A Liquid-Phase Continuous-Flow Peptide Synthesizer for Preparing C-terminal Free Peptides

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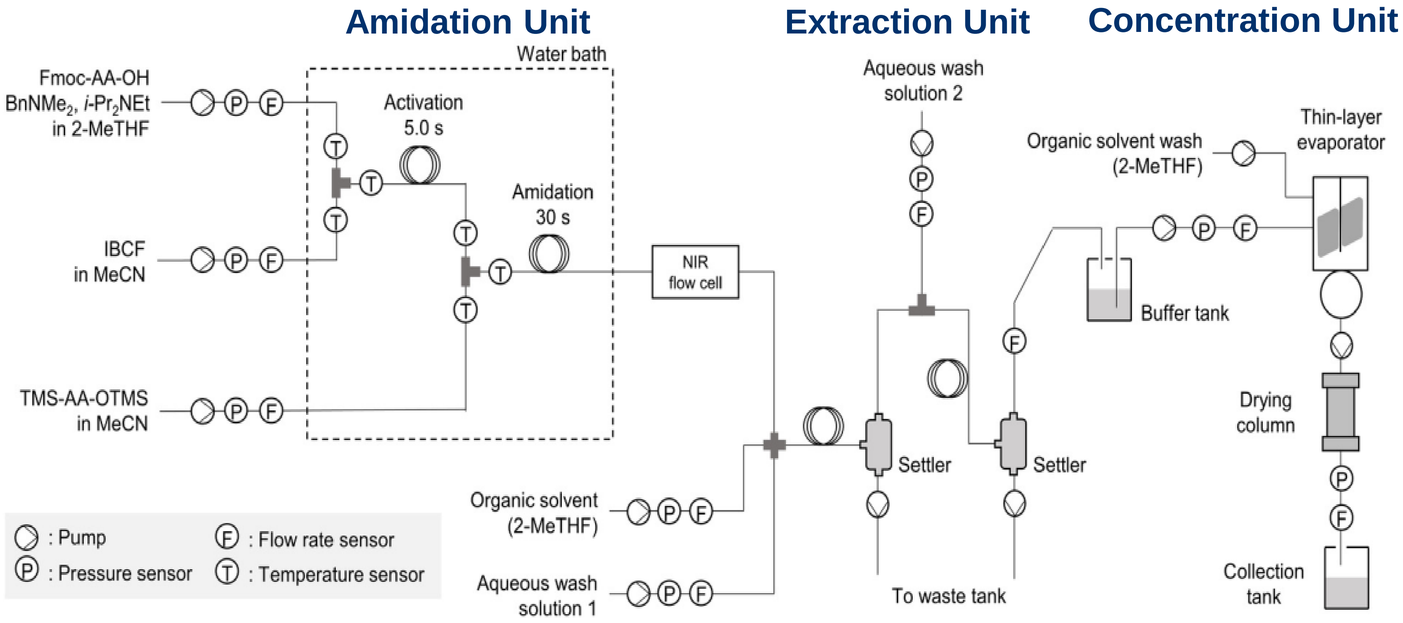
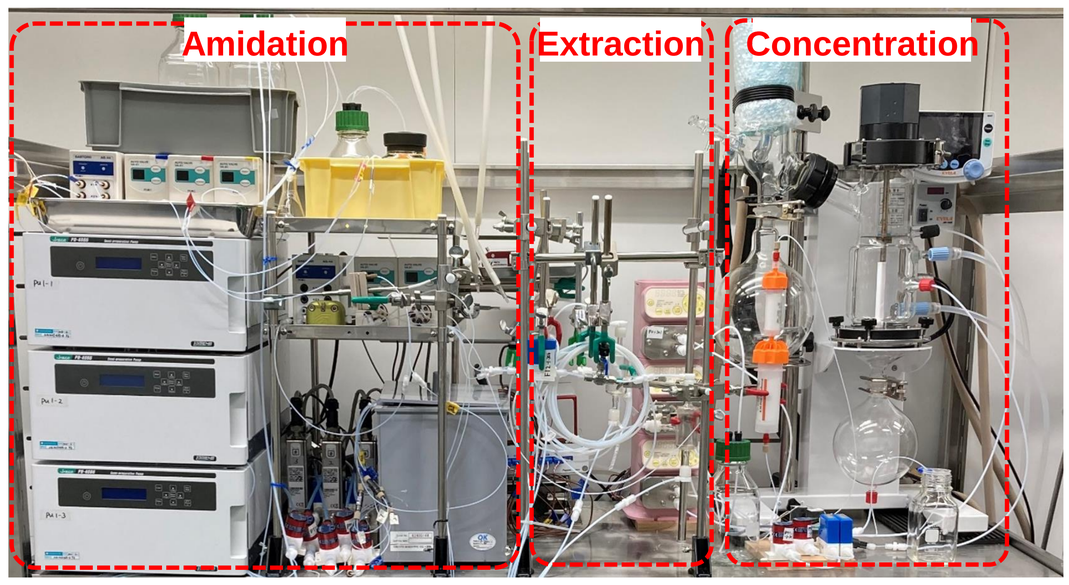
- ◆An Automated System for Continuous-Flow Liquid-Phase Peptide Synthesis with a C-Terminal Free Peptide Synthesis Method
- ◆Continuous Monitoring of Reaction with Process Parameters and NIR





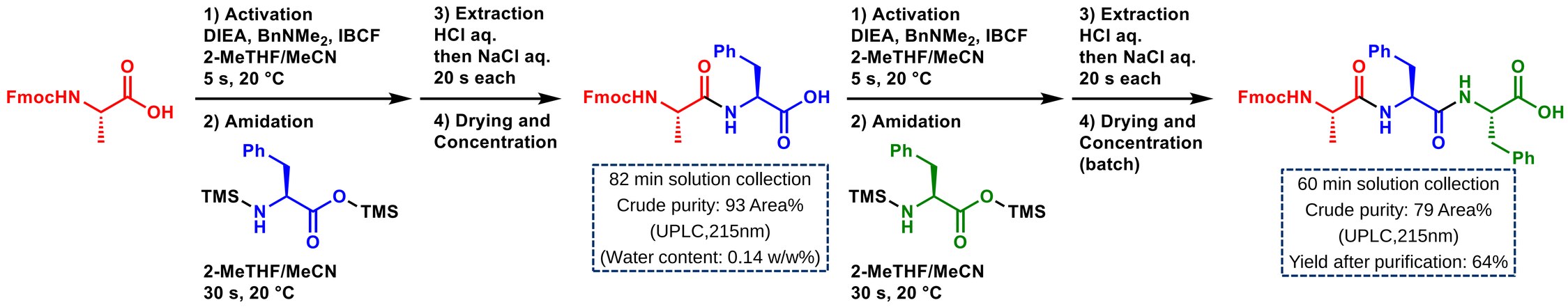
Schematic Diagram and a Picture of the System

◆The system was operated by the control unit developed in-house.

Introduction

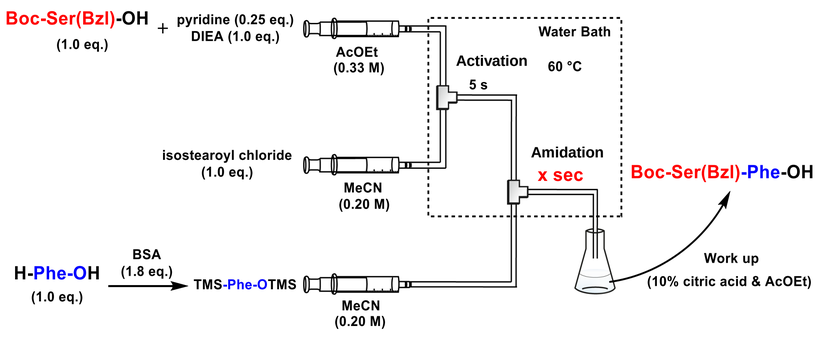
Conventional peptide production requires repeated amidation and deprotection steps that increase the amount of waste and the time and effort required. To solve these problems, we developed an automated continuous-flow liquid-phase peptide synthesizer for the preparation of C-terminal free peptides. A crude dipeptide and a tripeptide were synthesized using the synthesizer. The steadiness of the flow system was continuously monitored by measuring the process parameters, namely the flow rate, pressure, and temperature. The peptide synthesis was monitored using a near-infrared (NIR) sensor. This system enabled the first liquid-phase continuous-flow peptide synthesis, including aqueous workup and concentration, and the in-line NIR monitoring of peptide-bond formation. It will contribute to enhancing efficiency in peptide production.

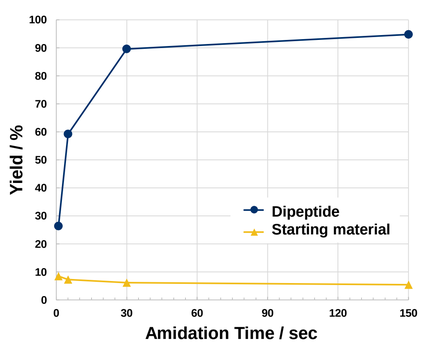
Procedure for the Preparation of Crude Dipeptide and Tripeptide



- ◆ The tripeptide was obtained (ca. 670 mL, purity: 79Area%) after 60 min of solution collection from the extraction unit.
- ◆ No purification of the dipeptide was needed prior to the tripeptide synthesis.

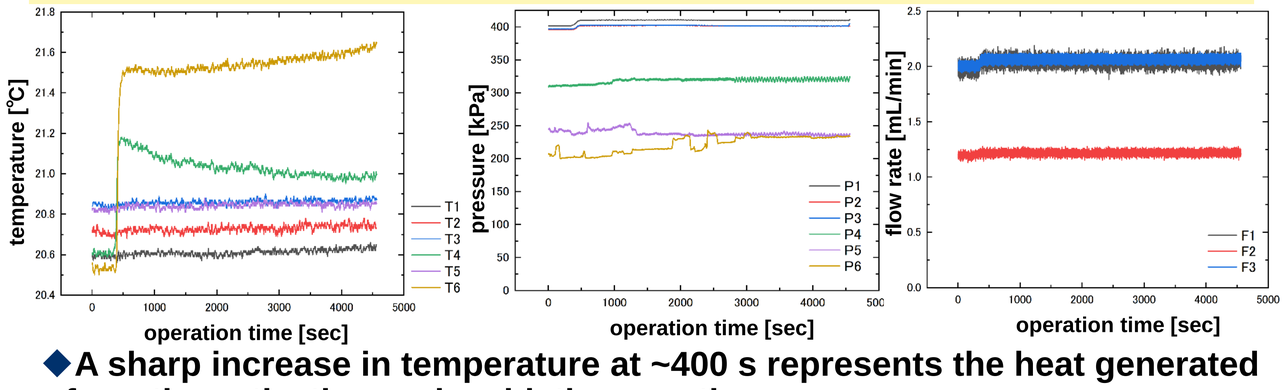
Optimization of Amidation Conditions





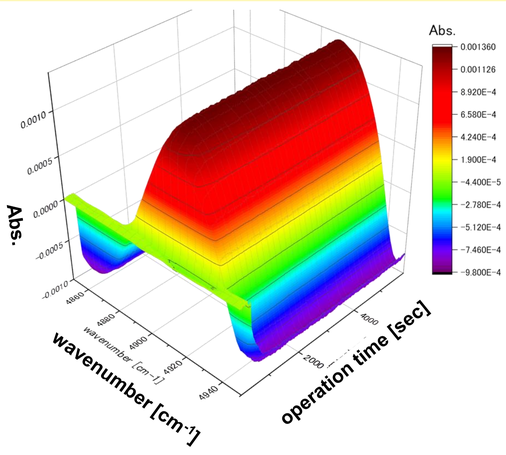
- ◆Amidation reaction completed in 30 s.

Temperature, Pressure, and Flow Rate Sensing Data

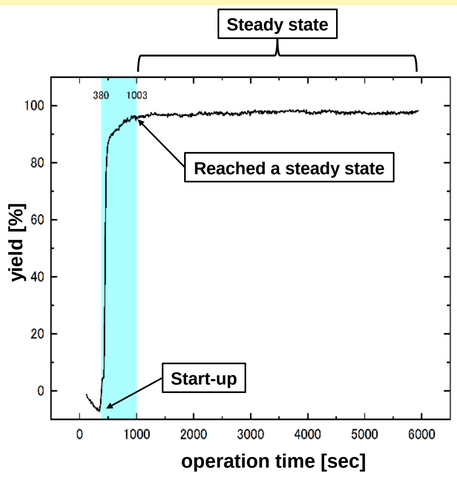


- ◆A sharp increase in temperature at ~400 s represents the heat generated from the activation and amidation reactions.
- ◆The pressure and the flow rate data represent the steadiness of the flow system.

In-Line NIR Monitoring of Amidation



The product yield was calculated from the NIR spectra using a calibration curve.



- ◆ NIR spectra were recorded every 8 s during system operation.
- ◆ The result showed the potential of in-line NIR measurements for real-time tracking of the steady-state condition of a continuous-flow liquid-phase reaction.

Conclusion

In this study, we developed a novel continuous-flow system comprising an amidation unit with a micro-flow reactor, an extraction unit with mixer-settlers, a concentration unit with a thin-layer evaporator, and a control unit. We successfully synthesized C-terminal-free peptides using our continuous-flow system. This research demonstrates an automated continuous-flow C-terminal free peptide synthesis with an integrated workup process and an adapted in-line PAT tool for the continuous manufacturing of peptides. We expect that our strategy will contribute to the development of efficient manufacturing processes for peptides as well as other small- and middle-molecule substances.

Reference)

1) H. Kurasaki, A. Nagaya, Y. Kobayashi, A. Matsuda, M. Matsumoto, K. Morimoto, T. Taguri, H. Takeuchi, M. Handa, D. R. Cary, N. Nishizawa, and K. Masuya, *Organic Letters* **2020** *22* (20), 8039-8043.
2) Y. Otake, K. Adachi, Y. Yamashita, N. Iwanaga, H. Sunakawa, T. Shamoto, J. Ogawa, A. Ito, Y. Kobayashi, K. Masuya, S. Fuse, D. Kubo, and H. Ito, *Reaction Chemistry & Engineering*, **2023,** Advance Article