Tag-Assisted Peptide Synthesis (TAPS)

Enabling Efficient and Sustainable Peptide Synthesis and Purification

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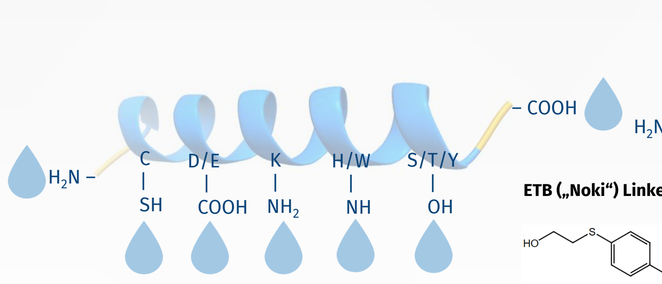
https://doi.org/10.17952/38EPS.2026.P005

Abstract

Discovery and production of synthetic peptides significantly progressed in the last years resulting in the fact that complex peptides like GLP-1 analogues are produced in large quantities for commercial consumption. However, peptide length, solubility, secondary structure and modifications challenge to develop robust synthesis processes. Especially aggregation-prone sequences can be handled more efficiently when adding semi-permanent solubilizing tags during synthesis and purification.

Herein we demonstrate how tags placed at the C-terminus of a peptide can help to manufacture a difficult peptide sequence in high yield and how such tags can be removed. Different tag linkers enable peptide acids or amides, and the choice of the linker may affect coupling efficiency, resin cleavage, purification, and tag removal.

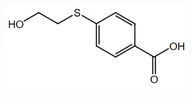
Potential Tag and Linker Positioning



Concept of C-Terminal Arg_6-Tagging with Cleavable Linkers

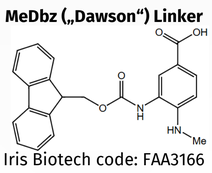


ETB („Noki“) Linker



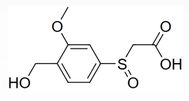
Iris Biotech code: RL-8715
Oxidative activation by mCBPA
Cleavage with DEA and piperidine

MeDbz („Dawson“) Linker



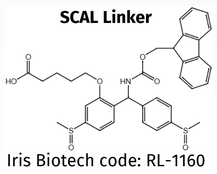
Iris Biotech code: FAA3166
Activation by acylation
Cleavage by hydrolysis

Mmsb Linker



Iris Biotech code: RL-3895
Reductive activation by NMe_4I
Cleavage with TFA

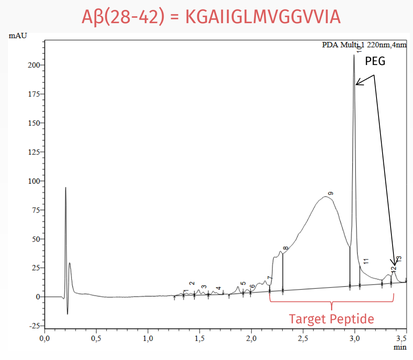
SCAL Linker



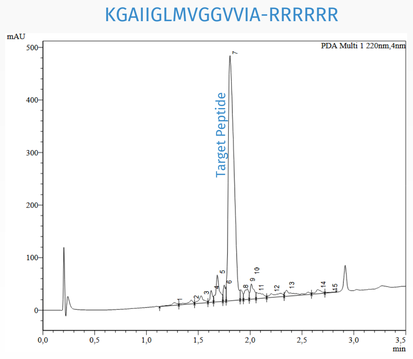
Iris Biotech code: RL-1160
Reductive activation by NMe_4I
Cleavage with TFA

Aggregation Suppression

Aβ(28-42) = KGAIIGLMVGGVVIA



KGAIIGLMVGGVVIA-RRRRRR



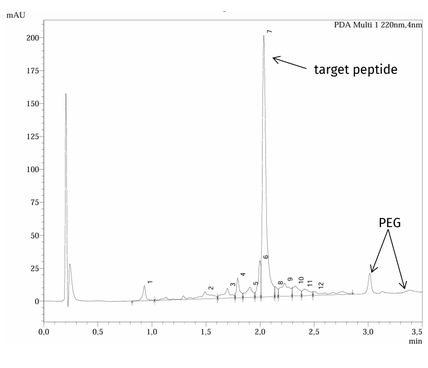
KGAIIGL-Nle-VGGVVIA-ETB(O_2)-R_6-NH_2 KGAIIGL-M-VGGVVIA-MeDbz-R_6-NH_2 KGAIIGL-M(O)-VGGVVIA-Mmsb-R_6-NH_2 KGAIIGL-M(O)-VGGVVIA-SCAL-R_6-NH_2

C-terminal Arg_6-tagging can convert the hydrophobic and aggregation-prone amyloid beta sequence 28-42 into a well synthesizable and separatable peptide.

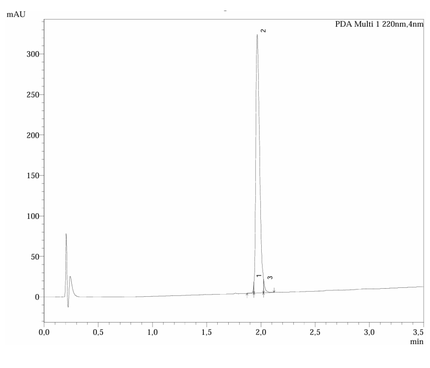
Results for Arg_6-Tagging with Different Cleavable Linkers

- **ETB (“Noki”) Linker** yielding peptide acids

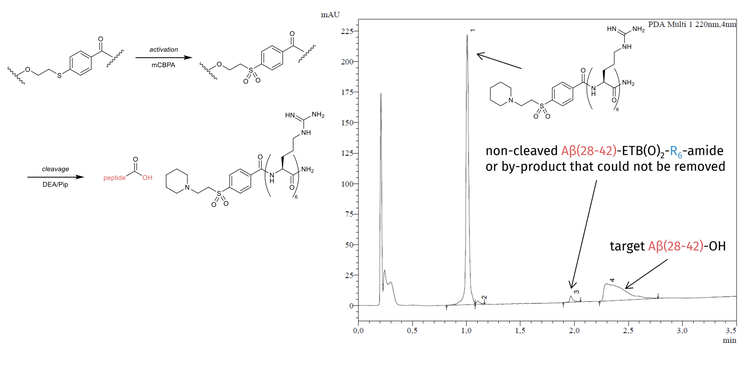
crude KGAIIGL-Nle-VGGVVIA-ETB(O_2)-RRRRRR-NH_2



purified KGAIIGL-Nle-VGGVVIA-ETB(O_2)-RRRRRR-NH_2



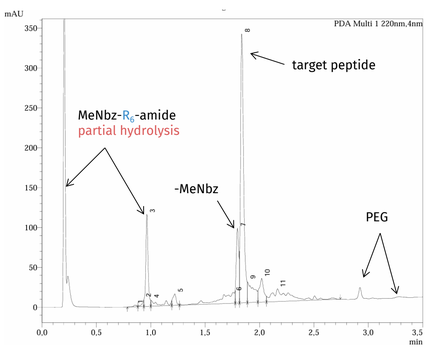
Tag removal by ETB cleavage to yield KGAIIGL-Nle-VGGVVIA-OH



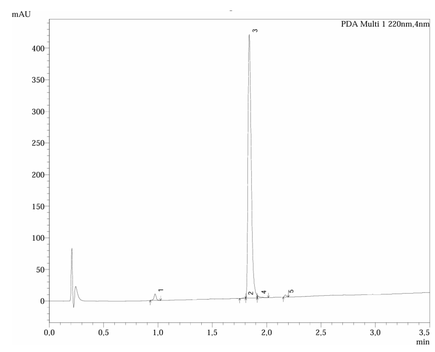
Linker and first amino acid coupling were difficult; slight tendency for diketopiperazine formation; linker activation on the resin and cleavage from the resin worked nicely; purification of the tagged peptide worked well with a little bit of sticking to the column; linker cleavage by piperidine released the desired peptide as a peptide acid.

- **MeDbz (“Dawson”) Linker** yielding peptide acids

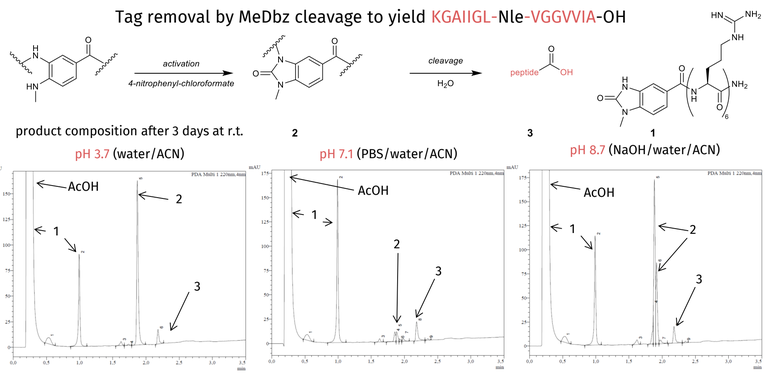
crude KGAIIGL-Nle-VGGVVIA-MeDbz-RRRRRR-NH_2



purified KGAIIGL-Nle-VGGVVIA-MeDbz-RRRRRR-NH_2



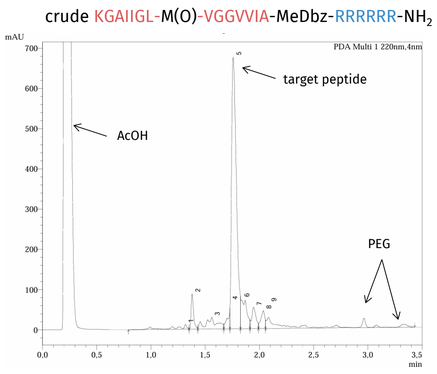
Tag removal by MeDbz cleavage to yield KGAIIGL-Nle-VGGVVIA-OH



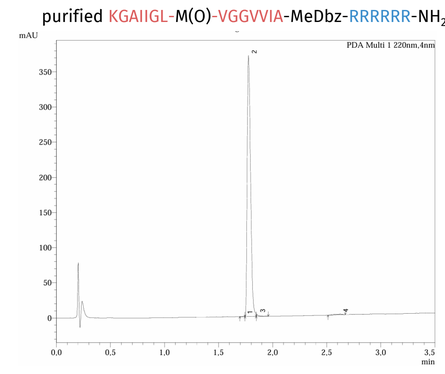
Linker activation on resin did not perform well – taking several repeats; premature cleavage and linker fragmentation reduced the yield of crude peptide; purification of tagged peptide worked fine; finally, the linker did not hydrolyze completely at any pH - even after days; in this prolonged time frame, the linker was rather decomposing than hydrolyzing, resulting in numerous undesired side products and low yield of desired peptide acid.

- **Mmsb Linker** yielding peptide acids

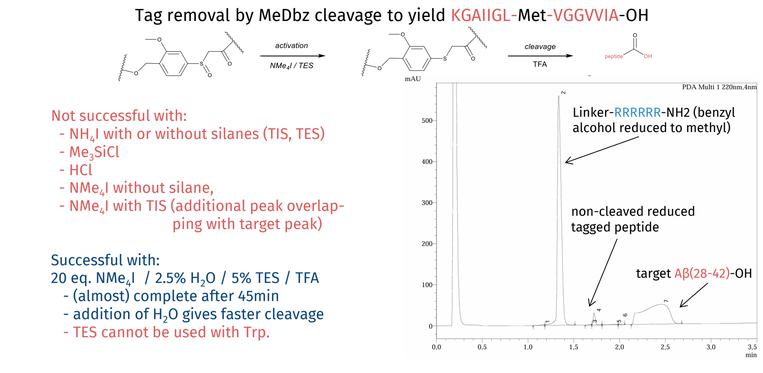
crude KGAIIGL-M(O)-VGGVVIA-MeDbz-RRRRRR-NH_2



purified KGAIIGL-M(O)-VGGVVIA-MeDbz-RRRRRR-NH_2



Tag removal by MeDbz cleavage to yield KGAIIGL-Met-VGGVVIA-OH



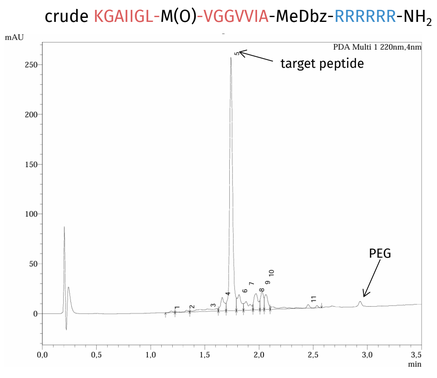
Not successful with:
- NH_4I with or without silanes (TIS, TES)
- Me_3SiCl
- HCl
- NMe_4I without silane,
- NMe_4I with TIS (additional peak overlapping with target peak)

Successful with:
20 eq. NMe_4I / 2.5% H_2O / 5% TES / TFA
- (almost) complete after 45min
- addition of H_2O gives faster cleavage
- TES cannot be used with Trp.

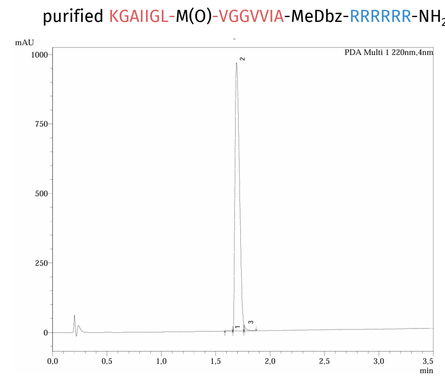
Mmsb linker coupling and peptide synthesis worked well; yield of crude tagged peptide and purification were satisfying; first standard protocols for linker cleavage did not work at all; finally, linker reduction worked with tetramethylammoniumiodide plus a silane (TIS), however producing an adduct peak overlapping with the target peak; this could be overcome using triethylsilane (TES) with tetramethylammoniumiodide, TFA, and some water;
note: TES is incompatible with tryptophan!

- **SCAL Linker** yielding peptide amides

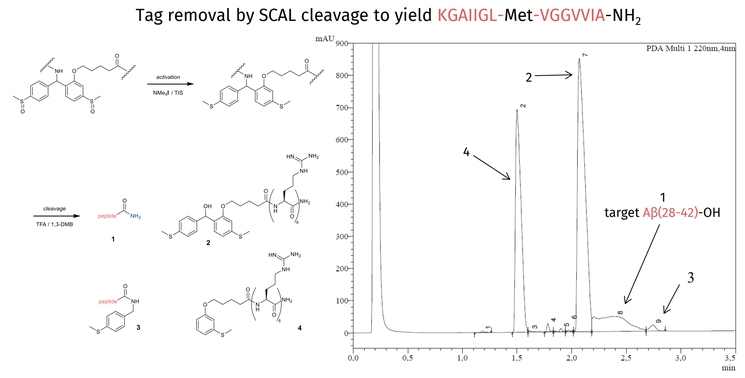
crude KGAIIGL-M(O)-VGGVVIA-MeDbz-RRRRRR-NH_2



purified KGAIIGL-M(O)-VGGVVIA-MeDbz-RRRRRR-NH_2



Tag removal by SCAL cleavage to yield KGAIIGL-Met-VGGVVIA-NH_2



SCAL linker coupling and peptide synthesis worked smoothly, yielding the largest quantities of crude and purified tagged peptide of the four linker variants; during linker reduction, Met(O) is reduced to Met; cleaving the linker, we found fragmentation behavior that is known also from other benzhydryl systems, yielding also a peptide still having “one half” of the former benzhydryl group attached (3); could be minimized by adding 1,3-dimethoxybenzene.

Summary

C-terminal Arg_6-tagging can strongly improve peptide yield and purity. However, removing the tag by cleavable linkers is not trivial and conditions have to be optimized case by case.

References:

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- An efficient Fmoc-SPPS approach for the generation of thioester peptide precursors for use in native chemical ligation; J. B. Blanco-Canosa, P. E. Dawson; *Angew. Chem. Int. Ed. Engl.* 2008; **47(36):** 6851-6855. https://doi.org/10.1002/anie.200705471
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