Green Liquid-Phase Peptide Synthesis (LPPS) Methodology for the Sustainable Production of Therapeutic Peptides

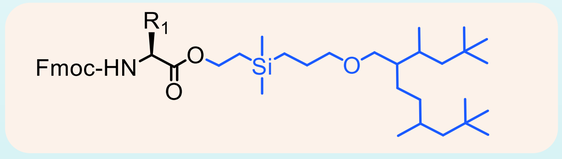
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Introduction

Solid-phase peptide synthesis (SPPS) remains the dominant approach for peptide production, but its very high process mass intensity (PMI, ~13,000) poses a significant challenge to sustainable manufacturing. In addition, the extensive use of N,N-dimethylformamide (DMF), a key solvent in SPPS, is now subject to increasingly strict regulatory restrictions in Europe. Tag-assisted liquid-phase peptide synthesis (LPPS) has therefore attracted attention as a low-PMI, DMF-free, and more sustainable alternative to SPPS. However, the linker moieties of most existing LPPS tags were originally designed for solid-phase synthesis, resulting in limitations such as acid instability and difficulties in chemoselective deprotection without side reactions. Consequently, these tags are not well suited for the preparation of fully protected peptide fragments for convergent synthesis or C-terminal modification. To address these challenges, we developed a new liquid-phase synthesis tag, FMSE, featuring a linker architecture specifically optimized for LPPS.

FMSE: Alkyl silane-based solubilizing tag



Stable under both acidic and basic conditions

- Compatible with Boc / Fmoc / Cbz chemistries
- Diketopiperazine (DKP) formation is effectively suppressed under Boc / Cbz conditions

Selectively cleavable

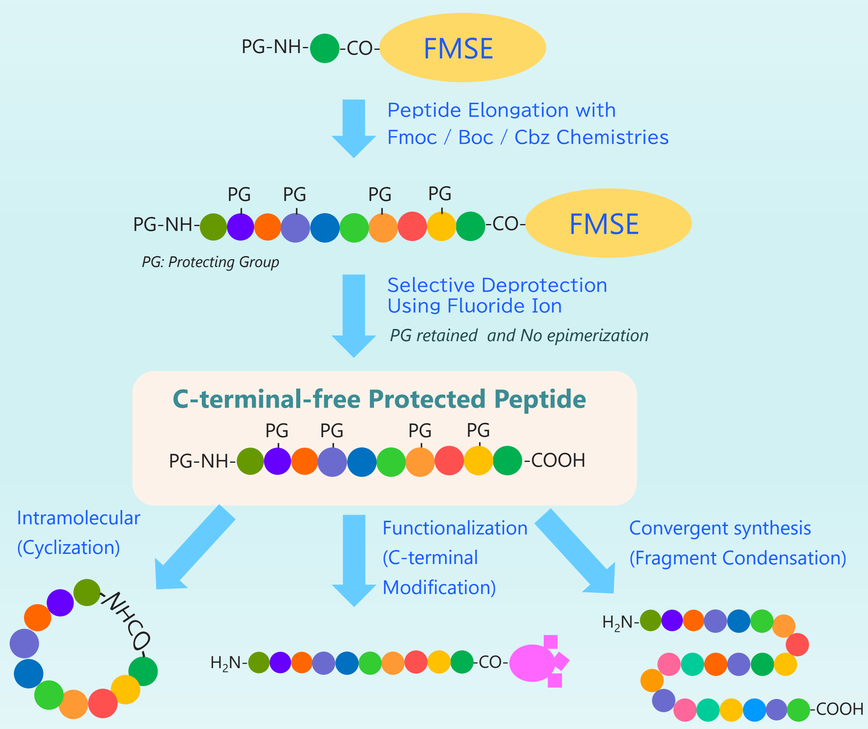
- Selective deprotection by TBAF treatment without loss of other protecting groups (except Fmoc) or C-terminal epimerization

*TBAF: tetrabutylammonium fluoride (fluoride ion source)

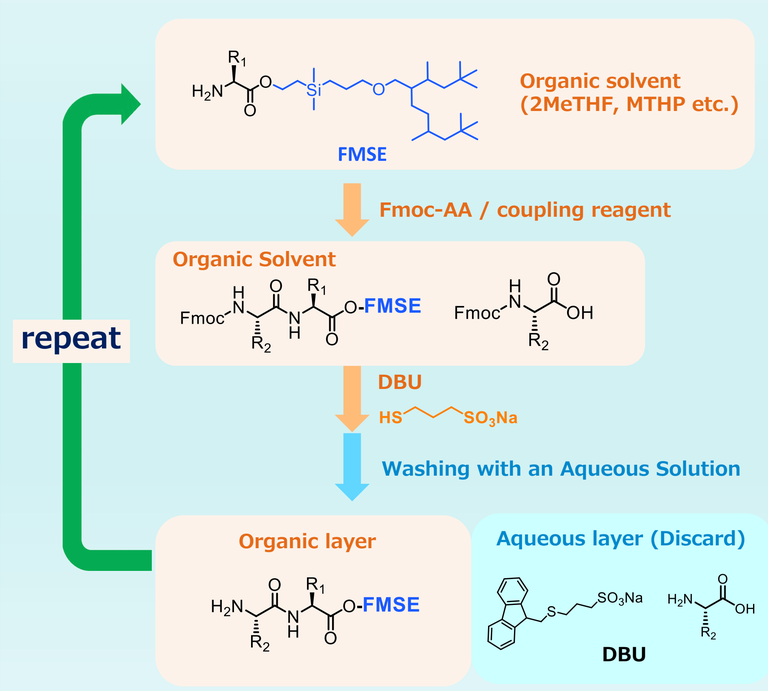
Effective solubilizing capability in green solvents

- Enables peptide elongation without regulated solvents such as DMF or NMP

General Scheme for Protected Peptide Synthesis and Utilization

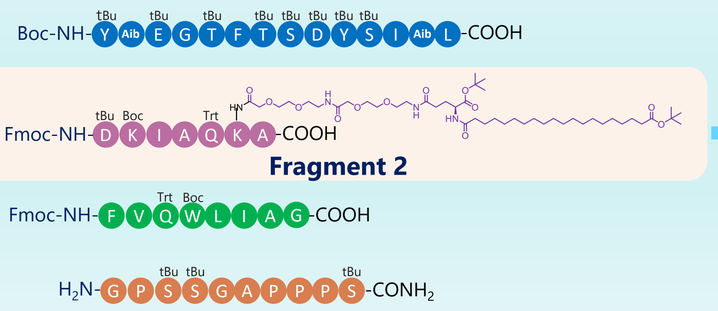


DMF/NMP-Free LPPS Synthetic Cycle Using the FMSE Tag

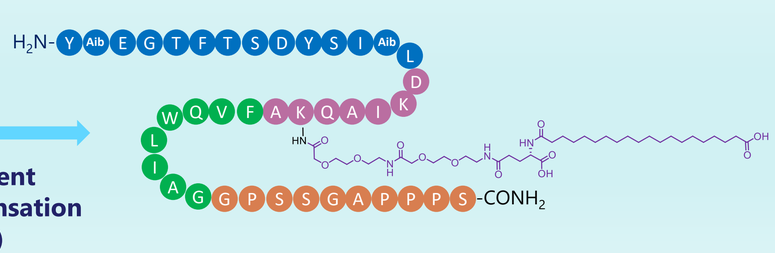


SPPS/LPPS Hybrid Strategy for Tirzepatide Manufacturing

Tirzepatide Fragments (SPPS synthesis)



Fragment condensation (LPPS)

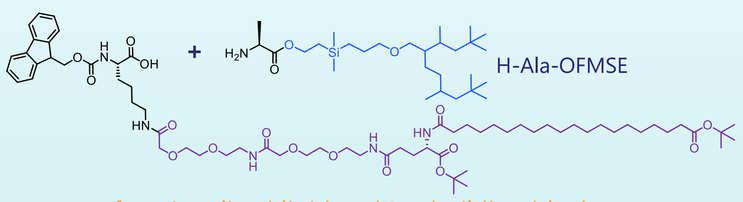


Key Challenges

- High PMI in SPPS-based fragment manufacturing
- Expensive pre-assembled lipidated Lys building blocks
- Large consumption of regulated solvents (DMF/NMP)

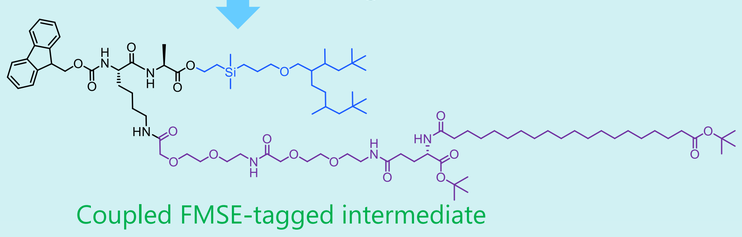
Org. Process Res. Dev. (2021) 25 (7): 1628–1636.

Issues in LPPS Coupling with Lipidated Lys Building Blocks



pre-functionalized lipidated Lys building block

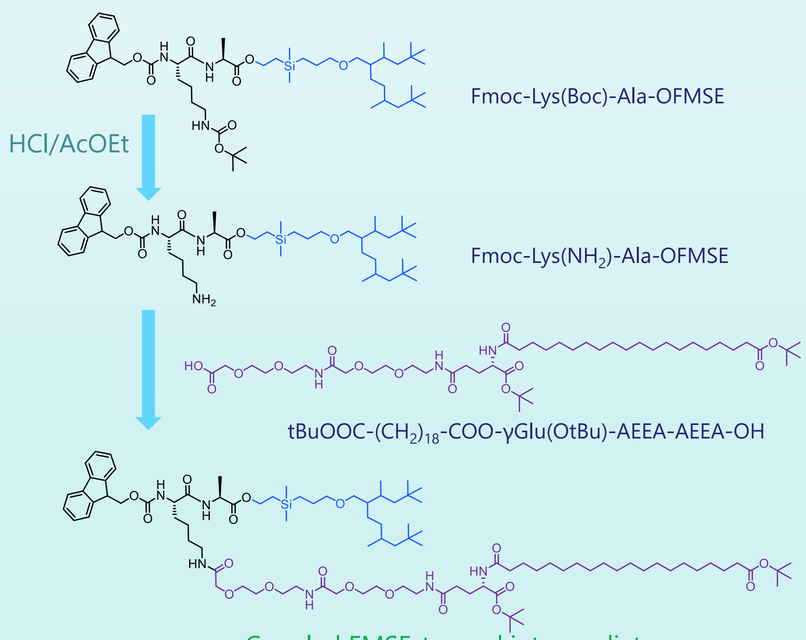
Coupling



Coupled FMSE-tagged intermediate

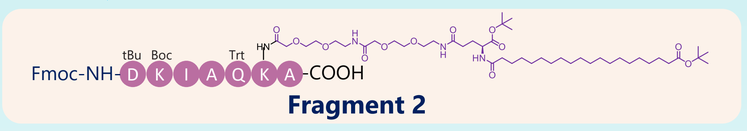
- Costly, pre-functionalized lipidated Lys building block
- Slow coupling of bulky Fmoc-Lys(Alkyl)-OH derivatives
- Poor separation of Fmoc-Lys(Alkyl)-Ala-O-FMSE from unreacted/excess Fmoc-Lys(Alkyl)-OH
- Separation remained difficult even after Fmoc deprotection to H-Lys(Alkyl)-Ala-O-FMSE and H-Lys(Alkyl)-OH

Alternative Strategy for Fragment 2 Synthesis



Coupled FMSE-tagged intermediate

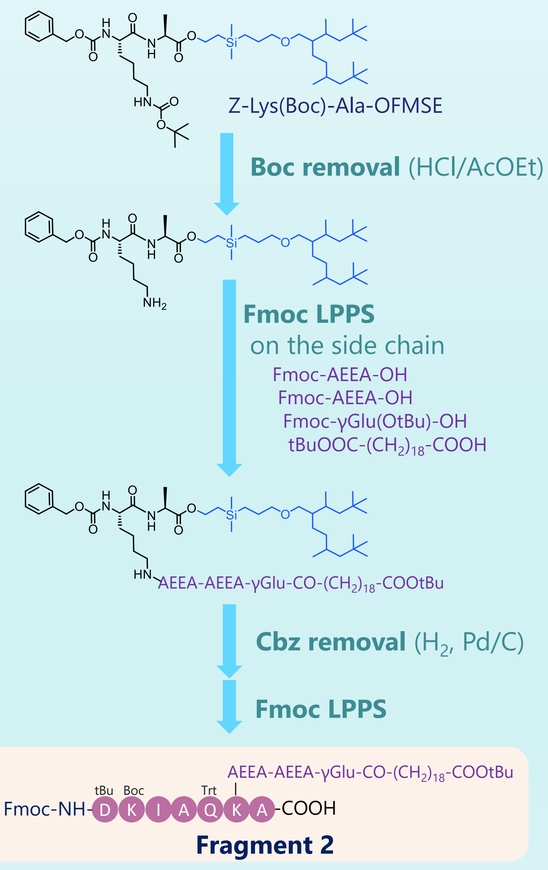
Stepwise LPPS and FMSE deprotection



FMSE-based stepwise side-chain construction

- Avoids costly pre-functionalized PEG–alkyl Lys building blocks
- Efficient coupling from less hindered Lys intermediates
- Enables clean aqueous workup with favorable phase separation
- Improves robustness in downstream peptide elongation

Bidirectional Extension for Lipidated Peptide Manufacturing



- Bidirectional elongation via Fmoc/Boc/Cbz chemistry
- Cbz removal after side-chain extension enables N-terminal LPPS
- Customized PEG–alkyl side chains without pre-assembled building blocks

Take-home Message

- FMSE enables DMF/NMP-free LPPS with low-PMI potential
- Fluoride-mediated FMSE removal provides protected peptide fragments without C-terminal epimerization
- Stepwise Lys side-chain construction expands access to lipidated peptide intermediates

SYNCSOL Company website

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