Advanced Peptide Synthesis Strategies for High-Purity, Complex modification and Scale-Up: A CRO Perspective

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Introduction

Peptide modification overcomes natural limitations like rapid degradation and poor bioavailability to enhance drug efficacy. Stability is improved via D-amino acids, cyclization, and terminal modifications, while conjugation techniques—such as PEGylation and lipidation—boost solubility, membrane permeability, and pharmacokinetic properties.

Challenges in Synthesis

Major challenges in synthesis/modification includes addressing complexity, specificity, selectivity and finding an optimized route for scaling-up

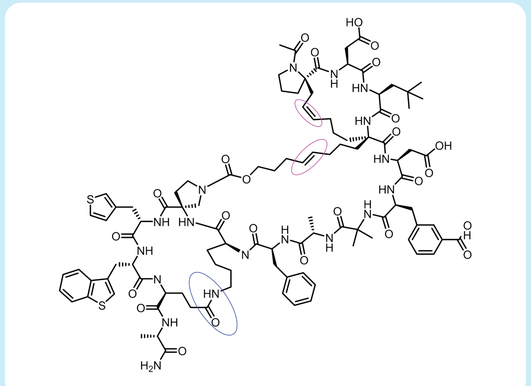
Role of the CRO in Peptide Development

We accelerate drug development pipelines by offering specialized expertise in the synthesis of complex peptide analogues, optimization of pharmacokinetic profiles, and the execution of comprehensive preclinical in vitro and in vivo studies.

- Cyclization Strategies: Cyclic peptides are crucial in drug development due to their superior stability, high binding affinity, and improved membrane permeability compared to linear peptides. We develop efficient, scalable, and orthogonal cyclization routes to synthesize complex cyclic peptides.
- Long Peptide/Small-Protein Synthesis: Chemical synthesis remains the most efficient approach—particularly when multiple unnatural amino acids must be incorporated in a defined sequence. Unlike mutagenesis-based approaches, chemical synthesis allows precise control over sequence composition and site-specific modifications. Fragment ligation techniques, such as Native Chemical Ligation (NCL) and Ketoacid-Hydroxylamine (KAHA) ligation, are used to construct complex, large peptides/small proteins with precise site-specific modification.
- Scale-Up Optimization: Developing robust, optimized methods to produce high-purity crude peptides for preclinical and clinical development.
- Peptide Conjugation: Enhancing therapeutic properties through the covalent attachment of moieties such as drugs, polymers, lipids, and fluorophores. Peptide-protein conjugation serves as a cornerstone for advanced drug delivery and targeted therapeutic development, utilizing specialized chemical conjugation (e.g., Cys-mediated) or enzymatic labelling via Sortylation.

Case Studies

Synthesis of Complex tri-cyclic peptide: WO 2022/261257



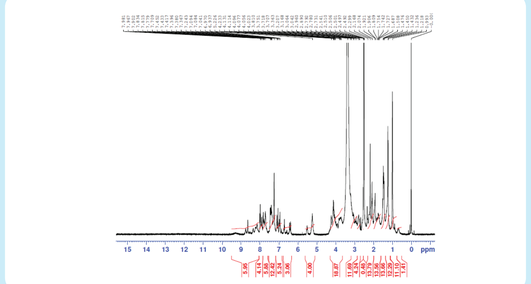
SYNTHESIS CHALLENGES

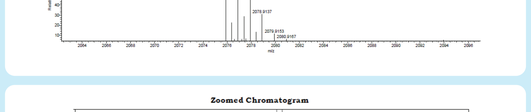
- Complex tri-cyclic peptide
- Low yield
- High quantity (>1g)
- Stringent purity requirement

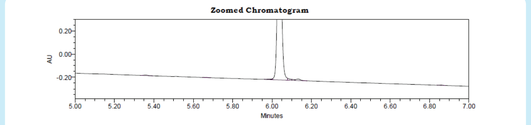
OPTIMIZATION STRATEGIES

- Optimization of C-C stapling condition
- Optimization of purification, two step purification
- First using 0.1% TFA then 10% ABC

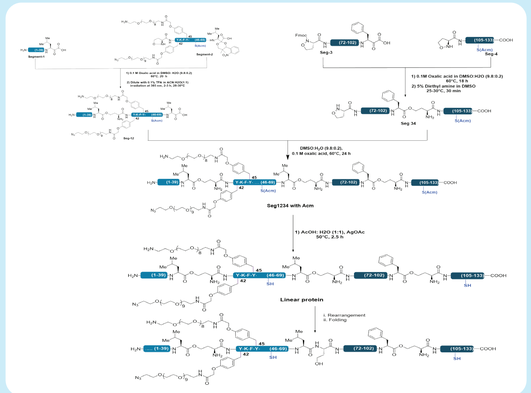
RESULTS







Chemical synthesis of small proteins with 133-151 AA



SYNTHESIS CHALLENGES

- Low yield
- High quantity (>10g)
- Aggregation
- Long peptide
- Stringent purity requirement

OPTIMIZATION STRATEGIES

- Employing KAHA ligation
- Double coupling
- Special resins
- Pseudoproline incorporation
- >10 g final peptide was synthesized

RESULTS

Seg-12

Seg-34

Seg-1234 with ACM

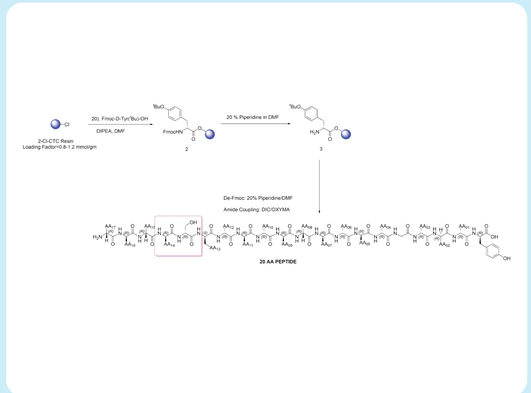
Linear Protein

Rearrangement and folding

Folding

Rearranged and folded protein

Non-GMP scale-up



SYNTHESIS CHALLENGES

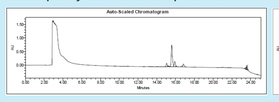
- Low yield
- High quantity (>1000g)
- Aggregation
- Low yield
- Stringent purity requirement

OPTIMIZATION STRATEGIES

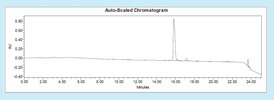
- Double coupling
- Special resins
- Pseudoproline incorporation
- Pseudo proline scan was done in three possible positions and one position was optimized.
- Crude purity improved from 50% to 84%
- Two different analytical methods were developed to separate two specific impurities (n-1 and diastereomer)

RESULTS

Crude purity-Before development-50.94%



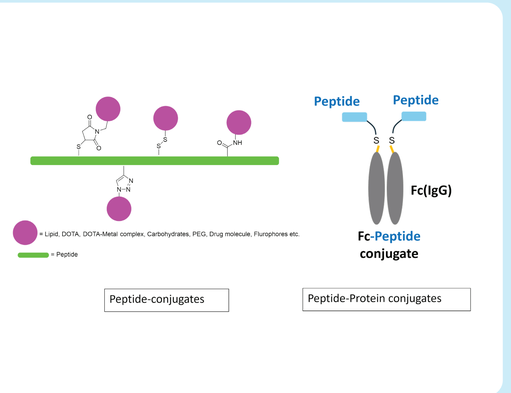
Crude purity-After development-84.56%



Final QC

(a) Chromatographic Purity by HPLC Method-1 (Area %)	>92.50	96.45
% of Diasteriomer		2.02
(b) Chromatographic Purity by HPLC Method-2 (Area %)		98.70
% of n-1 impurity		0.32
Chromatographic Final Purity by HPLC (Area %)*	>92.50	96.13 (96.45-0.32)
Acetate Salt content by HPLC	Report the result	4.16 (%w/w) (1.73 eq)
Moisture content by KF (% w/w)	<11.00	7.77
Assay by Q-NMR (% w/w)	Report the result	86.14
Assay after purity correction (% w/w)	Report the result	82.80

Peptide conjugation



SYNTHESIS CHALLENGES

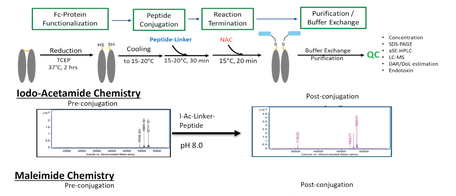
- Specificity and Selectivity:
- Structural Stability
- Reaching target DAR/DoL for peptide-protein conjugation
- Purification and characterization

OPTIMIZATION STRATEGIES

- Multiple approaches are adopted for peptide-conjugation including amide, click chemistry, di-sulfide, thiol-maleimide chemistry etc.
- For peptide-protein conjugation, optimization through cysteine directed thioether, thio-maleimide chemistry and through Site-Specific Enzymatic Conjugation by Sortylation.

RESULTS

Fc(IgG)-Peptide conjugation (Cysteine directed)



Site-Specific Enzymatic Conjugation by Sortylation of a Recombinant Protein with Peptides