

Optimization of IEDDA reaction parameters

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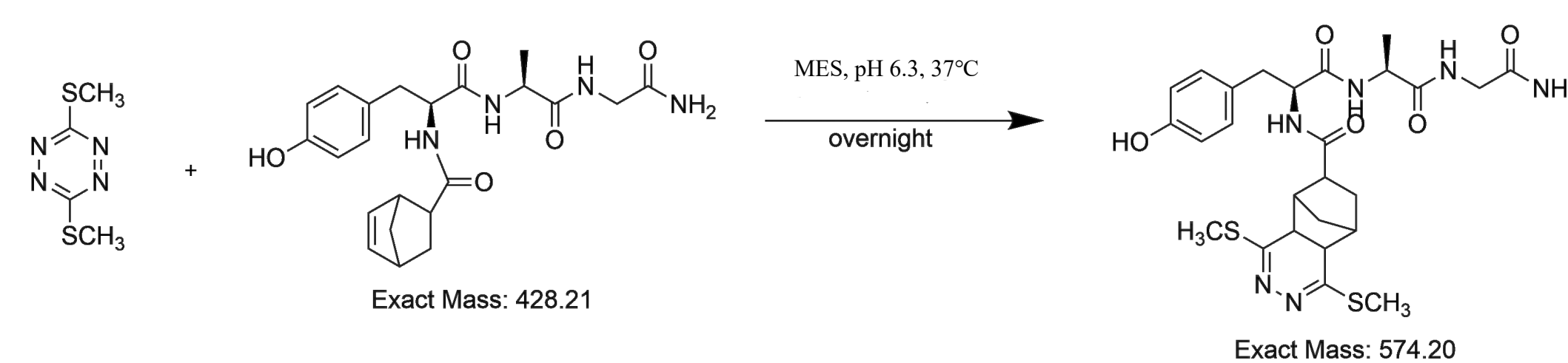
Introduction

Inverse Electron Demands Diels-Alder (IEDDA) reaction is a biorthogonal reaction that potentially can be used for various applications such as bioconjugation, imaging, screening etc. The reaction kinetics of the IEDDA reaction varies based on the nature of the substitutions on the tetrazine and diene. Incorporation of S, S-disubstituted tetrazine in peptides is synthetically much less challenging than the incorporation of an aromatic N,N-tetrazines, using the thiol functionality of Cys.

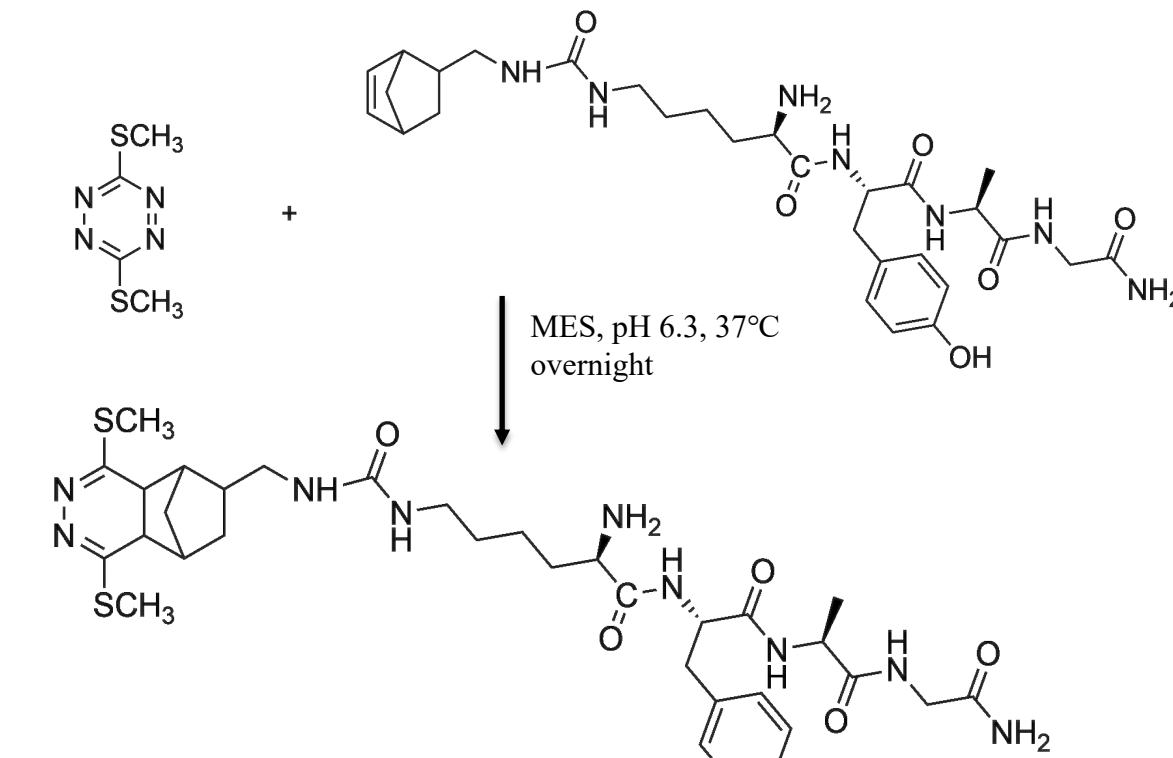
We have synthesized S, S-tetrazine cyclic peptides, and we want to study the reactivities of these peptides with different dienes, as there is very limited information about IEDDA reactions with S, S-tetrazine. We synthesized norbornene-containing or cyclopropene-containing dienes and incorporated them in peptides to increase the mass of the resultant products. The reactions of these peptides with S, S-dimethyl tetrazine are being studied under various conditions, to identify a diene with suitable reactivity that can be used for IEDDA reaction of S, S-tetrazine cyclized peptides with biomolecules like proteins.

Materials and methods

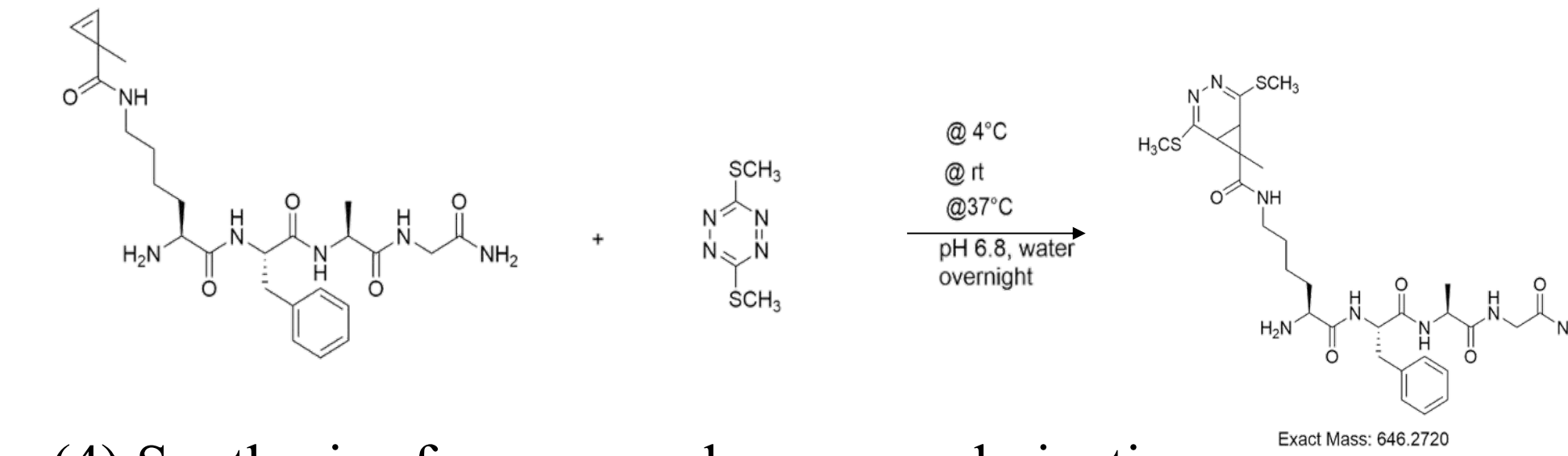
(1) IEDDA reaction with norbornene acid and tetrazine



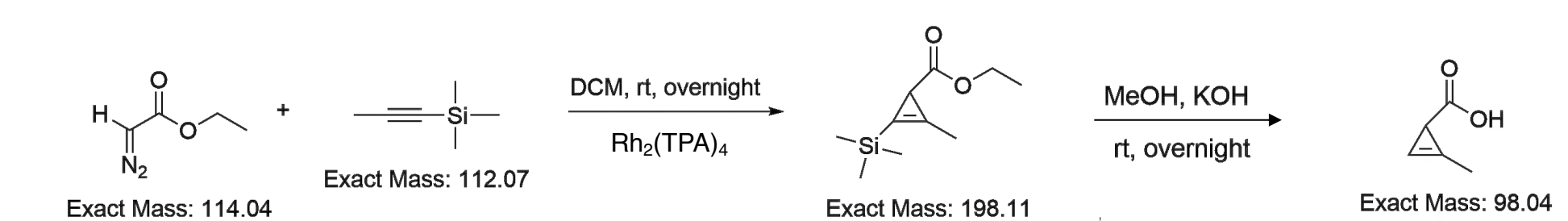
(2) IEDDA reaction with norbornene methyl and tetrazine



(3) IEDDA reaction with cyclopropene derivative and tetrazine

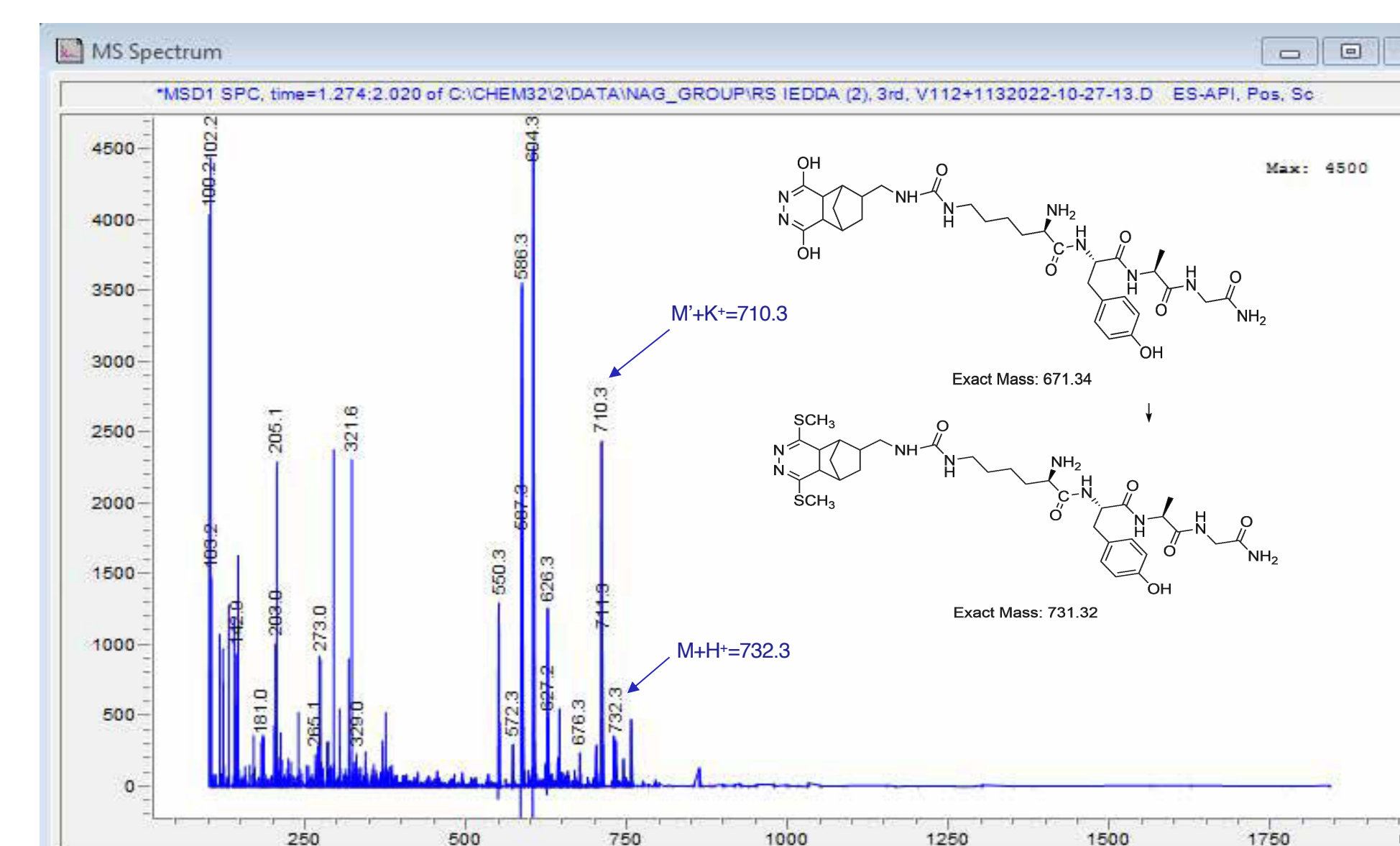
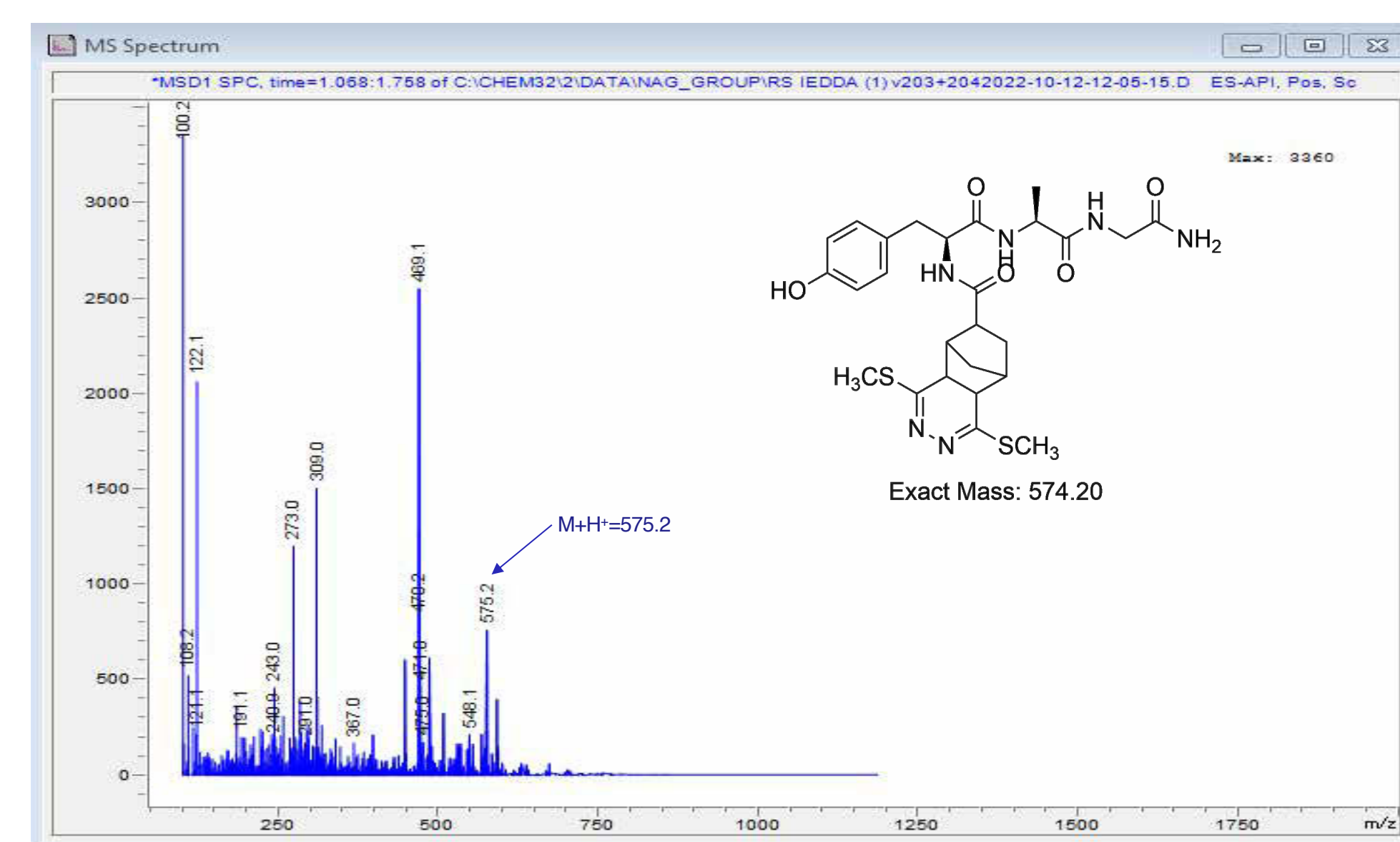


(4) Synthesis of a new cyclopropene derivative

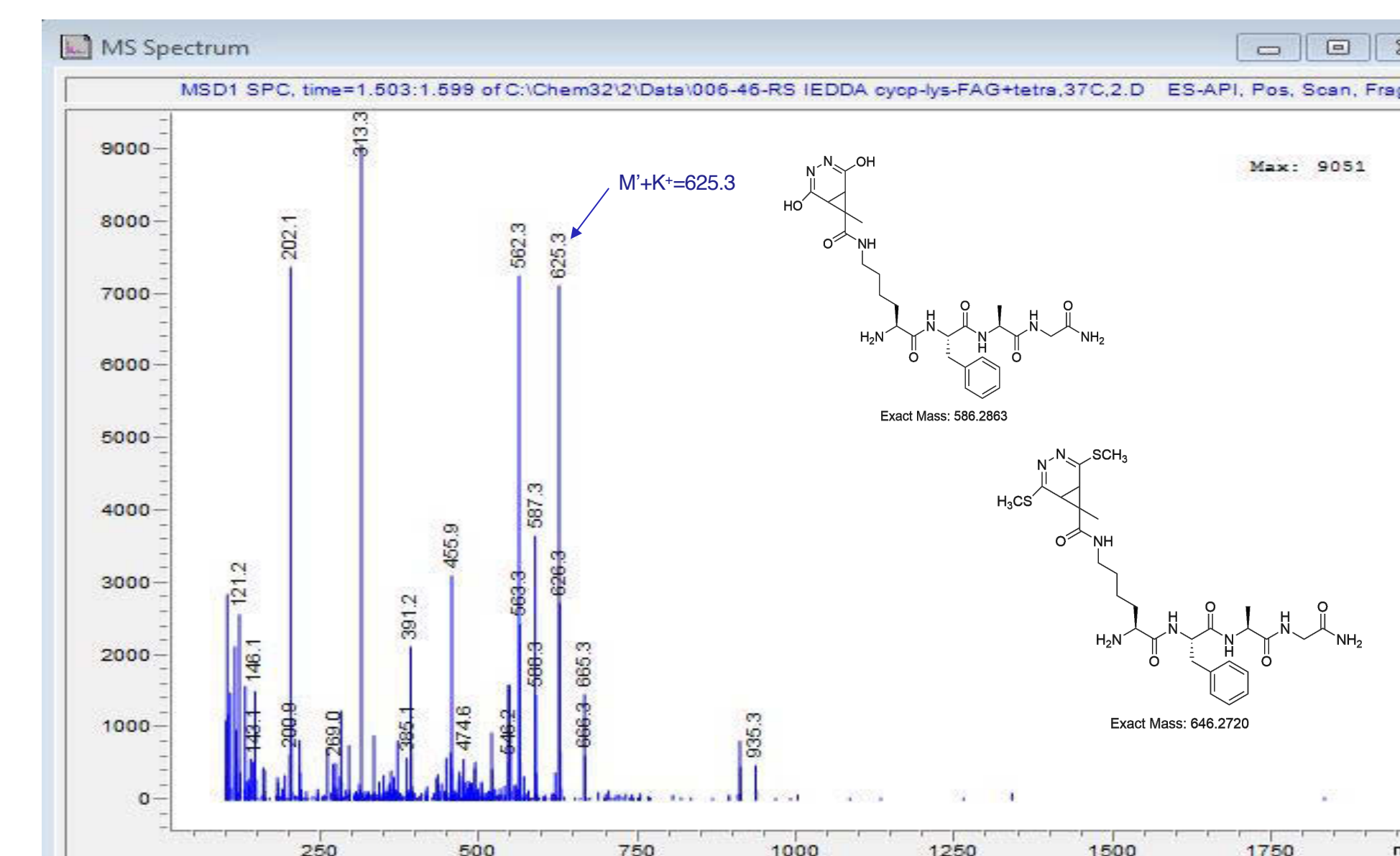
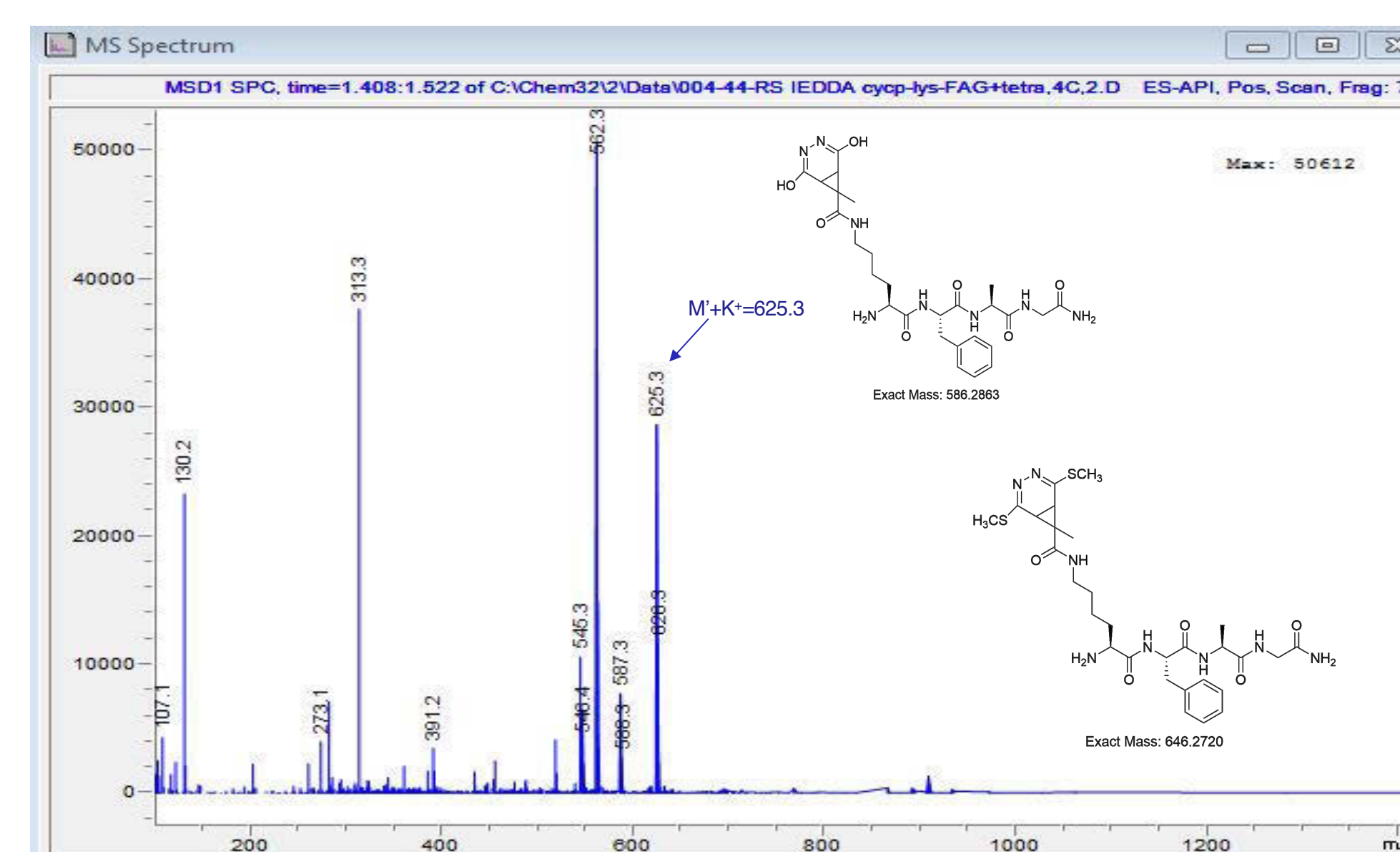


Results

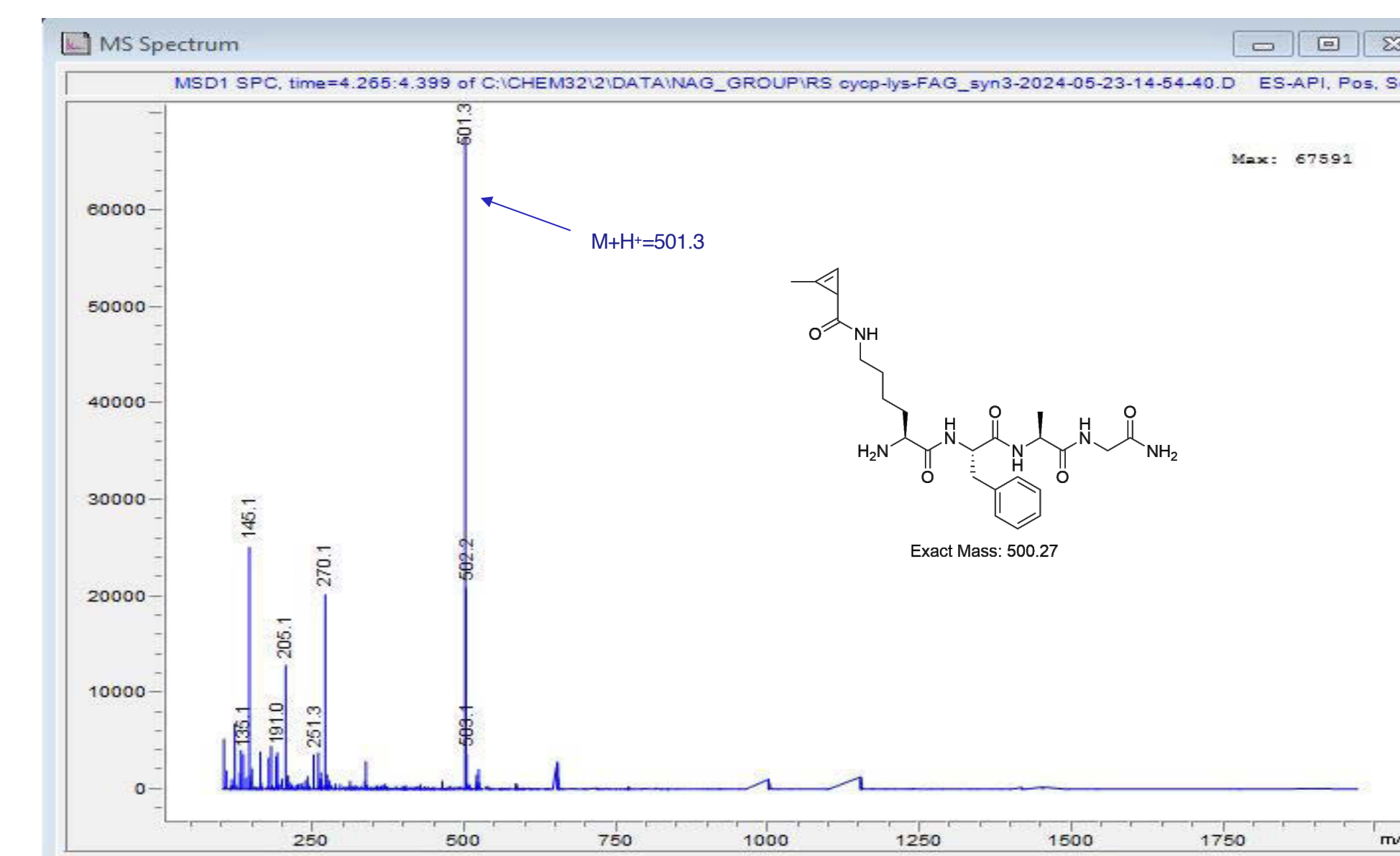
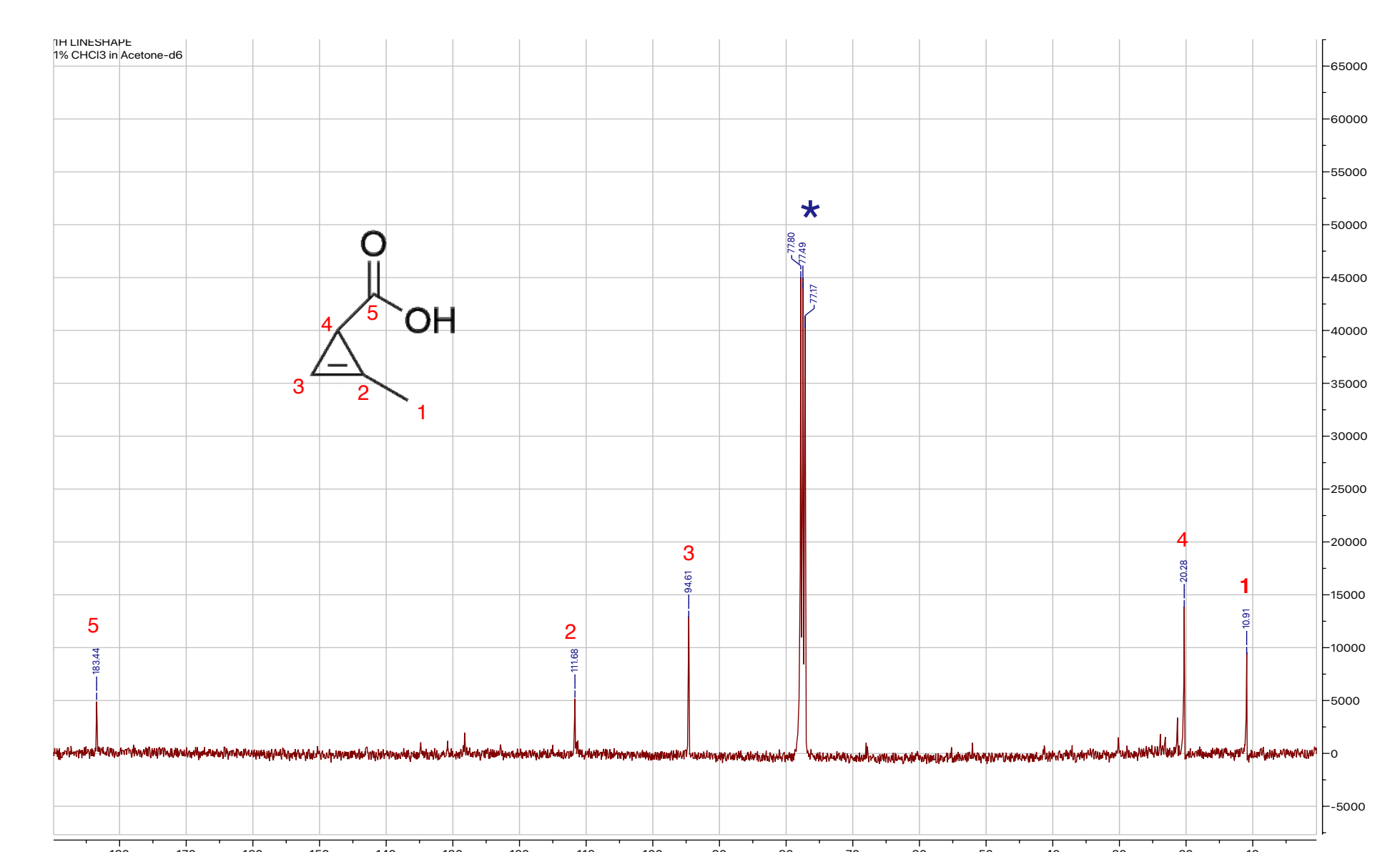
- (1) LC-MS result of IEDDA product. Dienophile: Norbornene-Lys-Tyr-Ala-Gly (1 eq), Diene: 3,6-Bis (methylthio)-1,2,4,5 tetrazine (5 eq).
- (2) Dienophile: Norbornene methyl-Lys-Tyr-Ala-Gly (1 eq), Diene: 3,6-Bis (methylthio)-1,2,4,5 tetrazine (5 eq).



- (3) LC-MS result of IEDDA product at 4°C (left), and 37°C (right). Dienophile: Cypc-Lys-Phe-Ala-Gly (1 eq), Diene: 3,6-Bis (methylthio)-1,2,4,5 tetrazine (1 eq).



- (4) ¹³C NMR result of 2-methylcycloprop-2-enecarboxylic acid and LC-MS result of Cypc-Lys-Phe-Ala-Gly



Conclusions

- Various dienophiles like norbornene-containing and cyclopropene-containing molecules have been synthesized successfully.
- IEDDA reactions with different dienophiles have been carried out.
- Color change was observed for IEDDA reaction of S, S-tetrazine with alkenes in solution but the correct product was not detected in LCMS analysis.
- IEDDA reaction with cyclopropene ring has shown more reactivity than norbornene
- Acidic pH may be leading to hydrolysis of the tetrazine.
- More reactive dienophile is going to be synthesized to occur the IEDDA reaction at room temperature to avoid hydrolyzation.
- More experiments in basic condition will be conducted to optimize IEDDA reaction conditions using cyclopropene derivative as a dienophile.

Literature cited

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Acknowledgments

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