Induction heating solid-phase strategy to improve the total chemical synthesis of ester insulin

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Introduction

From insulin, the first therapeutic peptide, to today's increasingly complex peptide therapeutics, advances in peptide chemistry have enabled the development of modified analogues with improved properties and clinical performance. This evolution, exemplified by insulin analogues and GLP-1 receptor agonists, highlights the growing need for efficient synthetic strategies capable of accessing complex peptide architectures.

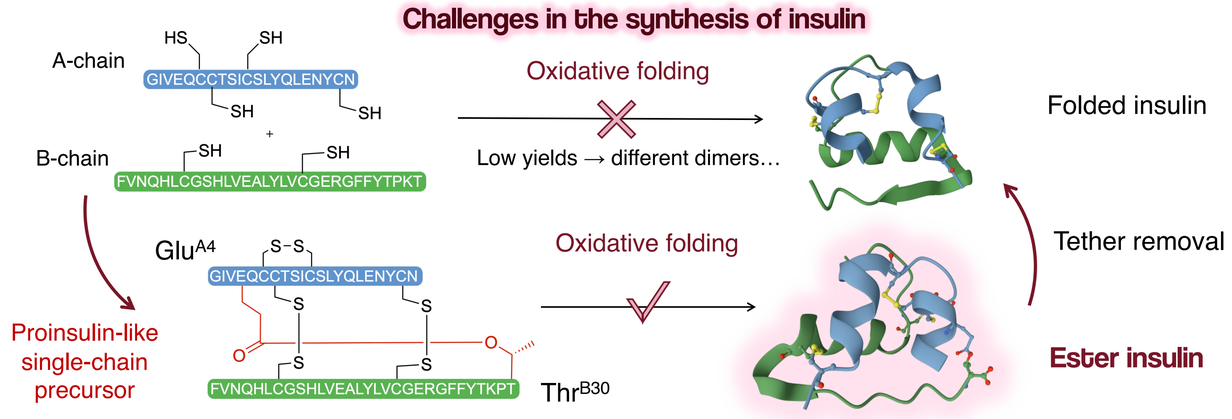
Recombinant production vs. Chemical synthesis

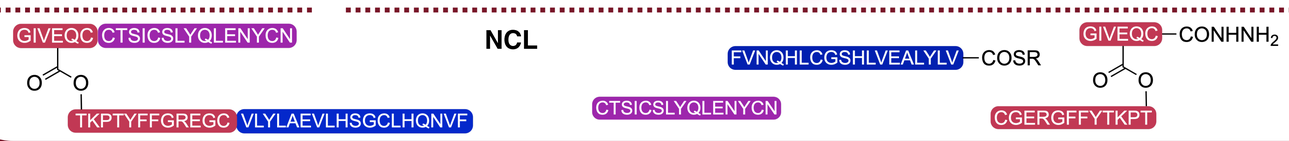
- Large scale production
- High purity
- Cost-effective
- Limitation to the twenty encoded amino acids

- Introduction of non-natural amino acids (e.g. Aib, D-amino acids, …)
- Introduction of chemical modifications (e.g. PEGylation, palmitoylation, …)

→ **Synthesis of new complex peptide therapeutics to improve**

Challenges in the synthesis of insulin





Induction – Assisted SPPS of peptide fragments



- Rapid and controlled temperature increase
- Significant reduction in synhesis time
- Enhanced reaction rates

Ester insulin as a demanding case study

- Accelerate and further optimize peptide fragment synthesis through **induction-assisted high-temperature solid-phase peptide synthesis**, using the PurePep® Chorus instrument.
- Refine the synthetic procedure and expand the understanding of the synthetic process, ultimately establishing more efficient and reproducible conditions.

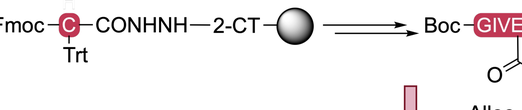
The increasing complexity of therapeutic peptides calls for more efficient and reliable synthetic strategies. Using ester insulin as a challenging case study, this work explores induction-assisted HT-SPPS to accelerate and optimize peptide synthesis, with the broader aim of developing approaches applicable to complex peptide therapeutics such as GLP-1 receptor agonists.

Beyond improving synthetic efficiency, this study also aims to understand an unwanted O-to-N acyl shift observed during synthesis, highlighting the importance of using a good model to optimize a synthetic process.

Induction - Assisted SPPS of peptide fragments

CTSICSLYQLENYCN

Induction Heating SPPS

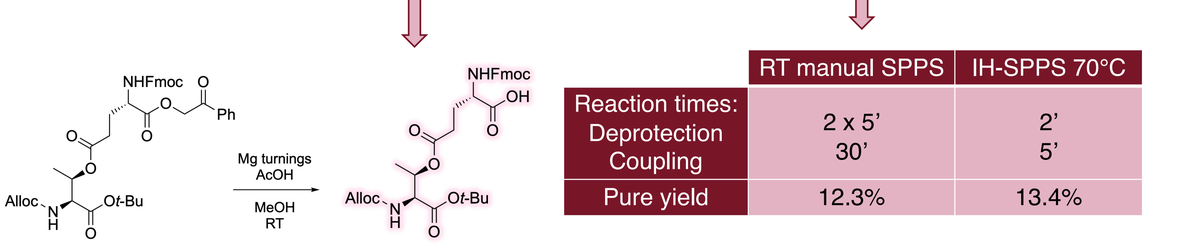


	RT manual SPPS	IH-SPPS 90°C
Reaction times: Deprotection / Coupling	2 x 5' / 30'	1' / 2'
Pure yield	18,7%	15.4%

>90% reduction in synthesis time!

FVNQHLCGSHLVEALYLV—COSR

$NaNO_2$ oxidation to MESNa thioester



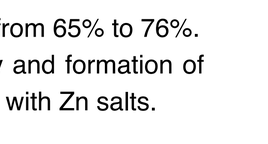
Induction Heating SPPS

	RT manual SPPS	IH-SPPS 70°C
Reaction times: Deprotection / Coupling	2 x 5' / 30'	2' / 5'
Pure yield	12.3%	13.4%

78% reduction in synthesis time!

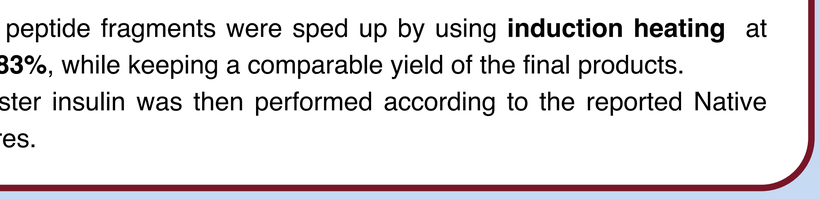
FVNQHLCGSHLVEALYLV

Fmoc-Leu-OH
Fmoc-Val-OH
Double couplings



Gly-Ser pseudoproline

GIVEQC CONHNH2
CGERGFFYTKPT





	RT manual SPPS	IH-SPPS 70°C
Reaction times: Deprotection / Coupling	2 x 5' / 30'	2' / 5'
Pure yield	12.3%	13.4%

80% reduction in synthesis time!

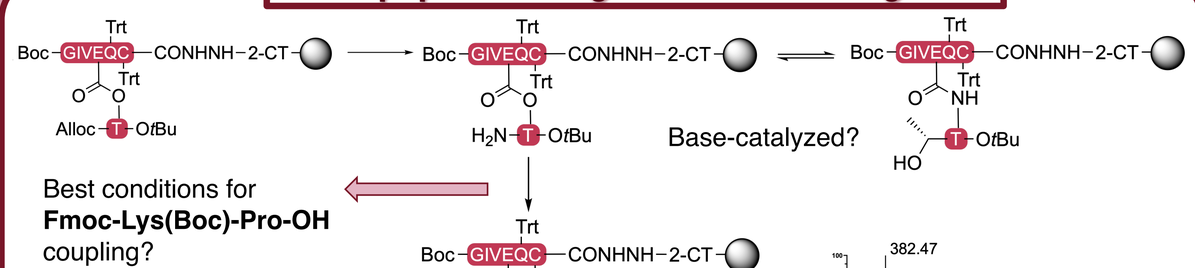
Zn → Mg for OPac ester removal

- From 78% to quantitative yield, bringing the total 5 step synthesis yield from 65% to 76%.
- Improved performance of Mg attributed to its higher reducing reactivity and formation of soluble Mg salts, avoiding stirring issues and ester cleavage associated with Zn salts.

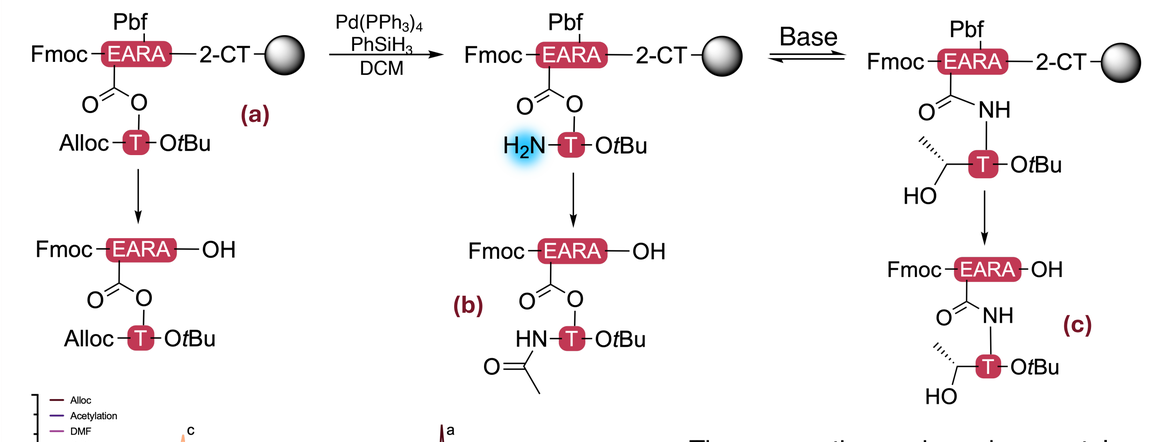
Conclusions

- The synthesis of the three peptide fragments were sped up by using **induction heating** at 70-90°C overall by around **83%**, while keeping a comparable yield of the final products.
- The synthesis of the full ester insulin was then performed according to the reported Native Chemical Ligation procedures.

Model peptide study of the O-to-N acyl shift



Model peptide



- The reaction is base-catalyzed (NMM > DIPEA), but also occurs in the absence of one.
- In the absence of a proper base, the catalyst of the shift is the α-amino group of neighboring O-linked Thr residues within the same solvent-swollen resin bead.
- When swelled, the collision frequency between peptide chains on the resin is comparable to that observed in solution.

Conclusions

Best conditions for **Fmoc-Lys(Boc)-Pro-OH** coupling:

- DIC and OxymaPure instead of HATU/HOBt and DIPEA
- Green binary mixture of EtOAc/DMSO 4:1 as solvent instead of DMF (traces of amine impurities)
- No wait time or drying after Alloc deprotection

References

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