Sustainable Scale-Up of GLP-1 Agonist Peptides through Green Solid Phase Peptide Synthesis

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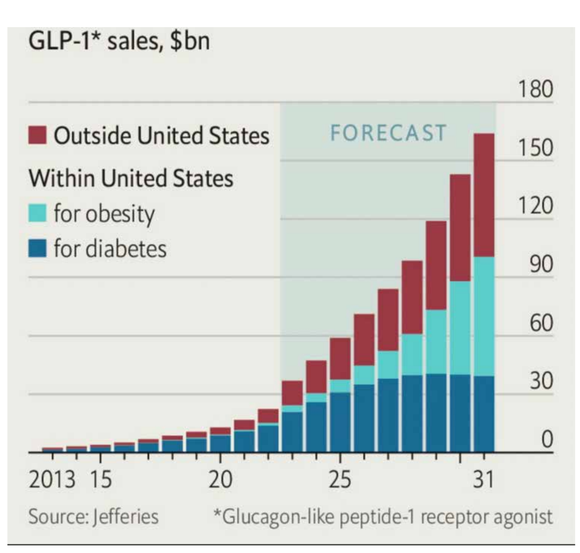
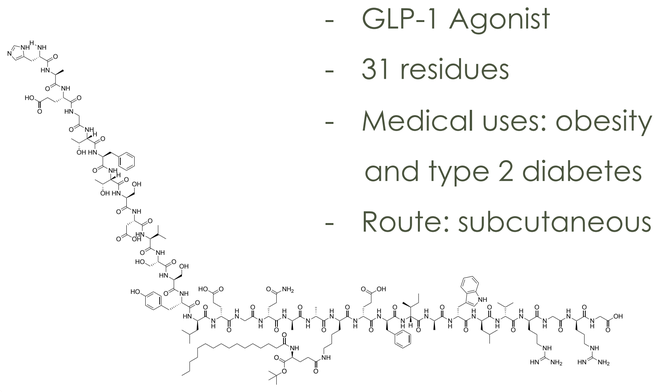
An insatiable Appetite

GLP-1 agonists are a transformative class of drugs used in managing **type 2 diabetes and obesity**, mimicking the GLP-1 hormone to enhance insulin secretion, reducing glucagon release, slowing gastric empty, and promoting satiety. Their dual efficacy in glycemic control and weight loss has positioned them as crucial in treating these conditions. Over the next decade, the GLP-1 agonist market is expected to grow significantly due to expanding indications, novel formulations, combination of therapies, and increasing recognition of their cardiovascular benefits. As pharmaceutical companies invest in research and development, these drugs are likely to become more accessible, revolutionizing chronic disease management and **positioning GLP-1 agonists as central to future metabolic and cardiovascular disorder treatments.**



Liraglutide: a case study for green SPPS



- GLP-1 Agonist
- 31 residues
- Medical uses: obesity and type 2 diabetes
- Route: subcutaneous
- Dosage: 0.6-3 mg daily
- Produced via traditional SPPS, using DMF and DCM and introducing a further orthogonality degree (Dde group) for a selective acylation on lysine

Experimental Set Up

Resin

Fmoc-Gly 2-CTC resin 0.33 mmol/g

Fmoc-Gly Wang TG resin 0.19mmol/g

Instrument

PurePep® Chorus
GYROS PROTEIN Technologies

Reagent System

Coupling: Fmoc-AA/DIC/Oxyma Pure 5/5/5 equivalents
Fmoc-Lys(Palm-Glu-OtBu)-OH/DIC/Oxyma Pure 2/2/2
in **BuOAc/DMSO** 7:3 (v/v)

Fmoc deprotection: 20% (v/v) Piperidine in **BuOAc/DMSO** (1:1)

Capping: 4M Pyridine + 4M Ac_2O in **BuOAc/DMSO** 7:3 (v/v)

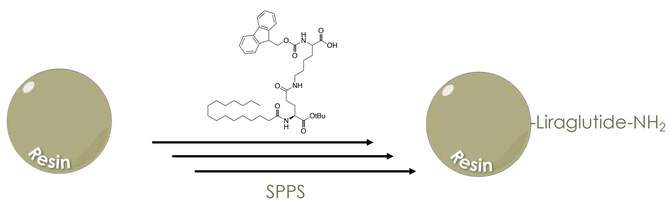
Main washing solvent: **EtOAc/DMSO** 8:2 (v/v)

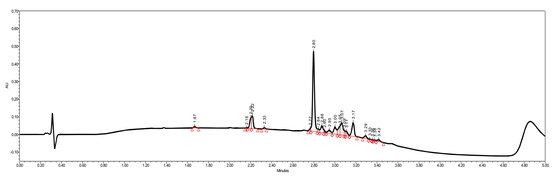
Coupling: r.t., 60' till 8th residue then 60'+60'

Fmoc deprotection: r.t., 5'+5'

Liraglutide green synthesis: strategy A

PEC® technology for purification





HPLC trace of a test cleavage of Liraglutide before PEC purification. Purity (%A/A): 45%

PEC-Linker coupling
4 eq. Linker
6 eq. Oxyma Pure
6 eq. DiPEA
16h, r.t. in BuOAc/DMSO 7:3

-Liraglutide-PEC-Linker

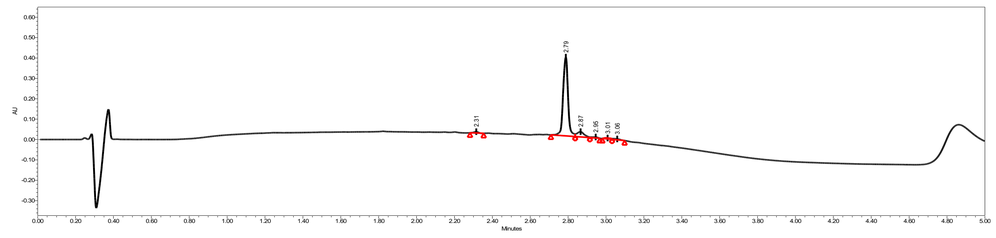
Cleavage
83% TFA
5% H_2O 5% EDT
5% Thioanisole 2% TIS
2h, r.t.
Et_2O, 10', -20°C.

Liraglutide-PEC-Linker

Immobilization
On functionalized Agarose beads
16h, r.t.

Liraglutide-PEC-Linker-Agarose bead

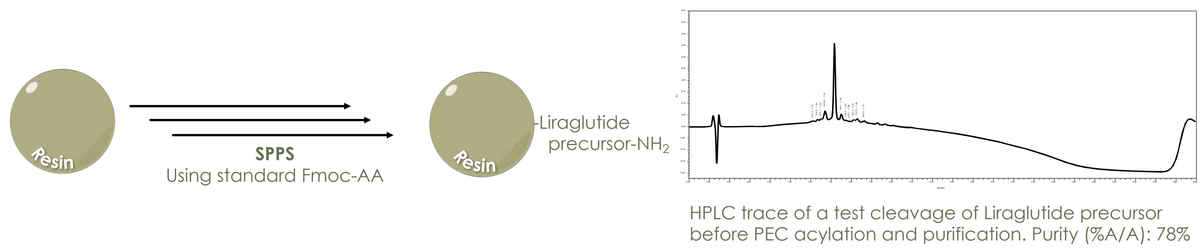
Release → **Liraglutide**



HPLC trace of Liraglutide after PEC purification, strategy A. Purity (%A/A): 83%, Overall Yield: 25%

Liraglutide green synthesis: strategy B scaled from 10µmol to 100µmol

PEC® technology for acylation and purification



HPLC trace of a test cleavage of Liraglutide precursor before PEC acylation and purification. Purity (%A/A): 78%

PEC-Linker coupling
4 eq. Linker
6 eq. Oxyma Pure
6 eq. DiPEA
16h, r.t. in BuOAc/DMSO 7:3

-Liraglutide precursor-PEC-Linker

Cleavage
83% TFA
5% H_2O 5% EDT
5% Thioanisole 2% TIS
2h, r.t.
Et_2O, 10', -20°C.

Liraglutide precursor-PEC-Linker

Immobilization
On functionalized Agarose beads
16h, r.t.

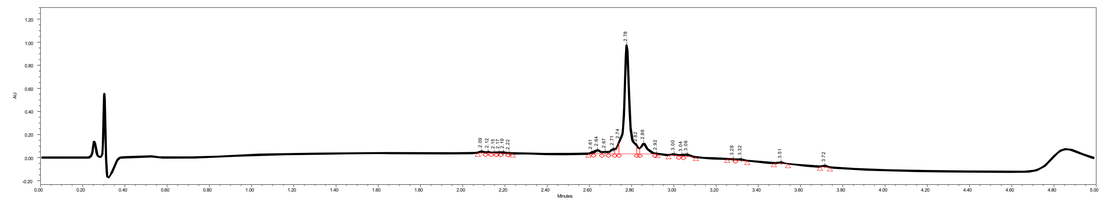
Liraglutide precursor-PEC-Linker-Agarose bead

Acylation → Liraglutide-PEC-Linker-Agarose bead

Acylation optimization

Building Block	Additive	Base	Time	Conv. (%A/A)	Purity after release (%A/A)	
Palm-L-Glu(OSu)-OtBu (4 eq.)	Oxyma Pure (4 eq.)	DiPEA (6 eq.)	5'	97	73	
	HOAt (4 eq.)	DiPEA (6 eq.)	PreActivation	99	78	✓
	Oxyma Pure (4 eq.)	Pyridine (6 eq.)	5 hours, r.t.	86	61	

Liraglutide-PEC-Linker-Agarose bead → Release → **Liraglutide**



HPLC trace of Liraglutide after PEC acylation and purification. Purity (%A/A): 78%, Overall Yield: 27%

Totally **scalable** on Gyros Protein Technologies SONATA + (work in progress)

No need of a further **orthogonality degree**

Green solvent mixtures

PEC® used for acylation and purification can be automated

Decreasing efforts for **final purification**

Suitable also for many **other GLP-1 agonist** (Semaglutide, Tirzepatide)

It can be futher improved by the use of **pseudoprolines**

The **excess** of expensive **building blocks** can be recovered and restored

References & Acknowledgments

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