

Synthesizing a Viable Indicator for *Staphylococcus Aureus*

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Introduction

- This research project aims to synthesize a viable indicator for *Staphylococcus aureus* (*S. aureus*).
- The V-8 protease produced by the *S. aureus* cleaves polypeptide bonds specific to glutamic acid.
- Glutamic acid-glycine dipeptide shows promise because glutamic acid acts as a strong quencher when bound to the dye, 2',7'-dichlorofluorescein (DCF). However, when the peptide bond of the dipeptide is cleaved, only glycine would be attached to the dye, so it would fluoresce because glycine is a weak quencher.
- When *S. aureus* is present, the dye will fluoresce.

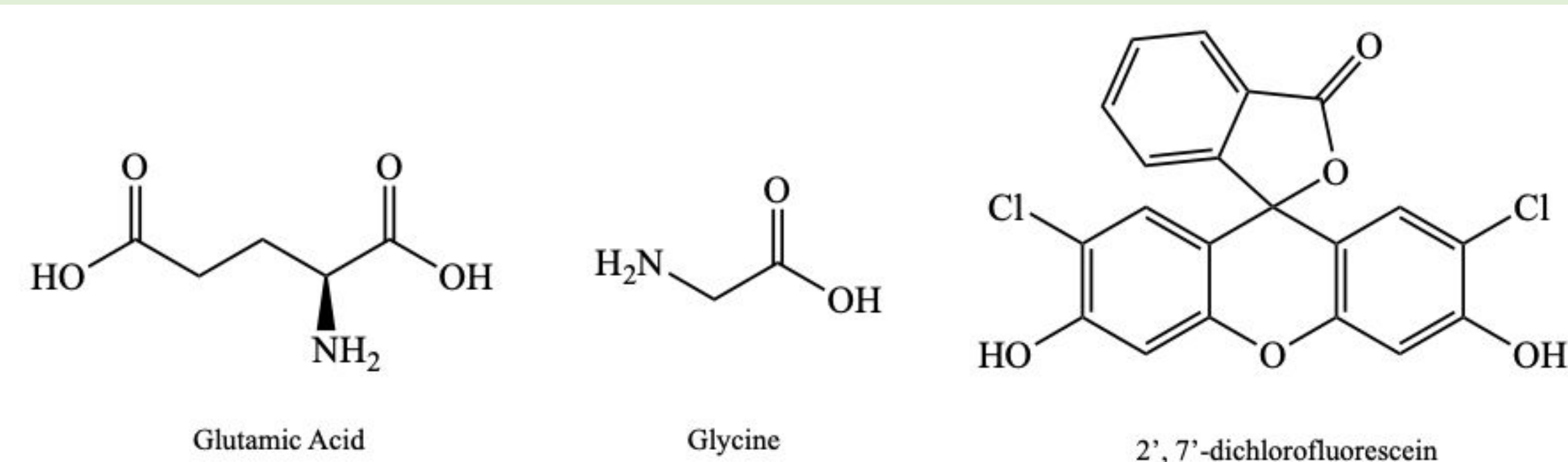


Figure 1. Chemical structures of glutamic acid, glycine and 2',7'-dichlorofluorescein.

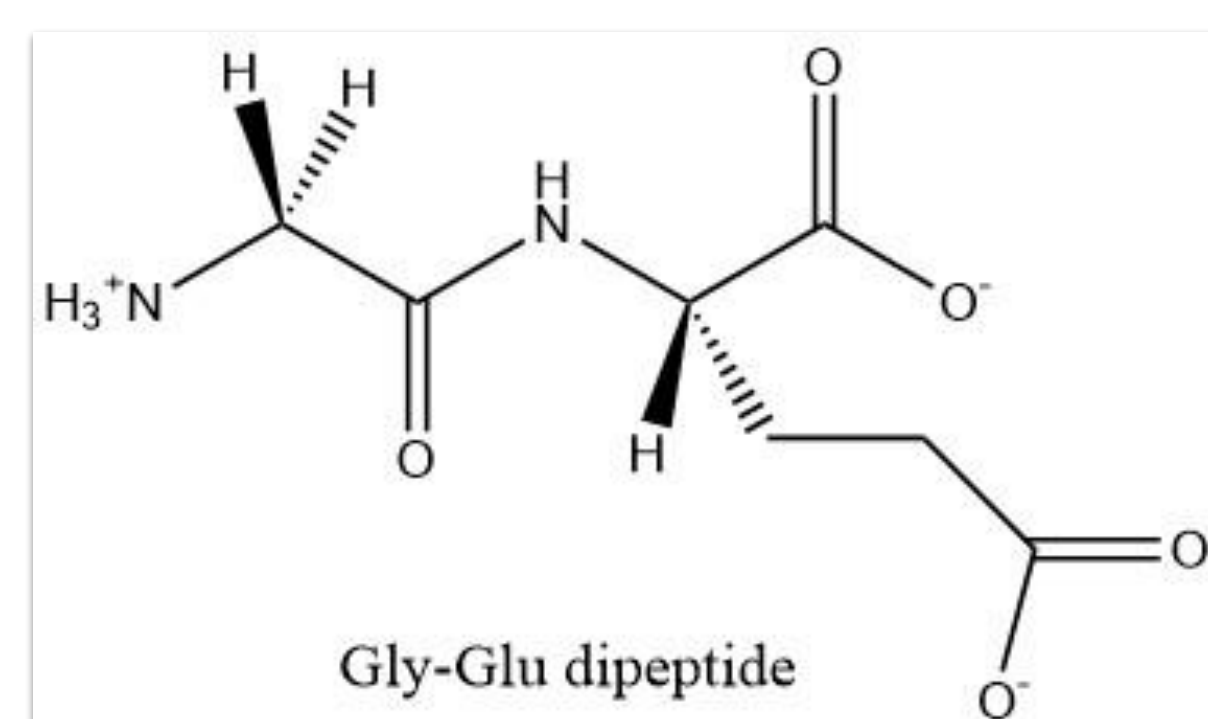


Figure 2. Structure of the glycine (Gly) glutamic acid (Glu) dipeptide.

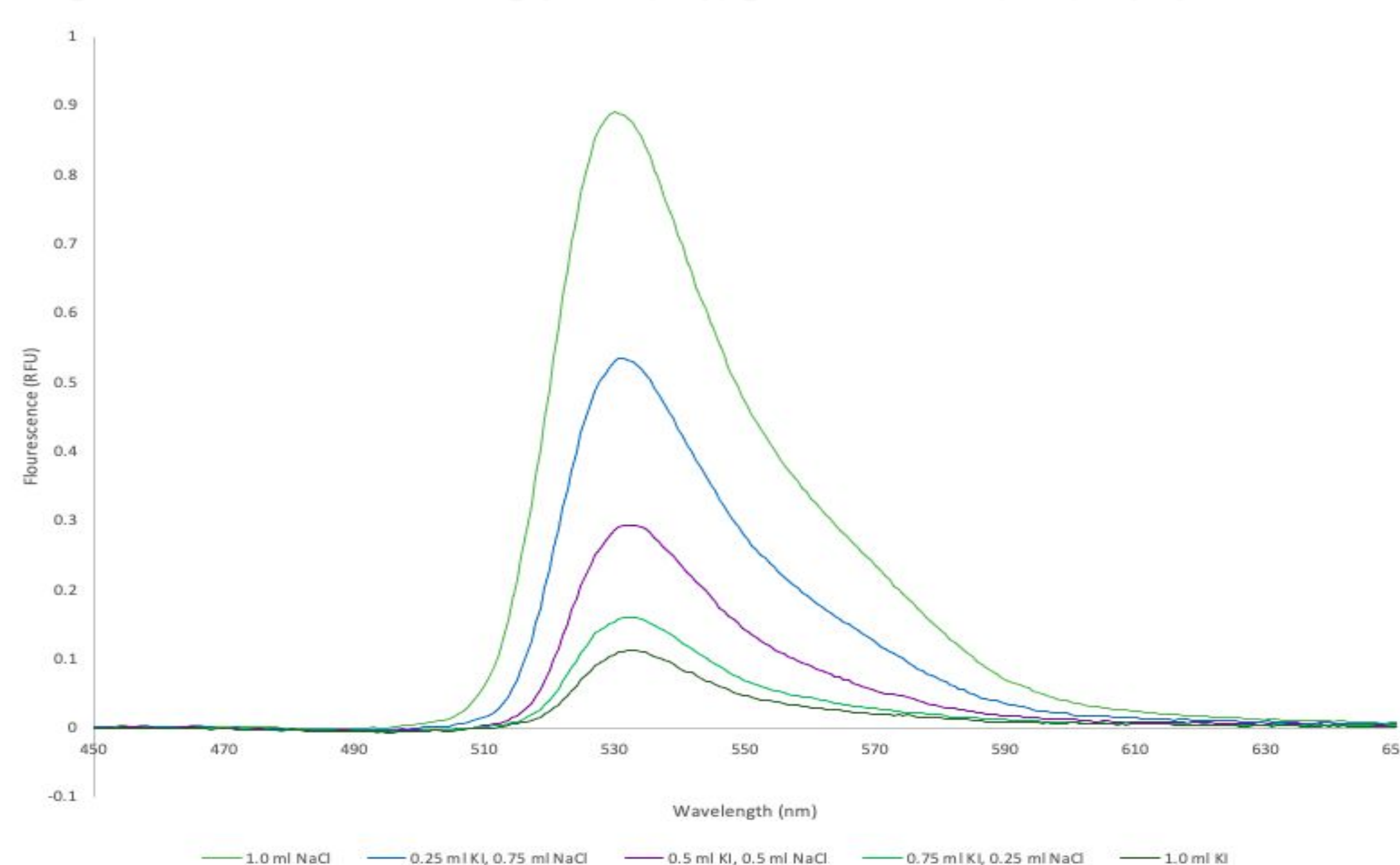


Figure 3. Fluorescence spectra of 1 M KI solution, 0.1 M NaCl solution, and 3 mL of DCF stock solution, demonstrating the quenching ability of KI on the dye.

Acknowledgements

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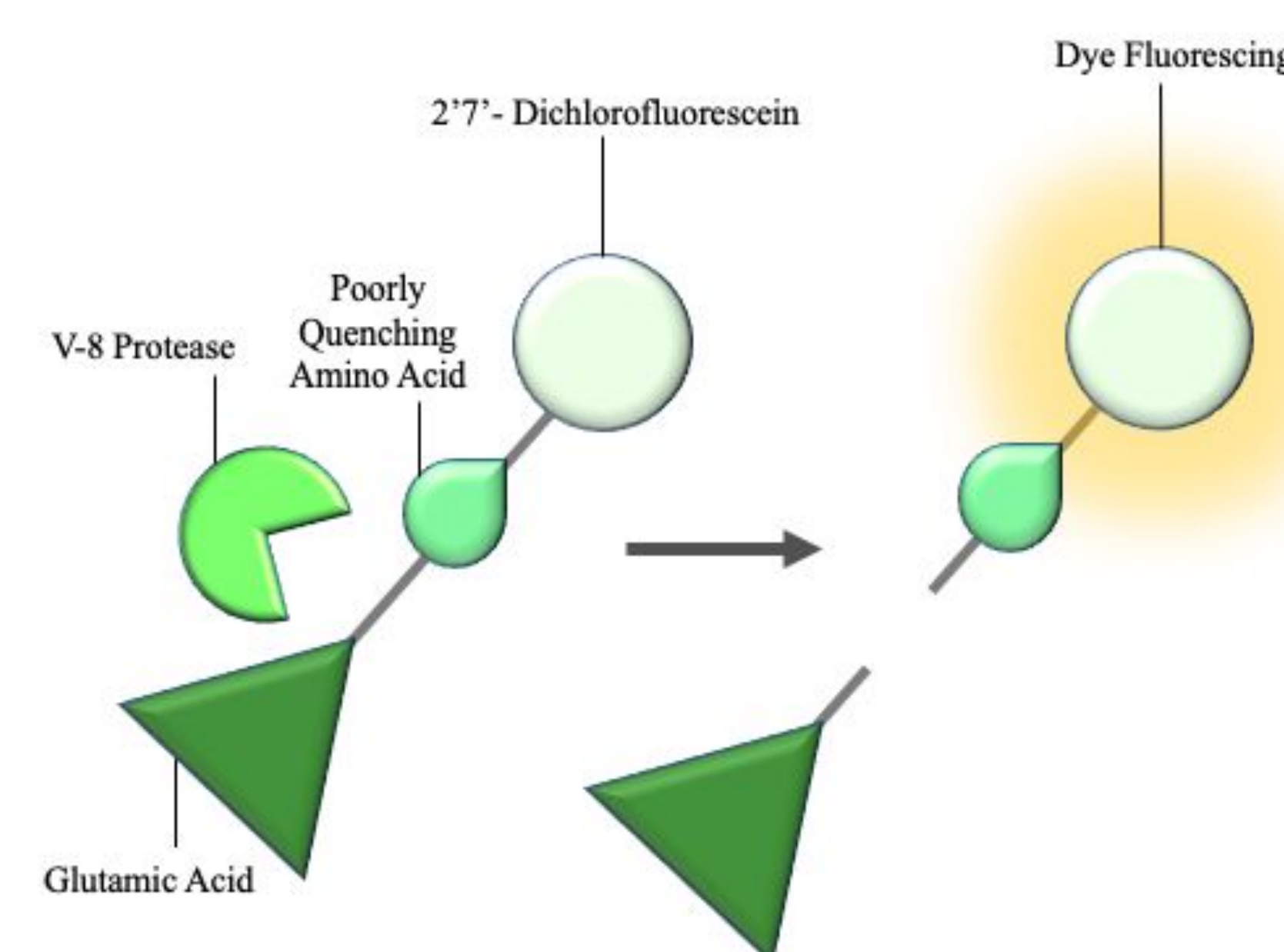


Figure 4. Glutamic acid, a strong quencher, bound to a poorly quenching amino acid (glycine) bound to a fluorescent dye, 2',7'-Dichlorofluorescein (DCF). The V-8 Protease enzyme specific to *Staphylococcus aureus* cleaves the bond between glutamic acid and the glycine. Cleavage of the bond results in fluorescence which indicates the presence of *S. aureus* (positive result).

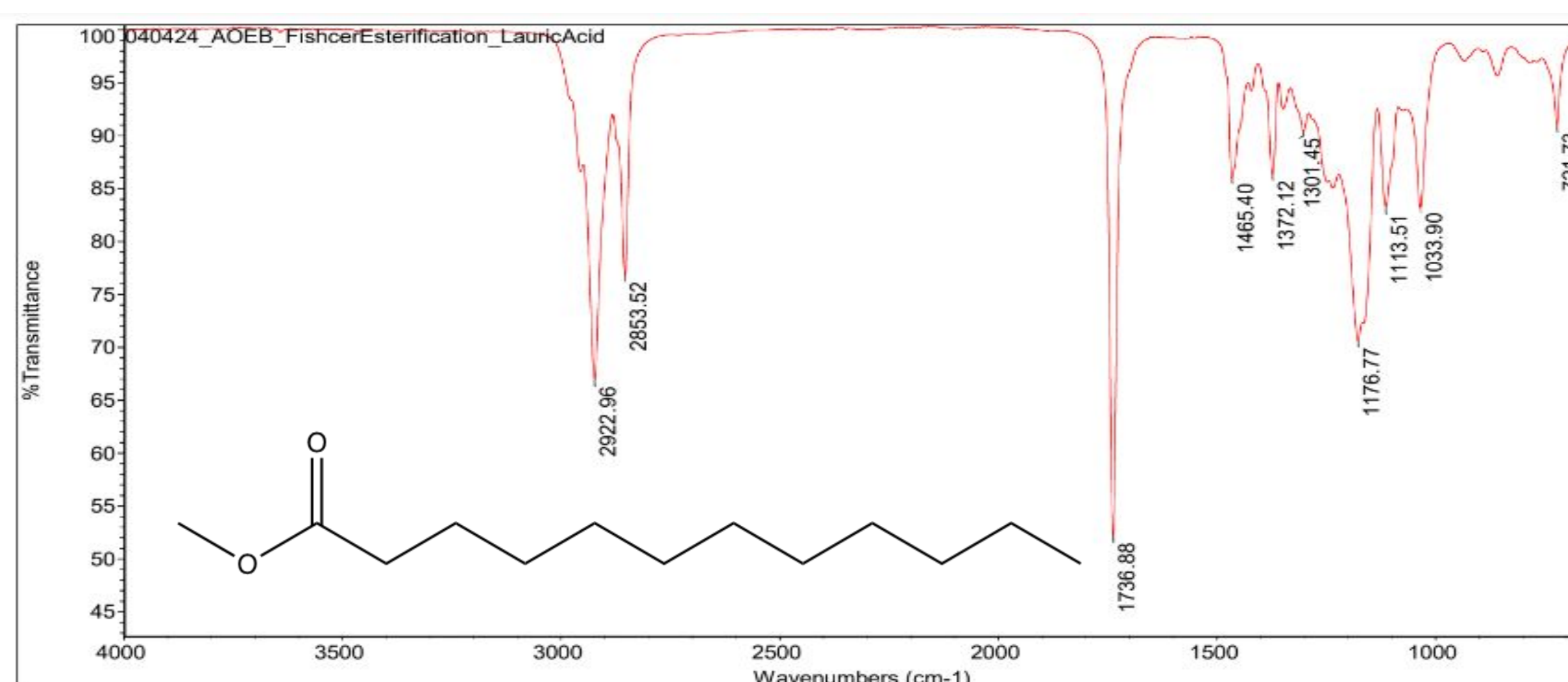


Figure 5. IR spectrum of esterified methyl laureate (structure shown). The lack of a strong and broad O-H stretch and the strong carbonyl ester peak at 1734 cm⁻¹ indicates the esterification was successful and supplies proof of method.

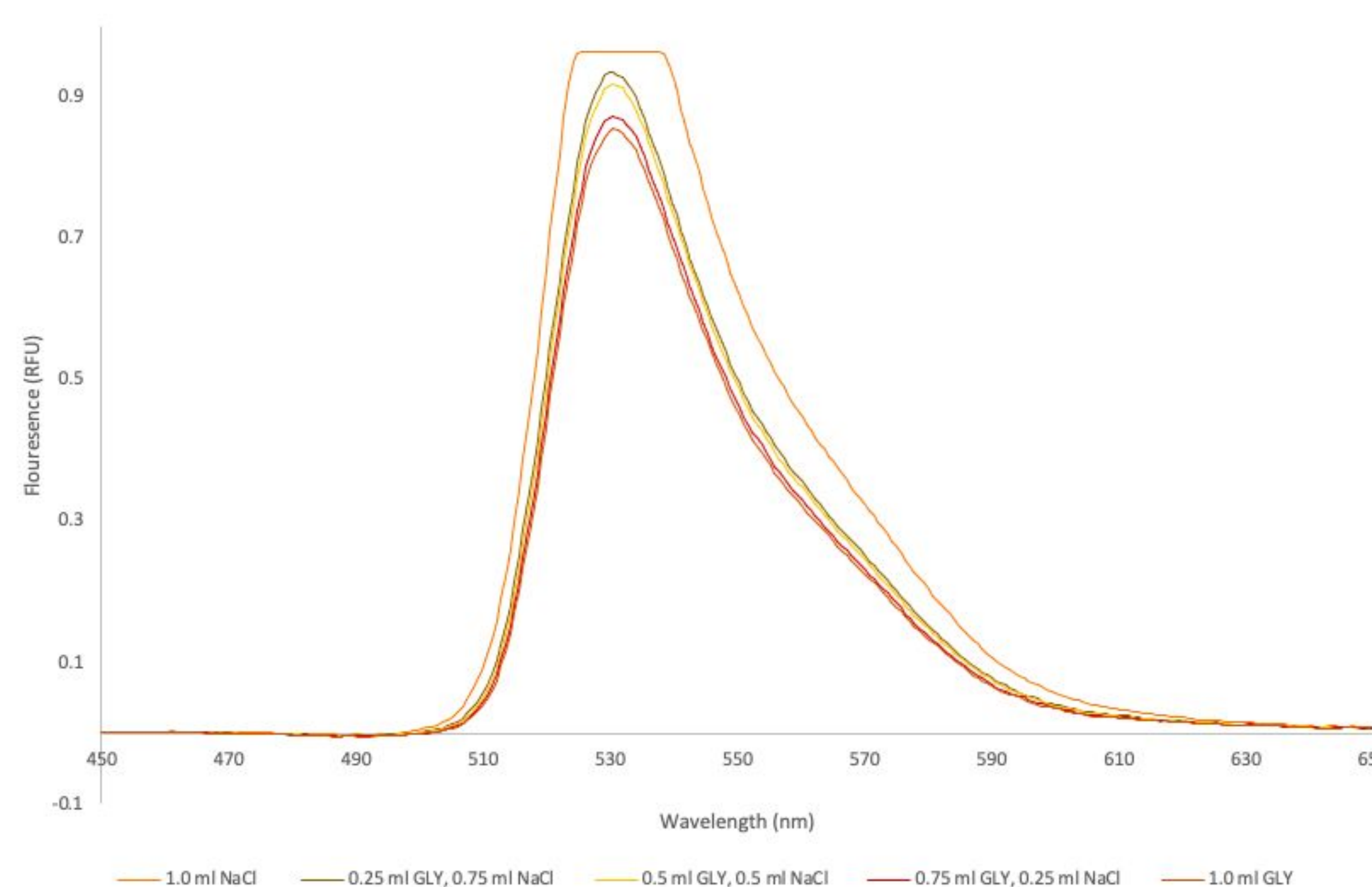


Figure 6. Fluorescence spectra of glycine solution, 0.1 M NaCl solution, and 3 mL of DCF stock solution, demonstrating glycine as a poor quencher.

References

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- (2) Sparano, B. A.; Shahi, S. P., and Koide, K. (2004) Effect of binding and conformation on fluorescence quenching in new 2',7'-Dichlorofluorescein derivatives. *Org. Lett.* **2004**, *6*, 1947–1949.
- (3) Crichton, J.; Kuga, K.; Breit, J.; Chennai, B.; Lieberg, J.; Rowley, J. *Staphylococcus Aureus* Indicator. Carroll College, Student Undergraduate Research Festival. Helena Montana. **2023**.

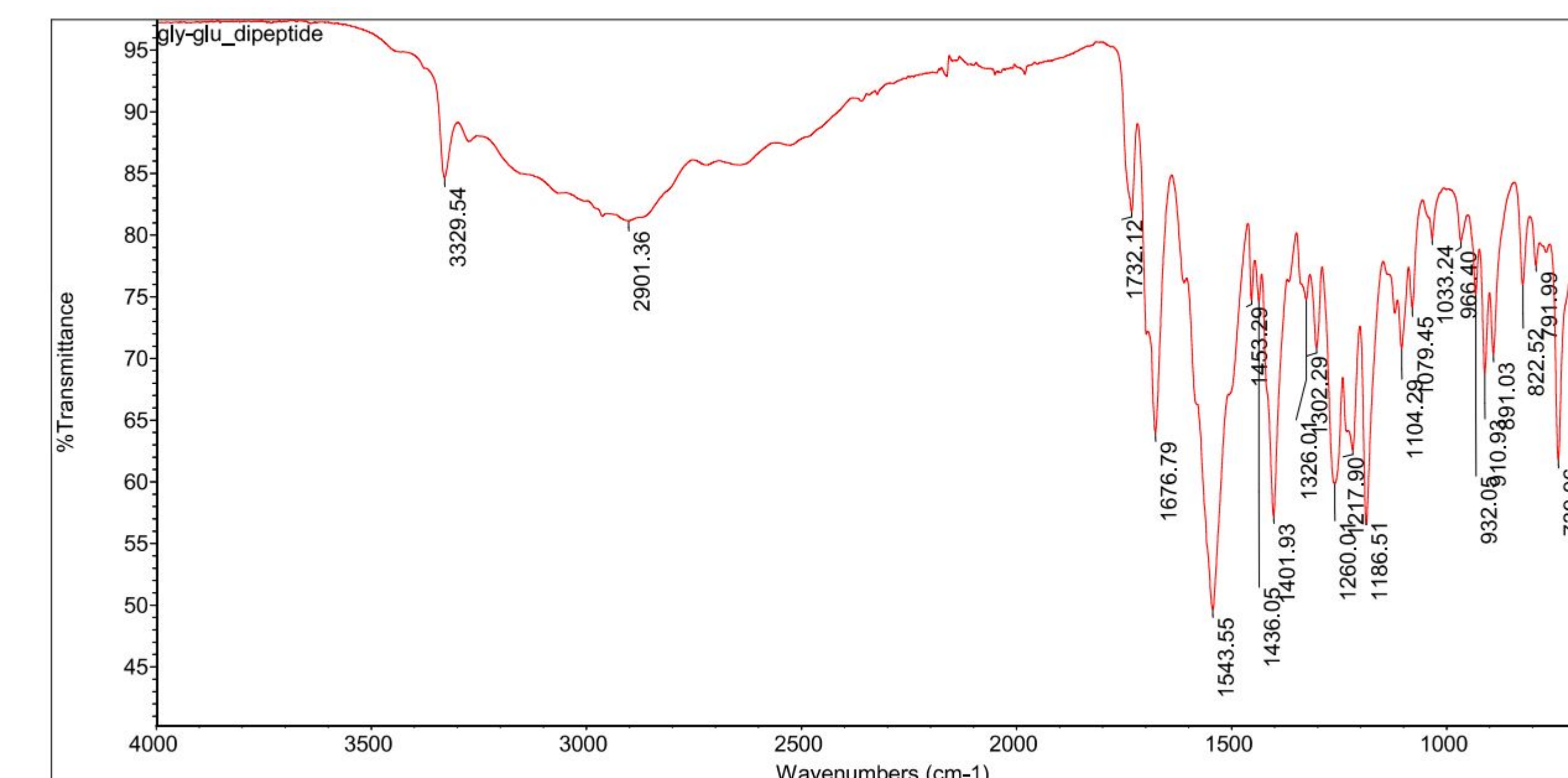


Figure 7. IR spectrum of pure Gly-Glu dipeptide. The large, broad peak from 2100-3500 indicates the presence of an alcohol.

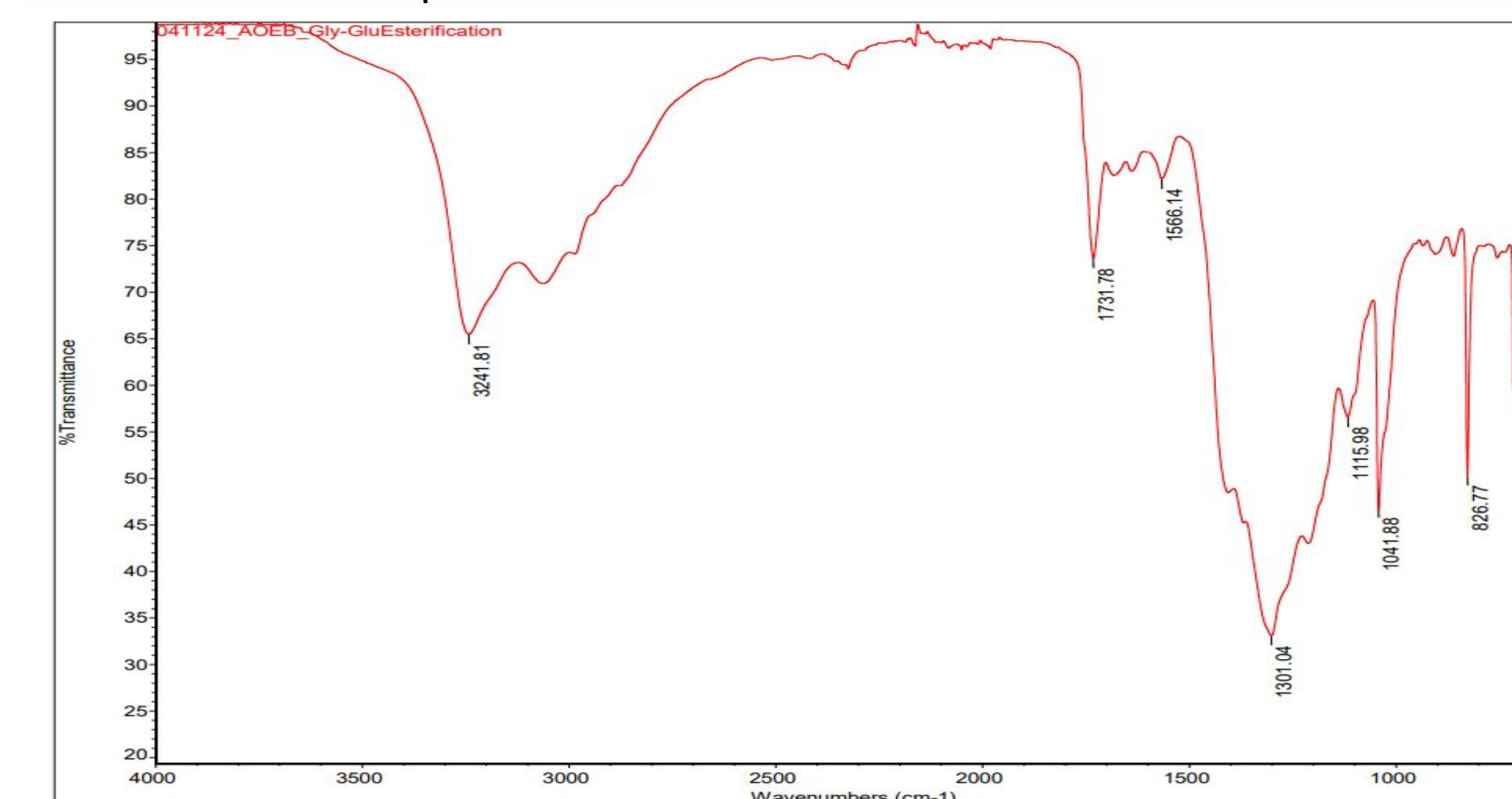


Figure 8. IR spectrum of partially esterified Gly-Glu dipeptide. The peak 2600-3500 is smaller than the peak of the dipeptide before esterification which indicates partial esterification, but the addition of protecting groups was not complete.

Discussion

- In the fluorometry experiment, glycine acted as a poor quencher (Fig. 6). Results indicating that glutamic acid is a strong quencher were unable to be replicated.
- Solutions were mixed using a 0.1 M NaCl solution for the fluorometer to prevent dipole moments from forming due to the polarity of H₂O which could have been affecting the atomic orbital shape.
- KI was tested as a strong quencher due to Iodine's large atomic radius to demonstrate proof of method.
- The carboxyl side chains on the dipeptide must be protected using an esterification reaction to ensure that the dye binds only to the glycine residue.
- Partial protection of the dipeptide was confirmed via IR (Fig. 7 & 8).

Future Work

- Repeat the glutamic acid fluorescence to replicate results of its quenching ability.
- Increase the molar equivalents of ethanol for the Fischer esterification of the dipeptide to yield fully-protected dipeptide.
- Once the dipeptide is protected, develop and proceed with a procedure to bind the protected dipeptide to DCF.