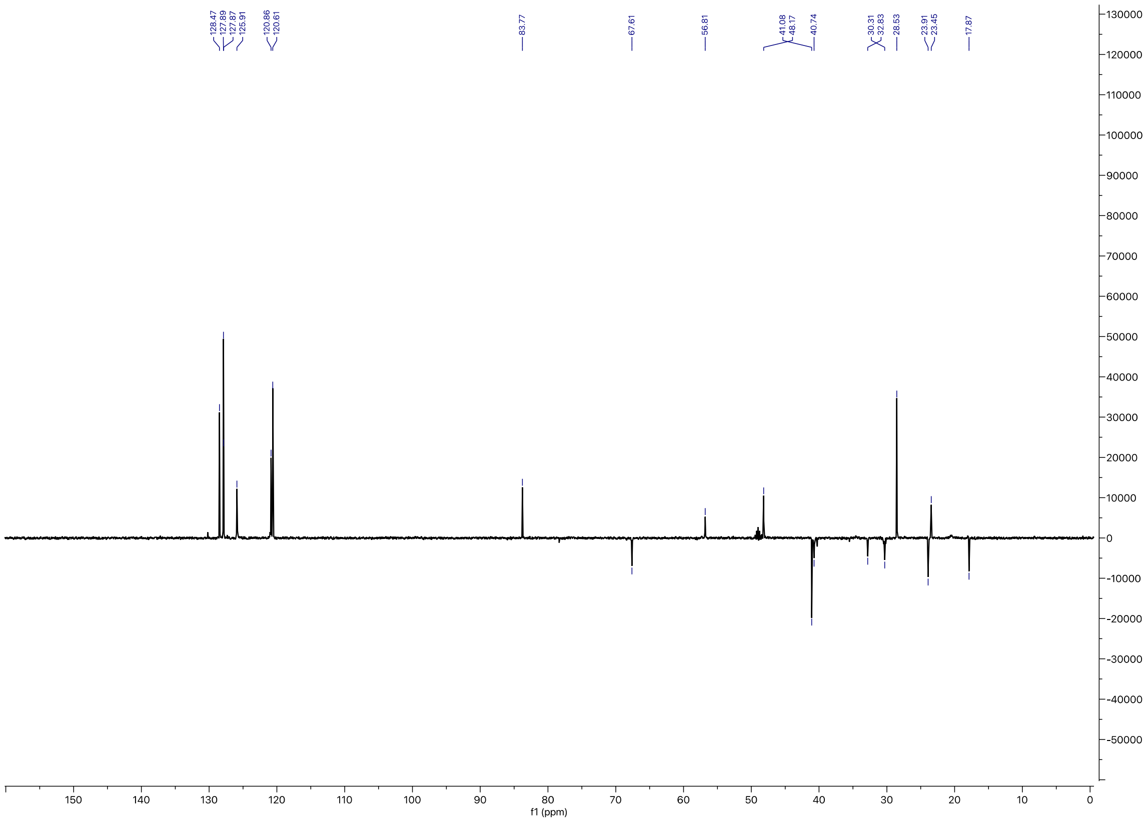


Figure S4: 1-c HRMS

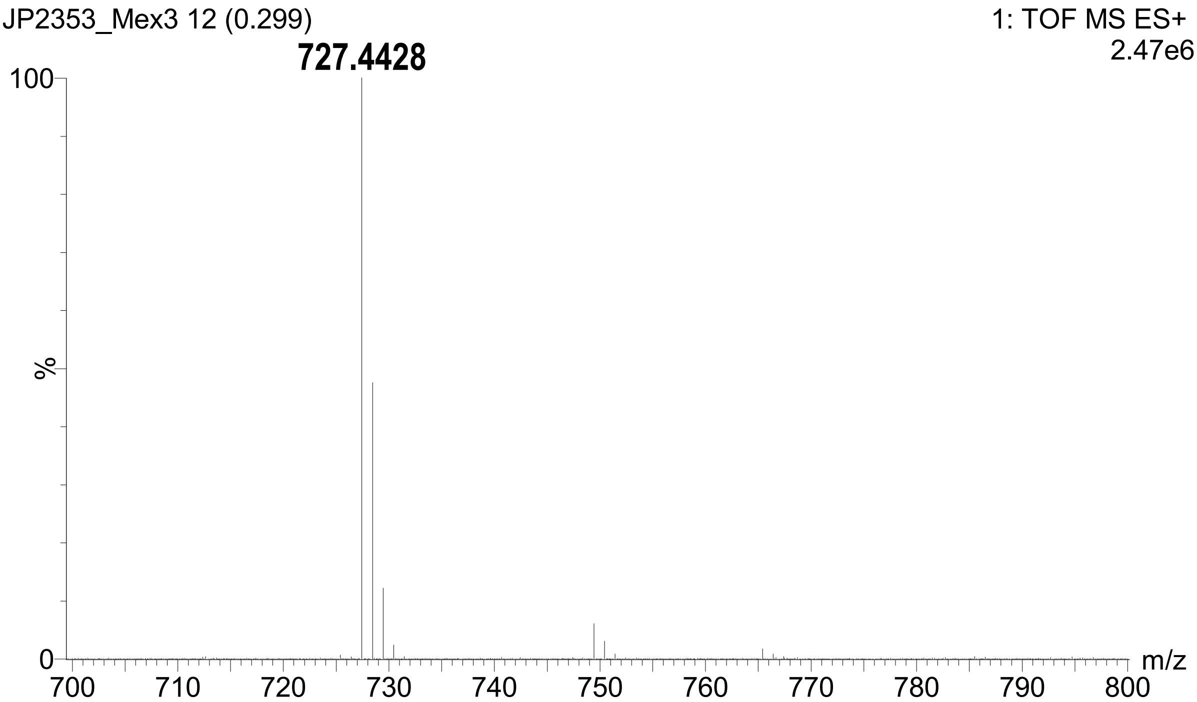


Figure S5: **1-c** ([1]H NMR)

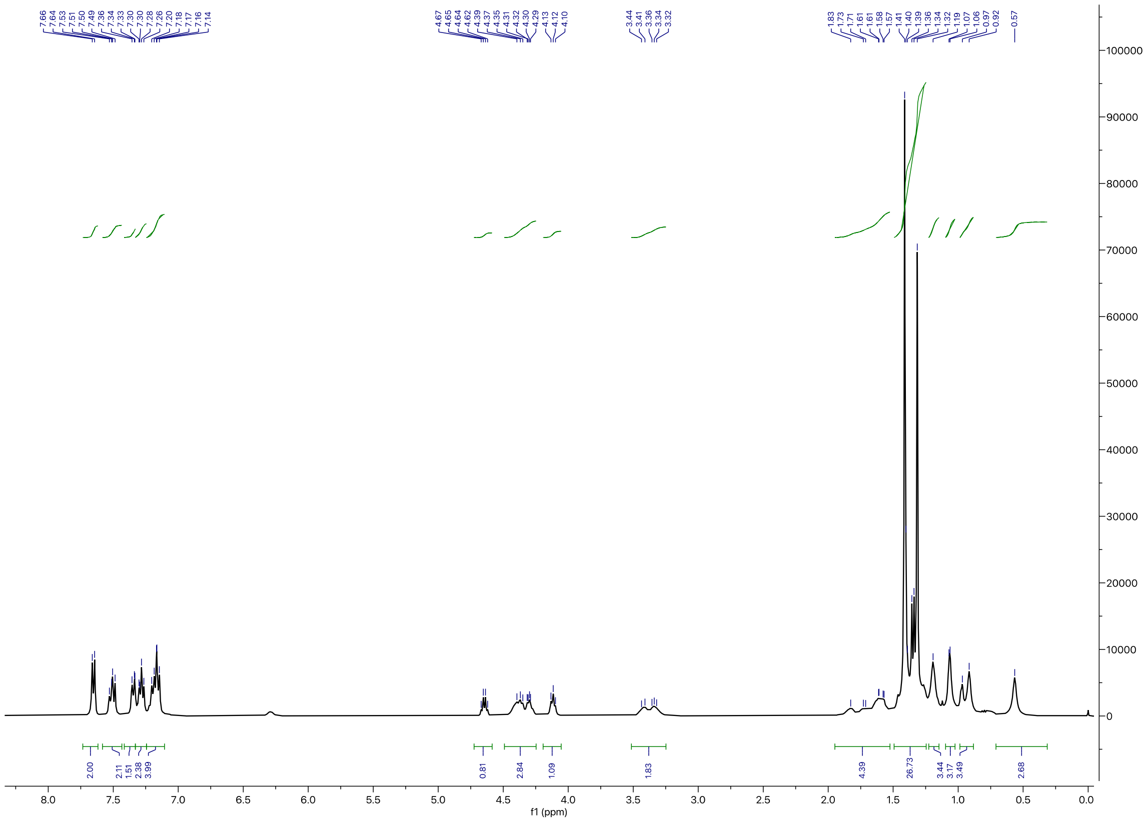


Figure S6: 2-c (^{13}C NMR)

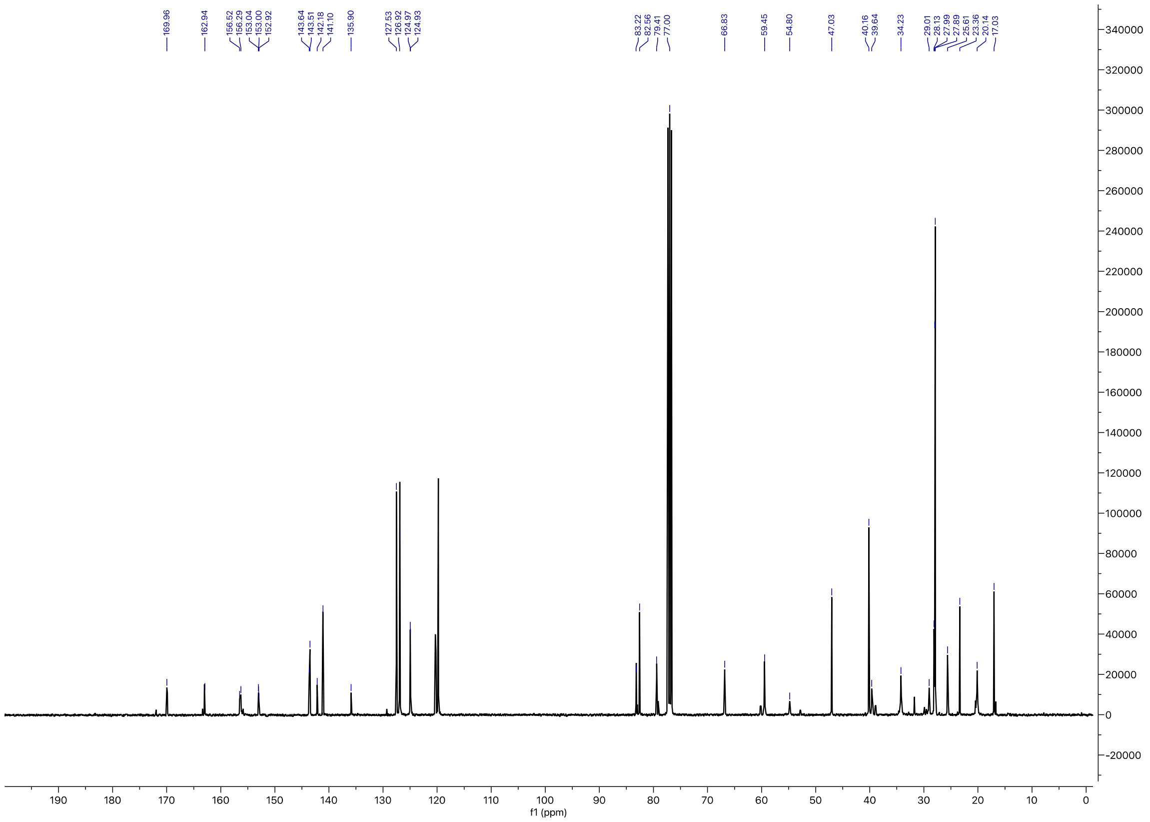


Figure S7: 2-c (^{13}C DEPT 135 NMR)

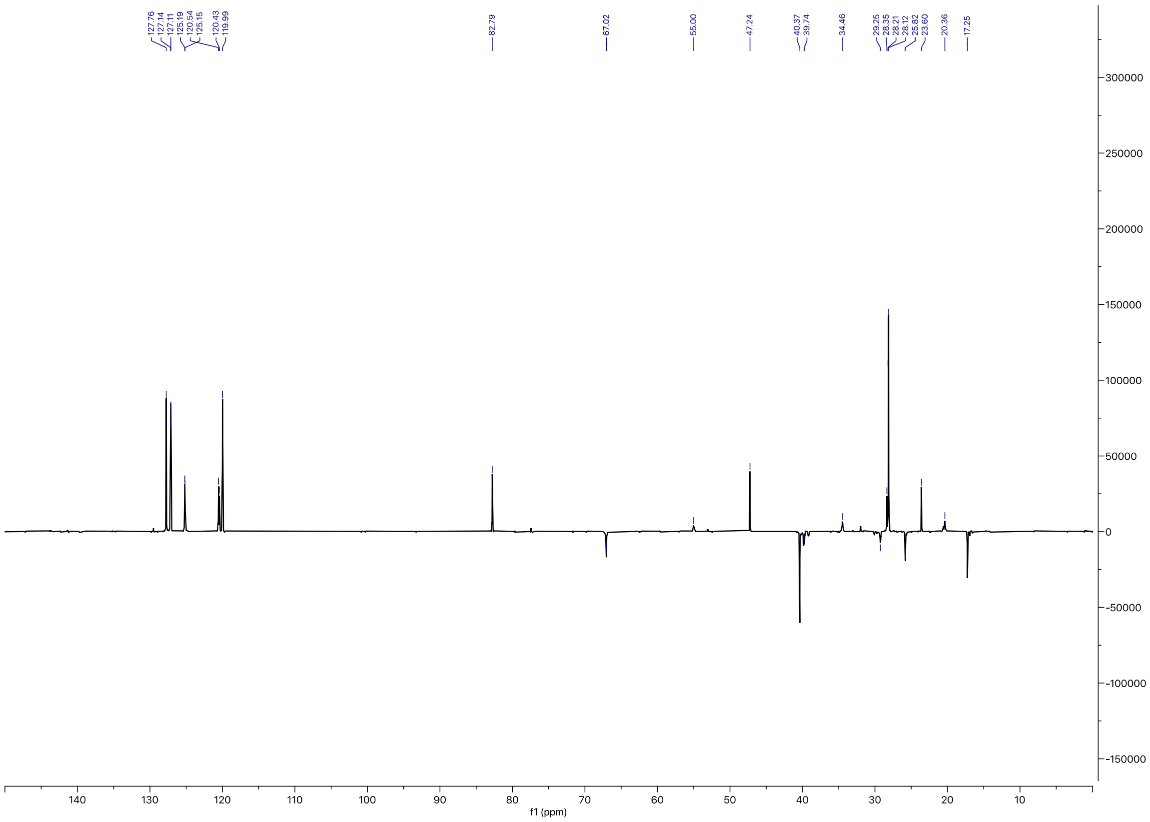


Figure S8: 2-c (HRMS)

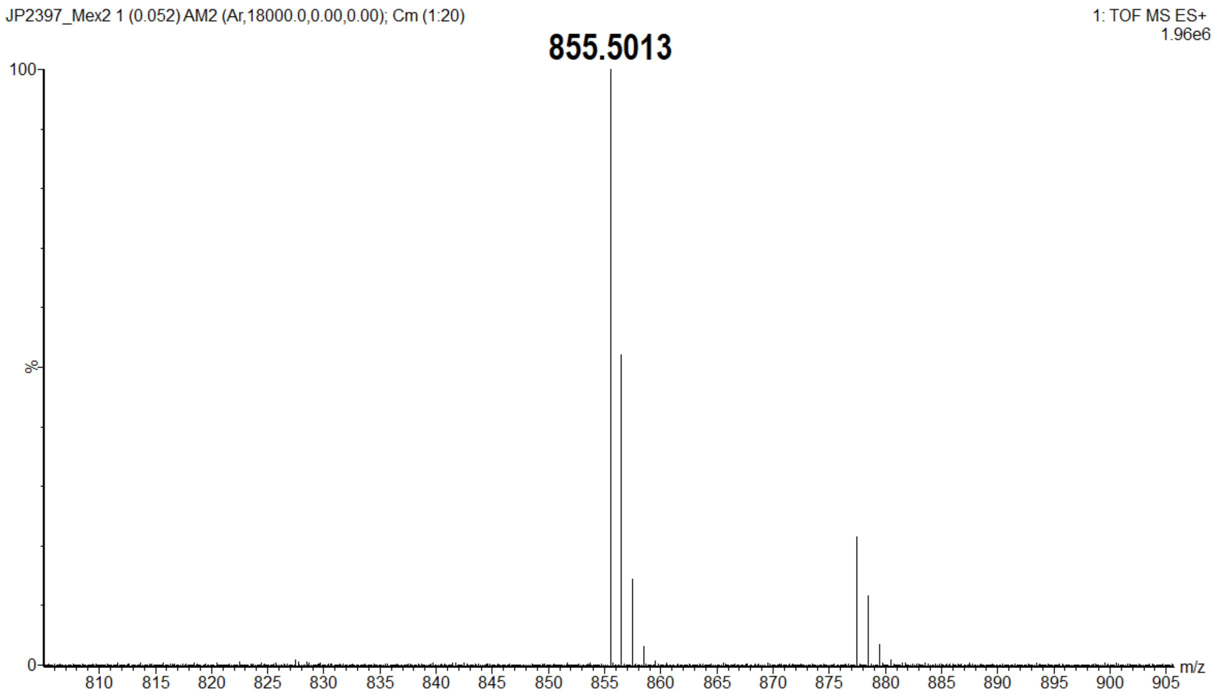


Figure S9: 1-b (^{1}H NMR)

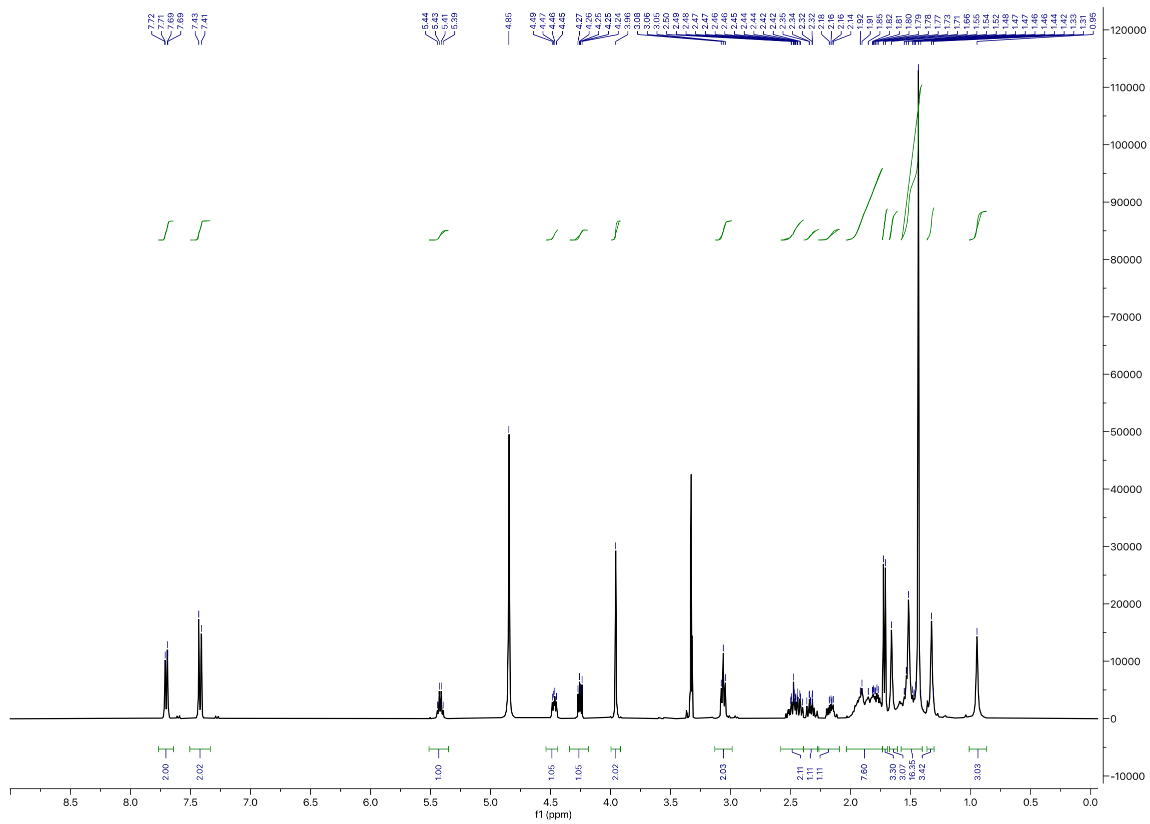


Figure S10: 1-b (^{13}C NMR)

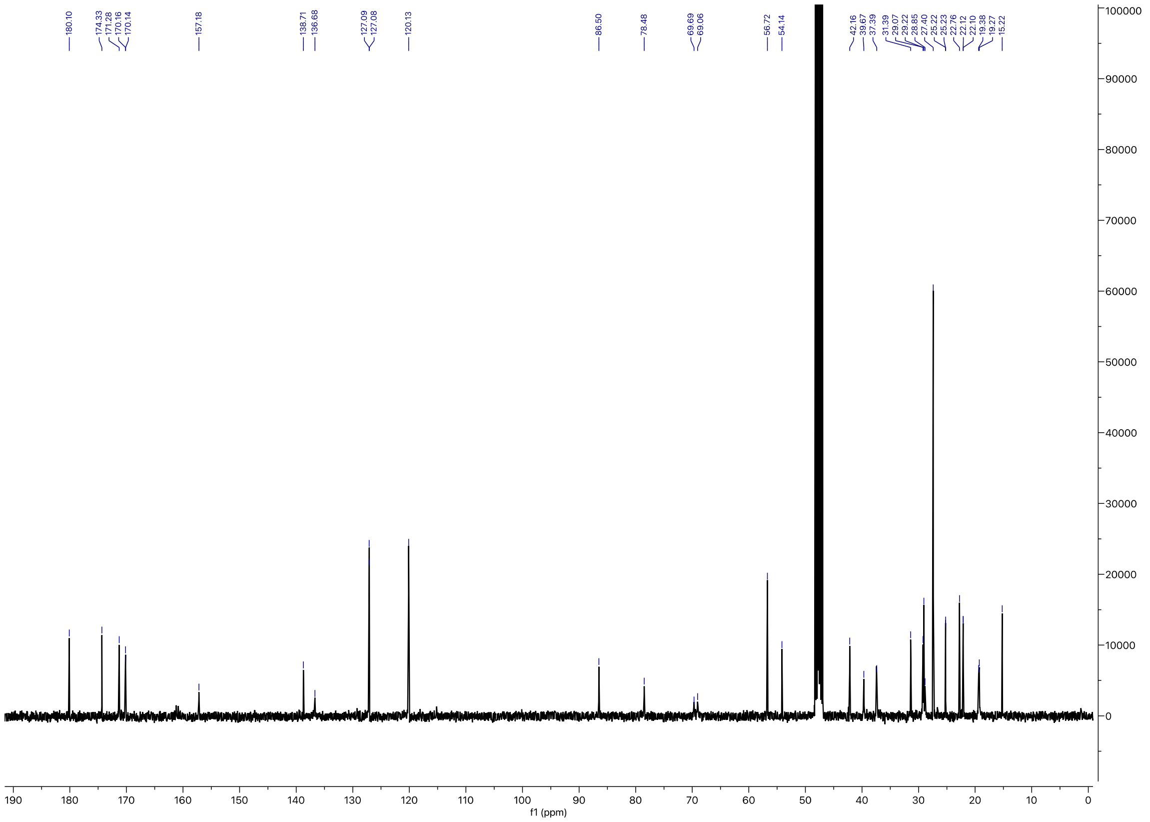


Figure S11: 1-b (^{13}C DEPT 135 NMR)

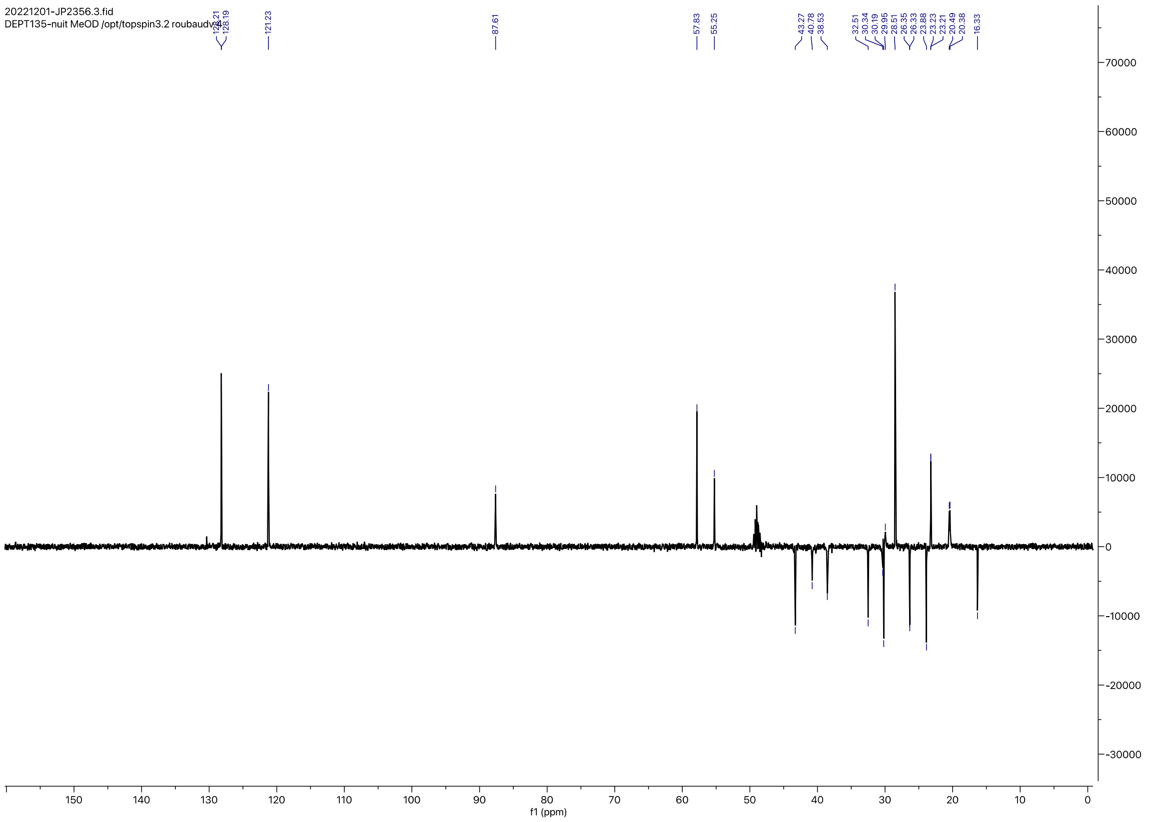


Figure S12: 1-b (HRMS)

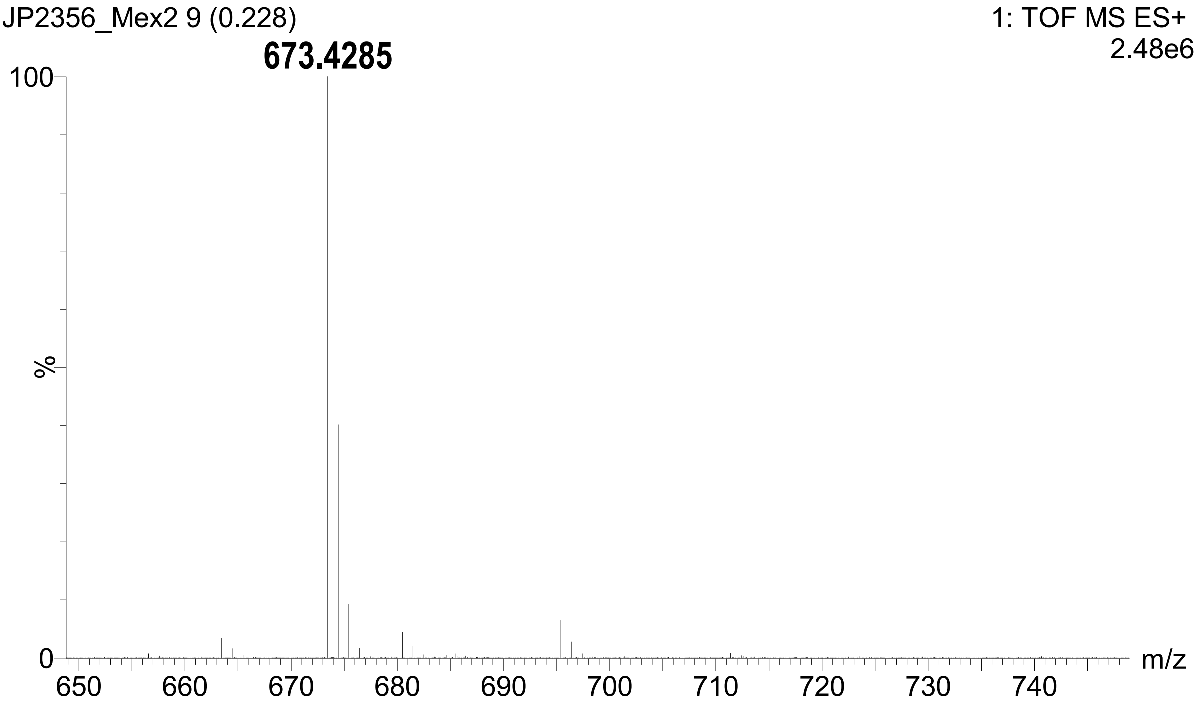


Figure S13: 2-b (^{1}H NMR)

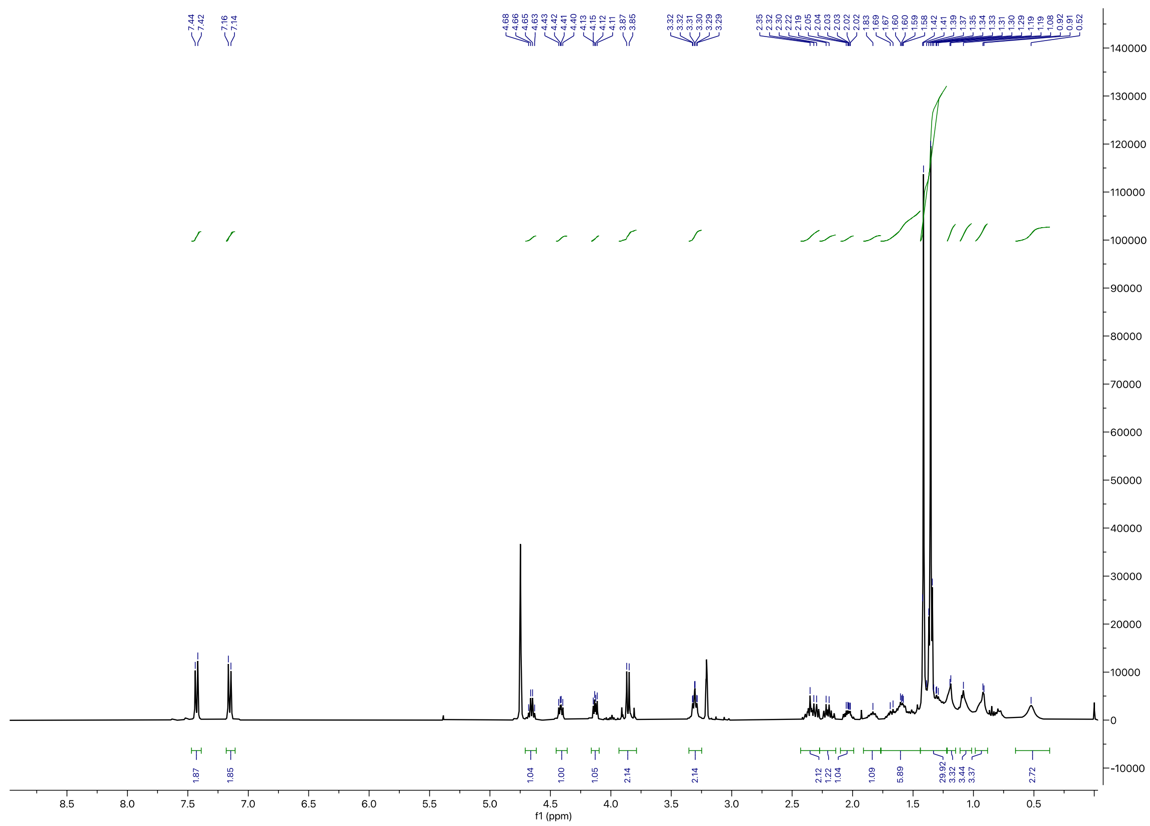


Figure S14: 2-b (^{13}C NMR)

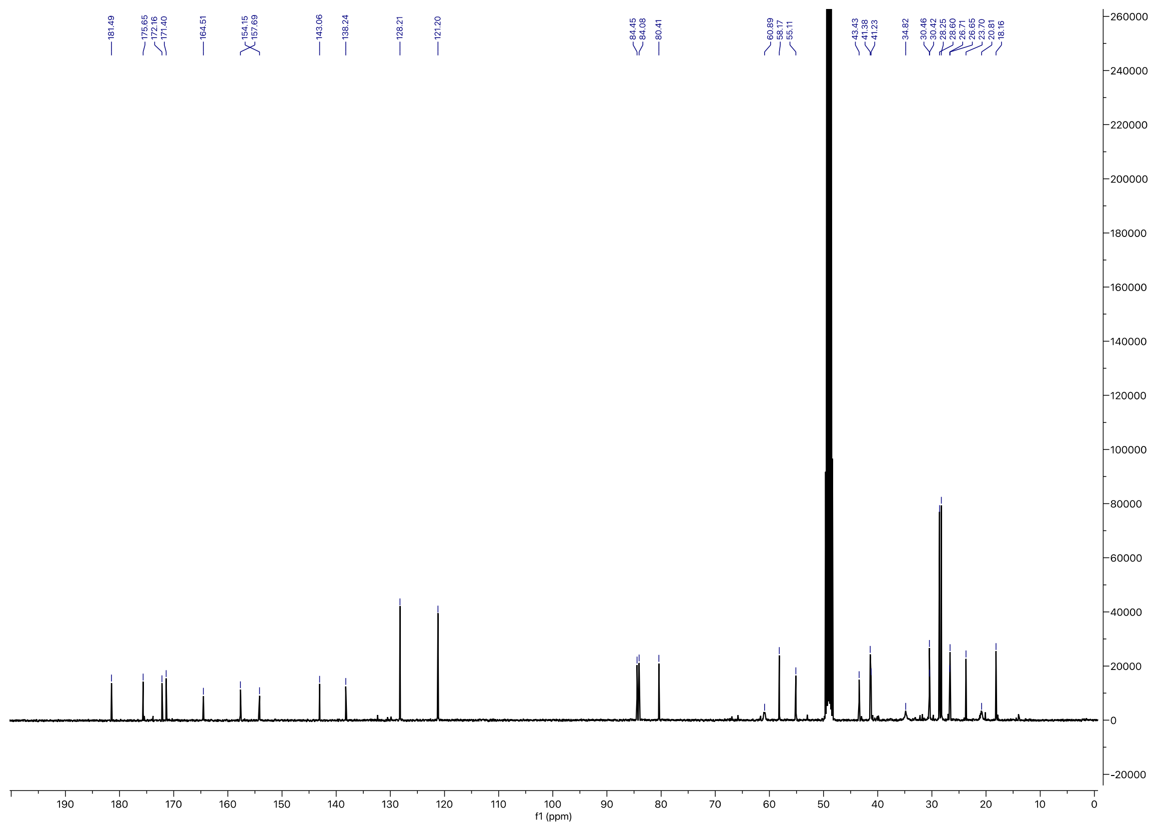


Figure S15: 2-b (^{13}C DEPT 135 NMR)

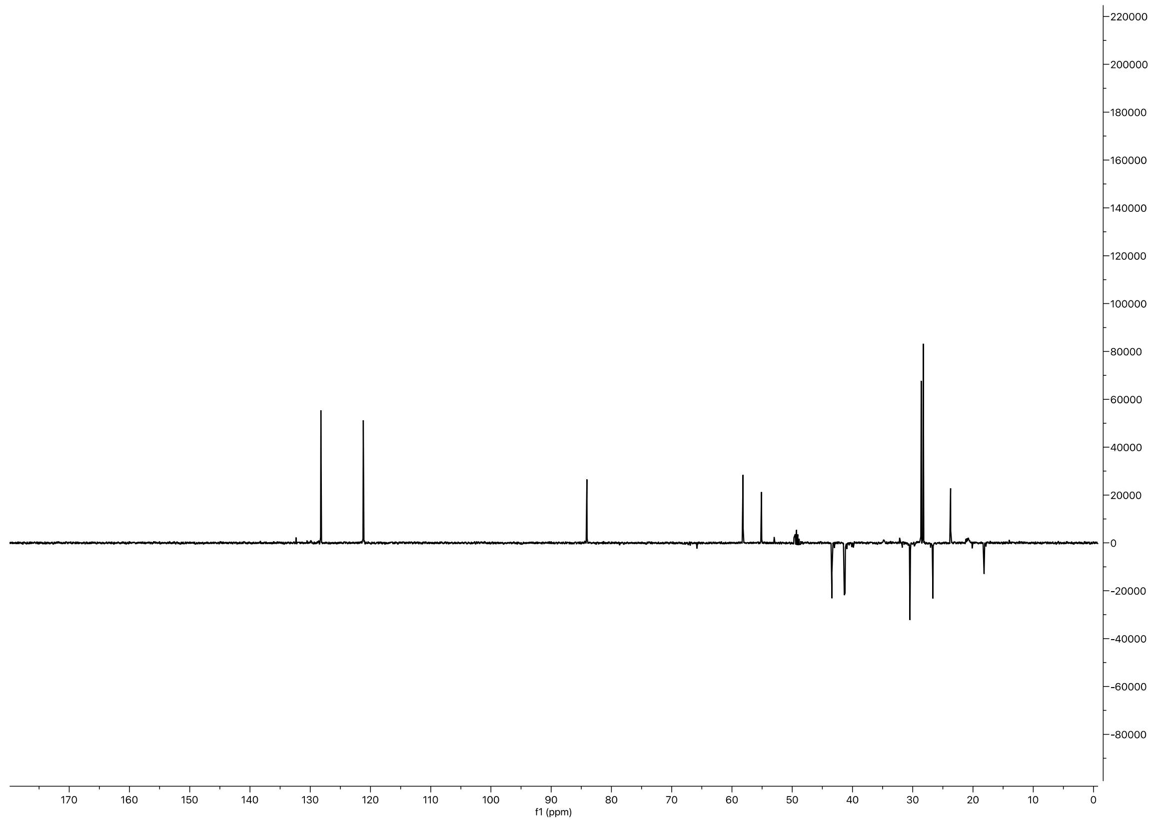


Figure S16: 2-b (HRMS)

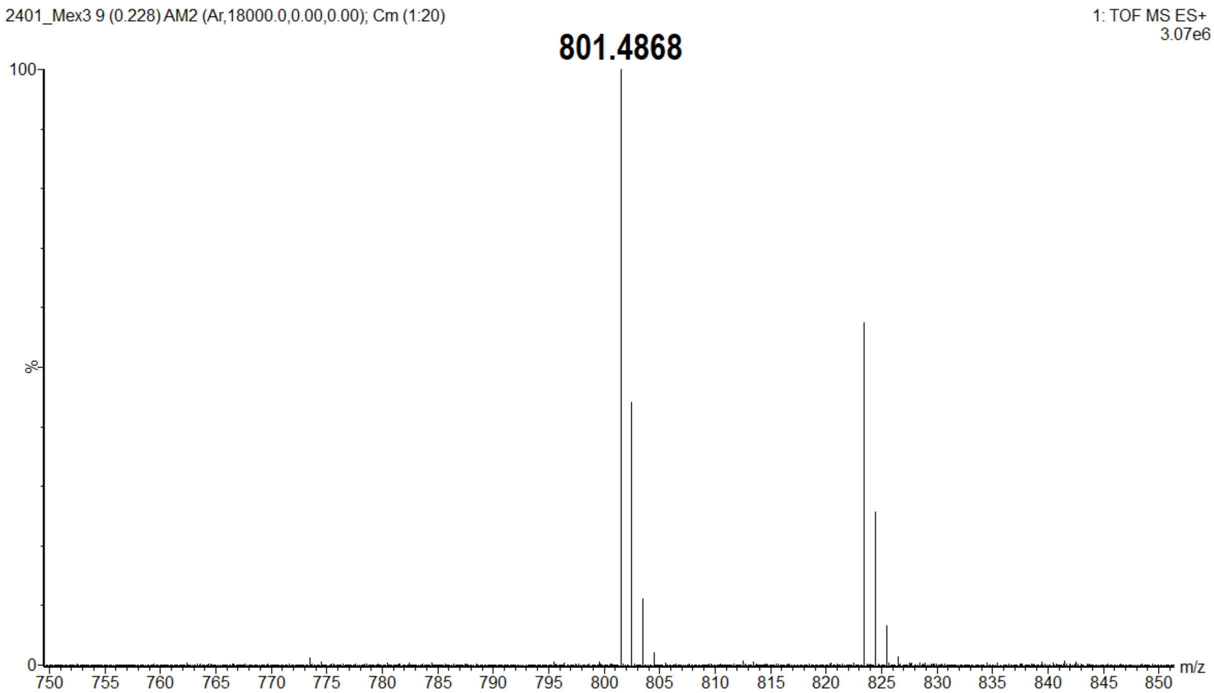


Figure S17: 1-a (^{1}H NMR)

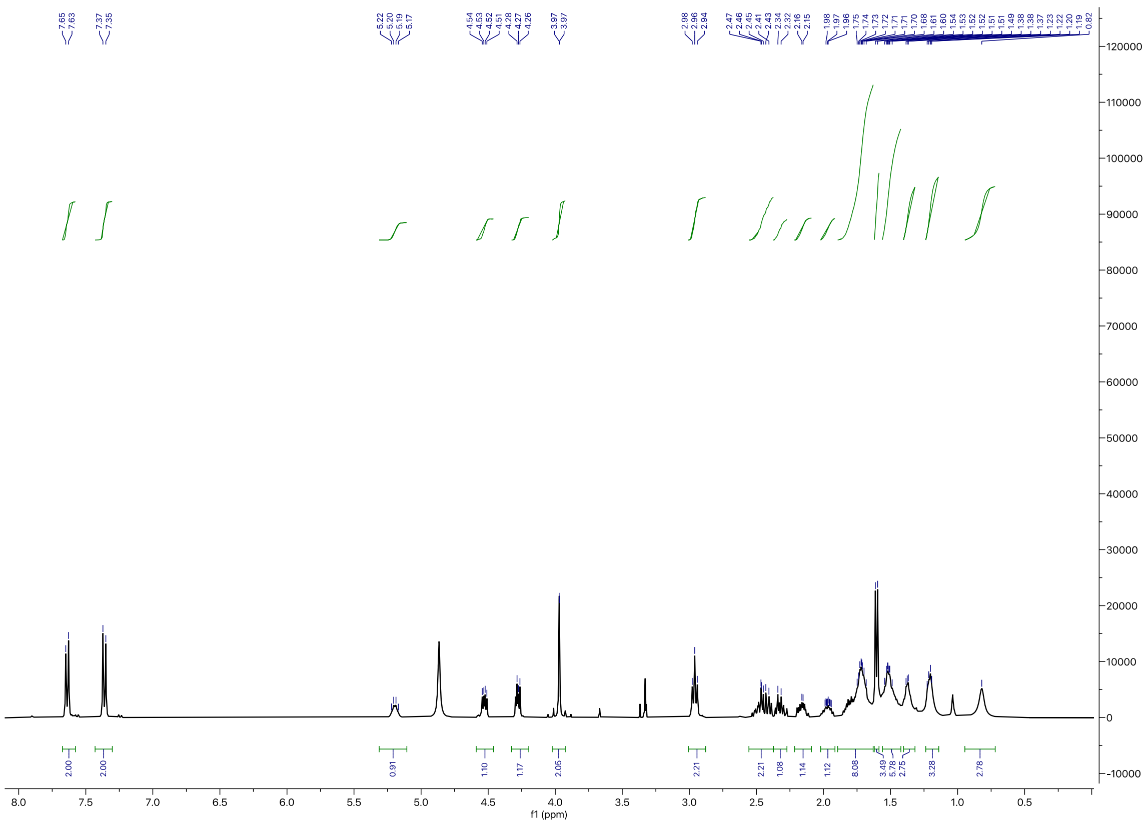


Figure S18: 1-a (^{13}C NMR)

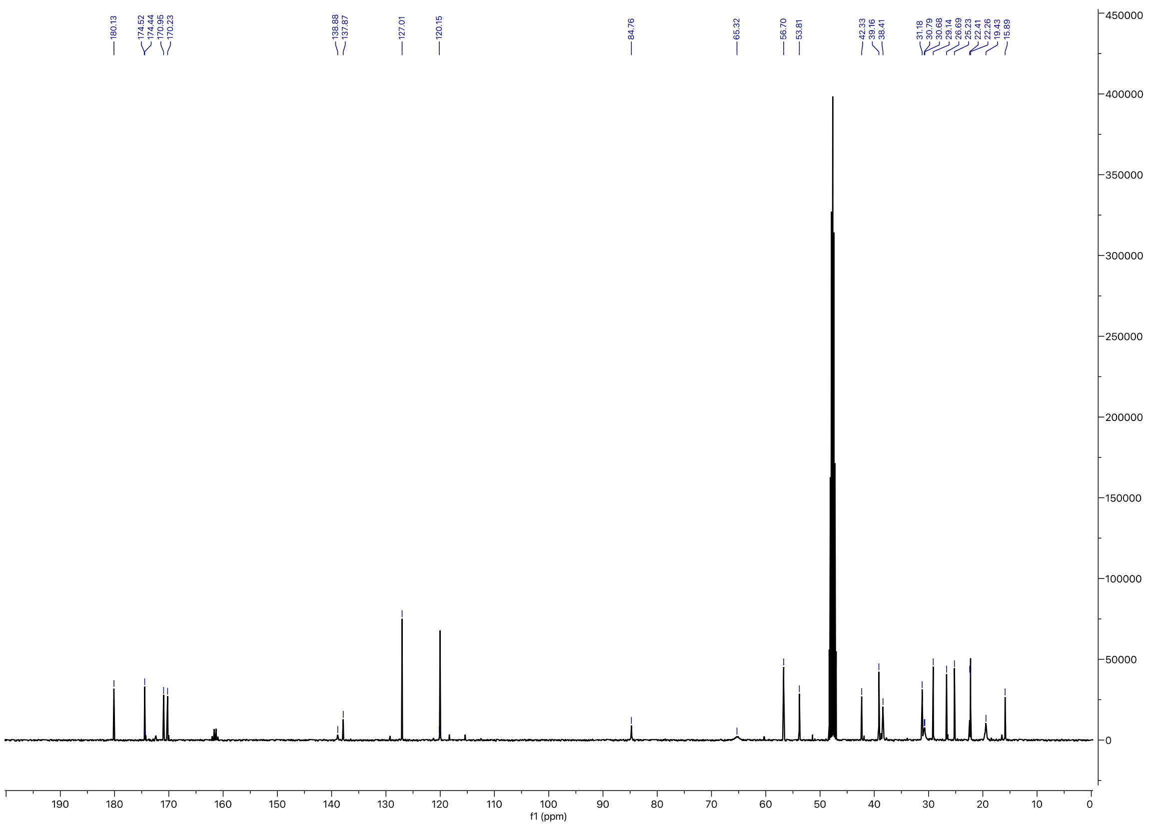


Figure S19: 1-a (^{13}C DEPT 135 NMR)

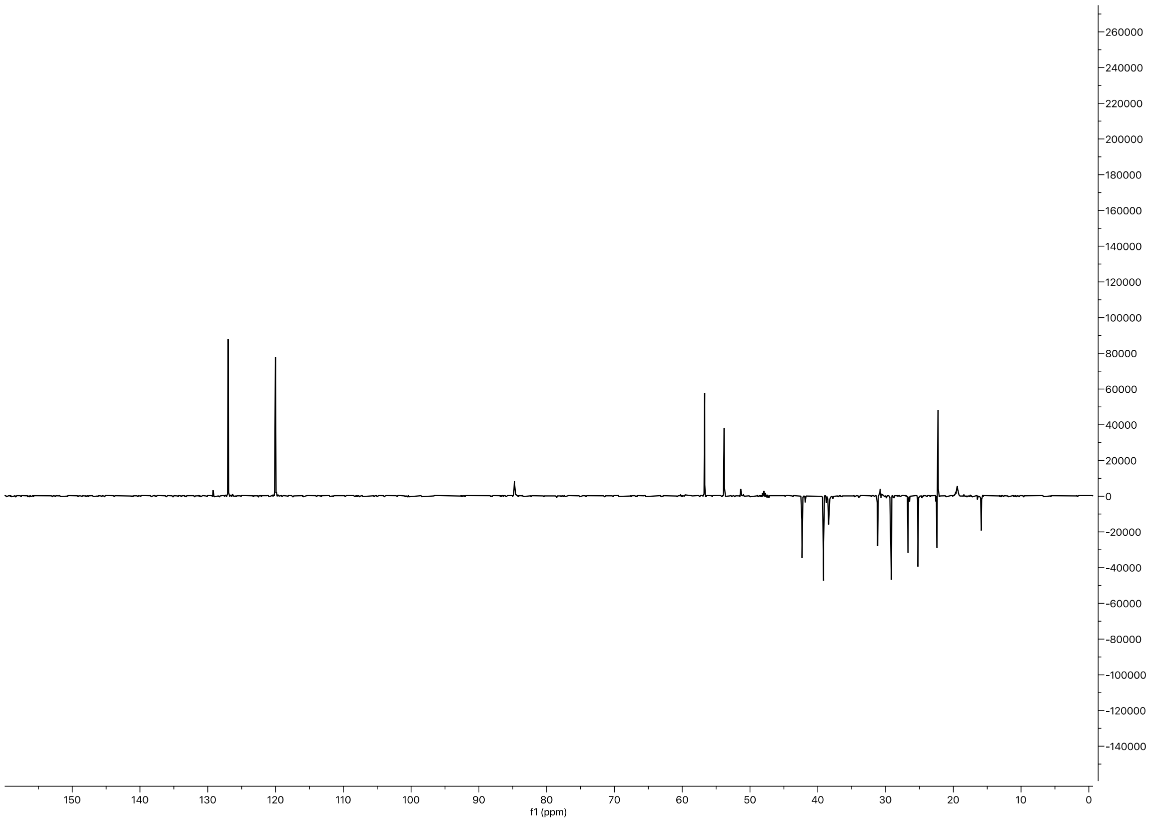


Figure S20: 1-a (HRMS)

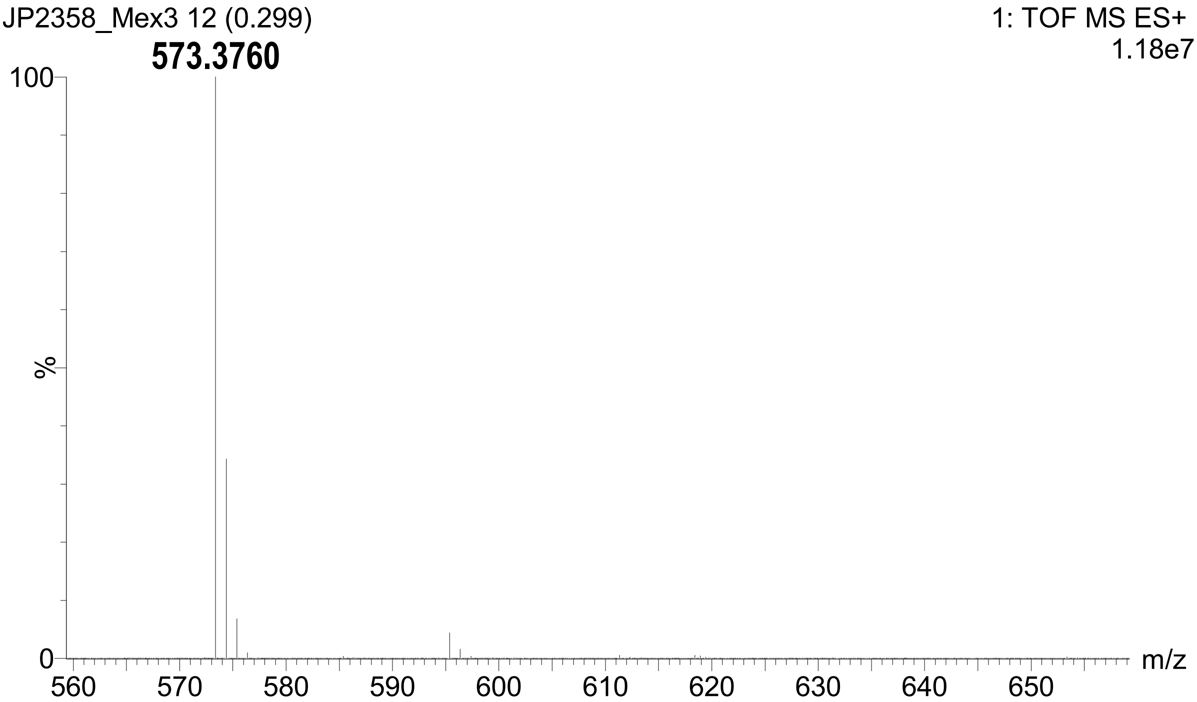


Figure S21: 2-a (^{1}H NMR)

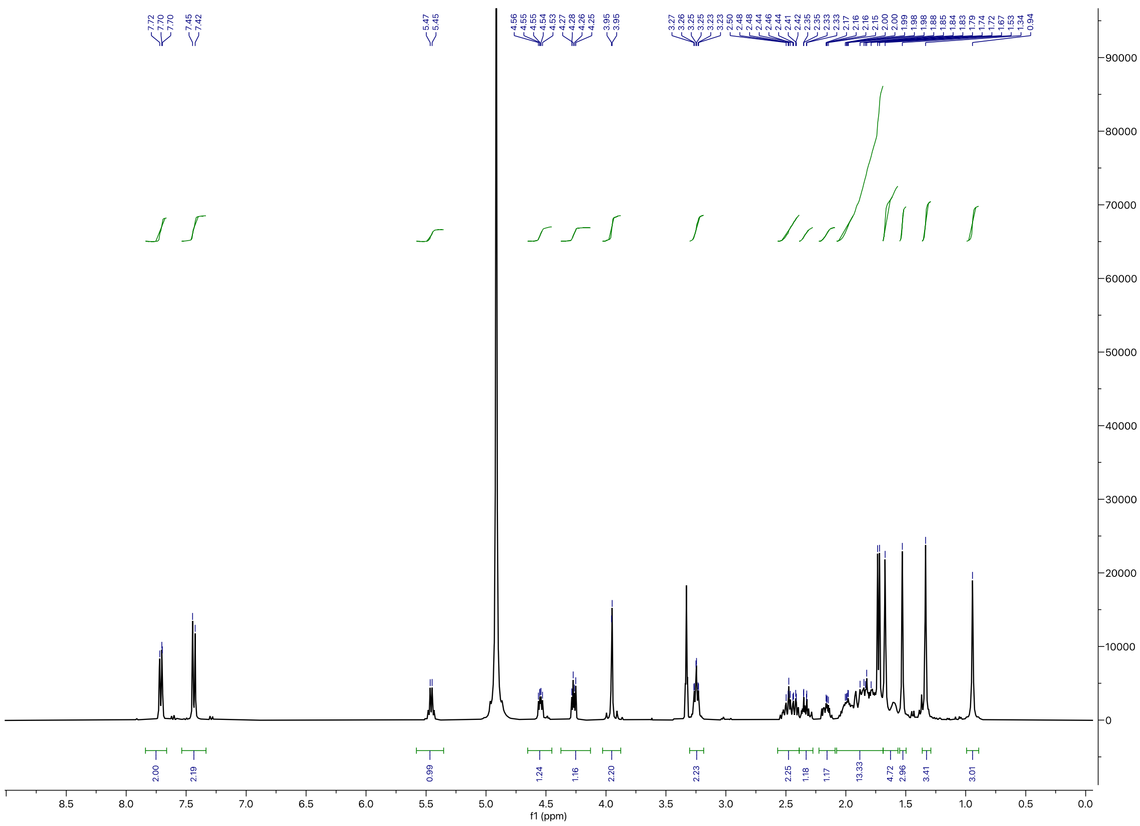


Figure S22: 2-a (^{13}C NMR)

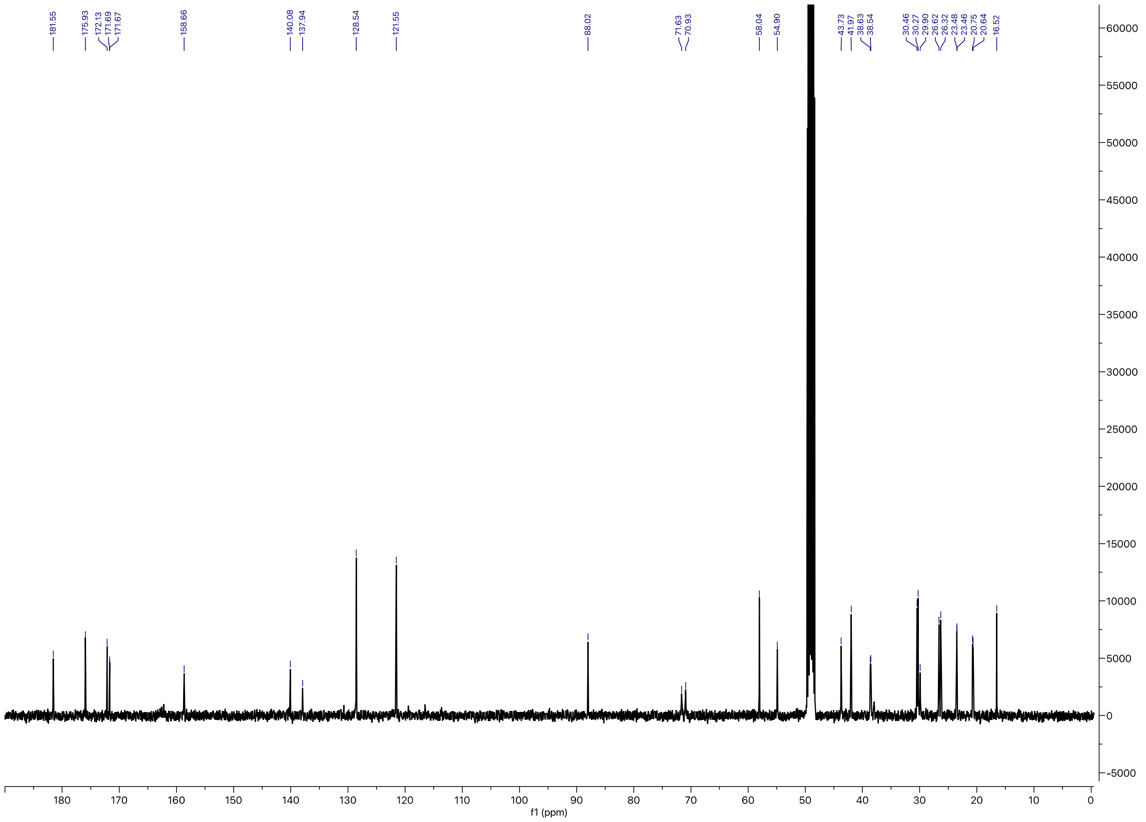


Figure S23: 2-a (^{13}C DEPT 135 NMR)

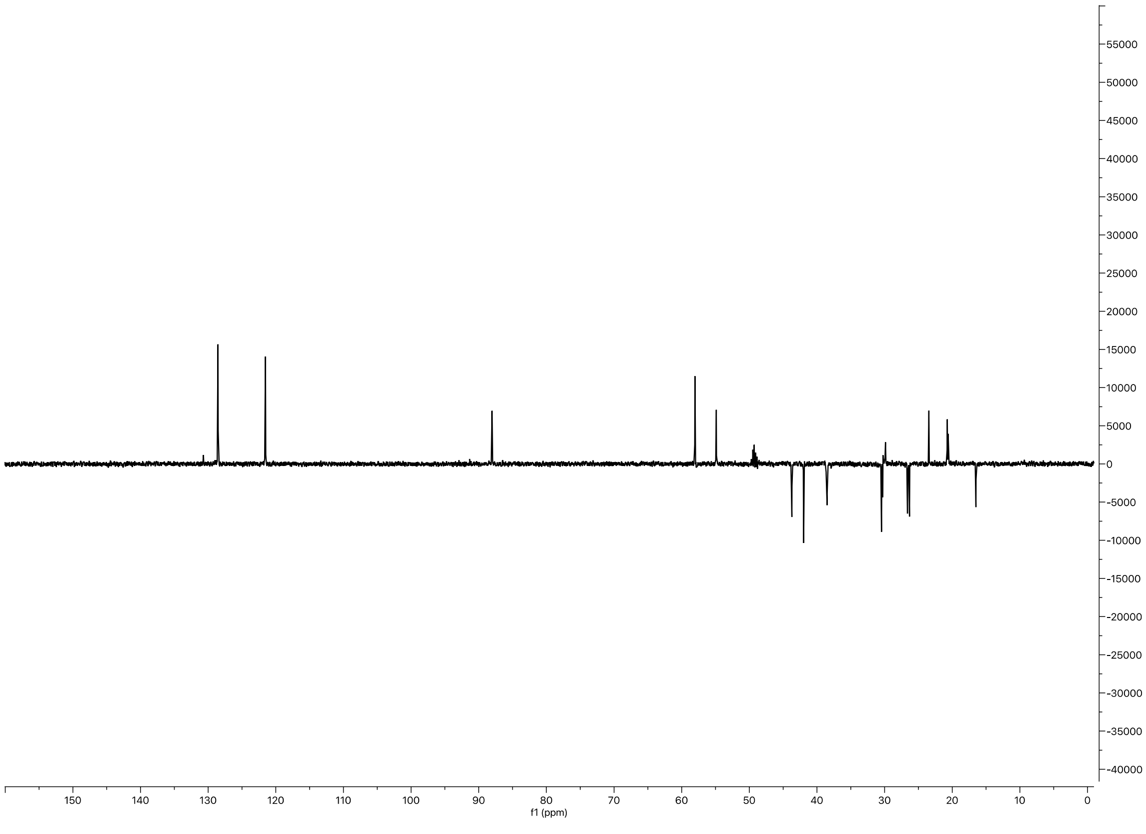
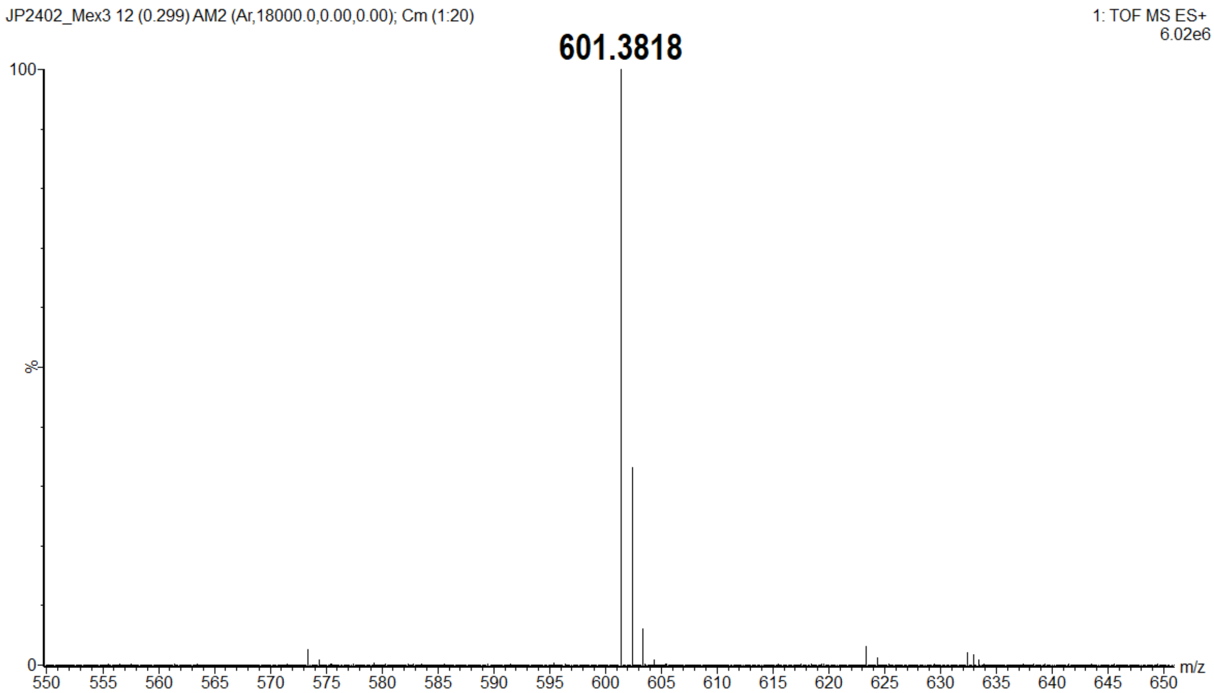


Figure S24: 2-a (HRMS)



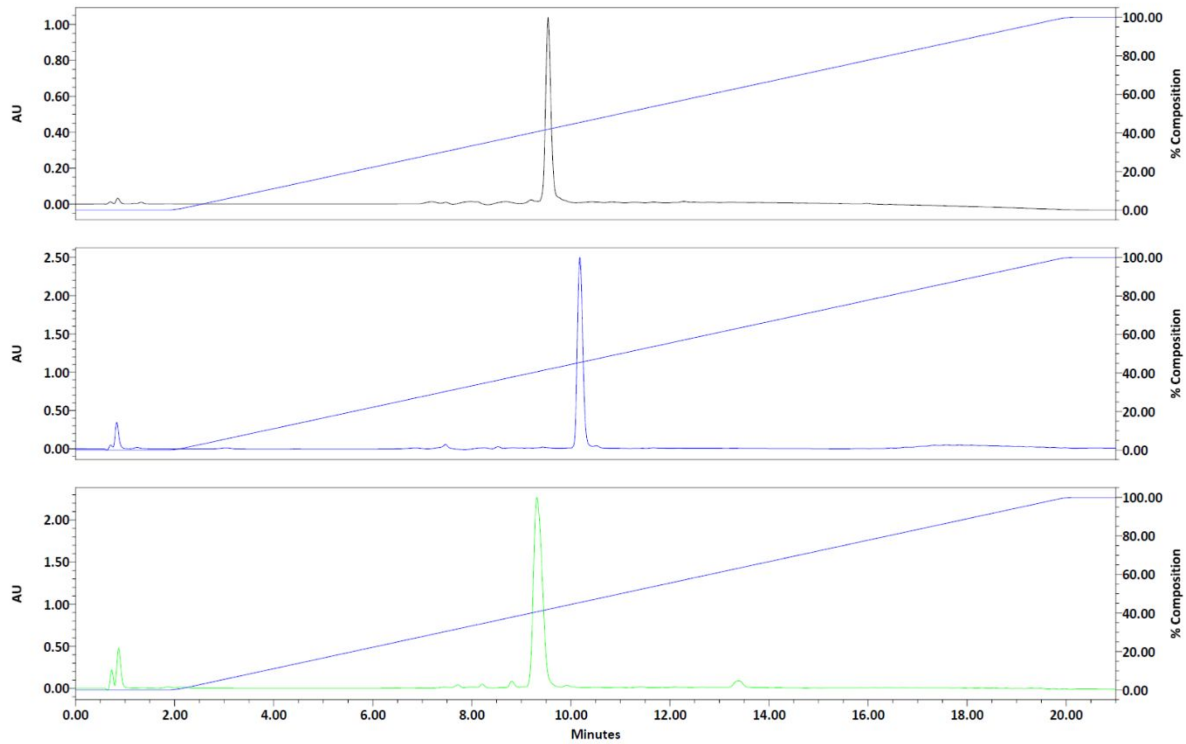


Figure S25: HPLC traces of pGlu-Gly-Lys-Anilide-TEMPO 1.a (Black) Succinyl-Ala-Ala-pro-Val-anilide-TEMPO (blue) pGlu-Gly-Arg-Anilide-TEMPO 2.a (green) for purity check. Detection at 214 nm. Column x-bridge BEH C-18 46x50 mm. Gradient: Solvent A (0.05% TFA in water) to solvent B (0.05% TFA in acetonitrile) in 20 minutes.

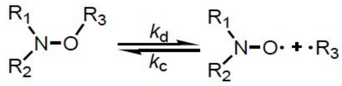
Kinetic Analysis of Alkoxyamine Bond Homolysis.

The kinetic homolysis for peptide-alkoxyamines reported in Table 1 was recorded in EPR using water as solvent. Table S1. Experimental temperatures (T), homolysis rate constants k_d, activation energies E_a in H_2O for peptide-alkoxyamine **1a** and **2a**.

Peptide-Alkoxyamine	*T (°C)* [a]	k_d (10^{-4} s^{-1})[a,b,c]	E_a (KJ.mol^{-1})[b,d]
1a	95	4.6	124.8
2a	95	2.7	126.5

[a]In water [b]Values measured for a mixture of diastereosisomers. [c]Given by Equation (1). [d]Given by Equation (2), and an averaged frequency factor was used A = $2.4.10^{-14}$ s^{-1}, R = 8.314 J.K^{-1}.mol^{-1}, k_d, and T are given in columns 2, 3 in Table 1.

The growth of nitroxide was recorded in the presence of an alkyl radical scavenger, i.e., O_2 here, to suppress the back reaction (k_c in Scheme 1), as already reported [1].



Scheme S1. Dynamic covalent bond in alkoxyamines: k_d stands for the homolysis rate constant and k_c stands for the re-formation reaction.

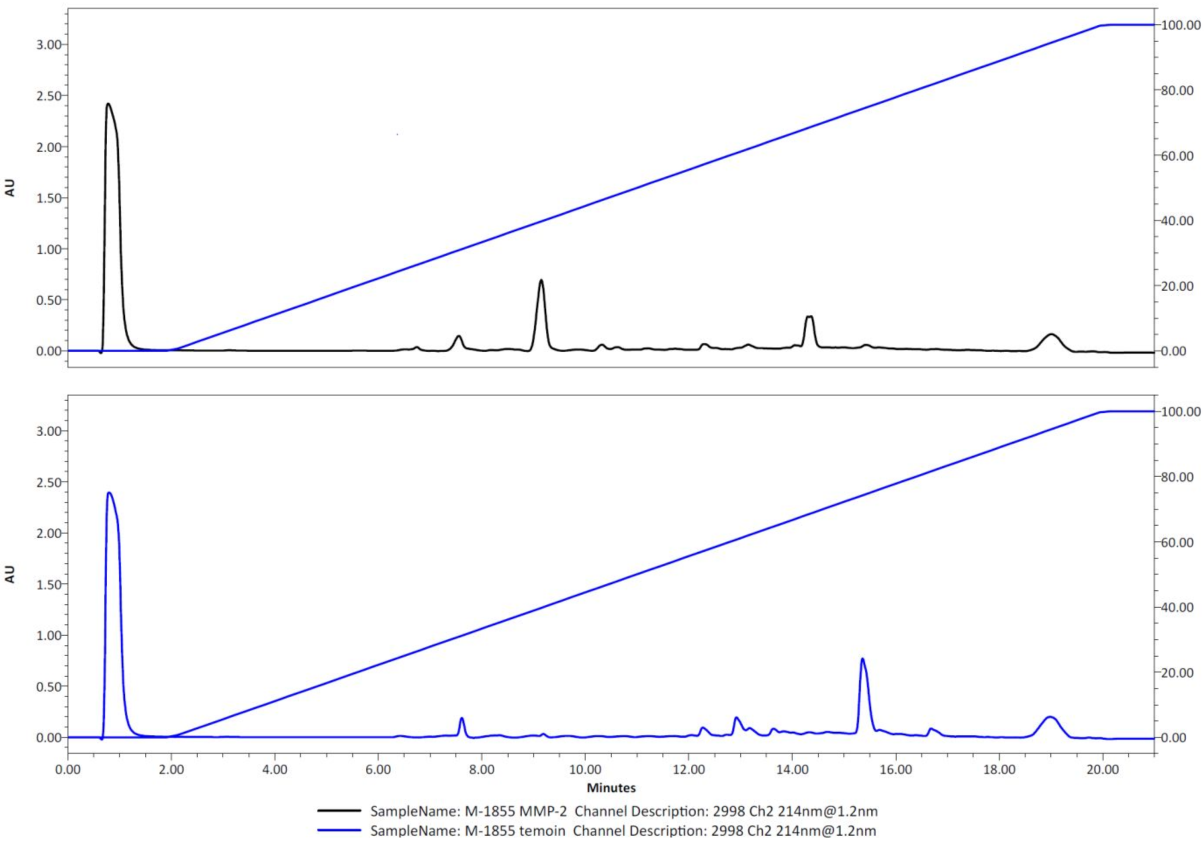
Homolysis rate constants kd are given by Equation S1 ([nitroxide]∞ = [alkoxyamine]0 = 0.1 mM), and the subsequent activation energy values Ea are given by Equation S2, as follows [1]:

$$ln\left(\frac{[nitroxide]_\infty - [nitroxide]_t}{[nitroxide]_\infty}\right) = -k_d \cdot t \qquad (1)$$

$$E_a = -RTln\left(\frac{k_d}{A}\right) \qquad (2)$$

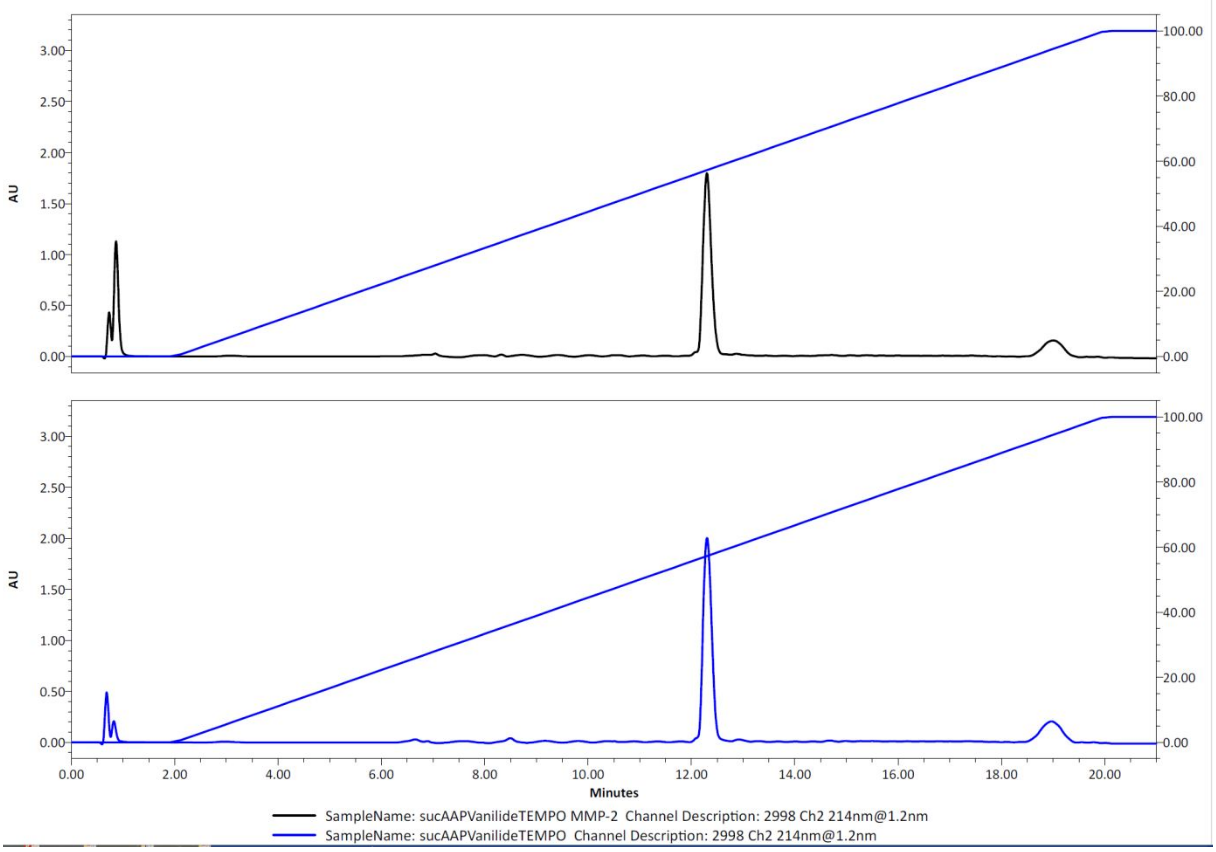
Figure S26: Proof for the inactivity of MMP-2 on suc-AAPV-Anilide-TEMPO.

Activity of MMP-2 sample was first verified by incubation the enzyme overnight at 37°C with a commercial substrate (M-1855, BACHEM) and running reverse phase HPLC:



The HPLC trace shows complete hydrolysis of the substrate.

Then suc-AAPV-Anilide-TEMPO was incubated overnight at 37 °C in the presence or absence of MMP-2.



No significant loss of the alkoxyamine is visible thus showing that MMP-2 does not hydrolyze the peptide from the prodrug.

References

[1] T. Reyser, T. To, C. Egwu, L. Paloque, M. Nguyen, A. Hamouy, J-L. S. gliani, C. Bijani, J-M. Augereau, JP. Joly, J. Portela, J. Havot, S.R.A. Marque, J. Boissier, A. Robert, F. Benoit-Vical, G. Audran, Alkoxyamines Designed as Potential Drugs against Plasmodium and Schistosoma Parasites. *Molecules*, **2020**, *25*, 3838.