Efficient Syntheses of N-Methylated Peptides

Prepared by Vapourtec Ltd

Coupling of N-methylated residues remains a major bottleneck in solid-phase peptide synthesis (SPPS). Fast-Flow SPPS (FF-SPPS) using Oxyma/DIC activation enabled efficient incorporation of challenging residues. Under optimised conditions deletion impurities were reduced from approximately 75 % to less than 3 %.

1. Background

N-Methylated peptides offer significant therapeutic advantages, including improved metabolic stability, membrane permeability and pharmacokinetic properties.

However, the steric hindrance of N-methylated amino acids makes them among the most difficult residues to incorporate efficiently during solid-phase peptide synthesis (SPPS), often resulting in incomplete coupling and deletion impurities.

This study demonstrates how optimised Fast-Flow SPPS overcomes these challenges, enabling the efficient synthesis of peptides containing N-methylated residues with high crude purity and minimal side products.

2. Experimental Set up

Peptide syntheses were performed using the Vapourtec Peptide-Builder under Fast-Flow SPPS conditions. A schematic of the experimental platform is shown in Figure 1.

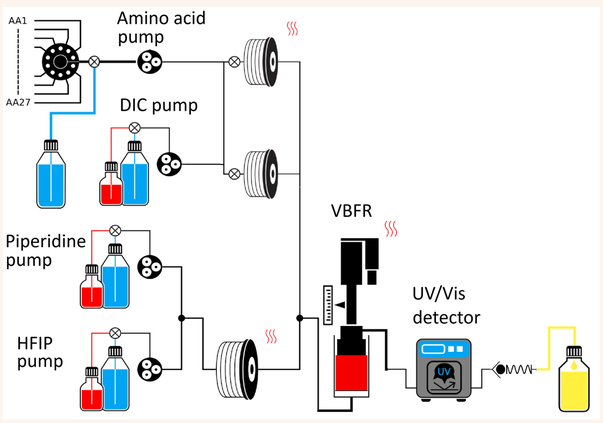

Figure 1: Schematic of Peptide-Builder

- Dedicated pumps for amino acid, DIC and piperidine delivery.
- Heated activation reactors coupled to a Variable Bed Flow Reactor (VBFR™).
- Accurate temperature and back-pressure control throughout synthesis.

Protocols

Standard Coupling Protocol
- Temperature: 80 °C
- Activation time: 1 minutes
- Contact Time: 1.2 minutes

Optimised Coupling Protocol
- Temperature: 90 °C
- Activation time: 4 minutes
- Contact Time: 4.8 minutes

Deprotection Protocol
- Temperature 80 °C
- Contact time: 30 seconds
- 60 eq. of Piperidine

3. Results and Discussion

Model Study

A model hexapeptide was designed to evaluate coupling efficiency of N-methylated residues.

H_2N-(Z^2)-(Z^1)-F-F-F-F-$CONH_2$

Symbol	Residue
Z^1	N-Me-Gly, Phe or N-Me-Phe
Z^2	Leu, Phe or N-Me-Phe

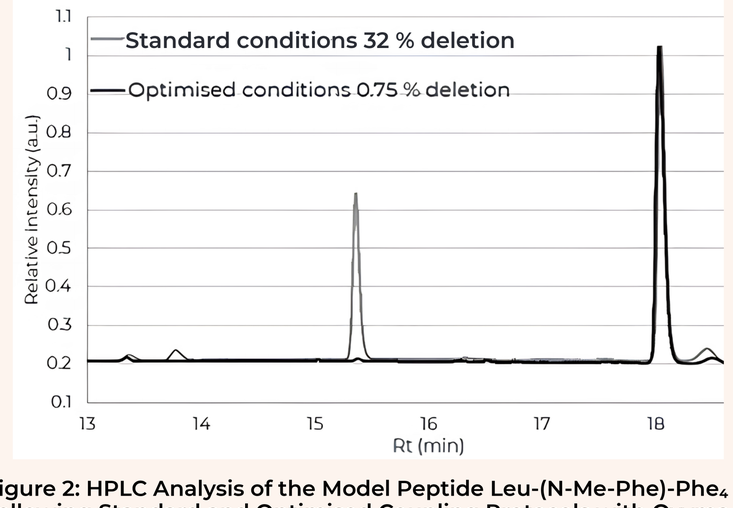

Figure 2: HPLC Analysis of the Model Peptide Leu-(N-Me-Phe)-Phe_4 Following Standard and Optimised Coupling Protocols with Oxyma

N-Methylated Residues Increase Coupling Difficulty

Table 1: Deletion Impurities Following Coupling of N-Methylated Amino Acids Using Standard SPPS Conditions

6-mer Peptide	Coupling Additive	Deletion (%)
LFFFFF	Oxyma/HOBt	<2 %
L(N-Me-F)FFFF	HOBt	75 %
L(N-Me-F)FFFF	Oxyma	32 %
L(N-Me-G)FFFF	Oxyma	<2 %
F(N-Me-F)FFFF	Oxyma	19 %
(N-Me-F)(N-Me-F)FFFF	Oxyma	60 %

- HOBt resulted in substantially poorer performance than Oxyma.
- Incorporation of N-Me-F significantly reduced coupling efficiency.
- N-Me-G remained readily accessible despite being a secondary amine.
- Consecutive N-Me-F residues represented the most demanding coupling.

Optimised Flow Conditions Restore Coupling Efficiency

Table 2: Deletion Impurities Following Coupling of N-Methylated Amino Acids Using Optimised Fast-Flow SPPS Conditions

6-mer Peptide	Coupling Additive	Deletion (%)
L(N-Me-F)FFFF	Oxyma	<2 %
F(N-Me-F)FFFF	Oxyma	<2 %
(N-Me-F)(N-Me-F)FFFF	Oxyma	<3 %

- Optimisation dramatically reduced deletion formation across all challenging couplings.
- The largest improvement was observed for the double N-Me-F sequence.
- Near-complete coupling was achieved without requiring extensive recoupling strategies.

Optimised Fast-Flow SPPS Restores Efficient N-Methylated Amino Acid Coupling Deletion impurities reduced from 75 % to <3 %

Case Study

Case Study 1: GLP-1 Fragment 16-mer

H_2N-L-E-G-Q-A-A-K-E-(N-Me-F)-I-A-W-L-V-K-G-$CONH_2$

Table 3 – N-methylated 16-mer case study information

Peptide	Crude Purity (%)
16-mer (natural AA)	90.5 %
16-mer with N-Me-F	89.5 %

- Substitution with N-Me-F produced essentially identical crude purity.
- No significant penalty was observed from introducing the N-methylated residue.

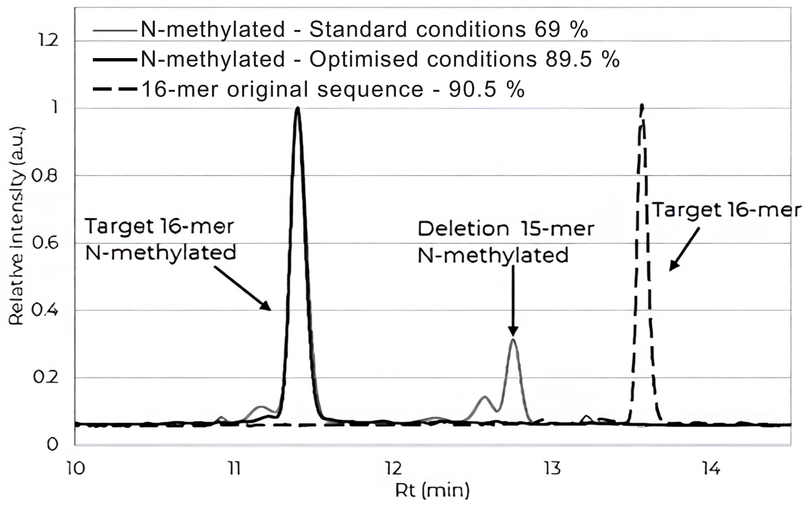

Figure 3: HPLC Comparison of Crude 16-mer GLP-1 Fragment Prepared Using Natural Amino Acids, Standard N-Methyl Coupling Conditions and Optimised Fast-Flow SPPS Conditions

Case Study 2: Multi-N-Methylated 10-mer

H_2N-A-P-(N-Me-G)-S-T-(N-Me-G)-Y-(N-Me-G)-(N-Me-G)-G-$CONH_2$

Table 4 – N-methylated 10-mer case study information

Peptide	Crude Purity (%)
10-mer with N-Me-G	86.7 %

- Multiple N-methylated residues were incorporated successfully.
- Consecutive N-methylated amino acids did not prevent efficient synthesis.
- High crude purity was maintained despite extensive backbone modification

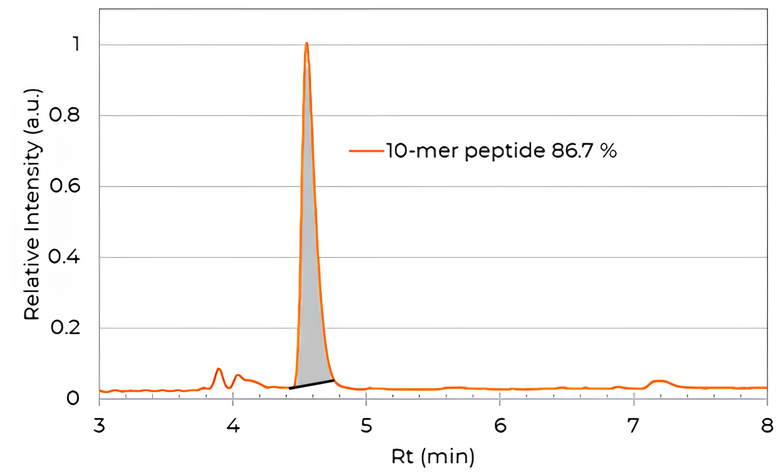

Figure 4 - Crude HPLC trace of N-methylated 10-mer with four N-methyl glycines (two sequential)

4. Conclusion

- Fast-Flow SPPS with optimised Oxyma/DIC activation reduced deletion impurities from as high as 75 % to below 3 %.
- The method enabled efficient synthesis of peptides containing single, consecutive and multiple N-methylated residues without recoupling.
- 89.5 % crude purity achieved for an N-methylated GLP-1 fragment, comparable with the 90.5 % achieved for the corresponding non-methylated sequence.
- A challenging 10-mer containing four N-methylated residues was synthesised in approximately four hours with 86.7 % crude purity.

References

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