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Development of a novel alternative method for liraglutide production

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Introduction

Liraglutide (Fig.1) is applied in type-2 diabetes and obesity. Although, solid-phase peptide synthesis (SPPS) strategies have been designed, most of them are expensive and many of them are difficult to scale-up. Furthermore, this long peptide is prone to aggregate during its elongation, thereby hampering the yield and purity of the product. Another disadvantage is the high cost of its purification by reverse phase high pressure liquid chromatography (RP-HPLC). In this work an alternative method for liraglutide production was developed to overcome the high cost and difficulties of current processes.

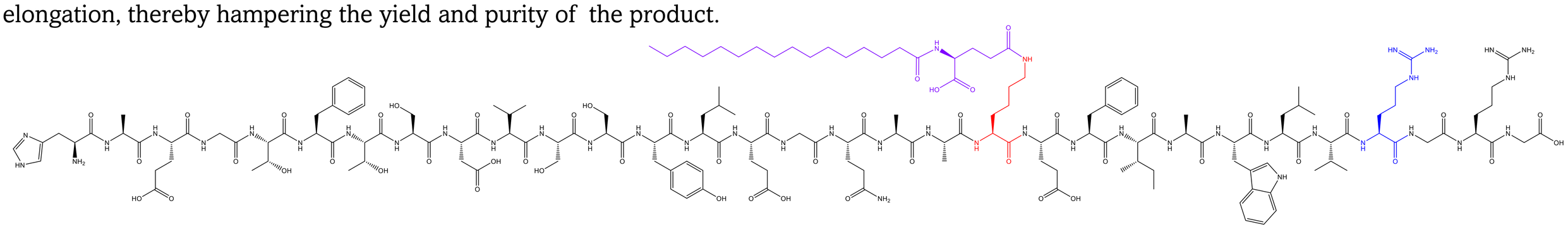

Fig. 1. Liraglutide sequence: His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys(γ-Glu-palmitoyl)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly

Materials and Methods

The peptide was elongated by Fmoc/tBu solid-phase peptide synthesis on 4-(Hydroxymethyl)benzoyl-ChemMatrix resin (HMBA-CM). To prevent peptide aggregation the chaotropic agent LiCl was used as an additive in the coupling and deprotection mixtures. Side-chain protecting groups were removed with TFA, leaving the unprotected peptide attached to the solid support. The resin was thoroughly washed to eliminate the contaminants. Next, peptide was released with NaOH. Finally, liraglutide was purified by hydrophobic interaction low pressure liquid chromatography (HI-LPLC) using a TOYOPEARL Phenyl-650M column (Fig. 2).

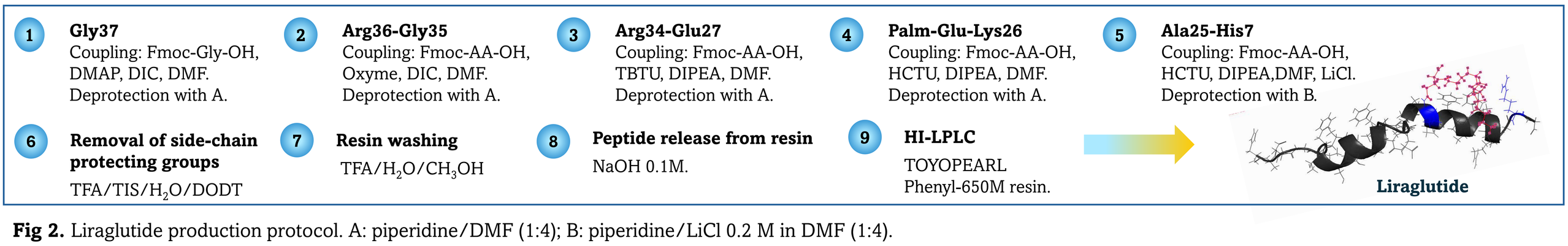

Fig 2. Liraglutide production protocol. A: piperidine/DMF (1:4); B: piperidine/LiCl 0.2 M in DMF (1:4).

Results and discussion

CM resin was suitable for the synthesis of this long peptide. LiCl was useful to prevent peptide aggregation. After side-chain protecting group removal, the resin was thoroughly washed and then the peptide was released from the resin. This two-stage procedure assures a high purity product. Afterwards, it was purified by HI-LPLC (Fig. 3) using an economic system with a peristaltic pump. The main peak was analyzed by RP-HPLC and showed a purity of 85% (Fig. 4). Its identity was analyzed by ESI-MS (Fig. 5). The main signals were $[M+4H]^{4+}$ (m/z=938.7) and $[M+3H]^{3+}$ (m/z=1251.3), which corresponded to the multiply protonated ions of liraglutide.

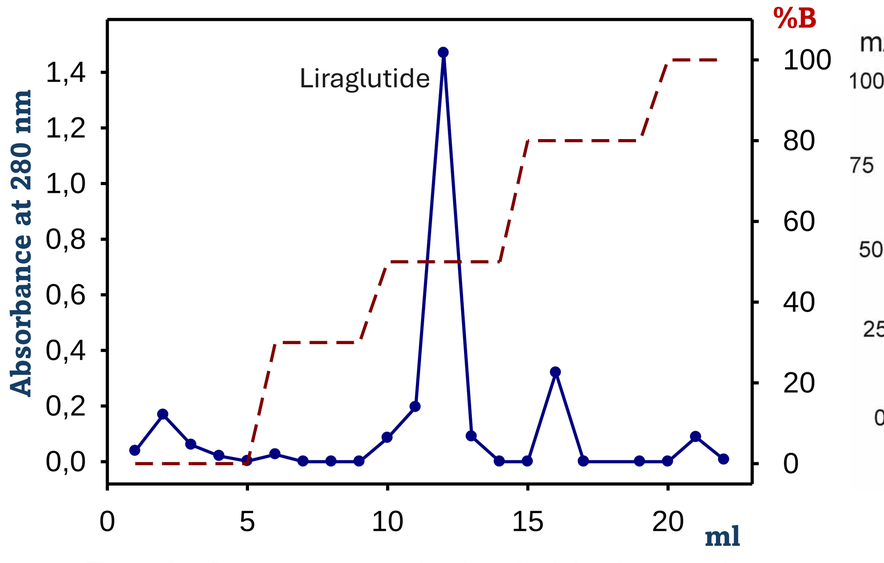

Fig. 3. Low-pressure hydrophobic interaction liquid chromatography. A: H_2O; B: Acetonitrile.

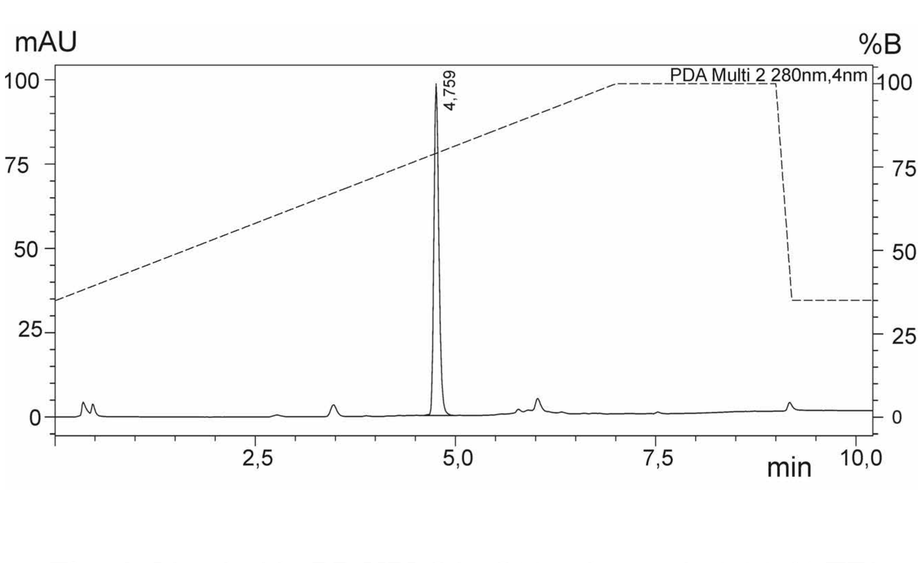

Fig. 4. Liraglutide RP-HPLC in C18 column. A: 0.045% TFA in H_2O. B: 0.036% TFA in acetonitrile.

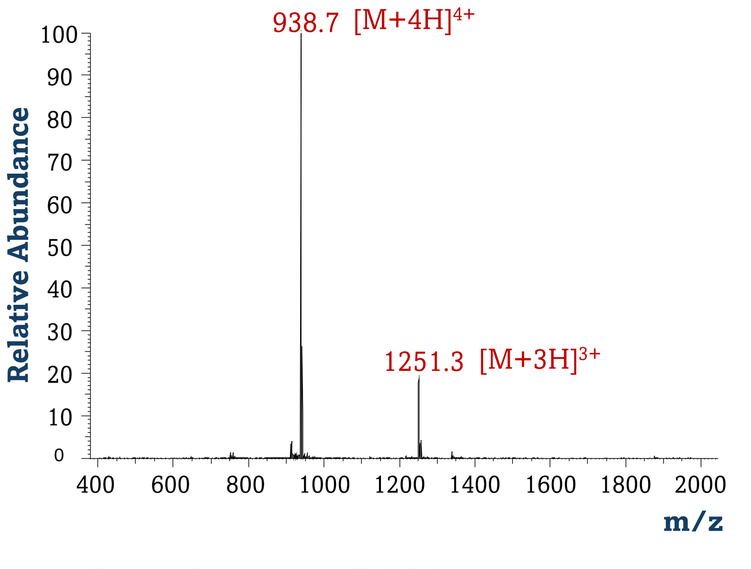

Fig. 5. Liraglutide ESI-MS.

Conclusions

This simple and economic method resulted in a product with high yield and purity as demonstrated by analytical RP-HPLC and ESI-MS while overcoming the high cost and difficulties of current processes. Although the SPPS has been performed manually with a domestic microwave, the method can be easily scaled-up by using an automated microwave peptide synthesizer. The present protocol can also be applied for the synthesis of similar incretin analogues.

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