

GAP peptide synthesis: Enabling efficient one-pot synthesis of tirzepatide fragments

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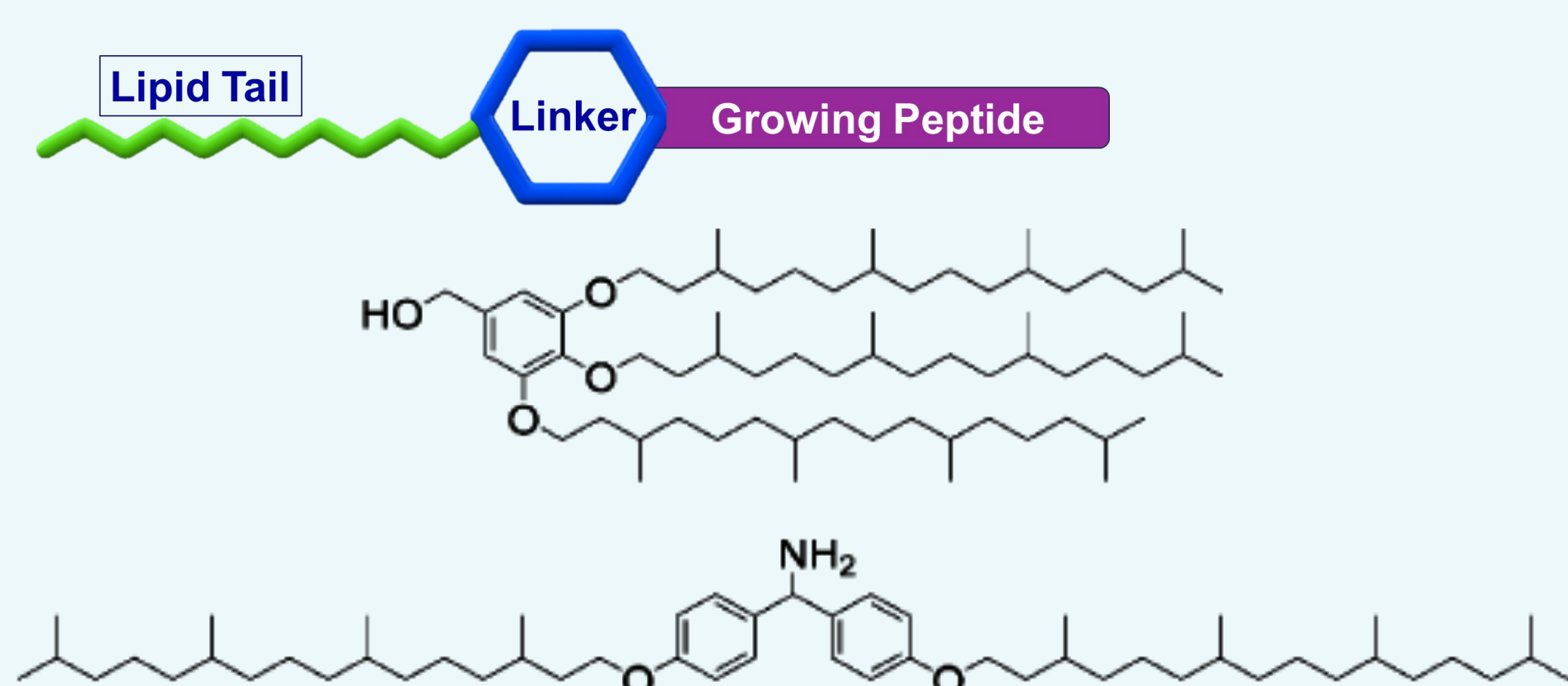
Background

Peptide manufacturing technology beyond solid-phase peptide synthesis (SPPS) is needed, due to the high reagent use, specialized equipment, limited global capacity, and cost of SPPS. GAP Peptide Synthesis (GAP-PS) is a type of tag-assisted liquid phase peptide synthesis (TA-LPPS) with enhanced solubility control, centered around the GAP Anchor, enabling efficient one-pot synthesis of peptide therapeutics with SPPS-like operability. Using tirzepatide as a model, fragments were synthesized through iterative coupling and deprotection cycles, realizing purity, yield, sustainability, and process economy benefits from the enhanced control. The approach achieved crude purities up to 96% and isolated yields of 98%, even for sterically demanding, hydrophobic sequences. GAP-PS offer a greener and more cost-effective route for assembling complex peptide intermediates.

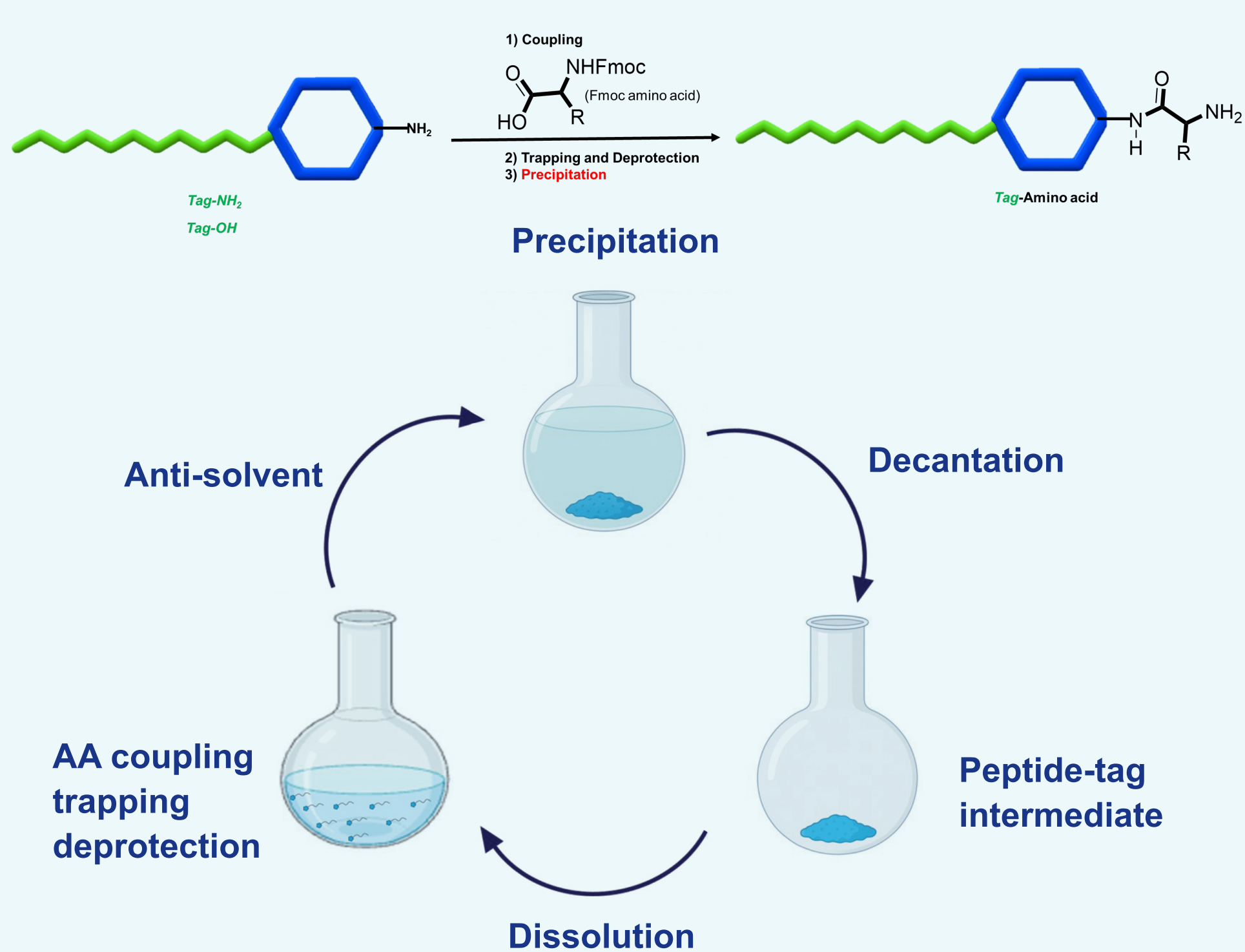
Tag-assisted liquid phase peptide synthesis

- Peptide synthesis on soluble tags in solution phase.
- Precipitation-based purification for peptide intermediate.
- Peptide synthesis:
Coupling → Trapping/Deprotection → Precipitation → Solvent Addition.

1. Tags structural composition

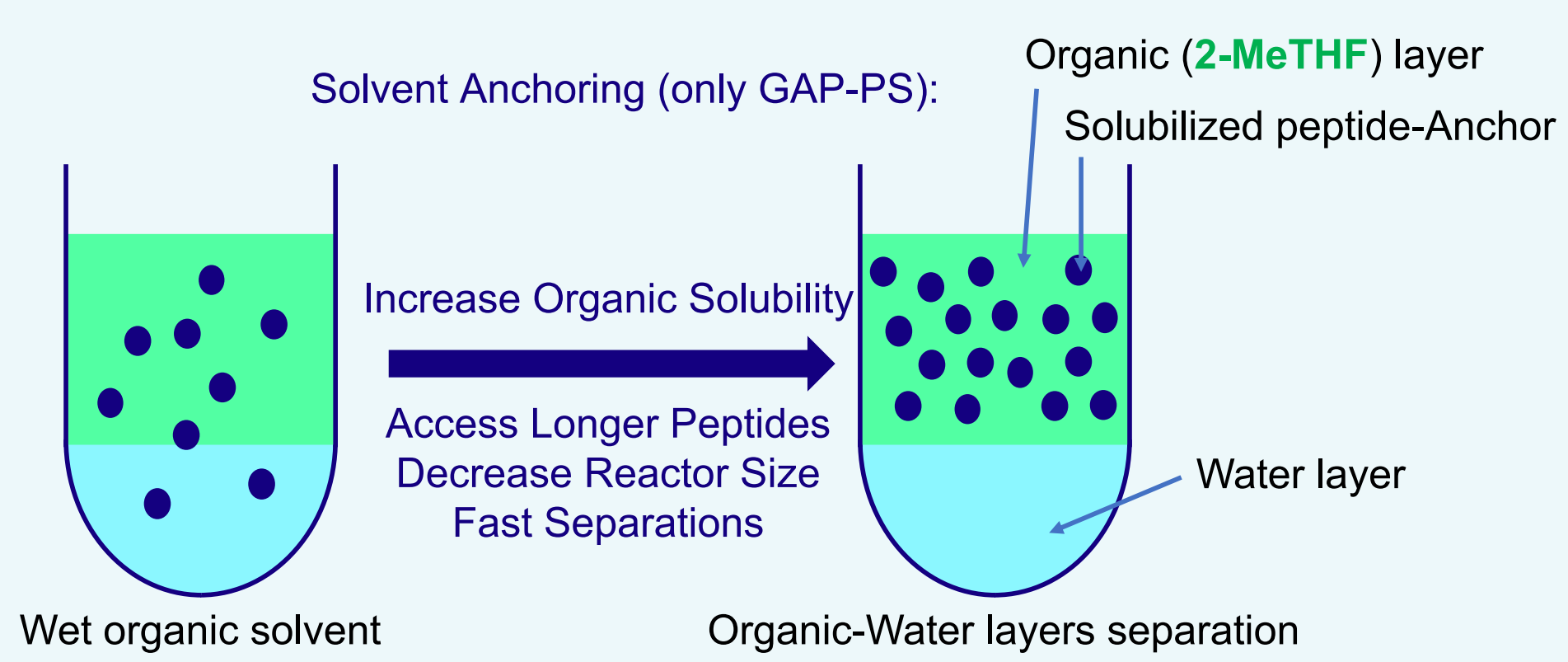


2. Peptide elongation



GAP peptide synthesis anchor design

- Include solvent anchor, good solubility control and solvent economical.
- Soluble Anchor, balance of hydrophobicity and hydrophilicity.



Conclusion and future work

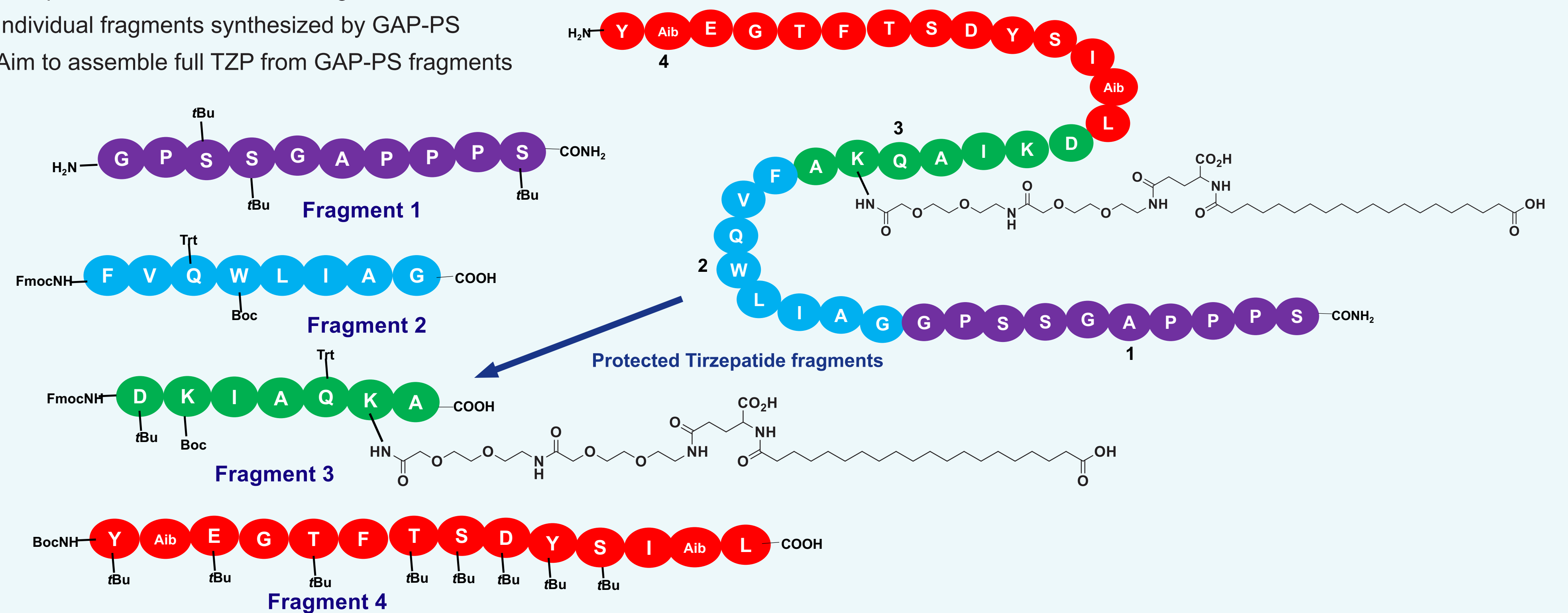
- Tirzepatide fragments 1, 3, and 4 synthesized on GAP Anchor.
- Future work include; fragment 2 synthesis,
→ Fragment condensation and purification of final tirzepatide.
→ Scale up to hundreds gram and kilogram scales.

Acknowledgements

We thank CRODA PHARMA for funding this work.

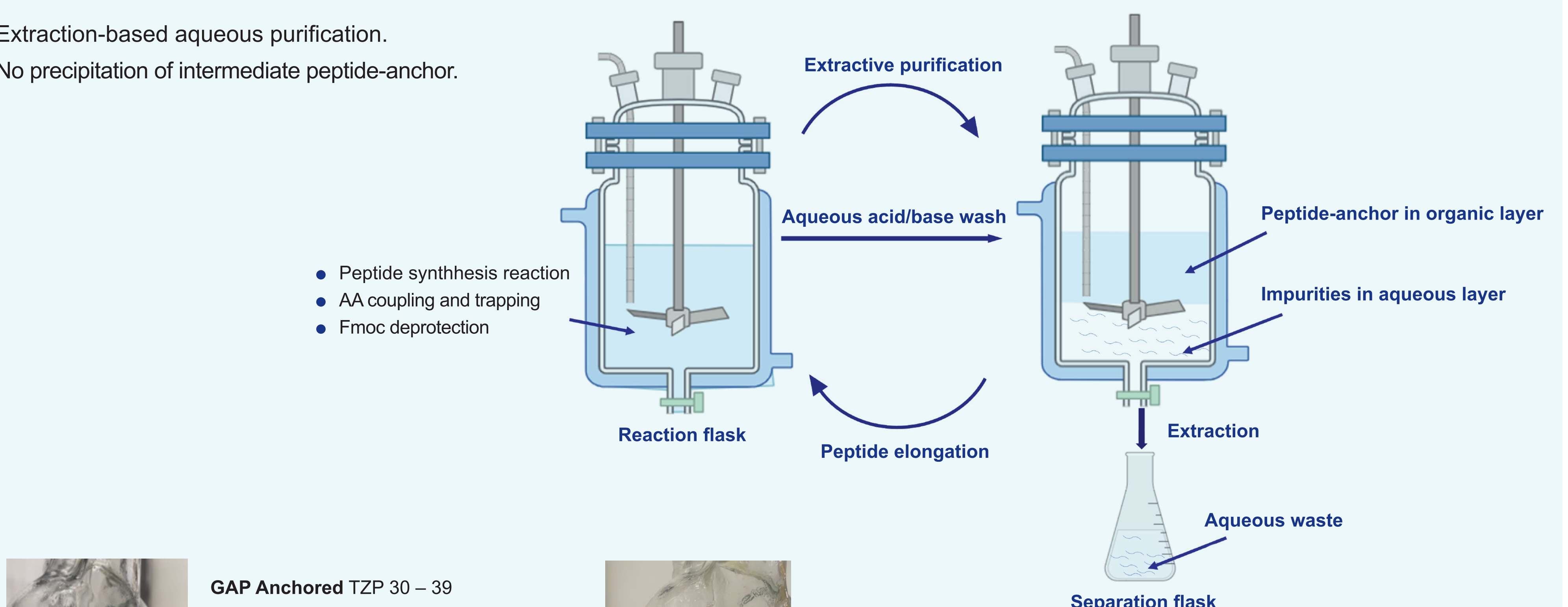
Tirzepatide fragmentation and synthesis by GAP anchor

- Currently, TZP is manufactured by Hybrid SPPS/LPPS
- Tirzepatide is broken into four fragments
- Individual fragments synthesized by GAP-PS
- Aim to assemble full TZP from GAP-PS fragments



GAP peptide synthesis process cycle

- Extraction-based aqueous purification.
- No precipitation of intermediate peptide-anchor.



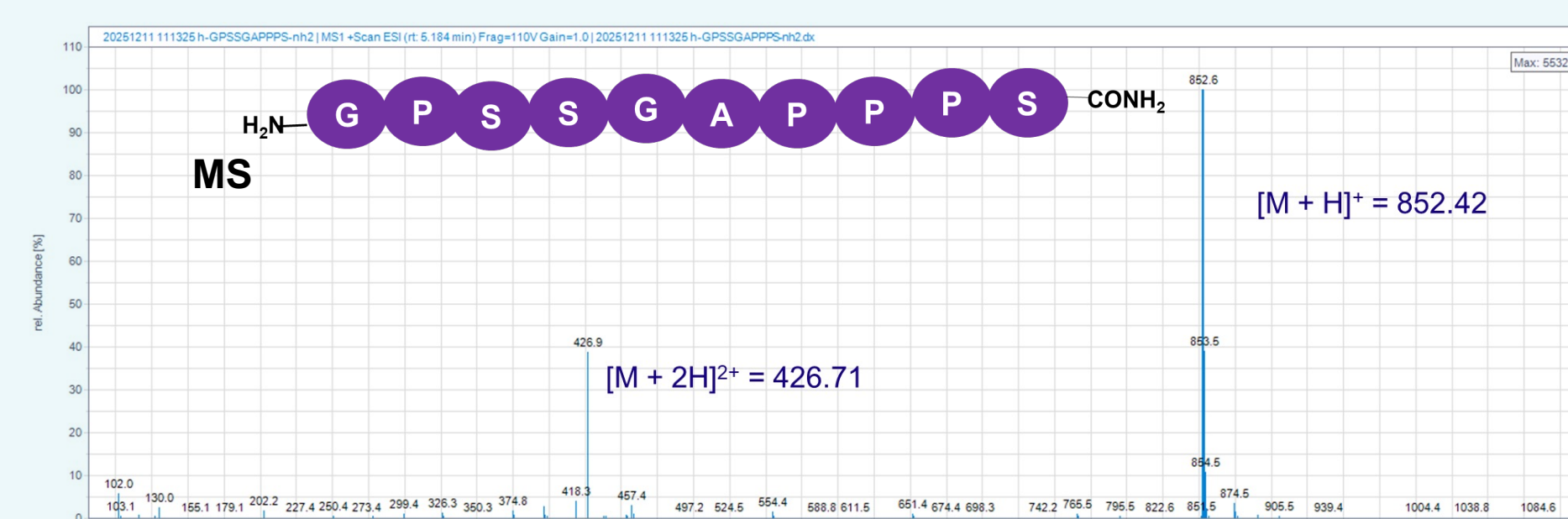
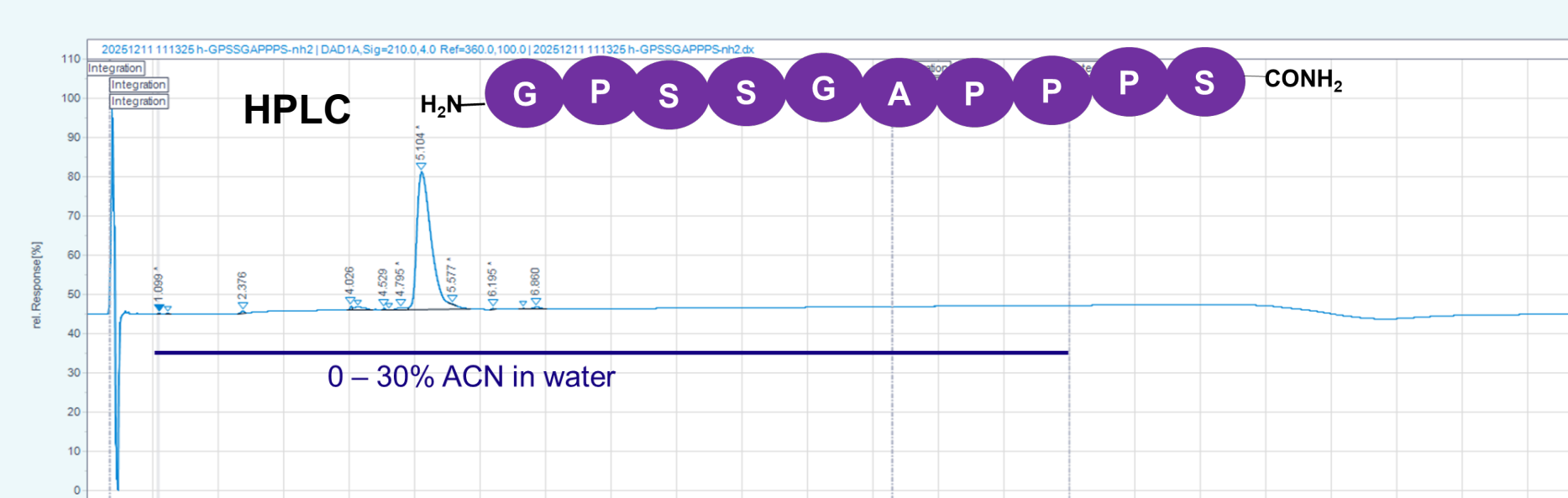
GAP Anchored TZP 30 – 39
GPSSGAPPSP-amide
NO optimization (1 FTE x 1 wk)
Made using Suberoyl-GAP Anchor
Single Fragment, 110 mg / mL
Now up to 160 mg / mL with optimization

GAP Anchored TZP 1-14
Boc-Y-Alb-EGTFTSDYSI-Alb-L-Anchor
NO optimization (1 FTE x 1 wk)
Single Fragment, 130 mg / mL
Now up to 200 mg / mL with optimization

GAP tirzepatide fragment synthesis results

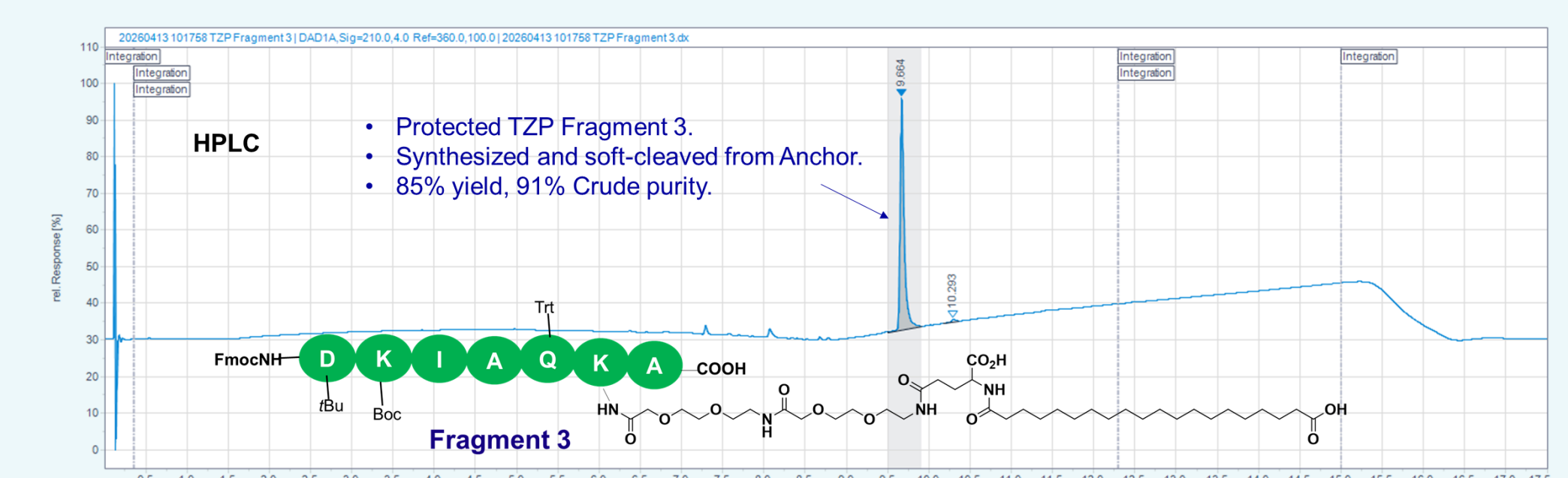
TZP fragment 1

- Synthesized, hard-cleaved and precipitated.
- 93% yield and 95% crude purity (No chromatography).



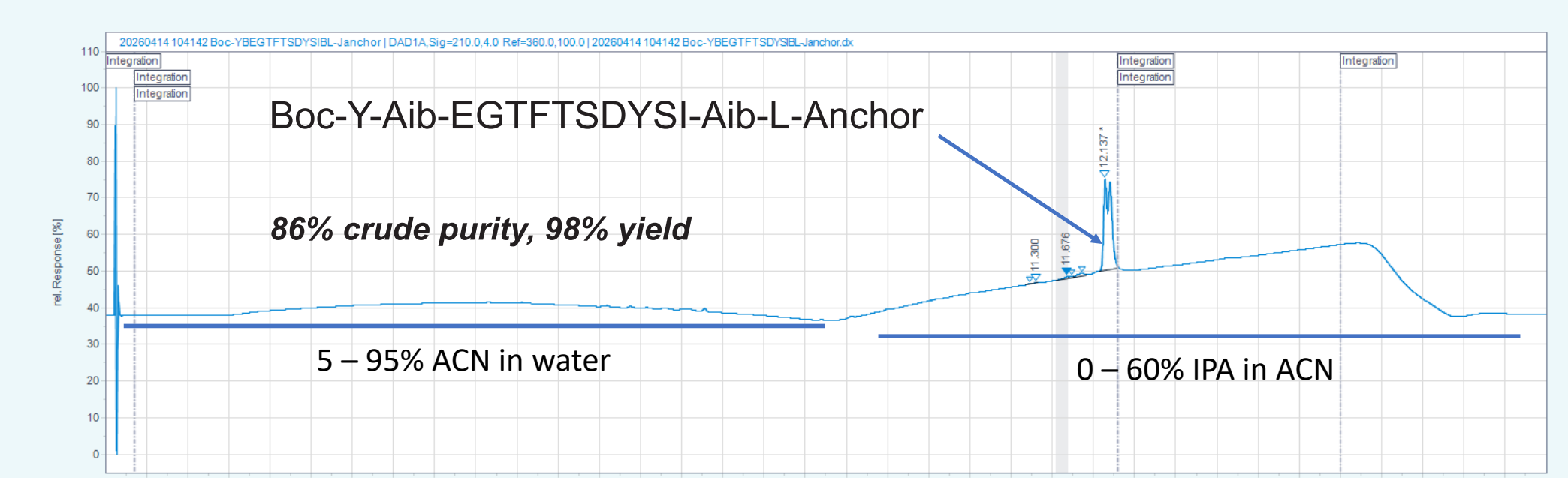
TZP fragment 3

- Synthesized and soft-cleaved, no chromatography. 85% yield, 92% crude purity.



TZP fragment 4

- Protected TZP fragment 4 synthesized on GAP Anchor.
- 86% crude purity, 98% yield



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