# Synthesis and Evaluation of Pyridyl Thioester-Based F-Tags for Rapid Analysis of Amino Acids by 19F NMR Spectroscopy

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Accurate and rapid analysis of amino acids is essential for metabolomics and disease related biomarker studies. 19F NMR spectroscopy offers high sensitivity and no biological background signal, but its performance depends strongly on the stability and reactivity of the fluorinated tagging reagent. Traditional N-hydroxysuccinimide based 19F-tags form stable amide bonds with primary amines, yet they undergo rapid hydrolysis in aqueous buffer, reducing labeling efficiency and reproducibility. This project aimed to synthesize and evaluate pyridyl thioester based 19F-tags as a more hydrolytically stable alternative for amino acid detection. 3-chloro-2-fluoro-pyridyl thioester F-tags were synthesized and characterized, followed by kinetic analysis of their reactions with selected amino acids under controlled buffer conditions. Rates of hydrolysis were measured by 19F NMR spectroscopy and compared to the NHS-ester systems. Solubility and spectral resolution were also assessed to determine suitability for rapid analysis. Additional tag variants were prepared to examine the effect of structural modifications on solubility. The data show that pyridyl thioester tags exhibit improved resistance to hydrolysis while maintaining efficient amide bond formation with amino groups. The data indicates comparable labeling performance, and 19F NMR spectra demonstrate clear, distinguishable signals for multiple amino acids. Overall, pyridyl thioester-based F-tags provide a more stable and reliable platform for rapid amino acid analysis by 19F NMR spectroscopy, supporting improved quantitative measurements in metabolomic applications.

**Awards Won:**
Third Award of $1,200