

Novel functionalized acylation reagents for Gly- and Lys-His tag acylation on proteins

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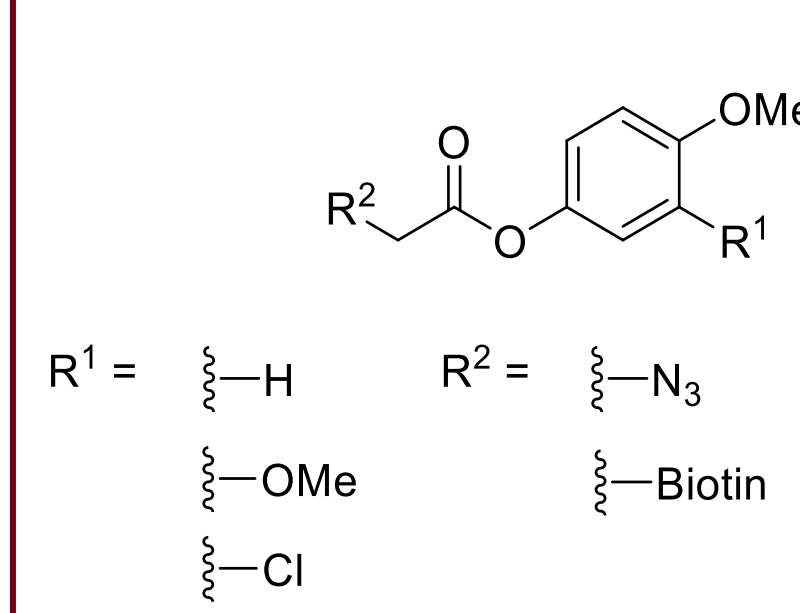
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ABSTRACT

Chemical modification of protein amines is often performed by acylation of amines using NHS-esters. Due to the high reactivity of NHS-esters, mixtures of products are formed. Highly selective chemical modification of a protein was performed using an N-terminal histidine based peptide tag, the Gly-His tag (GHHHHHH-), which has been developed in our lab. New acylating reagents which facilitate the introduction of functionalities such as biotin, fluorophores, or half-life extending moieties were designed and synthesized using well-known organic chemistry. This was demonstrated by the synthesis of an array of activated biotin esters. We compared them to our previous biotinylation reagent, and a biotin ester with X = H. The reactivity and solubility of the esters were tuned by varying the substituents on the α -carbon, X and Y, and including a carboxylate on the phenolic side. Our optimized esters reacted with very high selectivity towards GH₆-Protein X.

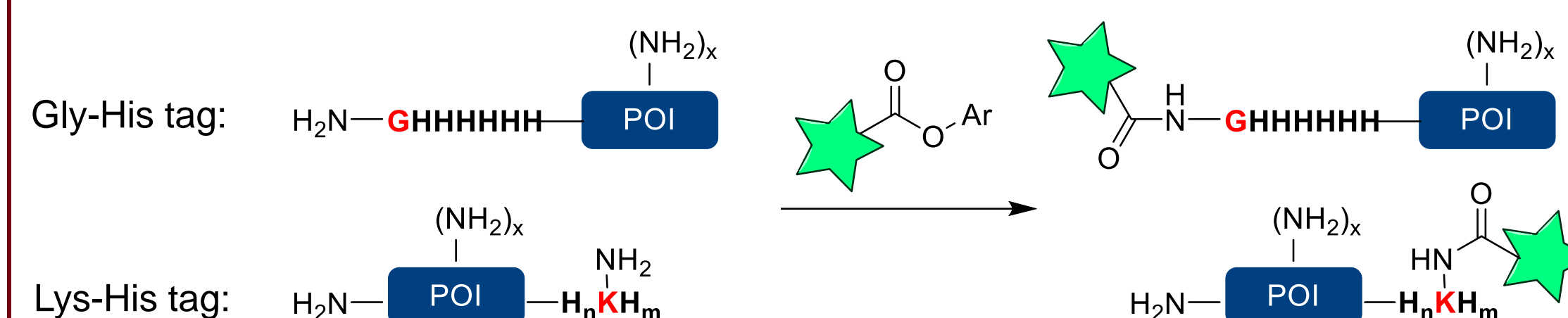
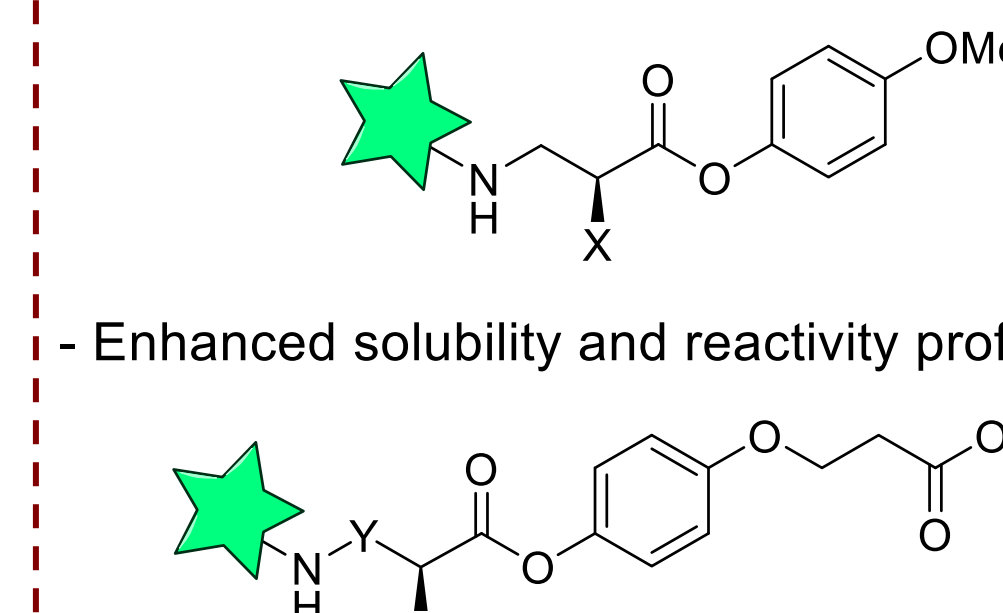
Previously:

4-Methoxyphenyl esters



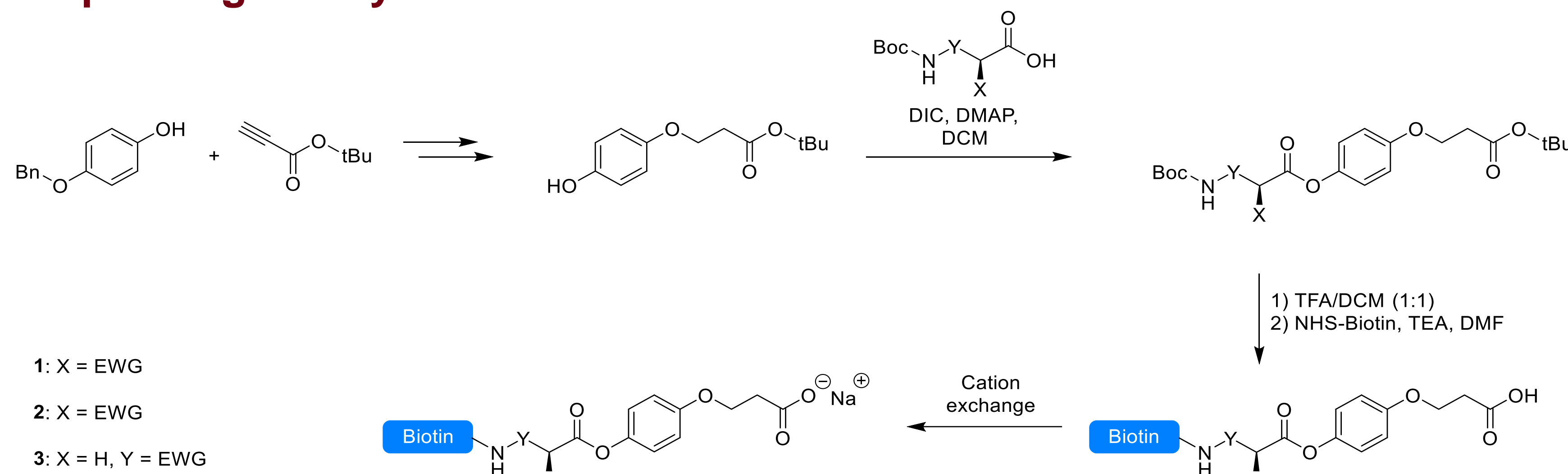
Here:

- Generic ester functionalization

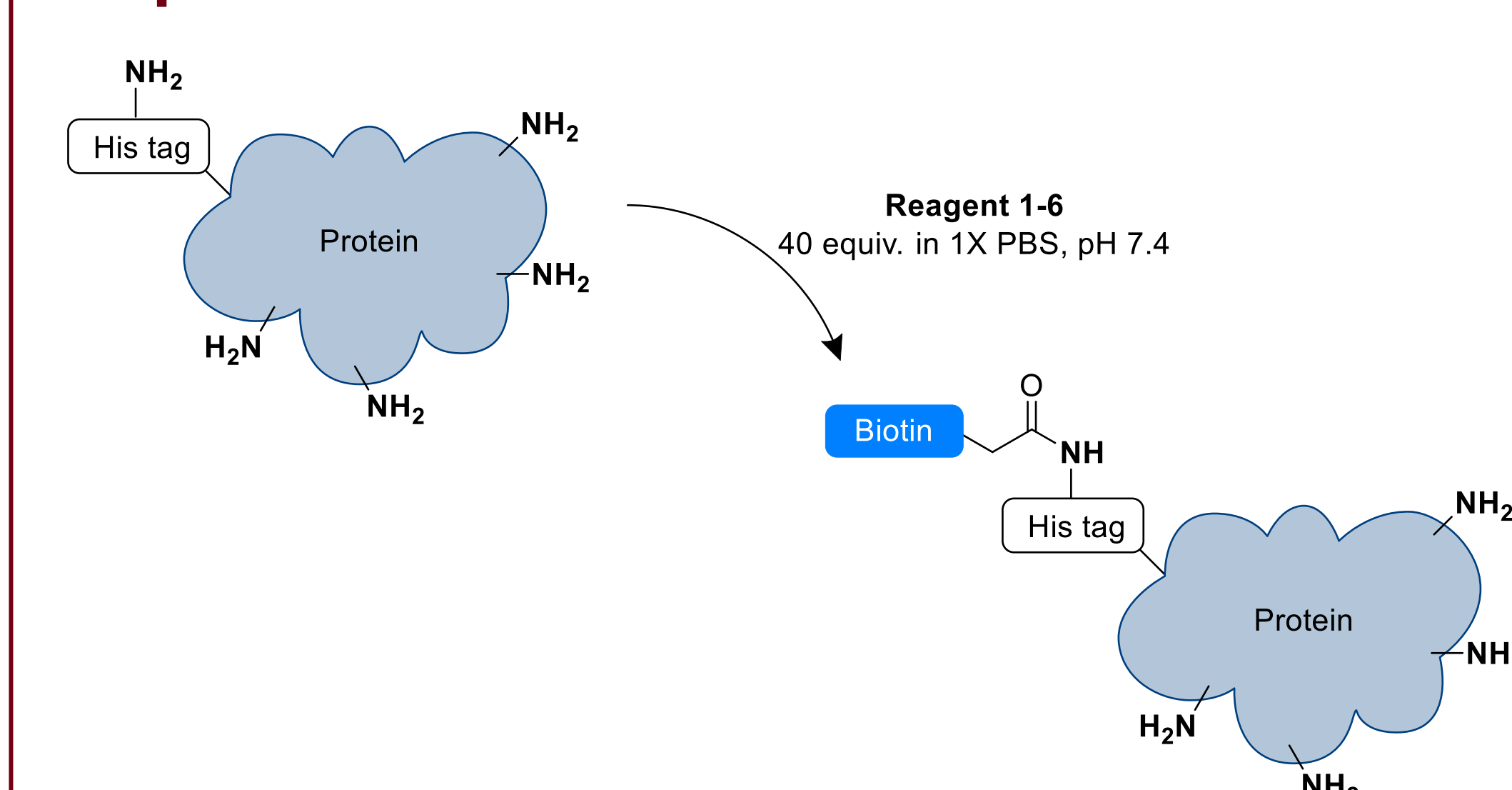


EXPERIMENTAL

Step 1: Organic synthesis



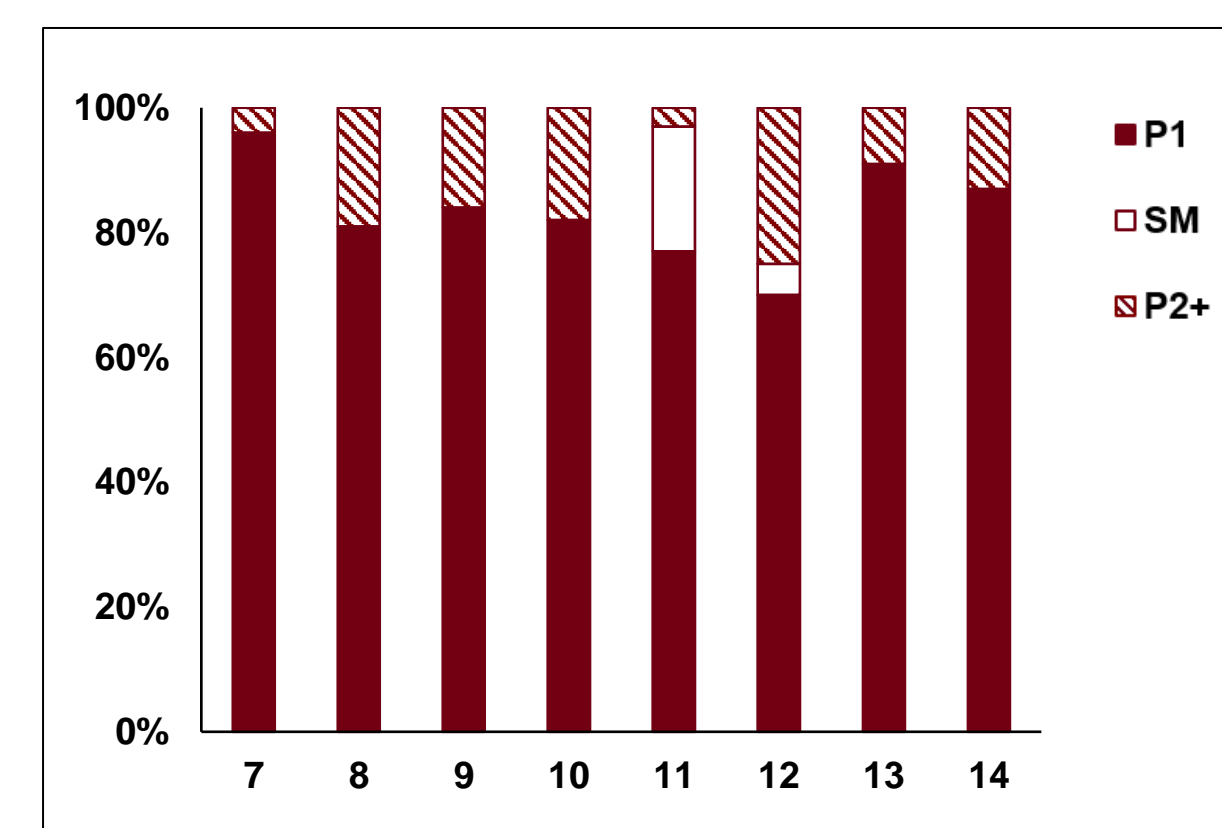
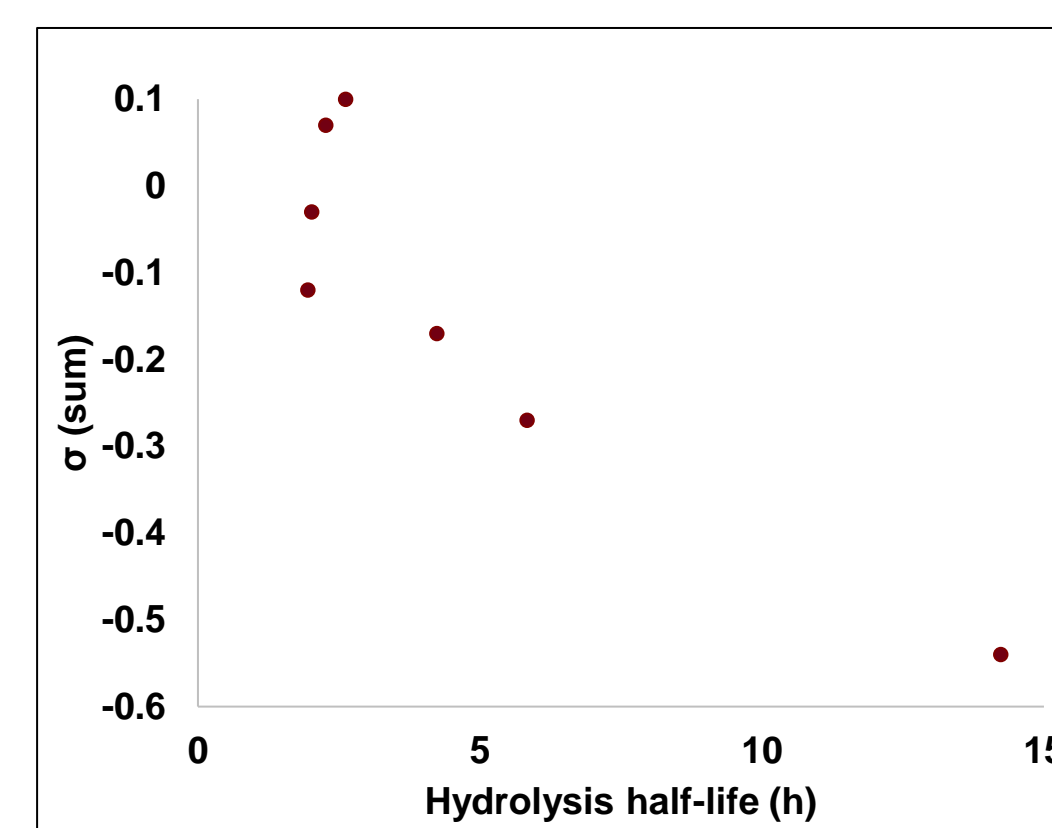
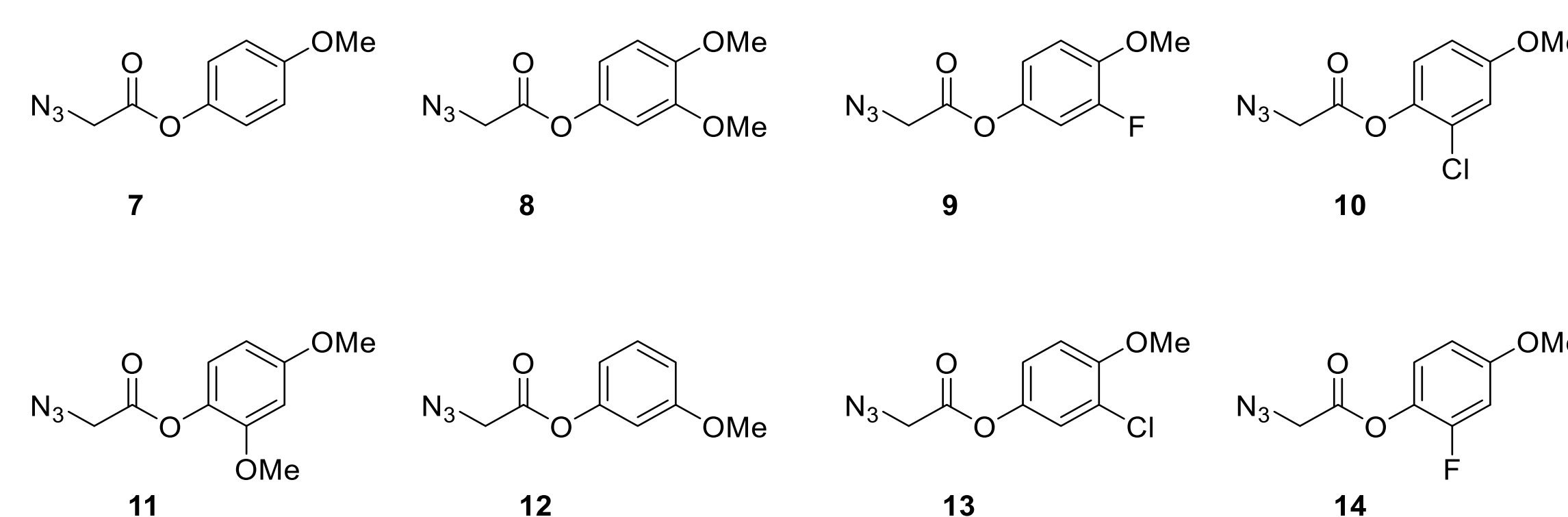
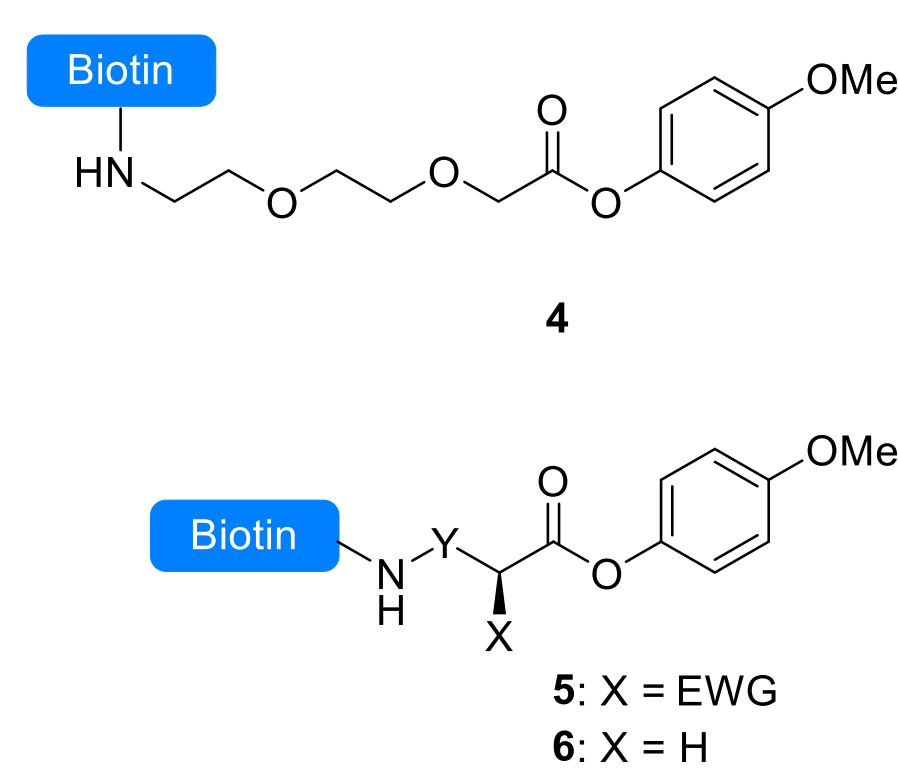
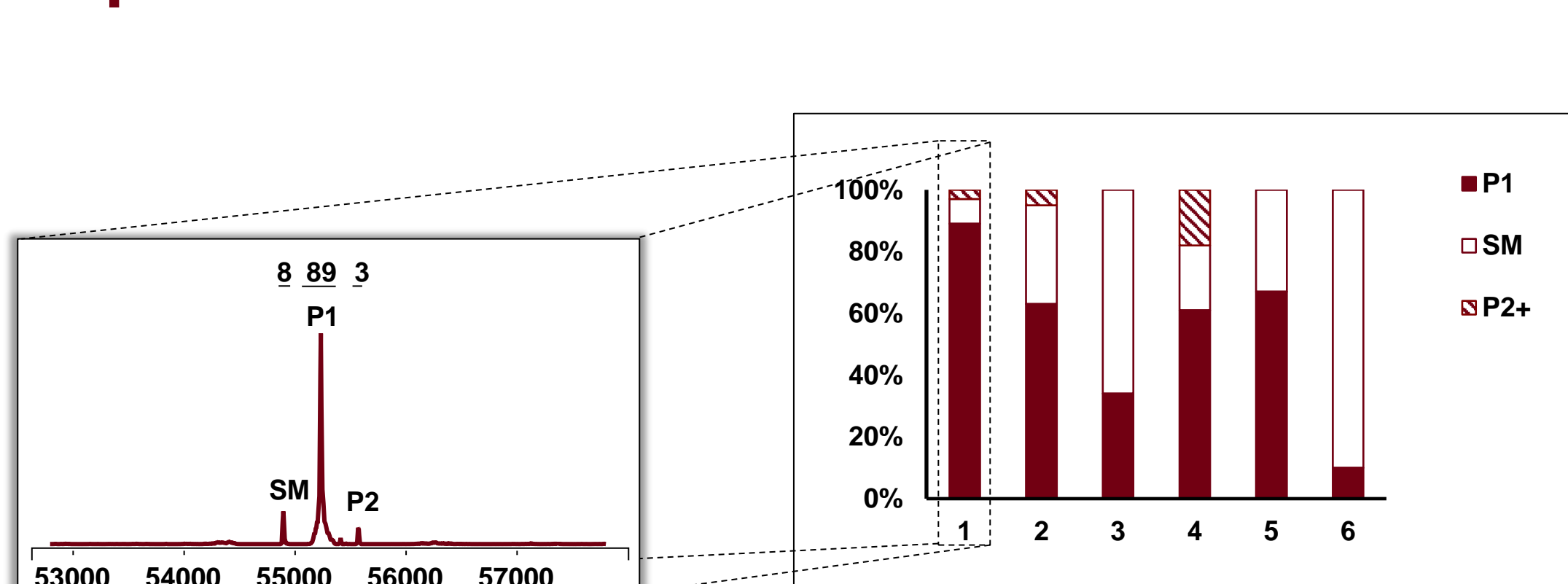
Step 2: Protein modification



RESULTS AND DISCUSSION

- Increasing the sum of Hammett parameters of the phenol substituents in the esters give a linear decrease in hydrolysis half-life of esters
- This also gave more non-specific acylation of model peptides

Improved biotin esters



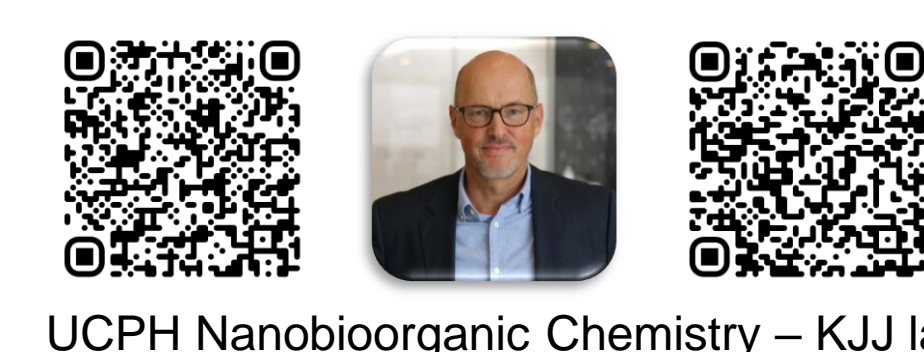
CONCLUSIONS

Esters with a higher sum of Hammett parameters of the phenol substituents hydrolyzed more rapidly and gave more formation of P2+. The increased electronegativity at the α -carbon in 5 relative to 4 and 6 resulted in enhanced formation of P1 and improved the selectivity of the protein acylation reaction. Incorporation of a carboxylate on the phenolic side of the ester increased the solubility and selectivity of the protein functionalization. Further, it did not affect the pH of the reaction mixture (data not shown). Esters with an EWG at the α -carbon showed the highest selectivity in Gly-His tag acylation of GH₆-Protein X. We expect an increase in the applications of our Gly- and Lys-His tag acylations following this development of a highly selective reagent for protein functionalization.

CONTACT



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References

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