Purification of sparingly soluble peptides by temporary solubilization

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Introduction

Many small to medium-sized peptides are sufficiently soluble in dilute aqueous acetic acid or aqueous buffer systems to be purified efficiently by RP-HPLC. On the other hand, due to aggregation even short peptides may be sparingly soluble in aqueous solvent systems impeding proper purification. To overcome this problem, Englebretsen et al. developed „solubilizing tails“ , i.e. constructs containing a large proportion of Lys or Arg and linked it *N*- or C-terminally by a selectively cleavable entity to the insoluble sequence, for Boc-SPPS [1]. Finally, the constructs were removed from the purified solubilized peptide with aqueous base.

Such a construct, attached to the *N*-terminus, could be introduced conveniently following SPPS. Accordingly, based on an N^{α}-protecting group described by Tesser et al. [2] we developed a linker moiety connecting tail and peptide, cleavable under much milder basic conditions precluding damage of the purified peptide. The synthesis of this compound is shown in Fig. 1. To test this type of modification, the synthesis of the poorly soluble HCV NS5B protein (407-419), SMSYTWTGALITP, was performed. The strategy based on this linker and the solubilizing tail $(Lys\text{-}Pro)_4$ is outlined in Fig. 2.

Peptides may also be temporarily solubilized by generating the isomeric depsipeptides (i.e., *O*-acyl isopeptidic bonds to Ser or Thr) [3-5] which rearrange to the desired peptide in the presence of bases. For comparison, this modification was also investigated in our model sequence following the protocol shown in Fig. 3.

The results of both approaches will be discussed in this presentation.

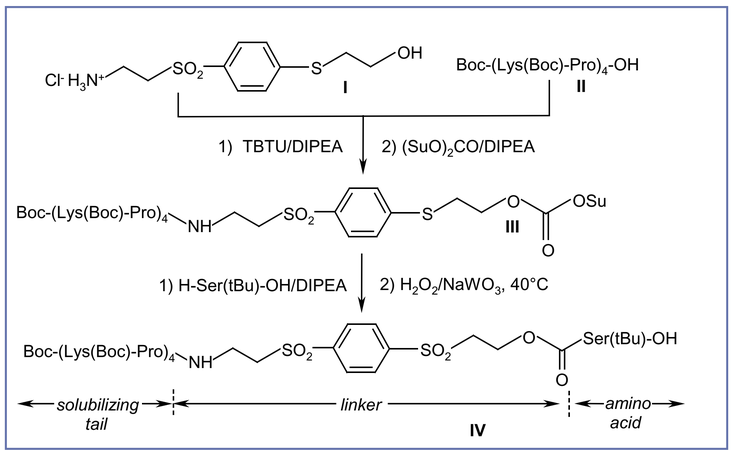

Fig. 1: Synthesis of the Ser(tBu) derivative of the solubilizing tail construct.

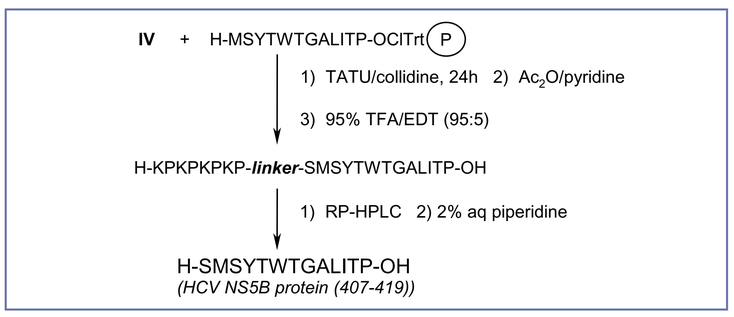

Fig. 2: Synthesis of HCV NS5B protein (407-417) applying temporary solubilization.

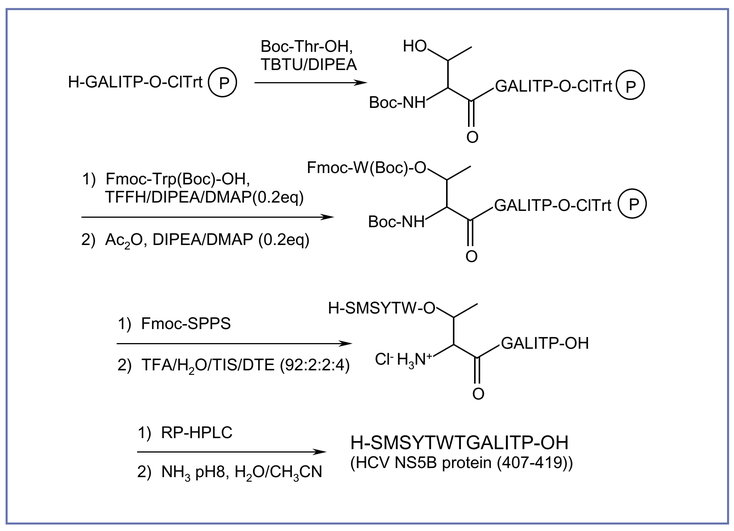

Fig. 3: Synthesis of HCV NS5B protein (407-419) via rearrangement of the Trp^{412}-Thr^{413}-isopeptide.

Results and Discussion

Solubilizing Tail

Thioether **I** was readily obtained from commercially available starting materials. Coupling of **I** to **II** under standard conditions followed by activation with di(succinimidyl) carbonate yielded the key intermediate **III**. **III** was then reacted with the *N*-terminal amino acid followed by oxidation with 35% $H_2O_2/NaWO_3$ in EtOH and coupling to the peptide resin. **IV** tolerates slightly basic conditions. Hence, it is compatible with routine coupling conditions applied during SPPS. Unfortunately, the tailed derivatives of Met and Cys cannot be obtained by this method due to their sensitivity towards oxidation. The inverse strategy consisting of oxidizing **I** (preceding removal of Boc) followed by coupling of the tail **II** had to be abandoned as attempts to obtain reactive carbonates from the intermediate Boc-$(Lys(Boc)\text{-}Pro)_4$-2-(4-[2-aminoethyl]-sulfonio)-phenylsulfonio-ethanol applying $(SuO)_2CO$, CDI or triphosgene in the presence of bases failed. Even weak bases generated the vinylsulfone from the carbonate.

The sparingly soluble HCV NS5B protein (407-419) SMSYTWTGALITP was chosen to test this type of modification. **IV** (2 eq) was coupled to H-MSYTWTGALITP-ClTrt-® using TATU/collidine in DMF. A pure product (HPLC: > 95%) was obtained from the crude water-soluble „tailed“ peptide (HPLC: 47%) by standard RP-HPLC followed by rapid cleavage of the tail with 2% aqueous piperidine. The desired peptide precipitated whereas the solubilizing tail-piperidine adduct remained in solution. A choice of representative analytical HPLC-chromatograms demonstrating the beneficial effect of solubilization is assembled in Fig. 4.

O-acyl Isopeptide

The motif Trp^{412}-Thr^{413} was chosen for incorporating the isopeptide link in the model peptide. Various protocols for esterification of secondary alcohols were tested. In most cases, low conversions and considerable amounts of the D-Trp-epimer (which was obtained independently as a reference) were observed. The highly active coupling reagent TFFH [6], which mediates the acylation of alcohols in the presence of DMAP via the acid fluoride [7] turned out to be the most efficient reagent evaluated in this study. The esterification of Fmoc-Trp(Boc)-OH to the β-hydroxyl moiety of terminal Boc-Thr^{413} applying TFFH/DIPEA and 0.2 eq of DMAP (15 h, at room temperature) resulted in a moderate yield and nevertheless, in an excellent optical purity, see Fig. 5A. The remaining unreacted hydroxyl groups were blocked by acetylation. Elongation of the peptide followed by acidolytic cleavage led to the isopeptide H-T(WTYSMS-H)GALIPT-OH together with the capped peptide (ratio approx. 2.3:1) and only traces of the D-Trp epimer. The crude material could be smoothly purified by RP-HPLC followed by ion exchange. In the presence of NH_3 at pH 8, the resulting isopeptide acetate readily rearranged to the native peptide. Analytical HPLC-chromatograms of the crucial stages of this synthesis are compiled in Fig. 5.

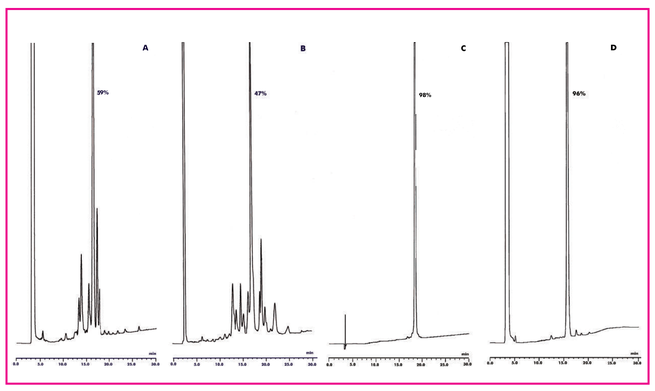

Fig. 4: Analytical HPLC-profiles of crude des-Ser^{407}HCV (A), crude tailed peptide (B), purified tailed peptide (C), and product obtained by treatment with 2% aqueous piperidine (D).
Conditions of HPLC - column: Bakerbond C_{18} 300Å, buffer: 0.1% TFA (A 10%, B 60% CH_3CN), gradient: 25%B to 55%B in 30 min, flow: 1ml/min, detection at 220 nm

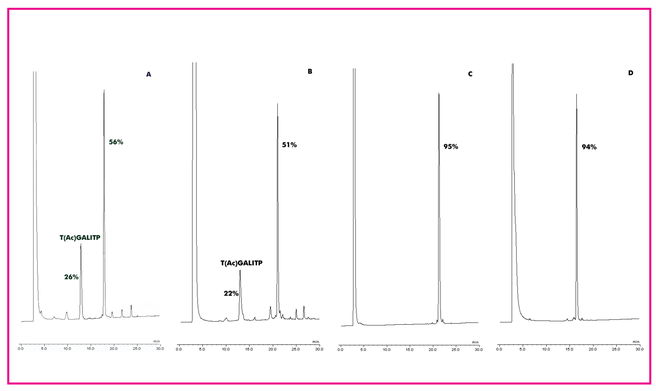

Fig. 5: Analytical HPLC-profiles of crude H-T(W)GALITP-OH(A), crude isopeptide (B), purified isopeptide (C), and peptide obtained by O-N migration (D).
Conditions of HPLC - column: Vydac 218TP54, buffer: 0.1% TFA (A 1%, B 100% CH_3CN), gradient: 15%B to 45%B in 30 min, flow: 1ml/min, detection at 220nm.

Conclusion and Outlook

The sparingly soluble model peptide HCV NS5B (407-419) was readily purified by RP-HPLC following either tagging or conversion to the isopeptide. Both methods permit the specific regeneration of the target product.

Albeit more laborious, the incorporation of the N^{α}-solubilizing tail via the linker to the *N*-terminal amino acid is more generally applicable and better scalable. For increasing its solubilizing efficiency, the tail may be elongated or modified. The chemistry of the linker described in this work precludes *N*-terminal Met or Cys. Hence, further linker moieties allowing the introduction of these *N*-termini are under investigation.

The isopeptide approach is more straightforward, but the necessity of Ser or Thr in the appropriate position limits this method. For a more efficient introduction of an isopeptide modification, the synthesis of the corresponding buiding blocks is recommended [8].

References

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Abbreviations

CDI	carbonyl diimidazole
TATU	2-(1H-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TFFH	tetramethylfluoroformamidinium hexafluorophosphate