Scalable PEC Purification Enables API-Grade Semaglutide with Minimal HPLC Intervention

PurePep®

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Introduction

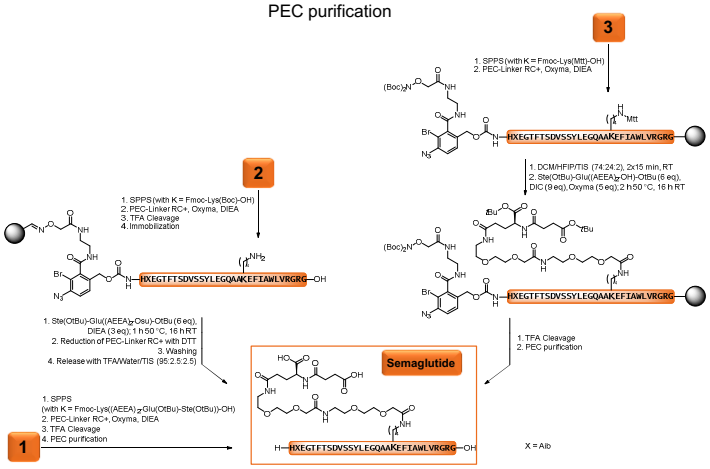
Rising demand for GLP-1 receptor agonists such as Semaglutide requires scalable workflows that connect method development, synthesis scale-up and efficient downstream purification. Here, three SPPS strategies were developed on PurePep® Chorus, upscaling the synthesis from 100 µmol to 1 mmol while reducing amino-acid excess. These strategies were evaluated as a basis for further scale-up to 8 mmol on PurePep® Sonata+. As processing crude material is difficult and chromatographic purification requires multiple runs, the catch-and-release based PurePep® EasyClean (PEC™)[1] technology was integrated as an orthogonal purification step to reduce the impurity burden between SPPS and a final preparative chromatography. The overall objective was not to eliminate HPLC, but to simplify final polishing to a single RP-HPLC run while maintaining high recovery and achieving >99% UV purity.

Three Routes – one Goal

Route 1
Pre-assembled building block
SPPS using Fmoc-L-Lys[(AEEA)$_2$-Glu(OtBu)-Ste(OtBu)]-OH followed by PEC purification

Route 2
Lys modification on PEC resin
SPPS using Fmoc-L-Lys(Boc)-OH followed by PEC purification and side-chain modification with the lipid moiety on PMA beads

Route 3
Lys modification on SPPS resin
SPPS using Fmoc-L-Lys(Mtt)-OH followed by side-chain modification with the lipid moiety on SPPS resin and PEC purification



Scheme 1. SPPS and PEC purification of Semaglutide, following the 3 different Routes

SPPS development and upscaling

The three Routes were first screened on PurePep Chorus in 100 µmol scale using 5 eq of amino acid per coupling at room temperature. Preloaded Fmoc-Gly-Trt-PS resin was used for routes 1 and 2 and Fmoc-Gly-Wang-PS resin for Route 3. Each SPPS cycle comprises of:

- Fmoc deprotection with DMF/piperidine (80:20), 2 × 5 min at RT;
- Single coupling with 5 eq AA, 5 eq Oxyma, and 7.5 eq DIC, 1 h at RT;
- Capping with DMF/acetic anhydride/pyridine (65:19:16), 1 × 5 min at RT.

After Fmoc deprotection and after capping, the SPPS resin was washed 4 and 3 times with EtOAc/DMSO (8:2) respectively.

Difficult coupling steps identified during this exploratory run were subsequently optimized using induction heating at 50 °C. More precisely, induction heating was used for the coupling of the following residues (from C- to N-Terminus): **8-16**, **20-31**.

The optimized methods were then upscaled to 1 mmol, while reducing the amino-acid, Oxyma and DIC excess to 3, 3 and 4 eq per coupling respectively to improve material efficiency. Crude UV-purity and SPPS recovery were used to assess whether and how synthesis performance would be affected by this adaptation.

Table 1. UV-purities and SPPS recoveries of the optimized and upscaled SPPS' of Semaglutide

	Optimized SPPS (5 eq AA)		1 mmol upscaled SPPS (3 eq AA)	
Route	Crude UV-purity (@210 nm)	SPPS recovery*	Crude UV-purity (@210 nm)	SPPS recovery*
1	67%	43%	49%	35%
2	63%	40%	47%	36%
3	61%	44%	48% ‖ 52%§	40%

* SPPS recovery calculated by dividing the obtained crude amount by the SPPS scale
§ Crude purity determined after modification of the lysine side-chain with the lipid moiety

PurePep EasyClean (PEC™) in 1 mmol scale

Semaglutide or its precursor was N-terminally modified with PEC-Linker RC+ and cleaved from SPPS resin using TFA/thioanisole/water/TIS (85:10:2.5:2.5) for 2 h at RT. With PEC2.0, the peptide was directly immobilized on aldehyde-functionalized PMA solid support from the cleavage mixture after addition of 35 vol% MeCN/pyridine (7:3). Non-immobilized impurities (i.e. capped truncations from SPPS) were removed by washing the PMA beads with water and DMSO. Subsequent DTT-mediated reduction and cleavage of the PEC-Linker RC+ with TFA/water/TIS (95:2.5:2.5) released the N-terminal free Semaglutide.

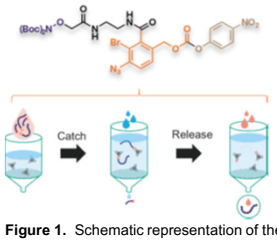


Figure 1. Schematic representation of the PEC purification with PEC-Linker RC+

Lysine Side-chain Modification

Routes 2 and 3 require orthogonal functionalization of the lysine side-chain on the PMA beads and SPPS resin, respectively (as shown in Scheme 1).

For Route 2, the lipid moiety was introduced during PEC purification while the peptide was immobilized on the PMA solid support. A freshly prepared NHS ester (6 eq) in NMP was reacted in the presence of DIEA (3 eq) for 1 h at 50 °C, followed by 16 h at RT.

For Route 3, the lipid moiety was introduced directly on the SPPS resin after coupling of PEC-Linker RC+. The Mtt protecting group was selectively removed by 2x treatment for 15 min with DCM/HFIP/TIS (74:24:2), followed by coupling of the lipid moiety (6 eq) with DIC (9 eq) and Oxyma (5 eq) in NMP for 2 h at 50 °C and 16 h at RT.

Purification Results

Route 3 provided the best overall balance of UV-purity and recovery. Following lysine side-chain modification on the SPPS resin and subsequent PEC purification, Semaglutide was obtained with **78% UV purity** and a **total recovery of 26%** relative to the 1 mmol synthesis scale.

An aliquot of the PEC-purified material was subjected to a single RP-HPLC polishing run, increasing the UV purity to **>99%** (Figure 2)**.** This demonstrates the potential of PEC to reduce downstream chromatographic burden while retaining HPLC as a single final polishing step.

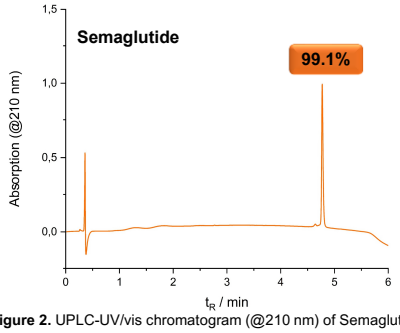


Figure 2. UPLC-UV/vis chromatogram (@210 nm) of Semaglutide after PEC purification and a single HPLC purification

Conclusion & Perspectives

Reducing the amino-acid excess from 5 to 3 eq during scale-up maintained comparable SPPS recoveries across all three strategies. At 1 mmol, Route 3 provided the highest crude UV-purity (52%) and SPPS recovery (40%) and enabled purification with PEC and a single HPLC run to achieve >99% UV-purity.

This route was therefore selected for further upscaling to 8 mmol on pilot scale synthesizer PurePep Sonata+ with additionally decreasing the amount of the lipid moiety needed for coupling on SPPS resin to 2 eq.

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SCAN ME

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References

[1] R. Zitterbart, et. al., Chemical Science, 2021

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