A GREEN APPROACH FOR THE SYNTHESIS OF DIFELIKEFALIN ACETATE

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INTRODUCTION

The production of Difelikefalin Acetate (Fig.1) by adopting green principles is presented here. Difelikefalin is a five amino acid synthetic peptide made up of D-isomers and the unnatural amino acid 4-Aminopiperidine-4-Aminocarboxylic acid. It was approved in 2021 by the US FDA to treat moderate to severe itching associated with renal disease in patients on dialysis.

Conventional SPPS methodology for Difelikefalin Acetate assembles the amino acids sequentially on a solid support, followed by a global deprotection using TFA, purification, and lyophilization. In our approach, we replaced TFA with HCl to effect the global deprotection of the resin bound peptide in Ethyl acetate resulting in the crude peptide as a chloride salt with a purity of > 90%. Our approach, reduces the need for larger volumes of anti-solvents during isolation of the crude peptide. These changes render the Neuland process (Fig.2) cost effective (Fig.3) when scaled up even on 0.25 Kg scale.

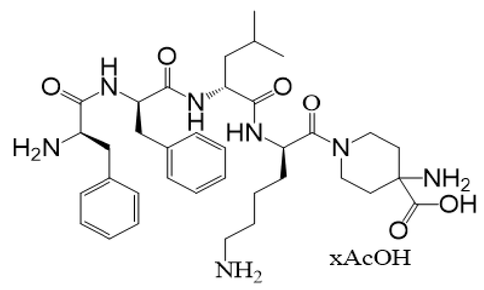

Figure 1: Structure of Difelikefalin Acetate

SPPS: Difelikefalin Acetate

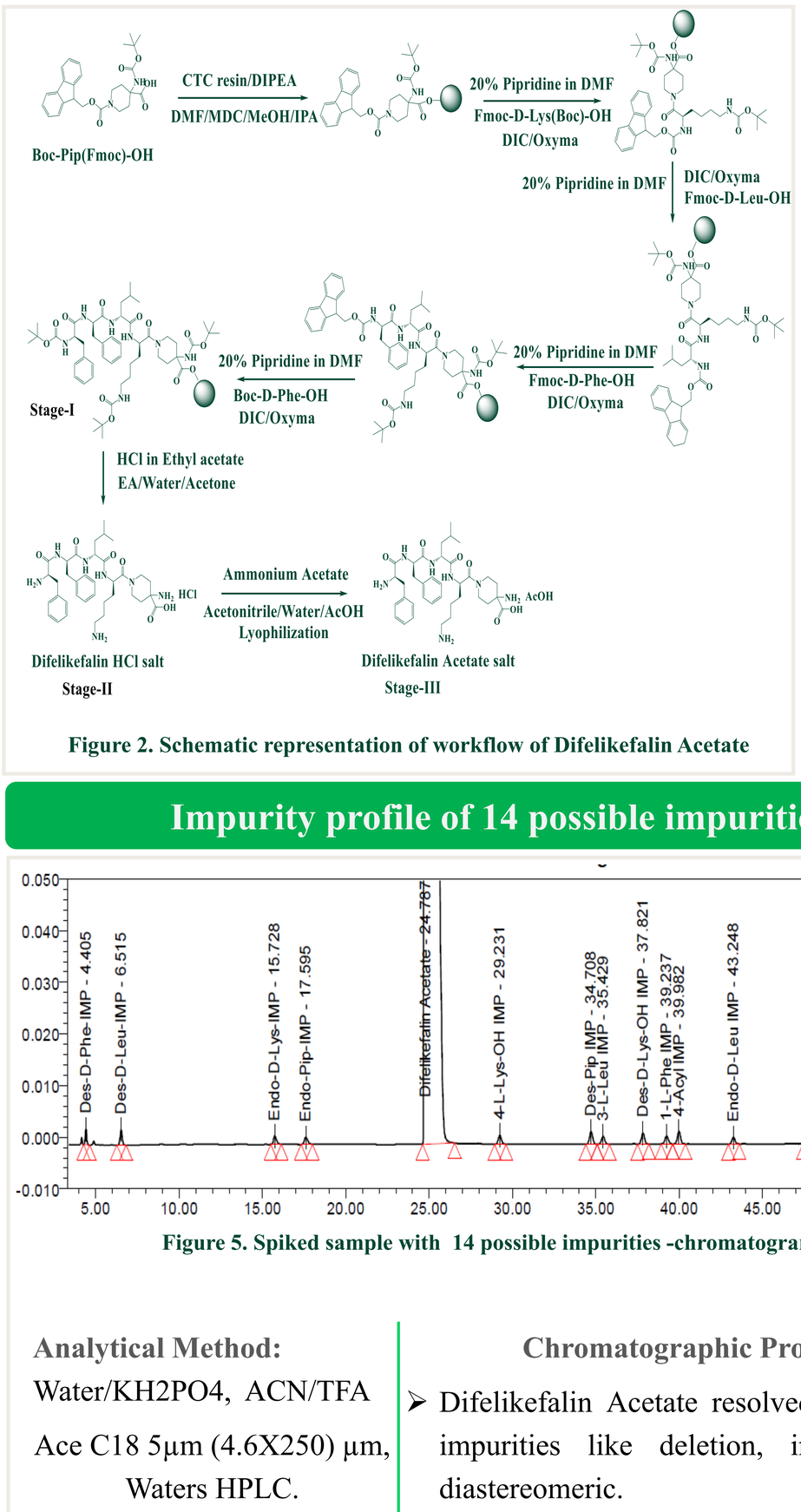

Figure 2. Schematic representation of workflow of Difelikefalin Acetate

Conventional Vs Neuland process

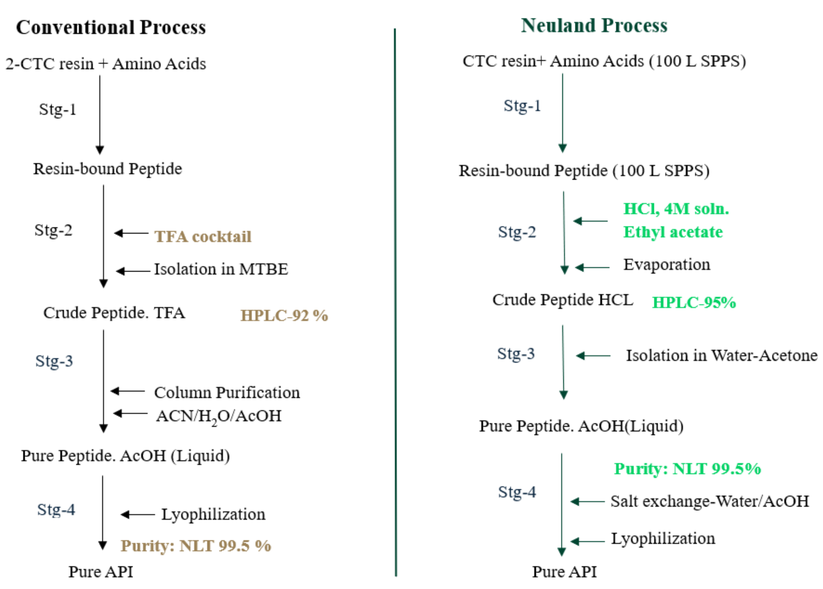

Figure 3. Process comparison

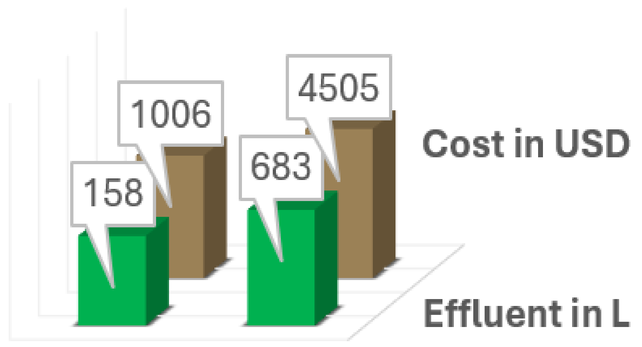

Figure 4. Effluent comparison NLL vs Conventional

Advantages:

- ✓ Less effluent generation
- ✓ Significant reduction of cycle time
- ✓ Reduces operational expenses (OE)
- ✓ Purity increases from 92% to 95%

Process comparison: Figures 3 and 4 illustrate the reduction in solvent usage and overall cost for synthesizing 1 kg of Difelikefalin using a greener methodology. This optimized process also enhances the crude peptide purity, increasing it from 92% to 95%.

Impurity profile of 14 possible impurities

Figure 5. Spiked sample with 14 possible impurities -chromatogram

Analytical Method:

Water/KH2PO4, ACN/TFA

Ace C18 5µm (4.6X250) µm, Waters HPLC.

Chromatographic Profile:

- ➢ Difelikefalin Acetate resolved from all 14 impurities like deletion, insertion, and diastereomeric.

Result:

API purity: 99.8%. All specified and unspecified impurities at levels below 0.05%.

Alternative greener approach for short peptide

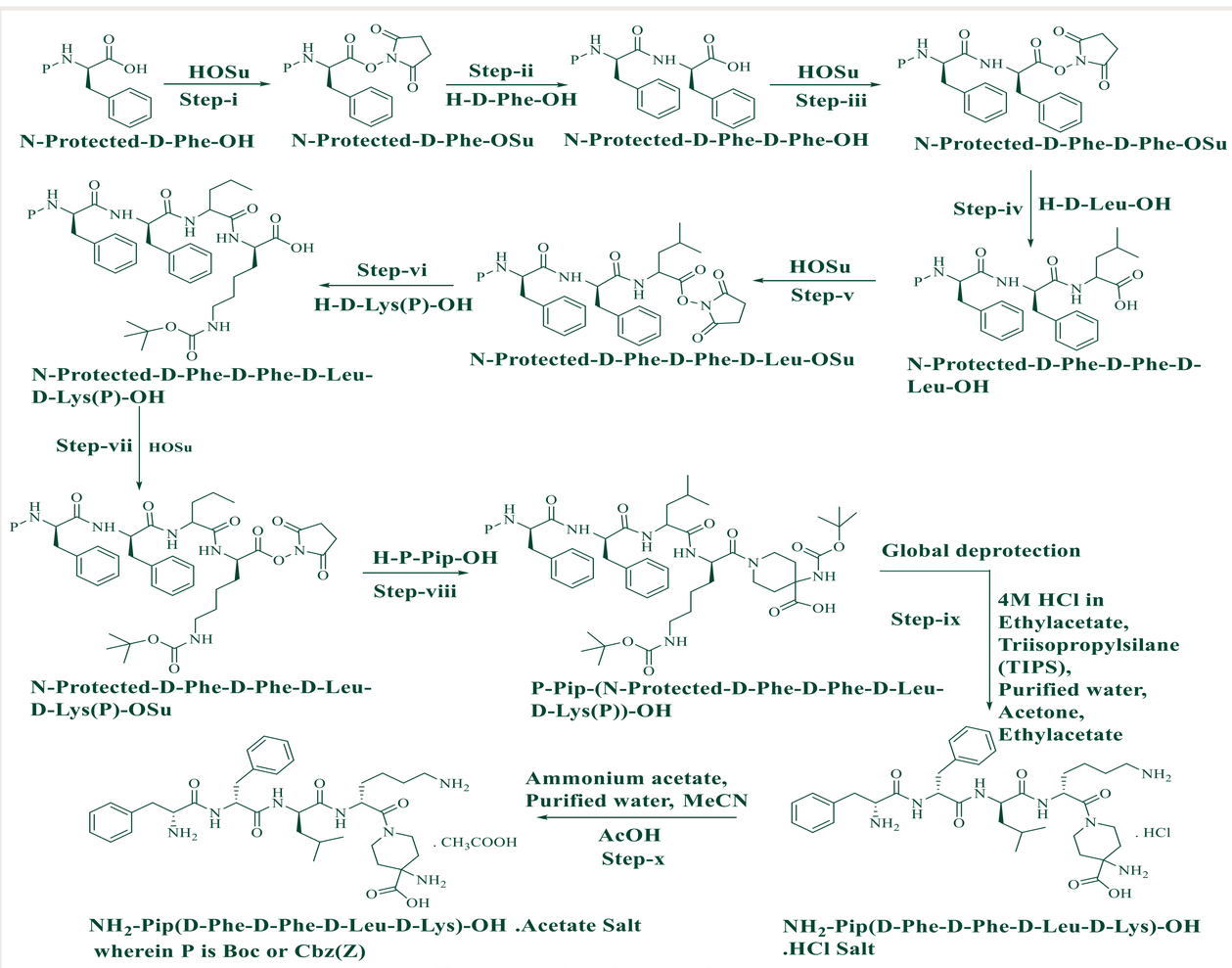

Figure 6. Synthesis scheme

RESULTS AND DISCUSSION

The present process introduces **hydrochloric acid (HCl) in ethyl acetate** as a reagent for **global deprotection** of peptides, replacing the traditionally used **trifluoroacetic acid (TFA)**.This has resulted into increase in the purity of crude peptide and which in turn has resulted to reduce the purification time of preparative HPLC, when compared to the prior-art process. The present invention is economically significant and eco-friendly.

A cost savings of $3.50 and 500 mL of solvent was achieved for every gram of product produced.

ACKNOWLEDGEMENT

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REFERENCES

1. Improved process for the preparation of Difelikefalin Acetate, IN 202241053596 (LPPS)
2. Improved process for the preparation of Difelikefalin Acetate, IN 202341026860 (HCl salt)
3. Improved process for the preparation of Difelikefalin Acetate, IN 202441028148 (LPPS)