Electrostatic assistance of NCL catalysis by 4-mercaptophenylacetic acid[1]

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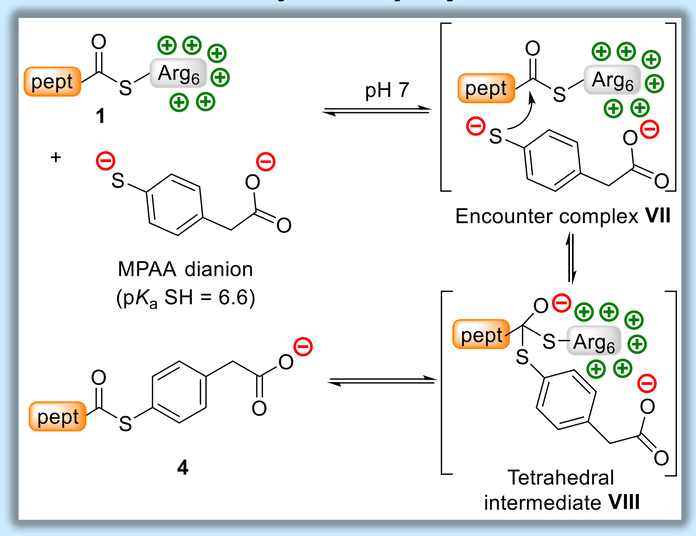
Principle of the electrostatic assistance of NCL catalysis step by MPAA

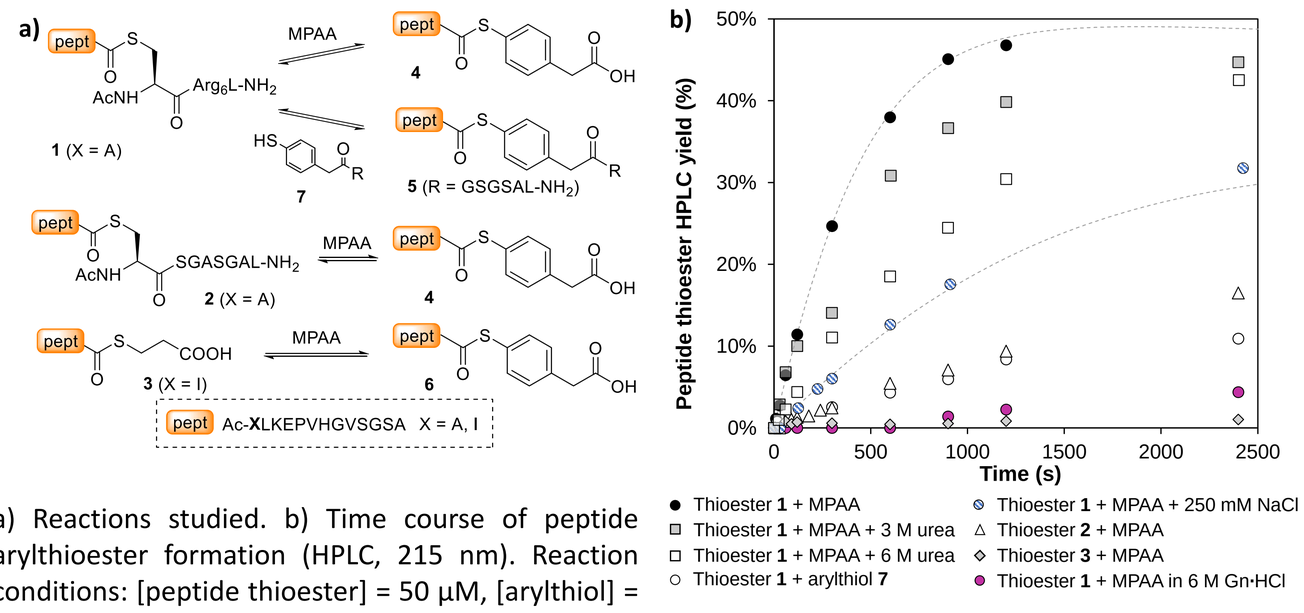


Introduction.

The native chemical ligation (NCL)[2] reaction is a powerful tool enabling the semi or total chemical synthesis of proteins[3]. The water-soluble aryl thiol 4-mercaptophenylacetic acid (MPAA)[4] is the gold standard catalyst for this reaction. In classical conditions and using peptide alkyl thioesters as acyl donors, it has to be used in large excess for achieving practically useful rates (up to 50-100 equivalents).

We report here that the rate of NCL using MPAA as catalyst is dramatically enhanced when the alkyl thioester features several arginines in the departing thiol from the thioester, due to the electrostatic assistance of the catalysis step (Figure →). By doing so, the electrostatically assisted NCL reaction ([e]NCL) proceeds very rapidly by using sub-stoichiometric concentrations of MPAA.

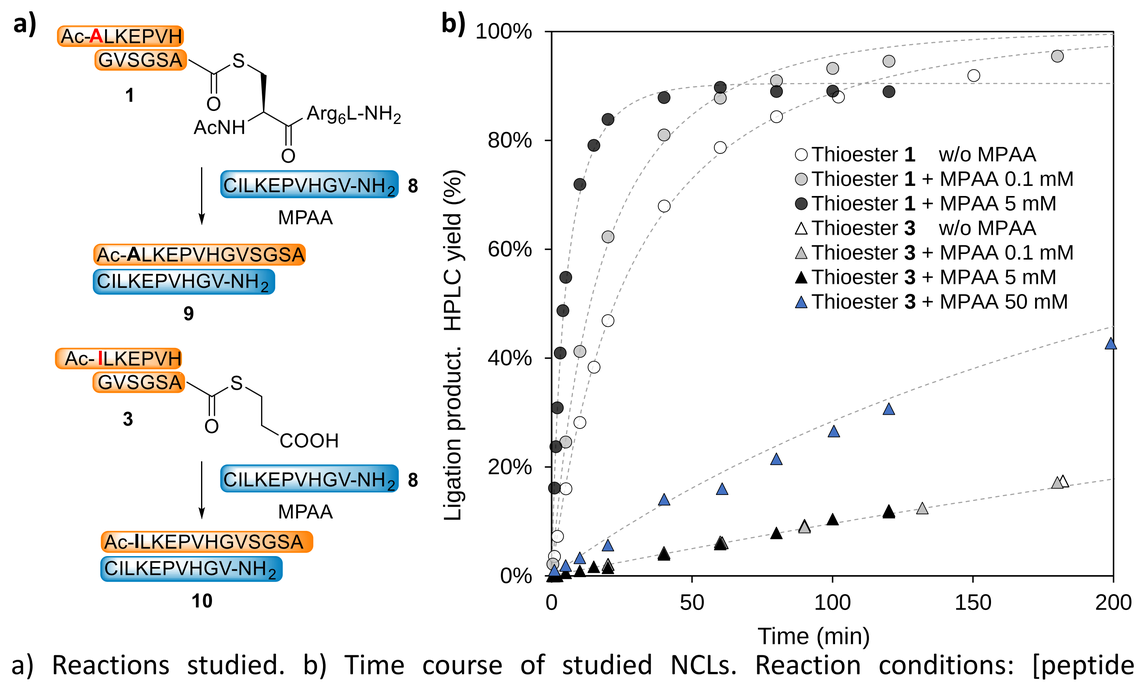
Electrostatic assistance of MPAA catalysis



a) Reactions studied. b) Time course of peptide arylthioester formation (HPLC, 215 nm). Reaction conditions: [peptide thioester] = 50 µM, [arylthiol] = 250 µM, 10 mM sodium phosphate buffer, pH 7, 19 °C. The dots correspond to the experimental data, the dashed lines to the fitting curves.

Guanidinium chloride denaturant inhibits the assistance, while urea is tolerated.

Electrostatic assistance of NCL



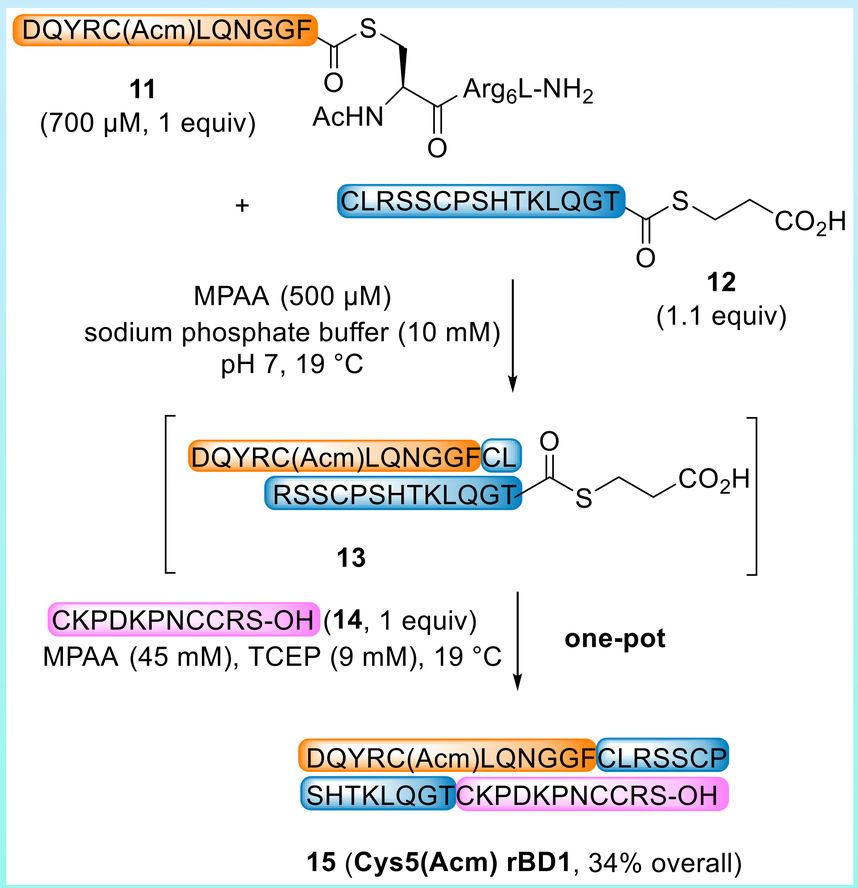
a) Reactions studied. b) Time course of studied NCLs. Reaction conditions: [peptide thioester] = 1 mM, [**8**] = 1,5 mM, 10 mM sodium phosphate buffer, pH 7, 19 °C. The dots correspond to the experimental data, the dashed lines to the fitting curves.

Due to the electrostatic assistance of the NCL catalysis step, NCL proceeds much faster than under classical conditions, while using only sub-millimolar concentrations of MPAA.

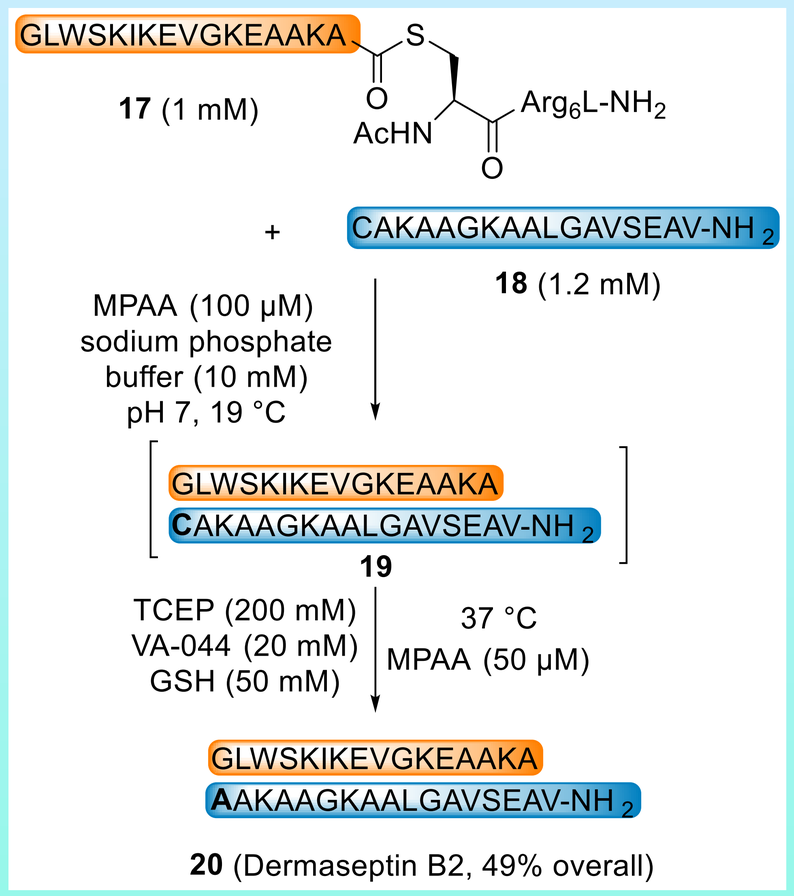
Applications.

[e]NCL enabled useful synthetic applications. Combining [e]NCL and classical NCL enabled performing one-pot kinetically controlled ligations. In another application, a one-pot [e]NCL/desulfurization process could be performed in one-pot because the sub-millimolar concentration of MPAA used for [e]NCL did not impair the desulfurization step.

One-pot kinetically controlled [e]NCL/NCL sequential ligation process



One-pot [e]NCL/desulfurization



Conclusion.

The catalysis of NCL by MPAA can be boosted by using the negatively charges present in the catalyst at neutral pH for enabling attractive electrostatic interactions with the positively charged thioester reactant. Introducing the positive charges in the departing thiol in the form of arginines makes the assistance traceless while bringing solubility to the peptide thioester reactant. [e]NCL proceeds very fast using only sub-stoichiometric concentrations of MPAA.

References.

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